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purchased poultry from the mentioned market who are thought to have inadequate knowledge in food hygiene, safety and microbiology. Therefore a study was carried out at the market to investigate the knowledge and perceptions of Mauritian consumers, on safe and hygienic handling of poultry and shed light on their domestic poultry preparation practices, and understand their attitudes and disposition towards poultry safety. A survey instrument was developed and administered, and data were collected during the period of June-November 2014. The results of this study showed that respondents often lacked knowledge of basic concepts in food safety, rendering them more prone to unsafe food practices. Moreover, poultry consumers, particularly the young demographic, were found to carry out unsafe food behaviors due to an optimistic bias, an illusion of control or habitual behavior. Poor regard to prevention of cross-contamination was noted. Lack of specific technical knowledge was estimated to be the central reason for unsafe behavior during poultry preparation. It was therefore recommended that education on food safety should start at an early age. Moreover, food labels should be designed to protect consumers from heath risks due to consumption of unsafe food and the media should wield a greater role in educating consumers on food safety.
Keywords: Food safety, Knowledge, Perceptions, Poultry, Consumers

[Full text-PDF] [XML] [DOAJ]

Research Paper

Determination of Crude Protein and Metabolisable Energy of Japanese Quail (Coturnix coturnix japonica) during Laying Period.

Agboola AF, Omidiwura BRO, Ologbosere EY and Iyayi EA.

J. World Poult. Res. 6(3): 131-138; pii: S2322455X1600018-6

ABSTRACT:
This study was carried out to determine the energy and protein requirements of laying Japanese quails. A completely randomized design of treatments comprising four dietary protein levels (18, 20, 22 and 24%) and three levels of metabolisable energy (3000. 3100, 3200 kcal/kg) in a 4 × 3 factorial arrangement was used.ÂÂÂÂÂ 144 Japanese quails aged 7 weeks were randomly divided into 12 dietary treatments with 3 replicates per treatment and each replicate with 4 birds. The experiment lasted for five weeks. The results of the study showed that there was no significant (P>0.05) effect of protein, energy or their interaction on feed intake, feed conversion ratio, hen day production, egg weight and egg number. However, protein as a single variable had a significant effect (P0.05) effect of protein, energy or their interaction on egg quality traits (yolk colour, yolk weight, albumen weight, shape index, shell thickness, shell weight, and haugh unit). However, birds fed 20% crude protein and 3000 kcal/kg metabolisable energy had better hen day production, number of eggs per bird and egg quality traits compared with birds on the other groups. Dietary protein increased egg production and egg weight, augmented by energy. The yolk colour was increased with increasing energy level. Therefore, the results of the experiment revealed that 20% crude protein and 3000 kcal/kg metabolisable energy could be used to obtain the best production performance and good egg quality traits of Japanese quails at the laying phase.
Keywords: Japanese quail, Production performance, Egg quality traits, Metabolisable energy, Protein.

[Full text-PDF] [XML] [DOAJ]

Research Paper

The Effects of Housing and Equipment Status on Egg yearly monitored Production Rates in Open Poultry Houses in Gezira State, Sudan.

EL-Dikeir N, ELBeeli MYM., Abdel-Rahim AM, Jadalla B and Mohamed Ali SA.

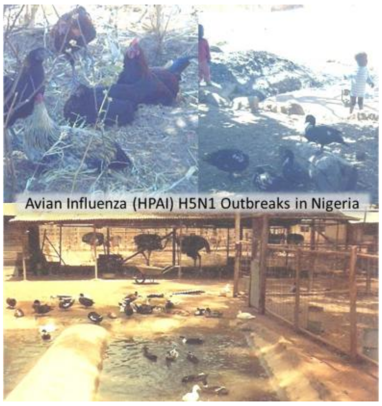
J. World Poult. Res. 6(3): 139-146; pii: S2322455X1600019-6

ABSTRACT:
This study was carried out in Gezira state, Sudan to investigate the effects of housing and equipment status on egg production in open layer houses. Data were collected through individual interviews (questionnaire) of 97 randomly selected among poultry farm owners. The height of 80% of north and south sides of wall were 50-100 cm in Almanagil, 76.5% in Alkamleen and 57%in south of the Gezira localities, while the height of the wall side at the east and west were (3-3.5m) in all (100%) houses in east of the Gezira, 77.8% in Alhasahesa, 60% in Almanagil and 47.1% in Alkamleen. The width was 5-8m in most poultry houses in Gezira State's localities surveyed. In Greater Medani, all the houses were at the width mentioned above while 76.4% and 73.5% in of those building were 5-8 cm in Almanagil, and Alkamleen localities respectively. The most of wall houses were not painted where 50% of those houses were with painted walls in east of the Gezira and 76% in Alkamleen locality. The most floor types were made of bricks. Flours with that type were 55.6% in Alhasahesa and 76.5% in Alkamleen locality. The layer of sand was thin in the major litter type of poultry houses surveyed in Gezira state localities though some houses were without litter, which affect birds' performance by low ventilation and insulation. Round feeders of 40 - 50 cm length were the majority feeders' type observed. In Alhasahesa 55.6% houses had that type of feeders while all houses surveyed had round feeders in east of the Gezira and Greater Medani localities. Oil containers were used as drinkers in most poultry houses surveyed. The troughs were with unsuitable height for hens to drink conveniently. The percent of house with that type of drinkers were 58.8% in Alkamleen and south of the Gezira localities. Birds/feeder and birds/drinker capacity varied between 50 and 75 birds. Clay pots were the mostly used egg nest type in the state. That type of nests were used by 88.2% of farm owners in Greater Medani to 100% in east of the Gezira, Alhasahesa and Almanagil localities. In average one egg nest was allotted to 15 hens. Yearly monitored egg production ranged between 60-70%.
Keywords: Layer, Production constraints, Housing, Equipment

[Full text-PDF] [XML] [DOAJ]

Research Paper

The Effect of Highly Pathogenic Avian Influenza (HPAI) H5N1 Outbreaks on Mixed Species Poultry Farms in Nigeria.



