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Poultry Farming

New Perspectives and Applications

Edited by Guillermo Téllez-Isaías



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Contributors

Brilliant Agaviezor, Shamal A. Al-Muffti, Mohammed A. Al-Badrani, Sérgio Eustáquio Lemos Da Silva Eustáquio Lemos da Silva, Nayane Lopes Ferreira, Vitor Simão da Silva, Renata Vieira Chaves Gabriel, Daniela Ribeiro Roldão, Vanessa Silva Miranda, Vagno Espíndola da Silva, Lucas Peraphan, Hafiz Ullah, Angélica Patricia Huertas Moreno, Gonzálo Téllez Iregui, Elena Amparo del Rocío Gómez Moreno, Andrea Patricia Varón Garcia, Pratap Sriram Sriram Sundar, Chandan Chowdhury, Sagar Kamarthi, Emmanuel-Ohagenyi Ifemma Justina, Simeon Ogochukwu Christopher Ugwu, Samuel Abanigbe, Mjabuliseni Ngidi, Temitope Ojo, Paul Orowole, George Agbele, Victor A. Palomino-Tapia, Omar Francisco Francisco Prado Rebolledo, Guillermo Téllez-Isaías, Arturo Cesar Garcia-Casillas, Juan Augusto Hernández Rivera, Luis-Miguel Gomez-Osorio, Ben Dehaeck, Carlos Cuello, Jenny-Jovanna Chaparro-Gutierrez, Sara Lopez-Osorio, Hanan H. Abd Elhafeez, Taymour M. El-Sherry, Catrin Sian Rutland, Mohamed A. M. Sayed, Solomon Ajide, Ibiyemi Opowoye, John Makinde, Zainab Bello, Afiniki Ahmadu, Moji Adeniran, Maryam Bot

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Meet the editor



Guillermo Téllez-Isaías received his DVM and MS in Veterinary Sciences from the National Autonomous University of Mexico (UNAM), and his Ph.D. from Texas A&M University, USA. He worked as a professor at UNAM for 16 years, eight of which he served as the head of the Avian Medicine Department, College of Veterinary Medicine. Dr. Téllez was president of the National Poultry Science Association of Mexico and is a member of the Mexican Veterinary Academy and the Mexican National Research System. Currently, he is a research professor at the Center of Excellence in Poultry Science, University of Arkansas, USA. His research is focused on poultry gastrointestinal models to evaluate the beneficial effects of functional foods to enhance intestinal health and disease resistance.

Contents

Preface	XI
Section 1 Poultry Management	1
Chapter 1 The Resilience Strategies of Smallholders' Poultry Actors <i>by Samuel Abanigbe, Mjabuliseni Ngidi, Temitope Ojo and Paul Orowole</i>	3
Chapter 2 Poultry Environment <i>by George Agbele</i>	11
Chapter 3 Antibiotics Alternatives in Poultry Production in Sub-Sahara Africa <i>by Brilliant Agaviezor</i>	23
Section 2 Poultry Nutrition	35
Chapter 4 Feed Sustainability and Efficiency <i>by Hafiz Ullah</i>	37
Section 3 Poultry Diseases	51
Chapter 5 From Understanding the Immune Response against Coccidiosis to the Use of Coccidia Vaccines <i>by Luis-Miguel Gomez-Osorio, Ben Dehaeck, Carlos Cuello, Jenny-Jovanna Chaparro-Gutierrez and Sara Lopez-Osorio</i>	53
Chapter 6 Effect of Fallowing on the Viability of <i>Salmonella</i> spp. in Poultry Facilities <i>by Sérgio Eustáquio Lemos da Silva, Nayane Lopes Ferreira, Vanessa Silva Miranda, Vitor Simão da Silva, Renata Vieira Chaves Gabriel, Daniela Ribeiro Roldão, Vagno Espíndola da Silva and Lucas Peraphan</i>	67

Chapter 7	77
Poultry Farming: New Perspectives and Applications Chapter – Parasitic Diseases of Chickens <i>by Mohammed A. Al-Badrani and Shamal A. Al-Muffti</i>	
Chapter 8	95
Autogenous Vaccines in the Poultry Industry: A Field Perspective <i>by Victor Palomino-Tapia</i>	
Section 4	
Breeding and Hatchery	115
Chapter 9	117
Natural Products as an Alternative to Formaldehyde for Disinfection of Fertile Eggs in Commercial Hatcheries <i>by Omar Francisco Prado Rebolledo, Arturo César García Casillas, Guillermo Téllez-Isaías and Juan Augusto Hernández Rivera</i>	
Chapter 10	135
The Relationship of Sperm Motility Pattern and Its Ability to Agglutinate with Vaginal Sperm Selection, Uptake in Sperm Storage Tubules and Competitiveness <i>by Mohamed A.M. Sayed, Hanan H. Abd Elhafeez, Catrin Sian Rutland and Taymour M. El-Sherry</i>	
Chapter 11	147
Bottleneck in Creating Layer Breeds of Chickens in Nigeria <i>by Emmanuel-Ohagenyi Ifemma Justina and Simeon Ogochukwu Christopher Ugwu</i>	
Section 5	
Processing	171
Chapter 12	173
Identification and Analysis of Safety Hazards and CCPs in a Chicken Meat Production Chain <i>by Angélica Patricia Huertas Moreno, Elena Amparo del Rocío Gómez Moreno, Gonzálo Téllez Iregui and Andrea Patricia Varón García</i>	
Chapter 13	201
Principles for Designing Green, Lean, and Smart Microfactories: Chicken as a Model <i>by Pratap Sriram Sundar, Chandan Chowdhury and Sagar Kamarthi</i>	
Chapter 14	221
Workable Alternatives to Conventional Inputs in Poultry Farming <i>by Solomon Ajide, Ibiyemi Opowoye, John Makinde, Zainab Bello, Maryam Bot, Afiniki Ahmadu and Moji Adeniran</i>	

Preface

Poultry farming has long been an essential aspect of human civilization, providing us with a consistent supply of eggs, meat, and other products. The world of poultry farming is rapidly evolving, and new perspectives and applications are shaping the industry like never before. *Poultry Farming – New Perspectives and Applications* sheds light on the dynamic changes taking place in this field and offers valuable insights to poultry farmers, researchers, and enthusiasts.

In recent years, advancements in technology, scientific research, and sustainable practices have revolutionized the way we approach poultry farming. This book brings together a collection of expert perspectives, innovative ideas, and practical strategies that reflect the current state of the industry and provide a glimpse into its future. Whether you are a seasoned poultry farmer or someone interested in exploring this field, this book will serve as a valuable resource.

Poultry Farming – New Perspectives and Applications covers a wide range of topics, including breeding and genetics, nutrition, housing and welfare, disease management, environmental sustainability, and emerging trends. Each chapter is written by a knowledgeable expert, drawing from their experience and expertise to provide comprehensive and up-to-date information.

One of the key focuses of this book is the integration of technology into poultry farming. From automated systems for feed delivery and environmental control to advanced data analytics and precision farming techniques, technology is transforming the way we manage and monitor poultry production. These advancements not only enhance efficiency but also contribute to improved animal welfare and environmental sustainability.

Another important aspect explored in this book is the growing interest in alternative and sustainable poultry farming practices. With the increasing demand for organic and free-range products, farmers are exploring new methods that prioritize animal welfare, reduce environmental impact, and promote consumer health. The book delves into these approaches, offering guidance on transitioning to sustainable production systems while ensuring profitability.

As the world population continues to grow, the importance of poultry farming in ensuring food security becomes even more crucial. This book acknowledges the challenges faced by the industry, such as disease outbreaks, market fluctuations, and regulatory changes, and provides insights on how to navigate them effectively. It also emphasizes the importance of biosecurity measures, responsible antibiotic usage, and best management practices to maintain healthy flocks and safeguard public health.

The contributors to this book are renowned experts and practitioners in the field of poultry farming. Their collective knowledge and experiences bring a wealth of information to readers, making this book an indispensable guide for anyone involved or interested in poultry farming.

It is our hope that *Poultry Farming – New Perspectives and Applications* serves as a catalyst for positive change in the poultry industry. By embracing innovation, sustainability, and best practices, we can collectively shape a future where poultry farming continues to thrive while meeting the evolving needs of society.

The editor expresses his sincere appreciation to all of the authors who contributed to this book for their hard work and dedication, as well as to the IntechOpen editorial team for allowing us to complete this project.

Guillermo Téllez-Isaías
Department of Poultry Science,
University of Arkansas Agricultural Experiment Station,
Fayetteville, AR, USA

Section 1

Poultry Managment

Chapter 1

The Resilience Strategies of Smallholders' Poultry Actors

*Samuel Abanigbe, Mjabuliseni Ngidi, Temitope Ojo
and Paul Orowole*

Abstract

Smallholder poultry actors play key roles in increasing food security and contribute significantly to the economy of both developed and developing countries. Despite their roles, they are a vulnerable group and mostly neglected by developmental programmes. As well, they account for most of the world's poor and hungry. Nevertheless, they continually strive to keep their activities directly as livelihood and indirectly as contributors to the society. They are challenged with; high cost of investment compare to slim margin on returns per unit, poor infrastructure; bad road network and public power supply, poor linkages to information, inputs, market, funding facilities and logistics for both input and output delivery, etc. Diversification into value addition, direct marketing of products using trust factors, investment in alternative power generation through cooperative society and community efforts in rural road development are observable resilience strategies used by these actors.

Keywords: resilience, strategies, smallholders, poultry, actors

1. Introduction

Poultry is an important subsector in the livestock sector of agriculture industry of any economy. It is significant in the aggregate economies of rural, peri-urban and urban livelihoods by contributing directly and indirectly to income and employment generations of all the actors along the value chain. Poultry in developing nations like Nigeria is a rare success story in the commercialization of Agriculture [1]. It is characterized by relative faster growth in consumption and trade volume than many other agricultural livestock sector, which include; chickens (local chickens, broilers, and layers), turkeys, geese, ducks, guinea fowls and pigeons [2]. Poultry is most advanced component of animal agriculture compared to other agricultural subsectors that are predominant with subsistence, low scale production capacity due to rudimentary engagements and the inability of actors to integrate science and technology into production.

Smallholder poultry is characterized with small units/capacity, investment outlay and income. Generally, its productivity is low and compounded by a lack of understanding of the interactions of interdependent enterprises [3] along the value chains as well as other adjoining commodities. The study of [4] exposed lack of participation

in commercial markets of smallholder poultry productivity as a major constraint, due to high transaction costs, shortage of quality labour, poor liquidity (low cash income) and limited access to credit and saving facilities, dearth of information and weak growth linkages. Also, the study of [5] affirmed the work of [6] that opined that lack of access to technologies and information, poor infrastructure and lack of access to markets and environmental factors were the key limiting factors to sustainable smallholder livestock operations. All these identified constraints are evidence in the day-to-day smallholders' poultry activities in developing nations.

However, smallholders' poultry actors have continually engage in their operations directly as livelihood and or indirectly as support to nutrient of households, gift to family and friends during festivals and major traditional rights, hence contributing to socio-economic needs of the society. One way, it can be infer that, this is an informal reasons or resilient strategy that these actors deployed to remain in smallholders' poultry activities despite the identified constraints to their productivity. Though, several developmental programmes and projects, nationally and internationally, such as the Poultry value chain of Agricultural Transformation Agenda (ATA) of Nigeria Federal Government, Smallholder's poultry care and business development in South-west by Global Alliance for Livestock Veterinary Medicines (GALVmed), African Chicken Genetics Gains (ACGG-NG) project in Nigeria implemented by International Livestock Research Institute (ILRI), Poultry value chain project by Foundation for Partnership Initiatives in the Niger Delta (PIND), etc., have been put forward to harness the potentials of rural households and smallholder poultry value chains, and also, help them to develop and implement coping mechanism in their operations.

2. The smallholder poultry actors

Conceptually, actors in any commodity value chains are people who actively engage in the processes of the commodities value chain. They are stakeholders who trade in a specific good; poultry in this context, they however, progresses up the value chain. It includes input, production (traditionally referred to as farming), processing, trading, and end users, which are often refer to as customers. Actors are the providers of activities or operations along the value chain. They can either be an individual or corporate organization. **Figure 1** shows a typical model of smallholder poultry value chain.

Along the chain, there are major value chain providers as well as subsidiary providers. For instance, a poultry input provider have, Seed (Day-old chicks, brood and sale, pullet, point of lay, etc.) suppliers, Feed (commercial feed, toll-miller, etc.) supplier and Equipment (simple poultry housing, medicament and veterinary, etc.) dealers. The production in the value chain is strictly performed by the smallholders' farmers, who transform raw inputs into primary products like broiler (meat poultry) and layers (egg poultry). Beyond these primary products, there are various secondary

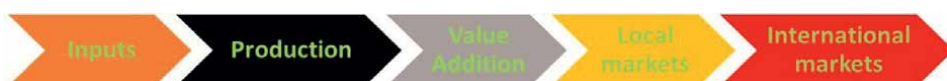


Figure 1.
Model of smallholders' poultry value chain.

products from poultry which the smallholders' poultry actors have been indirectly performed as supplementary livelihood through women and youth. Hence, another resilience strategy deployed to remain in poultry business. The value addition is occupied by processors who transform live broiler into freshly-processed and smoked chicken. Also, aggregate and transform the fecal or droppings of birds into organic manure, as well, the ovals, feathers and blood into livestock feed ingredient as protein source in the diet of livestock. Within the value addition and along other values in the chain is an important and significant logistic provider, who provides the services of transportation of input and output from one actor to another along the chain and to other commodity value chains, like vegetables, annual crops, etc. Sufficiently, this role is downplayed in the day-to-day business interaction of smallholders. It often caused damages to products due to bottleneck and high transactional cost that this service generates in developing nation. However, there have been strategy in area of cluster development and contract orientation between big companies and clusters of smallholders' poultry farmers to reduce the problem caused by logistic.

The local and international markets in the chain are knitted into distributors and consumers as twin-actors in Smallholders' poultry value chain. They are essential in providing the socio-economic outlooks for smallholders' poultry products. Distributors are those that showcase or those that give visibility to products. Hence, they enhance the economic index of all the other actors along the chain. They are creator and mobilizer of wealth. They link one actor to the other. They help communicate either good or bad of products along the chain. The distributor is often regarded to as aggregator and operates in stages or channels depending on the capacity of operators. Products distribution can be direct or indirect. Direct distribution involves just two players in the channel, that is, the producer and the consumers while, indirect distribution involves intermediaries (wholesaler, aggregator or retailers) before the products get to the final users. Smallholders' poultry farmers uses traditional market system of distribution which is also known as direct distribution channel to exchange their products for money with consumers. Consequently, distributor operates as business entity as sole-proprietor, partnership and or corporation. Their distribution space or market place can either be virtual or physical depending on choice of the users of the products. Consumer on the other hand is the user of smallholders' poultry products. They are the one that uses the products primarily as food and or as feed ingredients for livestock development. The aim of consumers is to derive optimal satisfaction from the products they purchase for themselves as well as for other auxiliary uses.

By and large, smallholder poultry has its root from rural poultry. The main actor, that is, the farmer can assume multi-dimensional roles as an entity. Besides poultry farming, he can acts as processor, marketer, transporter, and input provider, as well as consumer of the products (egg or meat). Smallholder poultry thus, contribute greatly to the livelihood of many resource-poor farmers and build household's asset [7]. It makes up about 80 percent of poultry population [8, 9] in low-income food-deficit countries and significantly contributes to; improving human nutrition, providing food (eggs and meat) with high quality nutrients and micronutrients; generating household income [7, 10] and savings, especially for women, thus enhancing the capacity to cope with shocks and reducing economic vulnerability [10]; providing manure for vegetable garden and crop production. As well as serving the socio-cultural and religious functions [7]. Notably, smallholder poultry actors are important stakeholders in increasing

food security and significant contributors to the economy of both developed and developing countries. Despite, they are a vulnerable group often neglected by development policy and they account for most of the world's poor and hungry [10].

3. The resilient strategies deployed by smallholder' poultry actors

3.1 The concept of resilient strategies

Resilience is the ability or capacity to recover quickly from difficulties or toughness [5]. The day-to-day activities of smallholders' poultry have some embedded risk factors within its system. These risks are operational as well as managerial inclined. Research and development have provided series of information on how to mitigate the operational challenges with the recent effort on climate change adaptability in livestock system. Also, there have been few managerial information on poultry productivity in which marketing system is key. The concept of cluster and cooperative development are mitigating strategies to market of smallholder poultry value chain. Thus, most operators within this sector have been adopting this market innovation.

In spite of these background, there are several interaction of complex concepts relating to challenges within smallholder poultry system. Thus, a single approach cannot be sufficient to unravel resilient strategies necessary for productivity of the sector. Hence, three theories were exposed by [5], which are socio-ecological system (SES) framework [11], the resilience theory [12] and technology affordances [13]. The SES framework suggests that a smallholder poultry system is made up of primary and secondary attributes. These attributes interact to produce specific outcomes [5]. Consequently, these attributes and interactions within the sectors are key determinants of the challenges faced by the actors and subsequently coping strategies they deployed. The work of [12] opined that applying the resilience theory helps to understand "individual variations in response to risk". Thus, resilience theory provided the understanding for smallholder poultry system actors to identify their system using SES framework, react to challenges and respond accordingly. Conclusively, availability and affordance of technologies [13] needed to support productivity is key to sustainability of smallholder poultry actors. Hence, access to technologies and information, infrastructure, environmental and markets knowledge are strong indicators to sustainable smallholder poultry value chain [5].

Resilience can provide a philosophical and methodological basis to address systemic risk [9] in a more useful way than traditional approaches based on risk management. Risk assessment and management are used to harden components of the systems affected by specific threats, yet such approaches are often prohibitively expensive to implement, and do not address cascading effects of system failure. Resilience approaches emphasize the characteristics and capabilities that allow a system to recover from and adapt to disruption. Therefore, resilience as the ability of a system to perform four functions with respect to adverse events: 1. planning and preparation; 2. absorption; 3. recovery; and 4. adaptation [9]. On the other hand, smallholder actors often lack the understanding of risk definition, which increases their susceptibility to all forms of risk [5, 14].

3.2 The challenges and resilient strategies of smallholder poultry sector (SPS)

In developing economy, studies have identified cost of investment, inadequate infrastructure, and access to technical information, inputs and support systems,

quality of manpower, nature of output pricing and informal market system as constraints to sustainable productivity of SPS. Lack of agribusiness management skill and poor access to information about the technicality of the poultry value addition process also compound the problems of sustainability of SPS. In some disadvantaged communities, market or price risk is high, such that, producers might not have access to buyers, hence, disposing the actors to sell products at any available price well below cost of production. Unpredictability of the system of production can also cause a challenge where products are of low quality and increase mortality rate which in turn lower income. Lack of policies direction, either directly or indirectly can lead to theft or direct loss of poultry inputs and output. Policies can as well be linked to poor management skill on the part of the owner. Traditional belief, in terms of ownership and gender understanding, where female member of household might not claim ownership of poultry stocks despite her role in caring for the poultry can predisposed SPS to weak or no productivity.

Consequently, these constraints have limit the livelihood of actors, it has impaired there status and made them vulnerable despite their continuous engagement in smallholder poultry activities over the years. However, these actors have been dealing with the minimum capacity that their limited capital and expertise can cope with as a resilient strategy. They do not want to overstretch their scale, sometimes, due to lack of knowledge, non-accessible agricultural loans and input linkages. On poor infrastructure, these actors have design community-effort or participation as a resilience mechanism to remain in the business of poultry production. In some farm settlement, they jointly own electric power generators through cooperative system to solve power need of their business. In some large farm community, they contribute to buy electric power generating transformers to satisfy the power needs. Also, they contribute to have accessible roads through regular grading and maintenance for ease of movement of their poultry inputs and outputs.

Recently, smallholders' poultry actors have started embracing membership in farmers' cooperative societies as a resilient strategy to indirectly access extension services to improve their agribusiness management skill, have access to input linkages, understand group dynamics for relationship among themselves and customers, as well as get information about pricing and market system, etc. The cooperative society has the potential to harness new system or technology from research and development agencies as well as private companies who are into service of poultry inputs suppliers or dealers. The cooperative society when organized and structured, has potential to benefit from cluster and off-taking/ contract agribusiness modeling for value addition, marketing and selling strategies of products all year round and linkages to inputs like feeds, drugs, day-old chicks (DOCs), etc. The cooperative society is also disposed to business information on the utilization of credit and thrift funding services to support members' business credit needs with single-digit interest rates. Beyond cooperative membership as resilience strategy, some smallholder poultry actors also engage in trading activities as supplementary livelihood to poultry farming business within their community. They do trade operations like selling other food items like rice, beans, smoked chicken and fish, eggs, groundnut oil, and other household foods within their communities in order to improve their daily revenue and achieve a higher social position. Also, some poultry farmers diversify into sales of poultry inputs like feeds, simple medicament and equipment and freshly processed chicken and or smoked chicken.

4. Conclusions

Smallholder poultry sector is significant in the aggregate economies of developing communities, providing employment, income and nutrients to households. The sector have various interrelated enterprises along the chain, which include, farming; raising of birds to produce broiler (meat) and layer (eggs), processor; transforming live bird into freshly processed meat, distributors; sales of poultry inputs like feeds, medicaments and equipment, logistic provider, etc. The individual that handles these enterprises are conceptualized as actors. The activities performs by these actors are faced with series of challenges. However, the following are some of the resilient strategies they deployed in coping with the sector:

1. Trading as supplementary livelihood to poultry business
2. Contract farming through cluster to maximize profit
3. Cooperative membership for group or community participation to access inputs, information and market of products
4. Maintaining and sustaining same scale of production for many cycles
5. Integrated farming system, where poultry farmers also engage in vegetable cultivation

Author details

Samuel Abanigbe^{1,2*}, Mjabuliseni Ngidi¹, Temitope Ojo³ and Paul Orowole²


1 Department of Agricultural Extension and Rural Resources Management, University of KwaZulu Natal, Durban, South Africa

2 Department of Agricultural Extension and Rural Development, Lagos State University of Science and Technology, Ikorodu, Nigeria

3 Department of Agricultural Economics, Obafemi Awolowo University, Ile-Ife, Nigeria

*Address all correspondence to: imolemi2013@gmail.com

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Chapter 2

Poultry Environment

George Agbele

Abstract

The title chapter focused on poultry environment “New perspective and Application”. Emphasis was on the need to create awareness on the nature of poultry environment in terms of controllable forces (vision and mission, leadership style, feeding, feeding pattern, labour union, organisational structure, and value system) and uncontrollable forces (temperature and humidity, technological, cultural, natural, political and legal environment). The interaction between the poultry farming and its environment was equally considered and the study revealed that a symbiotic relationship exists between the variables in the sense that both benefit from each other. The chapter also anchored on the socio-economic contribution of poultry farming to the environment to include meat and egg production, source of employment, source of income to the environment, tourist attraction, manure for agricultural purposes etc. The last section of the chapter handled general adaptive control mechanism to poultry environment. The study concluded that environmental forces are a challenge to poultry farming industries and recommended that poultry farmers/managers should be acquainted with environmental reports through scanning and analysis in order to enhance poultry farming sustainability.

Keywords: poultry, environment, macro element, micro element, adaptive mechanism

1. Introduction

The business plans, strategies and processes of poultry industry remain a mirage if the environment is not favourable. The potentiality of an environment can never be utilised if organisation (including poultry farm) fail to carry out environmental scanning and analysis. Poultry manager's decision could be nice in terms of achieving organisational goals, but many great decision makers failed to critically examine the workability of the decisions considering the constraints imposed by immediate business environment. Environmental factors are fundamental issues as far as continuous existence of enterprises are concerned especially in poultry farming.

Emphasis on this chapter is on poultry environment. The nature of poultry environment in terms of controllable and uncontrollable forces determines the extent the farm can go. No farm operates in isolation. Environmental factors are the sum of all the factors outside the control of management of company, the factors which are constantly changing and they carry with them both opportunities and risk or uncertainties which can make or mar the future of business firm [1]. A number of environmental factors appear to influence the operation of poultry farms. Some of these forces could be macro-element (uncontrollable), while others could be micro

element (controllable). As with many situations, management must assess the change in the environment in relation to the nature of the firm to determine whether the change is permanent or merely a short-term phenomenon, and the speed and extent of the change – that is, it likely to affect a large section of the firm? [1].

The issue of poultry environment is addressed majorly from two dimensions (external and internal dimensions), and how these challenges can be treated and probably the adaptive/control mechanisms to these environmental challenges to poultry farming in order to enhance effective poultry management and sustainability.

2. Dimensions of poultry environment

i. External factors (macro elements)

ii. Internal factors (micro element)

i. External factors: They are macro elements that is beyond the control of poultry managers. In other words they are uncontrollable variables.

Temperature and humidity: Environmental temperature and ventilation affect the performance of poultry farmers over the years and is only when the industry is current with the change in the environment that the effects can be averted. According to Ironkwe [2], the satisfactory temperature needed for the development of chicks ranges from 37°C to 39°C. Anything short of this becomes a threat to the industry. He further stresses that ventilation is very necessary in poultry farming and that average size egg (of about 58 g) consumes 5.113 of oxygen and emits 2.813 of carbon dioxide through the incubation period. Any environment that is short of sufficient ventilation is not advisable for poultry farming. Also humidity that is too low towards the end of incubation improves the emergency of chicks from their shells. On the other hand, if the humidity is too high the chicks may emerge with wet down feathers, in effect they are almost drowned in their shells [2].

According to Julian [3], under circumstances such as high heat, high humidity with reduced air flow or any other extreme stress the death rate due to physiological changes has increased.

Technology environment: Technology has turned the world into a global palour, so join the technology supper high way [4]. He further states that technology change is very dynamic, so it should be taken into consideration and provision should also be made for flexibility for future changes or expansion to meet up with the competitions and the new technology change. Technology as one of the environmental factors that is contending with expansion and development of poultry firms need to be properly looked into and addressed accordingly for better repositioning of poultry firms for economic development. As a result of global changes in technology, a business may automatically change their modus operandi. Change in technology affects poultry farming and business operation positively or negatively. Although technological effect on business is either a threat or opportunity. This has made companies to slow down or high their production capacity and this also follow that the quality and quantity of human resource must also be affected. When the quality of workers are changed it automatically affect the performance of the organisation in either ways.

The impact of technology on business organisation is enormous, (i.e. incubating and hatching of eggs) is going to be extremely impossible in this era of technology for any entrepreneur to grow without technological touch. How can we think of

effective marketing of poultry product without e-marketing (Internet marketing)? As a result of the internet marketing, instant and quality service are rendered by individuals, groups, organisation and companies. The ideal of technology must inculcated in poultry industry. This has made products to go beyond expectations of several firms. E-marketing is sometimes believed to have a wider range because it not only refers to the internet e-mail and wireless media, but in includes organisation of digital customer data and electronic customer relationship management (ECRM) systems [5]. E-marketing entails customer's relationship, sales, advertisement, publicity, promotion etc. infact; technology can catapults a business to an enviable height.

Technology is like two-edge sword. Opportunities and threats [6]. To Damaki [6], opportunities include new products, new markets, new services and new customers while threats consist of redundant, new technology, increased training cost, expatriates, obsolete and adaptability challenge. The level of technology in a society or a particular industry determines to a large extent what products or services will be produced, what equipment will be used, and how operations will be managed [7]. Business and productivity can be expanded quickly through technology in the modern world. Any business operation (including poultry operation) that neglects the role of technology is ready to remain static, which will consequently result to backwardness, so keeping abreast with the changes is an option.

Cultural environment: Hofstede [8] maintains that culture is a combine arrangement of the mind which distinguishes the members of one group or category of people from others. Culture in its general sense refers to how people do things and why they do it. Culture embraces how we play, dress, eat, work attitude, beliefs, our interactions with people, values, communication with people and even our behaviour. In other words, barely everything we do is affected or influenced by our cultural background or orientation.

Consequently, anything that influence a man's lifestyle will definitely affect everything around the individual even his business. Culture as a lifestyle is very influential, in every facet of organisational performance and even organisational culture affect employees behaviour. It involves standards and norms that prescribe how employee should behave in any given organisation [9].

Therefore, it is important that cultural values met on ground in any business environment should be appreciated by every poultry farmer. This could be different from group to another. Customer behaviour in a particular region is dependent on studying their social and cultural environment very deeply. The choice of goods and service are on people interest and environment shapes the values, behaviour, mind and aspiration of people. Poultry enterprises try to adapt to cultural value of the immediate environment for successful operations. For a successful intercultural interaction sentiment should be put aside and decide to imbibe the cultural values of your counterpart.

Intercultural services encounter, where the customer and service provider are from different cultures, is very common in service sector [10]. Language differences make communication difficult during business transaction. The authors further explain that such intercultural service encounters may be influenced not only by cultural differences but also by language barriers. Language (dialect) affect business performance significantly especially at the grassroots. Dialect differs from the tribe to another, community from community. This is how customers' reaction to product most times differs from community to another because of values for such produce based on their belief. Increased globalisation is forcing a growing number of business managers and employees to interact across linguistic boundaries [11].

Natural environment: The natural environment is very important factor that cannot be neglected when considering poultry performance in our business environment. Some of these natural environment elements may not be tamed by an individual, but rather must be appreciated the way they are and get used to it. According to Ngige [7], managers and business owners must pay attention to the natural environment if we are to preserve the world for future generation. Any business that pretend over these natural indicators may not go far. In any industrial and business environment the consideration of natural factors are very important to know the types of business that would survive in an area [12]. Isaken et al. [13] argue that work atmosphere in firms influenced employees participation in the creation of a creative climate. Climate in work place has impacted on employee motivation, behaviour, attitude and potentials, which in turn is predicted to influence poultry productivity [14]. According to Pelin and Fund [15], the climate or organisational climate is considered very important in the life of organisation due to its clear effect and relations to the various regulatory activities. It affects employees satisfaction and performance and, thus the success of the organisation and its ability to continue [16]. The climate of poultry farms must not be taken for granted.

Another indicator of natural environment is flood. The uncontrollable rainfall leading to flooding is also a challenge to poultry farming activities. This was experienced in Nigeria few year ago and mid this year. Indeed, the effect on performance of poultry farming industries was devastating to the extent that lives were lost not to talk of birds. This could happen again if proper care is not taking to avert it. Ngige [7] suggests that there is need for environmental scanning and monitoring to prevent this natural occurrence to an extent which may not be eradicated totally. As a result of this flood, CEO of poultry farms lost money and products, other also close down their business. Some business men relocated to the north because the effect on business activities was less compare to the south-east geo-political zones of the country.

Political environment: Political environment exact influence on the performance of firm irrespective of size, but politics is also components of other external risk and moreover, the political environment is often perceived to be outside of management control, making it difficult to define, predict and aligned with objectives [17]. Researchers also viewed political environment from different perceptions. The political environment is considered through the legal frame work where the organisation operates and this is done through the laws and regulations that guides the operations of the business in question. In planning for the success of poultry farm in any environment, the political situation of that environment is a significant factor to consider. Any organisation that hopes to succeed in any business environment must as a matter of necessity pay attention to political issues [18].

Again, political tensions and heat is created in different ways and by different groups of persons. All these risks can generate violence, directed towards firms' property and employees [17]. The most challenging issue about political environment is that it is not predictable, which makes planning difficult for managers. Multinational companies are grappling with political issues that sometimes surprise even the most experienced [19].

Legal environment: This components of the general environment are external to the firm as they can make broad long-term impact on the organisation [7]. There is no business over the ages that operate in isolation without the eye of the government on it through regulation and or policy put in place to regulate or monitor such business activity. The economic growth of every nation is very vital and the issue of legal environment and organisational performance (including poultry farms) is also very critical as far as economic development is concern. Nwizu [20] maintains that policy

is a guiding principles which governs action especially repetitive actions. It is a decision as to what should be done and how, when and where.

The legal environment includes all laws and legal regulations and policy framework refer to the relational system created between political power and business. This could be in form of commercial law, environmental law, pollution law, tax law, regulatory agents, proliferation of business law etc. From this perspective, we speak of the need to ensure climate of political and legal stability which may encourage and discourage business, avoiding the risk [21]. Legal environment constitute a very big challenge to free flow of business organisations.

Government creates rules and frame work in which enterprises are also to compete against each other favourably from time to time. Government also at the same time changes the rules and framework forcing enterprises to change the way they operate [22]. Attention of every organisation is needed all times regard the rules that concern the business. Readiness for adaptation to changes is a prerequisite for continuity in business existence irrespective of size and type.

- ii. **Internal factors:** They are referred to micro elements. They are the controllable internal variables of poultry farm. In other words, their effect or impact on the poultry operations can be influenced or adjusted unlike the macro elements.

Vision and mission: The vision and mission statement of poultry enterprises is a very strong platform for the success of the farm. Addressing the issue of poultry farming new perspective, the vision and mission of the leadership of the enterprise is very critical. The vision of a firm emphasises on the goal and objective of the firm, while the mission focusses on the overall purpose of the firm, procedures and methods to accomplish the vision of the organisation. In fact, is crystal clear that any poultry firm without any vision and mission is tending towards fruitlessness and unproductivity. That firm has no target or goal to accomplish.

The vision of organisation is like a propelling force that keeps the organisation going. Again these variables are like guiding principles. In our modern poultry farming, manager must ensure reliable vision for the farming operations. Both the leaders and lead must also ensure that the vision and mission statement is not treated with levity.

Leadership style: Transformational leadership style using organisational mechanism such as compensation, communication, organisational policies and procedures and methods create dynamic empowering culture with characteristics of active, strong and innovative [23]. Leadership style is an internal variable of an organisation that is very vital to the success of every enterprise. In addressing the issue of poultry environment, leadership pattern is very fundamental in the sense that is not possible for poultry organisation to stand and thrive if the leadership style is porous and unstable. Poultry managers must as a matter of necessity look into the welfare motivational aspect of their subordinate. Is very unfortunate that couple of decades now most managers do not see the need to carry their workers along rather than given specific attention only to the chicks. On the contrary, workers may not see the need to pay full attention to the chicks when adequate attention is not on them by the managers of the enterprise. There should be a defined communication and relationship between the poultry farm directors and their employees, and this in turn encourages productivity and effectiveness. Transformation leadership style in new ideas is known as one of the effectiveness leadership style [23].

Feeds: Birds given the right nutrients can never be compared with birds deprived of the right feeds in terms of weight, size, quality and disease resistant. Vitamins

deficiency has become a big challenge to so many poultry farms across the globe. Birds lacking this important vitamins look ruffled, drowsy, lack co-ordination as in encaphalomalial and encephalomalialities [2]. Feed as an internal micro element should be taken very seriously by poultry farming organisation in order to stand the test of time and in the competitive market. Besides, the type of feeds given to bird spreads better than the cost of advertisement in the market places. Good products sells itself. Bird feed with the require nutrients and vitamins give little stress and challenge in the area of marketing. Poultry farming enterprises must have the right perception for bird feeds. Adequate preparation must be put in place by consulting nutritionists in birds feeds, and this also open doors for the right counsel to famers regards the wellbeing of the bird for better performance in the area of meat and egg production.

Feeding pattern: Is one thing to have the right feeds and is a different thing entirely to administer the feeds according in terms of when and how the birds are to be fed. Every farm manager especially should be very observant. There is need for close study of the bird to know when required nutrient should be given. Bird feeds should not be stored for too long in order not to depreciate their nutritive contents of the feed and again to reduce the probability of mycotoxin build-up. In fact, practice feeding at cooler times of the day, for instance, early morning or in the evening. Feeding birds at the cooler times enables birds to make up for what they have not eaten during the day [24].

The categories of chicks grown at any point in time must be given the required quality and quantity of feeds (from 1 week old to 20 week old above). Enough water is equally necessary. Bird can be choked-up and die if not given water to drink (**Table 1**).

“The author further stated that poultry farm that had 2000 layers producing about 1800 eggs daily due to mismanagement of the farm manger, feed was not given to the birds for 1 day, the egg produced a day after it was reduced to 10 crates i.e. from 90% hen day production to 10%. It took almost 2 weeks of intensive proper feeding for the birds to return to about 70% hen day production.

Labour union: Poultry farms will do well in any environment when there is a good working relationship between the union and firm. There is no poultry farm or firm that can succeed in an environment and atmosphere of heat and pressure with the labour union. The labour union is like a middle personnel between the firm and the workers. They bargain collectively with the mangers for better working condition, wages and salaries for workers. The essence is to make sure that there is a smooth working relationship between the employers and employees and also in the environment that the firm is sited. The effect of good working relationship between firms and work force can never be overestimated. So many organisation has folded up as a result of non-chalant attitude towards maintaining a rapport with the labour union.

Age	Classes	Water litre/day
1–2 weeks	Chicks	0.8–0.12
3–6 weeks	Chicks	0.16–0.20
7–12 weeks	Grower	0.21–0.30
13–19 weeks	Grower	0.31–0.32
20 and above	Layer	0.38–0.40

Table 1.
Water consumption level for bird by [2].

Organisational structure: Organisational structure is important in providing guidelines on hierarchy, authority of structure and relationships, linkage between different functions and coordination with environment [25]. No manager can achieve set goal alone no matter strategically that individual might be or the type of orientation received in the area of management [26]. The structure that make provision for coordination and cross breeding of ideal among workers is an effective structure. Poultry manager must not fail to understand that employee ideas may be better in so many business operations. There are structures that welcome the utilisation of employee ideas for the betterment of the firm. Agbele and Onoriode further stated that resources of so many firms (including poultry farms) have been mismanage because the organisation itself was not strategic enough to sought for the corporate ideas of the employees. Over the years poultry farms have been managed without defined structure and that concept should be seen as obsolete if indeed poultry farms operations should thrive and stand the test of time.

Value system: In every business environment poultry farmers need to be guided by some ethical practices. There should be value principle. Value system represent practices, ethics and beliefs that guide organisation based on their conviction in achieving the mission and goals. Poultry farmers should maintain a level of ethics in their operations within the business environment, not doing things the way they like and what seems right to them but rather be done with conviction towards achieving set goals.

3. The interaction between the poultry farming and its environment

Actually, there is a symbiotic relation between poultry farming and its environment in the sense that they both benefit from each other (Table 2).

S/N	Environment	Poultry farm
1.	Provide workers for the farm	It provide employment to the immediate environment
2.	There is also the provision of task force in most of the poultry farming environment in order to maintain a cordial relationship between the community and the farm. They make sure that the poultry workers are not molested	Some poultry farming enterprises (especially the established ones) provide scholarship to the less privilege in the farming environment
3.	Flow of information. Current farming reports is always given to the immediate environment	Some communities are classified as attraction centres because of the poultry business that is operational in such environment
4.	Public relation officers is also constituted by most hosting communities standing as a middle personnel between them and the enterprise.	It provides meat and eggs to the host communities. Manure is also provided to the immediate environment to enhance and increase the production of other farm products

Table 2.
Relationship between Poultry Farm and the Environment.

4. Socio-economic contribution of poultry farming to the environment

- a. Meat and egg production.
- b. It serves as a source of employment to the environment.
- c. source of income to the environment.
- d. Tourist attractions (expertise attractions).
- e. Manure. This is used for agricultural purpose. It can be recycled on cropland or marketed.
- f. Offering. Contributing to the well-being of the immediate environment. Offering sales to the environment.
- g. Provision of school fees.

5. General adaptive/control mechanisms to poultry environment

1. Environment acquaintance: Poultry managers should be ready to scan the business environment from time to time. The scanning should follow by evaluation, formulation and implementation of policies that suits the changes in business environment. Try and get acquainted with the current business situation in the environment.
2. Understand your business environment: For instance, understanding the environmental culture is a very strong determining factor if you are to stay in a place or not. Is also a strong determining factor in deciding the type of business that will thrive in a place.
3. Forecasting: Predict and prepare for the future while focussing on the present.
4. Understanding and learn to manage it accordingly.
5. Improve the internal process of the organisation: Those structures and culture that is not improving performance can be adjusted and be improved upon. Organisation must not remain in a place for so long. Attention must be given to organisational activities internally and not only externally matching with competitors.
6. Adopt new method of thinking: Forget what you know and reason with others in the business environment. Accept their views because you may not be right all the time.
7. Analyse and watch the competitors: Advertising methods, promotion methods, early to market formula etc. know the weakness and capitalise on it to penetrate the market at once.

8. Embrace Technology, get updated at all time and follow new trends.
9. Accept changes, externally, internally and globally.
10. Frequent change of policies and programmes that result to political crisis must be avoided by the government.
11. Another adaptive mechanism to poultry environment is that managers of poultry farms must build their skills and keep on learning new ideas through seminars/ workshop.
12. Innovation as a business survival strategy: Poultry farmers that can innovate is bound to remain in the market. Improve on your skill to remain in market all the time. Somebody that is innovative and creative is not easily displaced with environmental challenges that always occur from time to time.

6. Summary of findings

The uncontrollable factors (temperature and humidity, technology environment, cultural environment, natural environment, political environment and legal environment) and controllable environment (vision and mission, leadership style, feeds feeding pattern, labour union, organisational structure and value system) has a positive significant influence on the operation of poultry farm. In other words, the success of poultry farming to a very large extend is dependent on the condition of poultry environment.

7. Conclusion


Environmental condition is very critical to the survival of poultry farming industry. Therefore, we can conclude that unfavourable environmental factors impedes the performance of poultry farming industry, while favourable and stable poultry environment encourages its performance for economic growth and development of every nation. We can also concludes that the effective operation of poultry industry is sensitive to controllable and uncontrollable factors.

Author details

George Agbele
University Of Delta (UNIDEL), Agbor, Delta State, Nigeria

*Address all correspondence to: george.agbele@unidel.edu.ng

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Chapter 3

Antibiotics Alternatives in Poultry Production in Sub-Sahara Africa

Brilliant Agaviezor

Abstract

The recent campaign for the exclusion of antibiotics and hormone growth promoters in livestock production has led to the increase in research and the use of ethnoveterinary medicine in livestock production. The use of ethnoveterinary medicine for smallholder poultry production in Sub-Saharan Africa has proven to be economical, culturally acceptable and ecologically sound since the natural products used are locally available. This chapter focuses on antibiotics alternatives used in poultry production in Sub-Saharan Africa for maintaining good health and improved performance. Antibiotics alternatives explained in this chapter include the use of probiotics, prebiotics, organic acids and medicinal plants. Different medicinal plants that cure bacterial diseases in poultry in Sub-Saharan Africa and their mode of preparation/administration were explained in this chapter. Despite the seemingly effectiveness of the antibiotics alternatives from plants source, there are some setbacks which include the inconveniences associated with the process of preparation and the difficulty in standardizing them. These setbacks need urgent attention.

Keywords: antibiotics alternatives, poultry, sub-Sahara Africa, probiotics, bacterial diseases

1. Introduction

The Sub-Saharan Africa is the geographical area and regions of the continent of Africa that lies south of the Sahara. These regions which include East Africa, West Africa, Southern Africa and Central Africa have witnessed increased population growth over the last decades. This population increase has necessitated increased demand for food (animal protein) of which poultry meat has contributed a greater percentage in meeting this need. A greater percentage of poultry production in Sub-Saharan Africa is still on an extensive management system except for a few large commercial farms. One of the greatest limitations to poultry production is poultry diseases. Diseases can wipe out a whole farm in a few days so farmers spend a fortune in vaccinating poultry birds against disease infection and in treatment of those infected already to reduce mortality. The impact of poultry diseases on poultry production cannot be over emphasized as it greatly increases the cost of production through cost of medication and losses incurred due to infections [1] estimated that the mortality of indigenous chickens up to 4 weeks old under extensive management system has been estimated

to be 53%. To reduce the cost of production, small holder farmers use herbal trees as antibiotics alternatives [2].

The small scale production and level of poverty in this region has encouraged the use of traditional medicine in managing the health challenges of these poultry birds. The use of traditional medicine to meet human and livestock health care challenges in Sub-Saharan Africa has being from time immemorial with various degrees of successes. It was estimated by [3] that about 80% of the people living in Africa depend on traditional medicine for themselves and their livestock. The recent campaign for the exclusion of antibiotics and hormone growth promoters in livestock production has lead to the increase in research and the use of ethnoveterinary medicine in livestock production [1, 4] reported that the use of ethnoveterinary medicine for smallholder poultry production in Sub-Saharan Africa is economical, culturally acceptable and ecologically sound since the natural products used are locally available. Furthermore, [5] confirmed that over the last 3 decades, researches have being conducted to developing antibiotics alternatives for maintaining the health of animals as well as their performance. There is therefore a need to make these medicinal plants, preparation and administration procedures available to other poultry farmers.

2. Importance of poultry to households and the economy of nations in Sub Saharan Africa

The importance of poultry to households in Sub Saharan Africa cannot be over emphasized as it serves as a source of food and income to several families [2]. Poultry products are accepted by all as there are no traditional or religious barriers to the consumption of poultry. Poultry provides meat and egg for human consumption. Poultry meat is good source of protein, phosphorus and other minerals, and of B-complex vitamins. Poultry meat is low in harmful fats, but high in beneficial monounsaturated fats—which make up about half of the total. In addition to poultry products as source of food and income, it is also a source of organic manure which is used in cultivation of crops especially vegetables. Furthermore, poultry feathers are used for pillow stuffing, diapers, insulation, upholstery padding, paper, plastics and feather meal. Backyard poultry farming has over the years contributed to a great extent to the agrarian economy of different countries in Sub Saharan Africa. Poultry production provides livelihood security for many households in this region in addition to securing the availability of food. Poultry production has also provided jobs to individuals especially youth and women. The indigenous poultry breeds in Sub Saharan Africa are well known for their adaptability as well as disease resistance. These breeds are also protected against predators using their plumage color.

3. Antibiotics alternatives used in poultry production in Sub Saharan Africa

Some of the reasons for the use of antibiotics alternative in poultry production are the toxic effect of antibiotics to humans who consume the poultry products from poultry raised with synthetic antibiotics, the problem of resistance to synthetic antibiotics by target parasites as well as the high cost of these antibiotics. However,

antibiotics alternatives are natural products that are cheap and environmentally friendly [6, 7] has reported the use of probiotics and prebiotics, extracts of plants, nutraceuticals like copper and zinc of antimicrobial peptides, antibiotics from chicken egg yolk, medium fatty acids, rare earth elements as antibiotics alternatives. Indigenous medicine is now recognized worldwide both by the rural populace and the urban elite as an important healthcare resource [8]. According to [8] a total of at least 35,000 plants species are widely used for medicinal purposes in Sub-Saharan Africa with some level of success which however needs to be standardized and improved upon using more scientific principles.

3.1 Probiotics

Probiotics have being used to reduce poultry enteric diseases [5]. Probiotics are live microbial feed supplements used to balance microbial population in the intestine through the production of compounds that displace pathogens from enterocytes and maintaining the pH in the gut of animals. Some of the microorganisms used as probiotics are *Lactobacillus acidophilus*, *Streptococcus faecium*, *Bacillus coagulans*, *Bifidobacterium bifidum*, *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Aspergillus oryzae* etc. [5]. Some of the advantages of the use of probiotics as antibiotics alternative are they inhibit the growth of diseases producing organisms. They also prevent digestive upsets and diarrhea due to bacterial invasion. Probiotics improve intestinal ecology and harmonize functions of the digestive system etc. [9] in their work while evaluating the dynamics of probiotics on immune response of broilers reported significantly higher antibody production ($P < 0.01$) in experimental birds fed probiotics as compared control ones.

3.2 Prebiotics

These are non digestible food ingredient/supplement that beneficially affects the host by selectively stimulating the growth of some or all of the non pathogenic organisms (bacteria) in the gut or colon of the animal. Prebiotics beneficially affects the host by stimulating the growth and activity of harmless bacteria, indicating a synergistic effect with probiotics. Prebiotics also help in inhibiting the colonization of pathogenic bacteria.

3.3 Organic acids

Organic acids have being used to reduce many pathogenic and spoilage organisms by lowering the gut pH. Some of the organic acids used are formic, lactic, citric, propionic and phosphoric acids. They have the ability of lowering the pH at which the activity of proteases and beneficial bacteria is optimized and the proliferation of pathogenic bacteria is minimized by a direct antibacterial effect destroying their cell membranes.

3.4 Medicinal plants

According to [4], Africa has so many medicinal plants that have being used over the years. They stated that there are about 3000 plants species used for treating various types of diseases in Southern Africa while there are about 10,000 medicinal plants species in Northern Africa. Natural medicinal products originating from

S/no	Plant antibiotics alternative	Country	Reference
1.	<i>Boswellia dalzielii</i>	Nigeria	[11]
2.	<i>Sclerocarya birrea</i>	Niger	[12]
3.	<i>Aloe vera</i>	Somalia	[13]
4.	<i>Carica papaya</i>	Cameroon	[14]
5.	<i>Peltophorum ferrugineum</i>	Togo	[15]
6.	<i>Capsicum spp</i>	Uganda	[16]
7.	<i>Aloe saponaria</i>	Southern Africa	[17]
8.	<i>Adenium multiflorum</i>	Zimbabwe	[18]
9.	<i>Lagenaria vulgaris</i>	Nigeria	[11]
10.	<i>Colocasia esculenta</i>	Kenya	[19]

Table 1.

Some antibiotics alternative of plant origin used in Sub-Saharan Africa.

herbs, spices and their products have being used as antibiotics alternatives to improving the wellbeing of poultry during production. The antimicrobial activates of phytochemicals vary from plant to plant and from region to region. Tannin act by deprivation of iron, binding with hydrogen or through interactions non-specifically with proteins of the bacteria. Some of the herbs used as antibiotics alternatives are garlic (*Allium sativum*), *Aloe vera*, Thyme (*Thymus vulgaris*), Tumeric (*Caurcuma longa*), Ginger (*Zingiber officinale*) among others. The positive effects of these herbs are due to the presence of essential oils, fatty acids, minerals, fiber, vitamin, protein and carbohydrates. Apart from the digestive and antioxidant properties of herbs, they exert the beneficial influence through antimicrobial, immunomodulating and antiparasitic effects.

Buena [10] has reported that guava fruit extract showed *in vitro* has antimicrobial activity against bacteria such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Shigella dysenteria*. The leaf extract is against *Staphylococcus aureus*. It was shown to antibacterial in another study and in addition to *Staphylococcus aureus* was also useful against *Streptococcus spp*. The leaves are rich in tannin, and have antiseptic properties. **Table 1** shows some medicinal plants used as antibiotics alternative for poultry in Sub-Saharan Africa.

4. Some bacterial diseases and their antibiotics alternatives in Sub Saharan Africa

4.1 Ulcerative enteritis

This disease is caused by *Clostridium colinum*. Chickens infected with Ulcerative enteritis will appear extremely depressed and will emaciate due to loss of appetite. Watery droppings with bad odor and mortality which is as high as 50 percent can be seen. **Table 2** shows the treatment of ulcerative enteritis using some medicinal plants as antibiotics alternatives in poultry.

