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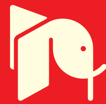
# Poultry
## An Advanced Learning

*Edited by Asghar Ali Kamboh*



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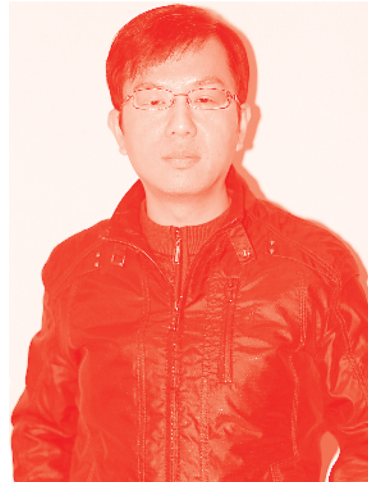

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Poultry - An Advanced Learning
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Edited by Asghar Ali Kamboh

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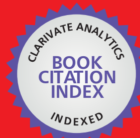


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# Meet the editor

Dr. Asghar Ali Kamboh received his PhD from Nanjing Agricultural University, China, in 2012. He has been working as an associate professor in the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University, Tandojam, Pakistan, since 2006. Currently, his research interests are antimicrobial resistance, microbial contamination of animal foods, plant antioxidants, bacterial pathogens of animal origin, and modulation of immunity and gut microbiota via dietary manipulation in poultry. He has published more than 80 articles in peer-reviewed journals and is an editor/editorial board member of many scholarly journals.

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# Preface

*Poultry - An Advanced Learning* is a unique compilation as it contains all advanced aspects of poultry production, i.e., from housing design/management to production parameters like production-improving strategies (from the use of traditional herbs to advanced biotechnological tools) and data analysis as well. Thus, this book will be of interest to poultry students, nutritionists, researchers, academicians, farm managers, statisticians, farm engineers/architectures, poultry producers, etc.

In the first chapter, Ayodeji Oloyo (Nigeria) and Adedamola Ojerinde (UK) jointly elaborate the housing designs of various types of poultry farms, particularly those that effectively mitigate the deleterious effects of summer stress. The authors have defined all architectural elements, including building orientation, roof slope, roof overhang, landscape, building height, building width, building length, etc. to design a naturally ventilated building for optimum poultry production. Moreover, the use of a tunnel and inlet ventilation system has also been described to sustain improved poultry production in extreme weather conditions.

In the second chapter, Emre Tekce and his colleagues from Bayburt University, Turkey, stress the importance of herbal extracts in poultry nutrition. The authors report the supplemental effects of essential oil mixture of various herbs on the fatty acid profile of broiler meat reared under heat stress. Herbal extracts significantly affect saturated fatty acids, including C14:0, C16:0, and C18:0; monounsaturated fatty acids, including C16:1, C17:1, and C18:1; and polyunsaturated fatty acids, including C18:2n-6c, C18:2n-6t, and C20:1n9 in the breast meat of broiler chickens.

The third chapter is written by Birendra Mishra and his team from the University of Hawaii, USA, on the involvement of hormones, genes/proteins, and their interaction for egg formation in the oviduct of laying hens. There are several genes (e.g., OVAL, TF, OVM, LYZ, COL10A1, etc.), hormones (e.g., FSH, LH, estrogen, etc.), and biological pathways (e.g., calcium signaling pathway, pantothenate and coenzyme biosynthesis, etc.) that trigger histomorphological and biochemical changes in the segments of the oviduct for egg formation. Estrogen regulates folliculogenesis, accumulation of yolk in the follicles, ovulation, and development of oviducts, while progesterone induces the ovulation of yolk from the ovary and development of oviductal glands.

Zhuanjian Li and his colleagues from Henan Agricultural University, China, wrote the fourth chapter of this book on the applications of advanced genomics in poultry production with more emphasis on the use of long noncoding RNAs (lncRNAs). lncRNAs have an important role in the regulation of gene expression at the transcriptional level, thus playing a vital role in many life processes, such as cell differentiation and proliferation, growth and development, organogenesis and tumorigenesis. The regulatory mechanisms of lncRNAs for muscle development, lipid metabolism, and immune modulation in poultry are well documented. Evidence has exhibited that lncRNAs could be a potential application for disease resistance and to improve sperm and egg production in birds.

Finally, yet importantly, the fifth chapter of this book is written by Akinlolu A. Olosunde from Obafemi Awolowo University, Nigeria, on the statistical analysis of poultry data. In this chapter, the author presents generalized exponential power distribution as an alternative to normal distribution commonly used in the analysis of agricultural data. Application of the probability density function is demonstrated in fitting poultry feed data. Moreover, the goodness-of-fit test is elaborated to show that it is a better substitute to normal distribution in applications.

Lastly, I would like to say thanks to all my supporters and well-wishers and my family members as well. Especially, I acknowledge the efforts of Dr. Liu for his review in the early stages of book production. I am also grateful to all authors for their excellent contributions that collectively appear in the form of this book. Last but not least, appreciations also go to the editorial staff of IntechOpen, particularly those related to this book project.

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Chapter 1

# Poultry Housing and Management

*Ayodeji Oloyo and Adedamola Ojerinde*

## Abstract

Majority of the people in the poorest regions of the tropics rely on poultry production as their major source of protein supply. However, poultry production is hindered by the harsh environmental conditions in this regions therefore, reducing the daily supply of protein. It is believed that understanding heat stress in birds by paying detail attention to the sources of heat generation in a poultry house can help manage the heat stress situation in this region. This text reviews the internal climatic conditions of the poultry houses, how the birds respond to them, and their implications for heat management in poultry production. Thus, it provides pertinent information for guidance on parameters for open poultry houses architectural design that ensures optimum climatic conditions that will alleviate heat stress problem in poultry production in hot and humid climate.

**Keywords:** heat stress, poultry house design, poultry production

## 1. Introduction

Poultry production has occupied a leading role in the agriculture industry worldwide in recent years. The compound annual growth rate of poultry protein between 2015 and 2025 is estimated to be +2.4% [1]. Asia, South America and Africa characterized by rapid urbanization, poverty and hot climate recorded the highest growth increment in poultry production [1, 2]. The trend of continuous growth of poultry production in those regions is obvious because it remains the fastest route to bridging the protein demand-supply gap.

Extreme weather conditions in the tropical regions of the world have proven generally detrimental to livestock production and is particularly of interest in chicken because of the latter's high sensitivity to temperature change [3–6]. Just like mammals, the avian species have the ability to regulate their body temperatures by losing or generating heat in response to environmental temperature. If the body temperature of a bird, which normally runs between 39.4 and 40°C, is allowed to increase, the bird will not perform well. Heat stress in poultry production had resulted in under-nutrition, stunted growth, reduction in egg production and size, laying of premature eggs and even death [7–11]. This problem is further compounded by the high body heat generated by genetically improved laying birds with increased metabolic activity resulting from the high rate of egg production [12–15].

Poultry housing design plays a vital role in the determination of the internal climatic conditions of the house for optimum health, growth and productive performance of the birds. Consequently, the type of poultry housing system employed by the proposed poultry farm is a function of the prevailing climatic conditions of the region where the farm is located. While open poultry house system has been adjudged a good method of housing in the tropical countries because of the

simplicity of its construction, ease of heat management and minimal management cost, the controlled housing system is the most common in the temperate regions of the world [16, 17].

## 2. Poultry birds and their thermoregulatory mechanism

Birds are warm-blooded 'homoeothermic' flighty feathered oviparous vertebrates that possess a high metabolic rate, with a normal breathing rate of 40–50 breaths per minute [17]. On the average, birds maintain an internal body temperature of between 39 and 42.2°C [18–20]. During hot weather, poultry birds maintain thermo-neutral temperature by losing heat mainly through conduction, convection, radiation and evaporative cooling [2, 20, 21].

Sensible heat loss through convection, radiation and conduction is only effective if the environmental temperature is below or within the bird's thermoneutral zone. However, evaporative cooling accounts for about 60% of the heat dissipated during body temperature regulation within the thermoneutral zone [2]. Sensible heat loss includes heat loss through opened surfaces such as wattles, shanks and other featherless areas around the neck and wings [21]. Heat loss for body temperature regulation through this process does not alter the bird's behavioral patterns, feed intake, or metabolism [22]. The effectiveness of sensible heat lost is a function of the temperature difference between the bird and its environment.

Where the environmental temperature exceeds 24°C evaporative cooling (latent heat loss) becomes the major method of dissipating heat in birds regardless the age [21]. Loss of heat through evaporative cooling at temperatures beyond the thermoneutral zone requires the bird to re-direct energy required for growth and development to panting. However, panting can lead to dehydration and respiratory alkalosis because of inadequate water supply and drop in blood pH due to excessive ejection of carbon dioxide [21]. During panting, evaporative cooling occurs when water evaporates from the respiratory system of the bird. However, this can be hindered by high humidity. This is problematic in high humid environments where poultry farmers employ evaporation cooling as the primary method of air-temperature reduction during the hot periods of the year [2].

Increasing the volume and velocity of air moving over birds enhances heat loss in birds due to convection, removal of heat trapped within the poultry house, and reduction of the effect of high humidity on evaporative cooling [2]. Simmons et al. [23], conducted a study that subjected 3 weeks old male broilers in a controlled environment for 4 weeks to a cyclic temperature of 25–30–25°C at varying wind speed of still air (<0.25 m/s), 2 m/s and 3 m/s. It was observed that the increased wind speed favored older birds in growth and development.

Water is an essential commodity in poultry production for the nutrients it possesses and its impact on feed consumption [24]. Nipple drinkers to provide cleaner water, reduce water spillage and labour for drinker cleaning has replaced the conventional open water system. May et al. [25], observed that chicken consumed more water when reared with conventional open water system in an experiment that compared the conventional open water system to nipple drinking. However, when these drinkers were used to rear chicken in a controlled room with air velocity of 0.25 and 2.1 m/s, birds in the higher air velocity with nipple drinkers did not differ from those on open water drinkers, but experienced increased weight gain and better feed conversion than birds at the lower air velocity [26]. Therefore, it is important to provide and maintain the required ventilation to ensure that the poultry house is conducive for the birds to regulate their body temperature by sensible heat loss.

## 3. Heat stress in chicken

Heat Stress is a general problem in the poultry industry, especially in the production of chicken meat and egg. Heat stress is experienced by chicken when the environmental temperature equals or rises above 26.7°C. At this temperature and beyond the birds begin to pant and can be detrimental to attaining the bird's optimum growth rate, hatching ability, egg size, egg shell quality and egg production. The problem of heat stress can be further compounded in a hot environment when the humidity rises. Heat stress has been reported to have adverse effect on broilers comfort, growth rate, feed conversion, and live weight gain [21].

In poultry production, the sudden exposure of birds to high temperature short periods is referred to as acute heat stress while exposure for extended periods is referred to as chronic stress. Chronic stress has deleterious effects on birds reared in open-sided houses, which is commonly used in the tropics. It has been reported to have adverse effect on growth and production efficiency, egg quality, meat quality, embryonic development, reproductive performance, immunity and disease incidence's in broilers, laying hens and breeders [21, 27–31].

## 4. Effects of internal climate conditions on chicken

It is important we understand the effect of internal climatic conditions of the poultry house on the birds, how the birds respond to them, and their implications on heat management for poultry production. The information will provide guidance on parameters for the open poultry house architectural design that will alleviate heat stress to ensure optimum poultry production in the tropics. The climatic factors of interest include temperature, relative humidity, air composition and velocity, and lighting condition.

### 4.1 Temperature

There is a huge debate on the ideal temperature range required for the various classes and age groups of chicken to attain optimum production. This could be because of other climatic factors such as humidity and wind velocity, which influence temperature change and previous adaptation of chicken to climatic change. Generally, chicken perform under a wide range of temperature regardless of its class (broiler, pullet or breeder) or age. However, exposure of chicken to high temperature has been reported to hinder the performance in chicken production [17]. It could also be further compounded by increased relative humidity for its negative effect on evaporative cooling [32].

Ketelaars [16] recommended a temperature of 30–32°C at chicken height for day old chicks. Thereafter, the temperature should be decreased by 3–4°C till the chicks are 4 weeks old as shown in **Table 1**. Daghir [19] reported that a temperature range

| Age of chicken (week) | Temperature range (°C) |
|---|---|
| 1 | 30–32 |
| 2 | 30–26 |
| 3 | 26–23 |
| 4 | 23–20 |
| ≥5 | 20 |

**Table 1.**
*Recommended temperature schedule.*

of 18–22°C is required for growing broilers. In other reviews done by Holik [15], it was concluded that birds are comfortable when environmental temperature is within the range of 18–24°C. However, it should be noted that the optimum performance of chicken is dependent on the market value of the product in relation to feeding cost.

It is a challenge to maintain the optimum production temperature in the tropics therefore, it is important that the poultry house designer pay considerable attention to temperature change.

### 4.2 Relative humidity

In a review done by Oloyo [17], it was reported that internal temperature above 26.7°C combined with high relative humidity adversely affected the feed efficiency, feathering, pigmentation, and weight gain of chicken. Furthermore, at internal temperature range of 35–37.8°C the birds' performances were poor regardless of the change in relative humidity. This means higher humidity can improve the performance of the birds at lower temperature. However, humidity must be controlled for it can provide habitat for microorganisms thus, exposing the birds to the threat of disease [18, 33].

Relative humidity has a strong relationship with temperature change. At the brooding stage, particularly in the earlier weeks the internal relative humidity may be low or too low because of the warming the chicken requires at that age or when the chicks are thirsty or hatched at higher temperature. Soon enough, the internal relative humidity increases because of the water vapor generated by the evaporative cooling act of chicken to regulate their body temperature as they grow [16]. Consequently, ages 3 weeks and above are very critical periods in chicken production regardless the class of chicken.

In Oloyo [17], it was reported that laying birds during brooding and after brooding require a relative humidity range of 60–80 and 50–70% respectively for optimum performance.

### 4.3 Air composition

The decomposition of bird's fecal material produces unpleasant and polluted gases, which include ammonia, carbon dioxide, methane and hydrogen sulphide. These gases are of particular interest because of their adverse effects on the performance of birds, cages, human poultry houses and the environment at large [16, 18, 34–38]. Consequently, for optimum production for chicken a concentration level of 25 ppm and not more than 2500 ppm for ammonia and carbon dioxide was recommended [18, 39]. It was recommended for good birds' health management that removal of fecal material from the poultry house should be done frequently to reduce the volume of gas emission [17].

### 4.4 Air velocity

High internal temperature can be controlled to an extent by varying the air velocity within the poultry house. Also, Air velocity plays an important role in convectional cooling and the regulation of air quality [2, 18]. In hot climatic regions, it is recommended that the ventilation capacity should be at least "5m$^3$ per chicken per hour, with inlets amounting to 1.5cm$^2$ per m$^3$ ventilation" [16]. Hulzebosch [18] reported that still air velocity (0.1–0.2 m/s) could be maintained if the temperature remains within 25–30°C. However, Lacy and Czarick [40], under the same temperature condition reported a better growth rate at 2 and 3 m/s air velocity respectively for broilers.

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In the quest to further understand the effect of air velocity on chicken, [23], factored the ages of chicken within the temperature range 25–30°C with varying air velocity. The study demonstrated that 6 weeks old broilers benefited from increased air velocity of 2 and 3 m/s than 4 weeks old broilers. This could be because of the high temperature required by younger birds at brooding stage.

### 4.5 Lighting

Lighting at early age in birds have little or no effect on hormonal system, it merely aids birds' activeness including feed intake, growth, and physical and physiological activities [15, 41, 42]. Subsequently, increase in lighting periods and light intensity may cause tiredness, cannibalism, immune responses, leg abnormalities and even death [41, 43–47].

The lighting program commonly used is the continuous lighting program of 16 hours light and 8 hours darkness and it has proven successful for overall chicken performance [15, 48–50]. However, it has been reported that alternating short light and dark period known as intermittent lighting enhances chicken performance [16, 51–54]. The continuous lighting program with a minimum light intensity of 20 lux is recommended at post-hatch stage (1–7 days old) to help the chick adapt to their environment and aid feeding [41]. Consequently, the light intensity is reduced to about 3–5 lux and intermittent lighting system is introduced for easy control of the birds' activeness for better performance and productivity [16, 41].

Birds reared under yellow, green, and blue light sources have been reported to have improved body weight compared to those reared under red and orange light sources [55–57]. Lewis and Morris [55] in a review concluded that the birds reared under blue light show docile trait while those reared under red light were more active and aggressive. In addition, it was noted that the red light improved sexual activities in birds.

## 5. Poultry housing system

The importance of the type of poultry housing system employed for chicken production cannot be over emphasized. It protects the birds from the harsh environmental climatic conditions, which may have adverse effect on the chickens' performance and productivity. In a poultry house, the overall heat generated is the sum of heat generated by the birds, the surrounding environment and biodegradation of fecal material [58–60]. Thus, the type of housing system to be used is a major determinant factor in the type of management to be adopted in the poultry farm. The housing systems used in the tropical region that is, naturally ventilated open housing system and mechanically ventilated open housing system are discussed here.

### 5.1 Naturally ventilated open housing system

The open poultry housing system has been identified with the tropical region for its simplicity, economic implications and ease of management of heat generation within the building through natural ventilation [2, 32, 61]. However, it is prone to the invasion of insect, rodents, birds and other small predators that can disturb the welfare, productivity and performance of chicken. In the quest to alleviate this problem, dwarf sidewalls are raised to the roof eaves with corrugated wire mesh to keep predators away. Also, gutter filled with insecticides to prevent the invasion of insects are built around the house. Discussed below are design considerations to be factored in when designing an open poultry house for optimum poultry performance and productivity.

*5.1.1 Building orientation*

In order to reduce the exposure of sidewall to direct to direct sun radiation the poultry house should be orientated in the east-west direction [2, 60]. This is very vital, because heat stress in birds can be hastened when they are exposed to direct solar radiation. Deep litter rearing may allow the birds avoid direct sunlight but this may lead to clustering or overcrowding of birds in an area of the house. Consequently, make cooling difficult and in severe cases this leads to stampede and even death [2].

*5.1.2 House width, length and height*

The east-west orientation of a poultry house may reduce the benefit of prevailing winds blowing from east or west. Therefore, Daghir [2] recommended that the width of the building should not exceed 12 m to prevent this problem. In addition, the problem of uneven air exchange rate and temperature within the building is eradicated.

Furthermore, the design must factor in the activities and services rendered by poultry farmers and professionals within the building. These activities may include transfer of chicken, feeding, de-pecking, waste management, vaccination, and so on. Therefore, longer pen house could be strenuous to maintain especially when the activities are carried out manually. Doors can be placed at interval of 15–30 m to make for easy circulation and service delivery [2]. Qureshi [32] recommended that for battery cages, it is rather advisable to factor in the number of tiers to be used. Two–tier cage system facilitates easy air exchange within the building whereas, three and four tier cage system can be problematic for air exchange. Therefore, it is recommend that rows of cages should not exceed three with center aisles not less than 1.2 m and a minimum height difference of 1 m from the ceiling.

*5.1.3 Roof slope*

A roof slope of 45° was recommended because the angle reduces the heat gain of the roof from the direct solar radiation; maximizes the distance of the bird from the heat accumulated under the roof; quick escape of the heat accumulated under the roof through ridge opening, maximizes air space to improve air exchange rate; and open space above for installation of equipment [2, 60, 62]. On the other hand, the slope in the insulated roof is dependent on the quality of the insulation.

*5.1.4 Roof overhang*

Roof overhang can be used to shade the sidewalls of a building from direct and indirect solar radiation. However, the length of the roof overhang is dependent on the height of the sidewalls [2]. Heat gain by the sidewall can be reduced to about 30% by roof overhang shading if properly applied at a roof slope of 45° [60].

*5.1.5 Ridge opening*

Naturally, hot air rises above cooler air due to difference in air density. Introduction of ridge opening can aid ventilation through stack effect in the poultry house. Adequate setback between buildings is required to prevent inadequate airflow and circulation [2, 61]. However, ridge opening has been reported to be ineffective in insulated poultry houses because of temperature uniformity within the house [63].

#### *5.1.6 Sidewall openings*

The sidewall consists of a dwarf wall built up to the roof eave with a permeable membrane such as a corrugated wire mesh and an adjustable curtain. A minimum height of 0.4 m is recommended to prevent the house from water seepage, direct and indirect solar radiation, pests and predators [2]. The corrugated wire mesh allows easy airflow within and outside the building, while the adjustable curtain is used to control the flow and air velocity. However, the curtain may be transparent or of varying colors to aid its use in managing intermittent lighting scheme [2, 15, 63].

#### *5.1.7 Building obstruction*

Adequate setback between buildings is required to prevent inadequate air exchange rates in building. Factors such as wind speed, wind direction and topography are major determinants for consideration in defining the optimal house spacing. However, the spacing between buildings can be determined by the expression below [63].

$$D = 0.4HL^{0.5} \tag{1}$$

where D, housing spacing (ridge of the closest wall of the next house); H, height of the adjacent building; L, length of the adjacent building.

Vegetation should be kept as minimal as possible and at average height to reduce the nest of wild birds and invasion of rodents and other predators. Also, the branch of trees should be kept at eaves level to prevent obstruction of airflow across the house [2].

#### *5.1.8 Roof, end-wall and sidewall insulation*

Farmers in the tropics have successfully used locally sourced materials such as thatched roof and bamboo as roofing materials for the construction of naturally ventilated poultry houses [32]. However, a minimum R-value of 1.25 $m^2$ C/W was recommended for ceiling insulation in naturally ventilated poultry house. Environmental temperature higher than 40°C would require a minimum R-value of 2.25 $m^2$ C/W [2]. The various methods of insulating poultry house ceiling include dropped ceiling, rigid board insulation, spray polyurethane insulation and reflective insulation [2].

#### *5.1.9 Cooling system*

Rooftop sprinklers have proven to be efficient for substantially cooling the roof [2, 60]. However, material of choice in this situation must be able to withstand the constant exposure to water [2]. Evaporative cooling in birds in hot weather can be subdued by using fogging system. With high water pressure it generates mist, which aids cooling in birds. However, the level of humidity within the house must be monitored for it could be detrimental to the health of birds at high temperature [2, 60]. Circulation fan eases heat stress by providing increased air velocity to increase convection cooling. Generally, circulation fans generate air velocity of 0.5 m/s or more and cover an area 15 times its horizontal diameter by five times its vertical diameter [2]. Furthermore, for effective use of circulation fans it should be installed at the center 1–1.5 m above the floor and tilted downward at an angle $5^{o}$.

#### *5.1.10 Vegetation*

Shrubs and grasses reduce reflective and direct solar radiation by shading and convection cooling [60]. Vegetation should be kept clean and trimmed to keep away

predators and pests [2]. The planting of tall trees along the sidewalls can provide a form of canopy to shade the sidewalls from exposure to direct or reflective solar radiation during the hot periods of the day.

## 5.2 Mechanically ventilated open housing system

The limitation of attaining adequate internal environmental conditions required for optimum birds' performance under extreme weather conditions has led to the use of the mechanically ventilated housing system. Also, the mechanically ventilated house provides more control over air exchange, wind velocity and wind direction [2, 16]. Mechanically ventilated system entails the use of either positive or negative pressure system. The negative-pressure system which is the most commonly used in mechanical ventilated house, expels air out of the building by fans through an air inlet system to create low pressure within the house to allow fresh air to rush in through the same air inlet system [2].

The negative-pressure systems can be achieved through inlet or tunnel ventilation. Inlet ventilation system uniformly distributes exhaust fans and air inlets across the house whereas, tunnel ventilation exhaust fans are located at one end and inlet pipes at the other end. This provides the tunnel ventilation with an advantage of greater air speed in turn creating more positive air exchange [2].