Akanbi OB, Ekong PS, Odita CI and Taiwo VO.

J. World Poult. Res. 6(3): 147-152; pii: S2322455X1600020-6

ABSTRACT:
The first outbreak of highly pathogenic avian influenza (HPAI) H5N1 virus in Nigeria was in 2006 and it involved different poultry species, mostly chickens in different ages, reared and bred on the same premises with some numbers of ducks, geese, turkeys and ostriches. To determine the effect of HPAI on mixed species poultry farming in the face of the ongoing 2015-2016 resurgent HPAI in Nigeria, data of confirmed 2006-2008 HPAI H5N1 outbreak in poultry were expressed as percentage proportions and used to produce spatial map using ArcGIS10.3 (ESRI®, USA) against some ecological features of the country. The outbreaks were more clustered in poultry farm dense areas especially in the northern states while very few clustering were observed around Important Bird Area and wetlands. A total of 177,996 (25.9%) on farm bird mortality was recorded from the selected outbreaks. From the backyard flock, the total mortality was 25, 915 birds (14.6%) and from the commercial flock, total mortality was 152, 081 birds (85.4%). The commercial flocks recorded higher mortality rate ($P < 0.0001$). In the single species flock, total mortality recorded was 173, 425 (25.5%) while in the mixed species flock, total mortality was 4, 571 (52.9%). Mortality rate was much higher in the mixed species flock ($P < 0.0001$) and ranged from 4.92 – 73.15% with the chicken-duck-turkey mixed flock farms having the highest rate (73.15%). Results show a higher risk of HPAI disease occurrence in multiple, mixed species poultry than in single species poultry production.

Keywords: HPAI, Mixed species, Nigeria, Poultry

[Full text-PDF] [XML] [DOAJ]

Research Paper

Variations in Morphometric Traits of Local Chicken in Gomoa West District in Southern Ghana.

Variations in Morphometric Traits of Local Chicken in Gomoa West District in Southern Ghana

Birteeb Peter T, Essuman Alfred K and Adzitey F.

J. World Poult. Res. 6(3): 153-160; pii: S2322455X1600021-6

ABSTRACT:
The study was undertaken to identify the variations among morphometric traits of local chicken in the Gomoa West district of Ghana. Thirteen body measurements namely Weight (WT), Body Length (BDL), Chest Circumference (CC), Thigh Circumference (TC), Shank Length (SL), Neck Length (NL), Wing Length (WGL), Head Length (HDL), Hip Length (HL), Wattle Length (WAL), Beak Length (BKL), Drumstick Length (DL) and Comb Length (CL) were taken on 500 birds and analyzed under general linear model to determine the fixed effects of sex, comb type, feather distribution and skin colour on variabilities in the traits. The male birds had significantly ($P < 0.001$) larger heads (NL=9.11 cm, HDL=6.59 cm, CL=5.23 cm etc.) and bodies (WT=1.19 kg, BDL=24.64 cm, CC=14.32 cm etc.) than their female counterparts. Cushion comb-type chickens were significantly ($P < 0.001$) superior to all other comb-type chickens in all head and body measurements. Feather distribution had significant ($P < 0.05$) influence on WT and BDL, as naked neck birds appeared superior. Birds with grey skin colour had significantly ($P < 0.001$) larger chest circumference than all other birds. These findings could be useful as selection criterion, thereby providing a basis for genetic manipulation and improvements of the local chicken in Ghana.

Keywords: Comb type, Measurement, Naked neck, Poultry, Skin colour

[Full text-PDF] [XML] [DOAJ]

Research Paper

Dietary Modelling of Nutrient Densities: Effect and Response in Different Growing Phases on Growth Performance, Nutrient Digestibility, Litter Quality and Leg Health in Turkey Production.

Dietary Modelling of Nutrient Densities on Growth Performance, Nutrient Digestibility, Litter Quality and Leg Health in Different Growing Phases of Turkey

Waseem Mirza M, Pirgozliev V, Rose SP and Sparks NHC.

J. World Poult. Res. 6(3): 161-190; pii: S2322455X1600022-6

ABSTRACT:
An experiment was conducted to explore the time bound (different growth phases) effect of different dietary nutrient densities i.e., different energy and protein concentration while maintaining the ratio between the two, all with the same ideal amino acid profile, on litter quality and leg health (footpad dermatitis (FPD) and hock burn (HB)), when fed to growing turkeys. The effects of dietary nutrient modelling on growth performance parameters, water intake and excretion, dry matter (DMD), organic matter (OMD), crude protein (CPD) digestibility coefficients and apparent metabolisable energy (AME) were also examined, when fed to growing turkeys in varying growth phases. At twenty-eight days of age one hundred and seventy five male turkeys (BUT 8) were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds in a pen, all pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consisting of 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases). Each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered ad libitum throughout the experiment. Five dietary treatments, containing either 77, 85, 100, 110 or 120% of the crude protein (CP) and metabolisable energy (ME) content recommended by the breed standard. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finishing at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). Nutrient density had a positive and linear effect ($P < 0.001$) on weight gain, feed efficiency and dry matter digestibility (DMD) whereas the effect of nutrient density on dietary protein digestibility (CPD) only approached significance ($P = 0.081$). As might be expected increasing nutrient density had a negative and linear effect on feed ($P < 0.001$) and water ($P < 0.01$) intake and did not affect the ratio between these two parameters. Increasing nutrient density had a positive effect on litter quality (linear ($P < 0.001$), with both the litter

moisture (P< 0.01) and the litter score decreasing (P< 0.001). Conversely litter ammonia concentration increased (P< 0.001) as nutrient density increased, similarly as nutrient density increased so did the prevalence of hock burn (P 0.05) of treatment on FPD. The results suggest that an increase in nutrient concentration can reduce the moisture content of the litter and so improve overall litter quality. However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health problems in turkey production.
Keywords: Nutrient density, Digestibility, Performance, Wet litter, Ammonia, Footpad dermatitis, Hock burn.
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Use of Organic Acids as Potential Feed Additives in Poultry Production