S/no	Plant	Plant part	Mode of administration
1	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
2	<i>Capsicum frutescens</i>	Fruits	Grind about 0.5 kg of fruit in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
3	<i>Pseudognaphalium luteoalbum</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
4	<i>Diospyros lycioides</i>	Roots	Used as decoction. Boil about 0.5 kg of roots in 20 L of drinking water. Allow to cool and administer to poultry for about a week

Table 2.
 Treatment of ulcerative enteritis using antibiotics alternatives.

4.2 Fowl cholera

Fowl cholera is caused by *Pasteurella multocida*, a bipolar, fluorescent, non motile rod shaped bacterium. There are three strains which are smooth encapsulated, mucoid and rough encapsulated. According to [1] the virulence is highest in smooth encapsulated and lowest in rough encapsulated. Chickens infected with fowl cholera have greenish-yellow diarrhea and fever that leads to increased water consumption. Loss of weight and rattling noised due to congestion in the airway will also be seen. **Table 3** shows the treatment of Fowl cholera using medicinal plants as antibiotics alternatives in poultry.

4.3 *Pasteurella anatipestifer* infection

This bacteria disease is caused by *P. anatipestifer* which is a gram negative rod bacterium. Clinical signs include sneezing, coughing and discharges from the eyes

S/no	Plant	Plant part	Medium of administration
1	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
2	<i>Capsicum frutescens</i>	Fruits	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
3	<i>Adansonia digitata</i>	Fruits	Break/crush about 0.5 kg and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
4	<i>Sclerocarya birrea</i>	Bark	Used as decoction. Boil about 0.5 kg of bark in 20 L of drinking water. Allow to cool and administer to poultry for about a week
5	<i>Boswellia dalzielii</i>	Young leaves	Grind/squeeze about 1 kg of leaves in 4 L of drinking water and administer to poultry for about a week

Table 3.
 Treatment of fowl cholera using antibiotics alternatives.

S/no	Plant	Plant part	Medium of administration
1	<i>Adansonia digitata</i>	Fruits	Break/crush about 0.5 kg and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
2	<i>Boswellia dalzielii</i>	Young leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
3	<i>Sclerocarya birrea</i>	Bark	Used as decoction. Boil about 0.5 kg of bark in 20 L of drinking water. Allow to cool and administer to poultry for about a week
4	<i>Nicotiana tabacum</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
5	<i>Pipper guineense</i>	Fruits	Break/crush about 0.5 kg of fruits and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
6	<i>Colocasia esculenta</i>	Tuber	About 0.5 kg of tuber washed and ground in a mortar, 2 L of water added and the mixture sieved. 3 drops are given once in the nostrils of each fowl for 5 days

Table 4.
Treatment of Pasteurella anatipestifer infection using antibiotics alternatives.

and nasal passages as well as greenish diarrhea, lack of coordination and death which range from 5 to 75%. **Table 4** shows the treatment of *Pasteurella anatipestifer* infection using medicinal plants as antibiotics alternatives in poultry.

4.4 Fowl typhoid

Fowl typhoid is caused by *Salmonella gallinarum*. Clinical signs include loss of appetite, increased thirst, lethargy and yellow-green diarrhea. Mortality ranges from 5 to 50%. **Table 5** shows the treatment of Fowl typhoid using medicinal plants as antibiotics alternatives in poultry.

S/no	Plant	Plant part	Medium of administration
1	<i>Pergularia extensa</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
2	<i>Aloe vera</i>	Juice	Add about 5 ml of juice in 4 L of drinking water and administer to poultry for about a week
3	<i>Carica papaya</i>	Leaves	Chop about 1 kg of leaves and mix with 10 kg of feed. Feed infected poultry with the leaves for a week
4	<i>Adansonia digitata</i>	Fruits	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
5	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.

Table 5.
Treatment of fowl typhoid using antibiotics alternatives.

S/no	Plant	Plant part	Medium of administration
1	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
2	<i>Capsicum frutescens</i>	Fruits	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
3	<i>Peltophorum ferrugineum</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
4	<i>Adansonia digitata</i>	Bark	Used as decoction. Boil about 0.5 kg of bark in 20 L of drinking water. Allow to cool and administer to poultry for about a week
5	<i>Cassia abbreviate</i>	Roots	Used as decoction. Boil about 0.5 kg of roots in 20 L of drinking water. Allow to cool and administer to poultry for about a week

Table 6.
 Treatment of colibacillosis using antibiotics alternatives.

4.5 Colibacillosis

This disease is caused by *Escherichia coli*. These bacteria invade and cause a secondary infection when chickens are stressed or already infected. Clinical signs of colibacillosis are depression, paleness, decrease in appetite and diarrhea. **Table 6** shows the treatment of colibacillosis using medicinal plants as antibiotics alternatives in poultry.

4.6 *Staphylococcus aureus* infection

This is a very common poultry bacterial disease. The bacterium liberates beta hemolysin and plasma coagulase that can hemolyze the blood and also cause it to coagulate. *Staphylococcus aureus* infection always occurs between 4 and 6 weeks of age. The symptoms are similar to that of cholera which includes depression, listlessness, fever and loss of appetite. Lameness and swelling of the foot can be implicated. Mortality could be as high as 60%. **Table 7** shows the treatment of *Staphylococcus aureus* infection using medicinal plants as antibiotics alternatives in poultry.

4.7 *Streptococcus* infection

This disease is caused by *S. zooepidemicus* and *S. faecalis*. The bacteria release toxins that contribute to their pathogenicity. Chickens infected in the acute form

S/no	Plant	Plant part	Medium of administration
1	<i>Adansonia digitata</i>	Bark	Used as decoction. Boil about 0.5 kg of bark in 20 L of drinking water. Allow to cool and administer to poultry for about a week
2	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
3	<i>Capsicum frutescens</i>	Fruits	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week

Table 7.
 Treatment of *Staphylococcus aureus* infection using antibiotics alternatives.

S/no	Plant	Plant part	Medium of administration
1	<i>Allium sativum</i>	Chopped bulb	Grind about 0.5 kg of bulb in 10 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
2	<i>Cucumis articulatus</i>	Fruit	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
3	<i>Capsicum eucalyptus</i>	Leaves	Grind/squeeze about 1 kg of leaves in 20 L of drinking water. Sieve to remove leaves particles and administer to poultry for about a week. Ground leaves could as well be added to poultry feed.
4	<i>Capsicum frutescens</i>	Fruits	Grind about 0.5 kg of fruit and soak in 20 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week
5	<i>Capsicum annum</i>	Chopped bulb	Grind about 0.5 kg of bulb in 10 L of drinking water. Sieve to remove fruits particles and administer to poultry for about a week

Table 8.
Treatment of streptococcus infection using antibiotics alternatives.

will be listless and feverish. There is loss of appetite and endocarditis in the chronic form (Table 8).

5. Some setbacks in the use of antibiotics alternatives in Sub Saharan Africa

According to [20] some of the major setbacks in the use of antibiotics alternatives in Sub Saharan Africa are the inconveniences associated with the process of preparation. In addition, some of these plants are seasonal and are not found for use at some times of the year. The major setback in the use of antibiotics remedies is that of difficulty in standardizing them. This is because the concentration of critical ingredients in these plants varies from one region to the other.

6. Conclusion

The use of antibiotics alternatives should be promoted through government policies and inclusion in curriculum of educational systems in Sub-Saharan Africa. This will create more awareness and acceptance among the citizens to encourage more usage and research to improve on the development and usage of antibiotics alternatives of plants origin. Further researches are needed under controlled conditions the check the efficacy rates, the active ingredients in all these plants used as antibiotics alternatives and their standardization.

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Conflict of interest


The authors declare no conflict of interest.

Author details

Brilliant Agaviezor
Department of Animal Science, University of Port Harcourt, Port Harcourt, Nigeria

*Address all correspondence to: brilliant.agaviezor@uniport.edu.ng

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Section 2

Poultry Nutrition

Feed Sustainability and Efficiency

Hafiz Ullah

Abstract

As the world population approaches the projected 10 billion thresholds in 2050, it is anticipated that global food demand, particularly for protein, would increase dramatically in the ensuing decades. In terms of absolute and relative growth rates, poultry has outpaced the other major meat types produced globally over the past 50 years. Poultry production is expected to continue to be a significant and expanding sector of meat production due to escalating global demand. Scientists need to rethink their approaches considering the rapidly increasing demand for poultry meat coming from both developed and developing countries worldwide. Several challenges impede the poultry industry's value chain. Production must be both socially and environmentally responsible in addition to being economically viable. Nutritional improvements for chickens will aid in addressing these problems. It is evident how crucial it is to use a holistic strategy to properly and sustainably transform feed into high-quality poultry protein. Regardless of the time of year, these high-yielding animals need to be able to consistently consume, digest, absorb, and convert enough nutrients to meet their genetic potential. To attain high consistency output with acceptable risk, this task will require improving the usage of existing technology, developing new technology, and expanding our knowledge and information network.

Keywords: poultry, feed efficiency, sustainability, nutrition, balanced diet

1. Introduction

The demand for poultry meat and other associated products has skyrocketed over the past few years. In 2020, there were 137 million tons of chicken meat produced globally, making it the most popular meat worldwide. Consequently, the chicken industry makes a significant contribution to the consumption of animal proteins, human nutrition, and global food security [1]. The world has seen a tremendous increase in the demand for poultry meat and other poultry feed with the advent of time. The trend of this growing demand will continue over time. It is not exaggerated to say that with the increase in demand, the production of poultry meat and egg has also increased in both developed and developing countries. In the next 20 years, it is predicted that a rapid increase in poultry production will take place in developing countries too because of rapid urbanization and the higher increase in animal protein demand. Globally, the poultry industry has grown quickly due to several factors, including improved knowledge of poultry feeding, genetic selection, and disease management. The main factor in improving egg and meat quality and quantity has been feed formulation [2]. For instance, the time it takes for a 2 kg meat chicken to reach the market has significantly

decreased from 63 to 35 days between 1976 and 2009, because of the efficient conversion of feed into poultry products. The need for feed and raw materials is significantly being impacted by this increase in poultry production. The key input for poultry production is feed; hence, low-cost, high-quality feeds must be readily available if poultry production is to remain competitive and expand to meet consumer demand for animal protein.

2. Feed system and poultry production

The poultry sector, historically, has passed through a system of evolution with three distinct phases: (i) traditional system of poultry production at home to meet the domestic need, (ii) semicommercial poultry production system, and (iii) industrial-scale production. Every system operates within a basic technological framework. The factors that set one production system apart from another are bird selection, husbandry, and feed systems. Depending on the approach implemented, different amounts of food, nutrition, and resources are needed to grow poultry.

2.1 Strategies in traditional systems

Most developing countries still raise poultry using traditional methods. The local birds raised in this system might be fed on household wastes, environmental materials (arthropods, mollusks, greens, seeds, etc.), agricultural residues, feedstuffs, and aquatic plants, as well as byproducts from nearby small industrial units. The struggle for feed resources in villages determines the survival and expansion of extensive poultry systems.

2.2 Feeding strategies in semicommercial system

Small- to medium-sized flocks of native or enhanced genotype birds and the purchase of at least half of the feed from industrial compounders define the semi-commercial poultry system. The feeding methods utilized in this approach include dilution of purchased feed with local feed, total mixing of local feed with commercial feed, and complete ration mixing on the farm.

2.3 Feeding strategies in the commercial system

Commercial production is dominated by developed nations, and it has recently become more prevalent in developing nations as well. This system makes use of vertically integrated production units and birds that have undergone genetic analysis. Feed is the core element of such a system, accounting for more than 60% of the costs of production. Productivity in such a system is reliant on the availability of a highly effective feeding system and the usage of nutritionally balanced and designed feed to meet the needs of the birds.

3. Feeding poultry with a balanced diet

Since most poultry species are omnivores, it is possible to combine several feeds to create the most useful final feedstuff. Except for a few geese and ostriches, which have well-developed digestive systems, most birds are sensitive to food because of

their digestive systems. Most birds have substantially shorter digestive systems than other animals. Food passes from the mouth through the cloaca in less than three hours in chickens with rapid growth. High-performing birds require nutrient-rich diets that are considerably easier to digest to compensate for their short digestive systems and quick digestion times. Nutrient balance is crucial under these circumstances [3].

Because genetic modifications have also altered the physiology of the birds, genetic selection cannot function alone. By altering dietary needs and nutritional management, only the genetic potential of the new strain can be met. The high genetic potential of the newly selected birds can only be achieved with the help of properly formulated energy and nutrient-dense feed. Poultry, especially growing birds, is exceptional in that any change in the composition of the diet has an immediate and noticeable impact on the performance of the birds. The poultry industry has successfully taken advantage of this phenomenon (**Table 1**) [4].

Nutrient	Unit	Meat chickens			Laying Hens
		0–3 weeks	3–6 weeks	6–8 weeks	
Metabolizable energy	kcal/kg	3200	3200	3200	2900
	MJ/kg	13.38	13.38	13.38	12.13
Crude protein	%	23	20	18	15
Amino acids					
Arginine	%	1.25	1.10	1.00	0.70
Glycine + serine	%	1.25	1.14	0.97	—
Histidine	%	0.35	0.32	0.27	0.17
Isoleucine	%	0.80	0.73	0.62	0.65
Leucine	%	1.20	1.09	0.93	0.82
Lysine	%	1.10	1.10	0.85	0.69
Methionine	%	0.50	0.38	0.32	0.30
Methionine + cysteine	%	0.90	0.72	0.60	0.58
Phenylalanine	%	0.72	0.65	0.56	0.47
Phenylalanine + tyrosine	%	1.34	1.22	1.04	0.83
Threonine	%	0.80	0.74	0.68	0.47
Tryptophan	%	0.20	0.18	0.16	0.16
Valine	%	0.90	0.82	0.70	0.70
Fatty acid					
Linoleic acid	%	1.00	1.00	1.00	1.00
Major minerals					
Calcium	%	1.00	0.90	0.80	3.25
Chlorine	%	0.20	0.15	0.12	0.13
Non-phytate phosphorus	%	0.45	0.35	0.30	0.25

Nutrient	Unit	Meat chickens			Laying Hens
		0–3 weeks	3–6 weeks	6–8 weeks	
Potassium	%	0.30	0.30	0.30	0.15
Sodium	%	0.20	0.15	0.12	0.15
Trace minerals					
Copper	mg	8	8	8	—
Iodine	mg	0.35	0.35	0.35	0.04
Iron	mg	80	80	80	45
Manganese	mg	60	60	60	20
Selenium	mg	0.15	0.15	0.15	0.06
Zinc	mg	40	40	40	35

[Source]: National Research Council, 1994.

Table 1.

Minimum nutrient recommendations for laying hens and meat chickens expressed as percentages or units per kg of food.

4. Recent advancements in poultry nutrition

The single biggest cost connected with raising poultry is feed. Therefore, nutritional research in chickens has focused on problems relating to finding impediments to optimal nutrient digestion and usage, as well as on methods for enhancing feed utilization. The knowledge of experts in other biological sciences, such as molecular biology, immunology, microbiology, histology, and microanalysis, is increasingly being blended with that of specialists in poultry nutrition. It is seen that most of the feed is not converted into animal products, and most of the feed goes as undigested waste. In most cases in broilers, though they are efficient in food digestion, 30% of the ingested feed goes undigested. This reveals that the effectiveness of feed utilization for animal products has improved [5].

Recent advancements in poultry nutrition have mostly focused on three domains: 1) gaining knowledge of the needs and metabolism of nutrients, 2) determining the number of nutrients and their availability in feed ingredients, and 3) designing diets at the lowest possible cost that successfully balances nutritional supply and demand. Precision feeding is the overarching goal to reduce expenses and increase economic gains. When there was uncertainty regarding the supply of essential nutrients, such as phosphorus and amino acids, or when dietary requirements were unclear, there was a historical inclination to over-formulate diets. This method is no longer permitted since it is wasteful and because excess nutrients excreted in the manure eventually become a source of pollution. Optimizing the efficiency of nutrient use involves fine-tuning meals to better meet the needs of the birds [6].

4.1 Nutrient requirement

Nutrient requirements are difficult to define since they are always changing and affected by a wide range of variables. Two main categories of variables determine nutrient requirements: those that are unique to birds, such as their genetic makeup, sex, form, and stage of development, and those that are present in their environment.

Precision in defining the criteria depends on accuracy in both areas. The characterization of nutrient requirements for various classes of chicken has significantly advanced owing to the improved uniformity of genotypes, housing, and husbandry practices across the poultry industry. It has made it possible to make significant advancements in the definition of nutrient requirements for various classes of chickens.

4.2 Identifying the nutrient profile and ingredient quality

Producers of poultry are constantly looking for ways to increase the types and quantities of the feed additives they can use in feed formulations. The prevalence of these possibilities is rising because of improvements in feed evaluation and nutritional analysis methods. The main purpose of the feed ingredients is to provide the nutrients that the bird consumes and uses for vital processes. Data on the ability of raw materials to deliver key nutrients are currently in abundance. However, the inherent heterogeneity of each raw material puts strain on the precise feed formulations. Data on variation (or matrices) for the main feed ingredients are available and used in feed formulation systems to increase precision. It is an important advance that quick diagnostics, such as near-infrared reflectance analysis, are now available to determine gross nutritional content and continually track changes in ingredient supply.

It is established that not all nutrients in foods are available for use in production and that some nutrients in foods are either excreted undigested or not utilized. As feed evaluation techniques advance, data on the availability of nutrients for chicken, particularly phosphorus and amino acids, have been growing. The greater use of digestible amino acid concentrations in feed formulations rather than the total amino acid concentrations, for instance, is a new trend. The use of digestible amino acid content is especially important in developing countries where highly digestible conventional products are not easily accessible, and diet formulations may include components with low digestibility.

By developing diets based on digestible amino acids, it is possible to increase the product categories that may be used and the proportions of alternative items that can be used in poultry diets. This assures more constant performance from the birds and improves formulation accuracy, which may also result in lower feed costs.

4.3 Feed formulation

Once the nutritional requirements have been determined, the following step is to blend products and supplements to meet those requirements. A balanced diet offering the proper amounts of nutrients that are biologically available is the aim of the formulation. Commercial food producers also strive to provide a healthy diet for the cheapest price possible. The production of the least-cost feed necessitates numerous mathematical calculations due to the variety of available feedstuffs and nutritional requirements. Over time, feed formulation has changed from the straightforward balancing of a few feed ingredients for a small number of nutrients to a computer-assisted linear programming system. With commercially available formulation software, stochastic nonlinear programming systems are currently becoming more and more common. The next stage is to combine products and supplements to meet the nutritional demands after they have been identified. Since the variation in ingredient composition is nonlinear, stochastic programs are the most efficient way to combat this issue.

5. Nonconventional feed resources in poultry

Over the years, there has been a massive rise in the consumption of chicken products, particularly poultry meat, and this trend is likely to continue. The developing world will account for a large portion of the rise in worldwide demand for chicken products [7]. The poultry industry's explosive growth has a substantial impact on the need for feed and raw materials. The demand for the four elements that make up conventional feed—maize, soybean meal, fishmeal, and meat meal—cannot be met, even with optimistic estimates. It is crucial to look into the use of locally available, alternative feedstuffs in feed compositions because it is predicted that the gap between local supply and demand for these traditional components will widen over the coming decades [8].

A wide range of alternative feedstuffs is available to all three poultry production systems. The semicommercial system and traditional family poultry systems (scavenging and backyard) hold the best possibility of properly utilizing these feeds. Only a portion of the feed requirement is met by commercial compounders; hence, the semicommercial technique allows for the mixing or dilution of purchased feeds with locally available, alternative feedstuffs. In local, low-input family poultry systems, alternative feeds can be used to supplement the feed foundation [9].

5.1 Nonconventional feed sources: Nontraditional feed resources

The term “nonconventional feed resources” (NCFR) refers to all feeds that either are not typically utilized in commercially manufactured livestock rations or have not historically been used in animal feeding. NCFR generally includes a range of feeds derived from perennial crops as well as feeds with both animal and industrial origins [10]. Single-cell proteins, feed material made from agro-industrial byproducts of plant and animal origin, palm press fiber (an oil palm byproduct), pallet oil mill effluent, and other innovative sources of feedstuffs have all been referred to be NCFR. Common NCFRs include agricultural byproducts, cereal grains, citrus fruits, farm-raised vegetables, and weeds that grow along the coastline [11].

5.2 Advantages of the nonconventional feedstuff

Nonconventional feed resources offer the following common advantages to poultry: (1) These are unutilized tangible resources from production and consumption; (2) they can take the shape of a solid, slurry, or liquid and are primarily organic. Their economic worth is frequently quite low; (3) fruit wastes that have sugars, such as pineapple pulp and banana rejects, are much more advantageous energetically; (4) some of the NCFRs are great sources of fermentable carbohydrates, such as cassava and sweet potatoes; (5) the majority of feeds derived from crops are bulky, low-quality cellulosic roughages, suited for feeding to animals, with high crude fiber and proteins.

5.3 Factors affecting the use of nonconventional feed

5.3.1 Nutrition-related aspects

Although alternative feeds are the most affordable option, using them has certain drawbacks as well. First, the quantity and quality of their nutrients are variable and irregular. Information on the availability of nutrients is scarce. Antinutritional

elements may also be present in some of the feeds. They also require the addition of supplements while using them.

5.3.2 Technical factors

Technically, nontraditional feed ingredients are not always available throughout the year. Such feeds are widely dispersed over the seasons of the year, and storage is costly. They are bulky for use, storage, and transportation due to their physical nature. Before using them, they need to be processed. There is a dearth of knowledge regarding their use in poultry digestion.

5.3.3 Socioeconomic aspects

Several important considerations can cast doubt on alternative feeds, including competition for human consumption and farmers' lack of interest because these products are of lower quality than other crops. If they are processed for further use, they are not cost-effective.

5.4 Strategies to overcome the nutritional challenges posed by the alternative feed

The following are the major criteria to overcome the nutritional deficiencies posed by nonconventional feed:

5.4.1 Evaluation of feed

One of the main factors preventing poultry feed suppliers from contemplating the use of alternative ingredients is the difficulty in evaluating the nutritional value of an ingredient because of the unavailability of suitable facilities for research and analysis. Over the years, there has been a lot of interest in assessing alternate feed resources, particularly from developing nations. However, frequent feed evaluation and constant updating of matrix values are essential for the effective use of these substances because there are so few published data on the digestible AA and apparent metabolizable energy (AME) of alternative feed ingredients.

5.4.2 Dietary planning using digestible amino acids

It is necessary to formulate feed based on metabolizable energy and digestible AA when fibrous and poorly digested components are being examined for usage. The amount of AA that can be digested varies depending on the component; some ingredients can be digested more easily than others. When diet formulas include a variety of alternative, poorly digested substances, the utilization of digestible AA is especially relevant.

5.4.3 Use of synthetic amino acids to compensate for amino acid specification

To increase the accuracy of feed formulations and fulfill the AA requirements, it is possible to effectively harness the differences in the AA digestibility of feeds. Nowadays, owing to the availability and utilization of feed-grade essential AA in synthetic forms, nutritionists may accomplish this. There has been a lot of interest in using decreased protein diets supplemented with synthetic AA to increase feed efficiency, decrease nitrogen and ammonia emissions, and ensure sustainable poultry production.

5.4.4 Commercial exogenous enzyme augmentation

Over the past two decades, the commercial use of biotechnology and the acceptance of feed additives in poultry nutrition have created numerous opportunities to improve nutrient uptake, feed efficiency, and productivity. Exogenous feed enzymes are conceivably the most significant ingredient to enter the chicken feed industry. Since glycanases (xylanases and glucanases) have become more readily available in the 1990s, non-starch polysaccharides (NSP) have no longer been able to inhibit the use of viscous grains such as wheat and barley in poultry diets. Regarding the substitute ingredients, feed enzymes can (i) make it possible to utilize some ingredients (which might not be possible otherwise), (ii) get rid of nutritional restrictions and allow for larger inclusion levels, and (iii) broaden the variety of ingredients used in feed formulations.

6. Feed sustainability and efficiency

6.1 Sustainability

The value chain of the poultry industry is hampered by several issues. In addition to being economically feasible, production must also be socially and environmentally responsible. Nutritional advancements for poultry will help to meet these issues. It is clear how vital it is to use a holistic approach to successfully convert feed into high-quality poultry protein in a sustainable manner. These high-yielding animals must be able to regularly consume, digest, absorb, and convert enough nutrients to reach their genetic potential, regardless of the time of year. The effective completion of this task will necessitate the increasing use of current technology, the development of new technology, and the expansion of our knowledge and information network to achieve high consistency production with acceptable risk [12].

According to Oladokun and Johnson [13], feed production accounts for 70% of the cost of producing eggs, over 50–85% of all life-cycle greenhouse gas emissions, 80% of energy use, and similarly significant proportions of other resource and environmental consequences. A focus on enhancing the sustainability of poultry feeds is unquestionably necessary given the growing awareness of the role that animal production plays in several sustainability concerns and the ongoing expansion of the egg industry [14].

6.2 The impact of feeding

In the future decades, it is anticipated that global food demand, particularly for protein, would rise significantly as the world population approaches the estimated 10 billion mark in 2050 [15]. Among the primary meat varieties produced around the world over the past 50 years, poultry has seen the highest absolute and relative growth rates [16]. Poultry meat is expected to continue to be the key sector of overall meat production due to rising global demand (**Figure 1**).

This tendency has been primarily fueled by the convenience, purported health benefits, and reduced price of chicken meat compared to red meat, in addition to concerns of culture and religion [17]. The poultry sector will be critical in ensuring food security for a rising world population [18].

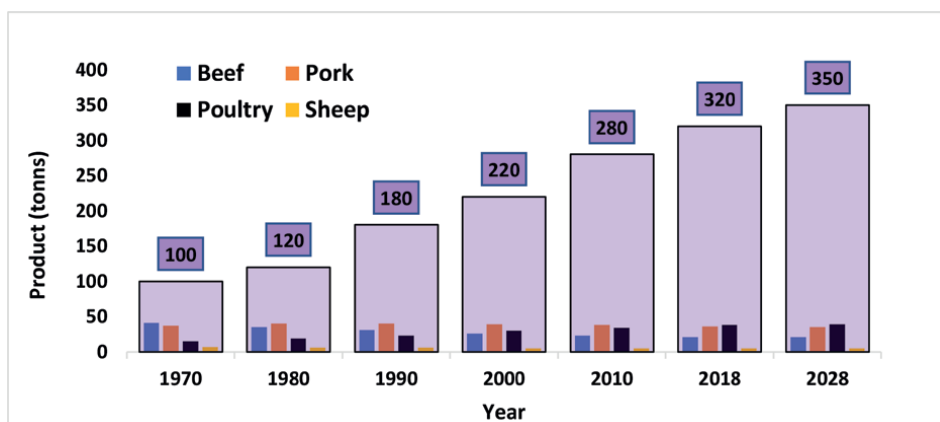


Figure 1. Global production of the four main types of products (beef, pork, poultry, and sheep); evolution from 1970 to 2018 and projection from 2018 to 2028 [Data source: FAO, 2020].

On the one hand, this gives a unique opportunity, but on the other, it also poses a significant challenge that must be overcome. Considering the growing public concerns about the pressure and competition for limited natural resources, loss of animal and vegetable biodiversity, the spread of antimicrobial resistance, as well as the environmental burden of livestock production, the concepts of “sustainable intensification” and “producing more with fewer resources” have been reinforced as refined strategies for feeding future generations [19].

6.3 Feed efficiency

The most popular technique to define feed efficiency (FE) in poultry is the feed conversion ratio (FCR), which assesses the correlation between feed intake and body weight gain for a specific growth stage. FE can also be viewed from a different perspective as a homeostatic process that determines the net result of “energy intake,” which is determined by voluntary feed intake and the efficiency of digestive processes (i.e., nutrient digestion and absorption), and “energy expenditure,” which is determined by maintenance requirements, particular nutrient redistribution mechanisms, and the rate of metabolic processes and intermediary metabolism in tissues and organs [20].

6.4 Broad benefits of feed efficiency

Higher FE means that less feed is needed per unit of production output from a practical standpoint (i.e., 1 kg of chicken meat).

6.4.1 Human food security

Any improvement in FE would promote food security for humans as feeding is a major production cost and would help the poultry industry remain economically viable.

6.4.2 Environmental impact

Advances in FE can reduce greenhouse gas emissions, which are mostly brought on by the production of feed crops, the transportation and processing of feed ingredients, and the conversion of natural ecosystems into farmed land [21].

6.4.3 Reduction of eutrophication

Furthermore, more productive hens have a higher ability to store dietary nitrogen and phosphorus, which reduce the excretion of nitrate and phosphate in manure and NH₃ emissions into the environment. Higher FE can thereby lessen the likelihood of eutrophication and acidification of poultry production [22].

6.4.4 Energy consumption, biodiversity conservation, and feed-to-feed competition

Improvements in FE can help with the conservation of animal and plant biodiversity, feed-to-food competitiveness, and energy utilization such as electricity and fossil fuels [23].

6.4.5 Impact on water utilization and climate change

Concerns about climate change and the pervasive effects of drought have made the impact of FE on water footprint more significant. Mekonnen and Hoekstra [24] estimate that the manufacturing of feed ingredients has the greatest impact on the industry's astonishing water use (4.3 m³ H₂O/ton of meat). Therefore, lowering the amount of feed needed per unit of output can lower the total amount of water used by the chicken meat supply chain, whether considering crop cultivation, the production of feed, or drinking water intake. How the increase in feed efficiency has led to improvements in other parameters is shown in **Figure 2**. Enhancing feed efficiency can help conserve both biological and non-biological environmental resources.

6.5 Additive-based feed improvement and sustainable expansion

According to EU Regulation 1831/2003, the field of feed additives has grown rapidly in recent years, giving rise to a wide variety of products with different specialties. The following feeds have been added to the formulas, which have improved the feed:

6.5.1 Precise amino acid addition

Dietary protein has always been a hot topic in chicken nutrition due to its value for bird performance and health, production costs, and environmental effects associated with nitrogen excretion [25]. One of the most challenging goals of the contemporary poultry industry is to reduce dietary crude protein concentrations in contrast to the current norms without impairing bird growth performance, FE, or health. Recent research has shown that such a reduction is possible but to a different extent provided the meal is kept at an appropriate level in terms of its amino acid profile to meet the demands of the bird [26].

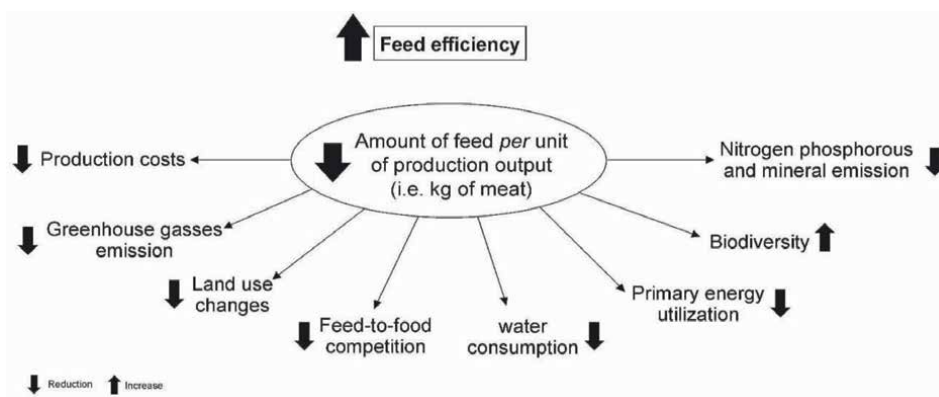


Figure 2.
Feed efficiency relationship with biotic and abiotic environmental domains.

6.5.2 Protease

By encouraging the activity of endogenous proteases, exogenous protease supplementation is a viable dietary method to increase dietary nitrogen absorption. Exogenous proteases have long been a component of enzyme combinations [27]. On the other hand, interest in this field of research has increased since the discovery of mono-component proteases ten years ago. Protease can enhance both growth performance and environmental impact indicators because it increases dietary nitrogen retention [28].

6.5.3 Phytase

The electronegative charge carried out by the phosphate groups in phytic acid has an antinutritional effect when the surrounding pH is close to neutrality. When phytic acid chelates remarkably large amounts of minerals (forming phytic acid salts called phytates), proteins, and carbohydrates in this condition, which is easily found along the chicken's digestive tract, insoluble complexes escape the digestive processes and are subsequently excreted with detrimental effects on animal performance and the environment [28]. However, the antinutritional effect of phytic acid can be limited by the enzymatic action of phosphatases, such as phytases, which rapidly hydrolyzes the esters bonds that support the phosphate groups (Vieira).

6.5.4 Trace Elements

The usage of trace minerals (TM) by the poultry industry, such as copper (Cu), manganese (Mn), and zinc (Zn), has sparked controversy due to the possibility of ecological damage. Inorganic salts like carbonates, oxides, or sulfates are utilized to add these nutrients to broiler diets because most products used to manufacture chicken feed do not contain enough TM [29].

7. Conclusion

It is abundantly clear from the current broiler system, which is characterized by a scarcity of natural resources and growing public concern over environmental impact

and animal welfare, that sustainable production intensification is the only pathway that the contemporary poultry industry can take to satisfy the rising demand for poultry meat. Given the benefits of enhanced diet utilization for environmental and economic sustainability, raising FE in poultry is currently a major goal. To do this, deep insight into the nutritional requirements of modern fowl is required. By reducing dietary nitrogen, phosphorus, and trace mineral excretion, feed additives can be used to boost overall productivity while addressing substantial environmental concerns. Additional research on the challenges is encouraged to further improve resource utilization, animal productivity and health, and production costs while safeguarding the environment. Therefore, a multi-actor approach combining breeding businesses, researchers, as well as poultry nutritionists and producers is essential to promote the sustainable increase of chicken production and accomplish the cherished aim of feeding future generations effectively and responsibly.

Conflict of interest

The author declares no conflict of interest.


Author details

Hafiz Ullah

Faculty of Biological Sciences, Department of Botany, University of Chitral, Pakistan

*Address all correspondence to: hafizullah@uoch.edu.pk

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Section 3

Poultry Diseases

Chapter 5

From Understanding the Immune Response against Coccidiosis to the Use of *Coccidia* Vaccines

*Luis-Miguel Gomez-Osorio, Ben Dehaeck, Carlos Cuello,
Jenny-Jovanna Chaparro-Gutierrez and Sara Lopez-Osorio*

Abstract

Avian coccidiosis is the most costly global poultry parasitic disease, which represents a threat to food production and sustainability. Coccidiosis is still ubiquitous even in modern poultry production systems. Protective immunity against coccidia does develop but differs for each *Eimeria* species and depends on the method of immunization and the immune response (including both early innate immune response by several proteins and professional phagocytes as well as acquired immune response with specialized cells). In addition, GALT is a master tissue in the immune response against coccidiosis because of its crucial functions: acquired immunity in both the cellular and humoral immune responses. Here, we present an extensive review on the immune response against coccidiosis and the use of vaccines as an alternative for consideration in integrated sustained coccidiosis control programs.

Keywords: *Eimeria*, innate immune response, acquired immune response, cytokines, live vaccines, precocious vaccines

1. Introduction

Avian coccidiosis is by far the most costly parasitic disease in poultry [1], and it may represent a threat to guarantee the supply for sufficient, safe, and nutritious food. According to some projections, the global population in 2050 will be 10 billion which will increase the demand for food production by 70% and therefore achieving global food security is a staggering challenge [2].

Coccidiosis is an infectious disease caused by protozoa, genus *Eimeria*. The parasite is host-specific and has a direct life cycle [3]. Birds become infected by ingestion of sporulated oocysts omnipresent in poultry houses. Once ingested by the chicken, the parasite invades and multiplies in the gastrointestinal tract, destroying epithelial cells [4]. The severity of infection will depend upon the number of infective oocysts ingested as well as the pathogenicity of the wild strains. Intensive methods of production of poultry favor the reproduction of *Eimeria*. Therefore, coccidiosis is a continuing problem requiring constant attention and, in the case of broilers, a need for continuous coccidiosis control tools [5].

Even today, coccidiosis is still ubiquitous, and it is generally accepted that, under the current production systems, coccidiosis control remains necessary [4, 6]. Coccidiosis is also one of the main triggers for other gastrointestinal disorders including necrotic enteritis, dysbacteriosis, *Salmonella*, among others [7–9].

Birds suffering with clinical coccidiosis will show typical signs such as diarrhea, bloody droppings, increased mortality, decreased feed intake, and impaired performance. Inadequate coccidiosis control may also result in impaired growth and an increased feed conversion ratio, even in the absence of obvious clinical signs (referred to as subclinical coccidiosis).

In a recent study, the global prevalence of clinical coccidiosis was estimated at 5% and subclinical coccidiosis at 20% of global poultry production [10]. This supports that, under current production systems, coccidiosis is still a major health and welfare issue, which needs to be controlled.

Synthetic anticoccidials were the first to be introduced in the market. The first paper on prophylactic use of anticoccidials was published in 1948 by Leland Grumbles and describes the continuous use of Sulfaquinoxaline for the control of coccidiosis in poultry [11]. After their introduction, synthetics were found to be very efficacious and were very popular. Up until 1971, they were the only available option for coccidiosis control as ionophores were only introduced in the 1970s.

The introduction of the first ionophore coccidiostat (monensin) in the 1970s has proven to be critical for the development of modern poultry production [12]. The use of ionophores has significantly helped in the development of poultry production and has improved the health and welfare of broilers (Report from the Commission to the Council and the European Parliament on the use of coccidiostats and histomonostats as feed additives, 2008).

As expected, suboptimal control of coccidiosis will result in the increased use of antimicrobials, some of which are medically important for human medicine.

2. Methodology

Google Scholar (<https://scholar.google.com>) and PubMed (<https://pubmed.ncbi.nlm.nih.gov>) scientific databases were used to search for articles published between the years 2000 and 2022 containing the keywords, “immune response” AND “coccidiosis” in combination with “broiler chickens,” “avian immunity,” “intestinal immunity,” “Coccidiosis Vaccines,” “*Eimeria* Vaccines.” Only manuscripts and book chapters in English or Spanish were included. Data from other animal species were also omitted except for the general overview of immune system. Data obtained in broiler chickens were grouped in tables including, the overview of avian immune response, peculiarities, intestinal immune response against coccidiosis and vaccines, type of *Eimeria* spp. infected, age at infection, among others.

3. A brief overview of the avian immune system

The immune system (IS) may be compared with a symphony orchestra in which a variety of molecules, cells, and tissues are finely organized to maintain the ideal state of homeostasis. In a nutshell, the IS may be defined as “A set of cells and molecules that defend the host against external (infections, trauma, among others) and internal aggressions (internal infections, autoimmunity, allergy as well as cancerous tumors)” [13].

The IS works as a passive system, meaning that it requires a threat to trigger an immune response (**Figure 1**). Once the IS is activated after the first contact with a foreign microorganism through the recognition of pathogen associated molecular patterns (PAMPs) and binding it with a variety of pattern recognition receptors (PRRs) the immune response is triggered. If innate immunity fails to eliminate the pathogen, adaptative immunity goes into action and activates more specific mechanisms to eliminate, obtain memory, and restore homeostasis [13].

Adaptative immunity comprises antigen presenting cells, lymphocytes (lym) including B and T cells as well as cytokines. There are fundamental properties of adaptative immune responses called cardinal features. Some include specificity, diversity, memory, nonreactivity to self (self-tolerance), and systemic localization (because of the ability of lym and other immune cells to circulate among tissues) [14]. There are two types of adaptative immunity: humoral and cell-mediated immunity which are mediated by different types of lym and work to kill different types of microbes [14]. Humoral immunity is conducted by molecules in the blood and mucosal secretions and is termed the secretory system [15].

T lym orchestrate cell-mediated immunity. Many pathogens can survive and replicate within the cells of the host. They are inaccessible to humoral response secretory molecules in these locations. As a result, cell-mediated immunity plays a role in the defense against this internal microorganism [14].

Protective immunity against a pathogen may be provided either by the host response (active immunity) or by transfer of secretory molecules that defend against the microbe. An important example of this form of immunity is the transfer of maternal antibodies by the bird to its offspring through the egg yolk, when the antibody is absorbed and enters the circulatory system, thus preventing or reducing clinical outcomes [16].

Among avian species, immune response in chickens is currently most studied followed by turkeys [17]. In theory, the avian immune response works similarly to the mammalian system. There are far more immunology studies conducted in mice compared with chickens. The use of pathogen infection models in mice has led to a greater advance of immunology understanding in mammals. Extrapolations from mammals to birds must be cautiously performed. A quote by the famous chicken evolutionist and immunologist Jim Kaufmann “chickens are not mice with feathers”

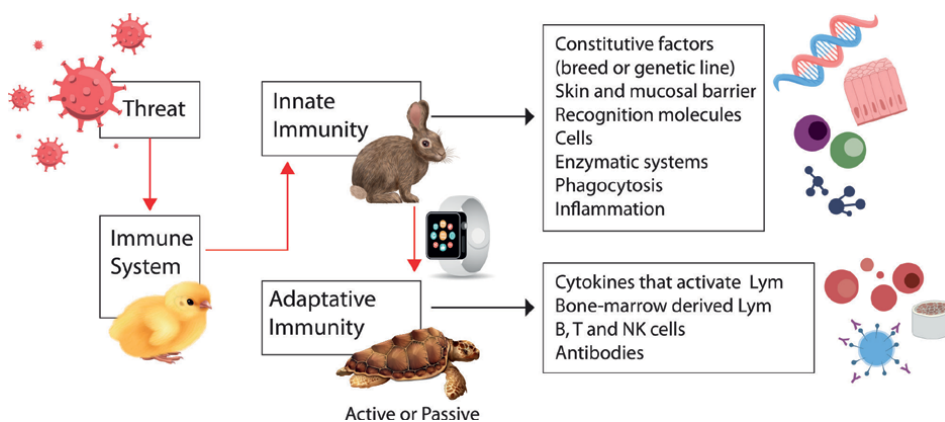


Figure 1. Overview and the appropriate logic of the immune system. See the text for details.

supports that the study of the avian IS is worthwhile [18]. Avian IS seems to be simpler than mammals. Although both do the same actions, different pathways are sometimes used [19, 20].

The most known difference is that Avian B lym are developed in the Bursa of Fabricius (BF), a unique bird organ, and not in bone marrow as in mammals [21]. Other important differences include the major histocompatibility complex (MHC), tumor necrosis factor (TNF) and its receptor (TNFR) superfamilies, chemokines as well as the interleukin (IL) 1 superfamily, where the chicken repertoire is smaller. There are other cases with the opposite relationship such as the immunoglobulin-like receptor family where the chicken repertoire is greater than that of mammals [22]. The full descriptions and details about the avian immune system are found elsewhere and are beyond the scope of this review [17, 19, 23].

4. Intestinal immunity in birds

The gastrointestinal tract (GIT) is a complex environment because it is responsible for the digestion and absorption of nutrients, is constantly exposed to pathogens, and harbors beneficial microbiota of the host [24]. In addition, the GIT is the largest immune and nervous system, which is constantly challenged with immunogens from different sources including food, foodborne, and infectious pathogens as well as microbiota [25]. These actions may sound like a biological paradox which can be explained as follows: the poultry host must simultaneously maintain homeostasis (or the absence of disease) with nutrient absorption, intestinal integrity, exclusion of harmful microbes, tolerance of beneficial microbiota, and shaping mucosa immune response [26, 27].

The structure of the GIT varies throughout the length of the gut. In a nutshell, the intestine is a pipe with a tubular structure surrounded by a linear layer of epithelial cells embedded in a basement membrane (**Figure 2**). It is also composed of columnar absorptive cells (enterocytes), enteroendocrine, goblet cells, as well as immune intestinal cells. Tight junctions are an intercellular complex protein system that connects epithelial cells. These compartments are organized in protruding villus structures to increase the surface area of absorption. These structures are composed of an epithelial layer, a core of underlying lamina propria (containing the microvasculature), and a thin layer of smooth muscle (muscularis mucosae). In the intestine, each villus is an absorptive unit [28]. There are also structures, known as crypts, which are defined as the site of stem cells with proliferating abilities for self-renewal and differentiation, thus maintaining homeostasis in the intestinal epithelium [29]. These crypts are interspersed in indentations. The villus crypt blocks may vary in their maturation stage in distinct locations along the intestine. There is a zone known as “proliferative” within the crypt where stem cells are located and divide to form daughter cells that migrate from crypt to villus and survive between 48 to 96 hours, after which they are sloughed into the lumen and die by apoptosis in the tip [30]. The time depends on the length of the villus and age of the chicken. During this migration process, the enterocytes acquire differentiated functions in terms of digestion, absorption, and mucin secretion [31, 32]. The intestinal mucosa is covered by mucus, a complex hydrated gel that protects epithelial cells from chemical, enzymatic, microbial, and mechanical damage. The epithelium and its mucus layer permit the selective movement of ions, nutrients, and water, but restrict the translocation of microbes and toxins from the lumen [33].

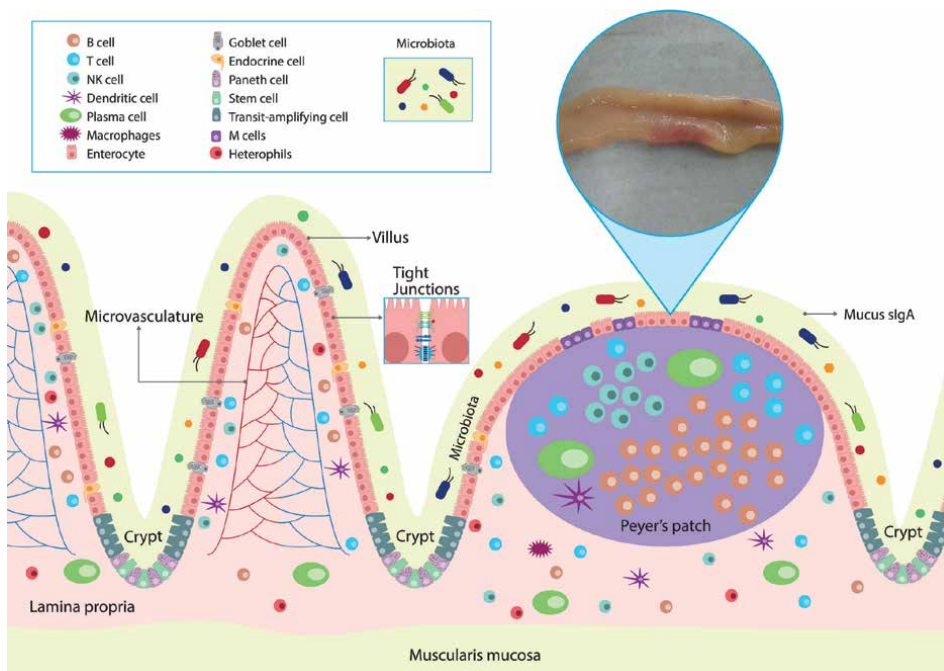


Figure 2.
 Schematic diagram of the architecture of intestinal immune cells.

The structures between the small and large intestine of the birds are quite different. While villus/crypt units are present throughout the whole small intestine, the large intestine has villus-like outgrowth structures, with a ruffled structure, known as folds. Hyperactive crypts are found within each folded unit [29].

Gut mucosa is exposed to food immunogens as well as microbiota antigens that are required for the processing of nutrients and the education of the local immune system early after hatching. As a result, there are organized structures which function as key organized elements of cells and molecules to defend the host against intestinal threats. These structures are known as Gut Associated Lymphoid Tissue (GALT). GALT is the largest compartment of the immune system and is comprised of lymphoid cells residing in the epithelial lining and distributed in the underlining in the lamina propria. In addition, there are specialized lymphoid structures. GALT's main role is to limit progression of systemic infection by detecting and destroying infectious agents in their early stages. In poultry, GALT encompasses esophageal tonsils, pyloric tonsils, Meckel's diverticulum, Peyer's patches, and two caecal tonsils (this is the most GALT important organ) [34, 35]. GALT is comprised of more immune cells than any other host tissue including different cell subsets and including most major cell populations found at other sites. These include heterophils, macrophages, DC, natural killer (NK) cells, as well as B and T lym (although the proportions of each cell type differ according to locality, microbial status, and age) [29].

The entire GIT is covered by a protective mucus consisting of Mucins family proteins which are produced by Goblet cells. Lysozyme, native microbiota, gastric juices, bile salts, as well as cationic peptides and other substances which act as a nonspecific defense are also important participants in the process [36]. Thus, GALT detects not only harmful pathogens as a potential threat of the intestine but also normal gut

microbiota and self-antigens that can elicit autoimmune responses. Therefore, a comprehensive study of the avian GALT is crucial to develop oral vaccines which can be alternatives to replace antibiotic growth promoters and immunomodulatory molecules that maintain intestinal homeostasis with the best performance [36].

5. Intestinal immunity against coccidiosis

GALT is a master tissue in the immune response against coccidiosis because of three crucial functions: acquired immunity development in both cellular and humoral immune responses (including antigen processing and presentation), antibody production and cytokine production [37]. Cellular immunity seems to be the most important effector mechanism against coccidial infection [38]. It is orchestrated by subsets of lymphocytes bearing either $\alpha\beta$ or $\gamma\delta$ T cell receptor (TCR) [39]. Natural infections of epithelial cells such as *Eimeria* infections, for which TCR $\gamma\delta$ cells are scarce in systemic circulation, they are commonly represented among IEL [40, 41]. Taking into account that *Eimeria* initiate the first contact with epithelial cells, it is tempting to speculate that IEL may be the first line of defense in response to *Eimeria* antigens which were processed and presented by major histocompatibility complex (MHC) expressed by epithelial cells [42]. Adaptive immune response against coccidiosis requires the involvement of these two pathways enabling proteins of MHC to be loaded with *Eimeria* epitopes. Only ligands expressed on the surface of antigen-presenting cells (APC) can activate T lymphocytes, which then execute effector functions, such as cytotoxicity, provision of help to B cells, and cytokine production [43]. In chickens, as in mammals, there are two subsets of lymphocytes classified by the system of cluster of differentiation (CD). These are CD4⁺ (known as T helper) and CD8⁺ (cytotoxic T cells). Adaptive immunity is highly dependent on T helper cells, and its activation is determined by MHC antigens [44]. Whereas CD8⁺ only recognizes peptides presented in the context of MHC-I molecules, T lymphocytes CD4⁺ recognizes peptides in the context of MHC-II molecules, and supports for co-stimulatory signals and other molecules. These molecular interactions underlying the regulation of the immune response between T lymphocytes and APC are known as immunological synapses [45].

This process is critical during the anticoccidial immune response in chickens. During the infection, the immune system inhibits parasitic development at three key stages in the *Eimeria* life cycle. The first is the sporozoite's search for binding sites in the epithelium cell, which allows it to penetrate the epithelium. While this is relevant, it is not particularly significant. Immune selection against life-cycle stages after the sporozoite stage may be more significant. Sporozoites are usually mentioned because immunity is so effective, but there are several studies that have studied later lifecycle stages and revealed that immunity can also inhibit multiple stages later in the life cycle.

