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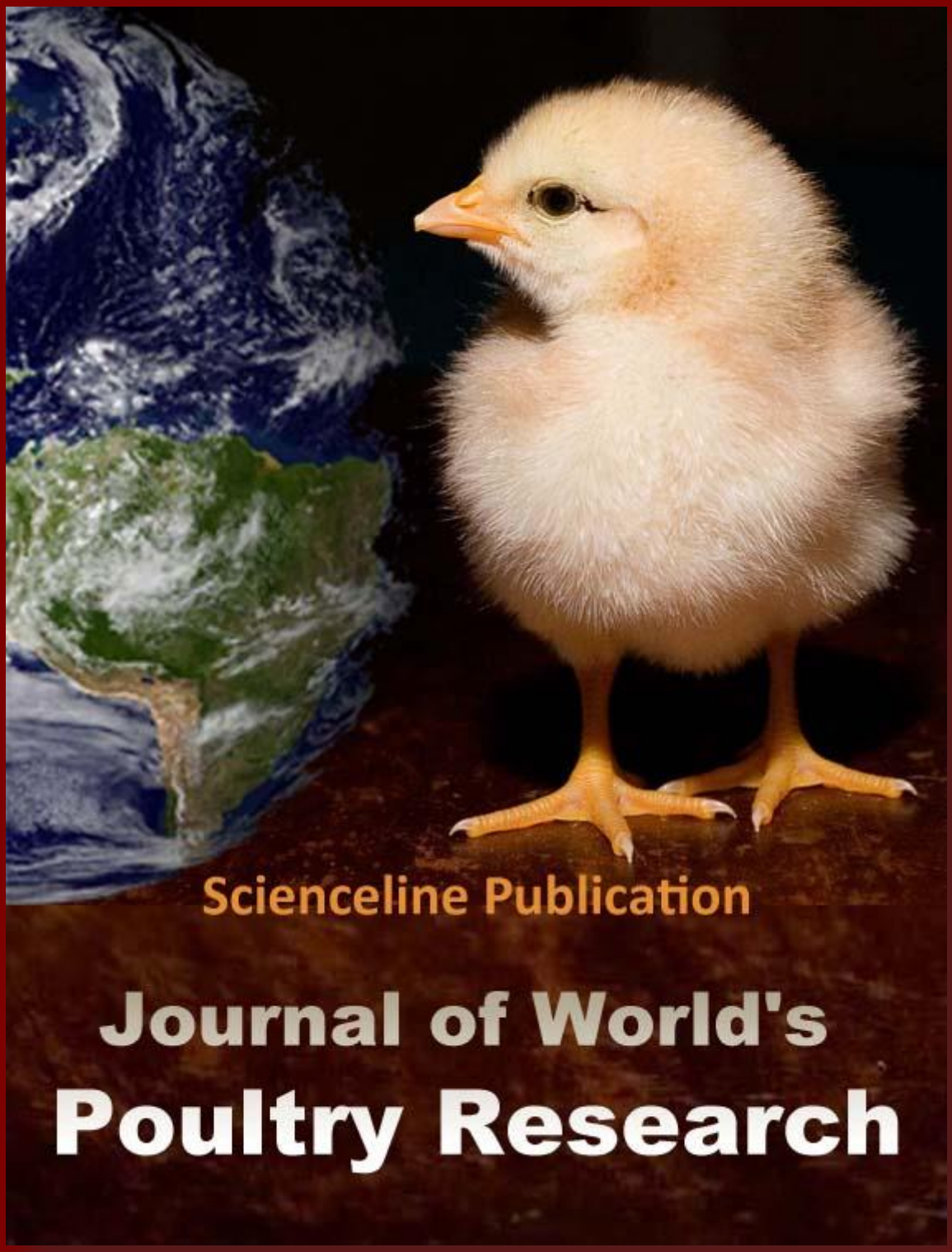

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other European countries. To expand this sector with respect to environmental concern some issues need to be considered very carefully such as effective poultry feed production system, energy consumption in both poultry production and processing area, manure management system, wastewater and odour management systems. If these issues are not handled carefully, several types of harmful effect will occur in both living and environment cycle such as water borne diseases, global warming and ozone layer depletion. The objective of this report is to give an overview of the current situation of poultry production in Ireland, policies and legislation related to poultry production and to show the way to expand this sector in Ireland in line with current ecological concern.
Keywords: Ecological and policy, Management of poultry-waste, Poultry and environment, Poultry production

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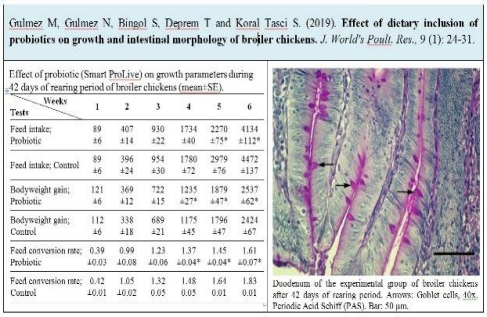
Research Paper

Effect of dietary inclusion of probiotics on growth and intestinal morphology of broiler chickens.


Gulmez M, Gulmez N, Bingol S, Deprem T and Koral Tasci S. (2019). **Effect of dietary inclusion of probiotics on growth and intestinal morphology of broiler chickens.** *J. World's Poult. Res.*, 9 (1): 24-31.

Effect of probiotic (Smart ProLive) on growth parameters during 42 days of rearing period of broiler chickens (mean±SE).

Tests \ Weeks	1	2	3	4	5	6
Feed intake; Probiotic	89 ±6	407 ±14	930 ±22	1734 ±40	2270 ±75*	4134 ±112*
Feed intake; Control	89 ±6	396 ±24	954 ±30	1780 ±72	2979 ±76	4472 ±137
Bodyweight gain; Probiotic	121 ±6	369 ±12	722 ±15	1235 ±27*	1879 ±47*	2537 ±62*
Bodyweight gain; Control	112 ±6	338 ±18	689 ±21	1175 ±45	1796 ±47	2424 ±67
Feed conversion rate; Probiotic	0.39 ±0.03	0.99 ±0.08	1.23 ±0.06	1.37 ±0.04*	1.45 ±0.04*	1.61 ±0.07*
Feed conversion rate; Control	0.42 ±0.01	1.05 ±0.02	1.32 0.05	1.48 0.05	1.64 0.01	1.83 0.01

Duodenum of the experimental group of broiler chickens after 42 days of rearing period. Arrows: Goblet cells, 10x. Periodic Acid Schiff (PAS). Bar: 50 µm.

Gulmez M, Gulmez N, Bingol S, Deprem T and Koral Tasci S.

J. World Poult. Res. 9(1):24-31, 2019; pii:S2322455X1900004-9

ABSTRACT
Probiotics are currently under investing the most valuable substances alternative to antibiotic growth promoters in poultry breeding practice. This research was performed to evaluate the effect of supplementing broiler drinking water with probiotics (*Pediococcus acidilactici* and *Bacillus subtilis*) at a concentration of ≥10^8 CFU/ml during 42 days of feeding period on growth performance and gut health. A total of 144 one-day-old Ross 308 broiler chicks (mixed gender) with an average initial BW of 42.3 g were used. The chicks were allotted to pens with 12 birds per pen and six replications per treatment with food and water provided *ad libitum*. Feed intake of Probiotic group was 4134 g, 338 g less than that of control group. Live weight of probiotic group was 2537 g and a 113 g more than that of control group. The feed conversion ratio of probiotic group was 1.61, 0.22 less than that of control group. The crypt depth of probiotic group (1110.46 ± 224.016 µm) was statistically deeper than that of control group (949.39 ± 114.166 µm) in ileum. Continuously use of probiotics in drinking water of commercial poultry flocks appears to be alternative to AGPs. The results of this study provide a greater understanding of the impact of long-life use of probiotics on broiler health and growth performances.
Keywords: Broiler, Gut morphology, Growth performances, Probiotics

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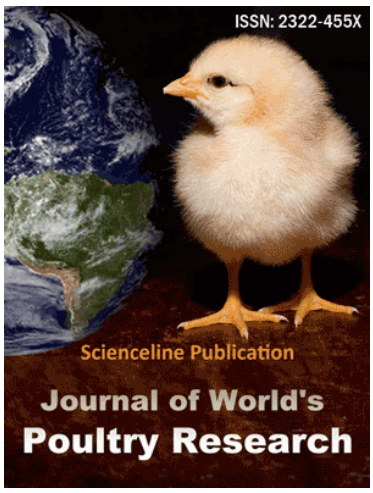
Journal of World's Poultry Research



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Homepage: www.science-line.com
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A Review on Fungal Fermented Cassava Pulp as a Cheap Alternative Feedstuff in Poultry Ration

Sugiharto Sugiharto

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, 50275, Indonesia
Corresponding author's Email: sgh_undip@yahoo.co.id

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ABSTRACT

In order to reduce the production cost, cassava pulp has been incorporated in chicken diets as an energy source. However, the use of such agro-industrial by-product may be confined by its higher fibre and lower protein contents. Improving the nutritional characteristics (lowering fibre and increasing protein content) through fungal solid state fermentation may be conducted to increase the inclusion level of cassava pulp in chicken rations. Apart from an energy source, fungal fermented cassava pulp (FCP) may also exert a beneficial effect on intestinal health of chickens, although further studies are needed to explore the functional benefit of FCP on chicken health.

Keywords: Chicken diet, Energy source, Fermented tapioca by-product, Fungal solid-state fermentation

INTRODUCTION

Feed cost has been a major component in chicken production, representing about 70% of the total cost of production. Generally, chicken diets are composed of several feedstuffs, and that energy-rich feedstuffs such as maize constitute more than half of the diets. Owing to this fact, any increase in the price of maize can potentially increase the chicken production cost and consequently, reduce the profit margin of farmers. Nutritionists are now exploring for unconventional energy-rich feedstuffs that may be used to reduce the proportion of maize in chicken rations. Among the alternative feedstuffs, cassava pulp has frequently been incorporated in chicken rations as a source of energy. On the other hand, the incorporation of such agro-industrial by-product in chicken rations may be limited by the high and low contents of fibre and protein, respectively (Khempaka et al., 2009). Fungal solid state fermentation could be one of the methods to improve the nutritional qualities and thus increase the inclusion level of cassava pulp in chicken rations (Khempaka et al., 2014; Sugiharto et al., 2017a). This present mini review elaborates the potential of fungal Fermented Cassava Pulp (FCP) as an energy-rich alternative feed ingredient for chickens.

Cassava pulp as an energy-rich alternative feedstuff for chickens

As a response to the increased price of maize, nutritionists are currently searching some cheap energy-rich alternative to partially replace the use of maize in chicken diets. As a by-product of tapioca industry, cassava pulp is quite cheap and abundantly available throughout the year, particularly in tropical countries. Cassava pulp contains true metabolizable energy (TME) of about 2,484 kcal/kg energy, therefore, it may be potential as an energy source in chicken rations (Khempaka et al., 2009). On the other hand, it should be noticed that the nutritional qualities of cassava pulp may vary depending on the varieties of cassava, manufacturing process to produce tapioca, the process of drying and contamination (with fibre, sand and soil) (Chauynarong et al., 2015).

Dried cassava pulp has been incorporated as an energy source both in broiler and laying hen diets (Table 1). Nevertheless, its maximum inclusion level differs in broiler and laying hen diets. Dried cassava pulp may be incorporated at higher levels in laying hen (15-20%) as compared to that in broiler diets (Maximum 11%). Similar with this case, King and Zeidler (2004) documented that

dried tomato pomace (contains 31% fibre) may be incorporated at 15% in laying hen diets, while such pomace may only be included in broiler rations at the maximum of 5%. The better capability of laying hens in degrading fibre than of broilers seems to be the reason for the higher fibre digestibility (and thus intake of fibre-rich feedstuffs) in laying hens. Moreover, laying hens seem to have lower energy requirement compared to broiler chickens. This makes laying hens more tolerant to cassava pulp (Diarra and Devi, 2015). Within each broiler and laying hens, the variations in the optimal dietary levels of cassava pulp also existed. In such case, several factors may determine the variation, including the nature (e.g., nutritional composition) of cassava pulp, dietary composition, energy to protein ratio of rations, strains of chickens and other experimental conditions.

Aside from the high energy content, cassava pulp generally contains high and low contents of fibre (13.6%) and protein (1.98%), respectively (Khempaka et al., 2009). According to Morgan and Choct (2016), most of the fibre in cassava pulp is counted as insoluble fibre. The latter properties may, therefore, limit the use of cassava pulp in poultry rations, as poultry has a very limited capacity in degrading the insoluble fibre. High fibre fraction in cassava pulp may also increase the bulkiness of the diet and therefore limit the capacity of the digestive tract of chickens. This condition may eventually reduce palatability and feed intake in chickens (Khempaka et al., 2009). To elevate the dietary inclusion level of cassava pulp, supplementation of enzymes have been conducted. Khempaka et al. (2018) have recently supplemented dried cassava pulp with mixed enzymes containing cellulase, glucanase and xylanase in laying hens. Such enzyme supplementation could increase the inclusion level of dried cassava pulp from 20% (Khempaka et al., 2016) to 30% (Khempaka et al., 2018) in laying hen diets. The activity of enzymes in degrading the fibre fraction of cassava pulp may increase the digestibility of such feed ingredient and thus increase the feed intake of chickens (Khempaka et al., 2018).