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ABSTRACT

Historically organic acids (OA) have been used by humans as natural food preservatives and hygiene promoters with regard to the microbial growth and to enhance freshness and shelf-life of edible food items. This characteristic of microbial growth inhibition of OA also makes them suitable replacement to antibiotic growth promoters in poultry. OA are chemically weak acids, which prevent or completely seize the proliferation and colonization of pathogenic bacteria in the intestine of birds. Thus, reducing the competition for the nutrients as well as production of harmful microbial metabolites. This in turn improves bird's performance and enhances the specific and non-specific immunity by improving the bird's intestinal epithelial layer. OA also help improving absorptive capacity of the intestinal cells by improving the crypt-villus structures as well as by improving the digestive secretions, thus influencing a boost in the digestion of proteins, carbohydrates and especially the minerals. This results in enhanced growth rate and feed efficiency in poultry. This comprehensive review about dynamics of OA revealed that this potential feed additive will be used as performance modifier in commercial poultry production, functioning as gut microbial modifier, immune modulator and nutrients digestion enhancer. This review updates the last decade's developments about OA in poultry production.

Key words: Organic acid, Antimicrobial activity, Digestibility, Performance, Poultry

INTRODUCTION

Increased growth rate and improved feed efficiency (Miles et al., 2006) along with prevention of sub clinical diseases are the main reasons why dietary antibiotic growth promoters (AGP) have been practiced during the last 50 years in poultry production. However, their constant use at low dosage develops resistance in the bacteria (Collignon, 2003) and residues in the animal products.There was a fear of transferring these antibiotic-resistant bacteria to humans via food chain (Dibner and Buttin, 2002), therefore, European Union (EU) banned the use of AGP in animal nutrition in 2006 (European Union, 2003 and 2005). Ban on the use of AGP in poultry feed resulted in a poorer production performances and there was a change in the microbial ecology in gastrointestinal tract of birds. However, Danish industry evidence showed little effects of this ban on the productive performance. This situation therefore, compelled animal nutritionists and researchers to search for other non-therapeutic alternatives for poultry feed such as organic acids (OA) (Panda et al., 2009), plant extracts, (Taylor, 2001) enzymes, probiotics, prebiotics, herbs and essential oils (Islam, 2012). The use of OA and their salts in the poultry production were considered as safe therefore, they were allowed to be used as feed additive by the European Union (Adil et al., 2010). Moreover, most of the research during last decade shows that OA are excellent promoters of growth performance and gut health in commercial poultry production (Sohail and Javid, 2016). Therefore, it is important to understand and highlight their importance, impact and mode of action, to be able to maximize the benefits when included in poultry diets.

Organic acids

Any substance that contains the R-COOH group in its structure and has acidic properties is called an Organic Acid (OA) and hence include fatty acid and amino acid. Chemically they are weak acids and contrary to mineral acids they do not dissociate completely in water. The pKa is a logarithmic measure of the acid dissociation constant, the most important property that categorizes the strength and affects the activity of OA. The lower or more negative the number, the stronger and more dissociable the acid. It is important for OA's antimicrobial properties that its pKa value should be in the range of 3-5 (Dibner and Buttin, 2002).Due to this partial dissociation, not allOA's have

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the ability to influence gut microflora or have antimicrobial properties. OA are short chained acids (C1-C7), consisting of either a simple monocarboxylic acids i.e. formic, acetic, propionic and butyric acids or ones containing carboxylic group at the alpha carbon like tartaric, lactic, malic, and citric acids, which exhibit antimicrobial properties. Acids like fumaric and sorbic acid also have antifungal properties. The OAs and their salts do not exhibit their beneficial effects solely through their antimicrobial activity in feed and GIT of birds but also act as performance enhancers in many ways (Al-Kassi and Mohssen, 2009). These include an improvement in the growth rate through increase in the digestion and absorption of different nutrients, improvement in crypt-villus structure i.e., crypt depth and villus height and width and stimulation of the digestive secretion of different organs.