The second stage is when sporozoites are placed within intraepithelial lymphocytes in the villus (IEL). Finally, sporozoites migrate from lamina propria to the crypt [46]. T cells are undoubtedly the protagonist in modulating anticoccidial immunity. Cytotoxic lymphocytes have been observed after a primary *Eimeria* challenge with the subsequent increase of interferon gamma ($\text{IFN}\gamma$) activating proinflammatory pathways to inhibit intracellular *Eimeria* parasite development in host cells [47]. Natural killer (NK) cells are also an important component of the intestinal immune response against coccidiosis [48]. Some subpopulations of NK mediate spontaneous cytotoxicity in chicken intestinal IEL underlying the statement that they are crucial for

intestinal immunity. NK cell activity depends on the infection stage, which decreases during early stages of infection, and recovers to normal levels 1 week after primary infection as well as in the early stages of secondary infection [49].

There are several cytokines and chemokines reported that play a predominant role during coccidiosis infection including IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IFN γ , transforming growth factor (TGF)- β 1, and tumor necrosis factor, among others [38, 50]. Despite the high number of cytokines described in the pathogenesis of the disease, IFN γ and IL-10 are the key cytokines for host protection and susceptibility against parasitic infections, respectively [51, 52]. Detrimental effects on the parasite have been reported as a result of IFN γ release. This is because of the inhibition of parasite invasion and survival in the host cell as well as the promotion of local inflammation [53], free radical production [54, 55], activation of antibody-dependent cell-mediated cytotoxicity [56] and/or the promotion of the release of cytoplasmic granules containing perforin and proteases [57]. IL-10 has an inhibitory role in the intestinal immune response due to the interference with Th1 response and this decreases the ability of the host to eliminate the parasite [58]. Therefore, IL-10 is a proposed mechanism of host evasion by *Eimeria*. IL-10 have different functions such as the inhibition of nuclear factor kappa B and the suppression of proinflammatory cytokines enrolled in parasite cleaning from the intestinal cells [59]. In the end, the balance between Th1 and Th2 responses is crucial to the outcome of the infection, and the cytokine network involved in the control of the immune response needs to be elucidated.

The role of humoral immunity against coccidiosis is still controversial, and there is more consideration paid to cellular immunity responses. Humoral immunity appears to play a minor role in resistance against infection. In one of the classical studies, in which the BF was removed, chickens were not affected after a secondary infection despite their ability to produce immunoglobulins [60]. During *Eimeria* infection, specific antibodies are produced, but they do not seem to be involved in controlling the infection [61] and immunoglobulin levels are not correlated with disease susceptibility [62]. IgA was also considered important as humoral protection against parasite invasion in earlier studies [63]. In a chicken kidney cell line model of *Eimeria* infection, caecal content from immunized chickens was co-cultured. Sporozoite invasion did reduce. However, there was no correlation in either antibody levels or the neutralization of sporozoites [64]. One of the major challenges has been to replicate *in vivo* results from the *in vitro* findings regarding the humoral immune response against Coccidiosis.

Immunoglobulins, therefore, do not appear to play an important role in protective immunity against Coccidiosis and cell immunity seems to be more crucial. Manuscripts underlying the key role of antibodies and humoral immunity as a protective mechanism against coccidiosis have been published, however [65]. It was determined that IgY antibodies injected systemically are capable of reaching the site of infection and effectively blocking parasite development in the intestine [66]. A positive association between antibody titers and protection [67] was also shown. In other studies, it was established that egg IgY from hens immunized with live infections of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* could be used as a feed additive to passively protect young chicks against all three species [68, 69]. The results described above support the concept that providing large amounts of protective antibodies to young chicks, through passive or maternal immunization, can interrupt the growth, development, and replication of *Eimeria*. Although antibody production is a mechanism to limit the propagation of several pathogens [65], T-cell

mediated response is the major criterion for the control of intracellular parasites such as *Eimeria* [39, 70].

6. Vaccines as a strategy to control coccidiosis

Vaccines provide an effective strategy for the control of coccidiosis in chickens and benefit the sustainability of the poultry industry worldwide [71]. The first vaccine against coccidia utilized a sporulated oocyst of a live *Eimeria tenella* wild type strain, and it was initially launched in 1950. This vaccine was based on the concept that low doses of oocysts over a number of days induced protective immunity against a homologous challenge [72, 73]. Current *Eimeria* vaccines are marketed and consist of live wild-type (virulent) parasites or live attenuated vaccines (precocious lines). Thus, up till now, there are more than 25 commercial anticoccidial vaccines utilized in poultry (reviewed in [73, 74]).

In breeders, vaccination programs based on live vaccines are tremendously useful and have been very successful. There are, however, some hurdles such as homogenous mass application to the flock. If the application is not done correctly, it may lead to suboptimal immunization and insufficient protection against the different *Eimeria* species. Even with homogenous mass application to the flock, there are additional hurdles which can lead to uneven application, triggering outbreaks [75].

A recent report showing the vaccine-induced immune response was published [76]. Briefly, three important findings were reported. First, *Eimeria* species can elicit an innate immune response by expressing TLR21 in macrophages through the recognition of pathogen-associated molecular patterns (PAMPs). Next, Coccidia vaccine induced a Th1 pattern characterized by proinflammatory cytokines and cell subsets in both systemic and local lymphoid organs. Second, *Eimeria tenella* induced the strongest activation of macrophages. Cellular analysis showed that vaccination led to an increase in macrophages and activated T cells (immunophenotypes CD8 + CD44+ and CD4 + CD44+). Other important effects were reported, including a decrease in fecal oocyst shedding as well as an improvement in body weight gain. However, this was not statistically different.

Precocious lines are defined as lines of *Eimeria* selected from a population that complete their endogenous life cycle in the host more quickly than wild-type parent strains. They are not only different because of an abbreviated life cycle but also by significant attenuation of virulence [77, 78]. Therefore, precocious lines are proposed as a successful strategy to control coccidiosis because they are less pathogenic than their parents, no adverse effects are observed in vaccinated birds and, despite their reduced multiplication within the intestine are able to stimulate protective immunity which is virtually as good as that induced by their pathogenic parent strains [75].

7. Conclusions

For more than 70 years, the main tools for the prevention and control of coccidia were performed using coccidiostats. As the number of available products is limited and no new molecules have been introduced in the last 30 years, it is a challenge to keep coccidiostats as effective as they were at their introduction to the poultry industry. Parallel to the advances in our knowledge of the avian immune system and the study of avian coccidiosis immune responses, strategies which can protect the birds

against different species of *Eimeria* are being considered to overcome health issues caused by coccidiosis.

The application of both types of vaccines (wild-type live strains and attenuated or precocious vaccines) are still a challenge due to mass application. Advantages and disadvantages of each vaccine exist. Therefore, it deserves continuous research and field work in different scenarios and facilities to identify effective control strategies for avian coccidiosis which will ultimately benefit the sustainability of the global poultry industry.

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Conflict of interest

L.M. Gomez-Osorio, C. Cuello, and B. Dehaeck are employees of Huvepharma N.V. which commercializes the vaccine Advent® against coccidiosis as well as anticoccidials. J.J. Chaparro-Gutierrez and S. Lopez-Osorio do not have any conflict of interest.

Author details

Luis-Miguel Gomez-Osorio^{1,2*}, Ben Dehaeck³, Carlos Cuello⁴,
Jenny-Jovanna Chaparro-Gutierrez¹ and Sara Lopez-Osorio¹

1 CIBAV Group, Universidad de Antioquia, UdeA, Medellín, Colombia

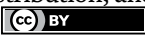
2 Country Manager Huvepharma, Colombia

3 Global Product Manager Anticoccidials, Belgium

4 Technical Manager LATAM Huvepharma, Ecuador

*Address all correspondence to: lgomezosorio@gmail.com

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Chapter 6

Effect of Fallowing on the Viability of *Salmonella* spp. in Poultry Facilities

Sérgio Eustáquio Lemos da Silva, Nayane Lopes Ferreira, Vanessa Silva Miranda, Vitor Simão da Silva, Renata Vieira Chaves Gabriel, Daniela Ribeiro Roldão, Vagno Espíndola da Silva and Lucas Peraphan

Abstract

Avian Typhoid, worldwide spread, is one of the principal diseases that devastate industrial poultry, causing serious economic losses to the poultry sector. The present study investigated the effectiveness of the fallowing technique, applied for a 149 days period, to a commercial poultry farm with a history of *Salmonella* Gallinarum isolation. Phenotypic detection of the pathogen in specific cultures was carried out from drag swabs collected from poultry facilities and equipment after the fallowing. An epidemiological inquiry was also carried out to verify the conditions of applied fallowing and to subsidize the laboratory tests. The bacteriological findings suggested that the fallowing technique in the period considered was not effective, for *Salmonella* spp. was isolated in 65% of the environmental samples. It was possible to infer that the sanitary-hygienic measures adopted in the fallow period were not effective, which requires new disinfection procedures and new bacteriological monitoring, besides an even longer fallow period. It was further concluded that the epidemiological inquiry is a valuable tool that ought to be adopted to investigate the relations between the epidemiological triad formed by *Salmonella*, the host, and the environment, while also being useful to support complementary exams, such as isolation in bacterial cultures.

Keywords: gallinarum, bacteriology, swabs, environment, epidemiological triad

1. Introduction

Salmonellosis of poultry are caused by bacteria of the *Salmonella* genus and are configured as one of the main systemic diseases or localized in the gastrointestinal tract of these animals, whose effects are associated with serious losses in poultry farming. Some of these diseases are zoonotic and, because of this, have a highlighted position in the exercise of public health surveillance worldwide. Despite all the technological development in the field of epidemiology and the normative regulations

regarding infectious diseases through prevention and control programs, which aim to preserve human health and poultry farms, the prevalence and notification of cases involving public and animal health are still increasing and worrying [1].

Starting from the increasing attention to the health of commercial poultry, public and private sectors have fomented the development of diagnostic and prevention instruments to reduce or eliminate *Salmonella* spp. from poultry flocks [2]. These bacteria are among the main pathogens involved in epidemics or outbreaks of diseases carried by food involving public health with vast dissemination, especially through eggs and meat, since poultry are the main reservoirs for the human species [3]. Thus, salmonellae stand out as one of the most pathogenic enterobacteria, responsible for intestinal and systemic damage and different degrees of mortality in their hosts [4].

The poultry industry has been constantly challenged by bacterial and viral pathogens, with serious economic losses. Among the infectious agents that challenge poultry production, the major responsible for losses in the sector is *Salmonella* spp.. Salmonellae belong to the family *Enterobacteriaceae* and can cause three specific diseases in poultry, including Avian Typhoid caused by *Salmonella* Gallinarum, Pulorosis caused by *Salmonella* Pullorum, and Avian Paratyphoid caused by the other serovars of the *Salmonella* genus [5].

Gastroenteritis and septicemia accompanied by elevated mortality in young birds are typically caused by the serotypes *Salmonella* Pullorum and *Salmonella* Gallinarum, which are transmitted horizontally and vertically. Cases caused by the other serovars, with the exception of serotypes Gallinarum and Pullorum, characterize paratyphoid infections, which can be transmitted by direct and indirect contact with individuals of the same species, such as birds themselves or with reservoirs represented by the reptiles, mammals, rodents, and the man [4].

Other *Salmonella* serotypes have public health importance, as are the cases of *Salmonella* Enteritidis and *Salmonella* Typhimurium. In these cases, the consumption of chicken meat and eggs is the most commonly described cause in the transmission of this bacterium to humans [6]. In man, these salmonellosis stand out as one of the main bacterial diseases which cause gastrointestinal disorders described worldwide [7]. Most cases of salmonellosis of importance in health surveillance are described as a self-limiting gastroenteritis characterized by diarrhea, fever, and abdominal cramps in humans. However, there are reports of mortality in children and the elderly. Furthermore, in the socioeconomic sphere, salmonellosis are described in association with absenteeism and medication expenses, besides the development of resistance to antimicrobials [8].

Over the years, the mechanisms and transmission pathways of salmonellosis in poultry have been a constant concern for the poultry industry. Given the epidemiological importance of poultry within the chain of transmission of *Salmonella* spp. to man, this study dedicates itself to questioning the presence of this agent in facilities aimed at poultry production, which represents a potential risk to public and animal health, with serious losses to the poultry agribusiness [6]. The detection of *Salmonella* spp. in commercial poultry flocks is probable, for this bacterium is widespread throughout the world, mainly in regions where there is a high poultry density [2].

In commercial farms, fallowing is one of the most commonly employed techniques to combat *Salmonella* spp. in contaminated farms. The fallowing method consists of the period between cleaning and disinfection of the poultry house and the housing of the next flock [9]. In counterpart, some authors define fallowing as a prophylaxis measure in epidemiology, represented by a period of time that must be applied to empty and disinfected premises to reduce the load of pathogenic microorganisms

and, therefore, minimize the microbiological challenge to the birds later housed in these premises [10].

Salmonellae are highly resistant bacteria in the environment and survive in poultry equipment and facilities for more than a thousand days [11]. Since this bacterium is responsible for great losses to the poultry sector and damage to public health, the present study is justified by the economic, social, and medical relevance of *Salmonella* spp. to society. Thus, it is necessary to analyze the effectiveness of fallowing used to promote its elimination from contaminated farms, as it is a widespread technique.

Considering the resistance in the environment and the survival in poultry equipment and facilities for long periods, it was questioned in this work the possibility of isolating *Salmonella* spp. in poultry farms with a history of *Salmonella* Gallinarum, after the completion of a 149-day fallow period. The possibility that after this period it would still be possible to isolate *Salmonella* spp. was admitted, given its characteristic of high viability or resistance to environmental conditions.

This study's overall objective was to investigate the environmental presence of *Salmonella* spp. bacterium in poultry houses of a commercial establishment destined for the confinement of poultry of the species *Gallus gallus*. The specific objectives were to verify the effectiveness of the fallowing technique applied for 149 days in poultry farms with a history of *Salmonella* Gallinarum isolation, to perform the isolation of *Salmonella* spp. in highly selective culture medium from environmental drag swabs, to characterize the isolated colonies in the culture medium, and to carry out an epidemiological inquiry in order to know the conditions of application of fallowing and the sanitary management practices adopted that are related to the epidemiology of *Salmonella* spp. in order to subsidize the bacteriological diagnosis.

2. Methodology

The environmental investigation of *Salmonella* spp. was carried out in three poultry houses of a commercial farm located in the city of Monte Carmelo, Minas Gerais, Brazil, destined for the confinement of poultry of the species *G. gallus*, suitable for meat production. The evaluated poultry houses had a history of *Salmonella* Gallinarum isolation in a previously housed flock and, because of that, were previously submitted to the sanitary emptying technique for 149 days, after the sanitary management of washing and disinfection. The determination of the sample amount was performed as recommended by the Normative Instruction No. 78 of November 3, 2003 [12], with some modifications. With the aid of 80 sterile swabs randomly numbered from 1 to 80, zigzag drags were made over the floor, feeders, feed boxes, fans, misters, drinking fountains, meshes, and roof structure. Concomitantly to the collections, the samples were placed in individual sterile flasks, which were placed in refrigerated Styrofoam boxes. The sample collection procedures were performed aseptically, using personal protection equipment to avoid contamination. After collection, the samples were transported under refrigeration to the Microbiology Laboratory from the Lutheran University of Brazil, where they were stored at a temperature between 2 and 8°C until bacteriological processing.

To perform the diagnosis through bacterial isolation, the collected samples were submitted to isolation in broths and culture means highly selective for *Salmonella* spp., following the recommendations of Ordinance No. 126 of the Ministry of Agriculture, Livestock and Food Supply (MAPA) of November 3, 1995 [13], and also according to Silva's description [14]. Initially, the stage of the bacteriological

analyses consisted of the infusion of the swabs samples in a non-selective enrichment broth. The swabs were inoculated into 20 mL of BHI broth, followed by incubation at the temperature of 35 to 37°C for 18 to 24 hours. At the end of the incubation in BHI broth, 2 mL of each sample in non-selective broth was inoculated in 20 mL of Tetrathionate broth and in 20 mL of Rappaport Vassiliadis broth, where they were incubated at a temperature of 42 to 43°C for 18 to 24 hours. For isolation, MacConkey and Brilliant Green agars were used. With the aid of a flamed platinum loop, samples in selective enrichment broths were streaked until depleted onto two plates, one containing Bright Green agar and the other containing MacConkey agar, followed by incubation at a temperature of 35 to 37°C for 24 hours. At the end of the plate incubation, the reading of the plates proceeded to observe the growth of colonies. On the MacConkey agar, colorless colonies were considered suggestive of *Salmonella* spp. while those that appeared rosy on the Brilliant Green agar were considered suggestive of bacteria belonging to the *Salmonella* genus. In addition to the research, an epidemiological inquiry was prepared according to Bannow [15], with some modifications, to survey the sanitary history of the farm and the sanitary conditions of washing and disinfection management. The epidemiological investigation was applied to the farm owner responsible for the production process, in form of a questionnaire.

3. Results and discussion

In the bacteriological isolation on plates, out of 80 samples of drag swabs submitted to diagnosis by plating, 52 samples showed growth of colonies with phenotypic characteristics suggestive of *Salmonella* spp. On plates containing MacConkey agar, there was a growth of colorless, plain, and circular colonies. Whereas on the Bright Green agar, the growth of isolated rosy, plain, and circular colonies occurred in the totality of positive samples. Therefore, when comparing the colonies obtained with the guidelines of Ordinance No. 126 of November 3, 1995 [13], 65% of the samples of drag swabs showed a growth suggestive of *Salmonella* spp. in the development of bacteriological diagnosis.

It is important to consider that the quantity of samples of drag swabs used in this study was defined based on Normative Instruction no. 78 from November 3, 2003 [12], with some modifications. The legislation in question recommends the use of 100 samples of swabs; however, only 80 were submitted to bacterial isolation. The modification does not seem to have influenced the bacteriological diagnosis results.

The isolation of *Salmonella* spp. from the poultry environment is representative of the risk of disease incidence that may result in financial losses related to industrial poultry production in Brazil, besides exerting an impact on the collective health of animals and humans [16]. In Brazil, these factors led the agency responsible for poultry health to establish the National Poultry Health Program and Ordinance 193 [17], which establishes standards for the prevention and control of *Salmonella* spp. in poultry and poultry products for human consumption [13]. Besides, Normative Instruction No. 50 of September 24, 2013, states that salmonellosis caused by *Salmonella* Enteritidis, *Salmonella* Gallinarum, *Salmonella* Pullorum, and *Salmonella* Typhimurium, when laboratorially confirmed, must be compulsorily reported to the official animal health service [18].

From a sanitary point of view, Payment and Riley [19] recommend that the longer the fallow period, the better the effectiveness of disinfection protocols. They also point out that the processes of cleaning and disinfection of poultry houses

associated with fallowing between flocks have shown to be extremely efficient in reducing the environmental persistence of *Salmonella* spp. Thus, when evaluating the epidemiological inquiry conducted in this research, it is important to stress that the fallow period applied and analyzed in this study was 149 days, and although it was longer than 4 months, it was still not enough to eliminate the pathogen from the environment.

Naturally, *Salmonella* species are eliminated in large numbers from the gastrointestinal tract of the infected birds and can remain in the fecal material for long periods, contaminating soil and water [20]. In particular, *Salmonella* spp. persist for more than 28 months in dry feces and dust. The fallow period evaluated in the present research was 149 days, and it is important to consider the notes by Gast [21], who states that the survival time of *Salmonella* spp. in chicken feces can be 9 days in the environment and that in the soil it can remain for up to 280 days.

According to Jaenisch [9], fallowing is a period that extends from the process of cleaning and disinfection of poultry facilities until the housing of the subsequent flock. The authors also state that the fallow period should be applied in a complementary way to the procedures for cleaning and disinfection of the facilities in order to enhance them, being determinant to achieve success in disinfection processes, especially the technical protocols related to the elimination of *Salmonella* spp. from poultry production farms. Furthermore, it becomes important to point out that the investigation carried out, through the application of an epidemiological inquiry and bacterial plating, revealed that the fallowing technique applied to three poultry farms with a history of *Salmonella* Gallinarum isolation was not effective, according to the bacteriological results.

The results obtained from the epidemiological inquiry presented important information related to the *Salmonella* Gallinarum transmission chain in broiler flocks. Based on the anamnesis performed with the owner of the poultry unit, the outbreak of Avian Typhoid was diagnosed on April sixteenth of the year 2014 and affected a flock of 33,000 birds housed in the same poultry houses where the drag swabs were collected in this research. As also described by Silva [14], the flock diagnosed with *Salmonella* Gallinarum was affected by 80% mortality and 60% morbidity, whose birds showed only clinical signs of inappetence and prostration on the bedding. At the time, all flocks housed on the farm were also affected and diagnosed with *Salmonella* Gallinarum. The flock diagnosed with *Salmonella* Gallinarum, as well as all other flocks diagnosed, was sacrificed and incinerated, depopulating all farm facilities. After the flocks were disposed of, the bedding from all the poultry houses underwent disinfection by fermentation or windrowing before being sent to the landfill in ditches. Similarly, the remaining feed was sent to the landfill along with the bedding.

It becomes relevant to point out that in the poultry houses with birds primodiagnosed with *Salmonella* Gallinarum, where the drag swabs were performed for the present study, a fallowing of 149 days counted from the end of the disinfection of the facilities was applied. According to Salle and Silva [22], the washing and disinfection of poultry houses and facilities are necessary for an effective control of pathogenic microorganisms and cannot be done randomly or irrationally, but with a scientific basis of knowledge. Thus, sanitation and disinfection measures were adopted in all poultry houses after the disposal of bedding and feed. For this purpose, the houses were swept and mechanically scraped to remove the remains of organic matter, such as remains of bedding, feces, and encrusted feed. Consequently, the facilities and equipment were thoroughly washed with high-pressure water, starting from the upper and ending with the lower portions of the facilities. The procedure was

repeated using water and detergent, and the action was waited for 30 minutes, followed by a third high-pressure wash. After the washing, the facilities and equipment were disinfected only once with Farmasept® Plus, whose chemical base consists of glutaraldehyde and benzalkonium chloride, at a dilution of 1.0 milliliter to 1.0 liter of water. After drying, hydrated lime was applied to the floor in the proportion of 500 g/m² of the premises. Workers' clothing and technical recording instruments were incinerated.

In this way, it is important to choose disinfectants that are ideal and compatible with the needs, taking into account the type of microorganism that one intends to control, the place and the object to be disinfected. For the disinfection of the poultry houses analyzed in this study, Farmasept® Plus was the disinfectant used in the recommended dosage in the datasheet of the laboratory producer. Sesti [11] points out that the chemical bases of these disinfectants are indicated for the elimination of bacteria of the *Salmonella* genus. Consequently, the results of the bacteriological exams suggest that the following process applied in association with the hygiene and disinfection process, up to the moment of the bacteriological analyses, was not effective in eliminating *Salmonella* Gallinarum from the installations and equipment, despite configuring itself as a prophylaxis technique to enhance the cleaning and disinfection processes applied in the environment.

Bannow [15] points out that the epidemiological investigation by inquiry in poultry production units is essential, for this practice has the purpose of performing an epidemiological triage pertinent to salmonellosis that affects poultry production and that can also affect public health. Through the epidemiological inquiry tool, it was observed that the birds affected by *Salmonella* Gallinarum did not present clinical signs suggesting infection by this pathogen. According to Oliveira [23], in general, birds affected by salmonellosis have mortality above the standard for the strain, presenting diarrhea, ruffled feathers, fallen wings, and dyspnea, in addition to depression and anorexia. However, Paiva [24] points out that infection by *Salmonella* spp. can develop asymptotically and sources of infection can become lifelong carriers. Also, by means of an epidemiological inquiry carried out on a farm suitable for broiler production, they found that a batch of infected birds did not present clinical signs suggestive of infection by *Salmonella* spp. [14]. Furthermore, according to Miranda [25] and Silva [14], the absence of *Salmonella* spp. isolation in plating may be associated with the high sanitary control that is applied to national poultry farms, as evidenced by the epidemiological inquiry in a poultry production unit studied.

The clinical manifestations of Avian Typhoid are usually observed in the adult stage of the host [23]. At this age, the birds show somnolence, with prostration, anorexia, diarrhea with yellow to greenish coloration, and a drop in egg laying, evolving to death in a few days. However, Bannow [15] describes the clinical occurrence of Avian Typhoid with isolation of *Salmonella* Gallinarum in two-week-old birds. In this way, it is important to consider that 35-day-old birds presented scientific epidemiological evidence that supports the research of *Salmonella* Gallinarum in young birds, even if they do not present clinical disease [26]. In this study, the epidemiological investigation also revealed that the researched farm is concerned with the health of its birds, which can be verified by noting that the farm uses a single-age housing system, controls the flow of vehicles and visitors, requires a bath for access to the facilities, has technical protocols for cleaning and disinfection of the aviaries, adopts following between flocks, and uses good quality feed and chlorinated water for bird consumption. Moreover, it is possible to observe that the farm was built in a region with low poultry density.

According to Fernandes [7], the lack of hygiene involving the environment and facilities favors the approach of synanthropic animals, such as flies, birds, vultures, and rodents, which may contribute to dispersing *Salmonella* Gallinarum throughout poultry farms. The authors also recommend the use of bacterial control methods, such as composting and dug and impermeable tanks for manure fermentation, to prevent the spread of *Salmonella* spp. They also highlight that the means of transporting poultry, manure, and eggs are configured as efficient ways to disperse the bacteria, especially when the vehicles enter production units without proper prior hygiene and disinfection. Furthermore, workers who move around poultry farms can act as a means of transmission of the agent of Avian Typhoid. They also orient that the creation of poultry flocks with multiple ages should also be avoided in a productive process. Moreover, according to Gast [21], vertical transmission should be an imminent and constant concern to avoid the introduction of *Salmonella* spp. in farms. Studies also point out that any negligence in the biosecurity program can lead to intrauterine transmission to the progeny. Therefore, infection-free flocks depend on specific and efficient prophylaxis measures to control, prevent, and eradicate *Salmonella* spp. through sanitary programs, which reflect directly on the health of the birds [27, 28].

Studies carried out by Silva [14] for the control of *Salmonella* spp. indicated that fallowing, applied as a prophylactic measure in poultry facilities with a history of isolation of *Salmonella* Gallinarum, configures itself as an effective technique for the elimination of this pathogen. In addition, they highlighted that the epidemiological inquiry was an efficient and recommended tool to investigate the epidemiological relations of the etiologic agent with the host and the poultry production environment. In counterpart to the present research, Silva [14] evaluated the effect of fallowing for a much superior period than the one considered in this work, which allows us to infer that longitudinal bacteriological studies are extremely important for the employment of epidemiology in poultry production.

4. Conclusion

The laboratory findings suggest that fallowing, applied for a period of 149 days to poultry facilities and equipment on a farm with a history of previous isolation of *Salmonella* Gallinarum in a previously housed flock, was not effective until the time of collection of environmental swabs. In this way, it becomes necessary to carry out new cleaning and disinfection procedures, as well as new monitoring through bacteriological exams, or even to extend the fallow period until the complete elimination of the pathogen from the environment. It was also concluded that the epidemiological inquiry is a valuable tool and should be adopted to investigate the epidemiological relations between the triad formed by *Salmonella* spp., the environment, and the host and is also useful to support complementary exams, such as isolation in bacterial cultures. Furthermore, it is possible to infer that longitudinal bacteriological studies of the environment are extremely important tools for the use of epidemiology in poultry production.

Author details

Sérgio Eustáquio Lemos da Silva^{1*}, Nayane Lopes Ferreira², Vanessa Silva Miranda³, Vitor Simão da Silva⁴, Renata Vieira Chaves Gabriel⁵, Daniela Ribeiro Roldão⁵, Vagno Espíndola da Silva⁵ and Lucas Peraphan⁵

1 Veterinary Sciences, Triângulo University Centre, Uberlândia, Brazil

2 Science and Mathematics Teaching, Lutheran University of Brazil, Canoas, Brazil


3 Microbiology Applied to Laboratory, Lutheran University of Brazil, Itumbiara, Brazil

4 Immunology and Microbiology, Triângulo University Centre, Uberlândia, Brazil

5 Graduating in Veterinary Medicine, Triângulo University Centre, Uberlândia, Brazil

*Address all correspondence to: sergiolemosvet@gmail.com

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Poultry Farming: New Perspectives and Applications Chapter – Parasitic Diseases of Chickens

Mohammed A. Al-Badrani and Shamal A. Al-Muffti

Abstract

Fowls and their eggs provide animal protein for human populations. Parasites are very common in fowls and heavy infection can affect the growth, and egg production, and cause death. Fowls during feeding pick the parasitic infective stage by ingesting contaminated food, and water. There are two groups of parasites infecting fowls: external (ectoparasites) and internal parasites (endoparasites). The clinical findings of the examined affected chickens showed that symptoms vary from healthy to subclinical symptoms. The main clinical signs were dullness, emaciation and weakness, hemorrhagic enteritis, congestion of ceca, mucoid and watery diarrhea. Besides. The research refers found 2 species of lice namely *Mencanthus stramineus* and *Goniocotes gallinae*. One species of soft tick, from genus *Aragas persicus*, was recorded. While internal parasites included different types of *Eimeria* oocysts. The current study did not reveal any blood parasites or *Cryptosporidium* oocysts in all of the examined fowls. Different types of intestinal nematodes which were recovered with *Subulura species* followed by large roundworms, *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria*. Regarding tapeworms, six species were recorded and identified, which were *Raillietina tetragona*, *R. echinobothrida*, *R. cesticillus*, *Fimbriaria fasciolari*, *Davainaea proglottina*, and *Amoebotaenia sphenoides*.

Keywords: chicken, Kurdistan-Iraq, Newcastle disease and poultry, poultry farming, parasitic diseases of chickens

1. Introduction

Domestic fowls are the most important protein sources of human populations in every part of the world. As is demonstrated that during the last 30 years, eggs and poultry meat were constantly increasing. Poultry industries make a significant contribution to improving the nutritional status and economic income of many countries of the world [1].

Animal welfare is a big problem in today's factory farming. And the widespread abuse of birds serves as an example of it. Every year, 9 billion chickens are grown and killed for food in America alone. Broiler chickens are the name given to chickens grown on factory farms for meat. They are kept in cramped, gloomy sheds. Despite the meat industry's best efforts to conjure up images of happy birds frolicking in green

fields, 99.9% of hens raised for food are kept in factory farms where they are deprived of access to sunlight and fresh air. And it confines them to filthy areas that serve as ideal breeding grounds for disease [2].

Parasitic infections of poultry are the major factors responsible for economic losses through a reduction in productivity and increased mortality. A lot of losses in poultry are linked to disease-causing pathogens such as viruses, bacteria and parasites. Poultry is subjected to a wide variety of diseases including Newcastle disease, salmonellosis, respiratory disease and a large number of ecto-endoparasites. Their diseases are often fatal resulting in high mortality and low productivity. Domestic fowls feed on different types of food materials, these materials include grains, fruits and insects which may harbor infective stages of parasites, particularly gastrointestinal helminths [3].

Gastrointestinal helminths of poultry are commonly divided into three main groups: nematodes, cestodes and trematodes. Nematodes are considered the most important group of helminths of poultry such as the species which belong to the genera *Capillaria*, *Heterakis* and *Ascaridia*. The most important genera of cestodes are *Railleitina* and *Hymenolepsis*. Regarding the types of trematodes, they are not very common like the other groups [4].

Gastrointestinal tract worms, in particular, are known to cause poor feed conversion and utilization that lead to emaciation and poor weight gains. Various ectoparasites are reported in the local fowls such as lice, fleas, mites and soft ticks [5].

In general, there is a need to understand the epidemiology of the various fowl parasites in order to plan strategies to increase the productivity of chickens [6]. Limited work has been done on ectoparasites and endoparasites of fowls in Iraq including the Kurdistan Region [7].

2. Types of ectoparasites in fowls

Ectoparasites of poultry are arthropods that live on the skin and feathers including lice, fleas, soft ticks and mites [8]. Ectoparasite problems may be controlled by:

- Cleaning of houses between flocks,
- Whole flock replacement rather than partial replacement,
- Using mesh to keep out wild birds,
- Rodent eradication program,
- Maintenance of manure in a dry condition to avoid flies breeding [9].

Members of the animal phylum Arthropoda, which is distinguished by having outwardly segmented bodies, jointed legs, appendages, and chitinous exoskeletons, include poultry ectoparasites [10].

One pair of antennae is linked to the head, three pairs of legs are attached to the thorax, and some mature insects have wings. These characteristics identify lice, flies, bugs, and fleas as members of the class Insecta [11].

Some ectoparasites of poultry such as lice eat the dead cells of the skin and skin acts as a medium through which they suck blood and from which they obtain shelter.

Lice may be closely confined to their hosts during their entire life cycle, while other parasites wander freely from bird to bird. Some are highly hosted specific while; some species may maintain host nonspecific relationships [12].

3. Types of internal parasites in chickens

3.1 Blood parasites of chickens

Birds may be hosts for a number of blood-inhabiting protozoan species and nematode worms which are transmitted by haematophagous arthropods [13]. Protozoan parasites include haemosporidia which belong to several genera such as *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Hepatozoon*, *Babesia*, and *haemoflagellates* that belong to the genus *Trypanosoma*. Most of the birds are susceptible to being infected with blood parasites and the prevalence rate, especially in the tropics, maybe more than 30%. Blood parasites vary in their host, both for the arthropod vectors and the vertebrate host, specificity. While some are restricted to a small number of host species, others can survive and reproduce in a wide variety of birds and arthropods [14]. Internal parasites are mainly classified into two groups (Protozoa and Helminthes). Protozoa include gastrointestinal and blood protozoans while helminths include three groups: Trematodes, Cestodes and Nematodes [15].

Many recent studies have focused on avian blood parasites as a model system for host–parasite interactions in evolutionary and ecological aspects. Extensive laboratory studies have been conducted describing their pathologies, especially for species of *Leucocytozoon*). Based on the current taxonomy, three species of *Leucocytozoon* and three species of *Trypanosoma* are found in the domestic chicken *Gallus gallus domesticus*, mainly in tropical and subtropical regions worldwide [16].

These are:

Leucocytozoon macleani.

Leucocytozoon caulleryi.

Leucocytozoon schoutedeni.

Trypanosoma numidae.

Trypanosoma calmettei.

Trypanosoma gallinarum.

All of these species are well distinguished based on the morphology of their blood stages and/or laboratory experiments documenting their transmission and life cycles. The pathogenicity of many species of *Leucocytozoidae* (Sporozoa, Haemosporida) in wild birds is unclear, many cases of mortality have been reported in domestic chickens and other poultry. The most common vectors of avian trypanosomes are arthropods that belong to the families Hippoboscidae, Culicidae, Ceratopogonidae, and Simuliidae [17]. In addition, dermanyssid mites have been identified as avian trypanosome vectors. Little is known concerning the pathogenic effects of trypanosomes in chickens although artificial infection with *Trypanosoma brucei* showed no obvious impairment of health. Previous accounts of blood parasites in chickens in Africa are relatively rare. In a study in Zimbabwe, 4 of 94 examined chickens harbored *Leucocytozoon sabrazesi*, and 5 of the 94 examined chickens harbored *L. macleani* in Ghana; however, no *Leucocytozoon* or *Trypanosoma* infections were detected [18]. Earlier studies showed *Leucocytozoon* species infected 55 of 163 (34%) examined chickens in Ibadan, Nigeria. In a study of 110 chickens observed in Anambra, and Nigeria, none was infected with blood

parasites. In Tanzania, it is reported that out of 150 chickens tested, more than 50% were infected with *L. schoutedeni* [19].

3.2 Helminthes of chickens

The name “helminths” is derived from the Greek word helmins or helminthos, a worm, and is usually applied only to the parasitic and non-parasitic species belonging to the phyla Platyhelminthes (flukes, tapeworms and other flatworms) and roundworms (Nemathelminthes). The helminths are invertebrates characterized by elongated, flat or round bodies. The flatworms or platyhelminths (platy from the Greek root meaning flat) include flukes and tapeworms [20]. Round worms are nematodes (nemato from the Greek root meaning thread which includes helminths have similar anatomic features that reflect common physiologic requirements and functions. The outer covering of helminthes is the cuticle or tegument; nutrients must be absorbed through the tegument. A helminths also has a head and tail end, and its tissues are differentiated into three distinct tissue layers: - ectoderm, mesoderm and endoderm [21]. Parasitic helminths of chickens are commonly divided into three main groups:

3.2.1 Nematodes

They constitute the most important group of helminth parasites of poultry both in a number of species and external damage they cause. The main genera include *Capillaria*, *Heterakis* and *Ascaridia* (**Figure 1**) [23].

A. galli is a parasitic roundworm belonging to the phylum Nematoda. Nematodes of the genus *Ascaridia* are essentially intestinal parasites of birds. Nematodes and cestodes are common GI parasites of commercial poultry. The parasites typically cause acute irritation and might occasionally result in bleeding. The gut lining may erode severely and cause death. Deep litter households may experience a serious problem with these parasites. Heavy infections may lead to decreased fertility, egg production,



Figure 1.
Small intestines of a broiler chicken impacted with Ascaridia galli [22].

and growth. Nematodes of poultry infection are widely distributed in different parts of the world, and numerous types of research have existed to prevent the mortality of poultry from parasitic diseases [22].

- **Ascaridia**

The genus *Ascaridia* was first established by Dujardin in 1845. Nematodes of this genus are hosts specific to the class Aves. A large number of species have been reported from fowl; the common ones are as follows: *A. galli* is a parasitic roundworm belonging to the phylum Nematoda. *A. galli* is the most prevalent and pathogenic species, especially in domestic fowl. It inhabits the small intestine and causes ascariidiasis, a condition that affects poultry, especially hens and turkeys, and is caused by severe worm infestation. In birds, *A. galli* is the biggest nematode. The body is cylindrical, creamy white, and semitransparent. The mouth is prominent and is bordered by three broad trilobed lips on the anterior end. There are teeth-like structures on the borders of the lips [24]. detailed in fully the anatomy of *A. galli*, a creature whose body is totally wrapped in a thick protein structure known as a “cuticle.” The cuticular alae are underdeveloped, and the cuticle is striated transversely over the length of the body. The dorsal lip has two noticeable papillae, and the sub-ventral lips have one each. The nematode’s sensory organs are these papillae. *A. galli* exhibits clear sexual dimorphism and is diecious. With a vulva opening roughly in the middle of the body, halfway between the anterior and posterior ends, and an anus at the back end, females are noticeably longer and more robust [25]. It is typical for females to have a blunt, straight tail end. Males tend to be smaller and shorter than females, and they have distinctively pointed, curved tails. To the rear of the body, there are 10 pairs of caudal papillae that are grouped linearly into distinct groups such as precloacal (3 pairs), cloacal (1 pair), post-cloacal (1 pair), and sub-terminal (3 pairs). All types of poultry are affected by the nematode, however, young birds under the age of 12 weeks frequently exhibit the most severe damage. Reduced egg production and weight depression in poultry husbandry are primarily caused by heavy infection. Intestinal obstruction can happen in cases of severe infections. In heavy infections, adult worms may move up the oviduct and be found in hens’ eggs, and sometimes they are also found in the feces of the infected birds [26].

- *Heterakis*

The genus *Heterakis* was first described and named by Dujardin in 1845. The parasites belonging to this genus are characterized by the following features: they are small worms with the anterior end bent dorsally, and mouth surrounded by three small equal size lips. Small white worms are found in the tip or blind ends of the caeca. The female measures 10–15 mm long and the male 7–13 mm long. Esophagus end in a well-developed bulb, containing a valvular apparatus. Pre-anal sucker well developed in males, papillae present, spicules unequal, uterine branches in the female opposite, vulva near the middle of the body, and eggs with thick and smooth shells [27], while studying six species of *Heterakis*, emphasized that they do not have certain points of interest, especially those related to the sense organs which reveal the possibility of identifying their larval forms since these organs are well developed in larvae The most important gastrointestinal nematode responsible for considerable production losses in poultry is *Heterakis gallinarum* [28].

- *Subulura*

Subulura also named *Allodaba*, which is still used as a synonym. *Subulura* genus usually has lateral cervical alae and mouth dorsoventrally elongated, vestibule with a thin chitinous lining or heavily chitinized, Esophagus dilated posteriorly and followed by a bulb. The male has a fusiform pre-anal sucker, located, a distance anterior to the cloaca and the caudal alae is slightly developed or absent. The caudal papillas are sessile and arranged in two longitudinal rows and the spicules equal in length. In females, the vulva is near the middle of the body and uterine branches diverge. The female may be oviparous or ovoviviparous and the eggs sub-globular and thin-shelled (**Figure 2**) [30]. *Subulura* infections in fowls are insignificant due to their low pathogenicity. Pathological changes suggestive of acute cecal hemorrhagic enteritis were recorded. Infection occurs in the cecae of fowl, turkey, guinea fowl, and wild-related birds in Africa, North and South America, and Asia.

- *Capillaria*

There are several species of *Capillaria* that occur in poultry in **Figure 3**. Male *capillaria* are 15–25 mm length and female *capillaria* are 35–80 mm long filamentous worms (females). Males only have one spicule, and many also have an early form of a bursa. The size of the eggs varies depending on the species; they contain bipolar plugs and thick shells. Important species include; *C. annulata*, *C. anatis* and *C. contorta*. *Capillaria annulata* and *Capillaria contorta* occur in the crop and esophagus. In the lower intestinal tract, there may be several different species but usually, *Capillaria obsignata* is the most prevalent. These species may cause thickening and inflammation of the mucosa. The life cycle of this parasite is direct. The adult worms may be embedded in the lining of the intestine. The eggs are laid and passed in the droppings. The created embryo will take 6 to 8 days, the eggs are infective to any other poultry that may eat them. The most severe damage occurs within 2 weeks of infection [29]. Approximately 1 cm (0.39 in) long, adult *Capillaria* are “threadlike” worms that are extremely thin.



Figure 2.
Anterior end Subulura sp. [29].



Figure 3.
Adult male of *Capillaria* sp. [31].

Their barrel-shaped eggs contain clear pugs on each pole and can only be seen under a microscope. There are various different *Capillaria* species, and each one infests a certain area of the chicken. Some species, including *C. annulata* and *C. contorta*, can infiltrate the esophagus and crop, thickening and inflaming the mucous membranes. Other pathogens, including *C. bursata*, *C. caudinflata*, and *C. obsignata*, attack the lower intestinal tract and cause inflammation, bleeding, and erosion of the intestinal lining. *Capillaria* can be lethal to the chicken if they are present in large enough quantities.

3.2.2 Cestodes

There are two important genera infecting chickens, *Raillietina* and *Hymenolepis*. *Raillietina* is the name of a genus of tapeworms that includes helminth parasites of vertebrates, and mostly of birds. The genus was named in 1920 by Louis-Joseph Alcide Railliet. of the 37 species recorded under this genus, *Raillietina demerariensis*, *R. asiatica*, and *R. formsana* are the only species reported from humans, while the rest is found in birds. *R. echinobothrida*, *R. tetragona*, and *R. cesticillus* are the most important species in terms of prevalence and pathogenicity among wild and domestic birds (**Figure 4**) [31]. There are many different species of tapeworms that can infect backyard poultry. The majority of these species are totally harmless, however, large numbers of tapeworms may cause weight loss and loss of egg production.

Raillietina tetragona occurs in the posterior half of the small intestine (ileum) of the chicken, guinea fowl, pigeon and other birds. It is cosmopolitan in distribution. It is one of the largest of the fowl tapeworms and the adults reach up to 25 cm in length. The scolex is smaller than that of *R. echinobothridia* and the rostellum is armed with one or two rows of hooks and the suckers are oval and armed. The genital pores are

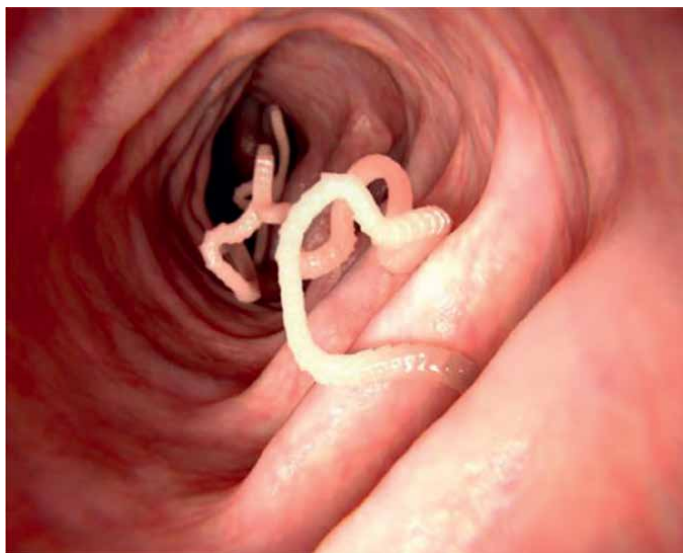


Figure 4.
Cestode in the small intestine of chicken [32].



Figure 5.
Scolex of Raillietina tetragona 336×448 pixels [32].

usually unilateral and the eggs are found in egg capsules each containing 6 to 12 eggs. The eggs are 25–50 μm in diameter (**Figure 5**) [33].

Raillietina echinobothrida is the most prevalent and pathogenic helminthic parasite in birds, particularly in domestic fowl (*Gallus gallus domesticus* Linnaeus). It requires two hosts, birds and ants, for completion of its life cycle [34]. The parasite is to blame for the chicken version of “nodular tapeworm sickness.” A typical tapeworm structure, the body of an adult *R. echinobothrida* is made up of a number of ribbon-like body segments that enlarge gradually from the anterior end towards the posterior. It is dorso-ventrally flattened, pale in color, extremely elongated, and fully covered in a tegument. The body can be up to 25 cm long and typically measures 1–1.5 cm in width. *Raillietina cestricillus* is very common throughout the world in domestic poultry, macroscopically is about 15 cm long and the anterior border of the segment is shorter than the posterior one. The scolex is cylindrical or nearly globular in shape and smaller in size [32] in **Figure 6**. Hosts. *R. echinobothrida* infections are observed in chickens and turkeys, tetragonal infections are most common in chickens, guineafowl, and pigeons, and domestic chickens are infected with *S. cestricillus*. The range of all three species is international. The worms are found in the small intestine, where the scolex is embedded in the mucosa, as their preferred habitat. 36 Morphology: *S. cestricillus* measures 9–13 cm, whereas *R. echinobothrida* and *R. tetragona* can grow to a length of 10–25 cm. All three species’ eggs are the same size, measuring 74×93 , however, the quantity of eggs in each gravid segment differs. The *R. tetragona* gravid proglottid has the most

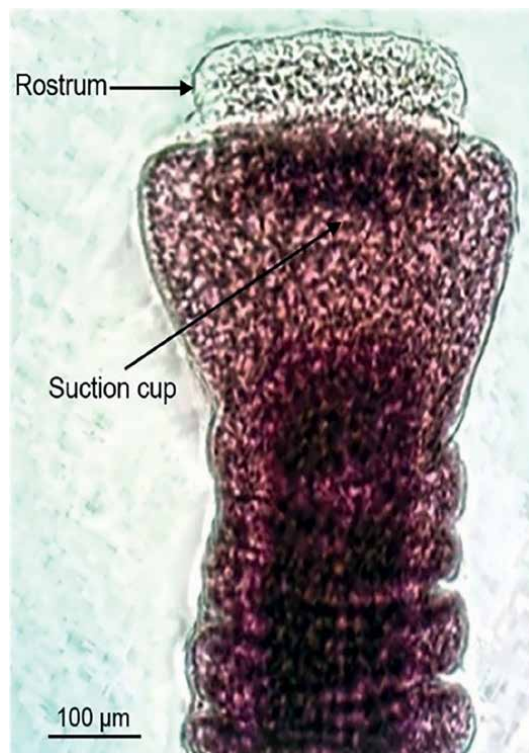


Figure 6.
Scolex of *Raillietinia cestricillus* 150×141pixels [32].

egg capsules overall. *R. echinobothrida* and *R. tetragona* have different gravid segment morphologies than *S. cesticillus* because the segments of the first two are replaced by numerous fibrous walled egg capsules, each containing several eggs, as opposed to the numerous thin-walled egg capsules, each containing a single egg, in *S. cesticillus*.

The family of tapeworms known as *Davaineidae* contains helminth parasites of vertebrates. This family has 14 genera, of which *Davainea* is the best known and has been the subject of the most in-depth research. Members of the family can be identified by the rostellum, which is a crown of mattock- or hammer-shaped hooks, present at the tip of the scolex. Suckers with spines encircle the rostellum on both sides. The most frequent hosts of these tapeworms are birds, though they can also be discovered in some cases in mammals. Small insects like ants serve as intermediate hosts. The intermediate hosts for *Davainea proglottina* are slugs and chickens (**Figure 7**) [35]. Clinical symptoms and pathogenicity: Despite its small size, *D. proglottina* is one of the more dangerous species, especially in young birds and especially if it happens frequently. Clinical symptoms include a lifeless appearance, sluggish movements, decreased weight gain, emaciation, dyspnea (breathing problems), leg paralysis, and death. It is possible to notice microscopic necrosis, hemorrhages, and thicker mucosal membranes.

Amoebotaenia sphenoids occur in domestic poultry and have a global distribution. It is a little tapeworm with dimensions of 2 to 3.5 mm in length and 1 mm in breadth. It is generally triangular in shape and has 20 segments. Earthworms serve as the cestode's intermediary hosts during its development. About 4 weeks after consuming infected earthworms, mature tapeworms are discovered in chickens. Even though this parasite does not cause any clinical symptoms, enteritis and wasting have been linked to it when there are significant infections present (**Figure 8**) [37–40]. It is small, up to 4 mm long, and roughly triangular in shape • The rostellum is armed • The genital pores usually alternate irregularly at the extreme anterior end of the proglottid margin • The uterus is sac-like and slightly lobed.



Figure 7. Adult stage of *Davainea proglottina*, 186×480pixels [32].

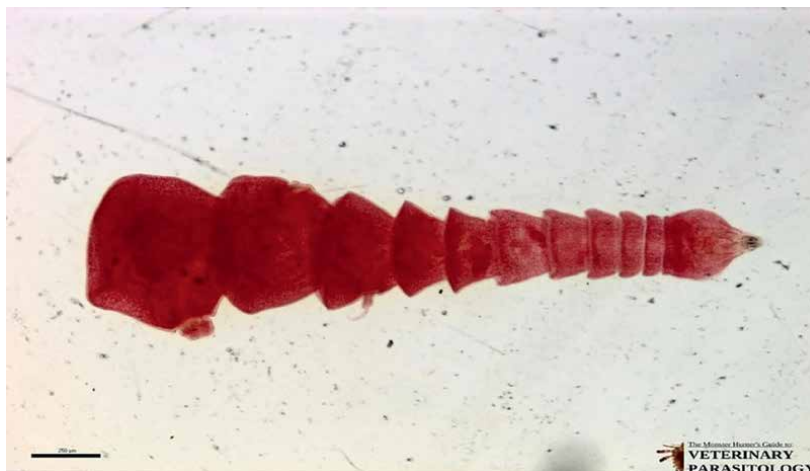


Figure 8.
Adult stage of Amoebotaenia cuneata = sphenoides 400×542pixels [36].