### *5.2.1 House construction*

For proper ventilation control, it is required that the house be highly insulated and tightly constructed [2]. However, the sidewall can be equipped with insulated adjustable curtains instead of solid wall for use in the cooler periods of the year or incase of power failure emergency. It is important to note that solid wall have higher insulation value that adjustable curtains.

### *5.2.2 Air exchange*

High external temperature coupled with the heat generated from the activities within the poultry house increases the temperature of the internal air. An effective mechanical ventilation system has to exchange the air quickly to ensure the internal air temperature maintains not more than 2.8°C difference from the external air temperature. The expression below can be used to calculate the appropriate exhaust fan required for effective ventilation [2].

$$\text{Building surface heat (watts)} = (A/R) \times (T_o - T_i) \tag{2}$$

where, A, area of the building surface ($m^2$); R, insulation value of the wall material ($m^2C/W$); $T_o$, temperature outside (°C); $T_i$, temperature inside (°C).

The value of $T_o$ is the hottest external temperature that is excepted of the external environment. However, when calculating heat gain for roof in a house with attic space, the value $T_o$ it is assumed to be 55°C whereas the $T_o$ value for ceilings with insulation directly below the roof is assumed to be 65°C [2]. On the other hand, $T_i$ is best assumed as 27°C to ensure comfort for birds. The value of R will be the overall sum of the insulation value of the wall section.

The total heat produced (sensible and latent) in commercial broiler is 7.9 W/kg while broiler, pullets and broiler breeders is 5.1 W/kg [2, 64]. The heat generated by birds is expressed below [2].

$$\text{Bird heat (W)} = \text{sensible heat (W/kg)} \times \text{weight of the bird (kg)} \quad (3)$$

where sensible heat, 50% of the total heat produced by birds.

$$\text{Total heat (W)} = \text{building surface heat (W)} + \text{bird heat (W)} \quad (4)$$

However, the air movement capacity to maintain 2.8°C between intake and exhaust air is expressed below.

$$\text{Air capacity } (m^3/h) = \text{total heat (W)} \times 3.4/2.8^{\circ}\text{C} \quad (5)$$

*5.2.3. Air inlet system*

There are a number of negative-pressure air inlet pipes used to control the internal climatic condition by controlling the entry location, speed and direction of fresh air. However, the exhaust fan determines how much air enters the house.

*5.2.3.1 Inlet speed*

The pressure difference between the internal and external environment determines the entry speed of fresh air [2]. However, the pressure is a function of the number and sizes of the air inlets. Therefore, the easy manipulation of differential pressure allows for possible control of airflow pattern within the building and of negative-pressure air inlet pipes used to control the internal climatic condition.

*5.2.3.2 Inlet area*

For easy control and distribution of air within the poultry house, the exhaust fan must develop a static pressure of about 12–25 Pa [2].

*5.2.3.3 Air inlet control*

Air inlet design should be located strategically as the direction of air depends on external climatic condition, age and class of the chicken. Normally, air inlets should be designed to direct air towards the ceiling at cooler time while another should be directed towards the floor during the hot periods of the year [2].

*5.2.3.4 Air inlet control*

Static pressure of about 12–25 Pa was recommended for easy control of the air inlet for a static pressure above or below that range can lead to supply of insufficient air velocity [2].

*5.2.4 Types of inlet ventilation system*

*5.2.4.1 Cross ventilation*

The exhaust fans are installed on one side while the air inlet pipes are along the other side of the poultry house. It is best suited to narrow poultry houses (less than 10 m) because it leads to difference in environmental conditions in the house with larger width [2].

*5.2.4.2 Sidewall inlet ventilation*

The exhaust fans are placed below the air inlet pipes on both sides of the building walls [2]. However, a distance not less than twice the diameter of the fan should be between the exhaust fans and the air inlet pipes. Air movement is directed towards the center, and drawn through the floor by the exhaust fans. It is also best suited for narrow house with not more than 12 m width [2].

*5.2.4.3 Attic inlet ventilation*

The exhaust fans are placed on the lower sidewalls while, air inlets are placed in the ceiling. This kind of ventilation requires proper ceiling insulation and it best suitable for hot climate areas. The ventilation method is greatly recommended for rearing laying hen [2].

*5.2.5 Air movement inlet ventilated house*

Fresh air enters through the air inlet pipes at a velocity of 3.5–6 m/s, however this velocity is quickly dropped to about 1 m/s depending on the size and type of the house. Hence, circulation fans are used to boost the air speed to ensure air movement is sufficient in the building [2].

*5.2.6 Tunnel ventilation system*

Tunnel ventilation system is designed to meet the specified air velocity and air exchange rate. However, the required air velocity is dependent on the class of birds in question. **Table 2** shows the recommended air speed required for rearing various classes of poultry birds [2].

*5.2.6.1 Tunnel fan capacity and air velocity*

The tunnel fan capacity is determined by the same method used for inlet ventilation system. Unlike the inlet ventilation system where the adequate air velocity is propelled by circulation fan, the required average air velocity within tunnel house is calculated by the expression below [2].

$$\text{Air velocity} = \text{tunnel fan capacity} / (\text{cross} - \text{sectional area of the house}) \times 3600 \qquad (6)$$

where air velocity, m/s; tunnel fan capacity, $m^3/h$; cross section area, $m^2$.

However, it is important to note that the cross sectional area of the house adversely affect the air speed within the house. Therefore, it is advisable to design narrow and long house with lower ceilings [2]. Consequently the expression below can be used to design the desired air velocity.

| House type | Air speed (m/s) |
|---|---|
| Broilers | 2.5–3 |
| Pullets | 1.75–2.25 |
| Broiler breeders | 2.25–3 |
| Commercial layer | 2.5–3 |

**Table 2.**
*Recommended air velocities in tunnel-ventilated houses.*

$$\text{Tunnel fan capacity} = \text{desired air velocity} / (\text{cross} - \text{sectional area of the house}) \times 3600 \quad (7)$$

where desired air velocity, m/s; tunnel fan capacity, $m^3/h$; cross section area, $m^2$.

In cases where there is land constraints, air deflectors can be installed houses with large cross-sectional area to reduce the cross sectional area within the poultry house. Air deflectors are curtains that extend from the ceiling not more than 2.5–3 m from the ground. Air deflectors have been reported to increase air velocity for a distance approximately 1.2 and 6–9 m upward and downwind of the deflector respectively. However, it is important to ensure that the air deflector exceed 2.5 m from the ground to have it from disrupting the performance of fans and air exchange rate by increasing static pressure [2].

#### *5.2.6.2 Air velocity distribution*

Normally, the air velocity in a tunnel house is assumed uniform across the house. However, it can vary slightly depending on the smoothness of the building surfaces, presence of poultry equipment and other obstructions that deflect air. The difference between the air velocity in the center and the side of the house can vary from 15 to 40% [2].

#### *5.2.6.3 Bi-directional tunnel house*

Generally, it is best to install the fans on one end and the inlet in opposite end to ensure the maximum air speed is achieved in the tunnel house. However in cases where the poultry house is over 180 m long and the air velocity required for airflow in one direction exceeds 3.5 m/s it is advisable to apply the bi-directional tunnel house system. The fans are located at end-walls of the building and the tunnel inlet at the center of the house. The air velocity in both direction is reduce to half of the required velocity while retaining the same air exchange rate to ensure the temperature difference between the inlet and the fan remains the same [2].

#### *5.2.6.4 Tunnel fan placement*

The fans can be installed at the end-walls or the sidewalls near the end, and this installation arrangement does not affect the performances of the fans. However, dead spot can be noticed when the fan is installed on the sidewalls as the width of the houses increase.

#### *5.2.6.5 Tunnel inlet opening*

In the absence of evaporative cooling pads, it is recommended that the inlet area should be at least 10% greater than the cross sectional area of the house. Meanwhile, the pad used determines inlet size for tunnel house with evaporative cooling pads. It is recommended that inlet opening on the sidewall should be installed as close as possible to the end wall. However, if the house width exceeds 15 m it is advisable to install the inlet openings on the end-wall [2].

#### *5.2.6.6 Cool weather inlet system for tunnel ventilated houses*

It has been recommended that tunnel ventilated system should be used in hot weather because cool weather reduce the air exchange rate. Consequently, it was

recommended that a minimum of 60% of the tunnel fan capacity should be controlled by the traditional inlet system before upgrading to tunnel ventilation for easy switch during cooler weathers [2].

*5.2.7 Poultry exhaust fans*

*5.2.7.1 Types of fans*

*5.2.7.1.1 Exterior and interior shutter fans*

It is the simplest type of exhaust fan. Its shutters are used to when the fan is not in use. However, the exterior shutter restricts airflow as air spins off its blades on contact. In the case of interior fan on the other hand, the shutters are on the intake side of the fan thus, lessening the restriction of airflow. It has bigger shutters, which allows for more air movement. Daghir [2] reported that airflow is increased by 5–10% compared to exterior shutter fan.

*5.2.7.1.2 Discharge cone fans*

It increases fan performance by 5–10% as it eases the transitioning of drawn towards the fans [2].

*5.2.7.1.3 Belt-drive fans*

The fans blades are driven by a simple pulley mechanism. It may be upgraded with an automatic belt tensioner to prevent belt slippage [2].

*5.2.7.1.4 Direct-drive fans*

The fan's blades are attached directly to the motor shaft eliminating the use of belt. They are less energy efficient compared to belt-driven fans [2].

## 6. Conclusions

Heat loss in birds through convection, radiation and conduction is only effective if the environmental temperature is below or within the bird's thermoneutral zone. Naturally ventilated open housing system has been explored in the tropics to improve the environment for optimum production in birds. Studies show that when the volume and velocity of air is increased heat loss is enhanced in birds through convection. Also, the proper consideration of architectural elements such as building orientation, roof slope, roof overhang, landscape, building height, building width, building length, etc. have been reported to enhance naturally ventilated buildings for optimum production in chicken. In addition, the incorporation of cooling systems such as fogging system, sprinkling system and circulation fan in naturally ventilated design house systems have proven positive in optimizing birds' performances in general.

Consequently, in cases where the environmental temperature is severely high and unbearable for birds the mechanical ventilated open housing system have been introduced. The use of Tunnel and inlet ventilation system have been reported to sustain improved birds' production in this regions regardless the extreme weather conditions. However, to design an effective, mechanically ventilated house due attention should be given when calculating the fan capacity of the house, heat

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generated by the birds, sizes of inlet, level of installation, positioning of inlet pipes and exhaust fan and finally the capacity of circulation fans required in inlet ventilated systems.

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Chapter 2

# Investigation of the Effects of Some Herbal Extracts Used in Different Ratios on Meat Fatty Acid Profile Level in Experimental Heat Stress Created in Broilers

*Emre Tekce, Bülent Bayraktar and Vecihi Aksakal*


## Abstract

Stress is the biological or external alteration of the organism against the factors that make it possible to achieve hemostasis or normal physiological balance. In our world, temperature increase due to climate change has become one of the most important stress factors in poultry sector. This research investigated the effects of essential oil mixture (EOM; *Eucalyptus globulus* Labill, *Thymus vulgaris*, *Cymbopogon nardus*, and *Syzygium aromaticum*) broilers adding to the drinking water under heat stress conditions. The fatty acid profile was evaluated. In a 42-day study, 400 Ross-308 male chickens (1-day-old) were randomly assigned to eight different groups ($n$ = 50), each containing five subgroups ($n$ = 10). As a result of the research, in stress-free groups 22°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2n\text{-}6t}$), and Cis 11 eicosapentaenoic acid (C 20: 1n9) increased, whereas MUFA, UFA, and behenic acid ($C_{22:0}$) reduced. However, in stressed groups, 36°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and arachidonic acid ($C_{20:0}$) decreased, increased the UFA ratio, and had no effect on the MUFA and PUFA.


**Keywords:** essential mix oil, broilers, heat stress, water drinking, fatty acid profile

## 1. Introduction

For the world population to have a balanced and nutritious diet, it is essential that animal food, and products obtained from them, be consumed. Improving food quality, quantity of the products, and nutritional value is an important tool used to not only feed an ever increasing world population but also enhance health and longevity [1]. according to who do not exceed 30% of people's daily energy needs however, not more than % 10 of these oils should be saturated fatty acid (SFA) and % 3–7 polyunsaturated fatty acid (PUFA) [2]. Because of the metabolic diseases and disturbances associated with nutrition (cardiovascular diseases, low-density lipoprotein (LDL), arteriosclerosis, and diabetes), the human population brought about an increased interest for the functional foods [1, 3].



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Stress is associated with endogenous (nutrition, rapid growth, sexual maturation period, and infection) and exogenous (climate, high-density insufficient ventilation) factors in living beings [4–6]. Heat stress is one of the most critical environmental factors in poultry all over the world. Stress disrupts the balance between oxidation and antioxidant defense systems, causing lipid peroxidation, protein structure, and consequently DNA oxidative damage [7, 8]. Stress responses in poultry occur mainly by activation of the hypothalamic pituitary adrenal axis and the orthotic nervous system. Furthermore, heat stress causes a series of physiological and metabolic changes in broiler chickens, such as high body temperature, rapid breathing, and respiratory alkalosis [8]. İn addition high ambient temperature, especially such as feed consumption, growth, immune system disorder, physiological effects show. Therefore, it has become necessary to develop alternative strategies and modulations in animal nutrition for animal feeding, disease prevention, and heat tolerance. Within this concept, antibiotics have been used in stockbreeding for over 50 years to protect animal health and increase the quality and quantity of the products. However, the European Union has banned the use of antibiotics as productivity enhancers because microorganisms have developed resistance against antibiotics and there is a risk of residue in the final products. Various alternative feed additives have been used to compensate for the production impacts of this ruling [9, 10]. In addition, a growing sensitivity toward human health, food safety, and environmental pollution has emerged among consumers despite the progresses in food production techniques and slaughterhouse hygiene in European Union countries [11, 12]. Therefore, many studies have been conducted recently with the aim of achieving organic products using natural feed additives as an alternative to synthetic additives.

In this context, people's interest in natural products has been increasing in recent years. At the beginning of these products are volatile fatty acids derived from medicinal aromatic plants. Primary metabolites (such as carbohydrate, fat, and protein) in medicinal plants have small molecule secondary metabolites (alkolites, essential oils, glycosides, flavonoids, and resins) that are not very important in plants, which are not vital in plants [13]. It has been stated that animals do not pose risks to human health after accumulation, drug resistance, and use in their tissues [14]. These essential oil fatty mix acids have been reported to have properties that include antimicrobial [15, 16], anti-inflammatory [17, 18], antiviral [19, 20], antitumoral [15], antifungal [21, 22], and antiparasitic [23, 24] effects. In addition, UYA obtained from medical aromatics plants; metabolic reactions and metabolism reactions in the organism and metabolism analysis is done in the body tissue quickly does not accumulate [25].

This study investigated the fatty acid profile benefits of an essential oil acidic mixture (EOM; *Eucalyptus globulus* Labill, *Thymus vulgaris*, *Cymbopogon nardus,* and *Syzygium aromaticum*) added at different levels to the drinking water of temperature-stressed broilers (22 and 36°C, respectively).

## 2. Materials and methods

### 2.1 Animals, experimental design, and feeds

Four hundred 1-day-old Ross-308 male chickens were placed in a 110 × 110 × 100 cm pen in the poultry unit at the Bayburt University Food, Agriculture and Animal Husbandry Application and Research Center, during 7 days of exercise and 35 days of fattening. On day 7 of the experiment, the animals were randomly assigned to eight groups (*n* = 50) (C (Control), EOM-250 (22°C 250 ml/1000 L), EOM-500 (22°C 500 ml/1000 L), EOM-750 (22°C 750 ml/1000 L), SK (36°C Control), SEOM-250 (36°C 250 ml/1000 L), SEOM-500 (36°C 500 ml/1000 L),

SEOM-750 (36°C 750 ml/1000 L)), each containing five subgroups ($n$ = 10). For each of the experimental periods, four treatments were prepared by supplementing the drinking water with 0 (control), 250 mL/1000 L, 500 mL/1000 L, and 750 mL/1000 L of EOM. During the study period was applied to the C, EOM-250, EOM-500 and EOM-750 groups at 22°C and SC, SEOM-250, SEOM-500 and SEOM-750 groups at 36°C. The nutritional content and ratios of broiler rations were shown in **Table 1**. Each experimental drinking water was offered to birds housed at either 22°C (normal temperature) or 36°C (heat-stressed conditions), and the birds with drinking water with 0 EOM were considered the control groups (22°C, positive control; 36°C, negative control). The feed used in this study was analyzed according to the standard AOAC methods [26].

### 2.2 EOM composition

The volatile oil of EOM (contained 26.70% durenol, 23.89% eugenol, 16.49% gamma-terpinene, 8.35% heptaethylene glycol, 6.42% hexaethylene glycol, 3.31% cymene, 3.08% pentaethylene glycol, 2.87% caryophyllene, 2.30% D-limonene, 2.18% beta-pinene, 0.95% eucalyptol) was provided from a commercial company in Ankara, Turkey.

#### *2.2.1 Poultry house temperature, humidity, and illumination*

The general temperature of the poultry house was maintained at 32–33°C during the first 2 days and at 27–28°C during the next 5 days. However, thereafter, a temperature of 36°C and humidity of 75–85% were applied to the groups subjected to

| Raw material | Starter (0–14 days) | Grower (14–28 days) | Finisher (28–42 days) |
|---|---|---|---|
| Maize | 52.70 | 54.60 | 58.12 |
| Maize gluten feed | 15.21 | 21.20 | 26.14 |
| Soybean residue | 26.35 | 18.90 | 10.65 |
| Dicalcium phosphate | 1.95 | 1.70 | 1.60 |
| Calcium carbonate | 1.18 | 1.10 | 1.04 |
| Sodium chloride | 0.31 | 0.31 | 0.31 |
| Sodium bicarbonate | 0.20 | 0.20 | 0.20 |
| Salt | 0.2 | 0.2 | 0.2 |
| Methionine | 0.50 | 0.50 | 0.44 |
| Lysine | 1.20 | 1.10 | 1.10 |
| Vitamin-mineral premix[1] | 0.20 | 0.20 | 0.20 |
| ME (kcal/ kg) | 3100 | 3150 | 3225 |
| Crude protein (%) | 24 | 22 | 20 |
| Crude oil (%) | 2.61 | 2.30 | 2.50 |
| Ash (%) | 5.19 | 4.63 | 3.85 |
| Moisture (%) | 13.20 | 13.20 | 13.20 |

*The vitamin-mineral premix provided the following (per kg of diet): vitamin A, 12000 IU; vitamin D3, 1500 IU; vitamin E, 50 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 4 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; calcium-D-pantothenate, 15 mg; folic acid, 1 mg; niacin, 25 mg; D-biotin, 0.115 mg; Co, 0.2 mg; Cu, 6 mg; Fe, 60 mg; K, 0.75 mg; Mn, 80 mg; Se, 0.15 mg; Zn, 60 mg.*

**Table 1.**
*Composition and analyses of the basal diet (g/kg).*

heat stress, and a temperature of 22°C and humidity of 55–60% to the other birds. Throughout the experimental period, all groups were housed under lighted (60 W) conditions constantly.

### 2.3 Lipid profile in meat and blood

Fatty acid composition of the 40 breast meat filets, 5 from each group, was measured using a gas chromatograph with flame ionization detector (GC-FID) provided from a commercial company in İzmir, Turkey. For the fatty acid and conjugated linolenic acid analysis of the collected samples, fat transmission to organic solvents within the samples was performed by homogenizing in chloroform:methanol mixture (2:1) using a homogenizer at 24,000 rpm. Samples were kept frozen until methylation, following Folch et al. [27]. Subsequently, 0.5 ml of the fat was taken from the frozen samples and placed in centrifuge tubes with 1 ml 2 N methanolic KOH solution and 7 ml n-heptane and then centrifuged at 5000 rpm for 10 min with a closed lid. The gas formed at the top of the centrifuge tube was collected and transferred to viola after being filtered with anhydrous $Na_2SO_4$. A capillary column (100 m, HP88) was used to separate the fatty acids in viola. Gas chromatography was measured using an automated injection GC-FID FID (HP-6890 N, Agilent).

### 2.4 Statistical analysis

The measures were all normally distributed and data are expressed as means and standard errors of the mean. Univariate general linear model was used to identify if differences existed in the fatty acid profile groups. Duncan multiple range tests were applied to identify differences among groups. All statistical tests were performed at 5% level of statistical significance by IBM SPSS statistics 20.0.