Antimicrobial activity of organic acids

Major objective of the dietary acidification in poultry is to reduce the pathogenic bacteria (Partanen and Mroz, 1999; Griggs and Jacob, 2005) or increase beneficial bacteria number, both in the feed and by influencing the gut or intestinal environment (Ewing, 2009), so as to support enteric health and growth performance. However, their magnitude of microbial activity in the gut depends on the physiological status of the organism as well as physicochemical characteristics of the environment (Ricke, 2003). Most common bacteria that affect the intestinal health of poultry are *Salmonella*, *Escherichia coli* (*E. coli*), *Clostridium*, etc. Though a very small effect but these bacteria compete with the host for the nutrients and produce different types of metabolites like ammonia and amines, possibly a result of amino acid deamination, hence leading to reduced growth of the poultry birds. So, by reducing the number of these bacteria, growth rate gets enhanced. OA can provide control over *E. coli*, *Campylobacte*r and *Salmonella*challenges in poultry (Chaveerachet al., 2002 and Heres et al., 2003). *Salmonella* infection in poultry mainly spreads through contaminated feed (Ao, 2005), therefore, an in-feed addition of OA will prevent the foodborne *Salmonella* species (Broek et al., 2003). Likewise, OA can be added in the water to keep it free from all type of microorganisms. Albuquerque et al. (1995) reported that out of 136 feed ingredient samples studied for the incidence of Salmonella,19.85% were contaminated with *Salmonella*. Acid-intolerant species such as *E. coli*, *Campylobacter* and *Salmonella* families are particularly affected by the actions of OA (Al-Kassi and Mohssen, 2009). Hinton et al. (2000) reported that low pH and higher number of lactobacilli lower the incidence of the *Salmonella* in crop of broiler chicks. Similarly in feed addition of formic acid reduces the foodborne infections of poultry (Humphrey and Lanning, 1988 and Rouse et al., 1988). The pH value in crop decreased (P<0.05) in the broiler chicken fed OA based diets (Adil et al., 2011). Dietary supplementation of formic and propionic acid laying hens also resulted in lowering of the pH of the crop and gizzard, this lowered pH has also been shown to kill the *Salmonella in-vitro* (Thompson and Hinton, 1997).As a consequence fowl typhoid can be prevented/controlled (Berchieri and Barrow, 1996). Izat et al. (1990) documented that dietary acidification with buffered propionic acid lessen the number of *E. coli* in the small intestine. A mixture of OA significantly lowers the total bacterial count especially gram negative bacteria in broilers (Gunal et al., 2006). The RCOO- anions produced from OA can hinder bacterial genetic regulation i.e., DNA and protein synthesis. Van Immerseel et al. (2006) reported that at low dose butyric acid can suppress genes responsible for the Salmonella invasion. In an *in-vitro* study Entani et al. (1998) reported that a media containing 0.1 percent acetic acid inhibited the growth of 17 strains of the bacteria including *Salmonella typhimurium* and eight strains of *E. coli*. Adil et al. (2011) reported addition of OA to the diets of broiler chicken significantly decreased (P<0.05) the caecal viablecoliform counts compared to the unsupplemented group. Butyric acid supplementation decreases the colonization of salmonella in the liver and spleen in broilers (Fernández-Rubio et al., 2009). Maribo et al. (2000a) found that benzoic acid supplementation in the feed of pigs resulted in significantly lower counts of lactic acid bacteria, *lactobacilli*, coliform and yeast throughout the entire GIT.

Mycotoxinsthat are the metabolites of fungi are the major threat to the poultry industry as these suppress the immune system; reduces the dietary energy contents as well as causing poor feed conversion and less growth rate etc. A variety of OA such as acetic acid, lactic acid, propionic acid, or blends of acids are used to help control mold contamination (Higgins and Brinkhaus, 1999 and Santin, 2010). For the *in-vitro* assay, paper discs soaked in a spore solution were placed on the surface of agar plates containing increasing concentrations of the respective OA. *In-vitro* efficacy of propionic, acetic, lactic, undecylenic, butyric, valeric, benzoic, and sorbic acid against the *Aspergillus spp*., *Geotrichum spp*., *Mucor spp*., *Fusarium spp*., *Penicillium spp*., and *Scopulariopsis spp*. indicated that mold inhibiting property of the valeric acid is highest, followed by propionic acid and butyric acid. These three acids completely inhibit the growth of above mentioned mold at the concentrations of not higher than 0.35%. All the other OA showed fewer mold inhibiting activity and the least activity was shown by the lactic acid. Fusarium was the most susceptible mold when comparing the efficacy of different OA on different molds (Higgins and Brinkhaus, 1999). Propionic acid and butyric acid with effective inclusion rates of 0.1% and 0.2% were equal in their efficacy to inhibit *Aspergillus spp*. and *Fusarium spp*., respectively. Maribo et al. (2000b) compared bactericidal activities of six different acids in the stomach and small intestine of pigs against coliforms. The order of bactericidal activities of different OA were as follows from higher to lower order: benzoic acid >fumaric acid > lactic acid > butyric acid > formic acid > propionic acid.

Antimicrobial activity of OA is highly affected by the surrounding pH as pH affects the dissociation of the OA (Cherrington, 1991). When pH is low, ionization of the OA will also be less. Undissociated forms of OA are lipophilic and can diffuse across cell membranes of bacteria and fungi (Partanen, 2001). Once internalized into the more alkaline pH of the cell cytoplasma they dissociate quickly into their constituent ions resulting in lowering of the pH (Young and Foegeding, 1993) and as a consequence disrupting the nutrient transport system and enzymatic reactions (Cherrington, 1991). Concentration of the hydrogen ions due to dissociation of the acids increases and bacteria try to pump out these protons (hydrogen ions) from the cell. This process requires energy, so the availability of energy for the proliferation lessens, resulting in bacteriostasis (Luckstadt and Mellor, 2011; Suiryanrayna and Ramana, 2015). This direct antimicrobial activity makes OA an excellent choice as feed and food preservatives as well as hygiene promoters.

Coccidiosis, an important manage mental disease of poultry, causing more than $3 billion worth of economic losses to the world poultry industry annually (Dalloul and Lillehoj, 2006) is caused by the Emeria; a genus of protzoal parasite. Abbas et al. (2011) studied the anticoccidal effects of acetic acid against the *Eimeriatenella* by using 1, 2 and 3 percent acetic acid; and 125 ppm amproliumin drinking water. Results showed that acetic acid lowered the oocyte score, lesion score and mortality percentage in broilers. These effects were more prominent at 3%level of acetic acid but there was no difference between 3% acetic acid and amprolium in preventing the coccidiosis. Further studies are necessary in this regard for understanding the anticoccidial effects of other OA. Microbial growth inhibitory properties of some OA are presented in table 1 and table 2.