3.2.3 Trematoda

Generally, infection with trematodes is not very common in domestic chickens. The only reference that dealt with trematodes was 309 heads of birds (83 chicken, 152 ducks, and 74 muscovy ducks) from two districts of Sukabumi and Serang, province of West Java, has been investigated for the presence of trematode infection. Chicken from Sukabumi had a slightly higher trematode infection rate than chicken from Serang. During the study was identified at least 13 genera of trematode were: *Apatemon sp.*, *Catatropis sp.*, *Cotylurus sp.*, *Echinostoma sp.*, *Hypoderaeum sp.*, *Notocotylus sp.*, *Opistorchis sp.*, *Paramonostomum sp.*, *Philophthalmus sp.*, *Prosthogonimus sp.*, *Psilochasmus sp.*, *Dendritobilharzia sp.*, and *Trichobilharzia sp.* The last two identified flukes were found in both ducks and Muscovy ducks but not in chickens [41].

4. Smart techniques for better poultry farming and management

- While constructing the farm's shelter should be in an east–west facing to avoid excess sunlight.
- Adequate space required to avoid overcrowding should make sure 2 sq.ft. of space must be maintained for each bird.

What is the main problem facing poultry farming?

Eradication, elimination, and/or control of foodborne and zoonotic pathogens present a major challenge to the poultry industry [42].

5. New proposals for poultry farming

- Chicken Farm (Meat Production) The broiler industry's sole goal is to raise chickens for their meat. Day-old chicks must be raised into adult birds until they have gained the proper weight and are prepared to be culled and sold.

- Farm - layer (Egg Production) In the layer sector, specialized hatcheries nurture birds to the point of lay before supplying them to egg producers, who are typically located close to feed sources and markets [43].

6. Practical part

Antemortem examination

The whole body of each chicken, including the skin and the feathers, was examined by the naked eye and with the aid of magnifying lens starting from the head to the legs including wings, thigh, and neck for the presence of ectoparasites.

Collection and examination of ectoparasites

Ectoparasites, which were visible in live chickens, were collected gently using thumb forceps. All the collected ectoparasites of each chicken were preserved in a test tube containing 70% ethyl alcohol until the time of identification. The legs of each chicken were carefully examined for the presence of any inflammatory lesion, and if it is present, skin scraping was obtained using a clean blade and the scraped sample was mixed with 10% KOH and examined under the microscope for the presence of mites. Ectoparasites were collected by spraying a commercial insecticide over all of the body and in areas where suspected lesions of ectoparasites were present the entire body and feathers were then gently rubbed over a white cloth with a strong light source.

Postmortem examination

Following the slaughtering of each chicken, the blood sample was collected directly in a sterile test tube containing EDTA anticoagulant for thin blood film. All the thin blood films were stained with Leishman's stain for the presence of blood parasites.

7. Facilities and supplies

- **Feeders**
 - include both hanging feeders for older birds and trays for chicks.
- **Waterers**
 - Similar to feeders, they must be strong to prevent tipping over and should be simple to refill. To prevent drowning, fill the chick waterer with stones.
- **Nutrition**
 - Offer grit in little and larger sizes depending on the age of the bird. Sands from streams include minerals and stimulate the gizzard physically. Calcium can be found in abundance in oyster shells.
 - It's crucial to include fresh green vegetable matter and hay chaff seeds to satisfy the higher nutritional needs of chicks and laying hens.

- Think about sprouted grains that you have grown yourself or chick starts that you have purchased.

- **Temperature**

- A heat source is necessary for chicks. For this purpose, heat lights and heat pads are frequently employed. When housing a lot of chicks in a brooder, heat lights can be fitted [44].

8. Conclusions

- The microscopic examination of droppings, intestinal scraping and cecal contents revealed the presence of different types of *Eimeria* oocysts.
- Blood parasites and *Cryptosporidium* oocysts were not recorded in all the examined chickens.
- The nematode *Sublura* followed by *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria*.
- Six species of tapeworms were recorded and identified, namely: *Raillietina tetragona*, *R. echinobothrida*, *R. cesticillus*, *Fimbriaria fasciolaris*, *Davainea proglottina*, and *Amoebotaenia sphenoides*
- This article strongly suggests that ectoparasites and endoparasites were very serious problems of domestic local breed chickens, so appropriate control strategies need to be devised in order to limit the effect of infections on their productivity.

A. Appendix


No.	Host (male or Female)	Area or provinces	Date	Type of Parasites	% Infection
1					
2					
3					
4					
5					
6					
7					

Author details

Mohammed A. Al-Badrani and Shamal A. Al-Muffti*
Department of Biology, College of Sciences, University of Duhok, Iraq

*Address all correspondence to: shamal.al-muffti@uod.ac

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Autogenous Vaccines in the Poultry Industry: A Field Perspective

Victor Palomino-Tapia

Abstract

Emergent economically important diseases affecting the poultry industry in the face of commercial vaccination programs in place might require custom-made vaccines to be controlled in the field. These custom-made vaccines (“autogenous”), albeit requiring less regulatory burden than fully licensed commercial vaccines, are restricted in their scope and field isolates and can only be used in particular areas/operations. This chapter summarizes field and research experience of the author with some viral and bacterial autogenous vaccine programs (e.g., Avian Reovirus, Fowl Adenovirus, Infectious Bursal Disease Virus, *Salmonella enterica* spp., *Escherichia coli*, *Clostridium* spp.), as well as commentaries on regulations, and adjuvant technologies used in the poultry industry.

Keywords: autogenous vaccines, vaccine candidate selection, challenge evolution, poultry, autogenous vaccine program considerations

1. Introduction

Disease control and prevention in industrial poultry production relies mainly on biosecurity and vaccination [1, 2]. Biosecurity focuses on decreasing the possibility of agent entry into poultry barns, or reducing the environmental agent load by cleaning, disinfection, prevention of contact with wildlife, and entry/exit control of personnel and equipment, thus decreasing the likelihood or severity of the infection and/or delaying the age of infection [1–4]. In contrast, vaccination focuses on decreasing the susceptibility to pathogens likely to cause economic losses [1, 5] and/or preventing colonization by food-borne diseases (e.g., *Salmonella enterica*) [6]. Commercial vaccines are designed to target major and constant threats for the industry, common to several poultry markets worldwide and, as such, demand production of a high number of dosages across the world that justify the costly process of research, development, registration, marketing, and testing of each master seed and final product serial release. Because these problems are constant for the industry and homologous challenges to classic vaccines are prevalent in the field, these products are expected to stay relevant for decades, thus covering their expenses and generating revenues for vaccine-producing companies for a long time. Examples can be found in different vaccines, including but not limited to Marek’s disease (MDV-Rispens, HVT-FC126; SB1), Avian encephalomyelitis (AE), Fowlpox (FP), Newcastle disease (NDV), Avian

Reovirus (ARV) (cluster 1.1), Infectious bronchitis (e.g., serotypes Massachusetts, Connecticut), Chicken Anemia Virus (CAV), all of them with long product life cycles that are still relevant today, after decades of use since research, development, and registration [7–9]. As of March 2022, in the US, there are more than 1000 active licenses for veterinary vaccine products [10]. However, if the challenge agent is shown to be antigenically different (antigenic variants) from the classic strains present in standard licensed vaccines, the immunity elicited by these licensed vaccines might be insufficient to prevent the economical consequences of the challenge. Thus, control of disease might require “emergency,” “custom-made,” “complex-specific,” or “farm-specific” autogenous vaccines made with isolates obtained from affected flocks [11–14].

2. Legal framework in the US and Canada

Vaccination is performed using: (1) classical standard licensed vaccines, which require extensive testing (i.e., safety, purity, efficacy, and potency) and thus are costly to develop (e.g., >2 million USD), and slow to license (e.g., 3 ± 10 years) [7–9]; (2) conditional licensed vaccines, which require moderate testing (i.e., safety, purity, and reasonable expectation of efficacy and potency) and are therefore less costly and require a moderate regulatory process; and (3) federal-licensed autogenous vaccines, which, by regulation, are inactivated and can include bacterial and viral antigens. These autogenous vaccines require basic testing (i.e., basic purity, basic safety) [15–20]. In addition to these three types of vaccines described, there is a fourth classification present only in the US—one that allows the production of live autogenous vaccine(s) that do(es) not require a federal licensing to be produced. This is based on a provision in the Title 9 Code of Federal Regulations Section 107 [20] that allows the owner of the affected animals or a veterinarian in the course of a state-licensed professional practice under a veterinarian-client-patient relationship to manufacture products (i.e., vaccines) without the need of a federal license for the exclusive use of the animals under the ownership of the manufacturer or under the veterinarian’s care. This provision requires communication and approval by the state veterinarian. It has been used by the poultry industry for control of significant problems caused by a pathogen’s infection that cannot be resolved by adjustments to existing vaccination programs with licensed vaccines nor can they be controlled by inactivated autogenous vaccines or the addition of such into existing vaccination programs. Consequently, these problems require the manufacturing of an in-house live autogenous vaccine for the exclusive use of the poultry operation [21]. Specific examples include but are not limited to: infectious bronchitis virus (IBV) variant causing heavy condemnation losses (i.e., airsacculitis) [22]; field Hemorrhagic Enteritis virus capable of breaking maternal antibodies levels, causing severe immunosuppression [23]; turkey coccidia [24]; *Mycoplasma gallisepticum* [25]; and others. Caution should be exercised whenever using these live autogenous vaccines as potency and purity issues are common [24]. There is no legal provision for this fourth classification (live autogenous) in Canada. A list of advantages and disadvantages of each type of vaccine is shown in **Table 1** as modified from [13].

Autogenous vaccines are approved when commercial vaccines are not available in the location or are not effective against the challenge; need to be approved by a registered veterinarian under a veterinarian-client-patient relationship, and do not interfere with existing local and federal legislation or programs such as

Vaccine type	Legal provision		Advantages	Disadvantages
	USDA	CFIA		
Standard license	9CFR §101–118	VBG 3.1–1	<ul style="list-style-type: none"> • Full studies on purity, safety, potency, and efficacy • Facilities need to be inspected and approved • USDA/CFIA confirmatory test (Seeds, cells, and product) 	<ul style="list-style-type: none"> • Very slow and expensive process (e.g., 3–10 years)
Conditional license	9CFR §102.6	NA	<ul style="list-style-type: none"> • Used in an emergency, absence of effective standard license vaccine, limited market, etc. • Full purity and safety studies on master seed • Reasonable expectation of potency and efficacy (in-vivo) • Not limited to selected operation(s) • Faster availability than a standard license vaccine, not as fast as an autogenous vaccine is properly placed under advantages. • Can lead to a standard license vaccine • Less stringent inspection of facilities 	<ul style="list-style-type: none"> • Requires years to license • Efficacy is still uncertain, although with evidence suggestive of acceptable efficacy • Potency test not required for each serial • Limited distribution
Federal licensed Autogenous	9CFR §113.113 VSM 800.69	VBG 3.13E	<ul style="list-style-type: none"> • Used in an emergency. Faster availability time of vaccines containing new isolates (6 months—1 year) • Basic studies on purity (no extraneous bacteria/fungi/yeast) • Basic safety (either lab animals or limited number of host animals) • Only inactivated microorganisms • Requires vet-client relationship • Less stringent inspection of facilities 	<ul style="list-style-type: none"> • Host animal safety, potency, and efficacy not well established • Limited distribution to selected operation(s), usually only flock of origin/complexes within company • Limited testing on seeds
Non-Federal Autogenous approved by state veterinarian	9 CFR §107	NA	<ul style="list-style-type: none"> • Lowest regulatory burden • Shortest implementation time (between isolation & immunization) • Can be used for manufacturing and usage of “Live Autogenous” 	<ul style="list-style-type: none"> • Requires state-per-state permission by state veterinarian • Poultry integrator/vet needs to produce their own vaccine (owned or rented facilities) • High likelihood of purity/quality issues

USDA—United States Department of Agriculture; CFIA—Canadian Food and Inspection Agency; VBG—Veterinary Biologics Guidelines; VSM—Veterinary Services Memorandum; NA—Not applicable.

Table 1.
Advantages and disadvantages of vaccine types based on licensing requirements.

eradication programs (i.e., high pathogenic avian influenza) or vaccination ban [26]—low pathogenic Avian Influenza (LPAI), not H5 or H7, from poultry or from other species that potentially affects poultry might find authorization for federal-licensed autogenous vaccine production [27]. Purity, safety, efficacy, and potency require to be tested at different degrees in each type of vaccine. Following definitions in Chapter 9 of federal regulations, Section 101.5, “Purity” refers that product should be “free of extraneous material (organic or inorganic) as determined by test methods or procedures established by Animal and Plant Health Inspection Service in Standard Requirements or in the approved Outline of Production for such product, but free of extraneous microorganisms or material, which in the opinion of the Administrator adversely affects the safety, potency, or efficacy of such product.” “Safety” is defined as “freedom from properties causing undue local or systemic reactions when used as recommended or suggested by the manufacturer.” “Efficacy” is the “[s]pecific ability or capacity of the biological product to effect the result for which it is offered when used under the conditions recommended by the manufacturer,” while “[p]otency” is referred to as the “[r]elative strength of a biological product as determined by test methods or procedures as established by Animal and Plant Health Inspection Service in Standard Requirements or in the approved Outline of Production for such product” [20]. The Animal and Plant Health Inspection Service (APHIS)- part of the United States Department of Agriculture (USDA)- through its Investigative and Enforcement Services (IES), has the task to investigate alleged violations of the statutes that govern vaccine manufacturing (including autogenous vaccine manufacturing) and will issue warning letters, settlements, and penalties upon failure to follow APHIS-administered laws [28].

3. Economic size of the industry

Autogenous vaccine market revenues in 2022 from across the world are estimated in to 129.6 million USD, and it is anticipated to reach to 231.6 million USD by the end of 2033, a growth of 5.4% compounded annual growth rate (CAGR) [29]. These estimations were based on the increased CAGR of 4.7% obtained between 2015 and 2022 and the increased rise in zoonotic disease incidence, rare infectious diseases, and variant emergence in livestock and companion animals [11, 29]. Furthermore, autogenous vaccines containing bacterial antigens help reduce the overall usage of antimicrobials in a complex [30] and are considered as alternatives to antibiotics in livestock [12]. This is important as judicious use of antibiotics and consumer demand for products without antibiotics such as “Raise without Antibiotics” (RWA) and “No Antibiotic Ever” (NAE) have led to a sharp decrease of antibiotics in clinical practice to limit the emergence of multidrug-resistant bacteria [31]. Growth estimates of the autogenous vaccine market might decrease if the industry reaches critical mass for researching and developing a standard licensed product. One example would be that of Avian Reovirus. Variants of this virus expanded through North America and Europe in the last 10 years, and only in the US, it is estimated to have a cost of more than \$US90 million per year in the broiler industry in culls and mortality alone, while losses in the turkey industry are estimated at \$US33 million per year [32]. Thus, vaccine manufacturers are encouraged to fund and develop licensed vaccines, hence the newly developed inactivated avian reovirus vaccine including serotypes 1, 2, and 3 from a vaccine

manufacturer in the US [33], which correspond to genotypes 2, 4, and 5 under Kant classification (Dr. Sellers, personal communication).

4. General considerations on autogenous vaccines

Before thinking about implementing an autogenous vaccine program in the field, the first step would be to study the characteristics of the field challenge (e.g., antigenic diversity, virulence markers), to evaluate if the current vaccination program is performed properly (e.g., review vaccination audits; ELISA titers, field and hatchery vaccination records), and to find out the presence of immunosuppression (e.g., aflatoxins, MDV, chicken anemia virus—CAV, IBDV), and whether the problem can be resolved by adjustments to existing vaccination programs with licensed vaccines. Also, it is paramount to understand the nature of the problem in the field - most of the times, a change in management can resolve the issue, make it more controllable, or synergize with other interventions, such as vaccination modifications or additions (e.g., autogenous vaccines). For instance, if having a Fowl Cholera challenge with a *Pasteurella multocida* from a serogroup different than the vaccines being used, the field veterinarian not only should review the vaccination schedule but also should evaluate the pest control program, as well as water sanitation, as possible sources of challenge. Review biosecurity and management, including but not limited to: downtime length, proper water sanitation, environment disinfection upon reception of baby chicks, proper disinfection of equipment, monitoring programs in place, and pest and insect control at the farm. After determining that the problem cannot be successfully controlled by management and/or modification of existing vaccination programs with licensed vaccines, an autogenous vaccine program should be considered for a company. Interestingly, there are some diseases that can be well managed with a program, such as Avian Reovirus and Fowl Adenovirus, but others that might or might not have success, perhaps due to unaddressed management/sanitary problems; limited immunity provided by inactivated vaccine; high number of serotypes in the field, which makes vaccine candidate selection difficult [34]; and/or high level of transfer of virulence genes (e.g., *Escherichia coli*, *Clostridium perfringens*, *Clostridium septicum*). Control of these agents might or might not benefit from an autogenous vaccine program, and in some cases, control by autogenous vaccines has been deemed as not a viable option for the industry [24]. Because of the high vaccine cost and high labor in most of North America and Europe, autogenous vaccines are most used in long-life birds, such as broiler & turkey breeders, layer breeders, and layers but not in broilers. The goal is to generate enough humoral immunity to protect the progeny during the first days of life. This is particularly useful in diseases in which infection early in life can cause important clinical signs later in life (e.g., Viral Arthritis, Inclusion Body Hepatitis, Infectious Bursal Disease). In countries with lower labor costs, for instance those in Latin America or Southeast Asia, the application of inactivated vaccines is still economically feasible in areas with need for control of fatal disease challenges (e.g., virulent Newcastle disease, Avian Influenza).

An estimate of autogenous vaccine order, personnel training, equipment investment, vaccination logistics, and monitoring costs, as well as the expected benefits from such program implementation, would have to be discussed with the upper management for a cost:benefit discussion of the program and final approval- including but not limited to: complex manager, live production

manager, broiler breeder manager, processing plant manager, and others. Upon approval of the program, the field veterinarian would find valuable the following considerations:

4.1 Vaccine candidate(s) selection

A good monitoring program is necessary for proper selection of vaccine candidates. Such a program should contain comparable data across all levels of poultry production. Although most molecular classifications of poultry diseases are standardized and results are interchangeable between reference labs (i.e., serotyping of Fowl Adenovirus, *Pasteurella multocida*, *Escherichia coli*); some other pathogens can be classified using different systems among laboratories (i.e., Avian Reovirus); thus, caution should be exercised as to where clinical data should be generated and analyzed, or in the case such data can be easily converted from one system to another [35]. In case of food-borne diseases, such as *Salmonella enterica* colonizing the gut of chickens, it is recommended that a multiple strategy, considering licensed killed and live vaccines, should be used [36]. Federally licensed autogenous vaccines can be used to tailor the vaccination strategy to further decrease prevalence and level of contamination of the carcasses at the processing plant [37, 38]. As several types of salmonella can be present at different levels of the poultry production [39–41], it has been recommended to consider *Salmonella enterica* isolates/serovars found at processing plant monitoring as autogenous vaccine candidates [42]. This is because these isolates have successfully overcome the different control strategies already in place and are the most likely to find their way to human consumers and cause food-borne illness.

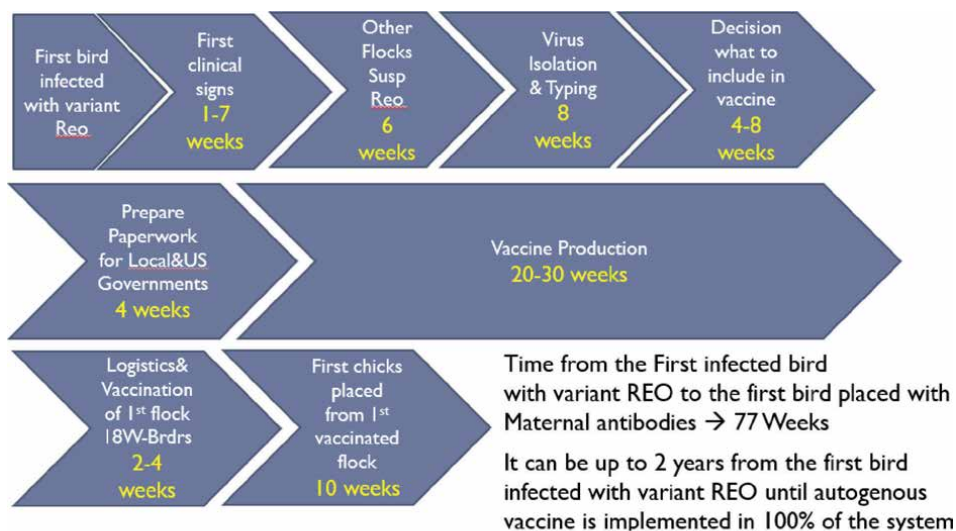
Another major problem when selecting the proper isolate in an ongoing autogenous vaccine program was the fact that isolation of the causative agents was extremely difficult once the program was in place. Because these agents could not be isolated, the isolates expired after 2 years of isolation and could not be used for the next batch. In the US, this problem was solved in the last Veterinary Services Memorandum (VSM) 800.69, and now isolates can be used up to 60 months from the point of isolation [43].

4.2 Production and implementation time

Federally licensed autogenous vaccines would require time for being produced (6–18 months Canada/4–9 months the US) and, once available, 6–12 more months to be fully implemented in a parent stock [33, 35]. Time variation would depend on the type of autogenous vaccine. For instance, autogenous bacterins would require less time for development than autogenous viral vaccines due to more testing on the master viral seed (up to two more months), availability of raw material (e.g., specialized media, SPF eggs), regulatory requirements (e.g., state regulations, country regulations, export-import paperwork), shipping scheduling (refrigerated truck for large orders over long distances), etc. Although an autogenous vaccine can be available for use in an extremely short time when compared with classic licensed vaccines, it still requires important time to be fully implemented; thus, it is crucial to get isolates in the autogenous vaccine that are representative of the challenge in the field (**Figure 1**).

4.3 Vaccine reactions

Birds react poorly to killed antigens (e.g., bacterial, viral) [44]. Thus, all killed vaccines in commercial poultry are adjuvanted for enhancing innate and adaptive



It is essential to get the right isolates!

Figure 1.
Timeline of avian reovirus autogenous vaccine design in Canada.

immune responses. The most common adjuvants used are based on mineral oil and aluminum hydroxide [45]; mineral-oil-based adjuvants usually exhibit a strong reaction at the site of application and stimulate a (predominantly) robust humoral reaction with effects that can last for months but take longer to mount (~ 4 weeks); whereas aluminum-hydroxide-based adjuvants last for a shorter period of time (several weeks) but take less time to mount (~2 weeks) [45]. In essence, a mineral-oil-based adjuvant is an incomplete Freund's adjuvant and can be acquired commercially or produced in-house [46]. Because of its ability to produce strong humoral titers, lasting effects, and high cost, oil-based vaccines are predominantly used in parent stock vaccine programs across poultry. A common oil-based adjuvanted vaccine is constituted by two phases: (1) the oil phase, which is composed by the oil adjuvant plus emulsifiers and surfactants constituting two-thirds of the total volume of the vaccine and (2) the aqueous phase, which is composed by the harvest containing the antigen or antigens—also known as “fractions,” which can be added from direct harvest or diluted in sterile media (e.g., 1X PBS, saline), and which constitutes the remaining third of the vaccine. These “phases” are processed and compounded in a specific way within parameters described in a document named as “Outline of Production,” which has received government approval. In short, both phases are sheared and emulsified following standard protocols in specific tanks for a predetermined amount of time to reach an emulsion with the given particle size distribution. Because of different fractions, different compounding of the aqueous phase, and small modifications to manufacturing procedures, there might be some unwanted variations in the quality of the emulsion. In general terms, in the experience of the author, a non-reactive oil-based vaccine would be within the following quality parameters: less than 10 microSiemens/centimeter ($\mu\text{S}/\text{cm}$) conductivity in a WTW conductivimeter or the Drop Test as described by Aucouturier *et al.* (indicating a water-in-oil emulsion) [47, 48], and a monomodal particle size distribution of 95% within 0–10 μm measured by a microscope [49] or outsourcing for assessment with

a Mastersizer device (Malvern, Worcestershire, UK) [50]. Emulsions with particle size distribution of 95% larger than 10 μm would be reactive and less stable and would stratify after a short settling; thus, small-sized particles are considered more effective than larger ones [50–52]. Furthermore, particles smaller than 10 μm are appropriate for direct uptake by antigen-presenting cells (i.e., macrophages, dendritic cells) [53, 54]. Although rare, in the event of an adverse local vaccine reaction, it is important to rule out all other potential vaccine management errors, such as cold application of vaccine—causing cold shock in the surrounding tissues, contamination of vaccine, vaccine tube manifold, needles, blunt needles, and harsh vaccine application [45, 55]. It is recommended to keep a sample of the same vaccine sent to the farm in which the adverse reaction is observed or a bottle from the same batch number for particle size testing. Another common vaccine reaction is that of hemorrhagic hepatopathy, which has been described with commercial and autogenous bacterins containing *Salmonella enterica* serovars, with high levels of LPS in the vaccine [56]. In this scenario, unknown seeds might be responsible for a higher than usual generation of LPS, which adds another level of complexity to the issue.

4.4 “Antigenic dilution” and potency issues

The term refers to “the more different antigens are included in the vaccine, the more diluted each individual antigen is within the serial” [33]. Autogenous vaccines are made from field strains that are not selected or optimized for the industrial propagation systems used for the vaccine manufacturing industry. One serial can include either bacterial or viral antigens. Both bacterins and viral autogenous vaccines contain at least one antigen; however, most include several (~2–5 antigens). Formulation examples for viral autogenous vaccines used in the field include: 2–4 antigens from different clusters of Virus A and 2–4 different serotypes from Virus B. For autogenous bacterins: 3–4 different serovars of Bacteria A (e.g., *S. enterica*) or 1–2 different serovars from two different bacteria (e.g., *Escherichia coli*, combined with *Riemerella anatipestifer*—usually used in ducks or with *Clostridium perfringens*—usually used in turkeys). An important concern from users is the limited space under the aqueous phase. Thus, the more different antigens are included in the vaccine, the more “diluted” each individual antigen is within the serial [33]. In short, most autogenous vaccines are produced in bottles of 0.5 L using an oil-based adjuvant at 0.25 mL per dose (2000 doses per bottle). This means that in 0.25 mL of dose per bird, two-thirds (~0.16 mL) would correspond to the oil phase (oil-based adjuvant plus emulsifier and surfactants) and one-third (~0.09 mL) would correspond to the aqueous phase, which contains the antigens. In this minuscule volume, antigens would have to be included at a proper antigenic concentration to elicit a satisfactory immune reaction, which most of the times require harvesting titers that might not be achieved by wild organisms because they are not adapted to industry propagation systems.

It is unclear what is the limit of antigens that can be delivered successfully at the same time. However, preliminary data show no significant negative effect on antibody levels when analyzing individual antigen vaccination versus application of all vaccines in a commercial broiler breeder program [57]. Thus, evidence suggest that antigenic level (potency) of an antigen is more relevant than the number of antigens in a particular vaccine. Although antigen at high concentrations in both viral and bacterial harvests can be diluted, only bacterial antigen can be cheaply concentrated. Viral antigen is more difficult to concentrate as it requires an ultracentrifuge that is labor-intensive and increases the costs of vaccine manufacturing. Therefore, most of

the times, viral antigens are not concentrated, and in some cases, these field viruses do not propagate in high numbers on the factory/lab production systems (SPF eggs, cell culture) and are added undiluted to the vaccine. This quantity of antigen might not be enough to elicit the strong immunity required by the program, and usage of multiple antigens in the aqueous phase could further “dilute” the already low titers in one individual dose [58]. Because vaccine manufacturing companies rarely share production details with clients, such as the quantity of antigen of each fraction in each serial, the efficacy of the autogenous batch should be indirectly measured in the field. The most common method would be by ELISA serological monitoring. Gamble and Sellers recommend to evaluate sera ELISA titers at 3–4 weeks after completion of the priming vaccines, at 6–8 weeks after the completion of the inactivated booster series, and at the end-of-lay in broiler breeders to create a good complex-specific baseline for Avian Reovirus [33]. The opinion of the author is that this sampling strategy can be used for the monitoring of other diseases as well (e.g., *Salmonella enterica* serovar Typhimurium and Enteritidis, Fowl adenovirus, etc.), though it might be influenced not only by the antigenic content of the autogenous vaccine but also by a live challenge at the field, vaccination errors, immunosuppression of the birds, and others, and it will only provide indirect, subjective information about the antigen content of the fractions included in the vaccine. Nucleic acids can be recovered from oil-based inactivated vaccines by separating the aqueous phase from the oil phase [59], so molecular techniques (e.g., qPCR or qRT-PCR) [60] might be researched and developed as a tool to indirectly assess the amount of antigen fraction included in the autogenous vaccine.

Potency issues relate with the issue of “antigenic dilution” and can be found more frequently in viral vaccines than in bacterines, as some field isolates propagate better in embryonated eggs (e.g., Avian Reovirus), or specialized differentiated tissues like spleen (e.g., Hemorrhagic enteritis virus) rather than some of the more common production systems used for their licensed counterparts (chicken embryo fibroblasts—CEF for Avian Reovirus S1133; or MDTC-RP-19 for HEV). Because of this different propagation ability, or growth potential in medias in the case of bacterial isolates, and lack of potency studies, it is common to have important variability between different isolates harvest titers, which can be translated into different antigenic levels of the vaccine fractions within an autogenous serial, potentially under-stimulating the immunity against some serotypes over others within the same vaccine. Thus, autogenous vaccines, even when containing the same isolate (from different harvests), may not share the same efficiency [61]. Other consequences of this issue would be the limitations of monitoring between one batch of vaccine from another as even viruses from the same cluster or bacteria from the same serotype may elicit important ELISA titer differences in the field. Other factors might obscure the meaning of these serological monitoring, as it can depend on other factors (e.g., priming, homologous/heterologous challenge, vaccination issues).

4.5 Order size of vaccine batch

Multiple vaccine manufacturing company acquisitions in the last decade and the search for scale efficiencies in volume production have caused that the minimal order for an autogenous vaccine in poultry be of 200,000 doses. This order is limited by the emulsification tank batch capacity of 50 liters with a formulation of 2000 doses per 0.5 L bottle at 0.25 mL per dose. This is important as small operations (across all live-stock industries and aquaculture, not only poultry) require lower number of dosages and would have to purchase a higher total order than the one required. This represents

an important market opportunity for a new competitor created by the large mergers of vaccine manufacturer companies in the last two decades.

4.6 Pathogen evolution

Multiple factors can influence the evolution of the agent in an operation with an autogenous vaccine program. These include but are not limited to: (a) agent mutation rate; (b) prevalence of the agent in the environment/resistance to disinfectants; (c) ability to prime and type of priming; (d) source of the challenge/reintroduction of pathogen; (e) level of agent shedding in vaccinated individuals or the progeny of vaccinated individuals.

- a) Agent mutation rate: mutation rate can be measured as substitutions per nucleotide per cell infection (s/n/c) for viruses and as substitutions per nucleotide per generation (s/n/g) for bacteria [62]. Findings by Sanjuan et al. showed that studied RNA viruses had a mutation rate of 10^{-6} to 10^{-4} s/n/c, with a genome size ranging from ~3.0 to 31.4 kilobases (kb); DNA viruses had a mutation rate of 10^{-8} to 10^{-6} s/n/c, with a genome size ranging from ~5.4 to 169 kb; and bacteria had a mutation rate of $\sim 10^{-11}$ to $\sim 10^{-9}$, with a genome size ranging from ~1700 to 5500 kb [62, 63]. In short, RNA viruses with their high mutation rate can generate variants in a short period of time, escaping the immunity generated by autogenous vaccines [33, 64], while DNA viruses are more stable and less likely to mutate and generate variants. Despite having the lowest mutation rate among the agents studied, some bacteria might use other strategies to escape the immunity elicited by vaccination by changing serotypes. This phenomenon is known as “serotype switching,” “capsule switching,” or “capsular switching” [65, 66] perhaps through a mechanism known as genetic exchange between loci [67, 68]. Preliminary evidence, showing highly related *Riemerella anatipestifer* isolates classified as different serotypes from before and after the implementation of an autogenous vaccine program (Palomino-Tapia and Nickel, unpublished), suggest that this mechanism might be responsible for common vaccination failures with this agent [69–71].
- b) Ability to prime and type of priming. Oil-based inactivated vaccines will generate a predominantly humoral immunity that can be measured in the bloodstream (sera) and is mainly systemic, with little to no local immunity in the mucosa (i.e., gastrointestinal, respiratory tract, ocular, nasal) [45], which are target tissues for many agents controlled by autogenous vaccines. Because there is no commercially available “live autogenous,” homologous to the autogenous vaccine able to “live prime,” memory B cells will not be stimulated properly, and titers will not be boosted. Examples of this can be found with autogenous vaccines containing FAdV, variant Avian Reovirus, and variant IBDV [45, 58, 72]. Lack of live priming is one of the reasons some companies apply an extra autogenous vaccination in the middle of broiler breeder production, just to have enough titers to cover the progeny until the end of the broiler breeder life. It is worth to mention the concept of “original antigenic sin” or “antigenic seniority” [73, 74]. In poultry, this effect has been found in agents with a high number of serotypes, such as Infectious Bronchitis virus [75] and Avian Influenza [73, 74]. In short, the immune response against an agent will be heavily influenced by the first serotype of antigen presented. Another disadvantage of

lacking of proper live priming to accompany an autogenous vaccine program would be the lack of proper mucosal immunity [45, 76].

- c) Resistance to disinfectants and environmental conditions. Agents resistant to physical and chemical factors might reduce the efficacy of an autogenous vaccine program. This is because the progeny might encounter higher levels of challenge if the barn is not properly composted, cleaned, and disinfected. This can cause a high early challenge that can overcome the protection given by maternal antibodies. Caution should be exercised when redesigning an autogenous vaccine program for very persistent disease agents, such as Fowl Adenovirus (FAdV). The field veterinarian should be very cautious to remove an FAdV serotype in farms characterized by repeated outbreaks with such serotype, as in the author's experience, it is highly likely the disease will come back after a few production cycles, despite some in-vitro [77–79] and in-vivo essays showing some degree of cross-protection between serotypes (e.g., FAdV 8a and FAdV 11) [80, 81].
- d) Source of the challenge/reintroduction of pathogen. Agents able to persist in areas of the barn (e.g., drinking water pipes-biofilm), and/or even persist or propagate in pests (e.g., rats, mice, darkling beetles), might reduce the efficacy of the autogenous vaccine program. This is because new variants or serotypes different from those contained in the vaccine might enter the barn environment and cause the flock to break; also, the fact of having another vessel in which to propagate without vaccination pressure would foster the propagation of the agents that evade the immunity produced by the program and might cause vaccine failure. Pest control and biosecurity to avoid contact with wild and domestic animals are also paramount in the control of agents such as *S. enterica* [36, 82, 83] and *Pasteurella multocida* [84, 85] so as to prevent the introduction of the pathogen into the barn environment.

5. Summary and conclusions


The recent increase in zoonotic disease incidence, rare infectious diseases, and antigenic variants from old diseases emerging in livestock and companion animals are the reasons for the continuous growth of the autogenous vaccine industry in the last 7 years. In the last ten years, the poultry industry has increased the usage of autogenous vaccines, leading to changes in legislation and vaccination programs. Currently, these autogenous vaccines are used extensively in North America as part of control programs—mainly in breeders. The use of autogenous vaccines requires constant monitoring for challenge, clinical case interpretation, and support for the vaccine manufacturing industry to develop new licensed vaccines to meet industry needs.

Author details

Victor Palomino-Tapia
Department of Laboratory Services, Poultry Health Services Ltd, Airdrie, Alberta,
Canada

*Address all correspondence to: victor.palominotapia@ucalgary.ca

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Section 4

Breeding and Hatchery

Chapter 9

Natural Products as an Alternative to Formaldehyde for Disinfection of Fertile Eggs in Commercial Hatcheries

Omar Francisco Prado Rebolledo, Arturo César García Casillas, Guillermo Téllez-Isaías and Juan Augusto Hernández Rivera

Abstract

Formaldehyde has been used in commercial hatcheries to cleanse eggs and prevent illness. However, formaldehyde's health risks and customer demand for eco-friendly products have spurred interest in natural egg disinfection. Formaldehyde-free natural materials sterilize viable eggs in commercial hatcheries. Formaldehyde's health and environmental dangers start the chapter. Modern hatcheries need safer and greener options. Natural egg disinfectants are next: plant-based extracts, oils, and acids. These natural chemicals' mechanisms, bactericidal properties, potential commercial hatchery pros, and cons are evaluated. The chapter also examines commercial hatcheries' natural disinfectant limits. Cost-effectiveness, efficacy against common diseases, application simplicity, and hatchery equipment compatibility are discussed. Regulations and uniform egg disinfection using natural agents are covered in the chapter. It emphasizes industry stakeholders, researchers, and regulators working together to promote natural alternatives. Finally, formaldehyde-free natural substances can disinfect viable eggs in industrial hatcheries. Studying natural product-based disinfection methods will increase their efficacy, safety, and feasibility. This book chapter concludes with natural alternatives to formaldehyde for cleaning viable eggs in industrial hatcheries.

Keywords: natural disinfectants, eggs, microbial contamination, hatcheries, formaldehyde

1. Introduction

Population growth varied social conditions, and economic differences in the world have an impact on food supply. Between 1960 and 2020, the world population increased from 3.0 to 7.8 billion, equivalent to 157%. Therefore, it is estimated that between 2020 and 2050, there will be a further increase of 2 billion inhabitants, so the impact on food security will represent a significant challenge. So much so that its

importance is already considered within the 2nd Sustainable Development Goal of the United Nations’ (UN) “zero hunger” concept [1]. For its part, the pandemic of Severe Acute Respiratory Syndrome Coronavirus 2, or SARS-CoV2 [2], confirmed the close connection between humans and animals; however, the phylogeny of the virus is still under investigation since the factors involved in its dispersal have not yet been fully resolved. Therefore, this example highlights the importance of the “One Health” concept as a unified and integrated approach that seeks to balance and sustainably optimize the health of people, animals, and ecosystems [3].

Given the global outlook on the deficit of food availability, table eggs represent a source of easily accessible, inexpensive, self-packed protein, which provides a source of highly digestible protein with a homogeneous balance of amino acids; thus, it is considered a food guarantee, since it has no religious barriers in its consumption, and has low production cost due to the high feed efficiency of the hens. Table egg production has increased significantly in recent years, with China contributing 1136.4 million cases of eggs, India 270.2, the United States of America (USA) 263.6, Brazil 146, and Mexico with 132.9 million cases (**Figure 1**), representing the countries with the highest production. It should be noted that each carton of eggs contains 360 units, equivalent to 30 dozen eggs. In 2018, world production was 76.7 million t; therefore, if this value is divided by the 7.6 billion people in the world, the result is a consumption of 161 eggs/person/year. The main consuming countries are Mexico with 23.7 kg *per capita*/year, Japan with 21.3 kg *per capita*/year, and Colombia with 20.3 kg *per capita*/year. Another significant fact is that consumption does not depend on large demographics, as China has a consumption of 255 eggs/person/year, India 76 eggs/person/year and the European Union (EU) with 210 eggs/person/year (**Figure 2**) [4].

After World War II, livestock production systems evolved. Before the war, production was done in the backyard for self-consumption; in the post-war period, agriculture faced a crisis, due to the low number of workers in the primary sector. In response, from the 1980s to 1990s, egg production via cage production systems increased. In that same decade of the 1990s, consumers requested that Livestock Production Units implement the concept of “Animal Welfare”, which is why the poultry industry producing table eggs implemented other production systems, which attempted to satisfy the five

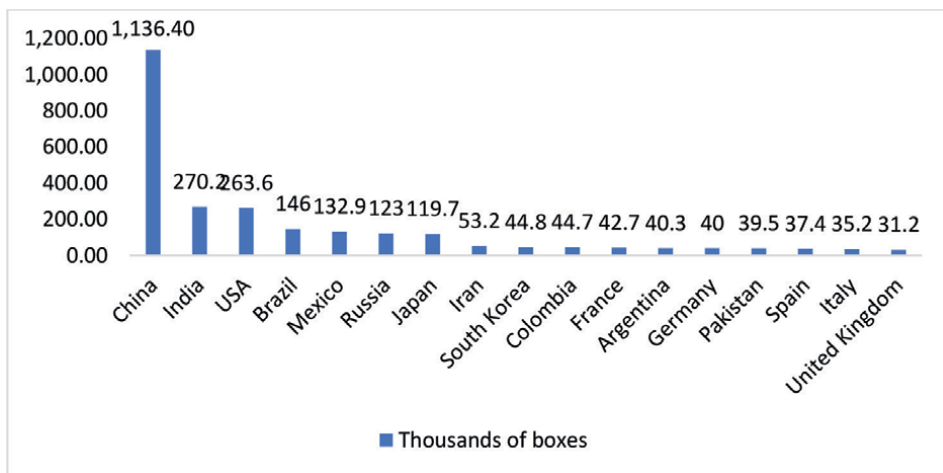


Figure 1.
Main table egg producing countries.

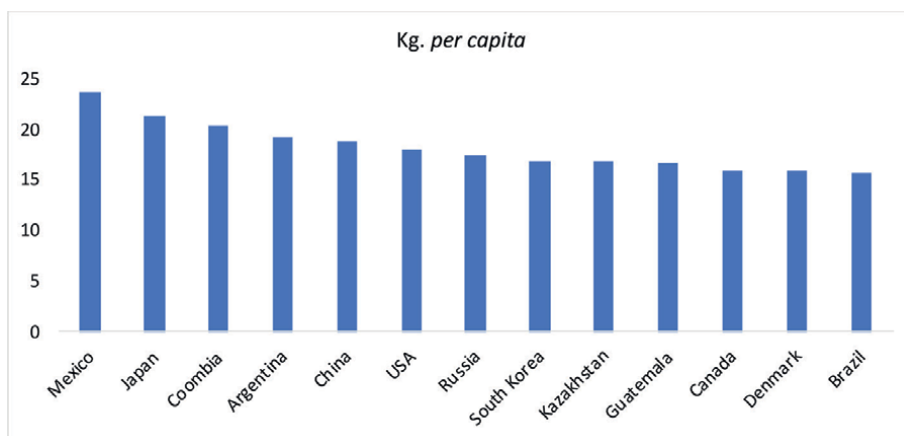


Figure 2.
Main fresh egg consuming countries.

freedoms: (i) absence of hunger, (ii) absence of thirst, (iii) possibility of movement, (iv) absence of fear, and (v) expression of natural animal behavior [5].

2. Natural egg defenses

Eggshells are the primary packaging and constitute the 1st defense barrier in containing microorganisms; the priority of maintaining their integrity and quality is of great importance for producers [6, 7]. The main component of the hull is calcium carbonate in the form of calcite (94%). Apart from CaCO_3 , there are other inorganic components in the shell: magnesium carbonate (<1%), calcium phosphate (<1%), and silicon oxide (<1%). The approximately 4% of remaining compounds are polysaccharides, various collagens, fatty acids, and water [8]. These components make the eggshell have a unique microstructure, where the CaCO_3 skeleton is characterized by a porous and rough structure with three levels of primary particles with approximately 10 nm. Calcite crystals are arranged in palisades and mammillary layers with different morphology and porosity, in addition to an absence of cell-directed assembly during calcification, compared to bone [9]. The mass of the eggshell is proportional to the egg mass and represents between 10 and 11% of the egg weight. In the eggshell, the cysteine-rich protein membrane, the mineralized layer, and the non-mineralized outer cuticle are deposited as the egg descends through the oviduct of the hens [6]. The eggshell membranes are synthesized during a period of 1.0–2.0 h, when the immature eggs travel through the proximal isthmus. Mineralized multilayers are formed in the distal isthmus and the shell gland over a period of 19–20 h. Finally, the cuticle is deposited on the eggshell in the uterus 1.5–2 h before oviposition [10]; and, at this time, the outer part of the eggshell is exposed to many contaminants that can harbor a wide range of microorganisms [11].

The cuticle covers the pores on the eggshell surface, thus forming a physical barrier against bacteria [12]; the chemical composition of the eggshell plays an important role by limiting bacterial contamination. Some antibacterial proteins (e.g., c-type lysozyme, ovotransferrin, and ovocalyxin-32) have been detected in eggshell; the open pores on the eggshell surface not only serve for gas and water exchange but are also

the route of invasion [13]. Consequently, eggshell thickness is an important factor for the ingress of bacterial contamination [14, 15]. In this regard, it has been shown that good shell cuticle quality can significantly reduce the opportunity for pathogen invasion and that the amount of cuticle as a hereditary trait can be an effective strategy to reduce the transmission of microorganisms in production poultry [6].

In order to reduce Enterobacteriaceae counts on the eggshell, in some countries, such as USA, Australia, Japan, and Sweden, eggs are washed with chemicals (e.g., sodium carbonate and sodium hypochlorite) [16]. This practice may damage or partially remove the cuticle, thus increasing the risk of bacterial ingress. Class A eggs should not be washed, due to potential damage to physical barriers, such as the cuticle. Good cuticle quality is of vital importance, as the safety of table eggs depends, to a large extent, on it. The cuticle and its degree of coverage are affected by many factors, such as the age of the hen, genetic background, rearing system, and egg storage conditions [17, 18].

Eggs can be contaminated at different stages from the production stage, through processing, cooking, and consumption. Transovarial or “vertical” transmission of microorganisms occurs when eggs are infected during their formation in the hen’s ovary. Horizontal transmission occurs when eggs are exposed to an environmental contaminant and microorganisms penetrate through the eggshell [13, 19].

In the past decade, Non-typhoidal *Salmonella* (NTS) caused an estimated 1.028 million cases, >19,000 hospitalizations, and 378 deaths in the USA, at a cost of \$3.3 billion [20]. Although NTS is frequently isolated in different foods of animal origin, poultry is considered an important reservoir, and contaminated poultry products are also a significant vehicle for human infection. There are >2400 recognized serotypes of NTS. However, not all are isolated from poultry; for example, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella heidelberg* are historically associated with poultry. However, *Salmonella kentucky* has positioned itself as the predominant serotype associated with U.S. poultry. This change in the population dynamics of *Salmonella* in U.S. poultry has a far-reaching implication for food safety [21]. The increase in multi-drug resistance (MOR) in *Salmonella* serotypes of both animal and human origin, and, in particular, resistance to important clinical antimicrobials, is an emerging concern worldwide [22].

3. Disinfectants based on natural products

Egg disinfection is a process that seeks to minimize the risk of contamination by microorganisms that can compromise both human health and egg quality, as well as the entire production chain of the poultry industry [23]. The disinfection process must ensure a good application of the disinfectant compound on the eggshell, which must be broad spectrum with the lowest toxicity rate. The mechanism of action must also be fast to avoid the dispersion of pathogenic microorganisms without generating high costs in the productive processes [24]. From the fundamental manufacture to the point of consumption, eggs and their markets must be subjected to control procedures aimed at achieving the appropriate level of defense for public health. An important aspect to consider is the marketing chain where egg collection, handling, storage, and transport must be supervised, either manually or automatically, with time and temperature also being taken into account [25, 26].

Studies have been conducted to determine the penetration of *Salmonella enteritidis* in various types of production systems, where *Salmonella* remains an

important transmission pathogen [19]. Therefore, many poultry companies are looking for new alternatives to the use of conventional disinfectants to protect fertile and table eggs from bacterial contamination [16]. In the case of fertile eggs, many hatcheries in different parts of the world have used formaldehyde as part of their disinfection routines; however, this element has genotoxic and cytotoxic properties, which can affect humans and chicken embryos, consequently causing irreversible effects from its inhalation. These effects depend mainly on the dose, exposure time, application method, and egg exposure period [27]. The problem with the use of formaldehyde lies in its concentration as a disinfectant, where at least 600 mg/m³ (489 ppm) is required, which represents a high exposure dose for workers [28], thus presenting the main reason to avoid its use in hatchery disinfection routines [16].

4. Vegetable extracts

Since the origin of civilization, plants have played an essential role in the development and well-being of civilization through their varied uses (e.g., food preservatives, flavorings, and dietary supplements to maintain human health) [29]. Plant extracts have been employed as safe and efficient remedies for ailments and diseases in traditional medicine. The active constituents of many plant extracts have been characterized and are publicly available, although there is little information on their antimicrobial actions [30]. The adoption of natural antimicrobial elements as egg disinfectants opens the door to their use as a safer alternative because they are biodegradable and non-toxic, compared to chemicals that are toxic, non-degradable, and corrosive. There are several methods used for oil extraction, such as the use of liquid CO₂ or microwaves, as well as low pressure distillation with boiling water or hot heat [31]. Among the most significant molecules are phenolic compounds: trans-cinnamaldehyde (an aldehyde found in cinnamon bark extract (*Cinnamomum zeylandicum*)), carvacrol extracted from oregano oil (*Origanum glandulosum*), eugenol (active ingredient of clove (*Eugenia caryophyllis*)), etc. These compounds showed rapid effectiveness in reducing *Salmonella enteritidis* compared to water-washed or chlorine-challenged eggs.

Yamawaki et al. [32] used phytochemicals products of secondary metabolites produced by plants with defensive properties against predators (e.g., caproic acid, caprylic acid, linalool, and pectin-based cuminaldehyde) to reduce *Salmonella heidelberg* on eggshells at a concentration of 1.0% alone or combined at 0.5% v/v with different storage times (0, 1, 3, 5, 7, 7, 14, and 21d) at 4°C. At the end of storage (21d), the lowest *Salmonella* counts were for caproic acid and caprylic acid at 1% pectin combination (2%) from 0d to 14d, and at the end of storage compared to untreated controls [16].

Capsicum essential oil, known as allspice oil, is obtained from the leaves of *Pimenta officinalis* Lindl. The main component is antimicrobial, and its application has proved effective against *Staphylococcus epidermidis*, *Proteus hauseri*, *Micrococcus yunnanensis*, and *Corynebacterium xerosi*. *In vitro*, it acts against *Listeria monocytogenes* and *Salmonella heidelberg* in turkey skin stored over short periods at 4 and 10°C, at a concentration of 0.5 or 1.0% [16, 33]. The compound extracted from clove oil (*Eugenia caryophyllis*), called eugenol, as well as trans-cinnamaldehyde, an aromatic aldehyde extracted from cinnamon bark (*Cinnamomum zeylandicum*), have shown antimicrobial effects on *Salmonella enteritidis* PT8 by interfering with several

genes associated with virulence, colonization, membrane composition, and transport ecosystems.