Table 1. The use of dried cassava pulp in chicken diets

References	Findings and recommendations
Khempaka et al. (2009)	Dried cassava pulp should be limited to 8% or less, as higher inclusion level may compromise the growth performance of broiler chickens.
Kumsri et al. (2009)	Dietary inclusion of 10% dried cassava pulp reduced weight gain of broiler chickens.
Ali-Mursyid et al. (2010)	Dried cassava pulp may be incorporated in broiler rations at a maximum of 11%, higher inclusion level may be detrimental for the growth of broilers.
Tang et al. (2012)	Feeding dried cassava pulp at a level of 25% resulted in poor growth performance in broiler chickens.
Triprugsachart et al. (2007)	Feeding dried cassava pulp up to 15% represented no detrimental impact on egg production of laying hens.
Chauynarong et al. (2010)	Dietary incorporation of dried cassava pulp up to 15% had no deleterious effect on egg production of laying hens.
Khempaka et al. (2016)	Dried cassava pulp can be included up to 20% in laying hen rations with no negative impact on productive performance, nutrient digestibility, and egg quality.
Khempaka et al. (2018)	Dried cassava pulp supplemented with mixed enzymes (cellulase, glucanase and xylanase) may be included in laying hen feeds up to 30% with no detrimental effects on nutrient digestibility, productive performance and egg quality.

Fungal fermentation to improve the nutritional characteristics of cassava pulp

Fermentation is a simple process using microorganisms to break down the complex substrates into simpler components (Sugiharto and Ranjitkar, 2019). Eventually, the degraded compounds maybe utilized maximally by the chickens. Depending on the microorganisms involved, supplements added, duration of the fermentation process and other fermentation conditions, there is a slight variation in the improvement of the nutritional quality of cassava pulp particularly with regard to protein and fibre contents. It appears from the documented studies that fungi (Filamentous fungi and yeast) are the most common microorganisms employed to ferment cassava pulp. The definite reason for such preference is not specifically known. There are several traits belong to fungi that may be exploited to improve the nutritional properties of cassava pulp, one of which is its fibrinolytic activity. A study by Mustafa et al. (2016) noticed that treatment with fungi was capable of degrading

the insoluble fibre (lignin and hemicellulose) resulting in reduced fibre content of the substrates. Such fibre degradation may be facilitated by the activity of extracellular cellulases produced by the fungi (Bhardwaj et al., 2017). The cellulolytic activity of the fungi may also transform the cellulosic compounds into protein, and therefore increase the protein content of materials. In such case, the conversion of fibre into protein-rich fungal biomass may be responsible for the increased protein content of the fungal fermented products (Asadollahzadeh et al., 2018). In addition, Bayitse et al. (2015) suggested that simple sugars may also be metabolized to protein resulting in an increase in protein content of the fermented products. Apart from the improved fibre and protein contents, fermentation has been known to result in the reduction of Hydrogen Cyanide (HCN) (Diarra and Devi, 2010).

To increase the protein content of FCP, supplementation using urea during fermentation has commonly been conducted (Table 2). During the fermentation process, urea may be used as a nitrogen source for the fungal growth (Bayitse et al., 2015). Such an increase in fungal biomass may thereby increase the protein content of the fungal fermented products. In most cases, fungal fermentation of cassava pulp has been carried out according to the solid-state fermentation method. This fermentation method is characterized by the low content of moisture in the substrates (Sugiharto and Rajitkar, 2019). Indeed, there is no specific reason on why solid state fermentation is more attracted to be employed in the fungal fermentation of cassava pulp. Yet, Gowthaman et al. (2001) suggested that solid-state fermentation may better support the fungi to grow on complex natural solid substrates without substantial pretreatment.

Table 2. Nutritional characteristics of fungal fermented cassava pulp

References	Microorganisms involved in fermentation	Supplement in fermentation	Characteristics of fermented cassava pulp
Lubis et al. (2007)	*Aspergillus niger*	None	Crude protein content increased from 2.21 to 3.58% and crude fibre increased from 11.2 to 17.0%
Thongkratok et al. (2010)	*Aspergillus oryzae*	Urea	Protein and amino acid contents increased by 17.4 and 15.1%, respectively
Animashahun et al. (2013)	*Penicillium* spp.	None	Crude protein increased from 2.39 to 3.25% and crude fibre decreased from 11.4 to 9.63% after 7 days of fermentation
Khempaka et al. (2014)	*A. oryzae*	Urea	Crude protein increased from 2.02% to 11.8%, whereas crude fibre decreased from 14.6% to 10.6%
Bayitse et al. (2015)	*Trichoderma pseudokoningii* (ATCC 26801)	Urea and ammonium sulphate	Protein content increased by 48.1% and 36.9% with supplementation of urea and ammonium sulphate, respectively
Sugiharto et al. (2015)	*Acremonium charticola*	None	Crude fibre decreased from 18.4% to 16.9%, while crude protein did not significantly change (compared to unfermented cassava pulp)
Sugiharto et al. (2015)	*Rhizopus oryzae*	None	Crude fibre decreased from 18.4% to 17.6%, while crude protein did not significantly change (compared to unfermented cassava pulp)
Sugiharto et al. (2016)	*A. charticola*	Urea	Crude protein increased from 2.14% to 11.3%, while crude fibre decreased from 25.6% to 20.8%
Sugiharto et al. (2016)	*R. oryzae*	Urea	Crude protein increased from 2.14% to 12.8%, while crude fibre decreased from 25.6% to 22.7%
Sengxayalth and Preston (2017)	*Saccharomyces cerevisiae*	Urea and di-ammonium phosphate	Crude protein increased from 9.5 to 18.4% and true protein increased from 2 to 12% dry matter
Okrathok et al. (2018)	*A. oryzae*	Urea	Crude protein increased from 1.98% to 13.3%, true protein increased from 0.98% to 12.4% and crude fibre decreased from 13.6% to 10.7%
Yafetto (2018)	*A. niger*	Ammonium nitrate	Crude protein increased by 22.61%

The use of fungal FCP in chicken diets

Fungal fermentation has been attributed to the improved nutritional qualities of cassava pulp. As a consequence, fermentation can increase the inclusion levels of cassava pulp in chicken rations. As shown in Table 3, FCP may be included in broiler and laying hen rations greater than that of unfermented cassava pulp. However, the levels of FCP inclusion may vary from study

to studies depending on the nutritional qualities of FCP, dietary composition and other experimental conditions. In most cases, there is a positive correlation between nutrient digestibility and feed intake in chickens (Sundu et al., 2006). In light with this, the improved nutritional qualities in FCP (especially the reduced fibre content) may be attributed to the reduced bulkiness and increased digestibility and thereby increased feed intake in chickens (Khempaka et al., 2014). Fermentation has been suggested to improve the palatability of products (Supriyati et al., 2015). In this respect, better palatability of FCP may be one of the reasons for the increased FCP intake in chickens when compared with the intake of unfermented cassava pulp. The toxicology of liver due to urea supplementation (during fermentation process) and the presence of toxic compounds such as HCN in cassava pulp may be a crucial point of consideration when using FCP as a dietary ingredient for chickens. In conjunction with Khempaka et al. (2014), we assessed the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators of liver health and found no change in the activities of these enzymes when feeding FCP as compared to control (Sugiharto et al., 2017b). The bioconversion of urea to fungal biomass protein (Bayitse et al., 2015) and the destruction of HCN during the fermentation process (Diarra and Devi, 2010) may implicate in safe inclusion of FCP in chicken diets.

Table 3. The levels of FCP inclusion in chicken rations

References	Recommended levels of FCP inclusion
Lubis et al. (2007)	*A. niger*-FCP-urea-zeolite may be used in diets up to 15% without negative impacts on growth performance and health of broilers.
Ali-Mursyid et al. (2010)	FCP could be included in diets up to 16.5% without detrimental effects on growth performance and nutrient digestibility of broiler chicks.
Khempaka et al. (2014)	*A. oryzae*-FCP can be included up to 16% in the rations with no deleterious effects on nutrient digestibility and retention, final body weight, carcass traits and biochemical parameters.
Sugiharto et al. (2017a)	*A. charticola*-FCP can be included up to 16% with no adverse effects on final body weight, digestibility and carcass characteristics of broiler chickens
Okrathok et al. (2018)	*A. oryzae*-FCP was safe to be included up to 24% in diets without deleterious effects on nutrient digestibility, egg production and quality and physiological conditions of laying hens

Functional properties of FCP as a dietary component in chicken diets

Fermented products have been attributed to functional properties such as higher lactic acid bacteria (LAB) and organic acids contents. These properties make FCP beneficial for the health of the gastrointestinal tract of chickens (Sugiharto and Ranjitkar, 2019). Regarding to the effect of FCP on chicken health, the published data are still scarce. A recent study by our research group revealed that *A. charticola*-FCP decreased coliform bacteria count in the ileum and increased butyric and propionic acid concentrations in cecal contents of broiler chickens (Sugiharto et al., 2017a). The capacity of LAB and organic acids in controlling the proliferation of potentially pathogenic bacteria such as coliform may be responsible for the reduced population of such pathogenic bacteria in the intestine of chickens fed FCP. To increase the functionality of fermented products, fermentation using probiotic microorganisms (as starter inoculum) has been conducted (Sugiharto and Ranjitkar, 2019). In this respect, fermented products may not only have improved nutritional qualities, but also contain higher numbers of probiotic microorganisms. Due to the limited data, the study on the functional effect of FCP on chickens needs to be extensively conducted.

CONCLUSION

Solid-state fermentation using fungi can be a simple method to improve the nutritional qualities of cassava pulp and thus increase the inclusion level of such cheap agro-industrial by-product in chicken rations. Further studies are needed to explore the functional benefit of FCP on chicken health.

DECLARATIONS

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Competing interest

I have no conflict of interest.

Consent to publish

I gave my consent prior to publication of this article.

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Haematological, Serum Biochemical and Histological Responses of Cockerels to Long Term Consumption of *Telfairia occidentalis* Leaves Extract

Temitope Oyekemi Onyekwereh[1], David Olusoji Adejumo[1], Kolawole Daniel Afolabi*[2], Friday Chima Nworgu[3] and Adenekan, Olusola Olubisi[1]

[1]*Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria*
[2]*Department of Animal Science, University of Uyo, Uyo, Nigeria*
[3]*Federal College of Animal Health and Production Technology, IAR&T, Moor Plantation, Ibadan, Nigeria*
*Corresponding author`s Email: kaydafl@yahoo.com

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ABSTRACT

Haematological, serum biochemical and haematological histological responses were studied in cockerels undergoing a long-term supplementation with *Telfairia occidentalis* Leave Extract (TOLE). Haematological and serum biochemical parameters investigated included haemoblobin, white blood cells, red blood cells, lymphocytes, heterophils, monocytes, eosinophils, total protein, aspartate aminotransferase, aspartate amino transferase and triglyceride. Histological changes associated with *Telfairia occidentalis* leaves extract on the kidney, liver, spleen and testes were also examined. Result showed that birds on TOLE has higher values for most of the haematological parameters studied which were significantly ($P<0.05$) higher than the control. Also the total protein, globulin and alanine aminotransferase were significant ($P<0.05$) for birds on TOLE having higher values while for triglycerides birds on the control treatment had the higher values which was significant. There were no significant changes in the albumin and aspayrtate aminotransferase. Histological changes showed mild to severe congestion in the spleen and testes of birds that received 120 and 150mL TOLE/L of water that also showed reduced germinal epithelium height and sloughing of the germinal epithelium respectively. Long term supplementation of TOLE for cockerel production should not exceed 60mL of TOLE per liter of water as the administration in excess of this can bring about tissue breakdown and reduced fertility. Animals suffering from blood loss can benefit from the administration of fluted pumpkin leaves extract as the extract increased erythron production.

Key wordS: Cockerels, Haematology, Serum biochemistry, Organ histology, *Telfairia occidentalis*, leaf extract

INTRODUCTION

Nigeria has rich genetic resources of cultivated semi wide and wild species of crops being used as traditional vegetables and different types are consumed by the various ethnic groups for different reasons (Fasuyi, 2006).

Nigeria has a wide range of savannah, tropical rain forest to coastal swampy vegetation where different permanent and arable crops are grown. Different fruits, vegetables and spices are also grown, that vary across localities as they are favorite delicacies and sources of vitamins, minerals and medicine (Fasuyi, 2006) in their diets. *Telferia occidentalis* is one of these vegetables that is commonly grown and eaten from the coastal area to other parts of the country (Imosemi, 2018). Regular consumption of these plant foods are associated with numerous health benefits rooted in their various physiological and nutritional constituents (Imosemi, 2018; Jimoh, 2018; Hunter and Fletcher, 2002). Plant leaf meal can offer a good alternative to synthetic drugs because they are cheap, readily available, safe, economical and biodegradable (Nneka, 2006). *Telfairia occidentalis* (fluted pumpkin) is a tropical vine grown in West Africa and highly reputed in traditional medicine (Badifu et al., 1995; Badifu et al., 1995). *Telfairia occidentalis* contains nutrients such as protein, carbohydrate, vitamins minerals and fibre (Fasuyi, 2006). It also contains oxalate, saponins, glycosides, flavonoids alkaloids and resins (Imosemi, 2018; Jimoh, 2018; Akubue et al., 1980). The nutritional content of *T. occidentalis* makes it desirable as dietary

supplements for humans. The diet preparation of air dried leaves of the plant significantly increased red blood cells counts, white blood cells packed cell volume and haemoblobin concentration in rats (Alada, 2000) while the dietary preparation made with sun dried leaves had no significant effect on haematological parameters in broilers (Fasuyi and Nonyerem, 2007), indicating that the potency of the plant depends on the method of preparation of the plant for consumption. Adaramoye et al. (2007) reported that *T. occidentalis* leaves have hypolipidemic effect and may be a useful therapy in hypercholesterolemia. It was found out that aqueous and ethanol extract of *T. occidentalis* could salvage and prevent free radical production and at the same time have antimicrobial properties (Oboh et al., 2010). In spite of the widespread use of *T. occidentalis*, there are scanty information on its various biological effects on cockerels. This study investigated haematological parameters, serum biochemical indices and histological changes of some organs associated with long term administration of *T. occidentalis* Leaves Extract (TOLE) on cockerels.