## 3. Results

**Table 2** shows the levels of monounsaturated fatty acids (MUFA), polyunsaturated fatty acid (PUFA), saturated fatty acids (SFA), and total unsaturated fatty acids (UFA) within the fatty acid profiles of samples collected from breast meat filets of broilers fed at 22 and 36°C. EOM; (*Eucalyptus globulus* Labill, *Thymus*

| | Fatty acid profile | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SFA | | MUFA | | PUFA | | UFA | |
| | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C |
| Control | 34.23 | 38.53 | 50.71 | 47.70 | 15.05 | 13.77 | 65.77 | 61.47 |
| EOM 250 mL/L | 38.48 | 35.60 | 43.17 | 51.15 | 18.14 | 13.26 | 61.31 | 64.41 |
| EOM 500 mL/L | 40.23 | 33.96 | 45.32 | 51.71 | 14.92 | 14.51 | 60.24 | 66.22 |
| EOM 750 mL/L | 33.10 | 34.52 | 52.12 | 50.00 | 14.76 | 15.48 | 66.89 | 65.48 |
| Source of variation (*P*-values) | | | | | | | | |
| Diet | 0.00** | | 0.16 | | 0.61 | | 0.71 | |
| Temperature | 0.10 | | 0.07 | | 0.07 | | 0.13 | |
| Temperature × Diet | 0.00** | | 0.01** | | 0.09 | | 0.00** | |
| Main effect means diet | | | | | | | | |
| Control | 36.38[b] | | 49.21[ab] | | 14.42 | | 63.62[a] | |

| | **Fatty acid profile** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **SFA** | | **MUFA** | | **PUFA** | | **UFA** | |
| | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** |
| EOM 250 mL/L | 37.04$^{b}$ | | 47.16$^{a}$ | | 15.71 | | 62.86$^{a}$ | |
| EOM 500 mL/L | 37.10$^{b}$ | | 48.52$^{ab}$ | | 14.71 | | 63.23$^{a}$ | |
| EOM 750 mL/L | 33.82$^{a}$ | | 51.06$^{b}$ | | 15.12 | | 66.18$^{b}$ | |
| Temperature | | | | | | | | |
| 22 °C | 36.51 | | 47.83 | | 15.72 | | 63.55 | |
| 36 °C | 35.65 | | 50.14 | | 14.25 | | 64.39 | |
| SEM | 0.32 | | 0.77 | | 0.49 | | 0.36 | |

*Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means.*Significant at 0.05 level, **Significant at 0.01 level, SEM = standard error of the mean.*

**Table 2.**
*Fatty acid profile of the experimental groups (7–42 days).*

| | **Fatty acid profile** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **C14:0** | | **C16:0** | | **C16:1** | | **C17:0** | | **C17:1** | | **C18:0** | | **C18:1** | |
| | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** | **22°C** | **36°C** |
| Control | 0.42 | 0.55 | 24.60 | 29.18 | 6.29 | 7.56 | 0.06 | 0.09 | 0.05 | 0.10 | 7.98 | 7.92 | 44.03 | 39.69 |
| EOM 250 mL/L | 0.67 | 0.51 | 28.26 | 28.03 | 6.56 | 7.84 | 0.14 | 0.09 | 0.07 | 0.09 | 7.95 | 6.51 | 36.15 | 42.92 |
| EOM 500 mL/L | 1.07 | 0.59 | 28.77 | 25.41 | 6.45 | 7.42 | 0.09 | 0.09 | 0.09 | 0.06 | 9.22 | 6.88 | 38.41 | 43.86 |
| EOM 750 mL/L | 0.38 | 0.30 | 25.29 | 25.58 | 7.34 | 7.02 | 0.07 | 0.09 | 0.06 | 0.06 | 6.72 | 7.02 | 44.39 | 42.56 |
| Source of variation (*P*-values) | | | | | | | | | | | | | | |
| Diet | 0.00** | | 0.00** | | 0.91 | | 0.18 | | 0.61 | | 0.08 | | 0.11 | |
| Temperature | 0.00** | | 0.37 | | 0.06 | | 0.73 | | 0.29 | | 0.02 | | 0.16 | |
| Temperature × Diet | 0.00** | | 0.00** | | 0.42 | | 0.08 | | 0.07 | | 0.06 | | 0.01** | |
| Main effect means diet | | | | | | | | | | | | | | |
| Control | 0.49$^{b}$ | | 26.89$^{b}$ | | 6.92 | | 0.08 | | 0.07 | | 7.95$^{ab}$ | | 41.87$^{ab}$ | |
| EOM 250 mL/L | 0.59$^{b}$ | | 28.15$^{c}$ | | 7.20 | | 0.11 | | 0.08 | | 7.23$^{ab}$ | | 39.54$^{a}$ | |
| EOM 500 mL/L | 0.83$^{c}$ | | 27.09$^{bc}$ | | 6.93 | | 0.09 | | 0.07 | | 8.05$^{b}$ | | 41.13$^{ab}$ | |
| EOM 750 mL/L | 0.34$^{a}$ | | 25.44$^{a}$ | | 7.18 | | 0.08 | | 0.06 | | 6.87$^{a}$ | | 43.48$^{b}$ | |
| Temperature | | | | | | | | | | | | | | |
| 22 °C | 0.63 | | 26.73 | | 6.66 | | 0.09 | | 0.06 | | 7.97 | | 40.74 | |

| | Fatty acid profile | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C14:0 | | C16:0 | | C16:1 | | C17:0 | | C17:1 | | C18:0 | | C18:1 | |
| | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C |
| 36 °C | 0.48 | | 27.05 | | 7.46 | | 0.08 | | 0.07 | | 7.08 | | 42.26 | |
| SEM | 0.02 | | 0.24 | | 0.26 | | 0.01 | | 0.01 | | 0.23 | | 0.69 | |

**Miristic: C14: 0, Palmitic: C16: 0, Palmitoleic: C16: 1, Heptadeconoic Acid ( Margaric): C17: 0, Heptadesenoic: C17: 1, Stearic: C18: 0, Oleic: C18: 1, Linoleic: C18: 2n-6c, Linoleic: C18: 2n-6t, Arachidic: C20: 0, cis 11 Eicosapentaoic Acid: C 20: 1n9, Behenic: C22: 0, lignoceric: C24: 0. Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means.* Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean.*

**Table 3.**
*Fatty acid profile of experimental groups.*

| | Fatty acid profile | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C18:2n-6c | | C18:2n-6 t | | C20:0 | | C20:1n9 | | C22:0 | | C24:0 | |
| | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C | 22°C | 36°C |
| Control | 14.49 | 13.39 | 0.56 | 0.38 | 0.31 | 0.32 | 0.34 | 0.35 | 0.28 | 0.17 | 0.56 | 0.30 |
| EOM 250 mL/L | 16.08 | 12.92 | 2.06 | 0.34 | 0.55 | 0.21 | 0.40 | 0.30 | 0.27 | 0.13 | 0.63 | 0.13 |
| EOM 500 mL/L | 14.47 | 14.15 | 0.44 | 0.35 | 0.35 | 0.26 | 0.38 | 0.37 | 0.16 | 0.25 | 0.57 | 0.50 |
| EOM 750 mL/L | 14.32 | 15.03 | 0.44 | 0.44 | 0.30 | 0.30 | 0.34 | 0.35 | 0.06 | 0.19 | 0.29 | 1.05 |
| Source of variation (*P*-values) | | | | | | | | | | | | |
| Diet | 0.80 | | 0.29 | | 0.45 | | 0.60 | | 0.12 | | 0.31 | |
| Temperature | 0.11 | | 0.15 | | 0.02* | | 0.25 | | 0.75 | | 0.85 | |
| Temperature × Diet | 0.16 | | 0.24 | | 0.05 | | 0.22 | | 0.01** | | 0.01** | |
| Main effect means diet | | | | | | | | | | | | |
| Control | 13.94 | | 0.47 | | 0.31 | | 0.34 | | $0.22^{b}$ | | 0.43 | |
| EOM 250 mL/L | 14.50 | | 1.20 | | 0.38 | | 0.35 | | $0.20^{ab}$ | | 0.38 | |
| EOM 500 mL/L | 14.31 | | 0.40 | | 0.31 | | 0.38 | | $0.21^{ab}$ | | 0.53 | |
| EOM 750 mL/L | 14.68 | | 0.44 | | 0.30 | | 0.35 | | $0.12^{a}$ | | 0.67 | |
| Temperature | | | | | | | | | | | | |
| 22 °C | 14.84 | | 0.87 | | 0.38 | | 0.36 | | 0.19 | | 0.51 | |
| 36 °C | 13.87 | | 0.38 | | 0.27 | | 0.34 | | 0.18 | | 0.49 | |
| SEM | 0.38 | | 0.22 | | 0.03 | | 0.01 | | 0.02 | | 0.08 | |

**Miristic: C14: 0, Palmitic: C16: 0, Palmitoleic: C16: 1, Heptadeconoic Acid ( Margaric): C17: 0, Heptadesenoic: C17: 1, Stearic: C18: 0, Oleic: C18: 1, Linoleic: C18: 2n-6c, Linoleic: C18: 2n-6t, Arachidic: C20: 0, cis 11 Eicosapentaoic Acid: C 20: 1n9, Behenic: C22: 0, lignoceric: C24: 0. Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means.* Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean.*

**Table 4.**
*Fatty acid profile of experimental groups.*

*vulgaris*, *Cymbopogon nardus*, and *Syzygium aromaticum*) essential oil added at the drinking water in various dosages (250, 500, and 750 mL/1000 L). The broilers exposed to heat stress showed increased SFA and UFA compared to the control groups, while there is no statistically significant effect on PUFA and MUFA.

**Tables 3** and **4** show the fatty acids profile influence of the addition of EOM to the drinking water of the broilers on the stressed and non-stressed groups. EOM; (*Eucalyptus globulus* Labill *Thymus vulgaris*, *Cymbopogon nardus* and *Syzygium aromaticum*) essential oil added at the drinking water in various dosages stressed groups 36°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and arachidonic acid ($C_{20:0}$) decreased, increased the UFA ratio, had no effect on the MUFA and PUFA.

## 4. Discussion

Fatty acid composition in broiler meat generally reflects the fatty acid profile in the rations. Thus, increased linolenic acid (n-3) levels in PUFA of the rations reduces saturated fat ratio in broiler carcasses. Particularly in monogastric animals, n-3 fatty acid added to the rations is stored in tissues without transformation within the ileum. Thus, with the change of broiler carcass fat compounds, omega 3 fatty acid, which is very beneficial for humans and plays an important role in preventing cardiovascular diseases, is maybe ingested with the broiler meat [28]. Some studies have shown that the main EOM components reduce SFA rate in serum and thigh meat samples linearly, i.e., MUFA, PUFA, n-3, and n-6 increase with increased EOM dosage, but do not affect breast meat samples [29], whereas other studies report that EOM dosage reduces SFA and PUFA in breast and thigh meat but increases MUFA rate [30]. Studies have shown that the increase in unsaturated fatty acid in broiler rations produces increased oxidation in the meat, whereas this oxidation is hampered by EOM alkaloids [31]. Studies on rats have reported that adding thyme and thymol increases PUFA rate within the brain; DHA, which is found in brain tissues; microsomes; mitochondria; and synaptic vesicles and affects behavior, memory, and motor skills, in comparison to control group, and that free radicals are inclined to increase with age [32].

Although saturated and unsaturated fatty acid levels in abdominal and subcutaneous tissues of heat-stressed broilers decrease, fatty acids in intramuscular tissues are not affected [33]. On the other hand, palmitic acid ($C_{16:0}$), a saturated fatty acid, increases under heat stress and palmitoleic acid ($C_{16:1}$) and linoleic acid ($C_{18:2n\text{-}6c}$), unsaturated fatty acids, reduce [33]. Furthermore, oleic acid ($C_{18:1}$) decreased, whereas linoleic acid ($C_{18:2n\text{-}6c}$), linolelaidic acid ($C_{18:2n\text{-}6t}$), and PUFA increased in heat-stressed broilers [34]. Compared to cows and pigs, chicken saturated and unsaturated fatty acid rates in intramuscular tissues are higher [35]. The current study on breast meat samples collected from groups without heat stress showed decreased MUFA and UFA ($P < 0.05$), while SFA increased compared to the control groups and there is no statistically significant effect on PUFA ($P > 0.05$). Groups with heat stress showed increased SFA and UFA ($P < 0.05$) compared to the control groups, while there is no statistically significant effect on PUFA and MUFA ($P > 0.05$).

The current study is consistent in terms of the effects of EOM dosage on saturated (SFA) and unsaturated (MUFA, PUFA, and UFA) fatty acids with some previous studies [29, 32, 34] but inconsistent with some others [30, 33]. This is probably due to lipid peroxidation being prevented by EOM in the fatty acids, which are highly sensitive to lipid oxidation, and other variations, such as time, method, and dosages of EOM addition to the water drinking and other differences in experimental materials and methods.

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Chapter 3

# Genetic and Hormonal Regulation of Egg Formation in the Oviduct of Laying Hens

*Birendra Mishra, Nirvay Sah and Sanjeev Wasti*

## Abstract

The chicken oviduct is a unique organ in which ovulated yolk transforms into a complete egg. Ovarian hormones induce the cellular and biochemical changes in the oviducts during the egg formation and oviposition. Estradiol regulates the folliculogenesis, accumulation of yolk in the follicles, ovulation, and development of oviducts. Estradiol also induces glandular development and expression of the genes responsible for egg white proteins. Progesterone induces the ovulation of yolk from the ovary, and development of oviductal glands. In addition, several genes are spatiotemporally expressed in the magnum for albumen synthesis and deposition around the yolk, in the isthmus for shell membranes synthesis, and in the uterus for eggshell biomineralization. This chapter highlights the involvement of hormones, genes/proteins, and their interaction for egg formation in the oviduct of laying hens.

**Keywords:** oviduct, hormone, gene expression, albumen, eggshell

## 1. Introduction

The poultry oviduct provides the biological environment for the egg formation and fertilization of ovulated oocyte. The hens are born with a pair of ovary and oviduct, however, the development of the right ovary and oviduct cease and gradually regress. The left ovary and the oviduct remain functional and contribute in the egg formation. The oviduct is a long tubular structure consisting of five functionally and histomorphologically distinct segments namely: the infundibulum (site of fertilization), the magnum (production of components of egg white), the isthmus (formation of the egg shell-membranes), the shell gland or uterus (formation of calcified eggshell), and the vagina (oviposition or egg laying). Following ovulation, the ovum passes through the entire length of the oviduct, where the constituents of the egg are secreted and deposited from respective parts of the oviduct. The yolk enters the oviduct, and in about 24–28 h, a complete egg is formed. While the egg traverses through the oviduct, each segment of the oviduct either produces a component of the egg or has a vital non-secretory role. Besides environmental, nutritional, and pathological conditions, oviductal functions also govern the egg production and quality. The formation of the egg inside the oviduct is highly complex and is under genetic and hormonal control. There are several genes and biological pathways involved in the egg formation [1, 2]. The purpose of this



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chapter is to provide updated information on the role of hormones, genes/protein, and their interaction that trigger histomorphological and biochemical changes in the segments of oviduct for egg formation.

## 2. Histomorphology and functions of the oviduct

The infundibulum in hens encloses the whole ovary and has two distinctions: the membranous and the muscular infundibulum. The membranous infundibulum covers the ovarian cluster, while the muscular infundibulum is lined by ciliated cells and acts as a passage for the yolk inside the oviduct. The egg remains for a very brief period (15–30 min) in the infundibulum and then descends in the magnum where albumen is deposited around it. The infundibulum, therefore, is also the site for any potential fertilization of the ovum. The magnum is the largest segment of the oviduct and produces the egg-white proteins which surround the yolk. The glandular epithelial cells of the magnum synthesize the different egg-white proteins, store them, and secrete only for the 2–3 h duration when the egg is present in it, whereas, the ciliated epithelial cells aid in egg transport. The egg-white is rich in protein and is the main source of nutrient for the embryo during development. It also contains some antimicrobial proteins that protect the embryo from pathogenic microbes. The albumen constitutes more than 60% of the total egg, so determines the egg weight and hatchling weight. Later, the egg moves down in the isthmus, the bridging segment between the magnum and the shell gland, where it remains for 1–2 h. In the isthmus, the outer and inner eggshell membranes (ESM) are formed around the egg albumen. The eggshell membranes are fibrous networks holding the jelly egg-white in the center and also provide the site for initiation of eggshell mineralization. After being enveloped by the ESM, the egg moves in the shell gland and sits there for nearly 18–22 h during which the calcite crystals are deposited on the ESM to form the eggshell. The eggshell is 95% calcium by composition and thus, is the main source of calcium for the growing embryo. The organization of the eggshell prevents the passage of external microbes inside the egg while allowing the movement of air inside the egg for the inchoate embryo to breathe. Eventually, after complete mineralization of the eggshell, the egg is held momentarily in the vagina. Pigmentation of eggs, in some birds, is completed in the vagina and finally, the egg is laid out.

## 3. Genetic regulation of egg formation

The egg formation is regulated through the spatiotemporal expression of genes/proteins and biological pathways in the segments of oviduct. The protein-coding genes expressed in the oviduct regulate the movement of egg, deposition of the egg constituents, and ensure the formation of quality eggs. The genetic regulation of egg formation in the oviduct is discussed below based on the genesis of each egg component.

### 3.1 Genetic regulation of albumen formation

The albumen, also known as egg-white, is the protein-rich jelly portion of a fresh egg. It is a composite of nearly 148 different proteins that are vital for the survival and growth of the chicken embryo. The fundamental proteins include ovalbumin (OVAL), conalbumin (TF), ovomucoid (OVM), ovomucin (MUC), and lysozyme (LYZ), among others. OVAL is a structural protein making up about 54% of the total egg-white protein. Ovalbumin X, a homolog of the OVAL protein,

has antimicrobial property [3]. TF also has some antimicrobial action [4, 5]. OVM is a trypsin inhibitor and an antimicrobial agent [6]. MUC is a mucoprotein having anti-bacterial and anti-viral activity [7, 8]. LYZ has some very well-known antibiotic effects. Most of these fundamental albumen proteins are synthesized in the tubular gland cells of the magnum. The amino-acids required for the genesis of these proteins are transported from the circulation across the epithelial membrane into the gland cells by special transporter genes; the solute carriers (SLCs). The expression of many SLC mRNAs is increased in the magnum epithelium during the egg formation (Sah et al., unpublished). The synthesis of OVAL, TF, OVM, and LYZ proteins occur in a single cell-type (gland cells) continuously at a proportional rate to their abundance in the egg-white [9]. The expression of *OVAL*, *TF*, *OVM*, and *LYZ* mRNA is upregulated in the magnum of laying hens during 4–23 h post-ovulation [10].

Once the egg enters the magnum, it creates a mechanical distention of the magnum wall which elicits the stimulus to trigger the release of the stored proteins. One such molecule that provokes the secretion of the proteins from the epithelial cells is relaxin (RLN3). Expression of *RNL3* mRNA is increased in the magnum with the presence of an egg in laying hens (Sah et al., unpublished). The renin-angiotensin system (RAS), besides its renal function, participate in the protein secretion-signaling pathway. The OVAL, TF, OVM, and LYZ proteins are released in secretory granules from the glands and deposited over the yolk. Some other proteins that get incorporated in the egg-white for its defense are avian beta-defensins, cystatin, and avidin [11–13].

### 3.2 Genetic regulation of eggshell membrane formation

The eggshell membranes are fibrous networks arranged in outer and inner layers interconnected with fibers making up a highly cross-linked fibrous meshwork. This meshwork provides the nucleation sites for the initiation of eggshell mineralization. Disruption in the formation and organization of these cross-linked fibers can negatively impact the eggshell strength [14]. The expression of several genes and proteins, when the egg is in the isthmus, is critical to the formation of the ESM. Collagens are the fundamental fibrous components of the ESM. The expression of *collagen X* (*COL10A1*) mRNA is higher in the isthmus of laying hens [14]. The collagen X proteins are homotrimer of α-1 chains secreted from the tubular gland cells of the isthmus [15] that provide the structural integrity to the ESM. Beside collagens, the ESM formation depends on other proteins such as fibrillin-1, cysteine-rich eggshell membrane protein (CREMP), lysyl oxidases, quiescin Q6 sulfhydryl oxidase 1 (QSOX1), and thioredoxin [1]. The fibrillin-1 is a microfibrillar glycoprotein whose mRNA is over-expressed only in the isthmus [14]. Fibrillin-1 gives the elastic nature to the ESM. The major constituency of cysteine in the ESM comes from CREMPs which are expressed most in the isthmus. The CREMP also has some antibacterial effect in the egg. Lysyl oxidases, on the other hand, are enzymes found in the ESM that mediate the formation of cross-links between collagen and ESM fibrillar proteins [16]. The QSOX1 protein also mediates the genesis of ESM meshwork and regulates the integrity of the ESMs [17]. The enzyme thioredoxin catalyzes the formation of disulfide cross-links between fibrillar proteins.

### 3.3 Genetic regulation of eggshell biomineralization

The chicken eggshell, the outermost calcified layer, is very critical for the safety of the eggs. The roles of several genes and proteins in synthesis and mineralization of the eggshell has widely been explored. The eggshell mineralization is activated with

the formation of calcite nodules on the outer ESM and is continued with deposition and elongation of calcium carbonate crystals. The mineralization process occurs in an acidic medium in the extracellular matrix uterine fluid. Matrix proteins such as ovocleidins, ovocalyxins, and osteopontin have well-established roles in the organization of the calcite crystals during eggshell calcification. Other localized proteins of the uterine epithelium such as, calbindin, calcitonin, otopetrin, and ATPases, as well, have crucial functions in ion-regulation across the uterine epithelium for the mineralization of egg.

For the eggshell formation, huge amount of calcium is required which is supplied partly through dietary sources and mostly through the mobilized calcium ions from medullary bones. The ion-transporting proteins, otopetrin-2, and ATPase 2C2, actively aids in the transfer of the $Ca^{2+}$-ions from the blood circulation into the uterine epithelial cells [1]. Calcium is also imported in the uterine epithelium passively via calcium-ion channels. Calcium-transporting ATPase (ATP2C2) and calcitonin-related polypeptide-β (CALCB) trigger the intracellular release of $Ca^{2+}$-ions from calcium reserve pools such as Golgi apparatus and endoplasmic reticulum [2]. The increased concentration of intracellular $Ca^{2+}$-ions in the uterine epithelium is maintained by calbindin 1. Calbindin-1 facilitates the transport of intracellular $Ca^{2+}$-ions to the extracellular matrix (ECM) in the uterine lumen [18]. Plasma membrane Ca-ATPases (PMCA) and sodium calcium exchangers (NCX) are the essential proteins necessary for the efflux of $Ca^{2+}$-ions into the uterine fluid [18]. Both PMCA and NCX transport one molecule of Ca-ion with a simultaneous import of one $Na^{+}$-ion in the uterine epithelium. ATPases such as ATP2B1 and ATP2B2 also transport $Ca^{2+}$-ions at the expense of $H^{+}$-ions import [2, 19]. The resultant increased in cellular $Na^{+}$-ions is offset with the efflux of those excess ions by ATP1A1, ATP1B1, and NKAIN4 but with the contemporaneous influx of $K^{+}$-ions in the uterine epithelium. Again, the elevated concentrations of K-ions are nullified by efflux via $K^{+}$-ion channel proteins such as KCNH1 or KCNJ2 [2, 19]. As such, the transport of $Ca^{2+}$-ions across the uterine epithelium requires the balance of $Na^{+}$-, $K^{+}$-, and $H^{+}$- ions which are regulated by ATPases, ion-channel and some other proteins. Bicarbonate ($HCO_3^{-}$)-ions are equally important in the mineralization of eggshell. The enzyme, carbonic anhydrase, catalyzes the formation of cellular $HCO_3^{-}$-ions from carbon dioxide and water. The $HCO_3$ -ions are then carried out into the uterine fluid by special transporter proteins, the solute carriers. These $HCO_3^{-}$-ions eventually combine with the free Ca-ions in the fluid bathing the egg to make calcite crystals.

Ovocleidins (OC) are eggshell matrix proteins which regulate the crystallization phenomenon in the uterus. The OC-17 catalyzes the mineralization of amorphous calcium carbonate to calcite crystals [20]. OC-116 regulates the organization of calcite crystals in the eggshell. Ovocalyxins (OCX) has three major member proteins which participate in eggshell mineralization. The OCX-32 controls the morphology of the calcite crystals and has a rather anti-mineralization function during the termination phase of calcification [21]. The direct role of OCX-36 in eggshell calcification has not been established, however, it protects the egg from microbial invasion [22]. Another member of the ovocalyxins, OCX-21, ensures the quality eggshell formation by providing a conducive environment [23]. Osteopontin, known as a secreted phosphoprotein, is also a negative regulator of calcification and determines the form and shape of the eggshell [24].

### 3.4 Ubiquitous proteins of the oviduct in the regulation of egg formation

Matrix metalloproteases (MMPs) are ubiquitous proteases that are known to degrade different extracellular matrix proteins (ECM) [25]. Cells in the body are surrounded by ECM, and cellular growth, proliferation, and differentiation are regulated by ECM degradation and remodeling through MMPs [25]. MMPs

are detected in the whole oviduct, and mostly in the magnum and uterus [1]. The cells of the magnum and uterus are highly secretory in nature, which require the proliferation of epithelium. The MMPs degrade the ECM surrounding the oviductal epithelium and help in cellular migration, proliferation, and differentiation [25]. Different MMPs (MMP-2, -7, and -9) are actively expressed in the oviduct during molting, while downregulated during the shift from immature to adult hens [26, 27]. Expression of MMP-1 and -10 is highest in laying hens in comparison to non-laying and molting hens (Sah et al., Unpublished). MMP-1 degrades interstitial collagens (type I, II, and III). MMP-2 degrades type IV collagens and induces angiogenesis. MMP-7 is also known as matrilysin which degrades casein, fibronectin, elastin and proteoglycans. MMP-9 is a gelatinase that also provokes the formation of new vasculatures [28]. MMP-10 is a stromelysin enzyme that can breakdown proteoglycans and fibronectins. The various matrix degrading roles of aforementioned MMPs ultimately ensures proper reproductive functions of the oviduct.

The solute carriers (SLCs) are another group of ubiquitous proteins found throughout the chicken oviduct. The SLCs are specialized molecular transporting proteins that are largely expressed on the plasma membrane. The expression of more than dozen of SLCs is evident in the oviduct [1, 2]. Several SLCs transport inorganic ions and amino acids in the magnum during albumen formation [29]. Some SLCs are mitochondrial carriers and are over-expressed in the uterus [19]. The SLCs are also upregulated at the uterovaginal junction to ensure the survival of the chicken sperm during storage [30, 31].