Table 1. The inhibitory effect of some organic acids used in animal nutrition on microbial growth.

Organic acid	Properties[1]		Growth inhibitory[2]		
	Molecular formula	Acid dissociation constant (pKa)	Bacteria	Yeast	Mould
Formic acid	HCOOH	3.75	++++	+	+
Lactic acid	$CH_3CHOHCOOH$	3.86	+	-	-
Acetic acid	CH_3COOH	4.76	++	+++	+++
Propionic acid	$CH_3CH2COOH$	4.87	++	+++	+++
Citric acid	$C_3H_5O(COOH)$	3.10-5.40	n.a.	n.a.	n.a.
Sorbic acid	$C_6H_8O_2$	4.76	+++	++++	++++
Benzoic acid	C_6H_5COOH	4.20	+++	++++	++++

[1]Adapted from Pölönen and Wamberg (2007); [2]adapted from Lassén (2007)

Table 2. Effects of different organic acids on various types of bacteria.

Organic Acid	Bacteria	Sample tested	Effect	Reference
Butyric acid	*Salmonella enteritidis*	Caecal colonization	Decreased total count	Van Immerseel et al. (2004)
Formic acid	*Salmonella*	Cloacal swabs and content	Not detected	Hinton et al. (1985)
Formic, propionic and acetic acid	*Campylobacter*	Boiler Feed	Decrease total count	Chaveerach et al. (2002)
Buffered propionic acid	*Escherichia coli*	Boiler Feed	Decreased the count	Izat et al. (1990)
Butyric acid	*Escherichia coli*	Caecum, small intestine and crop	Decreased the count	Panda et al. (2009)
Organic acid mixture	*Coliform*	Ileum and caecum	Decreased the count	Pirgozliev et al. (2008)
Malic acid	*Escherichia coli*	Intestine	Decreased the count	Moharrery and Mahzonieh (2005)

Table 3. Effectof different organic acids on the gastrointestinal tract of monogastric animals**.**

Organic Acid	Route	Effect on Intestine	Reference
Butyric acid	Feed	Increased the villus height	Adil et al. (2010)
Formic acid	Feed	Increased the villus height and crypt depth	Garcia et al. (2007)
Citric acid	Feed	Lowered the pH of digesta and gastrointestinal tract	Radcliffe et al. (1998)
Fumaric acid	Feed	Increased the villus height	Adil et al. (2010)
Lactic acid	Feed	Increased the villus height	Adil et al. (2010)
Butyric acid	Feed	Lowered the pH of crop and small intestine	Panda et al. (2009)

Effect of organic acids on gastrointestinal tract

Being the major organ responsible for nutrient digestive and absorptive phase, gastrointestinal tract plays a vital role in the chicken growth (Amit-Romach et al., 2004). It is also the largest reservoir of commensal bacteria and other microbes in bird's body. Therefore, epithelium of the intestine is the natural obstacle to the bacteria and toxic substances entering the body. Different pathogens, chemical toxins and stress conditions alter the permeability of this natural defense (Pelicano et al., 2005), by shortening of villus height and extension of intestinal crypt resulting in lower villi height to crypt depth ratio (Mista et al., 2010), aiding the invasion of pathogens and leading to inflammatory processes at the intestinal mucosa (Podolsky, 1993). This subsequently leads to increased cell turn over, decrease in villus height, and lowering of the digestive and absorptive processes (Visek, 1978). Dietary inclusion of organic acids are known to have strong antibacterial properties and beneficial effects on intestinal acidity and histomorphology, which are imperative to support enteric health and growth performance of poultry (Geyra et al., 2001 and Loddi et al., 2004). Evident from Adil et al. (2010) and Cengiz et al. (2012) study who reported that dietary inclusion of OA in broiler diets resulted in an increase in the villus height. Mista et al. (2010) reported that these histopathological changes in the small intestine can be averted through the use of short chain fatty acids; mainlyacetate, propionate and butyrate in mice. Similarly Fukunaga et al. (2003) while working on rats reported that short chain fatty acids can accelerate gut epithelial cell proliferation, thereby increasing intestinal tissue weight and resulting in changes in mucosal morphology. Effect of different OA on the gastrointestinal tract is presented in Table 3.

The proposed mode of action of OA is related to the reduction of intestinal pH (Waldroup et al., 1995), which might be followed by alterations in the intestinal ecosystem (Canibe et al., 2001).For example butyric acid supplementation of broilers diets @ 0.2, 0.4, and 0.6 percent, significantly decreased the pH of crop, proventriculus and gizzard as compared to control and furazolidone group, maximum reduction in the pH was recorded at 0.4 and 0.6% butyrate compared with 0.2% butyrate (Panda et al., 2009). Eventhough inclusion of 0.4% and 0.8% buffered propionic acid in broiler diets resulted in decreased total number of *coliforms* and *E. coli* in the small intestine of the bird however, it had no effect on intestinal pH (Izat et al., 1990). Likewise, acetic lactic and citric acid does not affect the pH of different intestinal segments (Abdel-Fattah et al., 2008).

OA salts such as ammonium formate and calcium propionate at the dose rate of 3 mg/kg diet can significantly improve intestinal villus height (Paul et al., 2007).Likewise, dietary organic acid in broilers at the age of 42 d resulted in a significant increase in villus width, height and area of the duodenum, jejunum and ileum region (Kum et al., 2010).

Short chain fatty acids are also believed to cause an increase in the plasma glucagon-like peptide 2 and ileal pro-glucagon mRNA, glucose trans-porter expression and protein expression, which are all signals that they can potentially mediate gut epithelial cell proliferation (Tappenden and McBurney, 1998).