MATERIALS AND METHODS

Preparation of *Telfairia occidentalis* leaves extract

A kilogram of freshly cut TOLE with leaf stalks were washed, drained, chopped and pounded in mortar and pestle. This was then squeezed and filtered with cheese cloth to obtain a homogenous extract of the *T. occidentalis* leaf extract (Nworgu et al., 2007). The extract was prepared at four days' interval and served to the birds fresh according to the treatments.

Experimental animals and management

A total of one hundred and sixty-two (162) Nera black cockerel chicks were weighed and randomly allotted to six dietary treatments that contains 30, 60, 90, 120 and 150mL TOLE per one liter of water (Nworgu et al., 2007) for B, C, D, E and F respectively. Treatment A served as the control with no extract. Each treatment was replicated three times with nine birds per replicate in a completely randomized design. The experiment lasted for 24 weeks. The birds were fed the same diets for the first eight weeks and later grower diet was given to the birds till the end of the experiment. The TOLE supplement was served at four days interval throughout the period of the experiment according to the treatment per liter of water and later clean water was served. Feed and water were served ad-libitum. Both the feed intake and weight gain were monitored. Other management practices such as routine vaccination, drug administration and maintenance of cleanliness within and outside the poultry houses were observed.

Collection of blood samples

Blood samples were collected at 24th week of the experiment. Six birds per treatment were randomly selected and bled via wing veins, for haemoglobin and serum biochemical analysis Serum was obtained by centrifugation and the serum samples were stored in deep freezers at minus 10^0c until analyzed.

Heamatological analysis

The Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Haemoglobin (Hb) concentration were determined using the Wintrobe Microhematocrit, Improved Neubauer haemocytometer and Cyanmethemoglobin methods, respectively (Coles, 1986). Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) levels were computed using the formula described by Jain (1986).

$$MCH = \frac{\text{Haemoglobin} \times 10}{\text{RBC}}$$

$$MCV = \frac{\text{PCV} \times 10}{\text{RBC}}$$

$$MCHC = \frac{\text{Haemoglobin} \times 100}{\text{PCV}}$$

Serum biochemical analysis

The serum biochemical assays were carried out using the standard chemical procedures. Total serum protein by Goldberg refractometer (Kohn and Allen, 1995), albumen by Bromocresol Green (BCG) method (Peters et al., 1982) while globulin was calculated according to Coles (1986). Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and triglyceride were determined using Randox test kits (Randox Laboratiries Crumlin, County Antrim, United Kingdom).

Histological studies

Histological studies of the organs and tissues were done according to procedures described by Disbrey and Rack (1970) and Drury and Wallington (1967). The kidneys, liver, spleen and testes of the cockerel were removed from the surrounding tissues. They were fixed in 10% formal saline, and after 72 hours the organs were dehydrated in graded alcohol (20, 30, 50, 70 and 95%) for five minutes, cleared in xylene and embedded in paraffin. The resulting blocks were completely sectioned and randomized. The selected sections were stained in haemotoxylin and eosin and the slides were examined at magnification of x400 under light or optical microscope.

Ethical approval

The study was conducted after approval of ethic and research committee of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Data analysis

The mean values obtained for the determination of various indices in respect of the six treatments were subjected to one-way analysis of variance (SAS, 2003). Treatment means were also separated by Duncan option of the software at p = 0.05 (5% significant level).

RESULTS

Data on the effects of TOLE on haematological parameters and serum biochemical constituents of cocks are presented in tables 2 and 3 respectively. The RBC, lymphocytes, heterophils, albumin, and AST values were similar (P>0.05) among the various treatment groups, while the PVC, Hb, WBC, MCV, MCH, MCHC, monocytes and Eosinophils were statistically different (P<0.05) across the treatments. Except for albumin and AST, all other serum parameters investigated were significantly (P<0.05) affected by the administration of the TOLE. For histopathological examination, there were lesion/necrosis in the liver and kidney of cocks on TOLE treatments while the spleen and testes did not show any visible lesion in all the treatments. However, for birds served 120mL and 150mL TOLE/L of water there were reduced germinal epithelia heights and sloughing of the germinal epithelia respectively (Table 4).

Table 2. Haematological response of Nera black cockerels served *Telfairia occidentalis* leaves extract at 4 weeks interval for 24 weeks

Treatments **Level of TOLE**	**A** **0 ml**	**B** **30 ml/l**	**C** **60 ml/l**	**D** **90 ml/l**	**E** **120 ml/l**	**F** **150 ml/l**	**SEM**
PCV (%)	35.20^{d}	37.22^{ab}	38.39^{a}	35.53^{dc}	36.58^{bcd}	37.11^{abc}	0.14
RBC (× 10^6/mm^3)	2.72	2.71	2.70	2.68	2.73	2.53	0.01
Hb (g/dL)	11.73^{c}	12.41^{bc}	12.79^{a}	11.84^{bc}	12.28^{abc}	12.63^{a}	0.05
WBC (×10^6/mm^3)	13.24^{ab}	12.45^{bc}	13.34^{ab}	13.92^{a}	12.16^{c}	13.55^{a}	0.06
MCV (μ^3)	131.32^{b}	137.38^{ab}	142.48^{a}	132.60^{b}	134.02^{b}	135.10^{b}	0.05
MCH (µµg)	42.87^{b}	45.78^{ab}	48.50^{a}	44.25^{b}	44.99^{ab}	45.03^{ab}	0.31
MCHC (%)	33.33^{a}	33.19^{ab}	32.97^{b}	33.33^{a}	33.33^{a}	33.33^{a}	0.21
Lymphocytes (%)	65.50	60.55	63.19	65.43	64.83	67.80	0.89
Heterophils (%)	29.00	35.36	30.36	28.33	29.52	31.33	0.87
Monocytes (%)	2.66^{abc}	2.86^{abc}	2.14^{c}	3.88^{a}	3.51^{ab}	2.28^{bc}	0.10
Eosinophils (%)	4.83^{a}	2.55^{b}	3.25^{ab}	3.25^{ab}	2.22^{b}	2.22^{b}	0.01

^{abcd} Means in the same row with different superscript are significantly (P<0.05) different. PCV =packed cell volume, RBC=red blood cell, Hb= haemoglobin, WBC= white blood cell, MCV= mean cell volume, MCH=mean cell haemoglobin, MCHC= mean cell haemaglobin concentration, SEM=standard error of means

Table 3. Serum biochemical response of Nera black cockerels served *Telfairia occidentalis* leaves extract at 4 weeks interval for 24 weeks

Treatments/ **Level of TOLE**	**A** **0 ml/l**	**B** **30 ml/l**	**C** **60 ml/l**	**D** **90 ml/l**	**E** **120 ml/l**	**F** **150 ml/l**	**SEM**
Total protein (g/dL)	3.88^{b}	4.24^{ab}	4.84^{ab}	4.97^{a}	4.98^{a}	4.26	0.08
Albumen (g/dL)	2.14	2.18	2.37	2.30	2.08	2.04	0.03
Globulin (g/dL)	1.61^{b}	2.06^{ab}	2.48^{ab}	2.67^{a}	2.91^{a}	2.08^{ab}	0.07
Albumin/globulin ratio	1.44^{a}	1.01^{b}	0.98^{b}	0.93^{b}	0.75^{b}	1.02^{b}	0.02
Aspartate amino transferase (IU/L)	91.60	102.40	101.86	97.28	94.86	96.10	1.28
Alanine amino transferase (IU/L)	7.96^{bc}	7.74^{c}	8.41^{ab}	8.00^{abc}	7.84^{bc}	8.06^{a}	0.05
Triglyceride (mg/dL)	202.84^{a}	163.57^{b}	160.52^{b}	149.42^{b}	158.62^{b}	154.92^{b}	1.54

^{abc}Means in the same row with different superscript are significantly (P<0.05) different. SEM=Standard error of means

Table 4. Histopathological effects of *Telfairia occidentalis* leaves extract served at 4 weeks interval for 24 weeks to Nera black cockerels on internal organs

Treatments / Organs	A 0 TOLE (mL/L)	B 30 TOLE (mL/L)	C 60 TOLE (mL/L)	D 90 TOLE (mL/L)	E 120 TOLE (mL/L)	F 150 TOLE (mL/L)
Kidney	No visible lesion	Wide spread coagulative necrosis of renal tubules	Severe generalized interstitial congestion.	Mild moderate loss and sloughing of renal tubular epithelium	Severe generalized interstitial congestion	Widespread coagulative necrosis of the renal tubules
Liver	No visible lesion	Portal congestion	Severe central venous congestion	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell	Severe portal congestion with periportal cellular infiltration by mono-nucleus cell
Spleen	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion
Testes	No visible lesion	No visible lesion	No visible lesion	No visible lesion	No visible lesion. Germinal epithelium height reduced	No visible lesion marked sloughing of the germinal epithelium

TOLE = *Telfairia occidentalis* Leaves Extract.

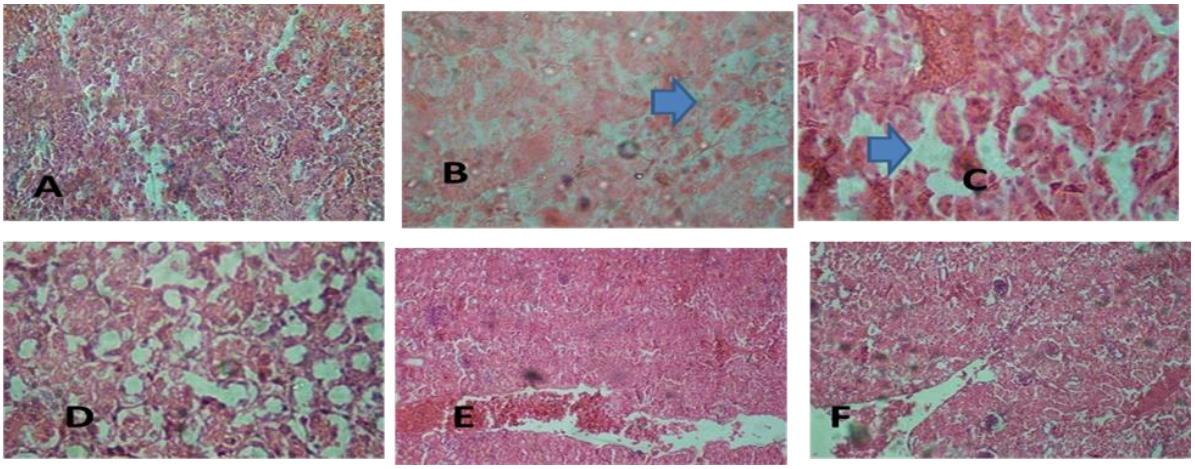

Figure 1. Micrographs of the kidneys of Nera black cocks served fluted pumpkin leaf extract at 4 weeks interval for 24 weeks A= 0mL/ TOLE/L (no visible lesions seen). B= 30mL/ TOLE/L (wide spread coagulative necrosis of the renal tubules (arrow); C= 60mL// TOLE/L (severe generalized interstitial congestion), D = 90mL/ TOLE/L (mild to moderate loss and sloughing of renal tubular epithelium), E= 120mL/ TOLE/L (severe generalized interstitial congestion), F= 150mL/ TOLE/L (widespread necrosis of the renal tubules. (mg× 400)

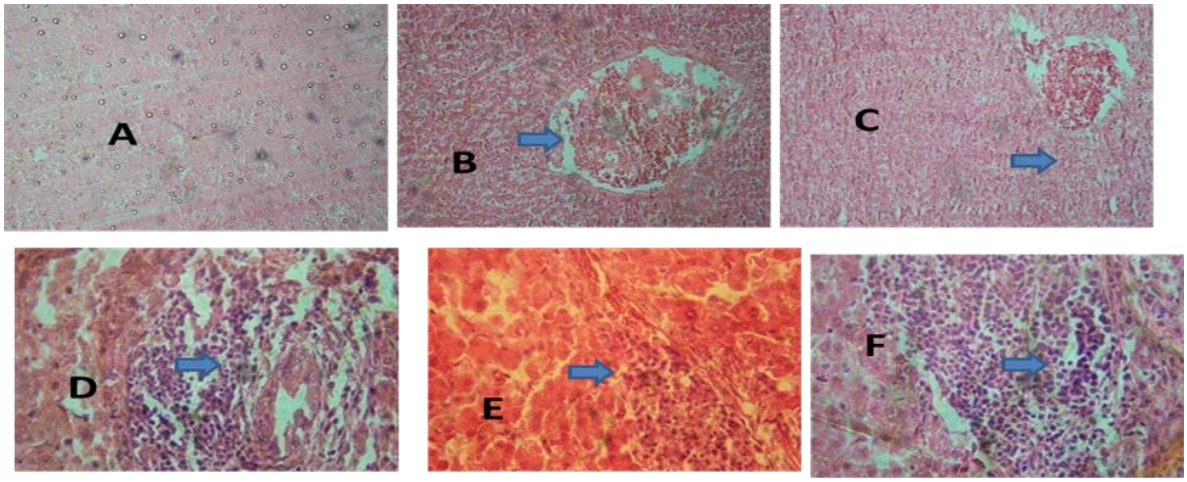

Figure 2. Micrographs of the liver of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks. TOLE = *Telfairia occidentalis* Leaves Extract. A: 0mL/L (No visible lesions seen), B: 30mL TOLE/L (Portal congestion (arrows), C: 60mL TOLE/L (Severe central venous congestion), D: 90mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells), E: 120mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells). F: 150mL TOLE/L (Severe portal congestion with periportal cellular infiltration by mono-nuclear cells) (Mg× 400).