## 4. Hormonal regulation of egg formation

Egg formation in the laying hen is an intricate process involving the interplay of different molecules and hormones. Hormones are of cardinal significant in every process of egg formation; from the development of the reproductive tract, ovulation, albumen synthesis, eggshell formation, and finally to the oviposition of eggs. Major hormones that play a crucial role in the egg formation in laying hens are discussed below.

### 4.1 Role of gonadotropin-releasing hormone (GnRH) in egg formation

The GnRH in hens is released from the hypothalamic/portal system in response to the photo-stimulation and rising concentration of the progesterone. Two chemical forms of the GnRH are present in the avian species: chicken GnRH-I (cGnRH-I) and chicken GnRH-II (cGnRH-II) [32]. These two forms of the GnRH play different roles in the avian species. GnRH-I is vital for stimulating synthesis and release of anterior pituitary hormones, GnRH-II, on the other hand, is involved in mating and courtship behavior [33]. GnRH in hens are regulated by catecholamine, vasotocin, vasoactive intestinal peptide, neuropeptide Y and opioid peptides [34]. Recently, we detected the GnRH receptor in the oviduct of laying hens; however, its functional role in the egg formation is completely unknown.

### 4.2 Role of gonadotropins in egg formation

The gonadotropins; follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are produced at the anterior pituitary in response to the GnRH from the hypothalamus. FSH in the hen is responsible for the recruitment and granulosa cell development of the small follicles. FSH acts mainly on the granulosa layer of the small yellow follicles, and the sixth (F6) to third (F3) largest follicles. It also

stimulates progesterone production in granulosa cells from F6 to F3 follicles [35]. Sustained plasma concentration of the FSH remains throughout the ovulatory cycle except for a small increase at around 12 h before ovulation [36]. The LH in hens, unlike other mammalian species, does not luteinize the follicles, rather they are involved in ovulation and steroidogenesis [37]. Plasma concentration of the LH peaks at around 4–6 h before ovulation (coincide with the peak rise of progesterone), whereas lowest plasma concentration of LH is observed at 11 h before ovulation [38]. The primary target for the LH is larger preovulatory follicles.

### 4.3 Role of estrogen in egg formation

Estrogens are mainly produced by the theca cells of the small follicles. The highest plasma concentration of the estradiol occurs 4–6 h before ovulation although some small rise in estrogen is also observed at 18–23 h before ovulation. Estrogen plays a crucial role in the egg yolk formation by stimulating the avian liver to produce the yolk precursor, vitellogenin and very-low-density lipoprotein, the primary source of yolk protein and lipid, respectively [39]. Estradiol also sensitizes hypothalamus to the positive feedback effect of the progesterone. Besides the essential role of estradiol for the growth, and development of the oviduct, it also regulates calcium metabolism for the eggshell formation and development of secondary sex characters. [37]. Albumen is mainly synthesized in the tubular gland cells in the magnum and comprises mainly of ovalbumin, conalbumin, ovomucoid, and lysozyme. Estrogen is found to be associated with the synthesis of these molecules and thus, plays a crucial role in egg-white formation [40].

### 4.4 Role of progesterone in egg formation

Progesterone along with its cognate receptor regulates the female fertility [41, 42]. Progesterone is mainly produced by the granulosa cells of the larger follicles (F1–F3). The peak plasma concentration of the progesterone occurs 4–6 h before ovulation [38]. During the time of preovulatory LH surge, only the largest preovulatory follicles secrete progesterone. This increase in progesterone creates a positive feedback response to the hypothalamus, which in turn increases the secretion of GnRH into the hypothalamic-pituitary portal system producing the surge in LH from the anterior pituitary. This LH causes rupture and release of yolk (ovum) from the mature follicles (F1). Progesterone is also associated with the avidin production, contraction of the myometrium and eggshell formation [41].

### 4.5 Role of androgens in egg formation

Androgen is produced in theca and granulosa cells of both small and large follicles. Peak preovulatory concentration of testosterone occurs 6–10 h prior to ovulation, whereas the highest concentration of the 5α-dihydrotestosterone occurs 6 h before ovulation [41]. Role of androgen in ovulation is still obscured. Androgen is found to regulate ovomucoid and ovalbumin gene expressions in the oviduct of the chicken [43]. Androgens also help in the development of the secondary sexual characters in hens such as growth and coloring of combs and wattle.

## 5. Conclusion

In conclusion, hormones are required for the timely ovulation of yolk from the ovary, and preparation of oviduct for egg formation. Gene expressions in the

different segment of oviducts help in the cellular remodeling, secretion, synthesis and transport of essential molecules for the egg formation. Understanding of this information will be helpful in developing persistence layers with quality eggs.

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## Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of these information.

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Chapter 4

# Regulatory Mechanism and Application of lncRNAs in Poultry

*Zhuanjian Li, Tuanhui Ren, Wenya Li and Ruili Han*

## Abstract

Long noncoding RNA (lncRNAs) are transcripts greater than 200 nt in length with decreased coding potential and are widespread in all types of biological organisms. lncRNAs can interact with protein, DNA and RNA, respectively, which may participate in the multilevel regulation of transcriptional, post-transcriptional and epigenetic modifications. It is well known that lncRNA, which length is single-stranded non-coding RNA molecule, plays crucial roles in animal growth, development, cell proliferation and differentiation, and other life activities. In this research, we review the regulation mechanism and current research status of lncRNAs in chicken economic traits and disease, which would contribute to further understanding the regulatory mechanisms and application of lncRNAs in poultry.

**Keywords:** chicken, long noncoding RNAs, economic traits, regulation mechanism

## 1. Introduction

In the past half century, poultry industry has made great progress with the research of poultry genetic improvement and feed nutrition. Poultry industry is the fastest growing industry in animal husbandry, and it is also the industry with the highest degree of scale, intensivism and the most complete industrial chain, which plays an important role in the development of the whole animal husbandry economy. Over the past 40 years, the rapid development of poultry breeding, feed and farming has contributed to unprecedented growth in global poultry production and productivity. Poultry production rose from 15 million tonnes in 1970 to 95 million tonnes in 2010, making it the second largest consumer of meat after pork, with egg production rising from 20 million tonnes to 68 million tonnes. There is no doubt that world poultry consumption will continue to rise in the future as the world population increases from nearly 7 billion in 2010 to 7.5 billion in 2020. According to the forecast, by 2020, the world poultry meat production will reach 122.5 million tonnes, egg production will reach 72 million tonnes. Global poultry consumption will grow by 2.5% a year by 2030, and developing countries by 3.4%, far outpacing other meat consumption. However, with the gradual improvement of people's living standards, the demand of consumers for meat and eggs no longer stays on the quantity, and puts forward higher requirements for the sense and flavor of meat and eggs. At present, people expect the prominent contradiction between the improvement of meat and egg quality and the decline of meat and egg quality, which has become a practical problem to be solved urgently in front of the broad masses of poultry breeders. Therefore, the research on egg and meat quality has

become the focus of breeding workers in poultry industry, and the research on meat and egg quality has become one of the hotspots in animal science research.

In recent years, geneticists and breeders have carried out breeding of new poultry varieties (lines) through modern biotechnology, such as molecular breeding and transgenic, focusing on the selection of poultry growth speed and meat and egg quality traits. To some extent, the performance of poultry is improved. However, for a biological trait, it is not only controlled by DNA level genes, but also regulated by mRNA level before and after transcription, and this level of regulation is more comprehensive, systematic and accurate. Long non-coding RNA (lncRNAs) plays an important role in regulating gene expression at the transcriptional level [1–5]. It is traditionally believed that lncRNA does not have the ability to encode proteins. The length of transcripts of RNA is longer than that of 200 nt, which exists widely in a variety of organisms [6]. lncRNA is involved in many levels of regulation such as epigenetic modification [7, 8], transcription and post-transcription [3–5]. But in recent years some studies have found that a small amount of lncRNAs can encode peptides [9–14]. These studies suggest that the coding capabilities of lncRNAs are far more complex than previously thought and need to be further studied and refined.

At present, lncRNA has been found to play a key role in many life processes, such as cell differentiation and proliferation [15, 16], growth and development [17], organogenesis [18], immune response and tumorigenesis [10, 19]. Its discovery opens a new chapter for human understanding of non-coding RNA, and provide a new way of gene regulation in animal cells, as well as complements the more rapid and effective regulation of target mRNA molecules at the level of RNA. Anyway, lncRNA identification and functional annotation provide a new perspective for us to study the molecular mechanism of the formation of important economic traits in poultry.

## 2. Overview of lncRNA

ENCODE's research shows that about 80% of human genome sequences can be transcribed, while less than 2% of the human genome is used for protein translation, and most of the remaining transcripts are non-coding RNA (ncRNA) [20]. The central principle of molecular biology is that genetic information is transcribed from DNA to RNA, and then translated into proteins by RNA [21]. However, transcriptome sequencing analysis shows that non-coding RNA (ncRNA) is not translated into protein, but through a variety of regulatory mechanisms to change gene expression, such as RNA interference or overexpression can affect gene transcription or translation. According to the fragment size, ncRNA can be classified as short sequence ncRNAs and long sequence ncRNAs [22, 23]. Long non-coding RNA (lncRNA) is generally defined as a class of weak or non-protein coding potential, low species conservation, the length of transcripts longer than 200 nt RNA molecules, widely exist in a variety of organisms [6]. Compared with the widely reported miRNA, lncRNA in small ncRNAs, the functional mechanism analysis is relatively less. Compared with protein-coding genes, lncRNAs has complex species and mechanism of action, lower expression of lncRNAs, space-time and tissue-specific, and low sequence conservation in species. According to different forms, lncRNAs can be divided into several types. lncRNAs has PolyA tail and no PolyA tail. The position in the genome relative to the protein-coding gene can be divided into five types: sense type (the transcript overlaps with the exon of the coding ability gene) and antisense type (the transcript originates from the reversal of the protein-coding gene, antisense), intron type (transcripts derived from intron, intronic) of protein-coding genes, Intergenic noncoding RNA (intronic) and bidirectional (bidirectional) (**Figure 1**) [23].

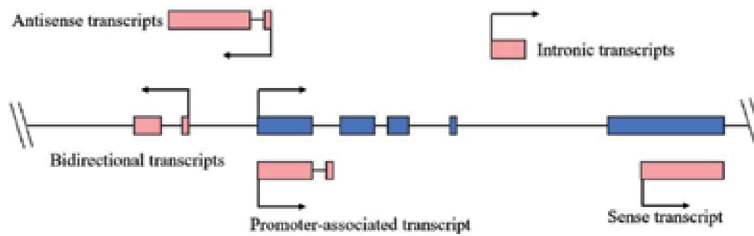


**Figure 1.**
*Classification of lncRNAs based on their genomic regions. Blue and orange are exons encoding RNA and non-coding RNA, respectively [23].*

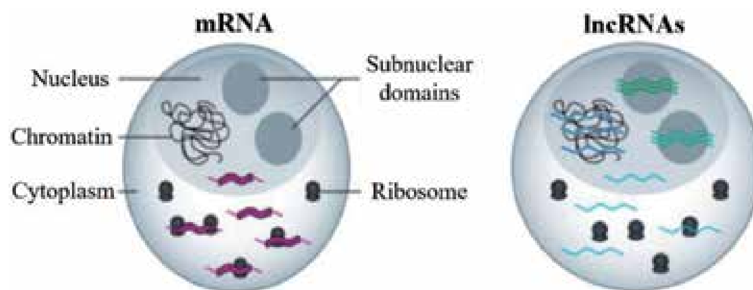


**Figure 2.**
*Subcellular localization of lncRNAs [27, 28].*

Most of the annotated lncRNAs are transcribed by polymerase II, so they can be capped, polyadenylated and cut like mRNA [2, 24]. Intergenic noncoding RNA (lincRNA) is transcribed in the intergenic region of the genome, while the natural antisense transcript, NATs is derived from a relative chain of protein-encoded genes. By far, lincRNA is the most abundant category of lncRNAs. Compared with mRNA, lncRNA may contain fewer exons than mRNA and usually have weak splicing and polyadenylation signals [25]. Similar to proteins, the function of lncRNA depends on the cell compartment in which they are located, and its localization information can provide some reference for predicting its function [26]. lncRNAs can be divided into cytoplasm and nuclear lncRNA, according to their cellular localization. Some lncRNAs are located in the nucleus and also exist in the cytoplasm. The different localization of lncRNA may play a different regulatory mechanism. lncRNAs, which exists in the cytoplasm, can bind through the interaction between the double strands of RNA, and then participate in the regulation of mRNA expression and maintain its stability. For example, Linc-MD1 is mainly expressed in the cytoplasm as ceRNA regulates the differentiation of skeletal muscle [17]. If lncRNA is located in the nucleus, it may have the ability to directly bind the target gene to inhibit or activate the target gene expression, and may also be involved in the recruitment of transcription factors or histone modified complex to mediate the regulation of gene expression. For Xist localizes the inactivated X chromosome in the nucleus and affects gene transcription by modifying the chromatin structure (**Figure 2**) [27, 28].

## 3. Biological functions of lncRNAs

Transcriptional, posttranscriptional and epigenetic modifications of lncRNAs in different cellular environments and biological processes have

regulatory effects on gene expression [24]. A large number of studies have shown that the mechanism of ncRNA, miRNA is through the complementary combination of RNA-RNA [29]. Compared with miRNAs, lncRNAs have longer sequences and more complex spatial structures, so they have more mechanisms and more information [30]. lncRNAs has many different mechanisms, including molecular decoy, molecular scaffold, molecular signal and molecular guide, which can regulate gene expression by cis-action, trans-action and antisense interference [31]. Although many of the functions and mechanisms of lncRNAs are still unclear, some of the mechanisms of lncRNAs that have been studied earlier have been fully elucidated. lncRNA has been reported to be involved in cell differentiation and proliferation, growth and development, and organ formation, immune response and tumorigenesis and other important regulatory processes [32].

### 3.1 Regulation of lncRNAs at transcription level

Transcriptional regulation is an important part of eukaryotic gene expression regulation, and it is the most important regulation mode of gene expression. lncRNAs can bind regulatory sequences to form DNA-RNA stable triple complexes to inhibit transcription. For example, the upstream promoter of DHFR gene of human dihydrofolate reductase can transcribe an lncRNA, and the transcribed lncRNA can form an lncRNA-DNA complex through the DNA sequence with the promoter, which can inhibit transcription [33]. The widespread transcription of enhancers and promoters indicates the core role of lncRNA in the regulation of gene transcription, and the way lncRNAs regulates transcription exists in a variety of mechanisms [34].

Proximal promoters can be transcribed into transcriptional programs with the function of recruiting RNA binding proteins, such as inhibiting the transcription of cyclin (Dl) in human cell lines [34]. The damage signal of DNA can induce the expression of lncRNA related to Dl gene, and co-regulate the activity of RNA binding protein (TLS). TLS can inhibit the activity of histone acetyltransferase p300 and CREB binding protein so as to inhibit the expression of Dl. Depending on the location, lncRNAs can positively or negatively regulate the expression of adjacent genes. When lncRNA is located near the upstream region of the protein-coding gene, the transcription of lncRNA may affect the binding of the transcript factor to the promoter region of the downstream protein-coding gene, such as the elongation of the transcription of lncRNA SRG1 in yeast, which occupies the downstream SER3 promoter region. Hinders the binding of transcription factors to the SER3 promoter, resulting in SER3 not being transcribed normally [35, 36], lncRNA acts as a coactivator to regulate the activity of transcription factors. In mice, the hyperconserved distal enhancer of Dlx6 gene transcribes lncRNA Evf2, Evf2 as a coactivator of homologous structural protein DLX2 and forms a complex with DlX2 [22]. lncRNA can also bind directly to transcription factors, promoters or polymerase II of genes, preventing gene promoters from binding with polymerase II to play a regulatory role [36, 37]. lncRNAs regulate transcription through several mechanisms as **Figure 3**.

### 3.2 Regulation of lncRNAs at post-transcriptional levels

lncRNAs can regulate the post-transcriptional process of mRNA through complementary binding sequences, including the selective variable splicing of mRNA precursors, acting as a sponge to adsorb miRNA, translation regulation, mRNA degradation mediated or maintenance of mRNA stability in **Figure 4** [24, 38].

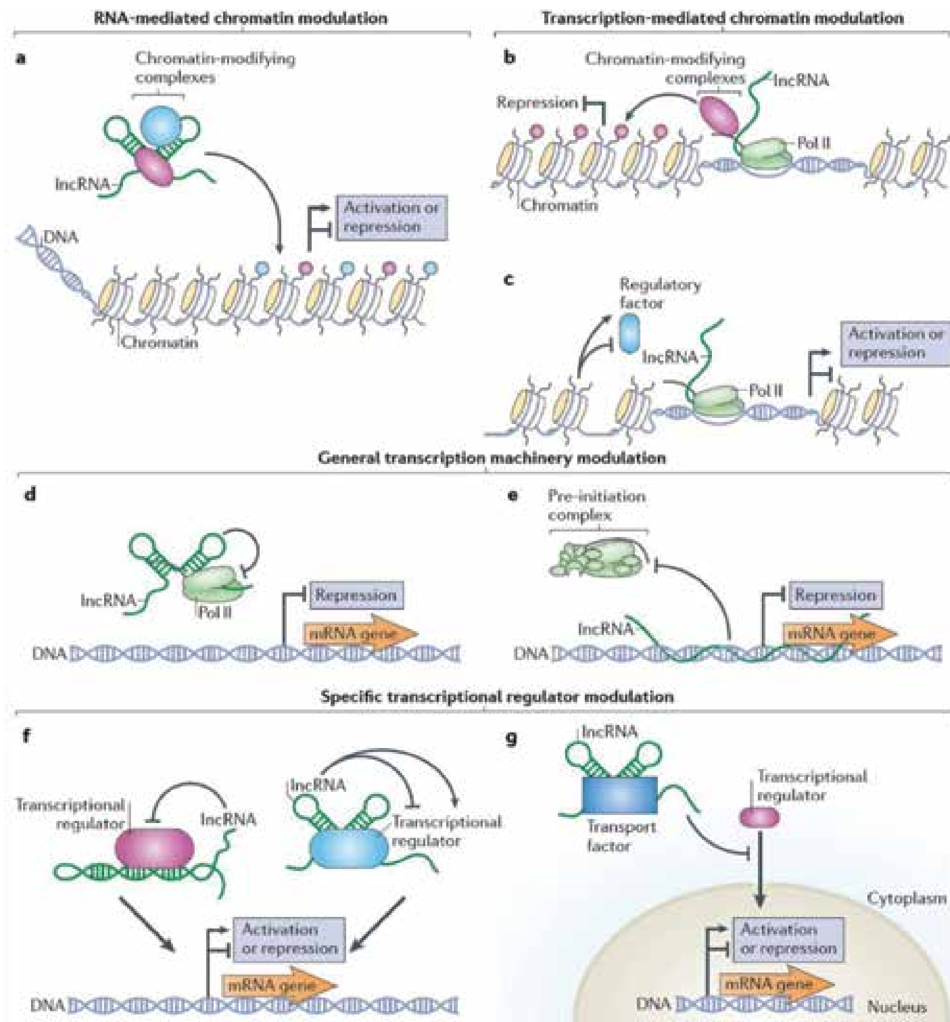


**Figure 3.**
*lncRNAs regulate transcription through several mechanisms. (a–c) Long non-coding RNAs (lncRNAs) can modulate chromatin through transcription-independent (part a) and transcription-dependent mechanisms (parts b and c). lncRNAs can modulate both the general transcription machinery (parts d and e) as well as specific regulatory factors (parts f and g). This picture is quoted from [38].*

### *3.2.1 lncRNAs combined with alternative splicing factor to regulate alternative splicing*

In previous studies, variable splice of mRNA was mostly reported. For example, the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is abnormally expressed in cancer, binds SR protein to regulate SR phosphorylation level in normal cells, and then regulate the variable shear of pre-mRNA, MALAT1 can also regulate the distribution of SR in cells, the absence of MALAT1 in cells will lead to the increase of SR protein level, giving priority to the form of dephosphorylation, thus changing the variable splicing mode of pre-mRNA [39]. Antisense lncRNAs can cover mRNA homeotropic elements (cis-elements) through the formation of double strand of RNA, which can directly regulate the variable splice of pre-mRNA. For example, antisense lncRNA Zeb2 (sip1) complements the 5′ splicing site of intron 5′ in zeb 5′UTR, which is a zinc finger Ebox homeotypic gene, and causes intron retention, which enables Zeb2 to be efficiently expressed and translated [40].

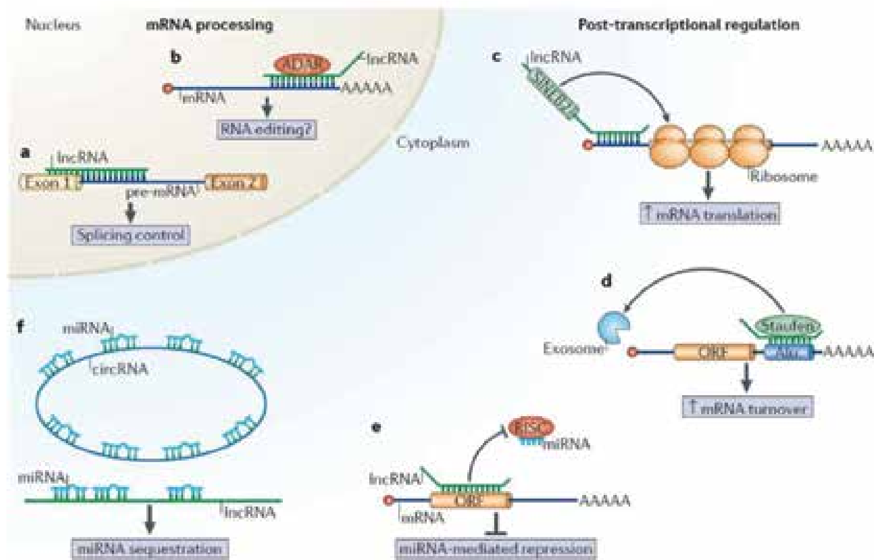


**Figure 4.**
*lncRNAs influence mRNA processing and post-transcriptional regulation. (a and b) Long non-coding RNAs (lncRNAs) can modulate mRNA processing. (c–f) lncRNAs modulate post-transcriptional regulatory events. This picture is quoted from [38].*

### *3.2.2 lncRNAs participates in the stability of mRNA and regulates translation*

Antisense lncRNA can form a protective mechanism of mRNA by binding to 3′UTR sites on mRNA that can be degraded by miRNA. For example, the natural antisense transcripts of beta-amyloid converting enzyme 1 (BACE1) gene, BACE1-AS, can bind to BACE1 mRNA to form a stable RNA double strand, which will block the recognition site of mRNA degradation mediated by miRNA, prevent BACE1 mRNA degradation, and lead to the up-regulation of BACE1 protein product expression [24]. In addition, lncRNAs can regulate the translation of mRNA, when RNA binding protein HuR or Ago2 is absent in cells, the stability of lincRNA-p21 is increased, and lincRNA-p21 binds to target mRNA and inhibits its translation [41].

### *3.2.3 Complementary binding of lncRNAs and mRNA to mediate their degradation*

The abundance of mRNA is an important factor affecting protein yield, which is mainly determined by the degradation rate and transcription quantity. In eukaryotic organisms, there is nonsense-mediated RNA degradation (NMD). RNA-binding protein STAU1 increases protein 1 by binding to NMD factors and recruits it to the 3′UTR of the RNA, which leads to the degradation of the RNA, this is known as the Stau1 mediated mRNA degradation mechanism [42].