Ginger, garlic, oregano, and cinnamon extracts, applied in 5% aqueous solutions, showed no differences in fertility, hatchability, embryonic mortality, body weight, or viability of the chicks during 14d of brooding. Regarding the incubation variables, ginger extract was the only one effective in preventing the growth of bacterial colonies [16]. On the other hand, when comparing oregano juice at a concentration of 50% diluted in distilled water at room temperature against fumigation with 100% formaldehyde in white Akbay breeders of 48 weeks of age, no differences were observed between disinfection groups on egg characteristics, eggshell microbial load, hatchability, embryonic death, body weight, weight gain, or feed conversion rate. However, weight loss was lower in formaldehyde fumigation versus oregano juice [34].

In terms of bacterial structure and susceptibility, Positive Gram have a peptidoglycan cell wall bound to other molecules, such as proteins or teichoic acid [35], and Negative Gram have lipopolysaccharide (LPS), which forms a barrier to the hydrophobic compounds that essential oils have in their outer membrane [36]. Therefore, Negative Gram are less susceptible to the effects of essential oils than Positive Gram [37]. However, it is important to note that the hydrophobic structure of essential oils can reach the periplasm of Negative Gram through outer membrane proteins (porins), where it travels slowly, followed by leakage of potassium into the extracellular space and loss of ATP [37–41].

The use of essential oils as preservatives may be limited by changes in the organoleptic characteristics of foods. However, in the disinfection of fertile eggs, their safety has been recognized, and their use is gaining more and more practitioners every day. Therefore, it is important to carry out studies on the minimum inhibitory concentration of essential oils to allow a balance between sensory characteristics and antimicrobial efficacy.

5. Propolis

Propolis is a sticky, gummy, resinous substance harvested by worker bees (*Apis Mellifera*) from the buds of certain trees and shrubs. The bees use it to seal parts of the hive. At least 200 compounds have been found in different samples of propolis (e.g., esters, fatty acids, flavonoids, terpenes, β -steroids, aldehydes, aromatic alcohols, sesquiterpenes, naphthalene derivatives, and stilbenes) [42, 43]. For centuries, propolis has been used as a medicinal agent to treat infections and promote wound healing [44]. Due to its broad antimicrobial effect, it has been used as an alternative preservative agent and as a protection for various agricultural products during their storage period [45, 46]. Propolis was used to reduce microbial activity in quail eggs stored for 7 and 14d, but it reduced hatchability and increased embryo mortality between 1 and 9d of incubation.

Oliveira et al. [27] conducted an experiment to evaluate the effectiveness of an alcoholic extract of propolis (15%) as a disinfectant for hatching eggs of Japanese quail (*Coturnix coturnix Japonica*). A low eggshell conductance in the control group (egg weight loss) and a decrease in the microbial load were obtained. Likewise, no differences in hatchability and embryo mortality were observed. Therefore, alcoholic extract of propolis (15%) can be used as a safe disinfectant in fertile quail eggs [16, 47].

6. Probiotics

Metchnikoff recommended, since the early twentieth century, the intake of beneficial microbes for health, particularly in the treatment of pathologies of the gastrointestinal tract. In 2001, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) officially defined probiotics as those live microorganisms that can confer a health benefit to the host, when consumed in adequate amounts [48]. Probiotics have had a considerable increase as an alternative over antibiotics used as growth promoters and pathogen control. This phenomenon has motivated the development of effective probiotic products for use in animal production [49, 50]. Prado et al. [50, 51] conducted an experiment where they evaluated an aerosolized probiotic formulation as a bactericidal method during incubation, compared against formaldehyde fumigation, where the results showed that the number of recovered non-selective aerobic bacteria and lactic acid bacteria increased in the incubation environment, thus suggesting the application of lactic acid bacteria in setters and hatchers. Likewise, lactic acid bacteria (*Lactobacillus acidophilus* and *Bacillus animalis*) administered *in ovo*, with the use of a commercial automated multiple egg injection system, have been used without affecting the hatch of fertile eggs. Although it recommends against administering *Bacillus animalis* at high concentrations of 105 and 106 CFU/mL, because they increase the number of chicks that bite and die, as well as contaminated eggs, the *Bacillus subtilis* strain is not recommended because it affects all stages of embryonic development, due to competition for nutrients or secretion of byproducts, such as bacteriocins, enzymes, and 2,3 butanediol, which is toxic to biological systems and damages the defense system and central nervous system [32, 52].

7. Chitosan

Chitosan is a modified natural carbohydrate polymer derived from the deacetylation of chitin; it is insoluble in water but soluble in weak, organic acid solutions [53]; it is part of the exoskeleton of crustaceans, cuticle of insects, algae, and fungal cell walls [54]; it has physical and chemical properties, including antibacterial activity; and it has a high degree of biocompatibility [55]. Chitosan is primarily used as a reinforcement in vegetation development, due to its anti-fungal properties. Chitosan is a biomaterial that can be used as a biofilm with a selective permeability effect for O₂ and CO₂ with good properties to effectively control pathogenic microbial growth. Its antimicrobial activity is dose-dependent, and it exhibits simultaneous cell membrane permeability to small components [56–58].

Prado et al. [51] developed a chitosan biofilm to preserve table and fertile eggs; chitosan concentrations were 0.1, 5, and 10%; table and fertile eggs were impregnated with chitosan and subsequently challenged with *Salmonella enteritidis*, then stored for 1, 24, 96, and 168 h at 4°C. The lowest concentration of *Salmonella enteritidis* was for the 5 and 10% concentrations in the table egg. For the fertile egg, incubation variables showed no differences for the different concentrations of chitosan [51].

From the most recent studies, chitosan has been used in combination with essential oils across a wide application in the food industry, although for applications in table and fertile eggs, there are no reports of its effectiveness [59]. Another combination has been with slightly acidic, electrolyzed water, as a protective alternative against bacteria present in the eggshell. However, this process damages the cuticle, so

after disinfection with slightly acidic, electrolyzed water, a chitosan-based coating was used to form a new, artificial cuticle to prevent loss of humidity and CO₂ from the damaged cuticle, which had positive effects on eggs stored at 25°C for 42d, without loss of internal egg quality [14, 26].

8. Organic acids

Organic acids, being natural products, have emerged as viable alternatives to formaldehyde for disinfection of fertile eggs in commercial hatcheries. These acids exist in a non-dissociated form and exhibit a measure of their dissociation through the K_a (acid dissociation constant) value. Organic acids are commonly found in nature and can be derived from various sources such as fruits, vegetables, and fermentation processes. Examples of organic acids include acetic acid (found in vinegar), citric acid (found in citrus fruits), lactic acid (found in dairy products), and formic acid (found in ants).

In their non-dissociated form, organic acids remain intact, allowing them to effectively penetrate the eggshell and target potential pathogens without harming the developing embryo inside. This characteristic makes them suitable for disinfecting fertile eggs in commercial hatcheries, where maintaining a sterile environment is crucial for successful incubation. The K_a value, also known as the acid dissociation constant, measures the extent to which an organic acid dissociates into its constituent ions in an aqueous solution. It provides an indication of the acid's strength and its ability to release hydrogen ions (H^+) when in contact with water. The higher the K_a value, the greater the extent of dissociation and the stronger the acid.

By considering the K_a value of organic acids, hatchery operators can select appropriate disinfectants that effectively combat pathogens while minimizing any potential adverse effects on the developing embryos. The choice of organic acid for disinfection can be based on factors such as its antimicrobial efficacy, safety, and compatibility with the hatchery environment. Overall, organic acids offer a natural and sustainable alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries. Their non-dissociated form allows for effective penetration of the eggshell, while the K_a value helps determine the acid's dissociation extent and strength, aiding in the selection of appropriate disinfectants for optimal hatchery operations.

Acetic, ascorbic, citric, formic, lactic, propionic, and peracetic organic acids are regularly used in food disinfection processes at concentrations of 0.05–2.5%, with no toxic residues [60]. Some organic acids, such as lactic, acetic, citric, and peracetic acids, are weak acids in solution, since one part of their molecule is dissociated [H^+] [A^-] and the other is not [A]. The ratio between the dissociated and non-dissociated part is expressed by the dissociation constant pK_a . By determining the acid concentration, pH and pK_a , the concentration of the non-dissociated acid present in the solution is established [61].

Lactic acid or its ionized form, lactate, known by the official nomenclature 2-hydroxypropanoic acid, is a carboxylic acid, with a hydroxyl group on the carbon adjacent to the carboxyl group. There are two optical isomers: D (–) lactic and L (+) lactic, as well as a racemic form consisting of equimolar fractions of the L (+) and D (–) forms. Unlike the D (–) isomer, the L (+) configuration is metabolized by the human organism [62]. It is a slightly brown liquid; it is the natural component of meat produced by post-mortem glycolysis; and it is used in carcass washing with doses of 2.5–5.0% at temperatures not exceeding 55°C with application before or after the carcass cooling stage [63].

Acetic or ethanoic acid of natural origin is present in most fruits. It is produced by bacterial fermentation, is present in all fermented products, and its commercial form (vinegar) has been used as a disinfectant since the beginning of civilization. Doses used range from 1.5 to 14.4% or 52°C in spray for 10s. Negative Gram bacteria are more susceptible to acids than Positive Gram bacteria [64].

Citric acid is the main organic acid in fruits, such as lemons, which contain between 7 and 9% citric acid on a dry weight basis. The three carboxylate groups of citric acid mono-hydrate have different pKa values ranging from 3.15, 4.78, and 6. At doses of 2–5%, it reduces the count of pathogenic bacteria [65]. The antimicrobial action is due to the dissociated form; being an anion, it is highly polar, so it does not cross the plasma membrane of microorganisms easily, but its non-dissociated form does cross the membrane [66]. The references found on the use of organic acids as antimicrobials only refer to their use in carcasses and parts of raw poultry, where they measured the effectiveness in reducing the native flora or inoculated bacteria that were mostly *Salmonella* or *Campylobacter*; in the case of the use of organic acids in the disinfection of the eggshell, they can demineralize the eggshell and eliminate the cuticle [67], which is why it is important to conduct experiments that consider the form of preparation, concentration, and measurement of cuticle integrity and calcium carbonate levels.

9. β -Glucans

Components of the cell wall of the yeast *Saccharomyces cerevisiae* have drawn interest in recent years, since their inclusion has had a positive impact on production parameters, due to their physiological effects on the intestinal digestive mucosa, by increasing the height of the jejunal villi [68]. The β -glucans are carbohydrates made of glucose polymers which provide the primary structure that is located in the wall of yeasts, fungi, algae, and cereal grains, such as oats and barley. Their structure can vary depending on the source and type of bonds present in the glucose polymers [15]. The backbone of β -glucans is formed via glucose molecules linked at carbon atoms 1 and 3 [69]. The six-sided glucose rings are connected to each other in linear or branched forms with glycosidic bonds, so the structure of these glycosidic bonds will affect the functionality of β -glucan molecules [70]. There are three structural types of these molecules: α -glucans, β -glucans, and mixed α,β -glucans. The configuration of glycosidic bonds and molecular mass are important for their characterization [71]. Fungal cell walls, which are mostly structural polysaccharides and glycoproteins, are the main source of various structural types of glucans [72].

The main biological activities attributed to medicinal mushrooms are due to the β -glucans present in their wall and in some plants. These substances are antitumor, immunomodulatory, antimicrobial, contraceptive, anti-inflammatory, prebiotic, and antioxidant [73, 74]. Supplemental β -glucans in poultry diet can enhance their innate defense by inducing intestinal colonization and invasion of internal organs by *Salmonella* [75]. The main biological properties of β -glucan (1,3/1,6) are the ability to form viscous solutions in contact with water and to form hydrogen bonds at different binding sites [76]. The β -glucan is soluble in water, although its solubility decreases with time, temperature, and pH. The highest solubility is reached at a temperature of 55°C [77]. The β -glucan has been evaluated to increase humoral response, productive performance, and viability, where an increase in serum IgA and IgG was observed [72].

10. Fructans

Inulin is a natural storage polysaccharide with many applications in food and pharmacology. It can be a low-calorie substitute for sugar or fats. It is widely distributed in plants and is present in the reserve carbohydrates of just over 30,000 plant products [78]. Inulin is not a simple molecule—it is a fructan which fructose units are connected by β -bonds (1, 2). The chain lengths of these fructans range from 2 to 60 units [79]. Inulin is a storage carbohydrate in many plants. It is found in fruits and vegetables (e.g., chicory, Jerusalem artichoke, artichoke, onion, leek, garlic, asparagus, banana) and in the stem of some cereals, such as wheat, as well as agave, which has been used for the production of distilled and undistilled alcoholic beverages [80]. Many biological properties have been found in *in vitro* and *in vivo* tests with antimicrobial, antifungal, antioxidant, anti-inflammatory, antihypertensive, immunomodulatory, antiparasitic, and anticancer activity [81, 82]. An important aspect to consider is that being a material rich in different compounds of interest for agroindustry, future research aimed at the isolation, purification, and protection of agave's secondary metabolites with environmentally-friendly processes is required, in addition to thoroughly investigating the development of products based on the use of pure metabolites or their extracts, evaluation of their activity and bioactivity, as well as experiments that allow determining applications to different areas of operation [83]. Regarding the application of agave fructans in the poultry industry, so far, they have only been used as prebiotics in broiler diets to improve performance and intestinal health [15, 84]. The use of natural alternatives as antimicrobials and disinfectants is increasingly arousing interest in the consumption of safe products, as well as the interest of scientists in offering natural alternatives to prevent the transmission of pathogens through food, such as those referred to here.

11. Conclusions

In conclusion, the utilization of natural products as an alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries offers a promising avenue for achieving effective and environmentally sustainable egg sanitation. This book chapter has highlighted the growing concerns surrounding the use of formaldehyde due to its potential health hazards, environmental impact, and regulatory restrictions. The exploration of natural alternatives has provided valuable insights into the efficacy and safety of various compounds derived from plant extracts, essential oils, and bioactive substances.

The research presented in this chapter has demonstrated that natural products possess remarkable antimicrobial properties, capable of effectively eliminating pathogenic microorganisms from fertile eggs. Furthermore, these alternatives have exhibited favorable characteristics such as biodegradability, low toxicity, and minimal residue accumulation, making them attractive options for commercial hatcheries seeking to adhere to stringent environmental regulations and consumer demands for sustainable practices.

While natural products offer numerous advantages, it is essential to acknowledge the challenges associated with their implementation. Factors such as product consistency, standardization, and cost-effectiveness must be carefully considered to ensure practicality and viability on a larger scale. Additionally, further research and development are required to optimize formulations, dosages, and application methods to maximize their efficacy and minimize any potential negative impacts.

Nevertheless, the potential benefits of using natural products as a substitute for formaldehyde in the disinfection of fertile eggs are substantial. By adopting these alternative approaches, commercial hatcheries can enhance their biosecurity protocols, improve animal welfare, and reduce the ecological footprint of their operations. Furthermore, the adoption of sustainable and environmentally friendly practices can foster positive public perception and contribute to the overall sustainability goals of the poultry industry.

In conclusion, this book chapter has shed light on the potential of natural products as a viable alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries. While there are challenges to overcome, the positive attributes of these alternatives make them worthy of further exploration and development. The incorporation of natural products into hatchery practices has the potential to revolutionize the industry by providing effective, safe, and sustainable disinfection solutions.

Author details


Omar Francisco Prado Rebolledo^{1*}, Arturo César García Casillas¹,
Guillermo Téllez-Isaías² and Juan Augusto Hernández Rivera¹

¹ Faculty of Veterinary Medicine and Zootechnics, University of Colima, Mexico

² Poultry Science Department, University of Arkansas, United States

*Address all correspondence to: omarpr@uclm.mx

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The Relationship of Sperm Motility Pattern and Its Ability to Agglutinate with Vaginal Sperm Selection, Uptake in Sperm Storage Tubules and Competitiveness

*Mohamed A.M. Sayed, Hanan H. Abd Elhafeez,
Catrin Sian Rutland and Taymour M. El-Sherry*

Abstract

To ensure survival, some unique features can be distinguished in birds that help them maintain reproduction. These features include the ability to store sperm for long periods within the utero-vaginal junction, a high sperm concentration per ejaculate, and polyspermy fertilization. Sperm face many challenges prior to fertilization. After copulation, most ejaculated sperm exit the female reproductive tract, and less than 1% continue in an attempt to achieve fertilization. In addition, egg size is substantially larger than sperm size because of the presence of the egg yolk. This results in a large number of sperm penetrating the egg away from the oocyte. These challenges have triggered evolutionary changes to maintain the existence of many species, such as the enormous relative size of the testis, which produces billions of sperm each day, and the ability to store viable sperm for long periods in the oviduct to ensure asynchronous fertilization. This chapter discusses several contemporary and sometimes controversial points regarding sperm behavior and their storage in the oviduct.

Keywords: microfluid device, rheotaxis, Sharkasi and danderwai chickens, sperm mobility, sperm selection, sperm storage tubules, sperm agglutination

1. Introduction

During natural mating, the rooster deposits its sperm into the hen's vagina, but a large number of sperm (more than 80%) are ejected shortly after copulation [1]. Furthermore, it has also been reported that only a small number of sperm (< 1%) inseminated into the vagina pass through and enter the sperm storage tubules (SSTs) [2]. Therefore, the vagina appears to be the primary site for sperm selection in avian species. It is believed that the sperm selection process is of utmost importance as it sorts the fittest sperm, allowing them to traverse and eliminate the non-fit sperm. This process is beneficial as it reduces embryonic mortality relative to what it could be

without this selection. Deep artificial insemination close to the utero-vaginal junction, performed shortly after oviposition, where the vaginal wall is flaccid, deprives the vagina of sperm selection. This has been reported to be associated with high embryonic mortality [3]. A number of researchers have described vaginal selection by limiting sperm migration to those sperm capable of progressive motility, eliminating dead and immobile sperm [4]. However, the mechanism by which the selection process takes place is still unknown.

1.1 Sperm motility and mobility

In chickens and turkeys, it was reported that the migration of spermatozoa, from the entrance to the vagina where they are deposited to the uterovaginal junction where they are stored, is achieved through their active motility [5]. However, this motility is not needed between the uterovaginal junction and the infundibulum because sperm are transported by passive displacement. According to this assumption, sperm transport through the vagina is critical and requires energy expenditure (for the flagellum oscillatory movement) to reach the uterovaginal junction and penetrate the sperm storage tubules. While other means such as the peristalsis movement of the oviduct and/or the movement of cilia may be responsible for the passive transport of the released sperm from the SSTs to the infundibulum. This has been demonstrated as dead spermatozoa inserted in the uterus are transported along the reproductive tract on inert particles, such as carbon powder [6, 7].

Moreover, hundreds of millions of sperm compete to traverse the vagina and motility is not the only determinant in winning this competition. Other factors such as velocity and progressive motility are included. Spermatozoa that move linearly but at a slow velocity, and those swimming in circles at high velocity, might not eventually achieve significant mobility and are unlikely to be competitive [8]. Therefore, it can be stated that not all motile sperm are mobile. Froman and McLean [9] developed an assay to measure sperm mobility in chickens using Accudenz solution in a cuvette with the spermatozoa layered on top of the solution and incubated at 41°C for 5 minutes. The researchers found that the sperm straight-line velocity (VSL) must exceed 30 $\mu\text{m/s}$ in order for the sperm to penetrate the Accudenz solution [10]. Sperm mobility is therefore defined as the net forward movement of sperm against resistance at body temperature [11]. This may indicate that spermatozoa must demonstrate progressive motility with $\text{VSL} > 30 \mu\text{m/s}$ to be capable of reaching the sperm storage tubules. Froman and coauthors [12] surmised that sperm mobility is the dominant factor in sperm selection within the vagina.

In heterospermic insemination trials, when a female is inseminated with ejaculates containing high or low sperm mobility from different males, the sperm cells from the first type of ejaculate fertilized most of the ova [5]. This means that a small number of low-mobility sperm are capable of reaching the SSTs and fertilizing a few ova. This proves that another factor interacts with sperm mobility in regulating vaginal sperm selection.

1.2 Mechanisms behind sperm transport

Rheotaxis has recently been considered an important factor controlling sperm transport in mammalian genitalia. Miki and Clapham reported that the sperm's ability to orient themselves in oviductal fluid flow secreted post-copulation to align against the flow direction and swim upstream is considered a significant factor responsible

for sperm guidance in mice [13]. How the fluid flow guides the sperm is still controversial. Some researchers have proposed that rheotaxis is an actively sensed process because fluid flow is sensed by mechanosensing channels on the sperm, while others have proposed that rheotaxis is a passive process and can be explained by the models of fluid mechanics. Sperm adjust their flagellar beating patterns in response to external stimuli during active reorientation. In response to the stimulus attractant, sperm bend their flagella asymmetrically and swim towards it. The asymmetric flagellar beating patterns are a result of sliding microtubules that are regulated by calcium and calmodulin. Thus, active sperm responses are always accompanied by calcium signaling and oscillations in intracellular Ca^{2+} concentrations. Zhang and colleagues [14] undertook quantitative analysis of human sperm flagellar behavior during rheotaxis-turning. The researchers did not observe significant differences in flagellar beating amplitude and asymmetry between rheotaxis-turning and freely swimming sperm in the absence of fluid flow. According to these observations, human sperm rheotaxis occurs passively through hydrodynamic interactions between the sperm flagellum and the surrounding fluid flow; therefore, no flow sensing is involved. Zaferani et al. [15] exploited the ability of viable sperm to swim against the flow and passively isolated motile sperm inside a corral from the semen sample using a microfluidic corral system. Medical infertility treatments and clinical trials require this kind of sperm sorting, which does not harm sperm structure and morphology. Unlike conventional methods which are labor- and time-consuming and involve more risks to sperm, the technique used by Zaferani et al. [15] eases the process of sperm sorting.

In birds, Parker [16] assumed that sperm pass through the oviduct by swimming against the ciliary current. Although it was proposed as the mechanism by which sperm ascend the oviduct in 1895 by Verworn [17] and its observation was noted in vitro in 1906 by Adolphi [18], studies on avian sperm rheotaxis are still lacking in the literature. In 1906 Adolphi observed that avian sperm exhibit positive rheotaxis when a slow current is generated in a thin layer of fluid contained between a coverslip and a glass slide [18]. Also, Wishart and Ross [19] observed in 1985 that chicken and turkey sperm show rheotactic properties by aligning themselves along the axis of a fluid current. More recently, El-Sherry and colleagues [20] fabricated a microfluidic device with a narrow channel cross-section approaching close to that of the sperm gland and forced tiny amounts of liquid inside the microchannel by applying hydrostatic pressure to generate a fluid flow (fluid flow = $33 \mu\text{m/s}$) to study the behavior of chicken sperm (**Figure 1**). The researchers observed that nearly half of spermatozoa showed positive rheotaxis [20].

Bakst et al. [2] reported that the cilia lining the lumen of the vagina beat in an abovarian direction. Through their activity, cilia direct luminal secretions to the cloaca. Sperm located in the troughs created between apposed mucosal folds get trapped in this secretory material and as a result only motile sperm propel themselves and/or are transported in an adovarian direction. A counter-current mechanism may facilitate the transport of sperm by moving oviducal fluid between longitudinally oriented folds towards the uterovaginal junction, while secretory material in the central vaginal luminal area is transported towards the cloaca [21].

To avoid inbreeding after mating, promiscuous birds can improve the genetic diversity of their offspring by selecting against related male sperm within the reproductive system [21]. When artificial insemination is used, the female's ability to prefer non-relative males disappears, which suggests that male phenotype as well as eye-sighting may influence sperm selection [21]. The female's tendency to bias her sperm selection in favor of nonrelative males by using a mechanism referred to as cryptic

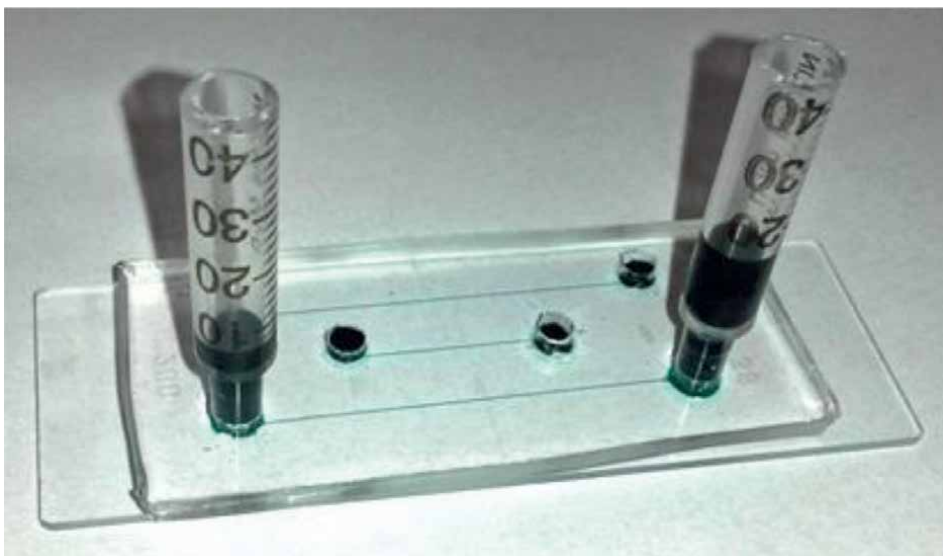


Figure 1. Hydrostatic pressure was applied to create fluid flow inside the microchannel where sperm rheotaxis was studied. Microchannels with dimensions of $200\ \mu\text{m} \times 20\ \mu\text{m}$ ($W \times H$) and a length of $3.6\ \mu\text{m}$ were employed [20].

female choice may be regulated through controlling the characteristics of the luminal oviductal fluid. It was reported that the efficiency of in vitro sperm rheotaxis is affected by fluid flow velocity, shear stress, and fluid viscosity. Increasing fluid flow velocity and shear stress induces more sperm to display rheotactic behavior, while increasing fluid viscosity act to decrease rheotaxis efficiency [14]. Consequently, a promiscuous female may bias her sperm selection in favor of genetically dissimilar males by decreasing the viscosity of her luminal vaginal fluid, thus causing it to flow more fluidly and increase rheotaxis behavior, which allows the sperm to swim across the vagina against the flow. The exact opposite happens in the case of genetically related males.

Once sperm cross the vagina and reach the uterovaginal junction, they enter the SSTs, where they are stored for a period of time which varies by species (from 2 to 10 weeks). This feature is unique to birds as it ensures the continuation of the fertilization process for a long time without the need for a number of repeated copulations, in the case of the absence of males, and also, if fertilization takes place a few hours before the egg is released, it ensures that the sperm remain alive inside the oviduct and are not expelled with the descent of the egg. How sperm remain alive without losing their fertilizing capacity for long periods inside the SSTs is still questioned.

Froman [22] proposed that sperm residency and egress from the SST can be explained on the basis of sperm cell motility. In accordance with the author's theory, sperm maintain their position by swimming against a fluid current generated by the epithelium of the SST, and egress when their velocity drops below the point at which retreated movement begins due to a lack of sperm's energy which makes the sperm swept by the fluid current force. Zaniboni and Bakst [23] confirmed the presence of aquaporin 2, 3, and 9 within the apical portion of the SSTs epithelial cells by immunocytochemistry. The authors reported that their findings support Froman's model of sperm residence in the SSTs. However, rather than Froman's assumption that SST fluid secretion is constant, the authors suggested that factors accompanying active

egg production modulate either the volume or the velocity of SST fluid secretion which would regulate the sperm residence and egress from the SSTs. None of the authors demonstrated the mechanism responsible for SSTs uptake of sperm, but we believe that rheotaxis is involved in this process.

On the other hand, Bakst [24] suggested that the resident sperm in the SSTs are metabolically inactive due to reduced oxygen consumption, which inhibits sperm motility and prolongs sperm storage duration within the SSTs. The authors attributed the decreased sperm oxygen uptake to an increased zinc concentration in the SSTs which acts as a metabolic inhibitor in turkey sperm. Similarly, but in a different way, Matsuzaki and Sasanami [25] proposed that avian sperm motility is suppressed within the SSTs and the resident sperm remain in a quiescent state which explains the prolonged sperm storage. After release from the SSTs, sperm motility is restored. Matsuzaki and coauthors [26] observed increased production and release of lactic acid in the SSTs under hypoxic conditions, which may suppress the motility of resident sperm. In this case, the significance of sperm rheotaxis is manifested during sperm selection and uptake but not during storage.

There has been evidence that resident sperm cluster together in agglutinated bundles. Head-to-head agglutinated sperm have been observed in the SSTs of chickens [27, 28], quails [2], and turkeys [29]. Because this pattern of sperm residency is common among domestic birds, the sperm agglutination mode was suggested as a plausible explanation for the prolonged storage period of sperm within the SST.

How does avian sperm agglutination occur? Is there a biochemical substance responsible for agglutination? Do all sperm agglutinate? Does sperm agglutination constrain sperm motility? Does sperm agglutination occur after arrival at the SSTs? These questions have been difficult to answer because it is not easy to monitor sperm inside the opaque oviduct.

The spermatozoal glycocalyx (glycoprotein glycolipid coating the sperm) is essential for gamete recognition and agglutination [30]. Froman reported that manipulating the spermatozoal glycocalyx by treating fowl spermatozoa with neuraminidase that hydrolyzes the α -glycosidic bonds resulted in decreased fertility without affecting sperm vitality [28]. It was suggested that neuraminidase manipulation of the glycocalyx perturbed sperm sequestration in the SSTs, which in turn decreased fertility. However, the authors could not overlook the possibility that neuraminidase treatment may have reduced sperm-oocyte recognition. To negate this possibility, they performed intramaginal insemination with neuraminidase-treated spermatozoa and found that fertility was not decreased compared to the controls. The authors concluded that manipulation of the sperm glycoprotein-glycolipid coat reduces fertility by increasing the rate at which sperm are lost from the SSTs through perturbing sperm sequestration in the SSTs but not through decreasing sperm-oocyte recognition.

Bakst and Bauchan [29] found small vesicles and membrane fragments in the SST lumen of turkeys, some of which were fused with the sperm membrane. The authors speculated that these particles may be involved in prolonged sperm storage. These particles are either secreted from the epithelium of SSTs, or produced and secreted from the male reproductive system, but their source remains unclear, as well as whether or not these particles are responsible for agglutination.

Grützner and coresearchers [31] reported that the epididymal epithelium of monotremes produces and secretes a specific protein(s) that is required for the formation of sperm bundles. In both short-beaked echidnas and platypus, Nixon et al. [32] found that an epididymal secreted protein, acidic cysteine-rich osteonectin; SPARC,

contributes to sperm bundle formation and that the dispersal of these bundles is associated with the loss of this protein.

El-Sherry and coauthors [20] provided the first detailed description of the sperm bundle characteristics in chickens. To overcome the difficulty of observing sperm behavior in the opaque oviduct, and to model chicken sperm motility inside the genitalia of chickens, the researchers used a microfluidic device which had a microchannel with a cross-section similar to that of sperm glands, and generated a flowing fluid with a flow velocity of 33 $\mu\text{m/s}$ to mimic the flowing secretions in the vaginal lumen area [20]. The authors observed that Sharkasi chicken spermatozoa form thread-like bundles composed of dozens of individuals immediately after ejaculation (**Figure 2**). A bundle is formed when a few sperm get close together, they start moving synchronously and wrap around one another, and then they adhere to an adhesive substance. This agglutinating substance is evident using scanning and transmission electron microscope (SEM and TEM; **Figures 3 and 4**) and also when Acridine orange-stained semen smears were examined under a fluorescence microscope (**Figure 5**). The agglutinated sperm bundles grew with time and could remain *in vitro* for hours before dispersing. The sperm bundles had a unique pattern of motility and were capable of sticking to any static or adjacent surface. The researchers found that the sperm bundle consists of two segments: the initial segment, which consists of the free heads of the agglutinated sperm, and the terminal segment, which consists of their tails and distal sperm (**Figure 6** and Video 1, <https://www.nature.com/articles/s41598-022-17037-x#MOESM4>). The free heads, at the initial part of the bundle, were observed to be responsible for the bundle motility due to their oscillatory movements, which drag the adhered distal segment of the bundle in a spiral-like movement. Long bundles had some free heads of adhered sperm



Figure 2. Two Sharkasi sperm thread-like bundles swimming in a dynamic environment parallel to the sidewall of a microfluidic channel of 200 μm . 20 μm dimensions (W, H) under phase contrast microscope. A magnification of $\times 400$ was used [20].

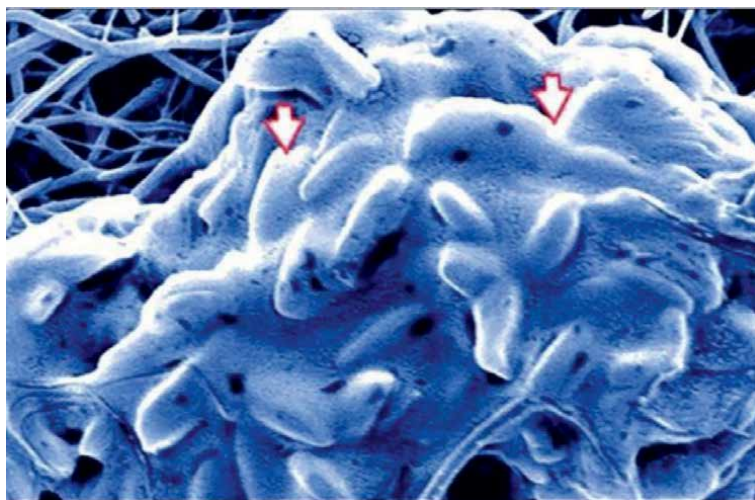


Figure 3. Scanning electron micrographs of agglutinating sperm. Multiple sperm showing adhered heads and wrapped tails with evident agglutinating substance [20].

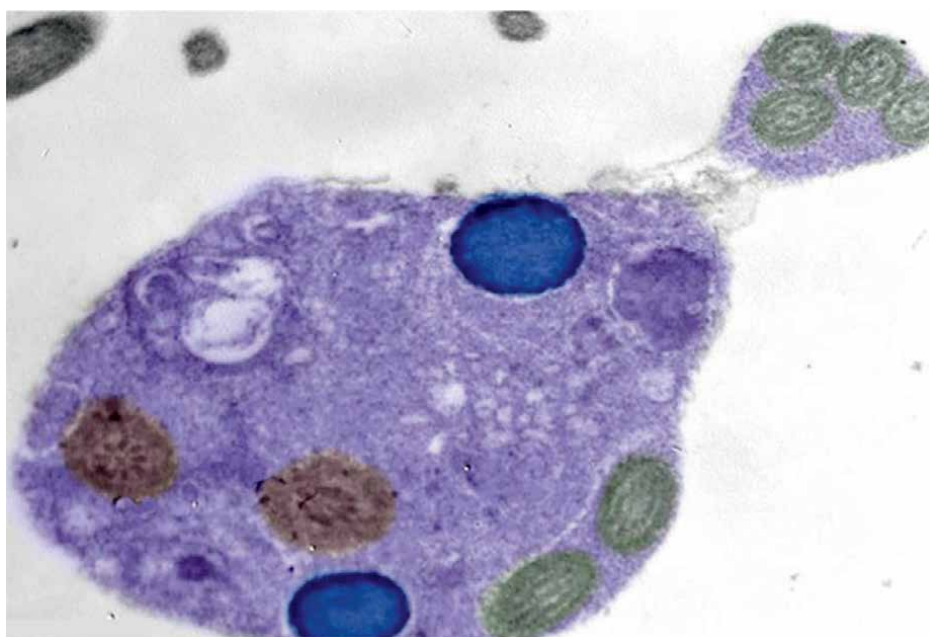


Figure 4. Digitally colored transmission electron micrograph of a sperm bundle in a cut section show the sperm heads with two nuclei (blue color) and flagellum (green color) present with the agglutinating material shown in a light purple color [20].

at the terminal segment that act as paddles and aid in bundle movement. Furthermore, the authors reported that sperm bundles showed rheotactic behavior and swam parallel to each other when present in a slow fluid flow; however, as the velocity of the flow increased they started to overlap and stick to any stationary object (microchannel sidewall) so as not to be swept away with the flowing current [20]. These findings are

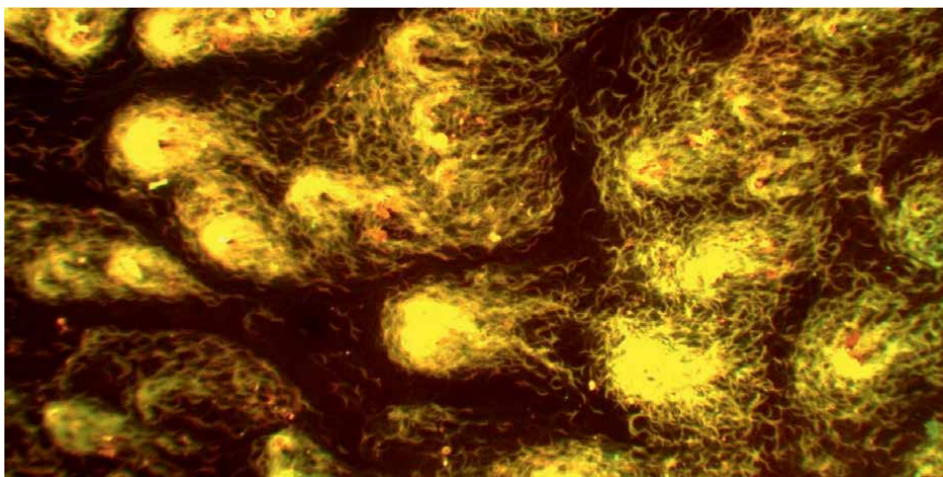


Figure 5. *Micrographs of sperm smears stained with Acridine orange showing sperm head aggregates coated with agglutinating material [20].*

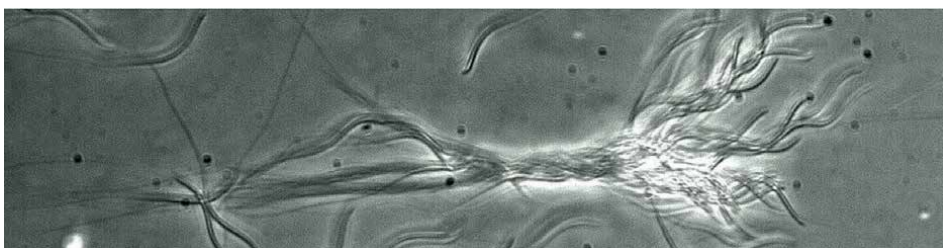


Figure 6. *An image of a developing sperm bundle under phase contrast microscope showing the initial segment of free heads and the terminal segment of adhered tails [20].*

important as they prove that sperm agglutination can occur before mating and that the agglutinating substance originates from the male reproductive tract. Consequently, the bundle formation is not subject to being confined in a small area (SSTs) due to pressure as suggested previously [33]. Additionally, sperm bundles are motile, showing positive rheotaxis, and are capable of sticking firmly in a dynamic environment with high flow velocity. Also, scanning electron microscopy of sperm bundles revealed that the sperm were coated with copious amounts of an adhesive substance particularly in their head region. This indicates that sperm heads adhere in immobile bundles that occur after reaching the storage site (SSTs).

In another study, Sayed et al. [34] demonstrated that the tendency of sperm to agglutinate varies between chicken breeds. This might be attributed to variations in the amounts of the agglutinating substance secreted from the male's reproductive tract. Through artificial insemination of Sharkasi and Dandarawi hens with semen pools containing equal numbers of sperm from Sharkasi (showing high sperm agglutination) and Dandarawi (showing low sperm agglutination), the authors studied the relationship between sperm competitiveness and sperm tendency to agglutinate. There were no significant differences between Dandarawi and Sharkasi in terms of sperm morphometric measurements, straight-line and curvilinear velocities, and progressive motility, but Sharkasi roosters fathered the majority of the offspring. It

was suggested that the higher tendency of Sharkasi sperm to agglutinate inside the SSTs, ensures longer periods of residency within the female, which in turn increases their chances of fertilizing more ova than Dandarawi sperm. Furthermore, Sayed et al. [34] reported longer fertility period in Sharkasi compared to Dandarawi chickens (22 vs. 14 days, respectively). It was suggested that Sharkasi sperm bundles remain in the SSTs for a longer time because they will spend more time completely dispersing compared to those in Dandarawi.

2. Conclusions

From the above-mentioned information, it can be concluded that intense sperm selection occurs in the vagina and that sperm mobility and rheotaxis are the determinant factors on the basis of which sperm selection in the vagina and sperm uptake in SSTs takes place. Sperm are capable of agglutinating in motile bundles having distinctive motility behaviors making them capable of clinging to adjacent surfaces. In the lumen of SSTs, sperm agglutinate in stationary bundles which prolongs sperm storage duration. Sperm gradually detach from the agglutinated sperm bundle and egress from the SSTs to ascend the oviduct and fertilize the ova. Therefore, sperm agglutination influences paternity outcomes when sperm from different males are present in a competitive situation because sperm bundles from males with a high tendency of sperm agglutination will remain in the SSTs for longer durations, and this gives them increased opportunities to fertilize more ova.

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Author contributions

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Conflict of interest

The authors declare no conflict of interest.

Author details

Mohamed A.M. Sayed¹, Hanan H. Abd Elhafeez², Catrin Sian Rutland^{3*}
and Taymour M. El-Sherry⁴

1 Faculty of Agriculture, Department of Poultry Production, Assiut University,
Assiut, Egypt


2 Faculty of Veterinary Medicine, Department of Cell and Tissues, Assiut University,
Assiut, Egypt

3 School of Veterinary Medicine and Science, University of Nottingham,
Sutton Bonnington, UK

4 Faculty of Veterinary Medicine, Department of Theriogenology, Assiut University,
Assiut, Egypt

*Address all correspondence to: catrin.rutland@nottingham.ac.uk

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Bottleneck in Creating Layer Breeds of Chickens in Nigeria

*Emmanuel-Ohagenyi Ifemma Justina and
Simeon Ogochukwu Christopher Ugwu*

Abstract

This study was carried out from a retrospective study of all undergraduate and postgraduate researches conducted on chickens in the Department of Animal Science of some Western and Eastern universities (University of Nigeria, Nsukka, and Federal University of Agriculture Abeokuta, Ibadan, and National Animal production institute, NAPRI, Zaria) in the northern part of Nigeria. The breeding strategies in some world-renowned breeding companies were also studied. The study further compared the various methods utilized for the creation of egg-type chicken by the researchers at the universities and institute with the methods adopted by successful breeding companies for the course of genetic improvement of a pure breed popularly known as the broiler or layer breed. The parameters used for evaluation included scope of breed of chicken (exotic or local), statistical model, heritability, and correlation, variance, mating systems, selection methods, uniformity of research environment, breeding methods (conventional and biotechnology), and emphasis of breeding goals. Finally, the study attempts to recommend a cheaper and a practicable plan to create a layer pureline. There is no gainsaying that the bottleneck of creating layer breed of chicken in Nigeria has been exhaustively evaluated in this study. The challenges comprises technical, financial, and inertia, and ineptitude of leadership elicits poor funding cum environment lacking the resilient approach and technical know-how ranging from inappropriate models to methods. We recommend firm breeding policy and adequate international funding for the proper alignment with the world chicken breeding strategies to help position Nigerian poultry industry to deliver its SDGs predicted goal of providing food security for the global populace by 2050.

Keywords: bottleneck, genetic improvement, layer, breed, Nigeria

1. Introduction

Man has practiced selective breeding since he first domesticated animals. He understood the idea that traits tend to be inherited not through formal education or from letters of books, but from his keen observation of resemblance of relatives. Man unconsciously performed selection, by choosing, for example, to retain particular productive or fertile animals longer than less productive individuals, if a choice became necessary [1].

Since the history of genetic improvement of poultry, ancient and modern breeders have availed themselves of two tools to bring about genetic improvement of these poultry breeds. Breeders simply change the gene frequency of the native breeds [2] by

- i. The choice of individual to be made parent, which is selection.
- ii. By control of the way in which the parents are mated, which constitutes inbreeding and cross-breeding.

Breeders have consistently adopted a systematic approach that combines inbreeding, cross-breeding, and selection for the improvement of economic traits such as growth and egg production. These breeding systems have global recognition, owing to its considerable merit in poultry industry for many years. Legates and Warwick further emphasized that highly inbred lines have positive potentials. This knowledge is applied in practice by breeders to take advantage of the genetic potential of highly inbred ones in the popular genetic game called heterosis or crossing of highly inbred lines. The use of inbred lines has resulted in enormous genetic gain, which aided most poultry companies in China, United States, Brazil, Russia, and India to maximize meat production and egg production, hence becoming top poultry-producing countries [3].

Another approach that modern breeding company has adopted today is popularly known as “broad breeding goals or multiple environment selection.” The new selection goal advocates shift from long-term selection of traits in only one environment to use a variety of environments, for selection purposes so that such breed of poultry would have improved performance in a wide range of growing environments across the globe sufficient to make profits [4]. Emmerson [5] reported that such selection strategies currently applied in Aviagen poultry (multi-environment and feed conversion rate) yielded nutritional response and profit in their birds.

The trend in livestock industry recommended by FAO involves whole genome sequence, DNA fragment analysis, microsatellite markers for investigating physiology of diseases, genetic relationships, and breed differentiation. This methodology provides information for preservation of gene pools and marker-assisted selection (MAS). Some breeders, like Dekalb breeders who applied DNA technique in poultry breeding in 1960s, blended B12 gene which has resistance against bacterial and viral diseases with the B21 genes that tended to give very good resistance against viral diseases. The outcome of the combination was that Dekalb developed a parent stock and commercial birds with optimum protection against a broad range of diseases. McAdam [4] reported that the Avigen UK breeding program was not left out in the technology. Avigen has adopted “broad breeding goals and genetic diversity as essential means to achieve a balanced progress in pedigree broiler lines and global market demand”. ISA breeders also have sequenced many of their chickens as well.

In Nigeria, selective breeding has been practiced by subsistent farmers, who retain hens that hatch more chicks and sell hens that had hatched few chicks or fail to incubate their eggs. Natural selection is the major force, since no elite farm with a planned breeding event seems to be found presently in Nigeria. Under the present dispensation, local farmers do not have easy access to improved chickens. Commercial farms import parent stocks that hatch their day-old chicks (DOC). Farmers depend on imported day-old broiler or layer chicks sold by commercial farms, who are profit

making companies. Importation of purelines has several limitations which include the following:

- i. Difficulty in incurring parent stock in many states which do not have strong international link with foreign poultry breeders. For instance, over 90% of commercial poultry farm with parent stocks are resident in Ogun state because of their proximity to Foreign affairs at Lagos state, while there are no commercial farms with parent stocks in the remaining 35 states. At the moment, farmers across 35 states of Nigeria depend on Ogun parent stock farms for their day-old chicks' supply.
- ii. Farmers purchase their chicks at an expensive cost leading to high cost of production and less net profit.
- iii. Nigerian poultry farmers incur huge total cost and minimal net profit than they would have made assuming Nigerian has an indigenous broiler and layer purelines.
- iv. Poor sustainability. Many government policy or international trade policy may lead to Bann on importation of poultry eggs or parent stocks.
- v. Scarcity of chicken meat and protein malnutrition are imminent problems facing a rapidly growing population whose poultry industry is stagnant.
- vi. The average performance of the Nigerian local chicken remains relatively (300 g) of meat in 8 weeks [6, 7], while broiler chicken yield 3,400 g of meat in 8 weeks and the fairly improved chicken (heavy ecotype) yielded 510 g. The local chicken lay an average eggs of 141/year (47 eggs in 4 months) [8], while the layer chicken lays up to 300 eggs/year in 1998.

Breeders efforts have consistently lead to improved growth performance of chicken. Changing broiler meat yield from 2 kg of meat in 13 weeks to 2 kg of meat in 6 weeks from 1960 to 2005 (% change of 60%) from 1960 to 2005. The change in layer egg production is from 230eggs/year to 300eggs/year (% change 30%) from 1960 to 2005 [9].

It is necessary to acknowledge that Nigeria has produced a breed, "Shika brown." It should also be stressed that the Shika breed has not entered the market till now and the breed has not got all the requirement to be classified a breed. Small population of "Shika brown" necessitated the intervention of West African Productivity Programme, (WAPP) Grants, a United Nation program dedicated to the multiplication of the "Shika breed" by Researcher and NGOs.

The ongoing researches in the Animal production departments of the Nigerian Universities and Colleges of Agriculture were again viewed alongside the activities of breeders in the US and some other developed countries. The following observations were made:

- i. Chicken meat reserved for festive periods. Despite ongoing researches since the past three decades, Nigerians still cannot afford chicken meat except during festive periods a situation that prevailed in the US before 1950s. Prior to World War II, chicken was reserved for special occasions. At that time the

arrival of visiting relatives meant roast chicken for dinner. Sunday dinner with the family was often graced with chicken and peas. Farm flocks were generally the domain of women and children to earn some cash-selling eggs. Flock sizes grew from a rooster and few hens to some flocks with 10,000 or more chickens, but it was not until the 1950s and 1960s when vertical integration of the broiler industry occurred and chicken factories with hundreds of thousands of birds appeared. Scarcity of chicken meat and protein malnutrition are direct effect of the failure of the Nigerian poultry industry to develop an indigenous pureline.

- ii. Some countries have over 50 breeds of chickens. Secondly, the studies so far in the Universities appears to be unproductive considering that at the moment Nigeria has not developed an indigenous pureline, when some countries have over 50 breeds of chickens. Modern poultry house now have 25,000 capacity man by one man, while modern farms now contract farms to farmer in and allow family members to maintain the farms [10].
- iii. US Per-capita broiler consumption is 82.3 pounds in 2010 and it continues to be more than beef and pork; US generated 6.8billion on broiler export and retail price per pound of broiler (\$1.8) continues to be less beef and pork (\$4.4 and \$3.2) respectively in 2010 [10].
- iv. Backward in advanced technologies. Researchers are yet to que into the recent trend in biotechnology and integrated breeding. A recent report by Ndofor-Foleng et al. [11] blamed low productivity of the local poultry resources on factors including the neglect of the local chickens by animal research scientists in preference for exotic breeds, insufficient feed supply and problem of diseases and social behavior [12], poor funding among other factors.
- v. A single poultry farm, like Tyson Foods Inc. located in USA, produces more chicken than Nigerian entire chicken population. Tyson is located in Springdale, Arkansas, and with revenue of US \$ 8.89 billion, producing 37.4 million birds, owns 34 slaughter houses, 115,000 employees, 36 hatcheries for the purpose of egg production. Nigerian poultry sector was estimated to be worth 700 billion naira (NGN) or around US\$3.4 billion [13].

Within the last few decades, the civilized countries like USA and China have experience amazing success in their poultry industry, producing several poultry breeds of fast meat-growing chickens, high egg-laying breeds, and highly productive dual purpose birds. These achievements were not possible without resilient visions, dynamics of breeding skills consisting of the conventional breeding method and biotechnology, feed intelligence (feed efficiency), among other factors. Today, the story of the American and Chinese poultry industry is both interesting and attractive. A multimillion Dollar business and international business transacted round the globe, poultry industry in many developed economy has also graduated from a one man business to a very complex and integrated system providing solution to unemployment. Improved poultry system has increasingly made substantially economic impacts of many developed countries in areas of job creation, salary and wages, export trade, per capita consumption, revenues.