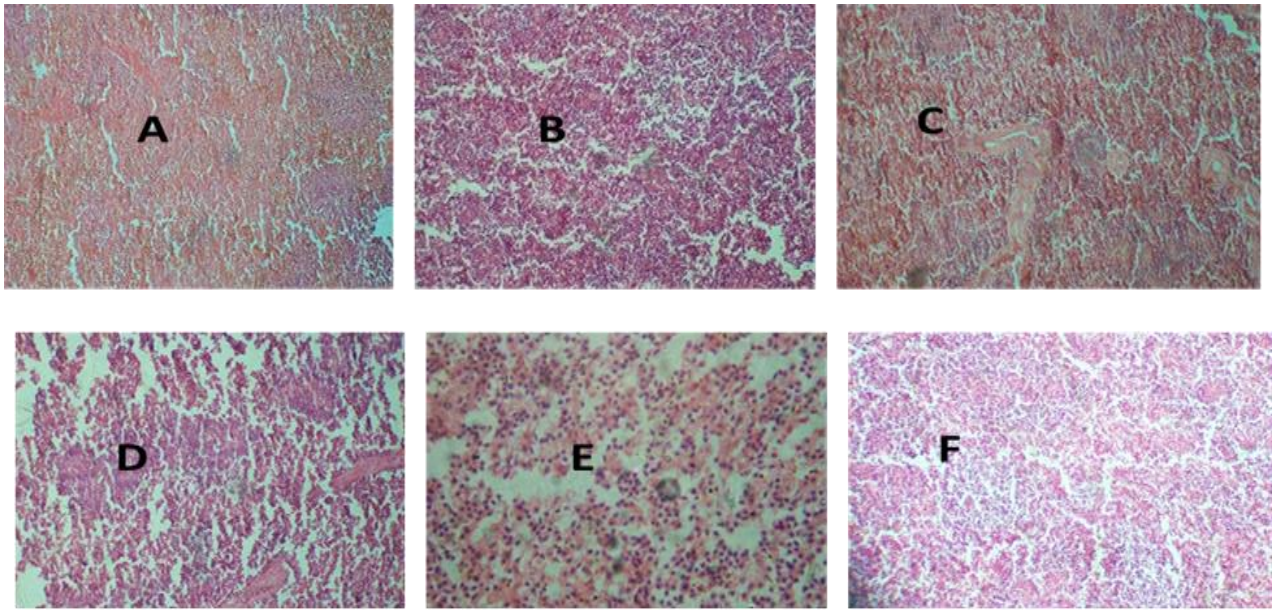

Figure 3. Micrographs of the spleen of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks A to F: No visible lesions seen, (Mg×400). TOLE = *Telfairia occidentalis* Leaves Extract. A= (0ml TOLE/L), B= (30mL TOLE/L), C= (60mL TOLE/L), D= (90mL TOLE/L), E= (120mL TOLE/L), F= (150 TOLE/L)

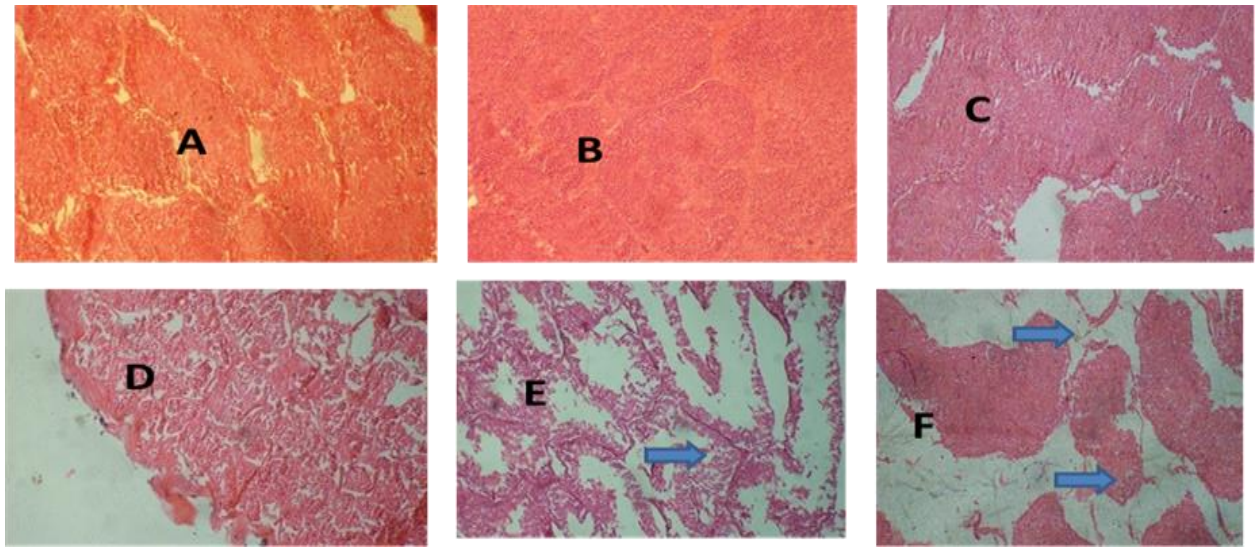

Figure 4. Micrographs of the testes of Nera black cocks served fluted pumpkin leaf extract at 4 week interval for 24 weeks A to D: No visible lesions seen, E and F: Marked sloughing of the Germinal Epithelium (arrows) (Mg × 400). TOLE = *Telfairia occidentalis* Leaves Extract. A= (0ml TOLE/L), B= (30mL TOLE/L), C= (60mL TOLE/L), D= (90mL TOLE/L), E= (120mL TOLE/L), F= (150 TOLE/L)

DISCUSSION

This study showed that the aqueous extract of the plant produced significant ($P<0.05$) increase in the levels of the PCV, Hb, WBC, MCV, MCH and monocytes when compared with the control. The best PVC (38.39%) and Hb (12.79 g/dL) were obtained for birds served 60 ml/l TOLE while the least parameters (35.20% and 11.73g/dL respectively) were obtained for birds on the control. This showed that animals suffering from low blood volume may benefit from the administration of this plant extract. This observation of increased level of erythron supports the earlier research findings that this plant has haematinic potency (Adedapo et al., 2002; Nworgu et al., 2007; Ifeanyi et al., 2014). The increase in the level of erythron produced by this extract may be due to the fact that the leaves of this plant are rich in many minerals (Burkill, 1994; Aletor et al., 1995; Archibong, 2002) high in crude protein, essential and non-essential amino acid in the leaf (Alabi et al., 2017; Fasuyi, 2007). This result agrees with

the study of Alada (2000) and lends validity to the use of the leaves in the treatment of anaemia (Gbile, 1986). Birds on treatment D recorded the highest value of 13.92 × 10^6/mm for WBC though it was not significantly different (P>0.05) from the values obtained for birds on treatments A, C and F but significantly different (P<0.05) from those on B and E. The WBC helps to defend the body against foreign organism or extraneous materials so the higher the WBC the better the ability of animal to fight diseases since the WBC values fall within the normal range as reported by Mitruka and Rawnsley (1981), it then suggests that the health status of the birds was not challenged by the administration of this extract.

Birds on treatment C recorded the highest MCV (142.48µ) value which was similar to the values obtained for birds on treatment B but different (P<0.05) from the values obtained for birds on treatments A, D, E and F. The highest MCH value of 48.50µg was also obtain for birds on treatment C though it was similar to the values obtained for birds on treatments A and D. The result obtained for the corpuscular constants in this study did not follow any particular trend and they were within the normal physiological range as reported by Mitruka and Rawnsley (1981) for normal cocks. The values obtained for the monocytes and eosinophils were significantly different but did not follow any particular pattern.

Serum biochemical investigations have been explored extensively to distinguish normal state from stress and diseased conditions in animals. Except for the albumin and AST, all other serum parameters investigated in this study were significantly (P<0.05) influenced by the administration of the TOLE.

The total protein, globulin and ALT increased with increasing levels of TOLE while triglyceride decreased with increasing levels of TOLE. The total proteins were higher for birds on the TOLE treatments than for birds on the control. This increase could be attributed to the additional protein supplied by the TOLE since the birds were on the same diets. This observation was similar to the report of Adedapo et al. (2008) and Alabi et al. (2017) who reported that aqueous extract of fluted pumpkin leaves caused a significant increase in the levels of the total protein and globulin of finisher broilers and rats respectively that were on fluted pumpkin leaves extract treatment. Although the albumin values in this present study were not significant (P>0.05), Nworgu et al. (2007) reported an increase in the albumin levels of birds on treatment F (150 mL/L) having the values of 10.6 IU/L. The slight increase in the serum enzyme activities suggest that there was little breakdown of muscle tissues on the birds on the TOLE treatments. The values of the triglycerides decreased with increasing levels of TOLE, this points to the ability of the extract to lower the lipids content as earlier reported by Adaramoye et al. (2007) and Nworgu et al. (2012).

Telfairia occidentalis leaves extract showed various cellular effects on histological characteristics of the tissues examined. The liver and kidney were the most affected while in the spleen and testes no lesion was observed. The hepatic lesion was characteristic by mild to severe portal congestion with periportal cellular infiltration by mono-nuclear cell and severe central venous congestion while the renal lesion were characterized by widespread coagulative necrosis of the renal tubule with generalized intestinal congestion and sloughing of renal tubular epithelium. This could be traced to the presence of alkaloids (Burkill, 1994) which have been observed with *Telfairia occidentalis*. This corroborates with the findings of Akubue et al. (1980) who reported venous congestion and in some cases burst vessels in the liver, spleen, lungs and heart of rats given aqueous extract of *Telfairia occidentalis* while Iweala and Obioda (2009) also reported irregularities in the liver of rats fed *Telfairia occidentalis* supplemented diet. Acute toxicity including hepatic toxicity have been reported (Akindele et al., 2018; Imosemi, 2018) in mice given *Telfairia occidentalis* up to 5000 mg/kg orally, with signs of decreased locomotion, calmness, writhing and increased breathing at higher doses of LD50 of intraperitoneal administration at 3000-5000 mg/kg. Although, there were no lesion observed in the testes in all the treatments, cocks on treatments E and F (120 and 150 mL TOLE/L) showed reduced germinal epithelium height and marked sloughing of the germinal epithelium respectively. The implication of this is disordered spermatogenesis and infertile/immature sperm cells. However, this effect is dose dependent since it was not observed at levels between 30-90 mL TOLE of water implying that, at higher doses of TOLE there was cell damage which could be detrimental to the fertility of cocks. This was similar to the findings of Adedapo et al. (2008) who reported testicular degeneration with severe disorganization of seminiferous tubules which were devoid of spermatic cells in rats served fluted pumpkin leaves extract. However, Iweala and Obioda (2009) reported the presence of large spermatogonia in the testes of rats fed *Telfairia occidentalis* supplemented diet while Nwangwa et al. (2007) reported a regenerative effect on the histology of rat's testes served the extract of fluted pumpkin leaves extract. Imosemi (2018); Sakpa et al. (2015) and Saalu et al. (2010) also testified to the

testiculo-protective attributes of TOLE at lower doses up to 200mg/kg in adult male Wister rats that showed increased sperm count, sperm viability and motility, enhanced spermatogenesis with elevated levels of testosterone and luteinizing and follicle stimulating hormones; but found aqueous TOLE to be testiculo-toxic at high dose of 800mg/kg body weight of male Wister rat.

CONCLUSION

Long term supplementation of TOLE for cockerel production should not exceed 60mL of TOLE per liter of water as the administration in excess of this can bring about tissue breakdown and reduced fertility. Animals suffering from blood loss can benefit from the administration of fluted pumpkin leaves extract as the extract increased erythron production.

DECLARATIONS

Competing interests

The authors declare that they have no competing interests.

Author's contributions

All the authors have made substantive contribution to the study from its design to implementation, collection of data, statistical analysis, writing reports and manuscript preparation.

Consent to publish

All the authors gave their informed consent prior to their inclusion in the study.