### *3.2.4 lncRNAs act as an adsorption sponge for miRNA*

miRNA is an important member of ncRNAs, which does not have a ORF box and has a short sequence of 18–25 nt [38]. For example, lncRNAs are used as molecular decoys for the specific adsorption of miRNA, which indirectly regulates the expression of miRNA target genes. This mode of action is called “sponge effect” of lncRNAs [24]. lncRNAs regulates miRNA by binding to miRNA responsive element MRE, and this competitive binding is mutually regulated. Such as some studies reported that a 3′UTR of lncRNAs contains a certain number of MREs, when the microRNAs are adsorbed by MRE of lncRNAs, they can act indirectly on

the microRNAs through trans or directly on the lncRNAs through cis [43, 44]. In addition, lncRNA can complement the sequence of the target gene and form ceRNA mechanism with miRNA, which can relieve the inhibition of the target gene. In adipocytes, ADNCR can act as an adsorptive sponge of miRNA-204, and then release the inhibition of miRNA-204 on its target gene SIRT1. The expression of SIRT1 increases and binds to the transcription co-inhibitors SMRT and NCoR, which led to the inhibition of adipogenesis [45].

### 3.3 Regulation mechanism of lncRNAs on epigenetics

#### *3.3.1 lncRNAs and DNA methylation*

In mammals, DNA methylation is a key form of epigenetic modification [46]. For example, X chromosome inactivated transcription (Xist) is the earliest discovered lncRNA. Its expression makes a large number of histone methylated, thus causing the X chromosome inactivation. In mammals, lncRNA deactivation can mediate X chromosome inactivation, which is also regulated by other lncRNAs, Tsix and Jpx encoded in the X chromosome inactivation center. Jpx can activate deactivation via cis or trans. Tsix changes the chromatin state through the action of cis, interferes with transcripts and regulates DNA methylation, resulting in silencing of Xist expression [24].

#### *3.3.2 lncRNAs and histone modification*

lncRNAs recruit DNA sequences or protein complexes related to chromatin modification to their adjacent genes and regulate adjacent genes via cis or trans. For example, lncRNA HOTMR can promote the trimethylation on lysine 27 of histone 3 by recruiting multiple comb inhibiting complex (PRC2), thereby inhibiting gene expression [47]. It is reported that lncRNA HOTAIR, which can act as a skeleton molecule, participates in epigenetic regulation by changing chromatin state. The 5′ and 3′ of HOTAIR can bind to different histone modified complexes respectively. Its 5′ can bind to PRC2 protein complex, and its 3′ can bind to CoREST/LSD1/REST protein complex. The 5′ of HOTAIR has demethylation function, while the 3′ has the function of promoting methylation, which regulates different methylation forms of different target genome proteins and then regulates gene expression [48]. Overexpression of lncRNA HOTAIR can reposition the PRC2 complex in the whole genome, induce silencing of specific tumor suppressor genes, and promote metastasis of breast cancer [49]. lncRNA ANRIL, which has similar function to HOTAIR, can change chromatin state by recruiting PRC2 and PRCl complex, decrease the expression of cyclin dependent kinase inhibitor 2A(CDKN2A) gene, enabling cancer cells to proliferate indefinitely [50].

#### *3.3.3 lncRNAs and chromatin remodeling*

Chromatin remodeling is mainly involved in the changes of nucleosome translocation, recombination and decreased stability. Nucleosome translocation and recombination can change the affinity between DNA regulatory sequence and transcription factors, and then affect the expression of related genes. lncRNAs can change chromatin structure through epigenetic approach, and can also affect chromatin conformation through direct binding [51–53].

In addition, lncRNAs can also participate in genomic imprinting. Interference with lncRNAs can alter chromatin condensation, suggesting that their binding may make the chromatin spatial structure more stable or facilitate chromatin/nonhistone binding [54]. So there are many other mechanisms in lncRNA that need further analysis.

## 4. The study of antisense long noncoding RNAs

Natural antisense transcripts, Nats, also called natural antisense RNA, is a complementary transcription product of sense RNA produced in natural organisms, which is transcribed by antisense strand in DNA double strand and processed by RNA polymerase II (RNA polymerase II) [55–59]. NATs can regulate the expression of mRNA and protein at the transcriptional and post-transcriptional levels [60]. The researchers classified NATs transcriptional initiation sites (TSS) as cis NATs (cis-form NATs) and trans NATs (trans-formNATs) [61]. Cis-NATs means that the TSS position of NATs is the same as that of sense chain mRNA, but it is located on antisense chain, and the nucleic acid sequence is completely reverse complementary to the sense chain gene mRNA [62] (**Figure 5a**). Trans-NATs means that NATs TSS is still antisense, corresponding to different exons or introns of the sense chain gene (different from the TSS position of the sense chain mRNA), but the nucleic acid sequence complements partially or completely with the sense chain gene mRNA (**Figure 5a**) [63]. In addition, the transcripts of sense and antisense chains can be divided into bidirectional transcripts and unidirectional transcripts [64]. The transcripts of sense and antisense strands can be divided into bidirectional transcripts and unidirectional transcripts according to whether the transcripts of the sense and antisense strands are derived from the same TSS in different transcriptional directions. In addition, we can refer to the relative position of NATs and sense chain gene mRNA to classify them. (1) Head-to-head overlap (head-head): partial or total reverse complementation of the 5′ regions (5′UTR) of sense and antisense transcripts; (2) tail-to-tail overlap (tail-tail): That is, the three untranslated regions

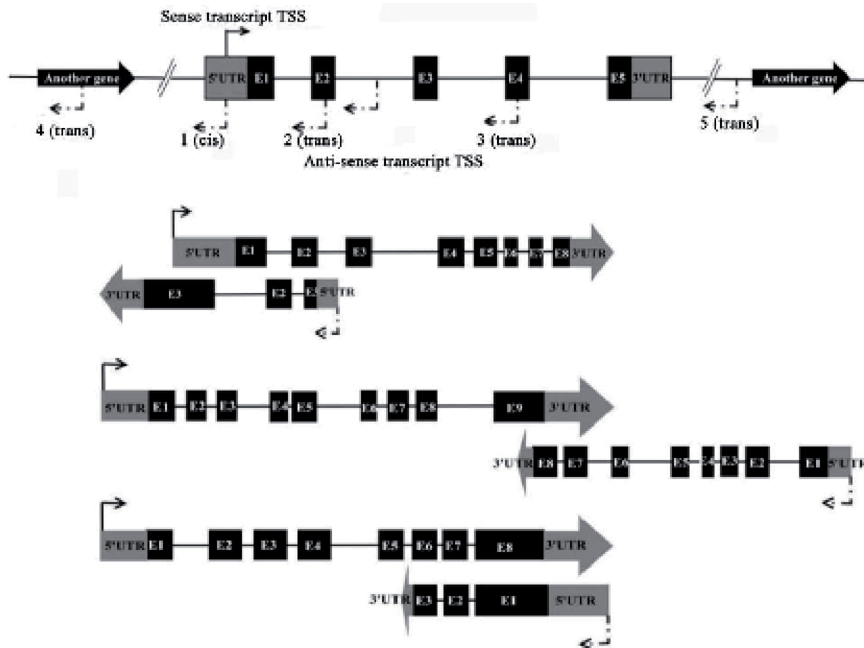


**Figure 5.**
*The classification of NATs. ■ is denoted as an exon, ➡ denotes transcripts of other genes adjacent to the gene, ↱ is expressed as a transcript of the sense chain. TSS, ⇠ is the TSS of antisense strand transcript. (a) The transcriptional initiation sites are different according to the antisense chain transcripts. Among them, 1 is cis-NATs, 2–5 is the trans-NATs transcribed by TSS in different position; (b) head-to-head overlap (head-head), drawing with DHRS4, AS1DHRS4 as template [59]; (c) tail-to-tail overlap (tail-tail): Drawing with WDR83, DHPS as template [60]; (d) complete overlap, drawing with GHR-S, GHR-AS as template [61].*

of sense and antisense chain transcripts, partially or completely reverse complementary; (3) complete overlap: that is, the sequences of the two transcripts are completely reverse complementary (**Figure 5b–d**).

Most of the NATs discovered so far are long-chain non-coding RNAs, which play a variety of important biological functions by regulating the expression of the corresponding justice chain protein-coding genes and has attracted more and more attention. Current studies have shown that there are three main mechanisms of NATs: first, antisense RNA and sense RNA form double-stranded RNA, through complementary regions of the sequence to regulate the stability and translation of RNA; For example, human Wrap53 regulates p53 in this way and plays a role in tumorigenesis [65]. Second, antisense RNA regulates sense RNA through epigenetic modifications, thereby participating in biological processes. For example, p15AS can regulate the expression of p15 gene and promote the proliferation of mouse embryonic stem cells by changing the methylation status of H3K4 (Histone 3 methylated at lysine 4) and H3K9 (Histone 3 methylated at lysine 9) in the promoter region of p15 gene [66]. Similarly, mouse BDNF-AS affects the proliferation of nerve cells and the development of nervous system by changing the modified state of H3K27me3 (the trimethylation on lysine 27 of histone 3) in the promoter region of BDNF [67]. Thirdly, antisense RNA protects and transports sense RNA, such as PTENpg1 antisense RNA, which transports PTENpg1 from the nucleus to the outside of the nucleus by forming a double strand with the mRNA of PTENpg1, thus avoiding the degradation of PTENpg1 and making PTENpg1 play a further role in the fine cytoplasm [68].

## 5. Regulation of muscle development by lncRNAs in poultry

The muscle content of animals is mainly determined by the total number of muscle fibers, muscle fiber length and muscle fiber size. Muscle content is also affected by different muscle fiber types [69, 70]. In general, chickens and mammals do not increase the amount of muscle fibers around a week before and after birth [71]. The formation of animal muscles depends mainly on the differentiation and proliferation of muscle cells in embryonic, while the growth of muscles is mainly dependent on the hypertrophy of pre-formed muscle cells in postnatal. In the study of chicken muscle development, the first systematic identification of lncRNAs using RNA-Seq to sample the transcriptome, the results identified 281 new intergenic lncRNAs in the chicken genome, these lncRNAs in general are less conserved than coding genes [72].

In recent years, there are some reports about the lncRNA regulated muscle development in chickens [73]. lncRNA pouMU1 was most abundant in the leg muscle and breast muscle tissue, and low or no expression in other tissues. The relative 1 day pouMU1 expression levels were significantly higher than the 6 and 16 weeks in breast muscle. Two mutations of g. 1198A>G and g. 1238-1239del/insGA combination in pouMU1 showed significant associations with leg muscle fiber width and leg muscle fiber roundness, and highly significant associations with leg muscle fiber girth and BW0. These results suggest that the pouMU1 gene may play an important role in early stage muscle development in chickens [32]. lncRNA-Six generated a micropeptide of about 7. 26 kDa was found to play an important role in the lncRNA-Six1 cis activity. Based on qPCR, the lncRNA-Six1 and Six1 mRNA were highly expressed in chicken breast muscle. Overexpression of lncRNA-Six1 promoted the mRNA and protein expression level of the Six1 gene, while knockdown of lncRNA-Six1 inhibited Six1 expression. lncRNA-Six1 overexpression promoted cell proliferation and induced cell division. Conversely, its loss of function inhibited

cell proliferation and reduced cell viability. Overexpression or knockdown of Six1 promoted or inhibited, respectively, the expression levels of muscle-growth-related genes, such as MYOG, MYHC, MYOD, IGF1R, and INSR. The results demonstrate that lncRNA-Six1 carries out cis-acting regulation of the protein-encoding Six1 gene, and encodes a micropeptide to activate Six1 gene, thus promoting cell proliferation and being involved in chicken muscle growth [74]. Taken together, these findings will contribute to further understanding the regulatory mechanisms of lncRNAs in chicken muscle development.

## 6. Regulation of lipid metabolism by lncRNAs in poultry

Abdominal fat is an important carcass trait of chickens. In the last decades, the overemphasis on selection for rapid growth rate leads to excessive fat accumulation in chickens, and excess fat deposition results in reduced feed conversion ratio and carcass yield [75]. The previous report indicated that lncRNAs can regulate adipogenesis and other processes associated with metabolic tissue in cattle and pig [45, 76]. However, little is known about the lncRNAs regulation mechanism during preadipocyte differentiation in the chicken. In recent studies, a total 3095 differentially expressed genes were obtained by pairwise comparison of preadipocytes at different times. The differentially expressed genes were involved in glycerolipid metabolism, and the mTOR signaling, PPAR signaling, and MAPK signaling pathways in chickens [75]. 2193 lncRNA genes were predicted in the chicken liver and adipose tissue by RNA-Seq, among which 1670 were stable expressed in the liver and/or adipose tissue, and which were divided into 177 intragenic and 1493 intergenic lncRNAs located between and within protein-coding genes, respectively. And the study observed similar structural traits between chickens and mammals, have significant synteny conservation but without sequence conservation. In addition, lncDHCR24, a novel lncRNA candidate involved in lipid metabolism, which is very high relevance with the DHCR24 gene that encodes a key enzyme of cholesterol biosynthesis [75]. In a second study, using RNA-seq sequencing, 4698 differentially expressed lncRNAs were obtained by pairwise comparisons of samples collected from preadipocytes at days 0, 2, 4, and 6 of differentiation, and 7 lncRNAs genes were differentially expressed in the entire differentiation process intramuscular preadipocytes, implying their importance in the intramuscular preadipocytes in Jinghai Yellow chicken [77].

## 7. Regulation of poultry reproduction by lncRNAs (sperm, egg production)

In the study of human disease and model animal reproduction, lncRNA plays an indispensable role is involved in early embryonic germ cell formation, early embryo implantation and development, and related the regulation of hormones [78–80]. lncLER gene is significantly highly expression in the liver tissue of Lushi chicken. The expression of lncLER was upregulated in the chicken liver and liver primary cells of chicken embryos by the estrogen treatment. The study indicated that the expression of lncLER is regulated by estrogen, and lncLER may play an important role in liver fat metabolism and yolk precursor synthesis in Lushi chicken [81]. Sperm motility is the most important indicator in evaluating roosters' fecundity. lncRNA play epigenetic roles in reproduction. In previous study, RNA sequencing was employed to analysis the testis transcriptome of 3 Beijing-you roosters of high sperm motility and 3 with low sperm motility. In total, 2597 lncRNAs were identified in the chicken testis, including 124 differentially

expressed lncRNAs. In addition, 544 mRNAs of differential expression were founded in the study. LOC428510 is the target gene of lncRNA MSTRG. 4081 by predict of software. LOC428510 and DNAH5 gene have similar structure, DNAH5 is an important motor protein of sperm movement, and mutation of DNAH5 may cause the sperm immobility. Meanwhile, the LOC428510 and lncRNA MSTRG. 4081 have co-expression characteristics by qPCR. These results suggested that MSTRG. 4081 and LOC428510 may be involved in the regulation of sperm motility in roosters [82]. MHM is a Z sex chromosome-linked locus adjacent to the DMRT1 gene, is methylated and transcriptionally silent in male chicken cells, but is hypomethylated and transcribed into a lncRNA in female chicken cells. In males, MHM mis-expression impairs gonadal expression of the testis DMRT1 gene and causes an increase in male-biased embryo mortality [83].

## 8. The role of lncRNAs in disease resistance breeding of poultry

Most evidence suggest that lncRNA plays a key role in the human disease, such as growth and metastasis of tumor cell [84]. There are also a few reports on lncRNA in disease research of poultry.

Gallid herpesvirus 2 (GaHV-2) is an oncogenic alpha-herpesvirus of chickens, previously known as Marek's disease virus (MDV-1) in chicken [85]. The RNA-specific adenosine deaminase (ADAR1) belongs to the ADAR family of proteins, which play a key role in innate immunity to viral infections. The ERL lncRNA, a new viral lncRNA, which is expressed during all phases of infection, and this lncRNA is a natural anti-sense transcript. This study showed that mdv1-miRM4-5p is the most strongly expressed miRNA of the mdv1-mir-M9-M4 cluster encoded by the anti-sense strand of the ERL lncRNA, promoted the overexpression of ADAR1 by downregulating the suppressor of cytokine signaling 1 [86].

Hyperpigmentation results in aberrant immune cell development in Silky Fowl, while, whether the melanocytes regulated B-cell proliferation in the bursa of Fabricius or not is unclear. The bursa of Fabricius development was relatively slower in Silky Fowl than in White Leghorn. Identified 4848 differentially expressed genes, 326 lncRNAs, and 67 microRNAs by the transcriptome analyses in the bursa of Fabricius of Silky Fowl. The annotation of the predicted targets indicate that differentially expressed lncRNAs were mainly associated with cell proliferation pathways, such as WNT, MAPK, JAK-STAT, and Notch signaling pathways. The lncRNAs and microRNAs can regulate the JAK2, STAT3, and IL-15 genes by predict in chicken. Thus, B-cell development in the bursa of Fabricius of Silky Fowl might be regulated and affected by lncRNA [87]. Avian leukosis virus (ALV) can causes substantial economic losses from increased mortality and decreased performance of chicken. Avian leukosis virus subgroup J (ALV-J) is harm the largest in the six subgroups of ALVs. Specific Pathogen-Free female chickens were infected with ALV-J or maintained as non-injected controls. Then, spleen samples were collected at 40 days and sequenced. There are differentially expressed 17 lncRNAs, 7 miRNAs and 864 genes has been identified in infected and non-infected birds. The mRNA with miRNA, lncRNA and virus genes identified key elements within the complex networks utilized during ALV response by co-expression network analysis [88]. In a second study, using high throughput transcriptome sequencing of HD11 and CEF cells infected with ALV-J, in total of 4804 novel lncRNAs were identified, including intergenic lncRNAs, antisense lncRNAs and intron lncRNAs. The results suggested that NONGGAT001975.2, NONGGAT005832.2 and NONGGAT009792.2 may be associated with immune response regulation by qRT-PCR analyses in vivo, and could function as novel biomarkers for ALV-J infection [89]. Se, as an essential micronutrient, plays a pivotal

role in various biological activities and Se deficiency induces disease of nutritional muscular dystrophy and exudative diathesis in chickens. A recent report showed that the increased expressions of IL-1β, IL-6, IL-8, and CCL4, and the decreased expressions of SCD, PPARα, PPARβ and PPARγ by silencing of ALDBGALG 0000005049 in chicken myoblasts. In addition, increased expressions of IL-1β, IL-6, IL-8, and CCL4 and inflammatory cell infiltration in microstructure of chicken muscles treated with Se deficiency were observed. This study revealed that downregulation of ALDBGALG0000005049 caused inflammation by regulating stearoyl-CoA desaturase in chicken muscle resulted from Se deficiency [90].

## 9. Study on antisense lncRNAs in poultry

Numerous studies have demonstrated that about 20% of the genes are capable of producing antisense transcripts (NATs) in the human cell transcriptome, and more than 70% of the transcription units in the mouse genome may produce NATs [25, 91]. Compared with human and mouse, the research of NATs is relatively lagging in chicken.

The programmed cell death 2 (Pdcd2) gene was assessed as a member of a highly conserved synteny on mouse chromosome 17. The identified Tbp-alternative Pdcd2-antisense transcripts maybe to play some regulatory role in gene expression, compared to the protein-coding function of the Tbp mRNA. The antisense transcripts are mostly localized in the nucleus and transcribed at a level sufficient to interfere with the transcription of the Pdcd2 gene. The conservation of Pdcd2/Tbp sense/antisense overlap in the mouse and chicken also point out their biological relevance. Moreover, the results suggest that at least some of the cDNAs identified in the sequencing projects labeled as noncoding RNAs are in fact incomplete alternative cDNAs of neighboring protein-coding genes. The conservation of alternative transcription of the Pdcd2 gene have different the biological importance in mouse, human and chicken [92]. Growth hormone receptor (GHR) play pivotal roles in human and animal growth. The mutation of GHR gene could lead to human laron type dwarfism and sex-linked dwarf chicken. A previous study identified an endogenously expressed long non-coding NAT, GHR-AS, which overlapped with the GHR mRNA (GHR-S) by a tail to tail manner. qRT-PCR analyses indicated that GHR-AS were highly expressed in chicken liver, and displayed increasing with the development of chicken from E10 to 3 w of age. Interfering GHR-AS could lead to GHR-S decreasing, accompanied with increasing of the H3K9me2, an inactive gene indicator, in the GHR-S promoter regions in LMH cells. RNase A protection experiment showed that GHR-AS and GHR-S can form double strand RNAs at the last exon of GHR gene in vivo and in vitro. Meanwhile, the expression levels of GHR-S and GHR-AS can be affected by DNA methylation. Compared the dwarfs with the normal chicken, the negative correlation trends were indicated between the GHR-S promoter methylation status and the GHR-AS expression levels. These results showed that GHR gene possessed NAT, and the results presented here further highlight the fine and complicated regulating mechanism of GHR gene in chicken development [57].

## 10. Conclusions

With the development of life sciences, the lower cost of RNA-Seq has become more and more popular in ncRNA research. In recent years, a large number of RNA-seqs have also been performed in livestock animals. The researcher used RNA-Seq data facilitates the analysis of the regulatory mechanisms of lncRNA in specific tissues, organs and cells. The study of lncRNAs fills the gap in the molecular

mechanism of life activities in organisms. lncRNA can play the functions of signal molecules, scaffold molecules, decoy molecules and guiding molecules. And lncRNAs participate in multiple levels regulation of epigenetic, transcriptional and post-transcriptional modifications.

At the present, there are few reports on lncRNA in chicken breeding. Compared with experimental animals, the lncRNA research of chicken are relatively backward, and any reports mainly focuses on the discovery and characteristic analysis of lncRNA in chickens. lncRNAs becomes one of research focuses in life sciences field and increasing lncRNAs has identified in human and mouse. However, there are many problems with the study of lncRNA related to poultry. lncRNA is not highly conserved among species compared with miRNA and mRNA, and it is difficult to learn from each other in species, there are few tools for studying advanced structures, and it is difficult to analyze the real-time dynamic changes of lncRNA in cells. In order to improve economic traits and accelerate breeding of chicken, the researchers could through the establishment of RNA libraries, the use of high-throughput sequencing technology and bioinformatics analysis methods to predict the sequence and structural characteristics of lncRNA, found many new lncRNAs; the candidate lncRNA was verified and determine its expression difference by Northern Blot and PCR technology. The functions of lncRNA were studied by in situ hybridization, overexpression, interference, qPCR, and in vivo experiments; and RNA pull down, RNA-RIP, ChIRP-seq and dual luciferase reporter systems were used to the mechanism of lncRNA. Optimization of these techniques can accelerate the study of chicken-related lncRNAs and dig out more functions. However, how to systematically and specifically study the function of lncRNAs in important economic traits of poultry, is a problem that researchers need to solve in recent years.

## Acknowledgements

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## Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 5

# Some Statistical Analysis of Poultry Feeds Data

*Akinlolu Adeseye Olosunde*

## Abstract

In this study we presented generalized exponential power distribution as an alternative to normal distribution commonly used in the analysis of agricultural data. The distribution which is more robust in modeling because of the present of shape parameters, which regulates it tails. Some of its mathematical and statistical properties are examined. The application of the probability density function is demonstrated in fitting poultry feeds data. The goodness-of-fit test was carried to show that it is a better substitute to normal distribution in applications.

**Keywords:** exponential power distribution, normal distribution, goodness-of-fit, poultry feeds data, Pearson's $\chi^2$ test, Kolmogorov-Smirnov test

## 1. Introduction

Cholesterol is a waxy substance that comes from two main sources: the liver and food intake. It has been noted that high level of cholesterol can block the arteries, decrease blood flow to other tissue in the body, thereby causing heart diseases [1]. Eggs have commonly been jettisoned owing to high cholesterol contents, therefore lowering consumption rate. But [1] further note that eggs are high quality source protein and other nutrients. It is also well known that eggs contain lecithin and phospholipids, necessary for the construction of brain cell membrane. In terms of feeding intellect, their value lies mainly in the quality of their proteins, they are actually rich in amino acids, essential in the production of the principal neurotransmitters. Hence, instead of a total boycott, it is better to reduce the risk associated with eggs consumption.