Effect of organic acids on immunity

Dietary OA play an important contributory role in the immune status of the bird. Reduction of subclinical infections (Humphrey and Lanning, 1988) and stimulation of the growth of beneficial bacteria may contribute to increased nutrient digestibility and a reduction in nutrient demand by the gut-associated immune tissue and microorganisms (Dibner and Buttin, 2002).

The immune mechanisms in birds are fairly similar with the mammals and are directly influenced by genetic, physiological, nutritional, and environmental factors (Sharma, 2003). The immune system of bird is complex and is composed of several cells and soluble factors that must work together to produce a protective immune response. Major constituents of the avian immune system are the lymphoid organs. Thymus and Bursa of fabricius are of utmost importance because these are involved in the development and differentiation of the T- lymphocytes and B-lymphocytes respectively (Qureshiet al., 1998). Functional immune cells leave the primary lymphoid organs and populate secondary lymphoid organs. Secondary lymphoid organs include spleen, gut-associated lymphoid tissues, gland of Harder, bone marrow and bronchial-associated lymphoid tissues (Sharma, 2003).

Citric acid supplementation enhances the density of lymphocytes in the lymphoid organs, so enhances the non-specific immunity (Chowdhuryet al., 2009 and Haque et al., 2010). Birds having the greater density of lymphocytes have stronger immune status to combat antigens (Khan et al. 2008). Wang et al., (2009) found that the dietary supplementation of phenylacetic acid increase the lymphocyte percentage in a short duration in layers. Organic acid supplementation causes hyperthyroidism and peripheral conversion of thyroxin (T4) to triiodothyronine (T3) which means that these birds have better immune competence and bursa growth (Abdel-Fattah et al., 2008). However, erythrocyte, leukocyte, eosinophil, heterophil and lymphocyte are not influenced by OA (Khosravi et al., 2010). Citric acid supplementation increases the bioavailability of Zn from the soybean meal in poultry (Boling et al., 2000b), a metal known for its immune enhancing properties (Kidd et al., 1996).

Dietary supplementation with acetic and lactic acid increases the serum globulin and decreases the albumin to globulin ratio (Rahmani et al., 2005; Abdel-Fattah et al., 2008). Globulin is a source of antibody production, so its serum level is a good indicator of immune responses and consequently better disease resistance (Griminger and Scanes, 1986). Das et al., (2011) and Houshmand et al., (2012) reported an increased antibody titer against Newcastle disease in broilers by dietary supplementation of OA.

Effect of organic acid on the nutrient digestibility

Protein and energy are the major factors influencing the performance of birds. Depending upon the regional location, protein in poultry diet can be supplied by animal and/or vegetable sources. Amongst vegetable protein sources, soybean meal remains the priority of animal nutritionists. However, there is a downside to it since it contains major anti-nutritional factors for poultry e.g., galacto-oligosaacharides, lectins and trypsin inhibitors; the major anti-nutritional factors present in soybean meal (Huisman and Jansman, 1991). Digestion of the protein in chicks is badly affected by the undigested galacto-oligosaacharides (Gdala et al., 1997) due to absence of α-1,6-galactosidase(Gitzelmann and Auricchio, 1965). Ao (2005) studied *in-vitro* effect of citric acid on the release of reducing sugar and α-amino nitrogen from soybean meal having different levels of protease and α-galactosidase. Results indicated that citric acid increases activity of both the exogenous galactosidase enzymes, thus enhancing the liberation of α-amino nitrogen and reducing sugars. Li et al. (1998) in an experiment using citric acid addition to the phytase supplemented swine diets reported a non significant improvement in dry matter, nitrogen, phosphorus and calcium digestibility. While other researcher (Dibner and Buttin, 2002; Omogbenigun et al., 2003; Suiryanrayna and Ramana, 2015) reported that organic acid supplementation in simple stomach animal diets resulted in an improved protein digestibility and energy availability by reducing microbial competition with the host for nutrients, endogenous nitrogen losses and production of ammonia. As OA increased the digestion of the protein, this consequently reduces the emission of ammonia and sulfur containing gases from the poultry house.

It is thought that reduction in the pH of digestadue to organic acid supplementation may increases the pepsin activity (Afsharmanesh and Porreza, 2005), resulting in enhanced protein digestibility (Gauthier, 2002).Pepsin proteolysis the proteins, thus producing the peptides which act as a strong stimulant for the release of hormones including gastrin and cholecystokinin (Hersey, 1987).These hormones then acts on pancreatic cells signaling them to release digestive enzymes. OA also act by increasing pancreatic secretions resulting in enhanced production of pancreatic juice (Smantha et al., 2009).As a consequence higher concentrations of trypsinogen, chymotrypsinogen A, chymotrypsinogen B, procarboxypeptidase A and procarboxypeptidase B are produced, which then lead to increased protein digestion (Kirchgessner and Roth 1982; Afsharmanesh and Porreza, 2005). Hume et al. (1993) studied the metabolism of propionic acid and found that 75% of this acid is used as energy source. Likewise Runho et al. (1997) reported improved metabolisable energy contents of broiler diets due fumaric acid supplementation.This proposes a correlation between energy levels and OA.

Thyroid hormones (Tri-iodothyronine) play a major role in regulating the oxidative metabolism in poultry. Any marked change in thyroid function (hypothyroidism or hyperthyroidism) will result in altered metabolic rate (Whittow, 2000). Abdel-Fattah et al., (2008) studied the effects of dietary organic acidification in broiler chicks using variable doses i.e., 1.5 and 3%, of lactic, citric and acetic acid to evaluate the effects on thyroid hormones and reported a significantly elevated serum Triiodothyronine (T3) concentration of organic acid fed broilers however, T4 levels were not significantly affected.