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Ecological Aspects and Policy Impact on Expansion of Poultry Production in Ireland (1995-2014)

AKM Sarwar Inam[1], Md Suzauddula*[2] and John Kearney[3]

[1]*Assistant Professor, Department of Nutrition and Food Engineering, Daffodil International University, Dhaka-1207, Bangladesh*
[2]*Research Associate, Department of Nutrition and Food Engineering, Daffodil International University, Dhaka-1207, Bangladesh*
[3]*Lecturer, Dublin Institute of Technology, Kevin Street, Dublin 2, D08 X622, Ireland*

*Corresponding author's Email: suzauddula34-506@diu.edu.bd; ORCID: 0000-0003-4475-8393

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ABSTRACT

Poultry meat is very popular in Ireland because of low cholesterol level. Ireland is in the top position for the consumption of poultry meat in whole Europe. Ireland emits 3.3 kg CO2- equivalent per kg of poultry for the poultry meat production which is the lowest amount among all the other European countries. To expand this sector with respect to environmental concern some issues need to be considered very carefully such as effective poultry feed production system, energy consumption in both poultry production and processing area, manure management system, wastewater and odour management systems. If these issues are not handled carefully, several types of harmful effect will occur in both living and environment cycle such as water borne diseases, global warming and ozone layer depletion. The objective of this report is to give an overview of the current situation of poultry production in Ireland, policies and legislation related to poultry production and to show the way to expand this sector in Ireland in line with current ecological concern.

Keywords: Ecological and policy, Management of poultry-waste, Poultry and environment, Poultry production

INTRODUCTION

Recently the department of agriculture, food and the marine announced that about 24 billion euro comes from agri-food sector in Ireland, and in the national economy, 6.3% gross value added, 10% of Ireland's total exports and 7.7% of total national employment are contributed by this sector (Teagasc, 2012). By exporting chicken in 2014 Ireland earned 310 million euro and in 2015 the earning increased to 320 million euro (Bia, 2016). The Irish poultry industry is a very important contributor to the agriculture and economies of Ireland. This industry is growing very fast. The annual consumption of poultry meat has been almost doubled between 1980 and 2000 (FAO, 2009). In the European Union, Ireland was the highest position in per capita consumption of poultry meat (McCarthy et al., 2004).

The poultry industries were contributing a lot to meet the increasing need for the cheapest and safe supply of meat and eggs since last decade (Kearney, 2010). Many new changes happened in this growing sector such as structural changes, land independent farming, intense productions etc. The main intention was to decrease the production cost and increasing supply which have been done by improving animal genetics, optimized nutrition, efficient operations and new integrated technologies (Paul, 2010). All these things have given rise to environmental concerns. This is now not only limited to production area but also amplify to environmental problems at local and global scales. Globally greenhouse gas emissions are one of the main problems in the world. The emission sources can be categorized as i) feed production, using for fertilizers to produce feed and even production processes of that fertilizer, ii) on-farm energy consumption, iii) energy consumption for transporting poultry or poultry feed, iv) emission from manure management system, v) energy consumption in poultry processing area, vi) emission from waste or by-product of poultry processing. Mainly six gases are responsible for greenhouse gases. They are CO_2, CH_4, N_2O, hydrofluorocarbon, perfluorocarbon and sulphur hexafluoride (IOPC, 2007).

The emission of first three (carbon-dioxide, methane, nitrous oxide) are higher for faecal matter and the concentrations depend on the ventilation efficiency and rate (JRC, 2010). In Ireland, the carbon footprint of poultry and egg production is low compared to other European countries. But from the ecological point of view in growing and to expand the poultry sector in Ireland utmost concern should be given at this field. In this review, current situation of poultry production along with European and Irish policies and legislation, carbon footprint, environmental issues and the prevention of the poultry waste with the expansion of this sector are discussed.

Poultry production in Ireland

In Ireland, four systems are maintained for poultry production. Those are intensive (commercial), free-range, label rouge and organic. Main differences between these production systems are shown in table 2 (Lampkin, 1997).

Table 2. Differences between poultry production systems-March 1997, Ireland

Production System	(a)Intensive		(b)Extensive
	Broiler[1]	Free range table birds[1]	Organic[2]
Minimum age at slaughter (days)	None, generally 39-45 days	56 (days)	81 (days) if not slow growing
Breed specification	None	None	None as such, but slow growing preferred
Max house stocking density (fixed housing)	34.0 kg LW/m^2	13 b/m^2 or 27.5 kg LW/m^2	6 b/m^2 (layers) or 10 b/m^2 (fattening) max 21 kg LW/m^2
Max house stocking density (mobile housing)			16 b/m^2 (fattening) max 30 kg LW/m^2
Flock size	Unlimited	Unlimited	4800 chickens, 3000 layers or 2500 turkeys per poultry house
Access to range	Not required	Continuous day time access require or all least half their lifetime	Weather permitting, for at least 1/3 or their life
Pasture allowance	None	1m^2/birds	So that not more than 170 kg/N/ha/yr
Feed specification	None	Finisher contains at least 70% cereals	At least 65% cereals, no synthetic amino acids, 100% organic ingredients. However, a derogation exists that allows 20% from non-organic sources

Source: (Lampkin, 1997)

Intensive production is a very common and commercial process. In this process, chickens are raised up in a controlled environment and high nutrient feeding system. Breast meat is very popular in Ireland. That is why breeders are preferred which give a high amount of breast meat. Normally it takes 35 to 56 days to reach the weight 3kg of chicken and in case of intensive egg production around 290 eggs can be collected per chicken per layer cycle.

Free range is also a popular system where chickens are allowed access to the outdoors. These birds take around 56 days to grow up. According to Teagasc (2006a) there are some regulations for free-range farming in Ireland. Those are i) chickens should be allowed daytime access for open-air running, ii) the ground should be covered with plant or grass, iii) the maximum stocking density has to be a thousand chickens per hectare, iv) an insulated house has to be made with a floor space of one-meter square per seven chickens (Teagasc, 2006a).

Label Rouge is a French pasture-based production arrangement. According to this system, there is some regulation for using feeding ingredients such as diets should contain 65% cereal. At six weeks age a ring is worn on the pullet's wing by the certifying organization. The density has to be 13 pullets per m^2. Normally chicken lays after 21 weeks when it will go through a complete light and nutrition program.

Organic table birds and layers are very popular in whole Europe. It must be maintained by the European council regulations and examined by certifying bodies of each country. In Ireland, there are three organic certification constitutions.

1. Demeter standards
2. Irish organic farmers and growers association
3. Organic trust (Teagasc, 2006b)

Around 29850 ha agricultural land was used for organic food in 2002 in (DAF, 2002). Most of the organic foods in Ireland are fruits and vegetables. Organic meat mainly beefs and lambs are occupied 25% of the organic food. Organic poultry and egg are negligible due to the limited supply (DAF, 2002). Table 1 shows the total number of producers and birds involved in organic poultry production in Ireland in 2002.

The market for organic poultry feed is very small. Hence only one supplier is available all over Ireland (Teagasc, 2006b). This feed is very expensive almost 80% expensive than normal poultry feed. This feed must be free from any types of genetically modified organisms. Chickens which are produced for organic meat production requires 81 days to grow up which is a lengthy procedure (Teagasc, 2006b).

Table 1. Total number of producers and birds in 2002, Ireland

	Total number of producers*	Total number of birds
Broiler hens	11	1,935
Laying hens	64	18,793
Turkeys	5	**

* Producers with poultry numbers or 10 or more** insufficient data. Source: Anon (2002)

European and Irish policies and legislation

In Europe union, the poultry meat production is directed by the European communities (fresh poultry meat) regulations 1996 (S.I. No. 3/1996) and council directive 71/118/EEC European Economic Community (EEC) (Directive, 1996). This regulation controls the premises approval, application of the health mark to poultry, hygiene and sanitary standard etc. European communities (marketing standards for poultry meat) regulations 2002 (S.I. No 440) cover the marketing standards such as labelling, grading by quality etc. (Magdelaine et al. 2008).

The Poultry hatcheries act 1947 and the poultry hatcheries regulations, 1959 controls about the breeding stock as they should be collected from permitted breeding sources. It also controls the inspection and blood testing to keep poultry free from diseases (HARRIS, 1973).

European communities (marketing standards for eggs) regulations, 254/1992 implementing regulation (EEC, No. 1907/90) and regulation (EEC, No. 1274/91) deals with egg grading, weighing, packing, labelling, transporting and marketing. The egg for incubation is not allowed for human consumption (EISB, 1992b).

European communities (egg products) regulations 1991 (S.I. No. 293 of 1991) and European communities (egg products) regulations 1992 (S.I. No. 419 of 1992) implementing council directive No. 89/437/EEC deals with hygiene, supervision, marking, marketing of egg products (IRL, 1991).

According to Ireland rules and regulation, if anybody is going to deal or trade poultry for commercial or non-commercial basis has to be certified with the department of agriculture, fisheries and food under the diseases of animals act 1966 (registration of poultry premises) order 2008 (S.I. No. 42 of 2008 as amended by S.I. No. 57 of 2011). And that person must be committed to the rules of biosecurity and record keeping requisites (DAFM, 2013)

The regulation for poultry manure is written in statutory instruments S.I. No. 378 of 2006. All the requirements are written here for that person who will apply for Integrated Pollution Prevention and Control (IPPC) license. The Environment Protection Act, 1992 is required for the following activities (EISB, 1992a).

1. Any type of emission cannot violate the air quality standard enumerated under section 50 of air pollution act, 1987.
2. Any type of emission cannot violate the quality standard of waters, trade effluents and sewage which is enumerated under section 26 of the local government (water pollution) act, 1977.
3. Any type of emission from the action of plant, methods or procedure cannot violate the rules of European communities act, 1972.
4. Any noise cannot violate the rule under section 106.

Environmental protection agency act 1992 (established activities) order, S.I. No. 279 of 2006 deals with poultry installation in Ireland (Intertradeireland, 2011). The key legislative concerned to poultry production in Ireland is actually characterized by the following standards:

1. International quality management standards as for example ISO 9001:2000
2. Hazard analysis and critical control points as summarized by Codex Alimentarius (1997)
3. Compatible national and Europe union legislative requirements involving European Commission(EC) 178:2002 and EC 852:2004
4. EN 45001 (1998) general requirements of bodies who are involved in product certification systems (Bord Bia, 2008).

Carbon footprint during poultry production

According to food and agriculture organization report "livestock long shadow; environmental issues and options" livestock production is one of the main issues for the environment. The report showed that around 18% Greenhouse Gas (GHG) emissions come from livestock (FAO, 2006). GHG is lower in pork and poultry for the adequate digestion process and inexistence of enteric fermentation process. And the GHG emission is lower in poultry comparing to pork. In the European Union considering product level 19 to 28kg CO2-equiv. per kg of meat (beef, sheep and goat) is the total GHG intensity. Comparing that amount poultry gives only 5-7kg CO2-equiv. which is less. The emission of CH4 and NO is high

for poultry production. Egg considerably gives lower carbon footprint. In Europe, union egg production causes a net emission of 2.8–3.2 kg of CO2-equiv. per kg of eggs. In Ireland, poultry meat production emits 3.3 kg CO2-equiv. per kg of poultry (Weiss and Leip, 2012).

Jacob (2009) showed the average GHG emission in a broiler industry. He stated that 5.5 tons CO2 emission happens for 1000 broilers marketed, 7.5 lbs CH4 emission occurs per thousand broilers marketed and 3.8 lbs N2O emission occur for thousand broilers marketed.

The carbon footprint of Ireland in the livestock sector is always lower than any other country in Europe union. Joint research center of the European Union commission published major research in which Ireland is rated amongst the best for the carbon footprint (Teagasc, 2011). The research entitled "evaluation of the livestock sector contribution to the Europe Union GHG emissions" demonstrated the carbon emission of livestock products, the production of feeds. It also reported the emission due to an input of mineral fertilizer, pesticides, energy etc. from this study, the poultry carbon footprint was 3.3 kg CO2-eq per kg of poultry where the average value of carbon footprint in European Union is 4.9 kg CO2-eq per kg of poultry (Teagasc, 2011).

From figure 1, it can be easily noticed that among the other European countries the emission of GHG in Ireland for egg production is really low. It is near about 2.5 kg CO2-equiv. per kg of product. From figure 2, it also can be easily noticed that the position of Ireland for poultry meat production is really not the alarming phase. Ireland is in the lowest position for the emission of GHG in case of poultry meat production

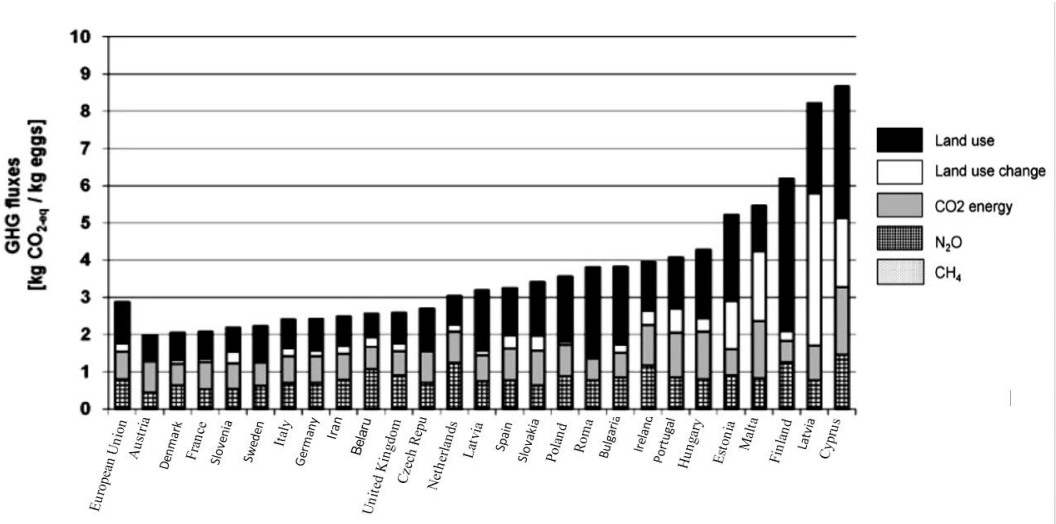

Figure1. GHG fluxes of eggs (in kg CO_2-equiv. per kg of product), European Union (27 countries), 2004 (Source: Weiss and Leip, 2012).