In order to alleviate the problem associated with consumption, an organic copper salt combination was used instead of the inorganic combinations currently used in preparation of poultry feeds. Ninety-six chickens were randomly selected and randomly divided into 2 groups of 48 each. Each group was subjected to the same general conditions and treatments, differing only in that while, the chickens in the first group were fed with inorganic copper salt, those in the second group were fed with inorganic copper salt. After 4 months the weight (gram) and cholesterol level (mg/egg) of the eggs yielded by the two groups were measured. The excerpt from the data used in this analysis is presented in the Appendix A courtesy of Federal University of Agriculture, Abeokuta.

Carrying out analysis of the data obtained from the laboratory study, it is a common assumption in literature to assume normal distribution in the analysis of agricultural data. This assumption is not always true especially in the current case. Therefore, we proposed to study a generalized form of normal distribution called



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the exponential power distribution and its multivariate extension, which contains normal distribution and others in the literature as special cases in fitting poultry feeds data. This unifying exponential power distribution is characterized by a parameter $\beta$ and a function $h(\beta)$ which regulates the tail behaviour of the distribution, thus making it more flexible and suitable for modeling than the usual normal distribution, while retaining symmetry properties. Finally we fit the generalized exponential power distribution as well as the normal distribution to data on eggs produced by chicken on each of two different poultry feeds (inorganic and organic copper salt compositions) and show that the generalized exponential power distribution fit is considerably better. We then use the Kolmogorov-Smirnov two samples one-tailed test to show that there is an increase in egg weights and decrease in cholesterol level when the feed is organic.

## 2. Exponential power distribution

The exponential power distribution is a class of densities which includes the normal and allows thick tails, Thus making it more suitable in modeling when compared with the usual normal distribution. In fact, it is a natural generalization of the normal distribution and also used in applications by [2–8]. They also presented a multivariate version of the exponential power distribution and [5] used this distribution to model repeated measurements. We present this distribution with a proposition. Codes were written in R environment to estimate the parameters of both the univariate and the multivariate version of the distribution see [7, 9, 10].

**Proposition 2.1** *let X be a random variable then*,

$$f(x;\mu,\sigma,\beta)=\frac{\beta h(\beta)}{2\Gamma\left(\frac{1}{\beta}\right)\sigma}\exp\left\{-\left[\frac{h(\beta)|x-\mu|}{\sigma}\right]^{\beta}\right\} \tag{1}$$

is a probability density function (p.d.f.) with three parameters $\beta > 0$, $\sigma > 0$, $\mu \in \mathfrak{R}$. The tail region is regulated by the function $h(\beta)$, which is positive for all $\beta > 0$.

If a random variable $X$ has the p.d.f (1) then its mth moments can be obtained from the relation

$$E(X^m)=\int_0^\infty\left(\left(\left[-1^m\left(\sigma(2z)^{\frac{1}{2\beta}}-\mu\right)^m\right]+\left(\sigma(2z)^{\frac{1}{2\beta}}+\mu\right)^m\right)\left(\frac{z^{\frac{1}{2\beta}-1}\exp^{-z}}{2\Gamma\left(\frac{1}{2\beta}\right)}\right)\right)dz$$

In addition, its central moment estimates Agro [11–14] are:

$$E(X)=\mu;E|X-E(X)|=\frac{\sigma 2^{\frac{1}{2\beta}}\Gamma\left(\frac{1}{\beta}\right)}{\Gamma\left(\frac{1}{2\beta}\right)};Var(X)=\frac{\sigma^2 2^{\frac{2}{2\beta}}\Gamma\left(\frac{3}{2\beta}\right)}{\Gamma\left(\frac{1}{2\beta}\right)}; \tag{2}$$

$$E(X-E(X))^3=0;E(X-E(X))^4=\frac{\sigma^4 2^{\frac{4}{2\beta}}\Gamma\left(\frac{5}{2\beta}\right)}{\Gamma\left(\frac{1}{2\beta}\right)}; \tag{3}$$

and Kurtosis=

$$\frac{\Gamma\left(\frac{5}{2\beta}\right)\Gamma\left(\frac{1}{2\beta}\right)}{\Gamma^2\left(\frac{3}{2\beta}\right)}. \tag{4}$$

The results indicate that the sample mean $\overline{X}$ is the estimate of the true mean $\mu$ while the shape parameter can be numerically obtained from the estimate of the kurtosis. Substituting shape parameter estimate into $Var(X)$ we estimate the scale parameter $\sigma$.

Also the log-likelihood function [14] for random samples $x_1, x_2, .., x_n$ from (1) is:

$$LogL(\mu,\sigma,\beta) = n\ln\left(\frac{1}{\sigma\Gamma\left(1+\frac{1}{2\beta}\right)2^{1+\frac{1}{2\beta}}}\right) - \sum_{i=1}^{i=n}\frac{1}{2}\left|\frac{x_i-\mu}{\sigma}\right|^{2\beta} \tag{5}$$

The derivatives of (8) with respect to $\mu$, $\sigma$, and $\beta$ are.

$\frac{\partial LogL}{\partial\mu} = \frac{\beta}{\sigma^{2\beta}}\left(\sum_{x_i\geq\mu}(x_i-\mu) - \sum_{x_i<\mu}(x_i-\mu)\right)$; $\frac{\partial LogL}{\partial\sigma} = -\frac{n}{\sigma}+\frac{\beta}{\sigma}\left|\frac{x-\mu}{\sigma}\right|^{2\beta}$; and

$$\frac{\partial LogL}{\partial\beta} = \frac{n}{2\beta^2}\left[\Psi\left(1+\frac{1}{2\beta}\right)+1\right] - \sum_{i=1}^{i=n}\left|\frac{x_i-\mu}{\sigma}\right|^{2\beta}\ln\left|\frac{x_i-\mu}{\sigma}\right|.$$

The expected fisher information matrix of EPD is

$E\left(-\frac{\partial^2 LogL}{\partial\mu^2}\right) = \frac{n\beta(2\beta-1)2^{1-\frac{1}{\beta}}\Gamma\left(1-\frac{1}{2\beta}\right)}{\sigma^2\Gamma\frac{1}{2\beta}}$; $E\left(-\frac{\partial^2 LogL}{\partial\sigma^2}\right) = \frac{2\beta n}{\sigma^2}$;

$E\left(-\frac{\partial^2 LogL}{\partial\sigma\partial\beta}\right) = -\frac{1}{\sigma\beta}\left(1+\Psi\left(1+\frac{1}{2\beta}\right)\ln 2\right)$; and

$$E\left(-\frac{\partial^2 LogL}{\partial\beta^2}\right) = \frac{n}{\beta^3}\left(1+\Psi\left(1+\frac{1}{2\beta}\right)+\frac{\Psi'\left(1+\frac{1}{2\beta}\right)}{2\beta}\right)+n\frac{(\ln 2)^2}{4\beta^3}\left(\Psi^2\left(1+\frac{1}{2\beta}\right)+\Psi'\left(1+\frac{1}{2\beta}\right)\right)$$

[10] developed codes in R programming environment to estimate these parameters from any given sample from (1), this also includes the parameter $\beta$ which has no explicit solution.

## 3. Data analysis

Normality assumptions are common in data analysis, but a close look at the normal and generalized exponential power Q-Q plot in **Figures 1** and **2**, though by the normal distribution is a reasonable fit, the tails seem to be shorter than expected and hence the $p$-values resulting from the usual tests based on normal assumptions cannot be trusted. On the other hand, the generalized exponential power distribution proves to be a much better fit for egg weights as well as cholesterol level in both groups. We now carry out estimation of the parameters of the distribution using the method of moments and maximum likelihood estimate (MLE). These methods were preferred because they have many optimal properties in estimation: sufficiency; consistency; efficiency; and parametrization invariance which are rarely found in other approaches (For details on MLE see [4, 9, 10] and the estimated values from the observations namely the means, standard deviation (s.d.), the $\beta$, Akaike information Criterion (AIC) as well as Bayesian Information criterion (BIC) are given in **Table 1** with the corresponding log-likelihood ( $\log(A)$) estimates. Since the explicit expression cannot be obtained for $\mu$ and $\beta$ in the estimation of maximum likelihood in the Section 2 we employed a numerical approach using a written code in the R software programme. The statistical computing environment [10], was supplemented with the package called “normp” downloaded into R file from the site http://cran.r-project.org/ and used to analyze the data. As earlier stated, though the normal distribution was a good fit to the sample data, but we have a better fit when we use the generalized exponential power distribution. This is evident when

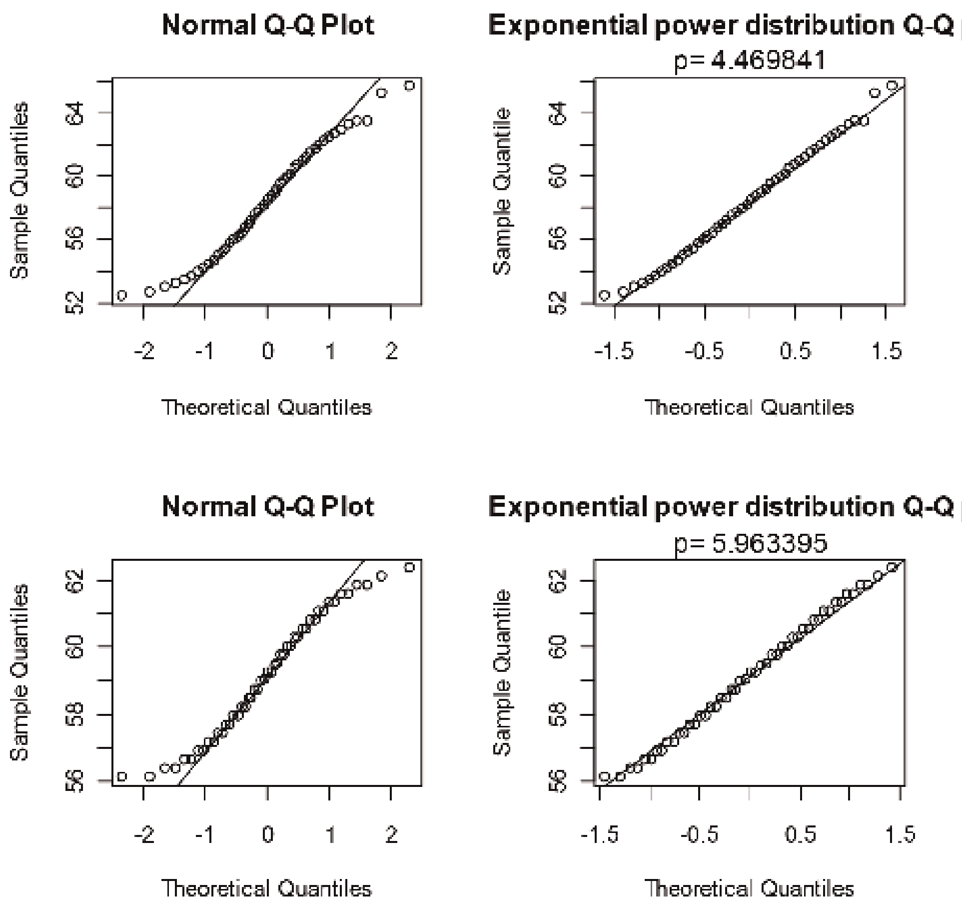


**Figure 1.**
*Top row are the Q-Q plots weight of the eggs for the inorganic copper salt, the first is the normal and the second is the Generalized Exponential distribution. The second row is the case of organic copper salt.*

comparing the results form the log-likelihood functions, AIC and BIC given in **Table 1** below, as well as the plots in **Figures 1** and **2**.

### 3.1 Hypothesis testing

We have used a numerical algorithm written in the R software environment to find estimates of the parameters giving the best fit for the data in appendix B. These estimates are reported in **Table A1**. We used the Kolmogorov-Smirnov two-sample test [15] for large samples to decide whether there is significant difference in the weights and cholesterol levels of the two groups. Clearly, if we find that there is a significant difference, then feeds should be changed to organic copper salts. Note that we have used a one sided Kolmogorov-Smirnov two-sample test [15], that is, the test compares the cumulative frequency distributions of the two samples and decides if the observed D indicates that they were drawn from different populations and one of which is stochastically larger than the other. Let $F_{n_1}(X)$ be the cumulative step function of the sample observations for the inorganic copper salt type and let $F_{n_2}(Y)$ be the cumulative step function of the sample observations for organic copper salt type. We test the null hypothesis that the two samples have been drawn from the same population against the alternative hypothesis that the values of the population from which one the samples was drawn are stochastically larger than the values of the population from which the other sample was drawn. In other words, for the eggs weights the null and alternative hypotheses are of the form

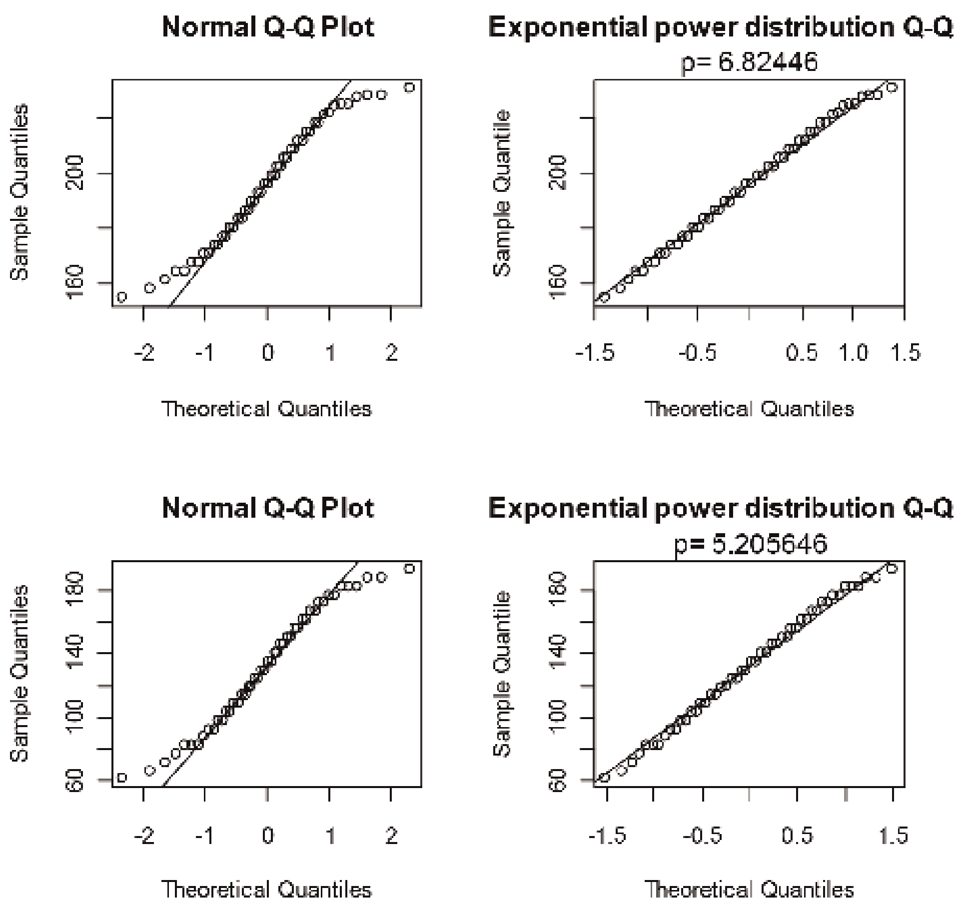


**Figure 2.**
*The Q-Q plots for the egg cholesterol content. The first row is the inorganic copper salt for normal and generalized exponential power distribution. The second row is the organic copper salt.*

| Density | Variables | Mean | s.d | Shape($\omega$) | h($\omega$) | log($\ell$) | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| Normal | Weight (inorganic) | 58.350 | 3.559 | Nil | Nil | −129.055 | 262.11 | 273.5948 |
| | Weight (organic) | 59.10 | 1.822 | Nil | Nil | −96.910 | 197.82 | 209.3048 |
| | Cholesterol (inorganic) | 195.728 | 21.907 | Nil | Nil | −216.282 | 436.564 | 448.0488 |
| | Cholesterol (organic) | 131.457 | 37.232 | Nil | Nil | −241.739 | 487.478 | 498.9628 |
| GEP | Weight (inorganic) | 58.507 | 4.371 | 4.470 | 0.281 | −126.028 | 258.056 | 275.2832 |
| | Weight (organic) | 59.118 | 2.306 | 5.963 | 0.168 | −91.850 | 189.700 | 206.9272 |
| | Cholesterol (inorganic) | 194.481 | 28.359 | 6.825 | 0.147 | −210.809 | 427.618 | 444.8452 |
| | Cholesterol (organic) | 129.720 | 46.327 | 5.206 | 0.192 | −237.448 | 480.896 | 498.1232 |

**Table 1.**
*Parameters estimation for eggs weights in Appendix B.*

$H_0 : F_{n_1}(X) = F_{n_2}(Y)$ and $H_A : F_{n_1}(X) < F_{n_2}(Y)$; also for the cholesterol levels while the null hypothesis remain the same, the alternative hypothesis is $H_A : F_{n_2}(X) < F_{n_1}(Y)$. We now define

$$D = maximum[F_{n_1}(X) - F_{n_2}(Y)]$$

The sampling distribution of $D$ is assumed to be a generalized exponential power distribution. It has been shown by [16] that

$$\chi_2^2 = 4D^2 \frac{n}{2} \tag{6}$$

(for number of observations are the same)

1. for the egg weights $D = 0.3125$ with corresponding $P[\chi_2^2 > 9.21] < 0.01$. Hence, we reject $H_0$ and conclude that the weight of the eggs fed with inorganic copper salt is less than the organic type.

2. for the cholesterol level which is the most important, we have: $D = 0.6875$ with $P[\chi_2^2 > 45.375] < 0.0001$. Hence we reject the null hypothesis and conclude that, using the organic copper salt type the cholesterol level significantly reduced.

## 4. Exponential power distribution table

Given a set of data, one of the statistical issues is to see how well the data fit into postulated model. This technique necessitates the corresponding table of the probability distribution for the proposed model. The cumulative distribution for the exponential power distributions is not in explicit form, but some numerical approach was used to produce the abridge version of the table of the cumulative distribution for quick use for those who are not familiar with code written to solve such problem with different values of shape parameter $\beta$ see Appendix C (**Tables A2–A6**). The table presented makes it workable to examine whether exponential power distribution is an appropriate model for any data set. Though we have the conventional testing method which is also discussed, one is Pearson's $\chi^2$ test and the other one is Kolmogorov-Smirnov test. An example in poultry feeds data and a simulation example are included, we compare the fitting with the normal distribution. To illustrate the use of the table, the cumulative distribution function (cdf) for a standardized random variable having (1) with real $\beta$ can be expressed has

$$P(X \leq x) = \int_{-\infty}^{t} \frac{1}{2\beta^{1/p}\left(1+\frac{1}{\beta}\right)} \exp\left\{-\frac{|x|^{\beta}}{\beta}\right\} \tag{7}$$

Thus, for each specified $\beta$, we can calculate the corresponding probability for each value of $t$. In the table, we present the corresponding probabilities for $t$ ranging from 0.00 until $P(X \leq x) \approx 1$ to 3 decimal places, with each increase in length by 0.01. We introduced Simpson rule in numerical computation coupled with R program developed by [9, 17]. We prefer Simpson's method compare to other methods because its guarantees the accuracy level of the table. The table is arranged as follows, if we wish to compute, say $x = 0.15$, the table in the appendix can used in the this way:

- $P(Y \leq 0.15) = 0.5910$, when $\beta = 0.5$
- $P(Y \leq 0.15) = 0.5695$, when $\beta = 1.0$
- $P(Y \leq 0.15) = 0.5583$, when $\beta = 3.6$

from the table we can see that the probability distribution of exponential power distribution depends on the shape parameter, $\beta$, and as $\beta$ increases the cdf changed. For example, the $P(Y \leq 3.0) = 0.9998$ remains the same at the accuracy of $10^{-4}$ for

$\beta$ ranging from 2.40 to 10.00. Therefore, the tables were truncated at some points, when the resulting values of $P(X \leq x)$ repeat the previous values for increase in shape parameter $\beta$. To check the accuracy of the table in the appendix, from our program we allowed $\beta = 1$ which of course gave the values for the cdf of Laplace distribution otherwise known as double exponential. Also, when $\beta = 2$ we have the values for the cdf of a random variable having a standard normal probability distribution function (not reproduce here, but available in many Statistical texts).

## 5. Goodness-of-fit tests for the exponential power distribution

In this section, we present two procedures for goodness-of-fit test for the exponential power distribution. One is *Pearson's* $\chi^2$ test and the other one is Kolmogorov-Smirnov test. These are two well-known tests in the literature to examine how well a set of data fits into a postulated model provided that the probability distribution of the postulated random variable is available.

### 5.1 $\chi^2$ procedure for exponential power distribution

Given a set of data $X_1, \ldots, X_n$. To carry out Pearson's $\chi^2$ test to ascertain if the data is well fit into exponential power distribution $EP(p_0)$, we proceed as follows:

- Partition the sample space into K disjoint intervals;
- Find the probability $\beta_k$ that an outcome falls in the $K$th interval under the assumption that the underlying population has an $EP(\beta_0)$ distribution. $\beta_k$ can be found using the table in the appendix, then $E_k = n\beta_k$ is the expected number of outcomes that falls in the $K$th interval in $n$ repetitions of the experiment;
- The $\chi^2$ test statistic with degree of freedoms $K - 1$ is then defined as

$$\chi^2 = \sum_{i=1}^{K} \frac{(N_i - E_i)^2}{E_i} \quad (8)$$

where $N_i$ is the number of outcomes that fall in the *ith* interval and $E_i$ is the expected number in the *ith* interval. The selection of $K$ follows the general rule in the application of Pearson's $\chi^2$ test.

If the $\chi^2$ value calculated from (8) is too large compared with the endpoint of $\chi^2_{k-1}$ at certain significance level, say 0.05 (commonly used) but on some occasion 0.01, it implies that the differences between the expected and the observed values are too large, then the assumption of exponential power with $p_0$ must be rejected. Other value of $\beta$ or even other models may need to be considered. If the calculated $\chi^2$ value is small, it implies that the data set fits well into the model. Therefore the model can be accepted at the significance level specified.

From this test procedure, conspicuously it is convenient to have the tables for practical purpose. For example, we can always compare the value of $\chi^2$ to see whether normal distribution or exponential power is a better fit for the data.

### 5.2 Kolmogorov test procedure on the exponential power distribution

Suppose we have a random sample $X_1, \ldots, X_n$ from a population with distribution function $F(x)$, we desire to see if a postulated exponential power distribution (with

specified $\beta_0$) can be used to fit the underlying population of the data. The null hypothesis can be stated as follows.

$H_0 : F(x) = G_0(x)$ for all $x$.

against the alternative.