Considering tremendous achievements recorded in major poultry companies' worldwide, it becomes imperative to "analyze what factors have made creation of layer Purelines difficult for Animal science departments of Nigerian Universities and Animal production institute, despite "long research" on poultry improvement." A retrospective study on methodology and skills adopted for creation of breed by researchers working at some Nigerian universities and Animal production institute have been compared to those of some breeding companies approach to improvement. The main objective of this study is to expose the bottleneck in the development of a pureline layer breed in Nigerian.

The specific objectives are as follows:

- To undertake retrospective analysis on approach implemented by Nigerian University research in poultry improvement.
 - To access the specific breeding methods of some breeding companies (Aviagen, Hubbard, Babcock, Dekalb, and Hendrix).
 - To compare approaches implemented by Universities and poultry breeding companies.
- To provide a more practical breeding plan for government and international funding.

1.1 Justification

1. The composition of the Nigerian chicken population will suddenly change from 80% subsistence farming to 80% intensive farming system initiating highly productive and efficient poultry sector.
2. The production of local chicken breeds in Nigeria, which is the largest economy in Africa, will play triple significant roles of stupendously increase local animal protein intake and per capita production and permanently address protein malnutrition in Africa where two out of every three individual are malnourished.
3. The Nigerian Elite farm will proffer substantial support for the growth of the poultry industries of other African countries in other that they may collectively meet FAO [14] prediction to meet the MDGs goal and provide global food security through the adequate management of their rich natural resources.
4. Establishment of Elite farms in Nigeria will increase foreign exchange earnings from the exports of Parent stock to other African countries and bring an end to foreign exchange spending on importation of Pullet Parent stocks and other poultry products.
5. It will be imminent to scale up to the poultry industries of civilized countries. For instance, it can gradually grow to Brazil, China, and European Union (EU), which in 2021 exported 14,400,000 metric tons 14,300,000 metric tons and 10,920,000 metric ton, respectively, out of 100,931,000 world chicken export. This is equivalent to N46,080 trillion (\$57.6 trillion), N47,760 trillion (\$57.2

trillion), and N34,880 trillion (\$43.6 trillion) annual export for Brazil, China, and EU, respectively, at the rate of \$4 per kilo of chicken meat.

6. A veritable option for diversification of Nigerian economy given the expected high positive economic impact accruing from a flourishing poultry industry. The exponential growth will turn the Nigerian poultry industry into a multitrillion business venture.
7. Unlimited job creation, which is a core goal of Animal science discipline, would be achieved through several breeding and several allied companies that will spring up.
8. Nigerian small-scale and large-scale poultry farming will be more profitable and more attractive, since the cost of production will be drastically reduced.

2. A review of the scope of genetic study in Nigerian universities and institution

Methodology

This studies was carried out through a retrospective study of all undergraduate and Postgraduate researches conducted on chickens at some selected Departments of Animal science of Western and Eastern Nigeria (University of Nigeria Nsukka and Federal University of Agriculture Abeokuta, Ibadan) and some works carried out on the “Shika breed” at the National Animal production Research Institute (NAPRI), Zaria. Researchers visited the Libraries of both universities for collection of all available research (project) works with chicken and genetic improvement as their main interest. The Proceedings of National Society for Animal production (NSAP) were used to source genetic studies conducted at NAPRI. The study further compared the various genetic methods adopted by these researchers for improvement with breeding companies. The genetic parameters considered include Heritability, Correlation, Variance, Cross-breeding, Inbreeding, Selection methods, Uniformity of research environment, and emphasis of breeding goals. Finally, the study attempts to recommend a cheaper and pragmatic plan to create a layer pureline.

Limitations

Some limitations of this work include the following:

- i. Limited research work materials in the area of genetic improvement of chickens of Nigeria.
- ii. Variation in environments.

2.1 Genetic studies at University of Nigeria Nsukka from 1980 to 2005

2.1.1 Post-graduate studies on improving chickens at University of Nigeria Nsukka (UNN)

The growth traits and estimates of heritability have been studied by Agbo, Ogbu [15], Ohagenyi et al. [16], Ndofor-Foleng et al. [11], Momoh and Nwosu [17], Ebangi and Ibe [18], Nwosu et al. [19] at the University of Nigeria Nsukka. The egg traits of the heavy and light ecotypes of the Nigerian local chickens were also estimated for their egg-laying traits

Traits	Ages WOA	Heritability estimates	Correlations estimates	Authors
Body weight	4–20	0.32–0.58	0.99 to 1.00	Ebangi and Ibe [18]; Ndofor et al. [23]; Ohagenyi et al. [16]; Momoh and Nwosu [17]
Body length	4–20	0.80–0.06		Ohagenyi et al. [16]; Momoh and Nwosu [17]
Shank length	4–20	0.14–0.80		Ebangi and Ibe [18]; Ohagenyi et al. [16]; Momoh and Nwosu [17]
Breast width		0.36–0.58		Ebangi and Ibe [18]
Average body wt gain	4–20	0.28–1.00	0.00–1.00	Ohagenyi et al. [24]
Thigh lengt,		0.13 to 0.52		Agu et al. [8] Agu et al. [8]
Back-width	4–20	0.23 to 0.40		Agu et al. [8]
Neck length	4–20	0.10 to 0.52		Agu et al. [8]
BW, EW EN Selection for 3 generations	0–39	0.13 ± 0.49 to 0.25 ± 0.31		Ogbu [15]
EN, EWAND BWFE Selection for 3 generations	0–39	0.12–0.20, 34–0.43 and 0.57–0.69		
EN, EWAND BWFE Selection for 3 generations	0–39	0.13–0.65		Oleforuh-Okoleh [20]

Table 1.
Heritability estimates of traits at University of Nigeria Nsukka.

by Agu et al. [8] and Oleforuh-Okoleh [20]. The population of the heavy ecotype local chicken has undergone six generations of selection by Ogbu [15] and Agbo. The studies by Asuquo [21] and Omeje [22] evaluated the growth traits of crossbred chickens. **Table 1** below presents the results of some postgraduate studies at UNN.

Heritability estimates varied and ranged from low to high. The studies indicated that the chickens varied significantly in all traits, an indication that mass selection was the best option for improving the Nigerian chickens. Positive genetic response obtained from six generations of mass selection conducted by Ogbu [15] and Agbo attested to this fact. Ohagenyi reported that 25 SNPs and diversity at the ghrelin (GHRL) locus of four Nigerian indigenous chicken populations.

2.1.2 Undergraduate research on chickens' at University of Nigeria Nsukka

Table 2 presents a summary of studies conducted by postgraduate and undergraduates students at UNN from 1980 to 2005 academic session. Various studies evaluating the performance of the Nigerian LC show that the LC although recorded low performance in growth rate, egg number, and egg size than the exotic chicken and their crosses had similar performance with exotic chicken in feed efficiency. The LC also laid its first egg earlier than the exotic.

Nine out of 19 studies on chickens from 1980 to 1985 academic sessions were on local chickens. Two were postgraduate studies, while 17 were undergraduate studies. During 1986–1990 academic sessions, 15 studies were carried out on chickens, 5 out of

Academic sessions	Postgraduate studies	Undergraduate studies	Exotic chickens	Local chickens
1980–1985	2	17	2	17
1986–1990	0	15	10	5
1991–1995	0	19	14	5
1996–2000	0	4	1	3
2001–2005	9	27	20	16

Table 2.
Students research on chickens’ at University of Nigeria Nsukka from 1980 to 2005 academic years.

which were on local chickens. During 1991–1995 academic sessions, 5 out of 19 studies on chickens were on local chickens. During 1996 to 2000 academic sessions, four studies were on chicken, while three are on LC. During 2001 to 2005 academic sessions, 20 studies were conducted on exotic chicken. Sixteen studies were on LC. Nine were postgraduate studies, while seven were undergraduate studies.

During the entire period of 25 years (1980–2005) of academic studies at the Animal Science Department of University of Nigeria, a total of 343 studies were carried out by both postgraduate and undergraduate students. A total of 122 out of the studies were on various types of chicken, and only 31 studies representing less than 10% of the entire study in the department was devoted to the local chickens. The result of this study agrees with [11] who blamed low productivity of the local poultry resources on the neglect of the local chickens by animal research scientists in preference for exotic breeds were on LC.

2.2 Genetic and genomic studies at University of Nigeria Nsukka from 2006 to 2020

The recent studies on chicken with genetic improvement as its main focus are also considered in this section for the purpose of illuminating how the research interest of scientists in these universities and Animal Production Research institute aligns with the global genetic improvement goals and genetic principles. Three cycles of mild selection are for three generations each for growth and egg traits using index selection, which lead to genetic progress in the egg and growth traits of the Nigerian heavy local chicken ecotype that was achieved in the last decade. Estimation of genetic parameter, breeding values, and inbreeding mating system have been employed within the period to achieve improved performance of the Nigerian chicken (Agu et al. [8], Ohagenyi et al. [25], Ohagenyi et al. [26], Ohagenyi et al. [27, 28], Okochi [29], Ezugwu, Emmanuel-Udeozor Ohagenyi et al. [30], Eze [31]).

This last decade has witnessed growing interest and involvement in advanced genetic improvement of the Nigerian chicken. Researcher have embraced genomic studies on local chickens comprising polymorphism of genes (Ghrelin, Ovocalyxin, Ovocledin, Growth factor, and Prolactin) and gene expression (toll-like 5 and NRAMP 1) (Ohagenyi et al. [27, 28, 32], Ikeh [33], Egom [34, 35], Tchoupou [36], Nwapku and [37]). These studies elicited genomic selection as an option for expedite the genetic improvement and creation of new chicken breeds.

The reports of heritability estimates on growth and egg traits of the local chickens and their crosses by Ohagenyi et al. [27, 28], Okochi [29], Ezugwu, Emmanuel-Udeozor Ohagenyi et al. [30], Eze [31], Olatunbosun [38], Olatunbosun [38],

Amusan [39], Akpan [40], Ebangi and Ibe [18], Ndofor et al. [23], Ohagenyi et al. [24] and Momoh and Nwosu [17] ranged from low to high, while genetic correlation were positive and significant in many combination of traits indicating that the local chickens and their crosses could be improved through mass selection.

The study further reveals that all local researches at UNN, Abeokuta, and Shika Zaria laid more emphasis on population performance [8, 17, 18, 23, 24, 38–40]. Many breeding companies lay emphasis on both group and individual.

2.3 Self-funded and on-going research on genetic improvement of Nigerian chicken population at UNN Federal University of Agriculture Abeokuta (FUNAAB)

It is with a deep sense of urgency and sorrowful heart that I cry out for assistance to TETFund innovation to save my genetically improved chicken population, a rare gene pool, that is being improved for six generations from 2018 till date from total wipe. I have lost 90% of this special chicken population, due to nonpayment of salaries, which has been my major source of fund for my genetically improved chicken.

It is highly commendable and interesting report that two on-going research teams dedicated to creating a breed of chicken were identified in the course of this study at UNN and Federal University of Agriculture Abeokuta (FUNAAB). These research team at the University of Nigeria Nsukka has doggedly embarked on self-funding of the genetic improvement of the Nigerian indigenous chicken by inbreeding, since 2018. The UNN genetic team is led by Dr. Ifemma Emmanuel-Ohagenyi has produced a rare gene pool that is being improved for six generations, while Prof A.O Adebambo led the genetic team of FUNAAB. The research team at Federal university of agriculture Abeokuta is driving the course of genetic improvement of local chicken through cross-breeding notwithstanding the general notion that breeding is the most expensive and technically advanced section of animal science. The resilience of these researchers has made remarkable progress in the improved populations.

This ongoing research is being self-sponsored by the grossly insufficient salary and my student's meager support. Six generations of the Nigerian chicken have been evaluated for growth and egg traits, genetic parameters, and genetic correlations. Information on estimated heritability and genetic correlation has informed the method of the selection, specifically index selection in this case. The superior individuals used as parents of the next generation have yielded genetic progress in meat and egg performance traits of the progeny during these generations. These researches at the University of Nigeria Nsukka Animal science farm has progressively increased the growth performance of the Nigerian chicken from 300, 400, 500 to 700 g at 8 weeks of age [27–29]. The egg traits of the inbred chicken have also witnessed genetic progress with a present egg weight of 60 g and egg number 210 egg per annum. The Ghrelin gene and Ovocalyxin gene polymorphism studies among Nigerian chickens have showed that the Nigerian chicken can be developed into broiler and layer breed through genomic selection [32]. The genetic progress recorded in the recent studies are indicative of the high predictive accuracy as well as trusted expertise of our adept research team and revealed that these fairly inbred chicken are not far from becoming a breed, if granted adequate local or international funding. **Figure 1** below shows the photo of the genetically improved chicken at UNN.

This work though driven by the passion to pencil Nigeria in the annals of history and save her the embarrassment of inability to boast of a standard breed of chicken



X

Parents of genetic group 1



X

Parents of genetic group 2



X

Parents of genetic group 3

Figure 1. Parents of Nigerian inbred chickens. Source: Parents of inbred chicken reared by Dr. Ohagenyi I.J., Okochi desire, Nwankwo favor, and Ijeoma Onyishi at the experimental poultry farm, University of Nigeria Nsukka.

despite its superfluous local chicken resource is earnestly daunting. This research has witnessed several setbacks including the following:

- i. Funding genetic improvement with a meager salary.
- ii. Shortage of finance to buy feed, drugs, vaccine, and payment of farm attendant.
- iii. Every production cycle of these five cycles have witnessed zero egg lay for a period of 2 months due to inability to buy feed.
- iv. Some superior sire and hens were lost at those moments of starvation.
- v. These are limiting factors to the timely realization of our ultimate goal of developing an indigenous layer and broiler breed in Nigeria.
- vi. Resistant strains of pathogen that defy most coccidiosis medications.
- vii. High probability for complete loss of gene pool, due to irregularity of salaries. For instance, my research team has lost 90% of this special chicken population, due to nonpayment of salaries, which has been my major source of fund for my genetically improved chicken.
- viii. Unavailability of hatchery.
- ix. Low hatchability.

2.4 Postgraduate studies on local chicken improvement at Federal University of Agriculture Abeokuta from 1980 to 2005

Heritability and genetic correlations: The genetic studies conducted at the Federal university of agriculture Abeokuta and University of Ibadan by Ige [41], Adeleke et al., Ogunsola [42], and Ajayi [43] showed that heritability estimates for body weight, body length, and breast girth were 0.30–0.62, 0.97, and 0.97, respectively. Heritability estimates for semen volume and motility ranged from 0.01 to 0.46.

The phenotypic correlation coefficients between body weight and other ranged from 0.13 to 0.92. Genetic correlation coefficient was high and positive and ranged from 0.40 to 0.99 and 0.43–0.99 between body weight and other traits [41, 42]. Highly significant correlation coefficients indicated that meaningful improvements can be made through selection of pair of traits that were positive and significant.

Crossbreeding: Several studies at the University of Nigeria Nsukka, University of Agriculture Abeokuta, and NAPPRI Zaria evaluated the performance of cross-bred chickens. The results of those studies revealed that the exotic and their crosses had better performance in body weight and semen volume than the local chickens (Adebambo et al. [44]; Akpan [40]; Akanni [45]; Adeleke et al. [46]; Adeleke et al. [47, 48], Adeleke et al. [49] and Sandaa et al. [50]).

The frizzling and naked neck genes conferred better feed conversion, growth rate, feed efficiency, and dressing percentage than the normal feathered chicken [43].

Cross-breeding/combining ability: Adebambo et al. [44] reported that Anak Titan had the best general combining ability (GCA) and some traits were found to be the most discriminating variables to separate the chicken genotypes [51].

Molecular technique: Little has been done on the molecular characterization of the indigenous chicken in Nigeria. It is, however, interesting to note that the two pioneer studies on molecular characterization of the indigenous chicken in Nigeria and DNA loop analysis at Abeokuta Adebambo et al. [52], Adebambo [52], and Ohwojakpor et al. [53] found no significant differences in genetic distance of indigenous chicken from three populations (Southwest, Northwest, and Northeast ecological zones) of Nigeria.

2.5 Genetic studies at National Animal Production Research Institute 2005

The program for breeding in Nigeria started in 1985 at the National Animal Production Research Institute, Zaria [54]. Some studies at Zaria have evaluated the performance of “Shika breed” under different nutritional regime [55–57]. Apno et al. [58] reported continuous differences in almost all the measurable parameters of Adamawa State chickens.

3. Genetic improvement strategies or attributes of world breeding companies

Breeding companies are formal institutions that pursue the genetic improvement of livestock as key goals. This process involves a conscious effort to improve the growth performance, reproductive, and fitness traits of the animals. This study is concerned with the breeding activities of poultry breeding industries. Their main targets comprised production of a meat type chicken, an egg type chicken or a dual purpose chicken. These processes require basically same strategies with limited differences, as they all follow same fundamental principles of genetics, which was revealed to the world by the study of Gregor Johann Mendel, the German Monk. No wonder then that most poultry breeding companies exhibit enormous affinity in breeding goals and attributes. Several characteristics of the world poultry breeding companies identified in this study are highlighted below.

1. **Complex selection index or genetic index:** All the information of economic value recorded on an individual basis is integrated into the pure lines genetic index evaluation by R&D staff using complex statistical analysis (Emmerson, [5]).
2. **High performance** of genetically improved flocks. According to ISA [59], each pure line bird is accountable for approximately 250 million commercial eggs.
3. **Welfare and sustainability:** Welfare and sustainability is prioritized by breeders. At a meeting that attracted 180 poultry industry experts and producers from around the world between 18 and 20 June 2013. Aviagen [60] outlined the need for welfare and sustainability components in broiler breeding.
- 4a. **Small beginnings:** Most breeding farm were not the richest at the beginning of their business career. Breeding companies started very small. Babcock Searcy

farm began on an acres with two 40x400ft laying houses, an egg room, and a tenant house. It grew to 500 acres after 30 years.

- 4b. **Few birds:** Babcock breeding operation was centered around four Cornish and six White Rock strains. These were the result of 6 years of selection and were the best lines that had evolved from the selection program [61].
5. **Profitable enterprise:** Babcock become one of the largest breeders of laying stock in the world during 1964 to 1974, with sales of their own of \$20 million a year and worldwide sales from franchise distributors of about \$80 million a year.
6. **Massive investment policy:** Most breeding companies have numerous diversifications. Babcock started with B 300 and later established pig, vaccines, and vaccine delivery equipment, and feed additives.
7. Breeding companies built a number of pens for best combining ability. Breeding companies as a rule tested for the best matching mating. These often yielded the best performance. Extensive buildings with numerous pens are built for this purpose. For instance, Babcock built 112 research pens for the purpose of evaluating the combining abilities of superior breeding chickens.
8. **International and multimillion dollar business:** Most world poultry companies export frozen chickens and poultry products to many countries. In 1991, the U.S. government helped sponsor the first shipments of frozen poultry leg quarters to the Soviet Union. Russian consumers called them “Bush legs” in honor of the first President George Bush [62]. ISA Breeder produced parent stock (PS) day-old chicks that are supplied to 300 distributors around the world [59]. Babcock sold parent stock to their franchisees all throughout the U.S. and all over the world from Ithaca [61].
9. **The most advanced scientific technology:** The industry employs the most advanced scientific technology available and is constantly seeking new methods (genomics and Radio Frequency Identification) to ensure wholesomeness and enhance quality for the consumer [62].
10. **Mission statement:** Every company has a mission statement and ways of achieving its mission statement. Babcocks has primary business philosophy yesterday, today, and tomorrow. “Supply the poultry businessman the type of breeding he wants at a reasonable price and deliver healthy birds on schedule to any customer anywhere in the world” [61].
11. **Diversified mating system:** Breeding companies systematically combined inbreeding, cross-breeding, and selection in each improvement plan [63].
12. **Test marketing:** Purelines are first tested marketed for years before released to open market by breeding companies. This practice was confirmed by the following report of the president of US Cobb-Vantress, A high meat yield, roaster type of chicken, the Cobb 700 has been test marketed in the USA for

more than 2 years, and parent stock is now available in limited quantities to customers around the world [64].

13. **Highly inbred line:** At Dekalb breeding company, Hendrix and Euribrid breeding companies, highly Inbred lines form the backbone of poultry improvement [63].

3.1 Challenges of breeding companies

Hubbard [65] lamented that the breeding industry is challenged by more and more difficult and complex circumstances consisting of the following:

1. To keep the Elite level purelines flocks under top conditions. One of the biggest challenges that geneticists are facing is running breeding programs where the Elite level purelines flocks are kept under top conditions (in order to maximize the expression of the genetic potential as well as guaranteeing a disease-free status) and, at the same time, breeding for robustness and the ability to perform under a variety of environments. This difficult equation is solved within.
2. Climate change and the aftermath of COVID-19.
3. To make the poultry industry more sustainable over time.
4. To be able to continue to feed the growing world population.
5. The strong increase in the cost of raw material.
6. Ongoing disease challenges around the world.
7. Increased pressure on animal welfare and use of antibiotics, and
8. Uncertainties about market developments in some regions/countries [65].

3.2 Challenges facing animal science researchers in Nigerian universities

Some of the problems facing researcher in the Universities and Colleges of agriculture have been highlighted by numerous authors in the past. These problems include the following:

1. Continuity.
2. Poor infrastructures.
3. Poor funding from government and industries.
4. All Animal improvement and Agricultural Policies are neither instituted nor effective.
5. Pathological problems.

6. High cost of feed.
7. Lack of state-of-the-art research skills.
8. Social behavior.
9. Preference for exotic breed.

4. Informal way of creating breed of chicken. (how novices can create breeds of chickens)

4.1 Things a novice must do after arrival

Beeken [66] has shown that novices can develop a new breed following the methods below.

1. Settle the foundation stock in as normal, and wait to see them happy in their new surroundings.
2. Ensure that the foundation stock has grown used to your new environment so that you have no fertility issues caused by stress.
3. Devote 1 year to hatching and rearing as large a quantity as possible to enable you to have the widest selection to choose during the next breeding cycle when you set up your next breeding pens.
4. Firstly cull any chicks showing serious faults or illness. By this action you will be breeding for vigor.
5. Closely monitor the youngsters regularly to enable you get familiar with the different rates of growth both in body and feather in other that you will identify the different stages and be able, with practice, to recognize the good from the bad at an earlier age.
6. Decision to show will also be much easier. And on the subject of shows is worth considering entering one in the late summer/autumn to get an idea of how they work and get yourself known, Do not be disappointed if your first effort do not result in a rosette, use it as an opportunity to talk to the judge, and get further advice on how your stock is doing [66].

4.2 Conventional breeding methods

The moment a breeder sets out to create a meat-type (broiler) or an egg-type (Layer) breed, it has an intention of changing the genetic composition of the population. This implies change of gene and genotypic frequency of that population. It can also be explained as changing the gene frequency of a superior gene from a low value (0.1 to 0.5) to a high value or fixation (1). Firstly, the breeder carefully considers the appropriate genetic model. The breeder examines the causal effects of the population variance to enable him determine the genetic parameters, consisting of heritability,

repeatability, and genetic correlations. Genetic parameters further illuminates the breeders decision on best selection option that is necessary for improved performance or genetic progress. The result of heritability estimates provides the framework for evaluation of the performance of individuals, popularly known as the breeding values. The breeding values ultimately reveal individuals of superior genotype that must be selected for necessary improves performance of genetic progress among the progenies.

4.3 Genetic gain or response (R)

- **Genetic Gain or response (R)** is simply a product of heritability and selection differential expressed in the following equation;

$$R = h^2 S \quad (1)$$

- Selection brings about genetic progress per generation. Offspring selected have better performance than the average population performance. This improved performance is known as selection differential, S.
- **Selection differential (S) = Mean Offspring - Pop mean**

4.4 Computation of a selection index

A statement of the breeding goal is the first information needed. The concept of merit based on a single trait must be replaced by merit based on combination of traits which are economically important and which have sufficient additively genetic variance to give a reasonable response to selection.

The worth, W, or value of an individual or group is defined as

$$W = (W - W) = W = (W - W) = W + a_1x_1 + a_2x_2 + \dots + a_nx_n = \{a_ix_i\} \quad (2)$$

where a_i are the relative increases in net worth expected from one unit of improved merit in the trait X, independent of the improvement in the other traits in w.

The additive genetic value for worth then becomes g_w in contrast to g for the I trait and it can be defined as

$$g_w = a_1g_1 + a_2g_2 + \dots + a_n g_n = \{a_i g_i\} \quad (3)$$

4.5 Development of breeding goals and breeding plans

In the words of Legates and Warwick, any breeder with the intention of developing or improving livestock must be able to mesh together the fundamental principles tampered with livestock experience and economic realities of animal production. Many early breeders like Babcock have confirmed this as the created purebred without academically specializing in genetics and animal breeding. Beeken [66] in his writings showed that even novice can create a pureline. Demonstrating the prerequisites for genetic improvement aided us to understand the reason behind the failure of Nigerian farmers, animal breeders, and animal scientists.

4.6 Prerequisites for genetic improvement

Two major conditions were underscored by the following.

Firstly, be able to assess the genetic merit of our present animals by keeping accurate records of performance of known ancestry.

Secondly, the influence of the animals with the desired genes must be extended and made available especially through AI and Embryo transfer.

Thirdly, developing a breeding goal embodied in an efficient Selection index, laying emphasis on the following factors,

- i. Consumer choice.
- ii. Prediction of population in future.
- iii. Per-capita income.
- iv. Competition of human and animal in cereal grains.
- v. Place emphasis on quality of poultry product to stimulate consumption and stand competition.
- vi. Admitting that the research and development costs of a poultry breeding operation are very high.
- vii. Think of the world as its market to be able to make sufficient profits.

5. Comparison of Nigerian institutions and world breeding companies on adopted strategies for genetic improvement of chickens

Given the numeric strength of the ongoing researches in the Nigerian Universities, there is no gainsaying that they have been actively involved in genetic research of their chickens. However, chicken breeding research in Nigerian Universities and institute has maintained basic genetic principles aspect in scope and operation of the breeding companies, although it has evaluated genetic merit of our present animals through artificial selection.

The result of this study showed that researchers at the Universities used adequate sample size, since breeding companies at the beginning used few animals of different breeds.

The findings of this study further showed close to zero funding of chicken breeding research; thus, personal funding is the available option for the majority. Consequently, few passionate breeders have remained attracted. Those who are passionate devise the approach of early breeders. They raise 20 to 50 chicks per generation. They often suffer great mortality, poor growth and delayed egg production emanating from shortage of feed, scarcity of drugs, and other environmental factors. They struggle to keep this small population, which were affordable and capable of creating generation of inbred lines, while eggs and chicks reared each generation would offset most cost of production. This approach, though may be efficient, slows down genetic progress.

No existing policy on funding was recorded in any of the chicken breeding researches in Nigeria, an aberration to breeding operation, which are known to be expensive.

Nigerian researchers use majorly SAS, SPSS statistical procedure, while breeding companies use complex animal models for analysis and bioinformatics for genetic improvement studies.

The result revealed the profit-driven goals upheld by breeding company in effort to sustain production. There seems to be no profit-based genetic researches at the universities.

Researcher at Nigerian universities and Institution has embraced genomic selection and conventional improvement strategies; however, it is far less comprehensive than the breeding companies.

Only a few performance traits are measured in Nigerian chicken breeding programs, while breeding companies measure up to 40 performance traits [63].

This study showed that very little attention to inbreeding and development of highly inbred lines by Nigerian universities and institute, although it is the basic element for breeders.

The study revealed that no research was reported on market testing of sibs in any of the universities, although it is a prerequisite before a breed enters a market.

Furthermore, this study showed that all breeding companies as a necessity bred many strains simultaneously [67]. However, no such attempt was found in Nigerian universities.

A comparative study of the method of chicken improvement at the Nigerian universities studied and the breeding companies showed that AI and Embryo transfer, which is the backbone of multiplication of foundation stocks in breeding companies [63] was not done at any of the Animal science department of the universities (UNN, FUNAAB and Shika Zaria).

The result of this study showed that ownership of breeding companies is passed from generation to generation, [63]. This is not common in the Nigeria institutions.

6. Summary and conclusion

There is no gainsaying that the bottleneck of creating layer breed of chicken in Nigeria has been exhaustively evaluated in this study. The challenges comprises technical, financial, leadership, policy, and pathological problems.

The inertia and ineptitude of leadership elicits poor funding cum environment lacking the resilient approach that characterizes breeding companies.

Finally poor technical know-how consisting of inappropriate models, inadequate number of performance traits, economically inefficient selection goals, wrong use of hybrid vigor, multiplication of foundation stock, absence of many strains, and poor AI facilities are the major factors responsible for the belated creation of layer breed in the Animal Science Department of the Nigerian universities.

6.1 Recommendations


We recommend further study on the scope of very recent chicken breeding researches in Nigerian universities and institution, a vibrant breeding policy and adequate international funding for proper orientation and alignment with the of world chicken breeding strategies to help position Africa and Nigerian poultry industry to deliver its SDGs predicted goal of providing food security and animal protein for the global populace by 2050.

Author details

Emmanuel-Ohagenyi Ifemma Justina* and Simeon Ogochukwu Christopher Ugwu
Faculty of Agriculture, Department of Animal science, University of Nigeria, Nsukka,
Nigeria

*Address all correspondence to: ifemma.ohagenyi@unn.edu.ng

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Section 5

Processing

Identification and Analysis of Safety Hazards and CCPs in a Chicken Meat Production Chain

*Angélica Patricia Huertas Moreno,
Elena Amparo del Rocío Gómez Moreno, Gonzálo Téllez Iregui
and Andrea Patricia Varón García*

Abstract

The identification of hazards in a food chain makes it possible to contemplate all the possibilities of contamination risk, in order to generate control and corrective measures in the processes involved. The above, in order to guarantee the safety of the food available to consumers. The objective of this work was to identify hazards (physical, chemical and biological) and CCPs in a poultry chain and to define the corrective and risk control measures applicable to the processes. The study was carried out through visits to the plants and sites that make up a chicken meat production chain, where we observed, inquired with the personnel involved in the processes, and reviewed all the required documentation. For the identification of hazards and associated risks, the HACCP methodology was used to identify the CCP (Critical Control Points). The main results showed that the most critical operations in the entire chain were disinfection of the carcass before pre-cooling and cooking of the feed before consumption.

Keywords: chicken, risk, safety, CCP, hazard, corrective and control measures

1. Introduction

Internationally, the production and consumption of chicken meat has been growing. FAO's 2016 report, which takes stock of the next 10 years until 2025, states that world meat production will grow by 16% by this date. This prediction is based on data obtained in 2015, which showed a 20% increase over the previous decade. The countries that will most significantly increase their production records will be the United States, Brazil, the European Union, India, and Russia [1]. In 2030, the world will need millions more tons of meat, especially poultry, whose consumption will almost quadruple according to FAO estimates.

On the other hand, chicken is considered a perishable food because of its high-water activity (Aw) and should be stored under conditions where microbial growth is slow or does not take place [2]. In addition, as chicken meat production increases,

so do food safety risks. Chicken meat is exposed to several types of hazards, such as biological, chemical, and physical hazards, which may be in the food and pose harm to the consumer's health when ingested [3]. In relation to biological hazards, these microorganisms can be pathogenic and cause foodborne diseases or STDs [4], depending on their pathogenicity and the number and concentration of bacteria in the product [5].

Foodborne diseases are a growing public health problem. It is estimated that every year, some 600 million people in the world—almost 1 out of every 10 inhabitants—fall ill from eating contaminated food and that 420,000 die from the same cause. Diarrheal infections, which are most commonly associated with the consumption of contaminated food, make about 550 million people sick each year and cause 230,000 deaths [6].

The objective of this work is to identify and analyze hazards and CCPs (Critical Control Points) in each of the stages that make up the poultry chain. Additionally, and based on the results, the corrective and risk control measures applicable to the processes are defined.

2. Methodology

The study was carried out by observing the production processes of the different plants (hatchery, fattening farm, slaughter plant, depressing plant, and consumption site), with information provided by the different actors in the chain (operating personnel of the company, consumers, company managers, experts, vendors, suppliers, transporters, among others), and by verifying all the documents required during the visits.

Some principles of the HACCP system were used as a tool, since it allows the identification of CCPs and the subsequent definition of corrective and control measures throughout the processes.

3. Results and analysis

The results are presented below according to the stated objective:

The chicken meat production chain is made up of the stages of incubation, fattening, slaughter, depressing, and consumption. For each of the plants and consumption site, data on the location of the plant such as altitude, surroundings, ambient temperature, and relative humidity are indicated, since these are environmental characteristics that can influence, justify, or be related to the hazards identified throughout the process in the poultry chain.

3.1 Hazard identification and CCPs

For the definition of CCPs, it is analyzed whether the operation exists specifically to eliminate or reduce a hazard, if contamination reaches unacceptable levels at that stage, or if a subsequent operation does not eliminate or reduce the hazard to acceptable levels. This is in accordance with the 1993 Codex Committee's Guide for the Application of HACCP.

The operations that make up each of the stages and the analysis of the hazards (physical, chemical and biological) and CCPs identified are presented in the following graphs.

Incubation:

Plant location data:

Altitude: 288 masl.

Surroundings: Vegetation (grass and trees). It is located 314m from a recreational club, 226 m from a Hacienda, 307 m from a hotel, 683 m from another hotel, and 338 m from a condominium.

Ambient temperature: 27.3–29.3°C (27.3–29.3°F).

Relative Humidity: 66.38%.

Figure 1 of the incubation stage is shown below:

The hazards identified in each of the operations of the hatchery stage are detailed in **Table 1**, as well as the CCPs:

As an open hatchery, it is exposed to environmental and pest contamination, since it is located in a rural area, surrounded by grass and trees, which increases the risk of biological contamination by animals or pests. With a climate between 27 and 29°C, there are more insects, crawling and flying insects, among others. In accordance with the above, there must be a rigorous cleaning and disinfection process *before* starting the processes.

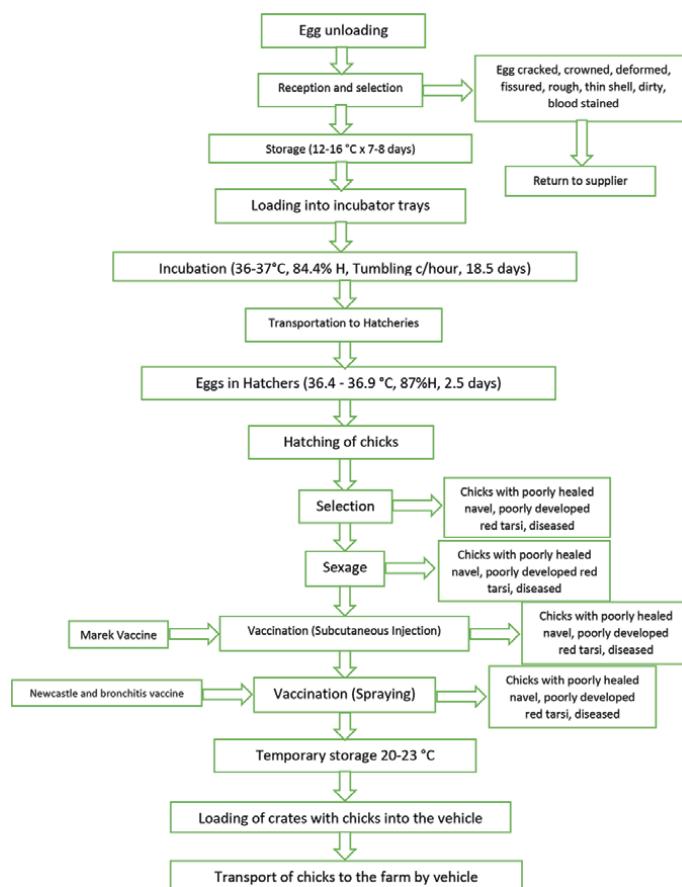


Figure 1. Incubation stage. Source: Own elaboration based on the visit to the hatchery.

Stage	Danger	Risk factor	PCC
Unloading and reception of eggs	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Egg contaminated by pathogens <p>Cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the vehicle, pallets, floors, walls, and roof, since these are activities to be carried out by the egg supplier or owner of the vehicle and which cannot be verified immediately, since there are no verifications using luminometers or similar techniques that generate immediate results and therefore immediate corrective actions.</p> <p>Cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the environment of the unloading site, personnel equipment, plastic buckets, hands, bucket transport carts, infrastructure, and poor operator hygiene practices. This is due to the fact that there are no disinfection process-verification techniques with immediate results for taking immediate corrective measures.</p>	No
Egg selection	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection of the sorting site, personnel equipment, plastic buckets, sorting tables, operator hands, bucket transport carts, infrastructure, and poor operator hygiene practices. Likewise, during the visit, no procedures were observed for disinfecting egg trays and sorting tables prior to receiving the eggs.</p> <p>Physical: Presence of feathers and dirt</p> <p>-Poor cleaning by the supplier. During the visit, the presence of these physical contaminants was evidenced during reception; however, they were rejected and returned.</p>	No
Egg storage	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection of the storage site, personnel equipment, plastic buckets, temporary storage tables, hands, bucket transport carts, infrastructure, and poor operator hygiene practices. Likewise, during the visit, no procedures were observed for disinfecting egg trays and receiving tables prior to receiving eggs for subsequent storage.</p>	No
Loading eggs into hatchery trays	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection of the loading site, personnel equipment, plastic buckets, operator's hands, incubator trays, bucket transport carts, infrastructure, and poor operator hygiene practices. Also, during the visit, some empty bottles of hand sanitizer were observed.</p>	No
Egg incubation	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of incubators and trays.</p> <ul style="list-style-type: none"> • Variation in the required temperature, ventilation, and humidity; however, the recording forms for these variables do not show large fluctuations, and the control of the equipment by the operators can be observed. 	No
Egg transport to hatcheries	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection of the loading site, personnel equipment, operators' hands, hatcher trays, tray transport carts, infrastructure, and poor operator hygiene practices. Also, during the visit, some empty bottles of hand sanitizer were observed.</p>	No

Stage	Danger	Risk factor	PCC
Eggs in hatchers and hatching of chicks	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of hatchers, trays, and operator's hands and poor hygienic practices. <ul style="list-style-type: none"> Variation in the required temperature, ventilation, and humidity; however, the recording forms for these variables do not show large fluctuations, and the control of the equipment by the operators can be observed. 	No
Chick selection	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection, disinfection of operators' hands, equipment, trays, selection line, and poor hygiene practices of operators. Also, during the visit, some empty bottles of hand sanitizer were observed.	No
Chick sexage	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the environment, hands, personnel, trays, sexage line, and poor hygiene practices of the operators. Also, during the visit, some empty bottles of hand sanitizer were observed.	No
Vaccination of chicks	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Incorrect application of the vaccine that does not guarantee the entry of the liquid into the chick's body and therefore is not effective against the disease. Cross-contamination due to deficient or inadequate environmental disinfection, disinfection of hands, equipment, trays, vaccination line, and poor hygiene practices of the operators. Also, during the visit, some empty bottles of hand sanitizer were observed.	Yes
Temporary storage of chicks	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection of the storage site, personnel equipment, plastic buckets, hands, bucket transport carts, infrastructure, and poor hygiene practices of the operators.	No
Loading of crates with chicks	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. Also, cross-contamination due to deficient or inadequate environmental disinfection of the loading site, equipment, plastic buckets, hands, bucket transport carts, infrastructure, and poor hygiene practices of the operators. 	No
Transport of chicks to farm	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. 	No

Source: Own elaboration based on the visit to the hatchery.

Table 1.
 Hazards and CCPs at the hatchery stage.

In egg incubation, the quality of the egg delivered by the supplier is very important, so that it is not contaminated by pathogenic microorganisms, since this determines the contamination from then on in the process.

Biological hazards during this stage are caused by deficient or inadequate disinfection practices, since the disinfectant is not correctly *dosed* in the water (milliliters of disinfectant per liter of water) or the retention times are not met, and therefore, there is no guarantee of effective elimination of unwanted microorganisms. Likewise, since immediate verifications are not carried out by means of techniques such as luminescence, the effectiveness of disinfection and corrective actions cannot be checked immediately. This, in addition to the fact that during the visit, there was no evidence of compliance with the processes of disinfecting egg trays and tables prior to reception, and some empty bottles of hand sanitizer were observed. All operations may be affected by cross-contamination due to the lack of hand disinfection, because they are performed manually with direct intervention of the operators.

The variation of temperature, humidity, and time in the incubators and hatchers causes a risk of growth of undesirable microorganisms; however, this equipment has sensors that regulate these variables, and during the visit, the operators were observed keeping records on paper forms for monitoring purposes.

Another risk factor for biohazards is the incorrect preparation of the subcutaneous vaccine or incorrect application of the vaccine, since the vaccine is prepared by plant operators. In relation to the correct application of the vaccine, this liquid has a green color so that this color can be observed in the application area, as evidence of the liquid entering the chick's body. For vaccination by spraying, there is a risk of exposing the chick to the vaccine for more or less time than allowed, since, although there is a timer, it is an activity performed by an operator.

At this incubation stage, the only CCP identified is *vaccination* since this vaccination operation has been specifically designed to eliminate a biological hazard such as Marek's disease, Newcastle disease, and bronchitis. The other operations (egg unloading, egg reception, egg selection, egg sorting, egg temporary storage, loading into setter trays, incubation, transport to hatchers, chick hatching, chick selection, chick sexing, chick temporary storage, chick crates loading to the vehicle, and chick transport to the farm) are not considered CCPs since they are not operations designed to reduce the risk of CCPs. The other operations (unloading eggs, receiving eggs, sorting eggs, selecting chicks, sexing chicks, temporary storage of chicks, loading crates with chicks into the vehicle and transporting chicks to the farm) are not considered CCPs, since they are not operations designed to eliminate a hazard or reduce it to an acceptable level, nor can contamination reach unacceptable levels in these operations.

In relation to *physical* hazards in the incubation stage, feathers and dirt are present on the eggs; however, they are checked and returned if these quality defects are present.

Fattening:

Plant location data:

- Altitude: 2625 masl
- Surroundings: Vegetation

- Ambient temperature: 14°C average
- Relative Humidity: 72%.

Figure 2 of the fattening stage is shown below:

The hazards identified in each of the operations of the fattening stage are detailed in **Table 2**, as well as the critical control points (CCP) based on the HACCP methodology:

When receiving the chicks, it is important to verify the state of health of the bird delivered by the transporter, so that it does not present diseases, since this determines the contamination from then on in the process.

Biological hazards occur at this stage due to the risk of contamination by pathogenic microorganisms either through contaminated water, contaminated feed, transmission by pests, ineffectiveness of the vaccine due to inadequate administration, or poor cleaning and disinfection processes of feeders, drinkers, curtains, and vehicle, among others, and non-compliance with biosecurity measures.

In this fattening stage, the CCP identified is *vaccination and feeding*, since in the case of vaccination, this operation has been specifically designed to eliminate a biological hazard such as Gumboro, Newcastle, and bronchitis diseases. And in the case of feeding, although this stage is not designed to eliminate or reduce a hazard, contamination can reach unacceptable levels, which are not reduced in subsequent operations. It is also possible to have intervention measures to reduce the danger of contamination by pathogens, through the provision of bacteriophages in the feed or similar. The other operations (unloading and reception of chicks, intake and fattening, feeding, loading and transport of chickens to the processing plant) are not considered CCPs, since they are not operations designed to eliminate a hazard or reduce it to an acceptable level, nor can contamination reach unacceptable levels in these operations.

In relation to *chemical* hazards, there is a risk of antibiotic residues in chicken meat, due to non-compliance with the withdrawal times of feed containing growth promoters or medicines given to the birds in case of illness.

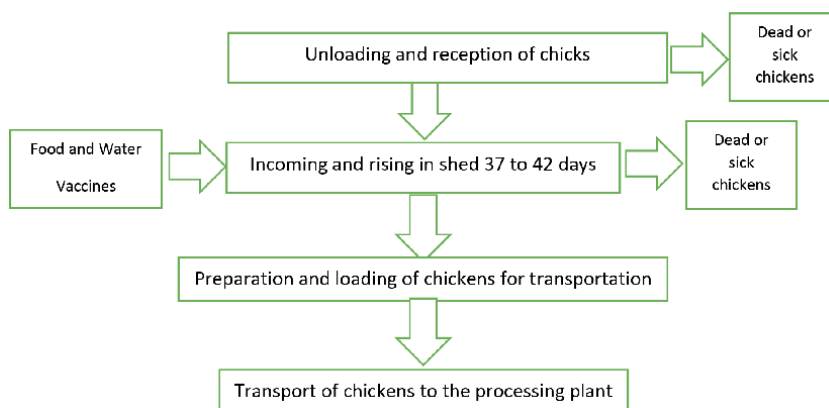


Figure 2. Fattening stage. Source: Own elaboration based on the visit to the farm.

Stage	Danger	Risk factor	PCC
Unloading and reception of chicks	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Pollution contaminated by pathogens or diseased. <p>Cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the vehicle, pallets, floors, walls, and roof, since these are activities to be carried out by the transporter and which cannot be verified immediately, since there are no verifications by means of luminometers or similar techniques that generate immediate results and therefore immediate corrective actions.</p>	No
Intake and fattening in sheds	Biological: Contamination by pathogenic microorganisms or diseases.	<ul style="list-style-type: none"> • Chickens that were received contaminated with pathogens or presence of diseases. • Transmission of diseases by pests (flies, rats, cockroaches) in the shed. • Feed and water supply. • Incorrect administration of the vaccine that does not guarantee the consumption of the beverage and therefore is not effective against the disease. <p>Cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the unloading-shed environment, personnel equipment, plastic containers, operator's hands, container transport carts, brooders, drinkers, feeders, swarf bedding, plastic curtains, infrastructure, and poor operator hygiene practices. This is due to the fact that there are no disinfection process verification techniques with immediate results for taking immediate corrective measures.</p>	No
Preparation and loading of poultry for transport	Chemical: Contamination by antibiotic residues. Biological: Contamination by pathogenic microorganisms	<p>Antibiotic residues due to non-compliance with withdrawal time of growth promoters or other medications fed to chickens.</p> <ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. Also, cross-contamination due to deficient or inadequate disinfection of the loading shed, personnel equipment, plastic containers, operators' hands, boots, overalls, drinking troughs, brooders, feeders, plastic curtains, container transport carts, infrastructure, and poor hygiene practices of the operators. 	No
Transport of chickens to the processing plant	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the vehicle: pallets, floors, walls, and roof. The above, because of another batch of chicken that was previously transported. 	No

Source: Own elaboration based on the visit to the farm.

Table 2.
Hazards and CCPs in the fattening stage.

Sacrifice:

Plant location data:

- Altitude: 2625 masl
- Surroundings: Paved streets. It is located 427 m from a gas station, 153 m from a chicken distributor, and 406 m from a butcher's area. In the surrounding area, there are restaurants, transport companies, distribution companies, butcher shops, meat distributors, and meat packing plants, among others.
- Distance to main road: Located on main road.
- Ambient temperature: 14°C average
- Relative Humidity: 72%.

Figure 3 of the slaughtering stage is shown below:

The hazards identified in each of the operations of the slaughter stage are detailed in **Table 3**, as well as the critical control points (CCP) based on the HACCP methodology:

The location of the slaughter plant in a cold climate mitigates somewhat the risk of contamination by undesirable microorganisms compared to slaughter plants located in warm climates. Likewise, being located in an urban area, the presence of domestic animals, pests, and flying crawlers is reduced compared to a plant located in a rural area with a warm climate.

A critical operation in the slaughter of poultry is the ante-mortem inspection, since there is a danger of biological contamination of birds with pathogenic microorganisms or diseases; therefore, it is necessary to certify disease-free animals from the farm.

Likewise, *biological* hazards during this stage are caused by deficient or inadequate disinfection practices, since the dosage of the disinfectant in the water (milliliters of disinfectant per liter of water) is not correctly carried out, or the retention times are not met, and therefore, there is no guarantee of effective elimination of undesirable microorganisms. Likewise, since immediate verifications are not carried out by means of techniques such as luminescence, the effectiveness of disinfection and corrective actions cannot be checked immediately. The above, in addition to the fact that during the visit, very poor cleaning and disinfection practices were observed, due to excess feathers in the plucking area, excess cuticles in the leg-peeling area, excess viscera and blood in the evisceration areas, viscera cooling tanks and viscera packaging. This is aggravated considering that there is always the risk of contamination with fecal matter coming from the crates where the live birds arrive and during the operations of unloading of birds, weighing of birds, ante-mortem inspection, hanging of birds, desensitization, slaughter, and bleeding and mainly during the evisceration process due to the rupture of the intestines.

The CCPs identified in the slaughter stage correspond to the *evisceration and disinfection of the carcass*. In the evisceration operation, specifically, if the intestine ruptures, given that, although the stage was not designed to eliminate or reduce a risk, contamination can reach unacceptable levels, due to contamination with fecal matter from the rupture of the intestine. In the disinfection operation, the step was specifically designed to eliminate or reduce a hazard; therefore, it is considered a CCP.

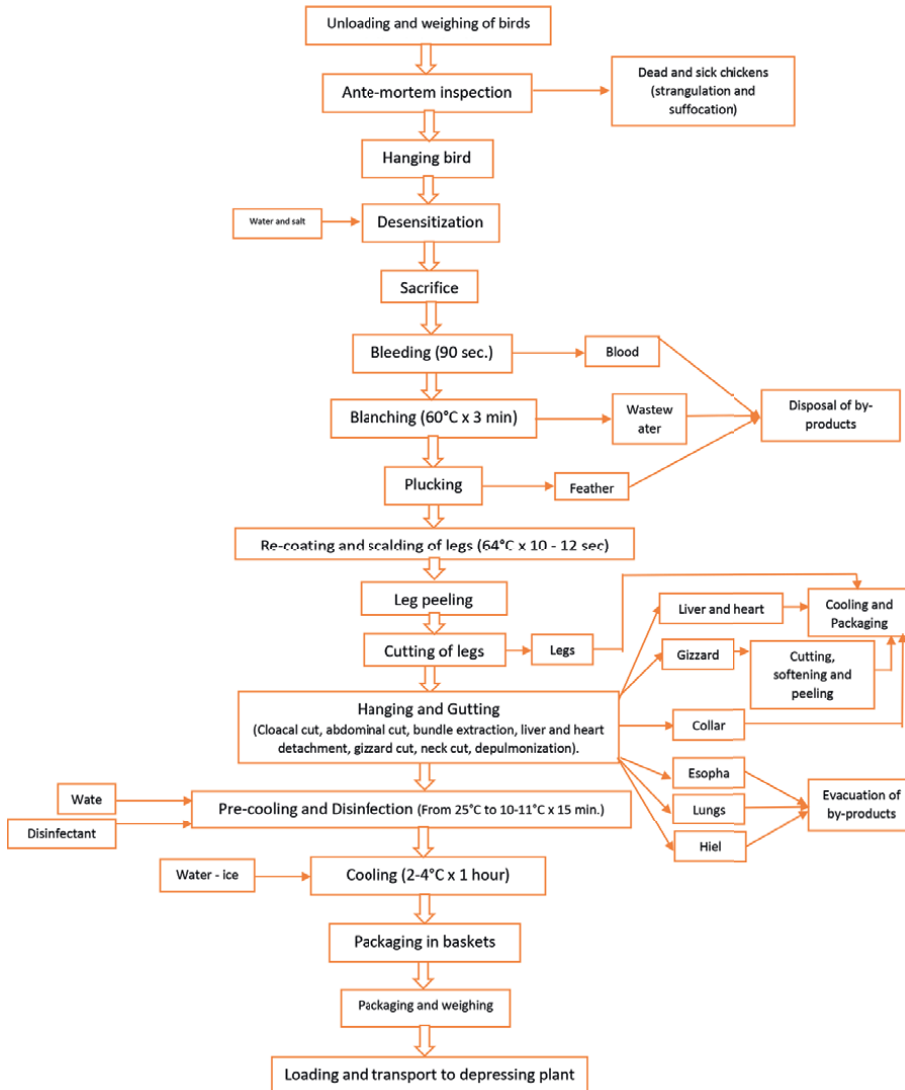


Figure 3. Sacrifice Stage. Source: Own elaboration based on the visit to the slaughter plant.