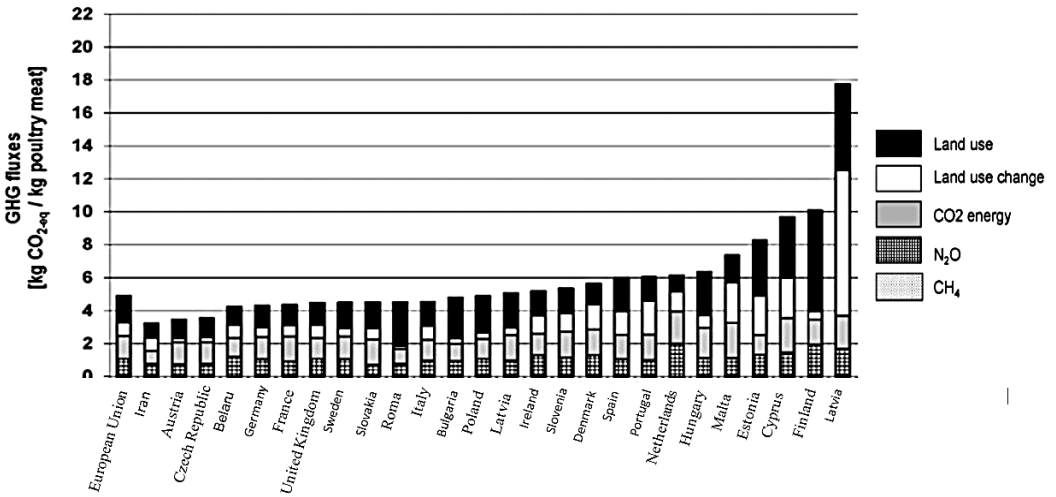

Figure 2. GHG fluxes of poultry meat (in kg CO2-equiv. per kg of product), European Union (27 countries), 2004 (Source: Weiss and Leip, 2012)

Environmental Issues

In a poultry processing area, some factors are very important to control environmental hygiene. The factors are exterior structure and grounds, interior structure, interior walls, ceiling and overheads, floors, drainage, doors, windows, lighting, water and water supply, knives, sterilizers, hoses and other equipment, extraction and ventilation, cleaning materials and storage, food trays, electronic fly killers, effluent treatment and complete waste disposal system (FAO, 1996).

Mainly two important parts are important for ecological aspects. One is the production of concentrated feed and another is the generation of GHG through chicken processing and transporting of processed product.

Environmental impacts of poultry feed production

The feeds for poultry consist of main cereals, soy, oilseeds and pulses. Intensive feed production effects negatively to the land and water. To produce high crop yield mineral fertilizer, pesticides and different herbicides has to be used intensively which pollute the environment. Smil (1999) reported that 30-50% of nitrogen fertilizer and 45% of phosphorus fertilizer is absorbed by the plant. For feed production in the world, about 20 million tons of nitrogen fertilizer is used and 36% of that feed is produced as poultry feed. This also causes serious air pollution because of the volatile nature of ammonia. Feed production also affects negatively in biodiversity. The increasing demand for feed requires expansion of cropland and thus it affects through the adaptation of natural habitats. Sometime overexploitation of fisheries is happening for producing the fishmeal for the poultry (Steinfeld et al., 2007).

Environmental impacts on poultry manure

The growing poultry industries are giving serious concern about poultry waste mainly poultry manure. Poultry litter is a very good origin of organic fertilizer. But this poultry manure creates some environmental problems such as nitrite that extracted into the groundwater, phosphorus from poultry manure mixes up with surface water bodies and various pathogenic organism released by poultry manure. The most important thing to the poultry manure management system is to keep the manure dry as early as possible (Moore et al., 1995).

Environmental impacts on air quality

In chicken production houses three gases are of greatest concern. Those are ammonia, carbon dioxide and carbon monoxide. Table 3 revealed that both long and short term CO_2 produce significantly had high amount than ammonia and Carbon monoxide. These gases negatively impact on human health.

Table 3. The following level has been observed for the protection of human health

Name of Gas	Long Term Exposure Limit (8 hours a day) ppm	Short Term Exposure Limit (10 minutes) ppm
Ammonia	20	35
Carbon monoxide	50	400
Carbon dioxide	3000	5000

Source: Bord Bia, 2008

Personnel-related to poultry production systems have to be aware of the location and production so that the air emission does not hamper the local environment. To establish a new production, house a producer can reduce problems related to the environment in many ways such as proper ventilation system, cover poultry litter, the proper management system of wastewater and waste packaging material, transporting through proper vehicle etc. Producers and processor should be careful because if they cross the limit they will need EPA license.

Prevention of poultry waste production

Industrial poultry waste management; in the poultry processing industry, various waste products are produced which have to be managed or processed for the safety of the environment. Some common practices are discussed here:

In a modern plant, flow-away systems are being used for fast and adequate processing. To reduce blood loss before killing stunning should be done. If stunning is not done blood may be spattered over a large area and deteriorate feathers. Dry cleaning is important before washing the whole receiving area. Feathers which are recovered can be disposed of or cooked by pressure to hydrolyze the keratin protein. Screened water should be used in the de-feathering operation. Feet, head, viscera and other parts which are inedible should be gathered for disposal or inedible analysis. Final evisceration wash water can be re-used for other unimportant sub-processes. The special nozzle should be used to reduce the wastage of water (FAO, 1996).

Wastewater management

Wastewater from the poultry processing industry causes a serious environmental impact. It mainly hampers the natural environment in three ways. The waste water contains a lot of biodegradable compounds which reduce the dissolved oxygen in the water. Water containing

reduced dissolved oxygen is very harmful to aquatic life. Eutrophication may occur due to the presence of macro-nutrients such as nitrogen, phosphorus in the water bodies. Excess algae growth and their consecutive dying release too much mineral in water which also affects harmfully to the aquatic life. Some effluent compounds directly harm aquatic life such as un-ionized ammonia (Demayo et al., 1982). In the poultry processing industry, the offal flume-water contains one-third of the total waste load. It has been reported that the average value of biological oxygen demand (BOD) is 3.4 kg per ton of LWK (live weight killed). The highest BOD is found in chicken blood around 4.5 kg BOD per ton of LWK. The detailed values are showed in table 4.

Scalding is the processing step in which maximum energy consumption takes place. The scalding tank which contains residues and feathers holds 0.6 to 3.1 kg BOD per ton of LWK. In the chilling process, the overflow water contains 0.4 to 2.5 kg BOD per ton of LWK. Final wash water holds 0.7 kg BOD per ton of LWK. About half of the BOD comes from cleaning operation in a poultry processing industry (FAO, 1996).

Table 4. Different components of poultry slaughterhouse, European Union, 1973

Components	Poultry slaughterhouses	
	Chicken Range (kg/Ton)	Turkey Range (kg/Ton)
Biological oxygen demand	3.3 - 25	1 – 9
Chemical oxygen demand	5.9 - 45	1.8 – 16
Kjeldahl nitrogen	0.15 - 12.2	0.4 - 1.9
Suspended solid	0.1 - 22	0.6 - 10.9
Phosphorus	0.054 - 2.5	0.034 - 0.2

Source: Verheijen (1996).

Manure management

Statutory Instruments S.I. No. 378 of 2006 deals with poultry manure management. The litter from the poultry farm should be stored in the litter storage shed. That shade should have 347-meter square floor area. Shed also contains a ditch for wash-waster holding. That ditch should be the 8.1-meter cube. Normally 1-meter cube space is needed for 600 kg of litter. A contractor will provide all the necessary things like machinery or labor to clean the houses and transfer the litter to the storage shed (EPA, 2009).

Emissions minimization

Emission to air, water or land that occurred for poultry housing can be prevented or minimized in many ways. Walls and roofs in the poultry house should be properly insulated and smooth enough for cleaning easily. The building should be waterproof. Forced air drying system should be used to dry manure rapidly on belts. This drying and stabilization will reduce flies. Gable fan and air inlet should be conducted electronically. Gable fan will be only operated when the temperature will rise more than 25 °C. Fans should be adapted with light filters to minimize emissions. Fans should be cleaned on a regular basis. Nipple drinker system should be used to minimize water wastage and to manage dry manure to reduce the emission of ammonia. Low energy lighting should be used. A review of housing and overall management should be carried out after 12 months and after following that review developed method should be implemented for the next year.

Manure should be dried instantly within 24 hours for volatilization of ammonia. Dried manure should be sent to manure store (Alberta, 2008).

Energy minimization

The artificial heating system is not commonly adapted for the layer farms because of high stocking density and low temperature. Common activities regarding energy requirement are heating the water in the winter season, distributing system of feed, ventilating the house, lighting (high energy consumption requires here), collecting and sorting of eggs and packaging. On the other way, in a broiler farm energy requires for the heating system which is being done initial phase, preparing and distributing of feed and ventilating of the house which is weather dependent.

To reduce the energy consumption for poultry production some methods can be applied, such as high-quality insulation in walls and roof, automatically ventilation and constant temperature controlled systems. On the other hand, in the feeding system, power consumption can be increased if high friction occurs. So effective and automated feeding system installation is necessary to minimize the energy consumption (Baxevanou et al., 2017).

Effective measures to monitor emissions

Emissions to air

Sometime incinerator should be used to burn carcass. Small incinerator (less than 50 kg per hour) should be used. The temperature inside the incinerator should reach to 85 °C for complete combustion. Monthly checking is necessary for the incinerator.

Emission to water

The dry cleaning system is used which remove completely the contaminated run-off. Sometimes it is passed directly from hard standing region to waste water tank. According to the code of good practice, the wastewater tank should be emptied to thoroughly clean up. Record keeping should be done with checking or emptying of the tank (Sparrey, 1994).

Emission to land

Discharging of ammonia and dust from the air and spreading manure on soil may be the reasons for emissions to land (Sharp, 2006).

Odour minimization

The odour of poultry waste may be minimized by taking some steps. Effective dry manure system is maintained with controlling humidity, temperature and prevent leakage at the water supply system. High standard of cleanliness should be maintained by regularly cleaning up deposited dust, the dead body removes and controlling internal weather condition. For preventing feed wastage storage bins should be sealed (Ranadheera et al., 2017).

CONCLUSION

Ireland is the highest position for consuming poultry meat among other European countries and on the other hand, this country has the lowest position for emission of greenhouse gases for poultry meat production. Here chicken meat production emits 3.3 kg CO_2-equiv. per kg of poultry. So the situation is not critical yet. But it is going to be critical soon if the necessary preventive measure will not be taken in the near future. Consumption of poultry meat is increasing daily. The new technologies are being used which consequently increase GHG. Intensive poultry production is popular here which gives fewer GHG compared to free-range poultry production. Through maintaining the rules and legislation for poultry farming in Ireland, it is very much possible for the poultry farmers to expand their business. Not only the poultry production industries but also the poultry processing industries need to be concern about ecological aspects. They produce the largest amount of BOD in the whole processing systems. Effective poultry feed production, energy consumption in both poultry production and processing area, manure management system, wastewater and odour management system all these are very importantly handled as these systems are directly affiliated to the environment. Proper knowledge on the current scenario of poultry production in Ireland with ecological concern and the proper guidance with European and Irish policies and legislation are the basic need to expand this sector.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

A K M Sarwar Inam and John Kearney designed the experiments and performed the experiments. A K M Sarwar Inam and Md Suzauddula analyzed the results, drafted and revised the manuscript. Finally, all authors have read and approved the final manuscript and consent to publish in JWPR.