$H_1 : F(x) \neq G_0(x)$ for at least one $x$.

where $G_0(x)$ denotes the cdf of $EP(\beta_0)$

$$D\big(F_n(x), G_\beta(x)\big) = \sup_z \left|F_n(x) - G_\beta(x)\right| \tag{9}$$

where

$$F_n(x) = \begin{cases} 0, x < X_{(1)} \\ \frac{i}{n}, X_{(i)} \le x < X_{(i+1)}, k = 1, \dots, n-1; \\ 1, x \ge X_{(n)}. \end{cases}$$

where $X_{(1)}, \dots, X_{(n)}$ in the expression of $F_n(x)$ are the ordered statistics of $X_1, \dots, X_n$. $G_\beta(x)$ at each sample points of $X_i$ can be found from the exponential power distribution table. In this case, the Kolmogorov-Smirnov test statistic D(.,.) is the maximum distance between empirical distribution function and postulated distribution function at the sample points. At significant level of $\alpha$, the test endpoint $d_\alpha$ for test statistic $D$ can be found from [15, 16]. The rule is that if the calculated $D$ is larger than $d_\alpha$ the postulated exponential power distribution function is too far away from the observed distribution function. Thus $H_0$ is rejected at $\alpha$ level of significance, otherwise, $H_0$ is accepted at the same significance level. To carry out this test, it is critical to find the $F_n(x)$'s for the postulated exponential power distribution. The table provide in this paper makes it possible for the implementation of this test.

**Example 1**: (Approximation of the exponential power distribution by the normal distribution). Normal distribution has been well known to be the limiting distribution for so many distribution in the literature. In this section with explore to what value of the parameter $p$ will normal give an acceptable approximation to data having exponential power distribution with parameter $p_i$. This will also examine the closeness between exponential power and normal distributions, using the Kolmogorov-Smirnov test of normality distance. Let $X \sim N(0,1)$ and $F(x)$ be the cdf, also let $Y \sim EP(\beta)$ and $G_\beta(y)$ be the cdf. The Kolmogorov distance between $F(x)$ and $G_\beta(y)$ is defined as

$$D\big(F, G_\beta\big) = \sup_z \left|F - G_\beta\right| \tag{10}$$

The values of $D\big(F, G_p\big)$ can be obtained from the tables in the appendix. The values of $D\big(F, G_p\big)$ from some selected $p = 1.6 - 4.4$. These are shown in the table below.

we observed from **Table 2**, that as $p$ increases $D\big(F, G_p\big)$ also increases, this implies that approximation by normal distribution becomes poorer with large

| $p$ | **1.6** | **1.8** | **2.2** | **2.4** | **2.6** | **2.8** | **3.0** |
|---|---|---|---|---|---|---|---|
| $D(F, G_p)$ | 0.0146 | 0.0072 | 0.0065 | 0.0180 | 0.0197 | 0.0226 | 0.0268 |
| $p$ | 3.2 | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 |
| $D(F, G_p)$ | 0.0311 | 0.0348 | 0.0384 | 0.0415 | 0.0447 | 0.0478 | 0.0504 |

**Table 2.**
*Kolmogorov distance between F and $G_p$.*

| Intervals | $n$ | $p_i$ | $EP(4.4)$ | *normal* |
|---|---|---|---|---|
| $(-\infty, -1.75]$ | 3 | 0.0033 | 3.3 | 40.1 |
| $(-1.75, -1.25]$ | 55 | 0.0540 | 54.0 | 65.5 |
| $(-1.25, -0.75]$ | 149 | 0.1524 | 152.4 | 121.0 |
| $(-0.75, -0.25]$ | 193 | 0.1924 | 192.4 | 174.7 |
| $(-0.25, 0.25]$ | 197 | 0.1958 | 195.8 | 197.4 |
| $(0.25, 0.75]$ | 196 | 0.1924 | 192.4 | 174.7 |
| $(0.75, 1.25]$ | 151 | 0.1524 | 152.4 | 121.0 |
| $(1.25, 1.75]$ | 52 | 0.0540 | 54.0 | 65.5 |
| $(1.75, \infty]$ | 4 | 0.0033 | 3.3 | 40.1 |

**Table 3.**
*Pearson's $\chi^2$ test.*

estimated $p$ from experimental samples. Large $D(F, G_p)$ is noticeable in all $p's$ when $t = 1.3$. Therefore, normal assumption in such case of large $p$ value may lead to error in conclusion. It should be noted that the significance of $D(F, G_p)$ also depends on the sample size.

**Example 2**: (Simulation from exponential power distribution). **Table 3** shows a simulation of 1000 samples from exponential power distribution with $p = 4.4$, where $n$ is the observed frequency in the *ith* interval, $p_i((a, b]) = P(EPD(4.4)))-$ $P(EPD(4.4) \leq a)$. and $Np_i$ and normal are the expected frequency in the *ith* interval for $EPD(4.4)$ and normal distribution, respectively. We obtained $\chi^2$ value of 0.4207 for $EP(4.20)$ with degree of freedom 9, thus $EP(4.20)$ is accepted as expected. However, the goodness-of-fit for $N(0, 1)$ gives an observed $\chi^2$ value of 832.559, which results in the rejection of $N(0, 1)$ model for the same data set. See **Table 3** for detail report.

## 6. The Kullback-Leibler information

The Kullback-Leibler (K-L) information function [14] can be used to discriminate between two distributions $F_\theta(x)$ and $F(x)$ of $= F_\theta(x); \theta \in \Theta$. It is defined as

$$I(\theta, \phi) = E_\theta\left\{\ln \frac{f(X;\theta)}{f(X;\phi)}\right\}; \theta, \phi \in \Theta \tag{11}$$

The family of $F$ is assumed to be regular.

**Proposition**: $I(\theta, \phi) \geq 0$ if and only if, $f(X;\theta) = f(X;\phi)$ with probability one.

**Proof**: Recall that $\ln x$ is the concave function of $x$ and by Jensen's inequality $\ln(E(Y)) \geq E(\ln Y)$ for every non-negative random variable $Y$, having a finite expectation. Accordingly, $-I(\theta, \phi) = E_\theta\left\{-\ln \frac{f(X;\theta)}{f(X;\phi)}\right\} = \int \ln \frac{f(X;\phi)}{f(X;\theta)} f(x;\theta)dx$ $\leq \ln \int f(x;\theta)dx = 0$ if both sides of the above equation is multiply by $-1$ we have that $I(\theta;\phi) \geq 0$. Also, if $P_\theta[f(X;\theta) = f(x;\phi)] = 1$ then $I(\theta;\phi) = 0$. *Q.E.D.*

It is worth noting that if $X_1, \ldots, X_n$ are identical and independent random variables then the $K_L$ information function say $I(\theta;\phi)$ is additive, that is,

$I_n(\theta;\phi) = E_\theta\left\{\ln \frac{f(X;\theta)}{f(X;\phi)}\right\} = E_\theta\left\{\sum_{i=1}^{n} \ln \frac{f(X_i;\theta)}{f(X_i;\phi)}\right\} = nI(\theta;\phi)$.

**Example 1**: let $F$ be the class of all normal distribution $\{N(\mu, \sigma^2), \mu \in R, \sigma > 0\}$. let $\theta_1 = (\mu_1, \sigma_1)$ and $\theta_2 = (\mu_2, \sigma_2)$.

The likelihood ratio is

$$\frac{f(x;\theta_1)}{f(x;\theta_2)} = \frac{\sigma_1}{\sigma_2} \exp\left\{ -\frac{1}{2}\left[ \left(\frac{x-\mu_1}{\sigma_1}\right)^2 - \left(\frac{x-\mu_2}{\sigma_2}\right)^2 \right] \right\} \tag{12}$$

**Example 3**: (Applications to poultry feeds data) Now consider the data in Appendix B, where cholesterol level $x_i$ of 48 eggs of chicken fed with organic copper salt are measured in $mg/egg$, where 5.20 is the estimated $p$ value for exponential power distribution and 131.457 and 37.232 are the population mean and standard deviation, respectively. Also for Normal we have 59.10 and 1.822 as the estimated mean and standard deviation, respectively. The ordered data set $x_i$ are given in **Table 4**, $z_i$ and $t_i$ is the standardized values for $x_i$ for $EP(5.20)$ and normal, respectively. $Z_i = P(EP(5.20 \le z_i))$ and $T_i = P(N(0,1) \le t_i)$ is the normal counterpart. We define $D_{EP}$ as the $\max(|Z_i - i/n|, |Z_i - (i-1)/n|)$ for $EP(5.20)$ and $D_N$ as $\max(|T_i - i/n|, |T_i - (i-1)/n|)$ for normal distribution. From **Table 4**, using Kolmogorov-Smirnov test, we find the corresponding $|D| = 0.061833$ for $EP(5.20)$ and $|D| = 0.77742$ for normal distribution. One can easily see that the fit of exponential power cdf is uniformly better than that of the standard normal cdf in this example. All these have been made possible using the table in the appendix. Details are provided in **Table 4**.

| $x_i$ | $z_i$ | $t_i$ | $Z_i$ | $\lvert D_{EP}\rvert$ | $T_i$ | $\lvert D_N\rvert$ |
|---|---|---|---|---|---|---|
| 60.73 | −1.489196365 | −1.899629351 | 0.0115 | 0.0115 | 0.5294 | 0.5294 |
| 66.03 | −1.374792238 | −1.757278685 | 0.0254 | 0.01627 | 0.5392 | 0.518367 |
| 71.33 | −1.260388111 | −1.614928019 | 0.0452 | 0.0173 | 0.5537 | 0.512033 |
| 76.63 | −1.145983983 | −1.472577353 | 0.0713 | 0.01203 | 0.5708 | 0.5083 |
| 81.86 | −1.033090854 | −1.33210679 | 0.1065 | 0.023167 | 0.5918 | 0.508467 |
| 81.93 | −1.031579856 | −1.330226687 | 0.1065 | 0.0185 | 0.5918 | 0.487633 |
| 81.93 | −1.031579856 | −1.330226687 | 0.1065 | 0.03933 | 0.5918 | 0.4668 |
| 87.16 | −0.918686727 | −1.189756124 | 0.1429 | 0.02377 | 0.617 | 0.471167 |
| 92.46 | −0.8042826 | −1.047405458 | 0.1829 | 0.016233 | 0.6469 | 0.480233 |
| 92.52 | −0.802987459 | −1.045793941 | 0.1864 | 0.02193 | 0.96492 | 0.77742 |
| 97.76 | −0.689878473 | −0.905054792 | 0.2284 | 0.020067 | 0.6814 | 0.473067 |
| 97.82 | −0.688583332 | −0.903443275 | 0.2284 | 0.0216 | 0.6841 | 0.454933 |
| 103.06 | −0.575474345 | −0.762704125 | 0.2707 | 0.0207 | 0.7236 | 0.4736 |
| 103.11 | −0.574395061 | −0.761361195 | 0.2747 | 0.01697 | 0.7236 | 0.452767 |
| 108.36 | −0.461070218 | −0.620353459 | 0.3182 | 0.026533 | 0.7676 | 0.475933 |
| 108.41 | −0.459990934 | −0.619010529 | 0.3182 | 0.01513 | 0.7709 | 0.4584 |
| 113.66 | −0.346666091 | −0.478002793 | 0.3613 | 0.027967 | 0.8156 | 0.482267 |
| 113.7 | −0.345802664 | −0.476928449 | 0.3613 | 0.0137 | 0.8156 | 0.461433 |
| 118.96 | −0.232261964 | −0.335652127 | 0.4088 | 0.0338 | 0.8669 | 0.4919 |
| 119 | −0.231398536 | −0.334577783 | 0.4088 | 0.012967 | 0.8707 | 0.474867 |
| 124.26 | −0.117857837 | −0.193301461 | 0.4563 | 0.039633 | 0.9247 | 0.508033 |

| $x_i$ | $z_i$ | $t_i$ | $Z_i$ | $\lvert D_{EP}\rvert$ | $T_i$ | $\lvert D_N\rvert$ |
|---|---|---|---|---|---|---|
| 124.3 | −0.116994409 | −0.192227116 | 0.4524 | 0.0149 | 0.9247 | 0.4872 |
| 129.56 | −0.003453709 | −0.050950795 | 0.4998 | 0.041467 | 0.9801 | 0.521767 |
| 129.6 | −0.002590282 | −0.04987645 | 0.4996 | 0.020433 | 0.9801 | 0.500933 |
| 134.86 | 0.110950418 | 0.091399871 | 0.5437 | 0.0437 | 0.0359 | 0.48493 |
| 134.89 | 0.111597988 | 0.09220563 | 0.5437 | 0.022867 | 0.0359 | 0.50577 |
| 140.16 | 0.225354545 | 0.233750537 | 0.5912 | 0.049533 | 0.091 | 0.4715 |
| 140.19 | 0.226002115 | 0.234556296 | 0.5912 | 0.0287 | 0.091 | 0.49233 |
| 145.46 | 0.339758672 | 0.376101203 | 0.6347 | 0.051367 | 0.148 | 0.45617 |
| 145.48 | 0.340190386 | 0.376638376 | 0.6347 | 0.030533 | 0.148 | 0.477 |
| 150.76 | 0.454162799 | 0.518451869 | 0.6778 | 0.0528 | 0.1985 | 0.44733 |
| 150.78 | 0.454594513 | 0.518989042 | 0.6778 | 0.031967 | 0.1985 | 0.46817 |
| 161.06 | 0.568566926 | 0.660802535 | 0.7253 | 0.058633 | 0.2454 | 0.4421 |
| 161.08 | 0.56899864 | 0.661339708 | 0.7253 | 0.0378 | 0.2454 | 0.46293 |
| 166.36 | 0.682971054 | 0.803153202 | 0.7681 | 0.059767 | 0.2881 | 0.44107 |
| 161.37 | 0.68318691 | 0.803421788 | 0.7681 | 0.038933 | 0.2881 | 0.4619 |
| 166.66 | 0.797375181 | 0.945503868 | 0.8136 | 0.0636 | 0.3289 | 0.44193 |
| 166.67 | 0.797591038 | 0.945772454 | 0.8136 | 0.042767 | 0.3289 | 0.46277 |
| 171.96 | 0.911779308 | 1.087854534 | 0.8535 | 0.061833 | 0.3621 | 0.4504 |
| 171.97 | 0.911995165 | 1.08812312 | 0.8535 | 0.041 | 0.3621 | 0.47123 |
| 177.26 | 1.026183435 | 1.2302052 | 0.8935 | 0.060167 | 0.3907 | 0.46347 |
| 177.26 | 1.026183435 | 1.2302052 | 0.8935 | 0.039333 | 0.3907 | 0.4843 |
| 182.56 | 1.140587562 | 1.372555866 | 0.9259 | 0.0509 | 0.4147 | 0.48113 |
| 182.56 | 1.140587562 | 1.372555866 | 0.9259 | 0.030067 | 0.4147 | 0.50197 |
| 182.56 | 1.140587562 | 1.372555866 | 0.9259 | 0.0116 | 0.4147 | 0.5228 |
| 187.86 | 1.25499169 | 1.514906532 | 0.9528 | 0.0153 | 0.4345 | 0.52383 |
| 187.86 | 1.25499169 | 1.514906532 | 0.9528 | 0.02637 | 0.4345 | 0.54467 |
| 193.16 | 1.369395817 | 1.657257198 | 0.9746 | 0.0254 | 0.4515 | 0.5485 |

**Table 4.**
*Kolmogorov goodness-of-fit test.*

## 7. Concluding remarks

We have proposed a generalized exponential power distribution, and studied some of its mathematical and statistical properties. We fitted this distribution to data arising from an experiment concerning the cholesterol level and weight of eggs. The Q-Q plots clearly show that the generalized exponential power distribution fits the data better than the usual normal distribution. Finally, hypotheses tests show that consumption of eggs from chicken fed with organic copper salt should not be boycotted for the fear of high cholesterol level. Therefore we recommend that the constituents of poultry feeds should change from the inorganic to organic combinations.

## Appendix A

See **Figures 1** and **2**.

## Appendix B

See **Table A1**.

| Inorganic copper salt | | Organic copper salt | |
|---|---|---|---|
| **Weight** | **Cholesterol** | **Weight** | **Cholesterol** |
| 52.67 | 164.23 | 56.08 | 56.08 |
| 53.17 | 167.42 | 56.34 | 56.34 |
| 53.67 | 170.6 | 56.61 | 56.61 |
| 54.17 | 173.78 | 56.87 | 56.87 |
| 54.67 | 176.96 | 57.13 | 57.13 |
| 55.17 | 180.14 | 57.39 | 57.39 |
| 55.67 | 183.32 | 57.65 | 57.65 |
| 56.17 | 186.51 | 57.92 | 57.92 |
| 56.67 | 189.69 | 58.18 | 58.18 |
| 57.17 | 192.87 | 58.44 | 58.44 |
| 57.67 | 196.05 | 58.7 | 58.7 |
| 58.17 | 199.24 | 58.96 | 58.96 |
| 58.67 | 202.42 | 59.23 | 59.23 |
| 59.17 | 205.6 | 59.45 | 59.45 |
| 59.67 | 208.78 | 59.75 | 59.75 |
| 60.17 | 211.96 | 60.01 | 60.01 |
| 60.67 | 215.14 | 60.27 | 60.27 |
| 61.17 | 218.33 | 60.54 | 60.54 |
| 61.67 | 221.52 | 60.8 | 60.8 |
| 62.17 | 224.69 | 61.06 | 61.06 |
| 62.67 | 224.85 | 61.32 | 61.32 |
| 63.17 | 227.88 | 61.58 | 61.58 |
| 63.43 | 228.03 | 61.85 | 61.85 |
| 65.67 | 231.06 | 62.34 | 62.34 |
| 65.15 | 228.01 | 62.11 | 62.11 |
| 63.43 | 224.83 | 61.85 | 61.85 |
| 62.93 | 221.65 | 61.58 | 61.58 |
| 62.43 | 218.46 | 61.32 | 61.32 |
| 61.93 | 215.28 | 61.06 | 61.06 |
| 61.43 | 212.1 | 60.8 | 60.8 |
| 60.93 | 208.92 | 60.54 | 60.54 |
| 60.43 | 205.74 | 60.27 | 60.27 |

| Inorganic copper salt | | Organic copper salt | |
|---|---|---|---|
| **Weight** | **Cholesterol** | **Weight** | **Cholesterol** |
| 59.93 | 202.56 | 60.01 | 60.01 |
| 59.43 | 199.37 | 59.75 | 59.75 |
| 58.93 | 196.19 | 59.49 | 59.49 |
| 58.43 | 193.01 | 59.23 | 59.23 |
| 57.93 | 189.83 | 59 | 59 |
| 57.43 | 186.65 | 58.7 | 58.7 |
| 56.93 | 183.46 | 58.44 | 58.44 |
| 56.43 | 180.28 | 58.18 | 58.18 |
| 55.93 | 177.1 | 57.92 | 57.92 |
| 55.43 | 173.72 | 57.65 | 57.65 |
| 54.93 | 170.74 | 57.39 | 57.39 |
| 54.43 | 167.55 | 57.13 | 57.13 |
| 53.93 | 164.37 | 56.87 | 56.87 |

**Table A1.**
*Observed data from inorganic and organic copper salt.*

## Appendix C

See **Tables A2–A6**.

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.5000 | 0.5087 | 0.5166 | 0.5239 | 0.5308 | 0.5373 | 0.5436 | 0.5495 | 0.5554 | 0.5609 |
| 0.10 | 0.5663 | 0.5716 | 0.5767 | 0.5816 | 0.5864 | 0.5910 | 0.5956 | 0.6000 | 0.6044 | 0.6086 |
| 0.20 | 0.6127 | 0.6168 | 0.6207 | 0.6246 | 0.6284 | 0.6321 | 0.6358 | 0.6393 | 0.6428 | 0.6463 |
| 0.30 | 0.6497 | 0.6530 | 0.6564 | 0.6594 | 0.6626 | 0.6656 | 0.6687 | 0.6717 | 0.6746 | 0.6775 |
| 0.40 | 0.6804 | 0.6831 | 0.6859 | 0.6886 | 0.6913 | 0.6939 | 0.6965 | 0.6991 | 0.7016 | 0.7041 |
| 0.50 | 0.7065 | 0.7089 | 0.7113 | 0.7137 | 0.7160 | 0.7183 | 0.7205 | 0.7227 | 0.7249 | 0.7273 |
| 0.60 | 0.7293 | 0.7315 | 0.7334 | 0.7355 | 0.7375 | 0.7395 | 0.7415 | 0.7435 | 0.7454 | 0.7473 |
| 0.70 | 0.7492 | 0.7511 | 0.7529 | 0.7547 | 0.7565 | 0.7583 | 0.7601 | 0.7618 | 0.7635 | 0.7652 |
| 0.80 | 0.7669 | 0.7686 | 0.7702 | 0.7719 | 0.7735 | 0.7750 | 0.7766 | 0.7782 | 0.7797 | 0.7812 |
| 0.90 | 0.7827 | 0.7841 | 0.7857 | 0.7876 | 0.7886 | 0.7901 | 0.7915 | 0.7929 | 0.7941 | 0.7955 |
| 1.0 | 0.7970 | 0.7982 | 0.7997 | 0.8010 | 0.8023 | 0.8036 | 0.8049 | 0.8061 | 0.8074 | 0.8087 |
| 1.1 | 0.8099 | 0.8111 | 0.8115 | 0.8135 | 0.8147 | 0.8159 | 0.8170 | 0.8182 | 0.8193 | 0.8204 |
| 1.2 | 0.8216 | 0.8227 | 0.8238 | 0.8249 | 0.8260 | 0.8271 | 0.8281 | 0.8292 | 0.8302 | 0.8313 |
| 1.3 | 0.8323 | 0.8334 | 0.8343 | 0.8353 | 0.8363 | 0.8373 | 0.8383 | 0.8392 | 0.8402 | 0.8411 |
| 1.4 | 0.8421 | 0.8430 | 0.8439 | 0.8449 | 0.8458 | 0.8467 | 0.8476 | 0.8485 | 0.8493 | 0.8502 |
| 1.5 | 0.8511 | 0.8519 | 0.8528 | 0.8536 | 0.8545 | 0.8553 | 0.8561 | 0.8570 | 0.8578 | 0.8586 |
| 1.6 | 0.8594 | 0.8602 | 0.8610 | 0.8617 | 0.8625 | 0.8633 | 0.8641 | 0.8648 | 0.8657 | 0.8663 |
| 1.7 | 0.8670 | 0.8678 | 0.8685 | 0.8692 | 0.8700 | 0.8706 | 0.8714 | 0.8721 | 0.8728 | 0.8735 |
| 1.8 | 0.8741 | 0.8748 | 0.8755 | 0.8762 | 0.8768 | 0.8775 | 0.8781 | 0.8788 | 0.8794 | 0.8801 |

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.9 | 0.8809 | 0.8814 | 0.8819 | 0.8826 | 0.8832 | 0.8839 | 0.8844 | 0.8850 | 0.8857 | 0.8863 |
| 2.0 | 0.8869 | 0.8875 | 0.8880 | 0.8886 | 0.8892 | 0.8898 | 0.8902 | 0.8909 | 0.8915 | 0.8920 |
| 2.1 | 0.8926 | 0.8931 | 0.8937 | 0.8942 | 0.8948 | 0.8953 | 0.8958 | 0.8963 | 0.8973 | 0.8974 |