With respect to *chemical* hazards, there is contamination by disinfectants, due to incorrect dosage (addition of more disinfectant than defined), longer retention time, or no rinsing (in cases where this should be done). This type of hazard is observed in the plucking process, since the equipment has spaces that are difficult to access, making cleaning and disinfection difficult and leaving disinfectant residues on the equipment. This risk is also present during the evisceration process, since carcasses that have any contact with dirty areas are disinfected, and during pre-cooling, where carcasses are disinfected prior to packaging. Finally, packaging contaminated with detergents or disinfectants may exist; however, this risk is low in the production plant, since there is evidence of packaging storage in separate rooms from the storage of all types of inputs, in compliance with Good Manufacturing Practices (GMP).

Stage	Danger	Risk factor	PCC
Bird discharge	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the vehicle, pallets, floors, walls, and roof. Likewise, cross-contamination due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the environment, rubber gloves, equipment, poultry houses, infrastructure, and poor hygiene practices of the operators. This is due to the fact that there are no disinfection process verification techniques with immediate results for taking immediate corrective measures. • Presence of fecal matter in the baskets where the birds are kept and on the bird itself. The above, given that during the visit, the presence of fecal matter was observed on the floor of the area. 	No
Weighing of birds	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, scales, gloves, equipment, operators' hands, poultry houses, infrastructure, and poor hygiene practices of the operators. • Presence of fecal matter in the cages where the birds are kept and on the bird itself. 	No
Ante-mortem inspection	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Birds with presence of pathogenic microorganisms, sick or dead. • Cross-contamination due to deficient or inadequate disinfection of the environment, rubber gloves, equipment, poultry crates, infrastructure, and poor hygiene practices of the operators. • Presence of fecal matter in the cages where the birds are kept and on the bird itself. 	No
Hanging of birds	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, rubber gloves, equipment, poultry crates, hanging tunnel, infrastructure, hanging hooks, and poor operator hygiene practices. • Presence of fecal matter in the cages where the birds are kept and on the bird itself. 	No
Bird desensitization	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, infrastructure, hanging hooks, water tank, and water. • Presence of fecal matter on the bird itself. 	No
Poultry slaughter	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, rubber gloves, operator equipment, slaughter line, infrastructure, knife, hanging hooks, and poor operator hygiene practices. • Presence of fecal matter on the bird itself. 	No
Chicken carcass bleeding	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, bleeding line, infrastructure, and hanging hooks. • Presence of fecal matter on the bird itself and in blood. 	No

Stage	Danger	Risk factor	PCC
Blanching of chicken carcass	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, scalding tank, hanging hooks, scalding water, infrastructure, and scalders. • Blood in the scalding water. • High variations in the defined temperatures and times; however, during the visit, the verification performed by the operators on the thermometers of the equipment was observed. • Inhalation of water from scalding if the bird is not dead. 	No
Chicken carcass plucking	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, plucking machine, hanging hooks, infrastructure, and plucking line. • Contamination by not washing frequently or removing feathers from the equipment. During the visit, the equipment was observed with excess feathers, as well as in the area. 	No
	Physical: Feathers	<ul style="list-style-type: none"> • Feathers that were not removed; however, the chicken carcass was observed to be approximately 98% clean. 	No
	Chemicals: Disinfectant Residues	<ul style="list-style-type: none"> • Residues of detergents or disinfectants in the plucking machine, since it has spaces that are difficult to access, making cleaning and disinfection difficult. 	No
Chicken carcass turning	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of the environment, hanging hooks, equipment, rubber gloves, infrastructure, and poor operator hygiene practices. This is due to the fact that hand disinfection was not observed on a constant basis or when there was a change of activity.</p>	No
	Physical: Hair	<ul style="list-style-type: none"> • Hair of handlers or operators. 	No
Scalding of carcass legs	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, scalding tank, hanging hooks, scalding water, infrastructure, and scalders. • High variations in the defined temperatures and times; however, during the visit, the verification performed by the operators on the thermometers of the equipment was observed. 	No
Peeling of carcass legs	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the environment, hanging hooks, infrastructure, and equipment (peeler). Also, cross-contamination with the by-product generated. This is due to the accumulation of residues (cuticles) on the floor and in the area in general. 	No
	Physical: Cuticles of legs	<ul style="list-style-type: none"> • Presence of cuticles due to deficient peeling. 	No
Cutting of carcass legs	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of the environment, hanging hooks, equipment, rubber gloves, knives, infrastructure, and poor operator hygiene practices.</p>	No
	Physical: Hair	<ul style="list-style-type: none"> • Hair of handlers or operators. 	No

Stage	Danger	Risk factor	PCC	
Hanging of chicken carcass	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the environment, hanging hooks, equipment, rubber gloves, infrastructure, and poor operator hygiene practices.	No	
	Physical: Hair	<ul style="list-style-type: none"> Hair of handlers or operators. 	No	
Gutting of chicken carcasses	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection, rubber gloves, personnel equipment, hanging hooks, infrastructure, knife, pneumatic sewer extraction gun, and poor operator hygiene practices.	Yes	
		<ul style="list-style-type: none"> Contamination with edible and inedible viscera generated. This is due to the fact that during the visit, viscera residues were observed on the floor that were not picked up immediately. 		
		<ul style="list-style-type: none"> Contamination due to gall breakage. 		
		<ul style="list-style-type: none"> Contamination due to rupture of the intestines. 		
		<ul style="list-style-type: none"> Contamination from spoiled viscera. 		
	Chemicals: Contamination by disinfectants	<ul style="list-style-type: none"> Delay of more than 30 minutes between bleeding and evisceration. Incorrect dosage or preparation. 	No	
Pre-cooling and disinfection of chicken carcasses	Physical: Hair	Hair of handlers or operators.	No	
		Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection, cooling tanks, or contaminated water.	Yes
			<ul style="list-style-type: none"> High variations in the defined temperatures and times; however, during the visit, the verification performed by the operators on the thermometers of the equipment was observed. 	
	Chemicals: Contamination by disinfectants	<ul style="list-style-type: none"> Incorrect dosage or preparation of disinfectants that does not guarantee disinfection of the carcass. Incorrect dosage or preparation of disinfectants. 	No	
Chicken carcass cooling	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection, cooling tanks, or contaminated water or ice.</p> <ul style="list-style-type: none"> High variations in the defined temperatures and times (higher than 4°C, measured in the center of the muscle mass); however, during the visit, the verification carried out by the operators on the equipment thermometers was observed. 	No	

Stage	Danger	Risk factor	PCC
Chicken packaging	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of the environment, packaging line, bags, rubber gloves, equipment, baskets, infrastructure, and poor hygiene practices of the operators. This is due to the fact that no hand disinfection was observed during change of activity.</p> <ul style="list-style-type: none"> Contaminated or dirty packaging due to improper storage. Temperature increase in the area (above 12°C). 	No
	Chemicals: Contamination with detergent or disinfectant residues	<ul style="list-style-type: none"> Packaging contaminated by inputs such as disinfectants or detergents due to improper storage. 	No
	Physical: Hair, plastics	<ul style="list-style-type: none"> Fractions or pieces of plastics from packaging. Hair of handlers or operators. 	No
Chicken packaging	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of the environment, packaging line, bags, rubber gloves, equipment, baskets, basket transport cart, infrastructure, and poor hygiene practices of the operators. This is due to the fact that no hand disinfection was observed during change of activity.</p>	No
	Physical: Hair	<ul style="list-style-type: none"> Hair of handlers or operators. 	No
Weighing of chicken	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate disinfection of the environment, scales, gloves, equipment, hands, baskets, infrastructure, and bags and bad hygiene practices of the operators. This is due to the fact that no hand disinfection was observed during change of activity.</p>	No
Loading and transporting chicken	Biological: Contamination by pathogenic microorganisms	<p>Cross-contamination due to deficient or inadequate environmental disinfection, rubber gloves, equipment, baskets,, basket transport cart, infrastructure, and poor hygiene practices of the operators. Also, cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof.</p> <ul style="list-style-type: none"> High variations in defined temperatures and times. In the dispatch area, a temperature above 15°C. 	No

Source: Own elaboration based on the visit to the slaughter plant.

Table 3. Hazards and CCPs in the slaughter stage.

In relation to *physical* hazards, feathers are present at the slaughtering stage and are removed by the plucking machine. Subsequently, at the end of the evisceration line and before pre-cooling, there is a process of visual verification of carcass quality, where any feathers that are still present are removed. Another danger is the cuticles that are removed from the legs and can become cross-contaminated with subsequent processes due to excess cuticles in the area without frequent cleaning of the equipment and the area; however, as mentioned, the carcass is visually inspected before pre-cooling. Fractions or pieces of plastic packaging may be present during the packaging process due to quality defects in the packaging supplied by the supplier; however, this risk is low, since there has been no evidence of such contamination by production plant personnel, and there have been no complaints from customers or consumers. Finally, for physical hazards, there are hairs, which can fall in any of the operations that take place outside the slaughter line tunnel and that have direct contact with food handlers, such as the operations of turning the chicken, cutting the legs of the carcass, hanging the chicken carcass, evisceration, packaging, and packing. However, this risk is low, since they wear a cap that completely covers their hair and ears, reducing this risk to a minimum, especially in the packing process.

Depressing:

Plant location data:

- Altitude: 2625 masl
- Surroundings: Paved streets. It is located 152 m from a gas station. In the surrounding area, there are banks, restaurants, bookstores, transportation companies, distribution companies, cab companies, and clothing stores, among others.
- Ambient temperature: 14°C
- Relative Humidity: 72%.

Figure 4 of the depressurization stage is shown below:

The hazards identified in each of the operations of the depressurization stage are detailed in **Table 4**, as well as the critical control points (CCP) based on the HACCP methodology:

The location of the depressing plant in a cold climate mitigates somewhat the risk of contamination by undesirable microorganisms compared to slaughter plants located in warm climates. Likewise, being located in an urban area, the presence of domestic animals, pests, and flying crawlers is reduced compared to a plant located in a rural area with a warm climate.

Biological hazards during this stage are caused by deficient or inadequate disinfection practices (when chicken or dams are dropped on the floor during all operations), since the dosage of disinfectant in the water (milliliters of disinfectant per liter of water) is not correctly carried out, or the retention times of the disinfectant are not met, and therefore, there is no guarantee that microorganisms will be effectively eliminated. In addition, during the visit, it was observed that a dam fell during the depressurization process and was not immediately collected for disinfection and reincorporation into the process. And in the marinating area, when placing the prey in baskets for later storage, one prey was observed to fall and was rinsed with water but not disinfected.

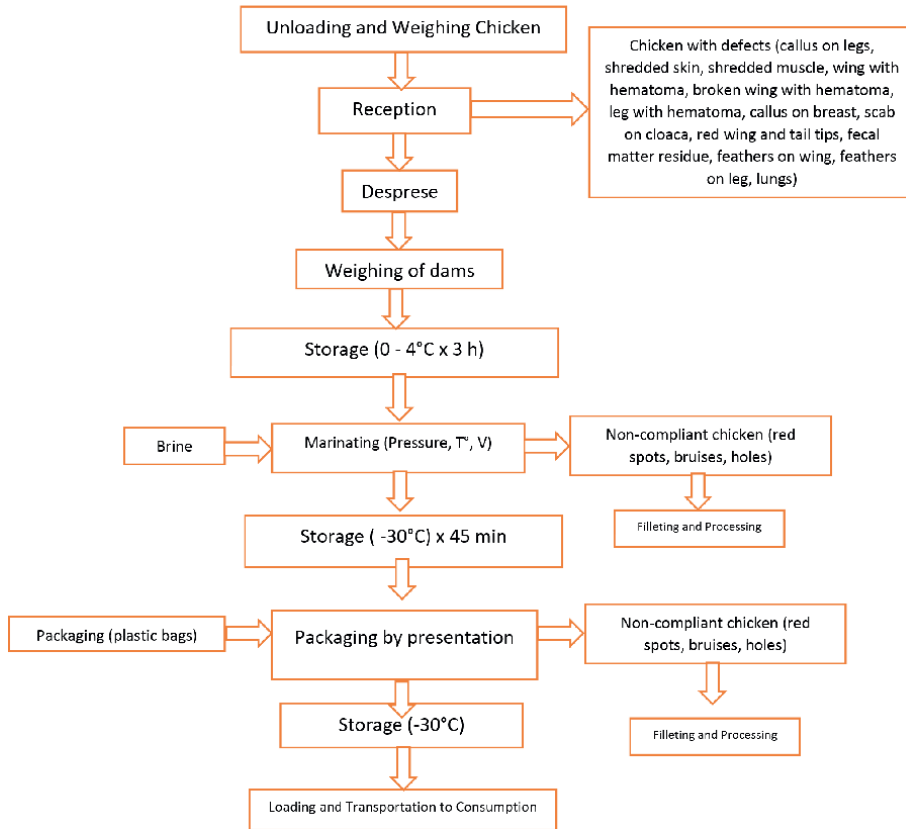


Figure 4. Despresing stage. Source: Own elaboration based on the visit to the depressing plant.

Temperature and time variations in refrigeration and freezing operations are a risk factor for the growth of undesirable microorganisms; however, during the visit, it was observed that the temperature of the cold rooms was monitored by filling out forms, thus demonstrating the control of the risk.

With respect to *chemical* hazards, there is contamination by disinfectants, due to incorrect dosage (addition of more disinfectant than defined), longer retention time, or no rinsing (in cases where this should be done). This type of hazard is observed throughout all the operations of the stage, since any chicken or prey that accidentally falls on the floor must be disinfected and incorporated back into the process. Likewise, in the depressurization and marinating processes, the cutter and marinating equipment has spaces that are difficult to access, making cleaning and disinfection difficult and leaving disinfectant residues on the equipment. There are also chemical hazards during the marinating process, specifically in the dosage used to prepare the brine. This is because it contains phosphates, stabilizers, and emulsifiers and is mixed with water in exact measures.

Finally, brine as raw material and packaging for final product contaminated with detergents or disinfectants may exist; however, this risk is low in the production plant, since there is evidence of storage of packaging in rooms separate from storage of inputs or raw materials and storage of cleaning and disinfection elements, in compliance with GMP.

Stage	Danger	Risk factor	PCC
Unloading of chicken in carcasses	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. Likewise, cross-contamination due to deficient or inadequate environmental disinfection, rubber gloves, equipment, baskets, basket transport cart, infrastructure, and poor hygiene practices of the food handler. This is due to the fact that there are no disinfection process verification techniques with immediate results for taking immediate corrective measures. 	No
Weighing of chicken carcasses	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the environment, scales, gloves, equipment, operators' hands, baskets, infrastructure, bagging, and bad hygiene practices of the food handler.	No
Chicken carcass reception	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> • Contamination by pathogenic microorganisms from slaughter. Cross-contamination due to deficient or inadequate disinfection of the environment, scales, gloves, equipment, hands, baskets, infrastructure, bagging, and bad hygiene practices of the food handler. • Chicken carcass contaminated with fecal matter residues, although in the last month, the control record forms do not show chicken with this defect. 	No
	Physical: Presence of feathers Presence of hair	<ul style="list-style-type: none"> • Deficient plucking during slaughter of the bird. The above, given that during the visit, this defect was observed in one (1) chicken of the sample that was verified. • Inadequate use of the cap by the food handler. 	No
	Chemicals: Excess of disinfectant (when a chicken falls on the floor, it is disinfected).	<ul style="list-style-type: none"> • Incorrect dosage in the preparation of the disinfectant. 	No
	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Cross-contamination due to deficient or inadequate disinfection of the environment, cutter, rubber glove, steel glove, equipment, basket, infrastructure, bag, and bad hygiene practices of the food handler. This is due to the fact that during the visit, it was observed that dams were falling to the floor and were not collected "immediately" for disinfection. • Increase in the temperature of the area (above 12°C) • Temperature increase of the product (above 5°C) 	No
Depressing of chicken carcasses	Chemicals: Excess of disinfectant (when a chicken falls on the floor, it is disinfected). Disinfectant residues.	<ul style="list-style-type: none"> • Incorrect dosage in the preparation of the disinfectant. • Residues of detergents or disinfectants on the cutter. 	No
	Physical: Presence of hair	<ul style="list-style-type: none"> • Inadequate use of the cap by the food handler. 	No

Stage	Danger	Risk factor	PCC
Weighing of dams	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the environment, scales, gloves, equipment, hands, baskets, infrastructure, bagging, and bad hygiene practices of the food handler.	No
Storage cooling of dams	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the cold room environment, walls, ceiling, and floors of the cold room; rubber gloves; equipment; baskets; fan; basket transport cart; and poor hygienic practices of the food handler. • Temperature increase, higher than –2 and 4°C.	No
Marinating of prey	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the environment, mixing tanks, marinator, hands, equipment, baskets, rubber gloves, basket transport cart, infrastructure, and poor hygiene practices of the food handler. During the visit, it was observed that a dam had fallen to the ground, which was only washed but not disinfected.	No
	Chemicals: Excess of brine (salt, phosphates, stabilizer, emulsifier) Contamination of the brine with chemical or cleaning products Excess of disinfectant (when a chicken falls on the floor, it is disinfected) Disinfectant residues	<ul style="list-style-type: none"> • Incorrect dosage in brine preparation. • Inadequate brine storage. • Incorrect dosage in the preparation of the disinfectant. • Residues of detergents or disinfectants in the sealing machine, since this equipment has several areas that are difficult to access and therefore difficult to clean and disinfect. 	No
	Physical: Brine mixing and marinating tank nuts fall out	<ul style="list-style-type: none"> • Incorrect preventive maintenance of the mixing and marinating tanks. 	No
Storage freezing of dams	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the cold room environment, walls, ceiling, and floors of the cold room; rubber gloves; equipment; baskets; fan; basket transport cart; and poor hygiene practices of the food handler. • Temperature increase, higher than –18°C.	No
Packing of dams	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection, table and packing tank disinfection, rubber gloves, equipment, baskets, basket transport cart, infrastructure, and poor hygiene practices of the food handler.	No
	Chemicals: Excess of disinfectant (when a chicken falls on the floor, it is disinfected) Packaging contamination	<ul style="list-style-type: none"> • Incorrect dosage in the preparation of the disinfectant. • Packaging contaminated by inputs such as disinfectants or detergents due to improper storage. 	
	Physical: Plastic bag waste	<ul style="list-style-type: none"> • Poor quality of packaging by the supplier. 	No

Stage	Danger	Risk factor	PCC
Storage freezing of chicken	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the cold room environment, walls, ceiling, and floors of the cold room; rubber gloves; equipment; baskets; fan; basket transport cart; and poor hygienic practices of the food handler. • Temperature increase, higher than -18°C .	No
Loading and transporting of chicken	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate environmental disinfection, rubber gloves, equipment, baskets, basket transport cart, infrastructure, and poor hygiene practices of the food handler. Also, cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. • High variations in defined temperatures and times. In the dispatch area, a temperature above 15°C .	No

Source: Own elaboration based on the visit to the depressing plant.

Table 4.
Hazards and CCPs in the depressing stage.

In relation to *physical* hazards, feathers are present in the depressing stage, which are a verification criterion in the reception of the chicken, and if they exceed the limit established in the sampling, the lot is rejected. Another danger is that fractions or pieces of plastic packaging may be present during the packaging process due to quality defects in the packaging supplied by the supplier; however, this risk is low, since there has been no evidence of such contamination by production plant personnel, and no complaints have been filed by customers or consumers.

Although there is a risk of nuts or elements falling from the marinating equipment and mixing tanks, the risk is low, given that they would not be incorporated into the product since the brine is injected through needles. In addition, at the exit of the sealing machine, the dams are manually placed in the baskets, where they are inspected, and any defects are removed. During the visit, it was found that preventive and corrective maintenance of the equipment is carried out at the frequency indicated by the supplier, and no physical contamination has occurred for the aforementioned reason. Finally, for physical hazards, there are hairs, which can fall in any of the operations that have direct contact with food handlers, such as unloading, weighing, receiving, depressurizing, and packaging operations. However, this risk is low, since they wear a cap that completely covers their hair and ears, reducing this risk to a minimum, especially in the packing process.

In the depressing stage, there are no CCPs, since none of the operations were designed to reduce or eliminate a hazard in the first place, nor can contamination reach unacceptable levels in these operations, given that during slaughter (pre-cooling and disinfection), a disinfection process was carried out on the carcass.

Consumption:

Consumption location data:

- Altitude: 2625 masl
- Surroundings: Paved streets. In its surroundings are located family houses, banks, restaurants, and neighborhood stores, among others.

- Ambient temperature: 14°C average
- Relative Humidity: 72%.

Figure 5 of the consumption stage is shown below:

The hazards identified in each of the operations of the consumption stage are detailed in **Table 5**, as well as the critical control points (CCP) based on the HACCP methodology:

Biological hazards during this stage are presented by deficient or inadequate disinfection practices (performed on utensils, tables, environment, i.e., everything that is in contact with the chicken meat) because the dosage of the disinfectant in the water (milliliters of disinfectant per liter of water) is not correctly carried out, or the retention times of the disinfectant are not complied with, and therefore, there is no guarantee that microorganisms will be effectively eliminated.

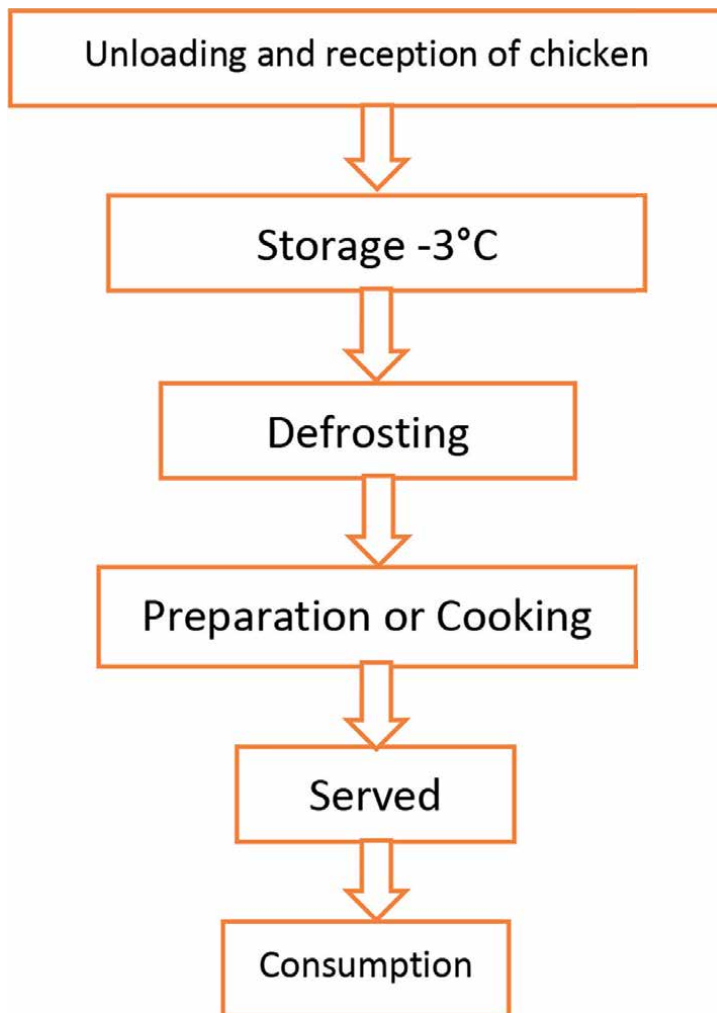


Figure 5. Consumption stage. Source: Own elaboration based on the visit to the consumption site.

Stage	Danger	Risk factor	PCC
Unloading and reception of raw de-boned chicken	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Contaminated chicken from the depressing plant. Cross-contamination due to deficient or inadequate disinfection of the vehicle, pallets, floors, walls, and roof. Likewise, cross-contamination due to deficient or inadequate environmental disinfection, rubber gloves, hands, equipment, baskets, bags, infrastructure, and poor hygiene practices of the food handler. 	No
	Physical: Presence of feathers Presence of hair	<ul style="list-style-type: none"> Deficient verification of the chicken carcass during packing. Inadequate use of the cap by the food handler. 	No
Storage freezing of raw dehydrated chicken	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of refrigerator, rubber gloves, equipment, and poor hygiene practices of the food handler. Cross-contamination by storage with other contaminated products. <ul style="list-style-type: none"> Temperature increase, higher than -4°C. 	No
Defrosting	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Incorrect thawing of the chicken (water at room temperature). Cross-contamination due to deficient or inadequate disinfection of the kitchen environment, rubber gloves, hands, equipment, and bad hygiene practices of the food handler. Temperature increase, higher than 4°C. 	No
Preparation or cooking	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the kitchen environment, pots, utensils, rubber gloves, hands, equipment, knives, and counters and poor hygiene practices of the food handler. <ul style="list-style-type: none"> Cooking of the food below 74°C in the center of the product. 	Yes
	Chemicals: Disinfectant residues	<ul style="list-style-type: none"> Incorrect dosage or preparation of utensil disinfectant. 	No
	Physical: Hair	<ul style="list-style-type: none"> Inadequate use of the cap by the food handler. 	No
Served	Biological: Contamination by pathogenic microorganisms	<ul style="list-style-type: none"> Cross-contamination due to deficient or inadequate disinfection of the dining room environment, cutlery, plates, hands, knives, equipment, and counters and poor hygiene practices of the food handler. Cross-contamination with contaminated raw food. Cross-contamination by utensils used with contaminated raw food.	No
	Chemicals: Disinfectant residues	<ul style="list-style-type: none"> Incorrect dosage or preparation of the disinfectant. 	No
	Physical: Hair	<ul style="list-style-type: none"> Inadequate use of the cap by the food handler. 	No
Consumption	Biological: Contamination by pathogenic microorganisms	Cross-contamination due to deficient or inadequate disinfection of the dining room environment, cutlery, plates, hands, knives, and dining room and poor hygiene practices of the consumer.	No
	Chemicals: Disinfectant residues	<ul style="list-style-type: none"> Incorrect dosage or preparation of the disinfectant. 	No

Source: Own elaboration based on the visit to the consumption site.

Table 5.
 Hazards and CCPs at the consumption stage.

Temperature and time variations in refrigeration and freezing operations are a risk factor for the growth of undesirable microorganisms; however, during the visit, it was observed that the temperature of the refrigerators was monitored by filling out forms, which shows risk control.

Finally, there is the cooking process, where there is a risk of not reaching a temperature of 74°C in the center of the product, and therefore, the elimination of pathogenic microorganisms would not be achieved.

The only CCP identified during the consumption stage is *cooking*, since, although it is not an operation specifically designed to eliminate or reduce a hazard, contamination can reach unacceptable levels, which are not eliminated later, given the immediate consumption of the food.

With respect to *chemical* hazards, there is contamination by disinfectants, due to incorrect dosage (addition of more disinfectant than defined), longer retention time, or no rinsing (in cases where this should be done). This type of hazard is observed throughout all stage operations, when disinfecting refrigerators, tables, utensils, pans, walls, floors, ceilings, cutting boards, knives, plates, spoons, forks, knives, and, in general, all elements that may come into contact with food.

In relation to *physical* hazards at the consumption stage, feathers are present, and if they are found, they are removed from the chicken prey; however, this type of finding is not frequent, according to the food handlers. Another physical hazard is hair, which can fall in any of the operations that have direct contact with food handlers, such as receiving, cooking, and serving operations.

3.2 Definition of corrective and risk control measures

According to the CCPs identified above, as well as what was evidenced during the visits made to the different plants or sites that make up the chicken meat production chain, the corrective and control measures proposed for the CCPs in each of the stages of this poultry company are defined below, starting with the hatchery stage, as shown in **Table 6**.

Table 7 shows the corrective and control measures in the fattening stage.

For the slaughter stage, corrective and control measures are presented in **Table 8**.

Stage	Risk factor	PCC	Control measures	Frequency	Corrective action
Vaccination	<ul style="list-style-type: none"> Incorrect application of the vaccine that does not guarantee the entry of the liquid into the chick's body and therefore is not effective against the disease. <p>Cross-contamination due to deficient or inadequate environmental disinfection; disinfection of hands, equipment, and trays; vaccination line; and poor hygiene practices of the operators. Also, during the visit, some empty bottles of hand sanitizer were observed.</p>	Yes	Verify the correct application of the vaccines by checking the back of the neck of the chick, which should be green, confirming the entry of the liquid into the bird.	Each time vaccination is performed	Pass again through the vaccination line.

Source: Own elaboration based on the visit to the hatchery.

Table 6.
Corrective and control measures at the hatchery stage.

Stage	Danger	Risk factor	PCC	Control measures	Frequency	Corrective action
Intake and fattening in sheds	Biological: Contamination by pathogenic microorganisms or diseases	<ul style="list-style-type: none"> • Cross-contamination by poultry fecal matter: due to deficient or inadequate disinfection (incorrect dosage of disinfectant and/or contact time) of the <i>shed</i> where unloading takes place, staffing of personnel, plastic containers, hands of operators, container transport carts, brooders, drinkers, feeders, chip litter, plastic curtains, and infrastructure, among others. • Presence of pests such as flies that act as a vehicle for transmission. <p>Deficient biosecurity measures and practices: these reduce the possibility of cross-contamination and consist of restricting visits, use of disinfected equipment, and shower baths for all personnel and visitors, among others.</p> <ul style="list-style-type: none"> • Contaminated food or water • Incorrect vaccination that does not guarantee protection against the disease. 	Yes	Verify compliance with biosecurity measures and cleaning and disinfection practices (curtains, feeders, drinkers, equipment, environment, and, in general, everything in contact with the chick) by the operators. Prevent the entry of flies. Use of bacteriophages, acids, or similar with food or drink. Use of potable water as drinking water for birds.	Daily	Change of equipment, new washing, and disinfection of feeding troughs, curtains, drinking fountains, overalls, and boots, among others. Installation of screens in the shed, since there are only curtains that do not fully cover the shed.

Source: Own elaboration based on the visit to the farm.

Table 7.
 Corrective and control measures at the fattening stage.

Table 9 shows the corrective and control measures for the depressurization stage:

As mentioned above, there were no CCPs in the depressurization stage; however, there are control measures in critical stages such as those involving temperature management:

Stage	Risk factor	PCC	Control measures	Frequency	Corrective action
Gutting of chicken carcasses	-Since in this operation, the viscera are removed, there is a risk of contamination with fecal matter from the cloaca and rupture of the intestines and viscera, where <i>Campylobacter</i> is naturally found. Likewise, there is a risk of cross-contamination due to deficient or inadequate disinfection of the environment and everything in contact with the carcass, including operators.	Yes	Avoid contamination of the carcass with fecal matter or viscera contents.	Continuously on the inspection line at the end of evisceration.	When contamination is visually evident, the entire carcass must be disinfected inside and out by immersion in disinfectant solution for the defined time and returned to the line.
Pre-cooling and disinfection of chicken carcasses	-Taking into account that this operation reduces the temperature from 25–10 °C by immersing the carcass in cold water for 15 minutes, <i>Campylobacter</i> cannot grow or multiply. -In this operation, the disinfection of the carcass is also carried out; therefore, there is a risk of incorrect dosage that does not guarantee its effectiveness and therefore the survival of the microorganism. Cross-contamination risks are present due to deficient or inadequate environmental disinfection, cooling tanks, or contaminated water.	Yes	Reduce the carcass temperature to 10°C. Correct dosage of the disinfectant used.	Measure the outlet temperature of the carcass every hour, verifying that it is at a maximum of 10 °C, and measure the temperature and pH of the water. Also, measure the line speed, confirming that the carcass lasts 15 minutes in the pre-cooling process. This is to ensure that <i>Campylobacter</i> is inactivated. Measure residual chlorine concentration.	If the carcass temperature and line speed are not as required, the water inlet temperature or line speed should be adjusted. Should the temperature or time increase, the skin conditions should be evaluated and a decision made whether to continue on the line or withdraw from the line. If the test strip indicates an incorrect dosage, the disinfectant must be mixed into the water again.

Source: Own elaboration based on the visit to the slaughter plant.

Table 8.

Corrective and control measures at the slaughter stage.

Stage	Risk factor	PCC	Control measures	Frequency	Corrective action
Storage cooling of dams	In case of cross-contamination, <i>Campylobacter</i> can survive in food at refrigeration temperatures for 1 to 3 weeks but does not multiply. Cross-contamination due to deficient or inadequate disinfection of the environment and of everything that comes into contact with the chicken carcass.	No	Maintain refrigeration temperature (4°C maximum).	Measure the temperature of the cold rooms every hour, in order to identify any deviations.	In the event that the monitoring report generates deviations, the chicken must be moved to another cold room, and the causes that generated the temperature increase (damage to refrigeration equipment, damage to the door of the room, damage to the thermometer, among others) must be identified, in order to take the necessary corrective or preventive measures.
Storage freezing of dams	Cross-contamination due to deficient or inadequate disinfection of the environment and of everything that comes into contact with the chicken carcass. In case of cross-contamination, freezing does not inactivate <i>Campylobacter jejuni</i> instantly; it can survive from 2 to 5 months at -20°C.	No	Maintain freezing temperature (-18°C maximum).	Measure the temperature of the cold rooms every hour, in order to identify any deviations.	In the event that the monitoring report generates deviations, the chicken must be moved to another cold room, and the causes that generated the temperature increase (damage to refrigeration equipment, damage to the door of the room, damage to the thermometer, among others) must be identified, in order to take the necessary corrective or preventive measures.
Storage freezing of chicken	Cross-contamination due to deficient or inadequate disinfection of the environment and of everything that comes into contact with the chicken carcass. In case of cross-contamination, freezing does not inactivate <i>C. jejuni</i> instantly; it can survive from 2 to 5 months at -20°C.	No	Maintain freezing temperature (-18°C maximum).	Measure the temperature of the cold rooms every hour, in order to identify any deviations.	In the event that the monitoring report generates deviations, the chicken must be moved to another cold room, and the causes that generated the temperature increase (damage to refrigeration equipment, damage to the door of the room, damage to the thermometer, among others) must be identified, in order to take the necessary corrective or preventive measures.

Source: Own elaboration based on the visit to the depressing plant.

Table 9.
 Corrective and control measures at the depressing stage.

Finally, in relation to the consumption stage, corrective and control measures are presented as detailed in **Table 10**.

Stage	Risk factor	PCC	Control measures	Frequency	Corrective action
Preparation or cooking	Cross-contamination due to deficient or inadequate disinfection of the environment and of everything that comes into contact with the poultry in dams during preparation. -If cooking is not done above 74°C, there is a risk of <i>Campylobacter</i> survival. This is because <i>Campylobacter</i> is unable to grow above 45°C.	Yes	Cook the chicken, reaching a temperature of 74°C in the center of the product.	Measure the temperature during cooking until the required temperature is reached.	If any of the measurements do not show compliance with the temperature, cooking should be continued, and the temperature should be measured again.

Source: Own elaboration based on the visit made to the consumption site.

Table 10.
Corrective and control measures at the consumption stage.

¹These control and corrective measures were the result of adjustments made with the advice of an expert on the subject (Andrea Varón^{10F}, Lead Instructor training FSPCA - Foreign Supplier Verification Programs - Train the trainer HACCP); however, to carry out their implementation, they must be validated in the different plants through methods that allow checking their effectiveness, especially those related to the use of disinfectants, where they must be verified through microbiological sampling. Likewise, the economic viability of their implementation must be evaluated by means of a cost–benefit analysis.

4. Conclusions

In the stages of the food chain of this poultry company, it was identified that biological hazards corresponded to contamination by pathogenic microorganisms; physical hazards to contamination by feathers, dirt in the eggs, presence of feathers, cuticles, hair, plastic, nuts, and equipment elements, among others; and chemical hazards to contamination by residues of drugs or antibiotics in the meat, residues of disinfectants and detergents in the equipment, and excess dosage of additives such as stabilizers or emulsifiers in the chicken brine. The identification of these hazards makes it possible to contemplate all the possibilities of contamination risk, in order to subsequently generate control and corrective measures in the processes involved.

CCPs along the food chain correspond to vaccination (incubation and fattening), feeding (fattening), evisceration and disinfection (slaughter), and cooking (consumption). These CCPs make it possible to establish critical limits, control frequencies, and corrective measures to be implemented in case of deviation of the variables.

¹ Manager of the company V&N Solutions, Food Safety Consultant, with 18 years of experience in the industry supporting companies to implement food safety management systems. Lead Instructor in Preventive Controls, trained in Foreign Supplier Verification according to FSMS Law and Train the Trainer HACCP. Technical Advisor for the Chicken Program at FENAVI in sanitary legislation and safety. Trainer in GMP and HACCP for authorities and professionals of the poultry and bovine industry in Colombia, Panama, Ecuador, Dominican Republic.

Based on the above, the critical operations in the entire chicken meat production chain are the pre-cooling of the slaughter stage where disinfection of the carcass is carried out, ensuring that the growth of pathogenic microorganisms is inhibited, and the preparation or cooking of chicken meat at the consumption stage, since microorganisms such as *Campylobacter* are destroyed at a temperature of 60°C for 12 seconds.

Deficient GMP practices were observed in the hatchery and slaughter plant, thus increasing biological risks. Depressurization and consumption are the stages where the best GMP practices were evident. GMPs are part of the prerequisite programs for the implementation of the HACCP system, which is why it is so important to comply with them rigorously.

For all stages in the chicken production chain, it is required that the eggs, chicks, poultry, chicken carcasses, or depressed chicken enter the process free of diseases or pathogens, that is, that they have a quality certificate that guarantees their safety.

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Author details

Angélica Patricia Huertas Moreno^{1*}, Elena Amparo del Rocío Gómez Moreno²,
Gonzálo Téllez Iregui¹ and Andrea Patricia Varón García³


1 Universidad Nacional de Colombia, Bogotá, Colombia

2 Pontificia Universidad Javeriana, Bogotá, Colombia

3 Universidad Jorge Tadeo Lozano, Bogotá, Colombia

*Address all correspondence to: aphuertasm@unal.edu.co

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Principles for Designing Green, Lean, and Smart Microfactories: Chicken as a Model

*Pratap Sriram Sundar, Chandan Chowdhury
and Sagar Kamarthi*

Abstract

Industrial revolutions have gone through four phases: steam, electricity, electronics, and Industry 4.0. Through all these four industrial revolutions, efficiency, productivity, quality, and automation have been greatly improved. However, the manufacturing processes created by humans have had disastrous consequences on the environment leading to a gigantic “climate change” problem. To mitigate climate change, engineers, and manufacturers all over the world have stepped up the research into cradle-to-cradle designs and sustainable manufacturing practices inspired by the designs and value cycles in nature. Bio-inspired designs have been gaining momentum to create products and manufacturing methods that are eco-friendly. All manufacturing (of a fruit, an organism such as a human baby) in nature happens in microfactories such as a womb, a leaf, a flower, or a chicken oviduct whose products are eggs. The product (egg) and the manufacturing process (chicken oviduct) are both green (eco-effective), lean (built with minimal resources), and smart (sensors and Internet of Things). Using a chicken as a model, this book chapter presents a set of metrics for green, lean, and smart attributes, which engineers can use to design products and microfactories.

Keywords: biomimicry, eco-efficiency, eco-effectiveness, lean, IoT, sustainability, cradle-to-cradle, microfactory design

1. Introduction

In the last three centuries, we have experienced four industrial revolutions. At the end of the eighteenth century, the steam engine powered the first revolution. Almost a 100 years later, electricity powered the second one leading to the proliferation of mass production lines. Nearly another 100 years later, the adoption of electronics, IT systems, and robotics sparked the third revolution [1].

The emergence of the fourth industrial revolution, labeled “Industry 4.0” or “I4.0” or 4IR,” was discussed for the first time in public at the Hanover Trade Fair in 2011 [2]. Since then, I4.0 has been revolutionizing industries by embracing the technologies offered by tools such as AI, advanced robotics, and cyber-physical systems.

Through these four industrial revolutions, efficiency, productivity, quality, and automation have greatly improved the delivery of products and services to customers. However, the manufacturing processes created by humans have had disastrous consequences on the environment due to “climate change.” To mitigate climate change, engineers and manufacturers worldwide have stepped up the research into cradle-to-cradle designs and sustainable manufacturing practices. Bio-inspired designs have been gaining momentum to create products and manufacturing methods that are eco-friendly.

Life has been thriving on the earth for 3.5 billion years. In just the past 200 years, starting with the invention of the steam engine, the four industrial revolutions ushered a pattern of destruction to our home called “The Earth.” We can see the dangers of climate change as portrayed by the documentary “Six Degrees” by National Geographic, as massive amounts of greenhouse gases are released into the atmosphere raising the average temperature of the Earth [3]. The other dangers are plastic pollution and loss of biodiversity. One recent book by Bill Gates starts with the chapter “51 billion to zero.” [4] It states,

“There are two numbers you need to know about climate change. The first is 51 billion. The other is zero. Fifty-one billion is how many tons of greenhouse gases the world typically adds to the atmosphere every year. Although the figure may go up or down a bit from year to year, it’s generally increasing. This is where we are today. Zero is what we need to aim for. To stop the warming and avoid the worst effects of climate change—and these effects will be very bad—humans need to stop adding greenhouse gases to the atmosphere.”

The conclusion is very clear: Our current design and manufacturing methods are unsustainable and dangerous to the environment and, therefore, to ourselves ultimately. We need to learn and imitate nature’s design principles and manufacturing methods.

The United Nations Department of Economic and Social Affairs has created a set of 17 Sustainable Development Goals (SDGs) as a blueprint for peace and prosperity in countries [5]. In the last few decades, companies across the world have been attempting to make their factories green, lean, smart, and green. “Green” refers to technologies and practices for sustainability. “Lean” refers to lean product design, lean manufacturing, and lean service. “Smart” refers to leveraging Industry 4.0 technologies. A seminal book “Biomimicry: Innovation Inspired by Nature,” by Janine M. Benyus led to the creation of the Biomimicry Institute [6]. Biomimicry looks to nature for solving design problems in a regenerative way. Biomimicry is about learning from nature and applying that knowledge to design, make and operate products, systems, businesses, and cities that are compatible with the sustenance of the earth. The author proposed nine principles of biomimicry: (1) Nature runs on sunlight, (2) Nature uses only the energy it needs, (3) Nature fits form to function, (4) Nature recycles everything, (5) Nature rewards cooperation, (6) Nature banks on diversity, (7) Nature demands local expertise, (8) Nature curbs excesses from within, and (9) Nature taps the power of limits [7].

Another book “Cradle to Cradle: Remaking the Way We Make Things” by William McDonough and Michael Braungart suggests several strategies to design products and systems that can be used and reused again and again, imitating nature’s circular economy to attain the principles of cradle-to-cradle life cycles [8]. The essential principles of cradle-to-cradle design emphasize a shift from humanity’s “cradle-to-grave”

to nature's "cradle-to-cradle" with a deep understanding of Technical and biological metabolisms. This requires a system that does not create monstrous hybrids such as landfills but plans for efficient separation of technical and biological nutrients and recycles them endlessly, just as nature does.

Similarly, Gregory Unruth, the author of the book "Earth, Inc.: Using Nature's Rules to Build Sustainable Profits," gives five eco-minded rules called "bio-sphere rules" for the sustainable design of products and processes [9]. These five rules are (1) Materials parsimony, (2) Value cycle, (3) Power autonomy, (4) Sustainable product platforms, and (5) Function over form [10]. They aim to create closed-loop business processes. Currently, we see a great interest in learning the principles from nature and applying them in design and manufacturing to realize nature's "cradle-to-cradle" approach to sustainability. In recent times research into bio-inspired design has been gaining momentum [11]. Thousands of new eco-friendly products are designed, developed, and patented [12]. A significant amount of time and resources are spent on nature-inspired biomaterials such as Chitin and Chitosan [13]. Innovations are happening in 3D printing (additive manufacturing) technologies to bring it closer to nature's manufacturing methods in terms of sustainability [14]. There is an urgent need to create a framework to achieve the sustainable development goals (SDGs) proposed by the United Nations Organization (UNO).

Nature produces a variety of products, such as grains, nuts, fruits, vegetables, herbs, wood, eggs, and meat, all in microfactories. A close observation of nature shows that all its products and manufacturing methods are green, lean, and smart. Most of the products of nature are manufactured in microfactories. For example, a plant manufacturing tomatoes, a bird producing eggs, and a womb assembling a baby. To expand on this discussion, we analyze a product of nature, the "egg," and the microfactory of nature, the "chicken" for their green, lean, and smart features.

2. Green, lean and smart product and production system: a framework

Everything that nature creates—for example, a chicken egg—happens in a lights-out factory [15]. Even a human baby is entirely created in the dark factory of the mother's womb. **Figure 1** presents an IDEF (Integration Definition) [16] model of the egg production process. There are four parameters in IDEF representation of a system: Input, output, mechanism, and constraints or controls. The inputs are cereal grains, water, air, minerals and vitamins, and feed additives such as antioxidants and organic minerals. The outputs are eggs, urine, and feces. The mechanism that converts inputs into outputs is the biological body of the chicken. Constraints and controls are the availability of resources such as chicken feed, water, and suitable living conditions.

The feed sustains the female chicken and aids in its growth. A chicken turns a portion of the feed into follicles in its ovary. These tiny follicles travel through the chicken's approximately 70 cm long oviduct. As a follicle travels through the oviduct, many parts of the egg, including membranes, albumen, chalazae, and shell, are added by processes similar to nano and additive manufacturing. The whole process is executed within the oviduct factory. This 70 cm-long microfactory typically produces an egg a day during the breeding season. Depending on the bird species and seasons, the number of eggs per clutch and the frequency of egg delivery vary.

A matrix with five parameters, as shown in **Table 1**, is to study, appreciate, and explore any object in Nature [17]. These five parameters will be used to study the

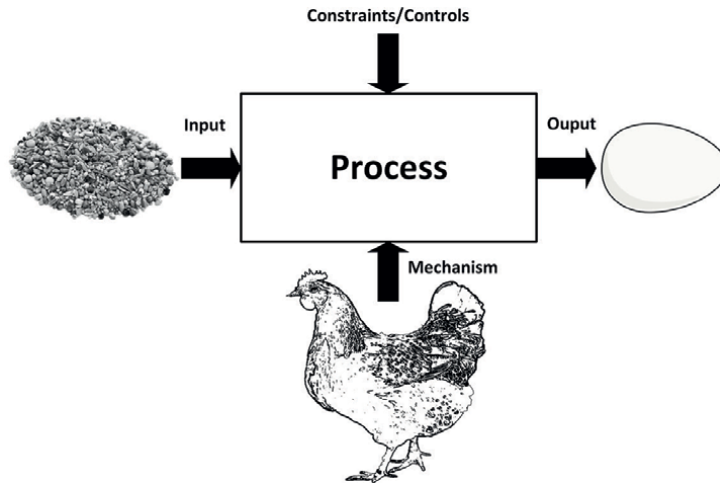


Figure 1.
Chicken input-output IDEF model.

Parameter	Form	Function
Model	Chicken Body. Egg shape (Ovoid).	Produce eggs. Provide a link between one generation and the next.
Metric	Green metrics. Lean metrics. Smart metrics.	Achieve eco-effectiveness. Use minimum materials, labor, and resources. Protect the egg and chicken body.
Mentor	Nature.	Provide insights.

Table 1.
Five parameters.

“chicken egg” and the “chicken body” as a “green, lean, and smart” microfactory to draw insights for the bio-inspired design of future products and factories.

Nature as Model: “Biomimicry, biomimetics, bio-inspired design” is a new science that studies nature as the model to imitate its ways and take inspiration from its designs and processes to find solutions to human needs. For example, taking inspiration from a leaf, scientists and engineers have created solar cells to meet human energy needs.

Nature as a measure (Metric): Nature has learned, through its 3.8 billion years of evolution, what works, what fits, and what lasts. Biomimicry uses ecological standards to benchmark our innovations.

Nature as a mentor: Biomimicry introduces a shift in thinking from “what we can extract from nature” to “what we can learn from nature.” As physicists, chemists, engineers, and biologists explore nature, they are discovering nature’s super-intelligent.

Form: Form is the visible shape or configuration of something. Or it is a particular way in which a thing exists or appears.

Function: The purposes for which a living or non-living thing exists. It implies a definite action or a particular kind of work (**Figure 2**).

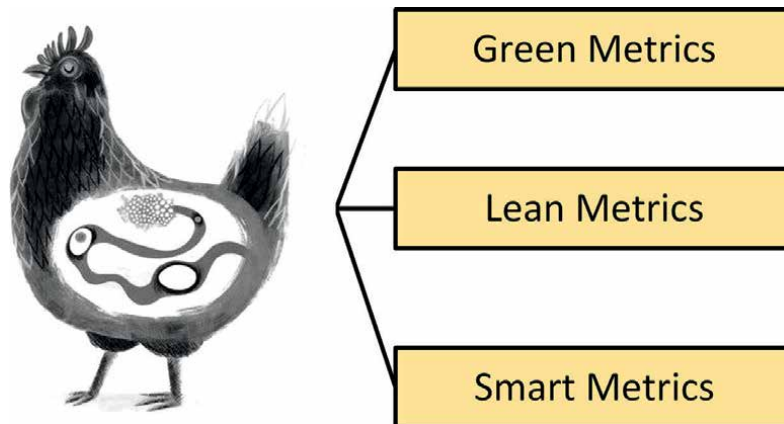


Figure 2.
Three sets of metrics of the egg and the chicken microfactory.

3. Green, lean, and smart product: chicken egg as a model

An egg is a reproductive unit that develops into a new individual like the one that produced the egg. Although very different from other cells of the chicken body, an egg is a single cell. The intelligence and love ensconced in an egg are infinitely mysterious for the following reasons [18]:

1. An egg is a large single cell.
2. An egg survives outside the animal's body, while no other cells can survive outside the body.
3. The egg is nature's remarkable and versatile invention that encapsulates everything necessary to create new life.
4. The egg has carried life from one generation to the next for millions of years.
5. There is no definite answer to the old riddle: "Chicken first or the egg first."