Minerals are crucial for normal physiological, structural and catalytic functioning of the body, (Underwood and Suttle, 1999) and therefore, must be supplied through feed. Minerals represent about 3.5% of the total body composition, of which 46% is calcium (Ca), 29% is phosphorus (P) and 24% included potassium (K), Sulphur (S), sodium (Na), chlorine (Cl) and magnesium (Mg). Minerals, especially Ca and P help to build bones and make them strong and rigid. Trace levels of iodine (I), iron (Fe), manganese (Mn) and zinc (Zn) are also included in the dietary mineral supplements to the poultry. OA reportedly increase the digestion of minerals in poultry. Citric acid (40 to 60 g/kg of diet) is very efficacious in improving P utilization in chickens fed on maize soybean meal diets and reduced the available phosphorus requirement by approximately 1 g/kg diet (Boling et al., 2000b). Boling et al. (2000a) also reported that the dietary citric acid supplementation increases the bioavailability of Zn to the chicks. Citric acid supplementation also increases the retention of Ca, P and Zn, thereby increased their levels in plasma (Brenes et al., 2003). Likewise acetic acid, citric acid and lactic acid increased the serum Ca and P (Abdel-Fattah et al., 2008). Adil et al., (2010) used butyric acid, fumaric acid and lactic acid in broiler diets and reported a significant increase in the serum concentration of Ca and P. Dietary supplementation of

OA resulted in chelation of anions of OA with the minerals making them less reactive with vitamins and more bioavailable to the birds (Li et al., 1998). There are many factors which affect the bone development e.g. genotype, age of bird, dietary Ca and P level, dietary vitamin D_3, dietary fiber content and type of feed ingredients. Monogastric animals consume diets composed mostly of oilseed and cereal grains that contain high level of P present in the form of phytic acid or phytate. The P in this form is generally unavailable to poultry due to low phytase activity found in the digestive tract (Cromwell, 1992). Many studies showed that OA can increase phytate P utilisation by poultry (Boling-Frankenbach et al., 2001 and Brenes et al., 2003). Maximum activity of microbial phytasecould be reached at lower pH values, thus it could be achieved by adding OA in the diet. Benzoic acid supplementation increase the uptake of the Ca by 0.85 g per day, retention of P by 0.74 g day, retention of K by 0.77 g day and plasma levels of the P in growing pigs (Sauer et al., 2009).

Pirgozliev et al. (2008) reported that birds fed organic acid supplemented diets excreted less mucin (measured as sialic acid (SA)), an indicator of endogenous losses, than birds fed supplemented diets. Increased concentration of SA in digesta or excreta is often connected to gut health problems (Reutter et al., 1982), thus dietary organic acid supplementation improves the gut health of birds.

Bone ash is the direct indicator of mineral deposition and bone strength. Citric acid supplementation at the rate of 6% to the broiler diet resulted in an increased bone ash of up to 43% compared to the groups fed non-supplemented diets (Boling et al., 2000b). Shohl (1937) observed a 61% increase in femur ash when rats consumed Ca and P deficient diets supplemented with citric acid/sodium citrate. Perhaps citric acid, a strong chelator of Ca, removes Ca from or decreases Ca binding to the phytate molecule, thus making it less stable and more susceptible to endogenous phytase.

Effect of organic acids on performance and profitability of poultry

The effects of OA on performance are not consistent for the poultry. As stated before quoting Ricke (2003), the magnitude of the organic acid response varies due to several reasons. OA increase the average live weight, daily gain (BWG), daily feed consumption and improves the feed conversion ratio (FCR) (Al-Kassi and Mohssen, 2009). Fumaric acid significantly increases the BWG (Skinner et al., 1991) at the rate of 0.5% and 1.0% without affecting feed in take in broilers and layers. Likewise, Patten and Waldroup, (1988) recorded a higher BWG in broilers with no effect on feed utilization when fed fumaric acid supplemented diets. Adil et al. (2011) reported that dietary supplementation with the butyric acid, fumaric acid and lactic acid at the 2 and 3% level each; resulted in higher final live BWG, improved FCR in broilers. Vogt et al. (1982) studied malic, sorbic, and tartaric acids (0.5 to 2%) in broilers and reported increase in BWG, with optimal levels of 1.12 and 0.33% for sorbic and tartaric acids, respectively and improved FCR. Izat et al. (1990) reported that formic acid, calcium formate and buffered propionic acid did not affect the feed utilization. Panda et al. (2009) studied the effect of butyric acid supplementation in broiler ration at the dose level of 0.2, 0.4 and 0.6 percent and documented improved BWG, FCR and a decrease in the weight and percentage of abdominal fat. Butyric acid was as much effective as furazolidone. Similarly body weight and FCR significantly improved by using 2% lactic acid in broiler diet (Versteegh and Jongbloed, 1999). Buffered propionic acid significantly improved the dressing percentage in female broilers and reduced abdominal fat in males at 49 days of age (Izat et al., 1990). Likewise Patten and Waldroup, (1988) suggested an increase in broiler production profitability through increased BWG when dietary supplementation of OA was adopted.

Contrary to the above findings Brown and Southern (1985) found that chick performance is not affected by the supplementation of citric acid and ascorbic acid. Supplementation of propionic acid depresses the feed intake and growth performance but similar results are not reported by the use of lactic acid (Cave, 1984). Though lacking any suggested reason for these effects, Alcicek et al. (2004) reported that dietary supplementation of the organic acid does not affect the feed intake and FCR at 21 and 42 day of age in broilers. Citric acid addition in the broiler diets does not have any significant effect on egg production, egg mass, egg size, feed efficiency, specific gravity of egg and body weight of laying hens (Boling et al., 2000a).