**Table A2.**
*Cumulative distribution table for exponential power at $p = 0.5$.*

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2.2 | 0.8979 | 0.8984 | 0.8993 | 0.8994 | 0.8999 | 0.9004 | 0.9009 | 0.9014 | 0.9019 | 0.9021 |
| 2.3 | 0.9029 | 0.9034 | 0.9038 | 0.9043 | 0.9048 | 0.9052 | 0.9057 | 0.9062 | 0.9066 | 0.9071 |
| 2.4 | 0.9075 | 0.9080 | 0.9084 | 0.9089 | 0.9093 | 0.9097 | 0.9102 | 0.9106 | 0.9111 | 0.9115 |
| 2.5 | 0.9119 | 0.9123 | 0.9127 | 0.9132 | 0.9135 | 0.9140 | 0.9144 | 0.9149 | 0.9152 | 0.9156 |
| 2.6 | 0.9160 | 0.9163 | 0.9168 | 0.9172 | 0.9176 | 0.9180 | 0.9183 | 0.9188 | 0.9191 | 0.9195 |
| 2.7 | 0.9199 | 0.9202 | 0.9206 | 0.9210 | 0.9214 | 0.9217 | 0.9221 | 0.9224 | 0.9228 | 0.9231 |
| 2.8 | 0.9235 | 0.9238 | 0.9242 | 0.9245 | 0.9249 | 0.9252 | 0.9256 | 0.9259 | 0.9262 | 0.9260 |
| 2.9 | 0.9269 | 0.9272 | 0.9276 | 0.9279 | 0.9282 | 0.9285 | 0.9289 | 0.9292 | 0.9292 | 0.9297 |
| 3.0 | 0.9301 | 0.9303 | 0.9304 | 0.9311 | 0.9314 | 0.9317 | 0.9320 | 0.9323 | 0.9326 | 0.9329 |
| 3.1 | 0.9332 | 0.9335 | 0.9338 | 0.9340 | 0.9343 | 0.9346 | 0.9349 | 0.9352 | 0.9355 | 0.9358 |
| 3.2 | 0.9360 | 0.9363 | 0.9366 | 0.9369 | 0.9351 | 0.9374 | 0.9377 | 0.9380 | 0.9382 | 0.9384 |
| 3.3 | 0.9388 | 0.9390 | 0.9393 | 0.9396 | 0.9398 | 0.9401 | 0.9403 | 0.9405 | 0.9407 | 0.9411 |
| 3.4 | 0.9413 | 0.9416 | 0.9418 | 0.9421 | 0.9423 | 0.9426 | 0.9428 | 0.9430 | 0.9433 | 0.9435 |
| 3.5 | 0.9438 | 0.9440 | 0.9442 | 0.9445 | 0.9447 | 0.9449 | 0.9452 | 0.9454 | 0.9457 | 0.9458 |
| 3.6 | 0.9461 | 0.9463 | 0.9465 | 0.9467 | 0.9470 | 0.9472 | 0.9473 | 0.9476 | 0.9479 | 0.9481 |
| 3.7 | 0.9483 | 0.9485 | 0.9487 | 0.9488 | 0.9491 | 0.9494 | 0.9495 | 0.9497 | 0.9499 | 0.9501 |
| 3.8 | 0.9504 | 0.9506 | 0.9507 | 0.9510 | 0.9511 | 0.9514 | 0.9516 | 0.9517 | 0.9519 | 0.9521 |
| 3.9 | 0.9523 | 0.9527 | 0.9528 | 0.9529 | 0.9531 | 0.9533 | 0.9535 | 0.9537 | 0.9538 | 0.9540 |
| 4.0 | 0.9542 | 0.9544 | 0.9546 | 0.9541 | 0.9549 | 0.9551 | 0.9553 | 0.9555 | 0.9557 | 0.9559 |
| 4.1 | 0.9560 | 0.9562 | 0.9563 | 0.9566 | 0.9567 | 0.9569 | 0.9570 | 0.9572 | 0.9574 | 0.9575 |
| 4.2 | 0.9577 | 0.9579 | 0.9580 | 0.9582 | 0.9583 | 0.9585 | 0.9587 | 0.9588 | 0.9590 | 0.9592 |
| 4.3 | 0.9593 | 0.9595 | 0.9596 | 0.9598 | 0.9599 | 0.9601 | 0.9606 | 0.9603 | 0.9606 | 0.9607 |
| 4.4 | 0.9609 | 0.9610 | 0.9612 | 0.9613 | 0.9615 | 0.9616 | 0.9617 | 0.9619 | 0.9620 | 0.9622 |
| 4.5 | 0.9623 | 0.9625 | 0.9626 | 0.9627 | 0.9629 | 0.9630 | 0.9632 | 0.9633 | 0.9635 | 0.9636 |
| 4.6 | 0.9637 | 0.9640 | 0.9640 | 0.9641 | 0.9643 | 0.9644 | 0.9645 | 0.9647 | 0.9648 | 0.9649 |
| 4.7 | 0.9651 | 0.9652 | 0.9654 | 0.9655 | 0.9656 | 0.9657 | 0.9658 | 0.9660 | 0.9661 | 0.9662 |
| 4.8 | 0.9664 | 0.9664 | 0.9666 | 0.9667 | 0.9668 | 0.9670 | 0.9671 | 0.9672 | 0.9673 | 0.9675 |
| 4.9 | 0.9676 | 0.9677 | 0.9678 | 0.9679 | 0.9681 | 0.9681 | 0.9683 | 0.9684 | 0.9685 | 0.9686 |
| 5. | 0.9687 | 0.9689 | 0.9690 | 0.9690 | 0.9692 | 0.9693 | 0.9695 | 0.9695 | 0.9696 | 0.9698 |
| 5.1 | 0.9699 | 0.9700 | 0.9698 | 0.9702 | 0.9703 | 0.9704 | 0.9705 | 0.9706 | 0.9708 | 0.9708 |
| 5.2 | 0.9709 | 0.9709 | 0.9711 | 0.9710 | 0.9713 | 0.9714 | 0.9716 | 0.9716 | 0.9718 | 0.9719 |
| 5.3 | 0.9719 | 0.9721 | 0.9721 | 0.9721 | 0.9723 | 0.9724 | 0.9725 | 0.9726 | 0.9727 | 0.9728 |
| 5.4 | 0.9729 | 0.9730 | 0.9731 | 0.9732 | 0.9743 | 0.9734 | 0.9735 | 0.9736 | 0.9737 | 0.9738 |

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 5.5 | 0.9739 | 0.9740 | 0.9741 | 0.9741 | 0.9743 | 0.9743 | 0.9744 | 0.9746 | 0.9746 | 0.9747 |
| 5.6 | 0.9748 | 0.9749 | 0.9749 | 0.9750 | 0.9751 | 0.9740 | 0.9753 | 0.9754 | 0.9755 | 0.9755 |
| 5.7 | 0.9756 | 0.9757 | 0.9758 | 0.9759 | 0.9760 | 0.9760 | 0.9761 | 0.9762 | 0.9763 | 0.9764 |
| 5.8 | 0.9765 | 0.9765 | 0.9766 | 0.9767 | 0.9768 | 0.9767 | 0.9769 | 0.9770 | 0.9770 | 0.9772 |
| 5.9 | 0.9773 | 0.9773 | 0.9774 | 0.9774 | 0.9776 | 0.9776 | 0.9777 | 0.9778 | 0.9779 | 0.9778 |
| 6.0 | 0.9780 | 0.9780 | 0.9782 | 0.9782 | 0.9783 | 0.9783 | 0.9785 | 0.9785 | 0.9786 | 0.9787 |
| 6.1 | 0.9787 | 0.9788 | 0.9789 | 0.9790 | 0.9792 | 0.9791 | 0.9792 | 0.9792 | 0.9793 | 0.9794 |
| 6.2 | 0.9794 | 0.9795 | 0.9792 | 0.9797 | 0.9797 | 0.9798 | 0.9799 | 0.9799 | 0.9800 | 0.9800 |
| 6.3 | 0.9801 | 0.9802 | 0.9802 | 0.9803 | 0.9804 | 0.9804 | 0.9805 | 0.9806 | 0.9806 | 0.9807 |
| 6.4 | 0.9808 | 0.9809 | 0.9809 | 0.9810 | 0.9810 | 0.9811 | 0.9812 | 0.9812 | 0.9813 | 0.9813 |
| 6.5 | 0.9814 | 0.9815 | 0.9815 | 0.9816 | 0.9816 | 0.9817 | 0.9817 | 0.9777 | 0.9818 | 0.9818 |
| 6.6 | 0.9820 | 0.9820 | 0.9821 | 0.9822 | 0.9822 | 0.9823 | 0.9823 | 0.9824 | 0.9825 | 0.9825 |
| 6.7 | 0.9826 | 0.9826 | 0.9827 | 0.9827 | 0.9828 | 0.9830 | 0.9829 | 0.9830 | 0.9814 | 0.9830 |
| 6.8 | 0.9831 | 0.9832 | 0.9832 | 0.9833 | 0.9833 | 0.9834 | 0.9835 | 0.9835 | 0.9836 | 0.9830 |
| 6.9 | 0.9837 | 0.9837 | 0.9837 | 0.9838 | 0.9839 | 0.9839 | 0.9831 | 0.9840 | 0.9838 | 0.9840 |
| 7.0 | 0.9842 | 0.9841 | 0.9839 | 0.9820 | 0.9835 | 0.9848 | 0.9844 | 0.9845 | 0.9846 | 0.9846 |
| 7.1 | 0.9847 | 0.9847 | 0.9848 | 0.9848 | 0.9849 | 0.9849 | 0.9849 | 0.9850 | 0.9850 | 0.9851 |
| 7.2 | 0.9851 | 0.9852 | 0.9852 | 0.9853 | 0.9853 | 0.9854 | 0.9854 | 0.9855 | 0.9855 | 0.9855 |
| 7.3 | 0.9856 | 0.9856 | 0.9857 | 0.9857 | 0.9858 | 0.9858 | 0.9859 | 0.9859 | 0.9859 | 0.9860 |
| 7.4 | 0.9860 | 0.9861 | 0.9861 | 0.9862 | 0.9862 | 0.9862 | 0.9863 | 0.9863 | 0.9863 | 0.9864 |

**Table A3.**
*Cumulative distribution table for exponential power at $p = 1.0$.*

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 7.5 | 0.9865 | 0.9865 | 0.9865 | 0.9866 | 0.9866 | 0.9866 | 0.9867 | 0.9868 | 0.9868 | 0.9868 |
| 7.6 | 0.9869 | 0.9869 | 0.9869 | 0.9868 | 0.9870 | 0.9871 | 0.9871 | 0.9872 | 0.9872 | 0.9872 |
| 7.7 | 0.9873 | 0.9873 | 0.9873 | 0.9874 | 0.9873 | 0.9875 | 0.9871 | 0.9875 | 0.9876 | 0.9873 |
| 7.8 | 0.9876 | 0.9877 | 0.9877 | 0.9878 | 0.9878 | 0.9878 | 0.9879 | 0.9878 | 0.9879 | 0.9880 |
| 7.9 | 0.9880 | 0.9881 | 0.9881 | 0.9881 | 0.9882 | 0.9882 | 0.9883 | 0.9883 | 0.9883 | 0.9884 |
| 8.0 | 0.9884 | 0.9884 | 0.9884 | 0.9885 | 0.9885 | 0.9885 | 0.9886 | 0.9884 | 0.9887 | 0.9887 |
| 8.1 | 0.9887 | 0.9887 | 0.9888 | 0.9887 | 0.9888 | 0.9888 | 0.9889 | 0.9889 | 0.9888 | 0.9892 |
| 8.2 | 0.9890 | 0.9891 | 0.9891 | 0.9891 | 0.9892 | 0.9892 | 0.9892 | 0.9892 | 0.9893 | 0.9893 |
| 8.3 | 0.9895 | 0.9894 | 0.9894 | 0.9895 | 0.9895 | 0.9895 | 0.9896 | 0.9896 | 0.9896 | 0.9896 |
| 8.4 | 0.9897 | 0.9897 | 0.9897 | 0.9898 | 0.9898 | 0.9898 | 0.9899 | 0.9900 | 0.9899 | 0.9900 |
| 8.5 | 0.9900 | 0.9899 | 0.9900 | 0.9901 | 0.9901 | 0.9901 | 0.9901 | 0.9902 | 0.9902 | 0.9902 |
| 8.6 | 0.9903 | 0.9903 | 0.9903 | 0.9903 | 0.9904 | 0.9904 | 0.9904 | 0.9905 | 0.9905 | 0.9905 |
| 8.7 | 0.9905 | 0.9906 | 0.9906 | 0.9906 | 0.9906 | 0.9907 | 0.9907 | 0.9907 | 0.9908 | 0.9908 |
| 8.8 | 0.9908 | 0.9908 | 0.9909 | 0.9909 | 0.9910 | 0.9909 | 0.9910 | 0.9910 | 0.9912 | 0.9910 |
| 8.9 | 0.9911 | 0.9911 | 0.9911 | 0.9911 | 0.9912 | 0.9909 | 0.9912 | 0.9912 | 0.9913 | 0.9913 |

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 9.0 | 0.9913 | 0.9914 | 0.9914 | 0.9914 | 0.9914 | 0.9914 | 0.9915 | 0.9915 | 0.9915 | 0.9915 |
| 9.1 | 0.9916 | 0.9916 | 0.9916 | 0.9916 | 0.9917 | 0.9917 | 0.9917 | 0.9911 | 0.9918 | 0.9918 |
| 9.2 | 0.9918 | 0.9918 | 0.9918 | 0.9919 | 0.9919 | 0.9920 | 0.9919 | 0.9921 | 0.9920 | 0.9920 |
| 9.3 | 0.9920 | 0.9920 | 0.9921 | 0.9921 | 0.9922 | 0.9921 | 0.9922 | 0.9922 | 0.9922 | 0.9922 |
| 9.4 | 0.9922 | 0.9924 | 0.9923 | 0.9923 | 0.9923 | 0.9924 | 0.9924 | 0.9924 | 0.9924 | 0.9924 |
| 9.5 | 0.9925 | 0.9925 | 0.9925 | 0.9925 | 0.9925 | 0.9926 | 0.9926 | 0.9926 | 0.9926 | 0.9926 |
| 9.6 | 0.9927 | 0.9927 | 0.9927 | 0.9922 | 0.9908 | 0.9931 | 0.9928 | 0.9928 | 0.9928 | 0.9928 |
| 9.7 | 0.9929 | 0.9929 | 0.9929 | 0.9929 | 0.9930 | 0.9930 | 0.9930 | 0.9930 | 0.9930 | 0.9930 |
| 9.8 | 0.9930 | 0.9931 | 0.9931 | 0.9931 | 0.9931 | 0.9931 | 0.9932 | 0.9932 | 0.9932 | 0.9932 |
| 9.9 | 0.9932 | 0.9933 | 0.9933 | 0.9933 | 0.9933 | 0.9934 | 0.9934 | 0.9934 | 0.9934 | 0.9934 |

**Table A4.**
*Cumulative distribution table for exponential power at $p = 1.0$.*

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.5000 | 0.5050 | 0.5100 | 0.5150 | 0.5195 | 0.5245 | 0.5290 | 0.5340 | 0.5385 | 0.5430 |
| 0.10 | 0.5475 | 0.5520 | 0.5565 | 0.5610 | 0.5655 | 0.5695 | 0.5740 | 0.5780 | 0.5825 | 0.5865 |
| 0.20 | 0.5905 | 0.5945 | 0.5985 | 0.6025 | 0.6065 | 0.6105 | 0.6145 | 0.6185 | 0.6220 | 0.6260 |
| 0.30 | 0.6295 | 0.6335 | 0.6370 | 0.6405 | 0.6440 | 0.6475 | 0.6510 | 0.6545 | 0.6580 | 0.6615 |
| 0.40 | 0.6650 | 0.6680 | 0.6715 | 0.6745 | 0.6780 | 0.6810 | 0.6845 | 0.6875 | 0.6905 | 0.6935 |
| 0.50 | 0.6965 | 0.7000 | 0.7025 | 0.7055 | 0.7085 | 0.7115 | 0.7145 | 0.7170 | 0.7200 | 0.7230 |
| 0.60 | 0.7255 | 0.7285 | 0.7310 | 0.7335 | 0.7365 | 0.7390 | 0.7415 | 0.7440 | 0.7465 | 0.7490 |
| 0.70 | 0.7515 | 0.7540 | 0.7565 | 0.7590 | 0.7615 | 0.7640 | 0.7660 | 0.7685 | 0.7710 | 0.7730 |
| 0.80 | 0.7755 | 0.7775 | 0.7800 | 0.7820 | 0.7840 | 0.7860 | 0.7885 | 0.7905 | 0.7925 | 0.7945 |
| 0.90 | 0.7965 | 0.7985 | 0.8005 | 0.8025 | 0.8045 | 0.8065 | 0.8085 | 0.8105 | 0.8125 | 0.8140 |
| 1.0 | 0.8160 | 0.8180 | 0.8195 | 0.8215 | 0.8235 | 0.8250 | 0.8270 | 0.8285 | 0.8300 | 0.8320 |
| 1.1 | 0.8335 | 0.8350 | 0.8370 | 0.8385 | 0.8400 | 0.8415 | 0.8435 | 0.8450 | 0.8465 | 0.8480 |
| 1.2 | 0.8495 | 0.8510 | 0.8525 | 0.8540 | 0.8555 | 0.8565 | 0.8580 | 0.8595 | 0.8610 | 0.8625 |
| 1.3 | 0.8635 | 0.8650 | 0.8665 | 0.8680 | 0.8690 | 0.8705 | 0.8715 | 0.8730 | 0.8740 | 0.8755 |
| 1.4 | 0.8765 | 0.8780 | 0.8790 | 0.8805 | 0.8815 | 0.8825 | 0.8840 | 0.8850 | 0.8860 | 0.8875 |
| 1.5 | 0.8885 | 0.8895 | 0.8905 | 0.8915 | 0.8930 | 0.8940 | 0.8950 | 0.8960 | 0.8970 | 0.8980 |
| 1.6 | 0.8990 | 0.9000 | 0.9010 | 0.9020 | 0.9030 | 0.9040 | 0.9050 | 0.9060 | 0.9070 | 0.9075 |
| 1.7 | 0.9085 | 0.9095 | 0.9105 | 0.9115 | 0.9120 | 0.9130 | 0.9140 | 0.9150 | 0.9155 | 0.9165 |
| 1.8 | 0.9175 | 0.9180 | 0.9190 | 0.9200 | 0.9205 | 0.9215 | 0.9220 | 0.9230 | 0.9235 | 0.9245 |
| 1.9 | 0.9250 | 0.9260 | 0.9265 | 0.9275 | 0.9280 | 0.9290 | 0.9295 | 0.9305 | 0.9310 | 0.9315 |
| 2.0 | 0.9325 | 0.9330 | 0.9335 | 0.9345 | 0.9350 | 0.9355 | 0.9365 | 0.9370 | 0.9375 | 0.9380 |
| 2.1 | 0.9385 | 0.9395 | 0.9400 | 0.9405 | 0.9410 | 0.9420 | 0.9425 | 0.9430 | 0.9435 | 0.9440 |
| 2.2 | 0.9445 | 0.9450 | 0.9455 | 0.9460 | 0.9470 | 0.9475 | 0.9480 | 0.9485 | 0.9490 | 0.9495 |

**Table A5.**
*Cumulative distribution table for exponential power at $p = 1.0$.*

| t | 0.0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.08 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2.3 | 0.9500 | 0.9505 | 0.9510 | 0.9515 | 0.9520 | 0.9525 | 0.9530 | 0.9535 | 0.9535 | 0.9540 |
| 2.4 | 0.9545 | 0.9550 | 0.9555 | 0.9560 | 0.9565 | 0.9570 | 0.9575 | 0.9575 | 0.9580 | 0.9585 |
| 2.5 | 0.9590 | 0.9595 | 0.9595 | 0.9600 | 0.9605 | 0.9610 | 0.9615 | 0.9615 | 0.9620 | 0.9625 |
| 2.6 | 0.9630 | 0.9630 | 0.9635 | 0.9640 | 0.9645 | 0.9645 | 0.9650 | 0.9655 | 0.9655 | 0.9660 |
| 2.7 | 0.9665 | 0.9665 | 0.9670 | 0.9675 | 0.9675 | 0.9680 | 0.9685 | 0.9685 | 0.9690 | 0.9695 |
| 2.8 | 0.9695 | 0.9700 | 0.9700 | 0.9705 | 0.9705 | 0.9710 | 0.9715 | 0.9715 | 0.9720 | 0.9720 |
| 2.9 | 0.9725 | 0.9730 | 0.9730 | 0.9735 | 0.9735 | 0.9740 | 0.9740 | 0.9745 | 0.9745 | 0.9750 |
| 3.0 | 0.9750 | 0.9755 | 0.9755 | 0.9760 | 0.9760 | 0.9765 | 0.9770 | 0.9770 | 0.9770 | 0.9770 |
| 3.1 | 0.9775 | 0.9775 | 0.9780 | 0.9785 | 0.9785 | 0.9785 | 0.9790 | 0.9790 | 0.9790 | 0.9795 |
| 3.2 | 0.9795 | 0.9800 | 0.9800 | 0.9800 | 0.9805 | 0.9805 | 0.9810 | 0.9810 | 0.9810 | 0.9815 |
| 3.3 | 0.9815 | 0.9815 | 0.9820 | 0.9820 | 0.9825 | 0.9825 | 0.9825 | 0.9830 | 0.9830 | 0.9830 |
| 3.4 | 0.9835 | 0.9835 | 0.9835 | 0.9840 | 0.9840 | 0.9840 | 0.9845 | 0.9845 | 0.9845 | 0.9850 |
| 3.5 | 0.9840 | 0.9850 | 0.9850 | 0.9855 | 0.9855 | 0.9855 | 0.9860 | 0.9860 | 0.9860 | 0.9860 |
| 3.6 | 0.9865 | 0.9865 | 0.9865 | 0.9865 | 0.9870 | 0.9870 | 0.9870 | 0.9875 | 0.9875 | 0.9875 |
| 3.7 | 0.9875 | 0.9880 | 0.9880 | 0.9880 | 0.9880 | 0.9880 | 0.9885 | 0.9885 | 0.9885 | 0.9885 |
| 3.8 | 0.9890 | 0.9890 | 0.9890 | 0.9890 | 0.9890 | 0.9895 | 0.9895 | 0.9895 | 0.9895 | 0.9900 |
| 3.9 | 0.9900 | 0.9900 | 0.9900 | 0.9900 | 0.9905 | 0.9905 | 0.9905 | 0.9905 | 0.9905 | 0.9910 |
| 4.0 | 0.9910 | 0.9910 | 0.9910 | 0.9910 | 0.9910 | 0.9915 | 0.9905 | 0.9915 | 0.9915 | 0.9915 |
| 4.1 | 0.9915 | 0.9920 | 0.9920 | 0.9920 | 0.9920 | 0.9920 | 0.9920 | 0.9925 | 0.9925 | 0.9925 |
| 4.2 | 0.9925 | 0.9925 | 0.9925 | 0.9925 | 0.9930 | 0.9930 | 0.9930 | 0.9930 | 0.9930 | 0.9930 |
| 4.3 | 0.9930 | 0.9935 | 0.9935 | 0.9935 | 0.9935 | 0.9935 | 0.9935 | 0.9935 | 0.9935 | 0.9940 |
| 4.4 | 0.9940 | 0.9940 | 0.9940 | 0.9940 | 0.9940 | 0.9940 | 0.9940 | 0.9945 | 0.9945 | 0.9945 |
| 4.5 | 0.9945 | 0.9945 | 0.9945 | 0.9945 | 0.9945 | 0.9945 | 0.9950 | 0.9950 | 0.9950 | 0.9950 |
| 4.6 | 0.9950 | 0.9950 | 0.9950 | 0.9950 | 0.9950 | 0.9950 | 0.9955 | 0.9955 | 0.9955 | 0.9955 |
| 4.7 | 0.9955 | 0.9955 | 0.9955 | 0.9955 | 0.9955 | 0.9955 | 0.9955 | 0.9960 | 0.9960 | 0.9960 |
| 4.8 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 | 0.9960 |
| 4.9 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9965 |
| 5.0 | 0.9965 | 0.9965 | 0.9965 | 0.9965 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 |
| 5.1 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 | 0.9970 |

**Table A6.**
*Cumulative distribution table for exponential power at $p = 1.0$.*

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