Consider a chicken egg manufactured by nature in the oviduct of a chicken. An egg has various parts, as shown in **Figure 3**, and each part fulfills one or more functions. All components of an egg are essential for its function. As a food source, an egg is a complete powerhouse. All parts of an egg are designed to support life and provide nourishment. With their unique combination of essential vitamins, minerals, fatty acids, and amino acids, it is hard to ignore the health benefits of eggs. All the parts of an egg are organic and upcyclable. Of more than 100 elements, nature chose to use just four—carbon, hydrogen, oxygen, and nitrogen—to produce all living things. These four elements, with the addition of a little sulfur and phosphorus, can account for 99% of the weight of all living things on the planet. The major parts of an egg and its functions are presented below.

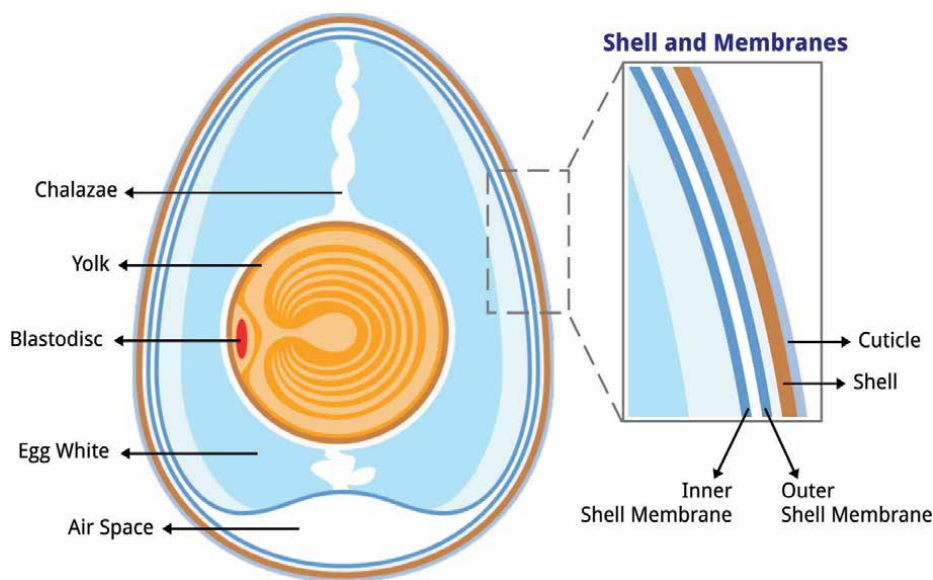


Figure 3.
Chicken egg and its parts.

3.1 Bloom

Description: Bloom, known as the cuticle, is the natural protective coating on the eggshell that seals the eggshell pores. Bloom dries and flakes off.

Functions: Seals off egg pores. Prevent the entry of harmful bacteria and dust into the shell. Reduces moisture loss from the egg.

3.2 Shell

Description: The chicken eggshell is 95–97% calcium carbonate crystals stabilized by a protein matrix; without the protein, the crystal structure would be too brittle to keep its form. The organic matrix is thought to play a role in the deposition of calcium during the mineralization process. The structure and composition of the eggshell formation require enough calcium deposition within hours, which must be supplied via the hen's diet. An eggshell contains between 7000 and 17,000 semipermeable pores. Shells come in an array of colors, from blues and greens to whites, browns, and often including specks.

Functions: The avian eggshell holds the parts of the egg and protects the egg against damage and microbial contamination. At the same time, it prevents desiccation, provides calcium for embryogenesis, and regulates gas and water exchange for the growing embryo.

3.3 Outer egg membrane

Description: The outer membrane is a translucent, film-like gel that nestles immediately next to the eggshell. Is partially made of keratin.

Functions: Outer membranes facilitate the porous activities of eggs. They operate as a bacterial barrier and air molecule vent permitting oxygen, nitrogen, carbon dioxide, and other gaseous particles to flow in and out.

3.4 Inner egg membrane

Description: Inner membrane is the second translucent protein barrier tucked right below the outer membrane. The inner membrane shelters the albumen (egg white). It is partially made of keratin, a fibrous amino acid. It is robust, water-insoluble, and microscopically dense, and acts as a sturdy protective shield.

Functions: This inner egg membrane is the strongest of the egg's protective layers. It blocks bacteria and holds the egg white and other contents together.

3.5 Air cell

Description: Air cell rest opposite the pointed end of an egg, nestled into the more rotund and spacious bottom curve. A freshly laid egg is hot at around 105°F. As the egg cools in the environment, the air cell is formed.

Functions: Air cell stores the oxygen required for a developing embryo. Without this oxygen pocket, a fertilized embryo cannot mature. Air cell assists in maintaining proper internal conditions for the egg. The cascade of chemical interactions that take place between the air cell gases and the rest of the egg's fluids and proteins rely on oxygen transfer for their stability and quality. Air pockets are universal and essential parts of an egg that keep it healthy and whole, with a stable shelf life.

3.6 Albumen

Description: Albumen, known as egg white, is a translucent fluid that makes up over 60% of an egg's interior weight. Albumen is 10% protein and 90% water. Egg white fluid consists of four segmented layers, with each alternating between a thin and thick consistency. This mix of consistencies provides protein-packed egg whites with a robust template that holds over 40 different amino acids. Chalaziferous White is the first and most central layer of the albumen. It rests around an egg yolk, restraining the yolk's movement to the center of the egg. Besides proteins, egg white contains micrograms of calcium, folate, choline, selenium, magnesium, phosphorus, and potassium; it does not contain fats.

Functions: It holds protein-based nutrients and compounds that aid in overall embryo growth if the egg was fertilized. During embryo development, folate and choline support cell growth, DNA replication, and hormone production. At the same time, calcium and magnesium build and activate hundreds of distinct enzymes to regulate blood sugar, blood pressure, nerves, muscles, and bone development.

3.7 Chalazae

Description: Chalazae are the long, stringy, fibrous little squiggles that run through and around an egg's yolk. Chalazae permeate the two ends of the yolk. It is made up of strong fibrous proteins.

Functions: They preserve the structure and safety of the yolk. They operate like yolk scaffolding, or like ropes that anchor the yolk's outer casing, supporting and balancing the yolk's movements.

3.8 Vitelline membrane

Description: This is a protective covering around the yolk. It is made up of two layers—the inner layer 1–3.5 μm thick, and the outer layer 0.3–0.5 μm . Vitelline membranes are made up of glycoproteins and other proteins.

Functions: Vitelline layer protects the yolk from cracking and seeping fluid inside the egg. It keeps the egg's central yolk separate from the albumen. A cracked internal vitelline membrane will destroy the egg. The vitelline membrane is also responsible for protein binding during the fertilization process. Without the signals and receptors held within its inner and outer layers, an egg cannot initiate the development of an embryo. It acts as a gatekeeper for hormones and substances to either pass into the yolk or remains blocked.

3.9 Yolk

Description: Egg yolk contains saturated fat, fatty acids, minerals, and fat-soluble vitamins A, D, E, B6, B12, Iron, Calcium, Phosphorous, Lutein, Zeaxanthin, Choline, and protein.

Functions: The major function of the egg yolk is to provide nutrients for a developing poultry embryo.

3.10 Blastodisc

Description: Blastodisc, also known as a germinal disc, is the embryo-forming portion of an egg with discoidal cleavage usually appearing as a small disc on the upper surface of the yolk mass.

Functions: A fertilized blastodisc (now called the blastoderm) grows and becomes the embryo. As it grows, the embryo feeds on the yolk as a food source.

Part name	Functions (verb + noun)
Bloom	Seal-off egg pores. Prevent entry of harmful bacteria. Prevent entry of dust. Reduce loss of moisture.
Shell	Hold all parts of the egg. Allow gas exchange. Provide calcium (to the developing embryo).
Outer egg membrane	Prevent harmful bacteria. Allow gas exchange.
Inner egg membrane	Hold egg-white and other contents. Block bacteria.
Air cell	Store oxygen. Give long shelf life. Aid in the growth of an embryo into a chick.
Albumen	Supply water and proteins (to the developing embryo).
Chalazae	Prevent yolk movement.
Vitelline membrane	Keep the yolk separated. Bind proteins (during the fertilization process). Allow or prevent hormones (during embryo development).
Yolk	Provide nutrients (to the developing embryo).
Blastodisc	Become embryo.

Table 2.
Summary of functional analysis of an avian egg.

Attribute	Description/specifications
Shape	Ovoid.
Weight	50–70 g.
Design Blueprint	DNA, a few nanometers in size.
Product BOM (Bill-of-materials)	About 15 major parts, about 1000 parts (counting proteins, fats, etc.).
Materials	Calcium, Oxygen, Hydrogen, Nitrogen, Water.
Calorific Value	70 calories (in a 50 g egg).
Composition	A 50 g (1.8 oz) medium/large chicken egg provides approximately 70 calories (290 kJ) of food energy and 6 g of protein. Boiled eggs provide significant amounts of several vitamins and minerals, including vitamin A (19% Daily Value (DV)), vitamin B12 (46% DV), riboflavin (42% DV), and vitamin D (15% DV), choline (60% DV), pantothenic acid (28% DV), zinc (11% DV), and phosphorus (25% DV).
Recyclability, Circular Economy	All parts are organic that are upcycled by nature.
Product recall	No customer complaints or product recalls.
Closed-loop system	The egg, when hatched, turns into a new factory (chicken) that can produce more eggs as per the blueprint (DNA) of the egg.

Table 3.
 Product attributes.

In **Table 2**, we summarize the functions of the parts of an egg. We use the standard notation of writing the function with a verb and a noun. For example, for the part “bloom” the verb is “seal-off” and the noun is “egg pores.” In **Table 3**, the product (egg) attributes are listed.

When we examine “egg” as a product for its green, lean, and smart attributes, the following conclusions emerge.

Green: All the materials that are used to manufacture an egg are organic. There are no toxic materials that might pollute or damage the environment. When an egg is discarded into nature, all the materials decompose by the actions of microbes except the shell which is made of calcium carbonate. Some animals and birds might consume eggshells as a calcium supplement. There is no waste. All the materials are upcycled.

Lean: Egg shape and all the parts are made of a minimum number of materials and labor to fulfill specific functions summarized in **Table 2**. It is evidently a lean design.

Smart: The materials in an egg protect the egg by preventing harmful bacteria at seven different levels in a hierarchical manner [19]. Its design allows good trade-offs between different functions; for example, the pores in the eggshell allow the passage of gas molecules but not liquid material.

4. Green, lean and smart microfactory: chicken body as a model

The oviduct of a chicken is a factory that produces eggs. An oviduct is the hen’s reproductive system. It is a long spiraling tube. There are five major stages in the manufacturing of an egg. These stages and the cycle times of each stage are shown in **Figure 4** [20]. The journey of the chicken egg starts as an egg yolk. First, a follicle or the oocyte (still unfertilized) is made in the ovary, and as it moves through the oviduct by a small distance, it may be fertilized internally (life is created) by a

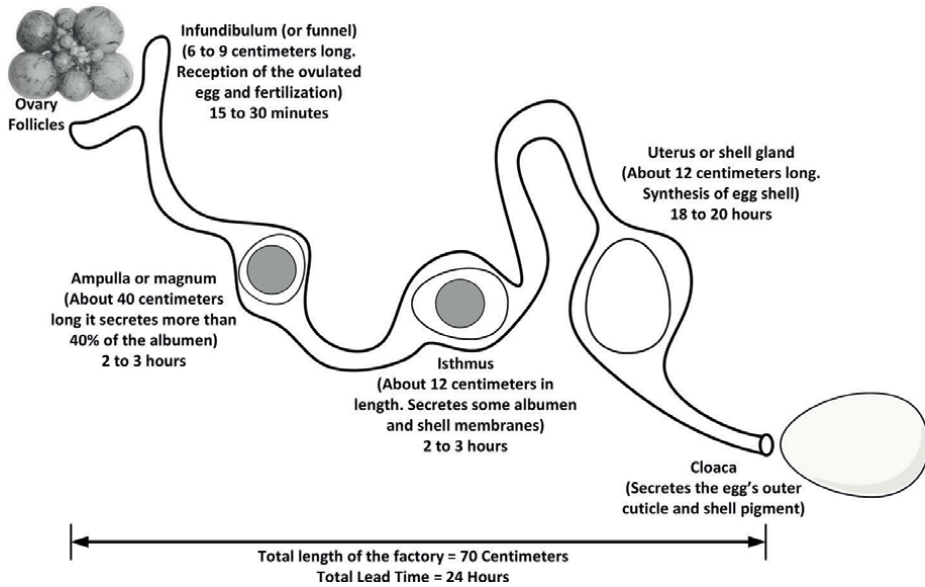


Figure 4. Manufacturing stages of a chicken egg production in oviduct.

sperm (stored inside the hen) and compacted into a spherical shape. Once the yolk attains a predetermined size and shape, its growth stops. This process takes about 30 minutes. The yolk continues down the oviduct (whether it is fertilized or not) and is covered with a membrane (called the vitelline membrane), structural fibers, and layers of albumin (the egg white). This part of the oviduct is called the magnum. As the egg goes down through the oviduct, it is continually rotating within the spiraling tube. This movement twists the structural fibers (called the chalazae to hold the yolk in the center of the egg, against the forces of gravity), which form rope-like strands that anchor the yolk in the thick egg white. This process of two chalazae anchoring each yolk, on opposite ends of the egg takes about another 2 hours. Then, two egg membranes made of keratin, are wrapped around the albumen to keep it in an ovoid shape. Then in the lower part of the oviduct, the synthesis of eggshell takes place, which takes about 20 hours. The shell is made of calcite, a crystalline form of calcium carbonate. Eggshell is not a solid wall, but porous with about 7000 to 17,000 holes. These pores allow the exchange of gases during the development stage. The cloaca secretes the egg's outer cuticle and shell pigment. Then, the egg is ejected out of the hen's body. Eggs are usually laid blunt end first. An air space filled with Oxygen forms when the contents of the egg cool and contract after the egg is laid. The embryo consumes this Oxygen as it grows into a chick during the hatching process.

Material transforms while moving through the tubular factory with minimum energy requirements in the conversion process. The oviduct is like a moving workshop, a silent and lights-out factory, where an egg is manufactured, at the rate of one egg every 24 hours. An egg, when hatched, transforms into a new factory (chicken) that can produce more eggs, with the egg's DNA blueprint. All parts of an egg are fully upcyclable. No part causes any damage to the environment (except the large-scale waste from the industrial poultries). A discarded egg putrefies and decomposes enriching the soil. Other birds and animals eat leftover eggshells to supplement their calcium intake. The chicken body and the "oviduct assembly line" are made of

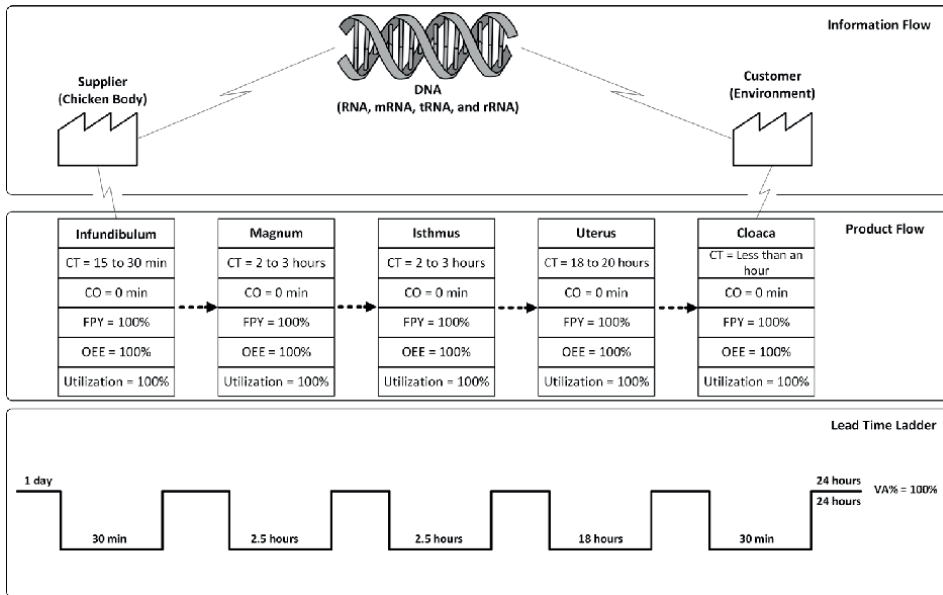


Figure 5. Value stream map (VSM) of a chicken egg production process.

biomaterials that are easily decomposed and upcycled. Considering that both the product (egg) and the manufacturing system (chicken body) are zero-waste systems, they can serve as models of a circular economy.

A Value Stream Map (VSM) of this manufacturing process in the oviduct is presented in **Figure 5** [21]. The inventory of protein, fat, calcium, and amino acids is approximately sufficient for just one day. The inventory in the chicken's body lasts for about a day, which is 24 hours. For the next day, for the next egg, fresh feed must be taken in by the chicken. There is no storage space for, say, many days of inventory. Some chickens might be fattened up, but there is a limit on how much a chicken can eat and store, which cannot be more than the inventory for a couple of days. From the inventory "turns" point of view, if a chicken is laying 400 eggs during the egg-laying period in a year, the number of inventory turns is 400. Because each egg is produced with a one-day worth of inventory, 400 is a very high number, far beyond what has been achieved by any human-built factory. The value addition percentage is also close to 100% which has not been matched even by the best lean manufacturers in the world. The physical, green, lean, smart, and operational attributes of this factory are summarized in **Tables 4–8**.

These characteristics listed in **Tables 4–8** clearly make it evident that the chicken body viewed as a microfactory is a green, lean, and smart manufacturing system. The chicken body is a lights-out factory with no workers, no supervisors, no machines, no tools, and no technology experts. The chicken body is made of environmentally benign biomaterials, and hence, it is green. All materials of a chicken's body are completely upcyclable. Millions of sensors in a chicken's body are connected to a central nervous system. When we apply the concepts of lean, Industry 4.0, or sustainability, we get answers that confirm that the chicken microfactory is extremely lean, highly automated, and 100% green. The lights-out score of this microfactory will be the highest compared to any human-built factory embracing the principles of green, lean, and smart. From the moment the first single-celled organism was born,

Metric/Attribute	Description
Shape	Long, flexible, and spiraling tube with an expandable cross-sectional area, and several bends to reduce overall space.
Factory length	60–70 cm.
Factory weight	2–5 kg (whole chicken).
Factory lifespan	5–10 years
Manufacturing process, tools, accessories, machines, furnaces, containers, etc.	The technology used is nanotechnology which involves bottom-up manufacturing with self-assembly, hierarchy, and massive parallelism; it is something more efficient than the current additive manufacturing (AD), or 3D and 4D printing technologies.

Table 4.
Microfactory physical attributes.

Metric/Attribute	Description
Material choice	Parsimonious biomaterial palette: Calcium, Oxygen, Hydrogen, Nitrogen, Water.
Energy	Renewable energy for the factory (chicken) comes from the grain produced by plants using sunlight.
Pollution	Zero pollution.
Upcyclability of factory (chicken) and product (egg)	100% upcyclable; the entire factory is made of organic materials which become food for animals and trees after the death of a chicken.
Power of platform	Nature uses the “Oviduct” platform for almost all bird species for producing eggs.

Table 5.
Microfactory attributes (green).

Metric/Attribute	Description
Process parameters	No high temperatures, no elevated pressures, or no caustic chemicals
Noise	No noise, no sound (zero decibel level).
Ambient lighting (electricity bill)	No lighting lights-out factory).
Maintenance	Self-repair.
Office staff	No offices or staff.
Material handling equipment	Muscles move the work-in-process (WIP) to the next stage.
Quality assurance staff	Built-in quality assurance with no quality control inspection.
Inventory turns	400 per year (approximately).
Cycle time (CT)	15 minutes to 20 hours.
Value addition (VA)	Close to 100%.
Kanbans	Leptin and Ghrelin hormones.
Changeover times (CO)	Not applicable (a chicken body is a focused factory producing a single product).
Overall equipment effectiveness (OEE)	Close to 100%.
Lead time (LT)	24 hours (for one egg).

Metric/Attribute	Description
First pass yield (FPY)	99.97% (3 Sigma).
Defect rate	Extremely low.
Scrap and rework	No scrap and no rework.
Visibility management (dashboards etc.)	A dark factory where chemicals control the flow of work-in-process (WIP).
Product recall	Close to zero.

Table 6.
Microfactory attributes (lean).

Metric/Attribute	Description
Sensors (Factory 4.0 and Industry 4.0)	Millions of sensors.
Connectivity (IIoT, PLCs, SCADA, etc.)	Central nervous system (CNS) of the chicken body.
Real-time information	Yes (to the chicken's brain).
Automation level	Highly automated.
Autonomy	Autonomous and self-cognitive factory.
Digital maturity score (strategy, operations, technology, culture, customer service, etc.)	100 (on a scale of 0 to 100).
Forecast accuracy	Close to 100%.
Order cycle time	24 hours for one egg.
Fill rate	Close to 100%.
Customer satisfaction	Close to 100%.

Table 7.
Microfactory attributes (smart).

Metric	Chicken microfactory (nature)
Size	Small and compact.
Cost	Very low.
Profit margin	About 30% on chicken meat and eggs.
Portability	Highly portable (one can carry the factory to wherever one wants to).
Scalability	Highly scalable. One can have one chicken or a hundred or even some thousands (in 2019 it is estimated that the leading countries produced 1225 billion eggs accounting for about 157 eggs per person per annum).
Upcyclability	The whole chicken becomes a part of nature as food and nutrients for other life forms after death.

Table 8.
Chicken body microfactory attributes.

about 3.5 billion years ago, nature has been using only green and lean principles in its creation. The key to nature's "lean" processes are nanotechnology and self-assembly which do not require enablers like machines, tools, workers, and supervisors. Nature's

factories are significantly more efficient than the best factories in our industrial world like Toyota, GE, Dell, or Apple. Nature's factories score much higher scores on "green, lean, and smart" metrics than the best modern factories. Similarly, nature's products are designed intelligently with nature-friendly materials, manufactured efficiently with no pollution, and upcycled completely after their useful life. In this sense, nature factories and products are perfectly created for the circular economy.

5. Green, lean, and smart factories

Lean manufacturing and later lean thinking have revolutionized the manufacturing and service sectors. Intellectuals and business leaders such as Frederick Taylor, Henry Ford, Sakichi Toyoda, Kiichiro Toyoda, Taiichi Ohno, Shigeo Shingo, Masaki Imai, Edward Deming, Joseph Juran, Kaoru Ishikawa, and James Womack have contributed to the knowledge of lean. The publications of the International Motor Vehicle Program (IMVP) [22] at MIT, Cambridge, MA, and the Lean Enterprise Institute (LEI) [23] have further promoted the implementation of lean across the globe. As lean thinking continued to spread to every country in the world, leaders have been adapting the tools and principles beyond manufacturing, to supply chain, logistics and distribution, services, retail, healthcare, construction, maintenance, and even government. The first report "Industry 4.0 and the Internet of Things" was published by Hannover Messe in 2013 [24]. With the advent of Factory 4.0 and Industry 4.0 technologies, lean is further fine-tuned to gain more productivity, efficiency, and quality. Hundreds of companies have been implementing Industry 4.0 technologies, and many smart manufacturing hubs are established all over the world. Recent advances in biomaterials and new technologies such as 3D printing and 4D printing [25] have been shifting design and manufacturing closer to a circular economy and bio-inspired manufacturing methods [26, 27].

Millions of products (grains, fruits, vegetables, fibers, eggs, etc.) in nature are manufactured in focused factories. For example, a tomato plant that produces vegetables, or an almond tree that produces nuts, or a bird that produces eggs are focused factories. The basic concepts and characteristics underlying the focused factories are simplicity and repetition that give consistent delivery performance [28]. Chicken body is like a focused factory which produces a single product (egg) at low cost, high quality, with consistent lead times, and with low investment. In this chapter, an attempt is made to look at the chicken oviduct as a model for sustainable design and manufacturing. The preliminary analysis presented in this chapter shows that "chicken microfactory" can serve as a benchmark "green, lean, and smart" metrics for human-built products and manufacturing systems.

6. Principles of microfactory design

Microfactory is a small-to-medium scale, highly automated, and technologically advanced manufacturing setup, which has a wide range of process capabilities [29]. A microfactory either refers to a local capital-lean facility used for the assembly of a complex product or a small manufacturing system (normally automated) for producing small quantities of products. The Mechanical Engineer Laboratory (MEL) of Japan proposed the term "microfactory" in 1990. Currently, microfactory describes the small-to-medium scale, highly automated manufacturers like Arrival Ltd., an

electric vehicle manufacturer headquartered in London, UK. The main advantages of microfactory are saving a substantial amount of space, energy, materials, time, and upfront capital costs [30]. Many companies are establishing microfactories leveraging new technologies. For example, Local Motors is a pioneer in establishing a microfactory for automotive production. In 2010, the company established its first microfactory for the commercial production of Rally Fighter cars in Phoenix, Arizona [31]. Microfactories have been built in many sectors, including automotive, apparel, consumer goods, food and beverage, electronics, and electronic waste recycling.

Microfactories are small high-tech manufacturing units located close to customers. These can even function as retail outlets for customized products. In the garment industry, some of the microfactories are producing clothes customized for the users. For instance, customers can send their preferred designs using the manufacturer's app and can receive a perfectly styled and fitted dress the next day from the manufacturer. The following are the benefits of microfactories:

- Capital costs are less.
- Distribution systems are less costly and more efficient.
- Mass customization is economically feasible.
- Investment risk is low.
- Breakeven volumes are low.
- Profit margins are high.

The supply chain complexity also gets simplified with microfactories responding to a pull market: only after getting confirmed orders from the customer, are the products manufactured. The following principles can be used in designing products and microfactories.

Products:

1. Use biomaterials.
2. Avoid toxic, non-renewable, non-recyclable materials.
3. Minimize the variety of materials.
4. Minimize part count.
5. Use generative design and 3D printing technologies.
6. Design for eco-effectiveness.
7. Use Internet-of-Things (IoT) to maximize the useful life of the products.
8. Design for disassembly, recycling, and upcycling.
9. Provide a product passport for tracking and recovery.

Manufacturing processes:

1. Design for eco-efficiency.
2. Use renewable energy to run the manufacturing processes.
3. Leverage digital technologies.
4. Use technologies such as product configurators, augmented reality, virtual reality, mixed reality, and Cobots.
5. Use the Industrial Internet of Things (IIoT), digital twins, and related cyber technologies.
6. Minimize transportation.
7. Locate microfactories close to the customer.
8. Leverage the technologies for mass customization.
9. Design for dismantling and reusing the materials and machines of microfactories.

Divergent, a company located in California, is a good example of a microfactory. It developed its own Divergent Adaptive Production System (DAPS) which is a complete software-hardware solution designed to replace traditional vehicle manufacturing. It is a complete modular digital factory for complex structures [32]. Given a set of digital requirements as input, the machine automatically engineers, additively manufactures and assembles any complex structure. The system can move seamlessly between manufacturing different vehicle models. To achieve the objectives of the circular economy, the World Economic Forum (WEF) has launched the circular car initiative [33]. The term “circular car” refers to a hypothetical vehicle with maximum material efficiency. This notional vehicle is expected to produce zero materials waste and zero pollution during the manufacturing process, product usage, and disposal. Many organizations have been exploring similar approaches toward a circular economy. For example, the production of rechargeable batteries, in their journey from mine to electric vehicles, poses significant social and environmental risks. The Battery Passport is created as a digital representation of a battery that conveys information about all applicable environmental, social, and governance (ESG) and lifecycle requirements based on a comprehensive definition of a circular battery [34].

7. Conclusions

In creating products and production systems, nature has been using design blueprints embedded in DNA, nano-biomaterials, nanomanufacturing, and self-assembly processes. The industrial revolutions in the past 200 years have thrown nature into disarray. Copying and imitating nature’s designs and processes can lead to green, lean, and smart products and production systems. For example, in all flowers, fruits’ beauty, function, and non-toxic decomposition coexist in their designs. In search of a solution to a problem, an important question to ask is, “WWND—What

Would Nature Do?” Keen observation and analysis of nature can lead to creative and sustainable innovations [35]. The solution to the industry’s attempts to solve complex sustainability issues is to look at nature. In this chapter, we examined a chicken egg and chicken body from the green, lean, and smart lens to present a framework that designers of products and production systems can use for learning and benchmarking human-designed products and human-built factories.

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Author details

Pratap Sriram Sundar^{1,2*}, Chandan Chowdhury^{3,4} and Sagar Kamarthi⁵

1 MIGM and PLIIM, Indian School of Business, Mohali, India

2 Operations Management, Indian School of Business, Mohali, India


3 Operations Management and Information Systems, Indian School of Business, Gachibowli, Hyderabad, TS, India

4 MIGM and PLIIM, Indian School of Business, Gachibowli, Hyderabad, TS, India

5 Data Analytics Engineering, Northeastern University, Boston, MA, USA

*Address all correspondence to: pratap_sundar@isb.edu

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Workable Alternatives to Conventional Inputs in Poultry Farming

Solomon Ajide, Ibiyemi Opowoye, John Makinde, Zainab Bello, Maryam Bot, Afiniki Ahmadu and Moji Adeniran

Abstract

The world's demand for poultry products is increasing at an alarming rate, therefore the need for innovations to mitigate the required inputs for sustaining this demand. The challenges of poultry farming attributed to their health, and nutritional requirement is vital to successful poultry production either at a subsistence or at a commercial level. Feed accounts for about 70–80% of the overall production costs. Meeting the nutritional requirements of poultry impacts positively on their health performance. Adequate feeding enables the birds attain table weight earlier than their counterpart that are poorly fed, which could be profitable or not as it depends on the costs of inputs in each production cycle. The essence of poultry farming to an investor is to make profit; however, should poultry farmers continuously make profit, they need to apply workable alternatives suitable for the conventional inputs such as protein, energy sources, plant extracts for orthodox medication, and brooding materials.

Keywords: poultry farming, alternatives, replacement levels, feed, profitability

1. Introduction

The world's population is likely to be 9.9 billion in the year 2050, which is 26.9% of the current population [1]. Agriculture generally plays a crucial role in enhancing food security and reducing poverty compared to other sectors of the economy [2]. It is regarded as unarguably important for economic growth and development, especially in developing economies [3]. Agriculture has been a vital component in the development of many countries in Europe such as Holland, England, and France [4]. The fundamental aspect of the livestock sector is poultry, and it is quite complex. There are many categories in the production cycle involving the breeder farm, hatchery, feed mill, and meat processing industry [5, 6] opined that the poultry industry has grown tremendously over the past five decades with a huge demand for its product. The poultry aspect of livestock production seems to have experienced the fastest growth and development in the livestock industry.

Poultry production is gaining tremendous awareness in less-developed countries as a result of the gap it bridges in protein supply and boosting socioeconomic growth and development in many countries [7]. The cheapest source of protein is from poultry meat and eggs that are easily accessed many impoverished people in Sub-Saharan Africa and South Asia [8]. In Kenya, 71% of the eggs, and meat produced and consumed is from the indigenous chicken, and this has a positive influence on the lifestyle and enhancing food security of those involved in subsistence farming [9]. In developing countries, livestock production constitutes 25–30% of the agricultural gross domestic product of less-developed countries and may increase by another 25% in about 20 years' time. The Nigerian poultry industry contributes about 25% of the agricultural domestic products, thereby ranking Nigeria the highest producer of eggs in Africa but fourth in broiler production [10]. The poultry industry has the potential of meeting the supply of animal protein in the short time basis [11]. The major constraint to the expansion of the poultry sector is feed, which accounts for about 70% of the cost of production [12].

Preston [13] reported that the limiting factor to researchers in the tropics is inability to proffer novel feed resources for monogastric animals. The development of cheap alternative feeding materials is currently receiving immense attention by entrepreneurs, nutrition scientists, and other researchers through evaluation of novel or unconventional feed resources such as agro-industrial by-products. This approach is to reduce the cost of production and competition among animals, industries, and humans for conventional foods/feeds without affecting the dietary and reproductive performance of the livestock.

Subtherapeutic use of antibiotics is used in the broiler industry to improve the growth performance indices and also reduce mortality [14]. However, there is an embargo by the European Union on the use of antibiotics as a result of the residues in poultry products and the increased bacteria antibiotic resistance [15], therefore the need for alternatives to improve poultry growth performance. The ancient use of some natural products has been gaining more acceptance [16]. The use of wood shavings/saw dust is not limited to the poultry industry, and there are other factories that use them as raw materials; in the same manner, the chemical contaminants from the used wood shavings and saw dust has necessitated the need for poultry farms to proffer alternative litter materials for commercial poultry production. The potential alternatives that could replace wood shavings without compromising the availability, cost, and ability to absorb and adsorb moisture include corn cob, straws, peanut hulls, rice hulls, newspapers, and gypsum [17].

2. Nonconventional feedstuff

The rivalry between man and livestock, especially the monogastric for the conventional feedstuff, has led to the need of proffering alternative feed resources by using novel feedstuff that can complement the conventional feeds competed for by humans [18]. The essence of poultry production is to convert feeds not edible by man or excess feed resources into table eggs and chicken meat. The anticipated surplus of feed ingredients during the harvest period for poultry production could barely be sufficient for the increasing human population in Nigeria, thereby aggravating the competition between the populace and the poultry industry necessitating the need for inclusion of novel feedstuff in poultry feeding [19, 20].

2.1 Suitable levels for replacing conventional feedstuff in poultry

2.1.1 Evaluation of selected agro-industrial waste wheat offal, maize offal, and rice bran in poultry diet

The use of agro by-products in feeding chickens varies in the growth performance of the birds mainly as a result of the feed quality, varieties, period of storage, and the atmospheric condition. Many authors had earlier reported the safety of agro by-products in the diet of broilers in the tropics [21, 22]. Makinde and Inuwa [23] reported the growth performance of grower's turkey fed agro by-products each at 15% involving wheat offal, rice bran, and maize offal. The growth indices (feed intake, weight gain, and feed conversion ratio) and carcass characteristics were similar with their counterpart fed the control diet. In conclusion, they affirmed that the dietary inclusion of 15% wheat offal has no detrimental effect on the growth, carcass, and health of the birds.

2.1.2 The use of graded levels of cassava peels in poultry

A lot of literature has shown that cassava peels can replace maize in poultry rations without any marked adverse effect on the performance of the birds. Although cassava has some anti-nutritional factors mainly cyanogenic glycoside, which could limit the use in poultry diet; however, different processing methods have being proffered such as soaking and sun drying, which has enhanced the use in poultry [24, 25]. Since the adoption of alternative feeds for livestock would likely meet such a requirement, cassava peel is found useful considering its large supply. According to Ogunwole et al. [26], dietary inclusion of cassava grits obtained from TME 419 and TMS 01/1371 varieties of cassava did not affect the growth performance of the broiler chickens. In the study conducted by Nwangwu and Ogah [27] on the effect of cassava peel meal on the hematological parameters of cockerels, the authors reported that dietary inclusion of 20–30% of cassava peel meal is the optimum level required to maintain homeostasis with a highly packed cell volume and hemoglobin levels in the blood. The higher hematological values recorded in the cassava peel meal-based diets with respect to the packed cell volume and hemoglobin values reflect a good physiological status of the birds.

2.1.3 The inclusion level of cassava grits in poultry

One of the potential alternative feedstuffs is cassava grits, and there seems to be paucity of information using cassava grits in layer's diet. Cassava grits are one of the by-products of cassava during the production of flour with a considerable energy content as its consumption is less competitive in view of cassava flour and maize as a suitable replacement for maize. Tewe [28] had earlier reported the use of cassava grits and chips as alternative sources of energy in poultry. Ajide et al. [29] reported the optimum level in which cassava grits could be used to replace maize in the diet of laying hens at 0, 20, 40, and 60% without affecting the production performance. The proximate composition of cassava grits was found to be high in energy and low in protein making it a suitable novel feed for replacing maize as an energy source in the diet of laying hens. The ether extract, ash, crude fiber, and nitrogen-free extract contents recorded in the test diet were 5.87, 2.76, 4.61, and adequate for meeting the nutrient requirement for laying hens as presented in **Table 1**. **Table 2** presents the production performance of the laying hens. The average daily feed intake of the

Dry matter	88.18
Crude protein	4.96
Ether extract	5.87
Ash	2.76
Crude fiber	4.61
Nitrogen-free extract	69.98

Table 1.
Proximate composition of cassava grits.

Parameters,%	TRE				SEM	P value
	0	20	40	60		
FI, g/bird/day	116.68	113.38	110.94	113.68	2.23	0.3995
FCR	1.47	1.73	1.60	1.62	0.14	0.6528
Egg prod., %	79.36	67.06	69.11	71.43	5.13	0.4035
Egg weight, g	57.13	62.62	60.72	57.69	1.60	0.4955

SEM = Standard error of mean; (P > 0.05); ADFI = Feed intake; FCR = Feed conversion ratio; Egg prod. = Egg production.

Table 2.
Effect of replacing maize with cassava grits on the performance of laying hens.

laying hens across the dietary treatments was not significantly ($p > 0.05$) affected by the introduction of cassava grits when the birds attained the age of 55–57 weeks. The same trend was observed in the feed conversion ratio, egg production, and egg weight. The pattern in the egg production revealed that the birds on the control diet recorded higher values but not statistically significant in relation to their counterpart fed the dietary cassava grits. The in-consequential effect of the cassava grits following the introduction in the diet of the hens from 20 to 60% implies that it may not impact negatively on the monetary returns of the farmers and could be leveraged upon during the off-season.

2.1.4 The use of different varieties of sorghum, millet, and residues for maize in poultry

Bulus et al. [30] reported the use of two different varieties of guinea corn and millets for replacing maize completely on the growth performance and nutrient retention in broiler chickens. Five dietary treatments were formulated involving the control, white guinea corn, yellow guinea corn, pearl millet, and finger millet as treatments 1, 2, 3, 4, and 5. The crude protein in the diets were 23.5%, 21.5% having a metabolizable energy of 2,800, 2,900 respectively at the starter and finisher phases. The birds on diet 4 (pearl) and 5 (finger) millets had higher final live weight, average daily weight gain in both phases. The best feed conversion ratio and cost per kilogram live weight were recorded in diet 4. The feed intake was higher in the birds fed the yellow guinea corn at the starter phase. The growth indices were generally lower in Diet 2 (white guinea corn) in comparison with other dietary treatments. The authors concluded that the use of millet and yellow guinea corn can be successfully used in replacing maize in the diet of broilers without affecting the growth performance parameters and nutrient retention.

Igwebuike et al. [31] evaluated the effect of replacing maize with spent sorghum grain on the performance of broiler finisher chickens. The growth indices revealed that the final live weight, average daily weight gain, feed intake, and feed conversion ratio were similar in the spent sorghum diet compared with the maize-fed control diet. The feed cost per kilogram was cheaper in the diet compounded with spent sorghum grain. It was concluded by the author that the profit margin in broilers fed spent sorghum grain was higher than the maize-fed control diet. Diarra et al. [32] substituted the use of wheat bran for millet bran, the authors concluded that the birds receiving the diet formulated with millet bran in place of wheat offal were not affected, and feed cost per kilogram was also cheaper.

2.1.5 The use of sesame seed meal as protein source in poultry

Sesame seed meal is a by-product of sesame after its oil extraction. Although sesame seed meal is lower in lysine, isoleucine, leucine, and valine when compared with soya bean meal, it has substantial levels of the sulfur-containing amino acids, especially methionine. Previous studies revealed that sesame seed meal can be used as substitute for corn and soya bean meal when synthetic methionine is included in the diet. It was reported that sesame seed meal can make up 10–12% of broiler diet without any side effect on the growth performance of the birds [33]. The crude protein, ether extract, soluble carbohydrate, and ash contents in the sesame seed were reported to contain 18–25%, 44–58%, 13.5%, and 5%, respectively [34]. Similarly, Olaiya and Makinde [22] conducted a study to determine the growth performance and carcass characteristics of broiler chickens fed different methods of processing sesame seed. In the experiment, five diets were compounded, diet 1 was the control, and the remaining diets 2, 3, 4, and 5 were processed by sun drying, roasting, boiling, and soaking. Each processing method was included in the diet at 15%, respectively. The final body weight and average weight gain were significantly influenced by the treatments. Birds fed the control, roasted, boiled, and soaked diets showed better feed utilization compared to birds fed the sun-dried diet. The average daily feed intake was significantly ($P < 0.05$) higher among birds fed soaked diet. The study concluded that birds fed diets containing 15% roasted and soaked sesame seed meal compared favorably with birds fed the control diet in terms of growth performance.

2.2 Common plant extracts and additives used for orthodox drugs in poultry production

The use of plants with medicinal properties is receiving global attention with respect to livestock production and human health due to the resulting resistances from the use of antibiotics in both humans and animals. Some of these microbes/bacteria have developed resistances posing a potential risk to the welfare of man and livestock [35]. Several phytobiotics have proven to be useful for improving growth, nutrient absorption, gut integrity, and immunity [36–38]. Presently, the use of herbs is not limited to humans alone but finding acceptance in many poultry farms. Low-income or subsistence farmers prefer the use of herbal medicines *vis a viz* the use of orthodox drugs in poultry farming, which are very expensive [39]. The call for restraint in the use of antibiotics for therapeutic and preventive measures against disease pathogens in poultry have necessitated the need for the use of alternatives that could serve the same purpose as it were in the application of antibiotics [40]. Some plants and their extracts have secondary metabolites that are useful in enhancing the

performance of the birds. The increasing cases of antibiotic resistances in livestock production are attributed to the low sensitivity of these disease parasites from the use of these conventional drugs [41].

2.2.1 *The use of herbal medicines for the treatment and control of gastrointestinal parasites*

The various herbs used in the control and treatment of gastrointestinal parasites has its foundation in ethnoveterinary medicine found relevant till date in different parts of the world [42]. Garlic, onions, and mint are found useful in treating animals or birds infected with gastrointestinal parasites. The leaves, flowers, and oil of a shrub (*Chenopodium ambrosioides*) with its origin in Central America are used as an anthelmintic [43].

2.2.2 *Plant and extracts used for alleviating Coccidiosis in poultry*

Some secondary metabolites in some plants and parts, such as the roots, bark, seeds, leaves, and stems containing alkaloids, tannins, terpenoids, saponins, and flavonoids, have therapeutic effects against coccidiosis. Coccidiosis is capable of wiping out a flock completely if not alleviated timely. Some strains of *Eimeria* spp. have developed resistances and insensitive to some orthodox coccidiostats and consequently leave their residues in the animal products. The inclusion of flaxseed either whole or the oil in the diet of day old chicks was found to decrease lesions associated with *Eimeria tenella*. There is a particular plant in India known as *Holorrhena antidysenterica* (kurchi) and it is antiprotozoal. Its extract is mixed with that of other plants such as *allium* spp and *berberis* making it a good coccidiostat [44–47]. Alicin, the main constituent of garlic, was extracted and was found to inhibit the sporulation of *Eimeria tenella* in an *in vitro* study [48–52]. The extract of green tea (*Camelia sinensis*) effectively constrains the sporulation of coccidial oocysts. The selenium and polyphenolic content in green tea was reported to deactivate the enzymes that enhance the sporulation of coccidial oocysts [51, 52]. The leaves of *Carica papaya* (pawpaw) also hinder coccidial oocysts [53, 54].

2.2.3 *Antibiotics and alternatives in poultry production*

Antibiotics are synthetic or natural compounds that are usually administered orally, topically, or parentally in humans and animals for the control and treatment of diseases [55]. The use of antimicrobial agents is dated back to the 1950s [56]. The administration in medicine has been found to be very impactful [57]. The prophylactic and therapeutic effects of antibiotics improve growth in livestock production [58]. There are some antibiotics that act as growth promoters; they are applied at low subtherapeutic levels to decrease or control the population of bacteria in livestock [59]. Clostridium, salmonella, and mycoplasma bacteria cause huge losses, which affect the profit margin in poultry business [56, 60, 61].

Researchers are poised to proffer credible alternatives to the use of antibiotics in poultry production with the aim of reducing or eliminating the residues in animal products and consequently its effect on human health [62–64]. The alternatives to be used in place of antibiotics should not be toxic to the animals, easily eliminated from the body and biologically available to the animals. The public health as well must not be at risk and environmentally friendly [64, 65]. There is an array of possible alternatives to the use of antibiotics in livestock production, and they include probiotics,

enzymes, phytogetic feed additives, and bacteriophages [66–68]. Phytobiotics are compounds extracted from the plant that improve the growth and performance of the animals. It is a useful alternative to antibiotics [66, 69]. The constituents in the phytobiotics are made of active organic compounds that could prevent or limit antibiotic resistances [70].

Certain secondary metabolites, such as polyphenols and polypeptides, are produced during the plant's metabolism; they have antimicrobial agents and exhibit immunomodulatory activities making them suitable phytobiotics that are used as feed supplements in poultry production [71, 72]. Phytobiotics promote growth, reduce stress in the chickens, and boost their immune system [73]. They also aid the activities of the intestinal microbiota, improve the uptake of nutrients, and prevent subclinical infections in poultry [74, 75].

2.2.4 *The use of essential oil as an alternative to antibiotics*

There are plants identified as important antibiotic growth promoters in poultry production [69]. Some herbs, spices, and essential oils have bioactive compounds and the method of processing engaged determines their effectiveness [76]. There are different parts of the plants that are used for the extraction of essential oils. Essential oils are natural, aromatic, and volatile oily fluids produced from the plants [77]. The combination of essential oils with other plant extracts from lemon grass, *Oreganum aetheroleum*, *Oregano* and thyme, carvacrol and thymol, garlic and *Oregano* oil, cinnamaldehyde and thymol, *Allium sativum*, *Echinacea purpurea*, and *Ocimum basilicum* oil is effective against parasites and bacteria. They have antioxidant properties and are used as growth promoters. Some essential oils exhibit antimicrobial activities against gram-positive and gram-negative bacteria, yeast, and mold as presented in **Table 1** in the comprehensive review on essential oils as green alternatives to antibiotics [78].

2.3 Alternative litter materials

There is need for considering other useable bedding materials for commercial poultry production in view of the high demand for wood shavings and saw dust as raw materials in some industries. The credible alternatives for consideration are those that have the ability to absorb moisture, relatively cheaper, and readily available. Maize cobs, rice hulls, and peanut hulls have been found to be useful in bridging the gap except for newspapers that is usually recycled but could be used where available [79].

2.3.1 *The use of rice hull as litter material*

Rice hull is produced from paddy rice during processing. It constitutes about 25% of the rice paddy [80, 81]. Rice hull is a complete waste as the disposal is usually a challenge in rice milling farms. This has necessitated its use for consideration as an alternative litter material for wood shavings and saw dust [82, 83]. The inclusion of rice hulls in poultry feed is limited because of the high silica and lignin content making it available for use as bedding material [84].

2.3.2 *The use of corn cob as litter material*

Corn is rated the highest cereal crop produced globally with a value of about 875 million tons annually [85]. Maize cob is a residue produced during the processing

of corn grains. The by-product accounts for about 200 kg per ton of grains threshed [86]. The use of corn cob is limited for use as building material and activated carbon [87, 88]. Maize cobs constitute more environmental problems in areas where corn is produced commercially [89]. The cellulose and hemicellulose content in maize cob is high except the lignin content that is low [90]. The ability for corn cob to absorb moisture makes it a high good bedding material for use in poultry [91, 92].

2.3.3 The use of shredded newspapers as litter material

Newspaper is found to be useful as litter material, and despite the availability of soft copies, there is still a substantial availability of hard copies. In areas, where not recycled, it is a good source of bedding material in poultry [93, 94]. The decomposition of paper is fast following its ability to absorb moisture [95]. The use of newspaper as litter material is not associated with any health impediment as it is free from dust, disease pathogens, and contaminants. Lien et al. [96] reported the need for newspapers to be processed into chips or smaller pieces in order to improve its ability to hold moisture and enhance evaporation.

2.3.4 The use of peanut hulls as litter material

The world's peanut produced was estimated to be 40 million tons as at the year 2015 [97]. The projected peanut hulls are 10 million tons amounting to 25% of the world's production having variable quantities of threshed kernels [98, 99]. In countries, where peanut is cultivated commercially, the hulls are usually discarded and allowed to decompose thereby making it suitable for different purposes [100].

3. Conclusion

In countries, where there is competition between man and livestock for conventional feed, especially poultry, certain practices could be engaged to enable the farmers make optimum profit due to the escalating prices of the feedstuff. Since the first 4 weeks or starter phase is critical in broiler production, farmers can feed *ad libitum* using quality conventional feed ingredients for feeding the birds to boost their growth. As the birds attain the finishing phase, the major feedstuff, especially the energy source, can be replaced with proven novel feedstuff that is readily available provided the phytochemicals, or secondary metabolites are treated below the levels they can exert detrimental effect on the performance of the animals such as soaking and sun drying for cassava peels. The use of protein feedstuff for birds at the finishing phase in broilers or even in layers is lower compared to energy source. Therefore, sesame seed cake to a level of 10–12% will not compromise the quality of the feed. For the laying hens, 20–60% cassava grits can conveniently be used in replacing maize in the diet of layers in the plateau or declining phase without affecting the performance of the birds. Residues from sorghum can equally serve as an energy source in poultry. Agro-industrial by-products, such as wheat offal, corn bran, and rice offal, could be included in the diet of broiler turkey to a level of 15% in the feed formulation. Some plant extracts having antiparasitic agents, such as onion, garlic, mint, *C. papaya* leaves, and flax seed, contain therapeutic effects against coccidiosis in poultry. In the same manner, some essential oils from *Oreganum aetheroleum*, mixture of *Oregano* and thyme, garlic and oregano, cinnamaldehyde and thymol, carvacrol and thymol,

and Lemon grass (*Cymbopogon citratus*) would serve as antibiotics in poultry production, thereby reducing the consequences of antibiotic resistance among humans. Litter materials, such as corn cob, rice hull, shredded newspapers, and peanut hulls, will serve as an alternative to wood shavings. The practice of these workable alternatives to feed, drugs, and litter materials will bring about a considerable reduction in the overall cost to poultry farmers and also promote healthy meat and eggs for consumption.

Author details

Solomon Ajide^{1*}, Ibiyemi Opowoye², John Makinde³, Zainab Bello⁴, Maryam Bot⁵, Afiniki Ahmadu⁶ and Moji Adeniran¹

1 Department of Animal Science, Landmark University, OmuAran, Nigeria

2 Institute of Food Security Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Nigeria

3 Department of Animal Science, Federal University, Gashua, Nigeria

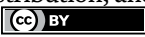
4 Department of Animal Production, University of Ilorin, Ilorin, Nigeria

5 Federal College of Animal Health and Production Technology, Vom, Nigeria

6 Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

*Address all correspondence to: ajidesolomon@gmail.com

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