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The Effect of Dietary Inclusion of Probiotics on Growth and Intestinal Morphology of Broiler Chickens

Murat Gulmez[1*a], Nurhayat Gulmez[2], Seyitali Bingol[3], Turgay Deprem[4] and Serap Koral Tasci[4]

[1]*Asilcag Trading Ltd. Nicossia, Cyprus.*
[2]*Near East University, Faculty of Veterinary Medicine, Department of Histology and Embriology, Nicossia, North Cyprus*
[3]*Kafkas University, Faculty of Medicine, Department of Histology and Embriology, Kars, Turkey*
[4]*Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embriology, Kars, Turkey*
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[*]Corresponding author's Email: mgulmez@hotmail.com; ORCID: 0000-0003-3888-6815

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ABSTRACT

Probiotics are currently under investing the most valuable substances alternative to antibiotic growth promoters in poultry breeding practice. This research was performed to evaluate the effect of supplementing broiler drinking water with probiotics (*Pediococcus acidilactici* and *Bacillus subtilis*) at a concentration of $\geq 10^8$ CFU/ml during 42 days of feeding period on growth performance and gut health. A total of 144 one-day-old Ross 308 broiler chicks (mixed gender) with an average initial BW of 42.3 g were used. The chicks were allotted to pens with 12 birds per pen and six replications per treatment with food and water provided *ad libitum*. Feed intake of Probiotic group was 4134 g, 338 g less than that of control group. Live weight of probiotic group was 2537 g and a 113 g more than that of control group. The feed conversion ratio of probiotic group was 1.61, 0.22 less than that of control group. The crypt depth of probiotic group (1110.46 ± 224.016 µm) was statistically deeper than that of control group (949.39 ± 114.166 µm) in ileum. Continuously use of probiotics in drinking water of commercial poultry flocks appears to be alternative to AGPs. The results of this study provide a greater understanding of the impact of long-life use of probiotics on broiler health and growth performances.
Key words: Broiler, Gut morphology, Growth performances, Probiotics

INTRODUCTION

Globally, antibiotic resistance is a growing public health issue (Gaggia et al., 2011). This emerging issue will likely lead to even greater challenges that will requisite innovative solutions in order to provide even greater public health protection. The goal is to enhance the knowledge base of alternative antimicrobials currently under investigation and to identify additional materials for potential strategic use in food animal production, leading to better protection of human health (Hume, 2011).

Antibiotics as growth promoters in animal feeds have been used in the member states of the European Union during the last 50 yr. However, the EU banned antimicrobial growth promoters (AGPs) in Jan 2006. So, the poultry industry has aimed to develop new strategies in order to maintain animal health and performance on a commercial scale (Castanon, 2007; Vahdatpour and Babazadeh, 2016). Accordingly, scientific efforts have been focused on the development of new non-antibiotic health and growth promoters for use in poultry breeding (Griggs and Jacob, 2005; Nikpiran et al., 2014). Such as from AGPs, it is expected from non-AGPs that they should promote digestive tract health to increase disease resistance in chickens (Istiqamah et al., 2013; Mahdavi et al., 2013; Afsharmanesh and Sadaghi,2014; Li et al., 2014 and Chughtai et al., 2015).

As a source of non-AGPs, extracts of some medicinal or aromatic plants, probiotics, prebiotics, Competitive Exclusion (CE) cultures, organic acids, specifically fermented feeds or their extracts has been used (Lee et al., 2001 and Prukner-Radovcic and Grozdonic, 2003). Except for these attempts, intensive efforts have been spent in developing dietary fibers, bacteriocins, strain specific phages and vaccines to combat many significant enteric diseases (Heres et al., 2003; Vandeplas et al., 2008 and Latha et al., 2016).

Probiotics are live microbial complements that provide beneficial effects on the host due to improvement in the intestine's microbial equilibrium (Fuller, 1989 and Giannenas et al., 2014). Sharma et al. (2012) demonstrated

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them as "the emissaries of health from microbial world". The efficacy of these products is often due to specific microbial ecological factors that alter the competitive pressures experienced by the microbial population of the gut (Callaway et al., 2008 and Lutful Kabir, 2009). Many researchers have suggested that probiotics and CE cultures can reduce colonization of pathogenic bacteria in chickens' intestines (Lee et al., 2001; Wolfenden et. al., 2007 and Schneitz et al. 2016).

Feed efficiency, feed conversion ratio, survival rate and weight gain rate are considered as the main parameters for evaluating effectiveness of non-AGPs in scientific studies, either via comparison with AGPs or using them alone (Mehr et al, 2014; Olnood et al., 2015 and Zhang et al., 2015). Gut histopathology, blood and digestive parameters are used as comparison parameters in such studies (Mehr et al, 2014; Agboola, 2015 and Ştef et al., 2015). Varieties of non-AGPs recommended for use in drinking water or in combination with poultry feed (Swiatkievicz, 2014 and Abu Akkada et al., 2015). Many studies have revealed the effectiveness of non-AGPs as growth promoters, providing alternative to AGPs to improve chicken growth indices (Landy and Kavyani, 2013). From the literature, it appears to be difficult to come to a consensus as to the best method to probiotic application for broiler chickens on an industrial scale. So, a liquid probiotic was used (Smart ProLive, a commercial preparation) in non-chlorinated drinking water once a day. So, it is aimed to investigate the effects of on growth performance and some histological changes in large intestines of broiler chicks during a period of 42 d of breeding.

MATERIALS AND METHODS

A total of 144 one dayold Ross 308 broiler chicks (mixed sexes) with an average initial BW of 42.3 g were allotted to pens with 12 birds per 1 m^2 cages in a 20 m^2 environmentally controlled room (32 to 24 °C and 65% relative humidity) flock. Six control (control group) and six experiment (probiotic group) cages were placed on the ground as two parallel lines. Four kg of wood shavings was used as bedding material for each cage. The light regime was 23 h light and one h darkness. The temperature in the flock was 32°C at the beginning of the experiment and was gradually reduced to 21°C at 21 d. Broiler chickens were vaccinated with live attenuated vaccines against Newcastle Disease Virus (NDV) Avinew© VG/GA strain (Merial-Lyon-France) at day 7 and day 26. No antimicrobial agent was applied to the birds. Birds were only subjected to 3 routine vaccination applications at different intervals and vaccines were applied to all the birds via drinking water.

The birds were provided with *ad libitum* feed and drinking water during the entire experimental period. All diets were taken from a commercial Ross 308 broiler chicken breeding farm. Chickens were feed with four structures of compound feed according to the recommendations in the growth boom for Ross 308 hybrid, namely: pre-starter, starter, grower and finisher in the following sub-periods: from hatching to 10 d, from 11 d to 24, from 24 to 35 d and from 36 to 42 d. The feed compositions are given in table 1. Control group was fed with basal diet and de-chlorinated drinking water. Probiotic group was fed with basal diet and de-chlorinated drinking water with 0.1 % Smart ProLive. Live weight and feed consumption ratio (FCR) were recorded weekly until 42 d (slaughter d).

Table 1. Composition of basal diets (g/kg) used for feeding of broiler chickens.

Composition of basal diets (g/kg)	**Feeding periods (day)**			
Ingredients	**0-10**	**11-24**	**25-35**	**36-42**
Maize	440	392.22	361.55	357.42
Soybean Meal (46%)	210	84	21	0
Soybean (Full fat)	150	200	270	267
Wheat	85.6	197	247	267
Maize gluten	54.5	24	0	0
Limestone	16.6	9.4	8.6	8.4
Soybean oil	15	5	5	3
$CaHPO_4$	12.3	5.1	3.6	3.7
L-Lysine HCl	4.1	3.89	2.58	2.76
DL-Methionine	2.94	2.67	2.35	2.18
$NaHCO_3$	2.44	3.4	3.14	3.85
NaCl	2.16	1	1.2	0.62
L-Threonine	1.13	0.78	0.78	0.85
Meat-bone meal	0	60	60	70
Vegetable oil	0	5	10	10
Vitamin premix [a]	0.2	0.2	0.2	0.2
Mineral premix [b]	2	2	2	2
Choline chloride	1	1	1	1
Nutrient level				
Metabolizable energy (MJ/kg) [c]	3037	3140	3225	3220
Crude protein (%)	23.56	20.9	19.2	18.9
Lysine (g/kg)	1.45	1.3	1.15	1.13
Methionine (g/kg)	0.7	0.61	0.53	0.5
Calcium (g/kg)	1.25	0.96	0.92	0.95
Available phosphorus (g/kg)	0.68	0.67	0.64	0.65

[a] Vitamin premix provided 1 kg of diet with: vitamin A, 10.800 IU; vitamin D3. 2160 IU; vitamin E, 15 IU; Vitamin K3, 1.0 mg; vitamin B1, 4 mg; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin 25 mg; vitamin B6, 8 mg; folic acid, 0,4 mg; vitamin B12, 0.08 mg; biotin, 0,15 mg. [b] Mineral premix provided 1 kg of diet with: I, 0,35 mg; Se, 0,15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg. [c]Metabolizable energy was obtained by calculation.

Enumeration of total aerobic bacteria in the probiotic source: Smart ProLive sold in the form of 5 l plastic container (as seen on the label, it contains ≥1×10^{11} CFU/ml probiotics as *Pediococcus acidilactici* and *Bacillus subtilis*) was analyzed for its total viable bacterial count. A 10 ml sample was mixed with 90 ml sterile saline solution (0.9% NaCl) and the10-fold increment serial dilution technique was conducted according to Maturin and Peele (2001). One milliliter of the homogenized suspension was then transferred into 9 mL of 0.9% saline solution (NaCl) and serially diluted from 10^{-1} to 10^{-8} by using the same saline solution tubes. From the last three diluted samples, 0.1 mL each was plated on the appropriate agar medium for enumeration of live bacterial population. After colony count, bacterial load was calculated as CFU/ml.

Histological measurements

At the 42th d of the trial period, all the birds were weighed individually and sent to a local commercial broiler slaughterhouse for the routine slaughter process. Electrically stunned birds were slaughtered. For histological examination, fragments from duodenum, ileum and ceca were taken from the individuals of six experimental variants in each of Probiotic Group and Control Group after the commercial slaughtering process. The fragments of the intestine were fixed in neutral formalin (10%), then dehydrated in increasing ethylic alcohol solutions (70^0, 80^o, 90^o, 100^o) and clarified in two baths of benzene and put in paraffin. The sectioning of the paraffin blocks was carried out using a manually rotary microtome (Ştef et al., 2015). The slides were stained with Periodic Acid Schiff (PAS) and Hematoxylin Eosin (HE) then examined by light microscopy. Micrometer in microscope was used for histometric measurements (Luna, 1968).

Statistical analysis

Data were analyzed using SPSS v.16.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by independent sample t test. Mean values were considered significantly different at $P<0.05$. Data are expressed as mean values ± SD (standard deviation).

Ethical approval

Direct collection of tissues and organs from freshly slaughtered birds was carried out in strict accordance with the recommendations of Kafkas University, Kars, Turkey for the care and use of laboratory animals. Also, slaughtered chickens were humanly handled.

RESULTS

There was no mortality or physical injury during the trial period of 42 d. After a 42 d of breeding period, each bird in probiotic group consumed 4134±112 g feed and it was 338 g less than that of control group (4472±137 g). Live weight of each bird in the probiotic group was 2537±62 g and it was 113 g more than that of Control Group (2424±67 g at 42nd d). The FCR of probiotic group was 1.61±0.007 and it was 0.22 less than that of control group (1.83±0.012). As seen in the table 2, feed consumption, weight gain and FCR results of probiotic group were superior to that of control group during all the breeding period. After total mesophilic aerobic count of the probiotic source, it is confirmed that it contained at the concentration of 1 x 10^{11} CFU/ml (data has not been shown).

The figures 1 and 2 represent the guts histological structures. There was no difference between probiotic group and control group aspect of histological structure, which were lymph follicles, goblet cells, crypt, submucosa and mucosa, in all parts of small intestine. The histometric differences between the two groups are given in table 3. Although the crypt depth in duodenum and in ceca of probiotic group and control group were statistically similar to each other, there was a significant difference between the two groups in ileum. The crypt depth of probiotic group (1110.46 ± 224.016 µm) was statistically deeper than that of control group (949.39 ± 114.166 µm) in ileum. Mucosa thickness of probiotic group in ceca and ileum appeared to be thicker than those of control group in those parts of the intestine (Table 3). There was no statistically significant difference between the two groups in the thickness of the duodenum mucosa (Table 3).

Table 2. Effect of probiotic (Smart ProLive) on growth parameters during 42 days of rearing period of broiler chickens (mean±SE).

Tests \ Weeks	1	2	3	4	5	6
Feed intake; Probiotic	89 ±6	407 ±14	930 ±22	1734 ±40	2270 ±75*	4134 ±112*
Feed intake; Control	89 ±6	396 ±24	954 ±30	1780 ±72	2979 ±76	4472 ±137
Bodyweight gain; Probiotic	121 ±6	369 ±12	722 ±15	1235 ±27*	1879 ±47*	2537 ±62*
Bodyweight gain; Control	112 ±6	338 ±18	689 ±21	1175 ±45	1796 ±47	2424 ±67
Feed conversion rate; Probiotic	0.39 ±0.03	0.99 ±0.08	1.23 ±0.06	1.37 ±0.04*	1.45 ±0.04*	1.61 ±0.07*
Feed conversion rate; Control	0.42 ±0.01	1.05 ±0.02	1.32 0.05	1.48 0.05	1.64 0.01	1.83 0.01

*P<0.05.

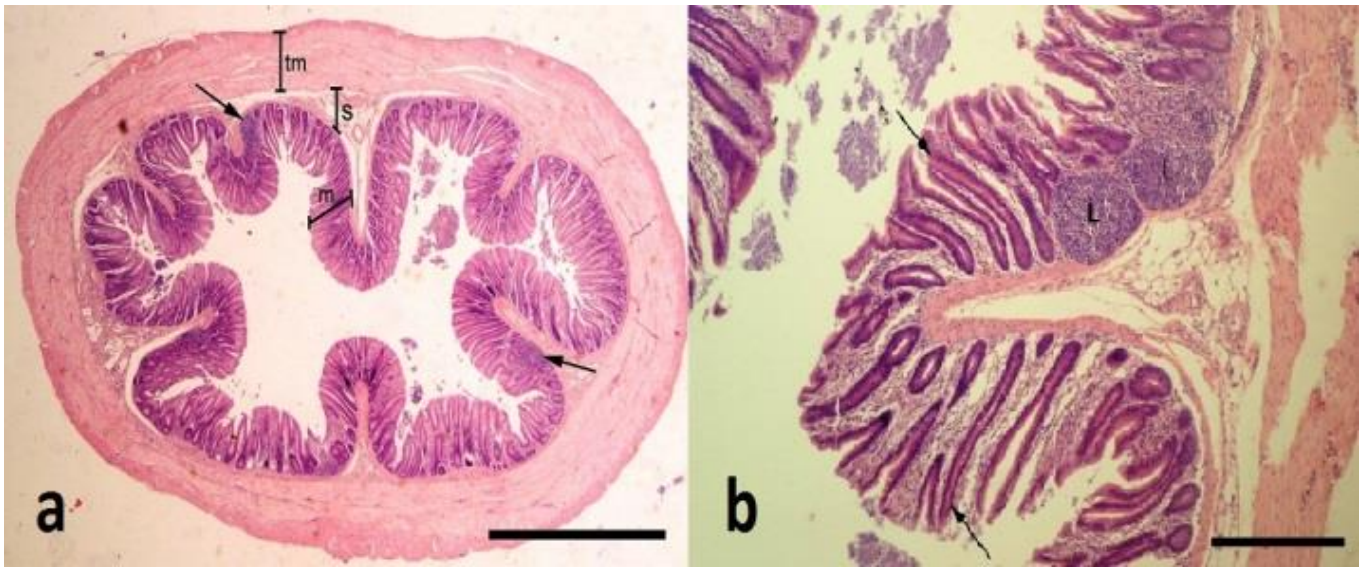

Figure 1. **a**) General view of the cecum in the experimental group of broiler chickens after 42 days of rearing period, 4x.Arrows: Lymph follicles, tm: tunica muscularis, s: submucosa, m: mucosa. Haematoxylin and eosin stain (H&E) Bar: 1000 µm; **b**) Cecum of the experimental group, 10x. Arrows: Crypt, L: lymph follicles. H&E. Bar: 200 µm.

Table 3. Effect of probiotic on crypt depth and mucosa thickness in gut segments of broiler chickens after 42 days of rearing period.

Tissue	Groups	Crypt depth (µm) ± SD	F	Mucosa thicknes (µm) ± SD	F
Doudenum	Probiotic	1622.5 ± 347.0	0.666	1965.1 ± 333.4	0.37
	Control	1445.7 ± 318.7		1721.6 ± 326.3	
Ileum	Probiotic	1110.5 ± 224.0	9.305*	1325.2 ± 222.8	8.78*
	Control	949.39 ± 114.2		1144.9 ± 129.0	
Ceca	Probiotic	306.8 ± 65.1	0.275	402.8 ± 109.5	4.73*
	Control	273.8 ± 59.3		353.1 ± 63.1	

*P < 0,05.

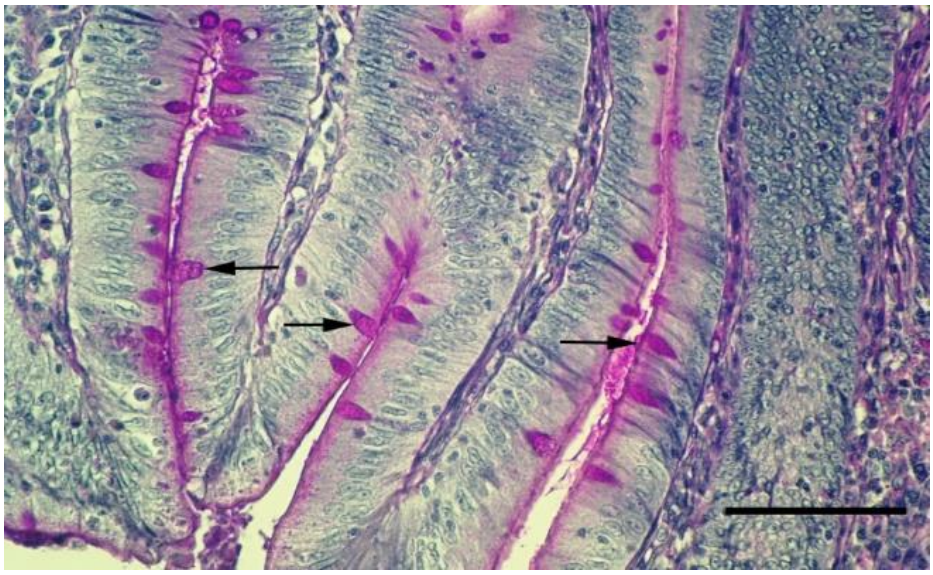

Figure 2. Duodenum of the experimental group of broiler chickens after 42 days of rearing period. Arrows: Goblet cells, 40x. Periodic Acid Schiff (PAS). Bar: 50 µm.

DISCUSSION

Antibiotics used in the field of veterinary application have been found to be responsible for the global antibiotic resistance problem. Currently, there is an alarming situation regarding antimicrobial resistance (Tellez et al., 2012). In 2013, the G8 summit was dedicated this subject alone and at the end of the summit the conclusion was that the situation had demonstrated as "an alarm state". The G8 ministers released a joint statement on June 14, identifying antimicrobial drug resistance as a "major health security challenge of the 21st century" (Davies, 2013).

Competition of good and bad flora demonstrates the health of the digestive system, and accordingly health of the total body (Ghadban, 2002). AGPs are used to suppress bad flora and allowing good flora to dominate in the intestines. Ban of antibiotic growth promoters in feeds from 2006 in the EU countries, many other countries have gradually adapted their regulations. After the ban, a rapid search has started to find new growth promoters to replace AGPs (Hume, 2011). In last 20 years, many studies have been conducted on new natural gut health promoters such as probiotics, prebiotics, CE cultures, direct feed microbials, fermented feeds, organic acids, essential oils. Probiotic are defined as a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). Given the lack and hazards of antibiotics, including reduction of microbiome diversity and antibiotic resistance, the use of probiotics instead of antibiotics is becoming increasingly more acceptable. (Nami et al., 2015). Probiotics, prebiotics and CE cultures have demonstrated to be the good natural digestive system promoters (Ghadban, 2002 and Nami et al., 2015). It is also pointed out by this study that probiotics can be used in broiler meat production as gut health enhancer and growth promoter.

Many studies have been conducted to determine, the efficacy of non-AGPs on the health and growth performances of meat chickens. Nevertheless, it is not easy to make a comparative evaluation on the subject (Applegate et al., 2010). Due to differences in breeding conditions, feed and water quality, and probiotic and CE culture type, confirmative results between scientific studies has not easily been demonstrated (Otutumi et al., 2012). Variations in the effects of probiotics on growth performance of broiler chickens may be attributed to differences in the strains of bacteria used as dietary supplements (Angel et al., 2005; Timmerman et al., 2006; O'Dea et al., 2006; Lutful Kabir, 2009; Blajman et al., 2015 and Olnood et al., 2015). Differences between physical and environmental conditions of the trials may also bias the results from these studies (Olnood et al., 2015). Also, an accurate dosage of administration has not yet to be established despite the wide use of probiotics (Khan et al. 2013; Li et al., 2014; Abu-Akkada and Awad, 2015 and Getachew, 2016). A continuously giving the probiotics via drinking water to the broiler chicken at whole breeding period may be more trusted way for taking maximum profit from it.

Adding to feed is the most commonly used method for administering probiotic preparations to broiler chickens in poultry production. Nevertheless, feed-type probiotic products rarely produce optimum results in pelletized diets usually fed to broilers (MacDonald and Wang, 2011). Probiotic bacteria incorporated into crumbles have an increased lifespan than those in pelletized feed (Eckert et al., 2010). Only spore forming probiotic bacteria can successfully survive in pelletized feed. Thus, the best natural solution to challenge the stability non-spore forming probiotic bacteria may be used in drinking water. However, chlorinated water can decline viability of the organisms rapidly (Raevouri et al., 1978). It is also in agreement with the researchers who mentioned that the best way of the giving non-spore forming probiotics to the broiler chickens may be the rote of drinking water.

Nurmi and Rantala (1973) have demonstrated that intubation in to the crop is probably the most satisfactory method for delivering a gap precise dose of probiotics to the animal. However, this route is not an applicable way on an industrial scale. Blankenship (1992) suggested that spray application of probiotic cultures, either on the environment of the birds or on the litter material seems to be an effective way of administering probiotic cultures. This way can also be applied during the first d of life of the chickens in industrial production practices, and it appears not to be easy and practical to apply at the farm level during rearing period.

The results of researches available in literature involving probiotics are very variable, several factors can interfere with the results, such as the type of probiotic, its action mode, its interaction with the host and breeding environment. There are few studies that demonstrate the usefulness of probiotics or CE cultures on growth performances (Ştef et al., 2015; Abu- Akkada and Awad, 2015 and Getachew, 2016). Almost all of the other studies have demonstrated at least one positive effect including growth promotion of probiotics on the broilers (Mehr et al., 2014; Ritzi et al., 2014; Agboola et al., 2015; Zhang et

al., 2015; Schneitz et al., 2016 and Erdogmuş et al., 2018). In this study, probiotic use had less feed consumption (338 g), more weight gain (113 g) and less FCR (0.22) than Control group (Table 2). The results have demonstrated that an efficient result of continuous use of a fresh liquid probiotic source at appropriate dose via drinking water appears to be alternative to AGPs. The performance results were significantly affected by probiotic use.

The Figures 1 and 2 represent the gut morphological structures. No differences were determined or superiority between the groups when examined the duodenum samples. There was no difference between probiotic group and control group aspect of histological structure, which were lymph follicles, goblet cells, crypt, submucosa and mucosa, in duodenum. The histometric differences between the two groups are given in table 3. There was no statistical significance in the crypt depth of duodenum and ceca between the groups. But, crypt depth of probiotic group (1110.46 ± 224.016 µm) was statistically deeper than that of Control Group (949.39 ± 114.166 µm) in ileum. Mucosa thickness of probiotic group in ceca and ileum appeared to be thicker than those of Control Group (Table 3). Present results are in aggreement with many other researchers who mentioned positive effects of probiotics on the gut health and accordingly growth performances (Giannanes et al., 2014; Ştef et al., 2015; Zhang et al., 2015 and Erdogmus et al., 2018). A good histological development in the ileum and ceca in the probiotic group chickens may contribute to understand the BWG and FCR efficiencies in the group compared to Control Group.

CONCLUSION

Based on the findings of the present study, it may be concluded that a continuously inclusion of a good blend of probiotics at 10^8 CFU/ml dose in drinking water may successively improve the performance and gut health of commercial broiler chicks. Therefore, under the conditions of the present study, it can be recommended that using a freshly produced liquid microbial growth promoter in the non-medicated and de-chlorinated water could prove highly beneficial for the local broiler producers. It could be suggested that further research work should be performed to comparatively evaluate the effectiveness of freshly produced liquid live microbial cultures with other powder forms both as applications in drinking water and rations as-post pellet applications. So, replacement of AGPs with non-AGP microbial cultures, of broiler meat industry and public health safety issues could be more lessened.

DECLARATIONS

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Author`s contributions

All authors participated equally in making the design, support with sampling andinterpretation of results, and writing the paper.

Competing interests

The authors declare that there is no conflict of interest.

Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

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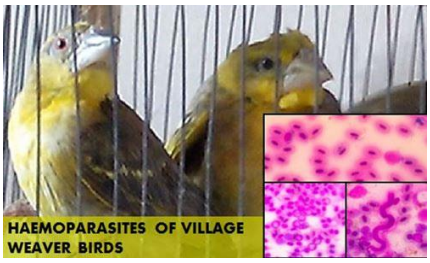

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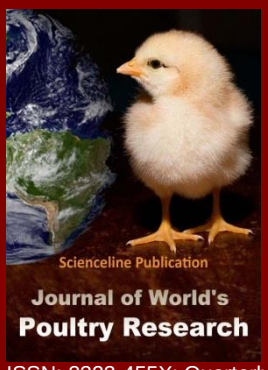

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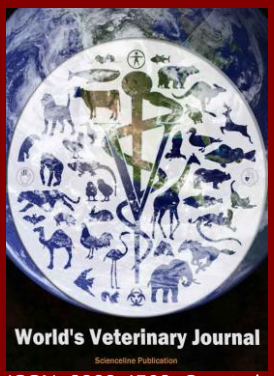

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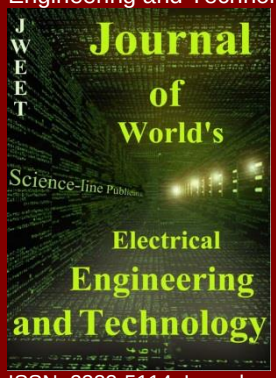

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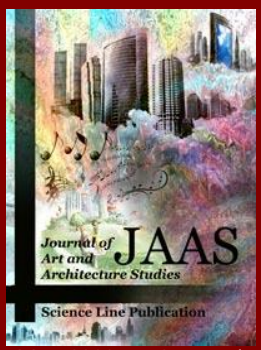

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