Meat preservation

Consumer interests regarding natural and certified organic foods are increasing. These consumer preferences increased the demand for bio-preservation of the food. OA are one of the best food preservatives(Ewing, 2009). Contaminated poultry meat causes the food borne diseases in humans. More than 76 million citizens in USA became ill by ingesting food especially meat products contaminated with pathogenic bacteria (Mead et al., 1999) which resulted in 1600 deaths (Callaway et al., 2003). Short chain OA are commonly used food preservatives and there is an increasing trend of bio-preservation of food in European countries as these can be used safely without creating residual effects. Lactic or acetic acid reduced the potential of *Campylobacter* in carcass or meat (Cudjoe and Kapperud, 1991). Addition of formic and propionic acid in the broiler feed causes sub-lethal damage of *Salmonella* resulting in the incomplete colonization (Thompson and Hinton, 1997). Poultry meat is preserved in order to prevent contamination, as contaminated poultry meat cause many foodborne diseases in humans (caused by microorganisms such as *E. coli*, *Clostritdium perfringens*, *Clostridium botulinum*, *Campylobacter jejuni*etc.). Some fungi like *Aspergillusflavus* and *Aspergillus paraciticus* also produce different type of diseases by producing toxins (Prange et al., 2005). Out of these, *Salmonella* is a major foodborne pathogen associated with poultry meat because fecal material and dirt from feathers and the hide, as well as dirt of processing equipments can contaminate the carcasses during slaughtering and packaging operations. Due to high pH (5.5-6.5), water activity (0.98-0.99) and enriched nutrient profile, fresh poultry meat is highly perishable and provide favorable environment for growth of food contaminating microorganisms (Acuf, 2005). *Salmonella gallinarum* and *Salmonella enteritidis* are frequently found in poultry and poultry products but rarely cause illness in humans (Braden, 2006). *Salmonella typhimurium*is the most common serotype associated with laboratory confirmed illness cases (CDC, 2009). Therefore, in this scenario OA can be used as potential hygiene promoters, where they lower the pH and also act as a complexing agent for ions, thereby inhibiting microbial growth (Ewing, 2009).

Environmental and economic challenges of using organic acid in poultry

All in all the usage of OA on the basis of above mentioned properties not only makes them a good choice for poultry production but also ensures a lower biological, environmental and economic overhead compared with other available supplements. For example enhanced nutrient digestibility will have nutrient sparing effect which along with better production performance will also lower the losses, therefore reducing the risk of environmental pollutionfrom animal production (Lückstädt and Mellor, 2011). This is particularly true for a reduction in nitrogen and mineral related environmental issues from poultry facilities (Dibner and Buttin, 2002; Riemensperger, 2012). Therefore their usage in poultry production is economically justifiable.

Possible adverse effects of using organic acid

However, there were few concern raised by the scientists regarding the adverse effects of OA supplementation on organoleptic properties (the appearance and texture) of poultry meat (Dickens and Whittemore, 1994 and Dickens et al., 1994). There is also an environmental concern for the disposal of waste water from poultry units using OA supplementation along with a fear of the emergence of acid-resistant pathogens (Fabrizio et al., 2002). Gabert and Sauer (1995) noted a reduction in ileal digestibility of both CP and amino acid when diet was supplemented with fumaric acid in growing pigs.

CONCLUSION

OA inhibit the growth of pathogenic bacteria, especially zoonotic bacteria, e.g. *Campylobacter*, *E. coli* and *Salmonella*, in the feed and gastrointestinal tract of poultry which is of great importance with respect to poultry health. They also cause reduction in the microbial load on poultry meat products. OA improve the mucosa growth, villus height and width, crypt depth and decrease the intestinal pH. They also boost the immune system and the digestibility of protein, carbohydrate and minerals, thus enhancing the growth performance of poultry. Therefore, OA can be meritoriously used as a replacer of the antibiotic growth promoters in poultry.

Competing Interests
The authors declare that they have no competing interests.

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Comparative Study on Diphtheritic, Cutaneous and Systemic Forms of Natural Avipoxvirus Infection in Chickens

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ABSTRACT

Avipoxvirus of the subfamily Chordopoxvirinae is known to cause fowl pox infection in chickens. The disease manifest as Cutaneous, diphtheritic, systemic and oncogenic forms in birds. The former two being the most frequent forms of the infection and occurring in chickens. Twelve cases of fowl pox virus infection in chicken flocks over a 5-year period were reviewed to describe the pathologies and the forms of pox virus infection observed in Bauchi and Plateau States in Nigeria. Three forms (cutaneous, diphtheritic and systemic) of fowl pox virus infection were investigated in indigenous and commercial backyard chicken breeds at different ages and with infection during different period of the year using gross- and histo-pathological features. Our findings showed that the cutaneous form was most common in Bauchi and Plateau States in north-eastern and north-central Nigeria respectively. Rather than the mixed Cutaneous and diphtheritic form previously reported, we observed a new co-occurrence of a mixed Cutaneous and systemic form of fowl pox virus infection in a young cockerel chicken. Also, there seems to be no seasonal variation in the occurrence of fowl pox virus infections in the chicken flocks in the study area, a notion responsible for fowl pox virus vaccine demands in the country. Therefore, present study suggest a routine fowl pox vaccination program for susceptible chicken flocks as all the flocks reported in this study had a history of unvaccinated status with the exception of the indigenous chicken which is rarely vaccinated against any infectious or contagious disease in the country.

Key words: Comparative, Fowl pox virus, Infection, Chicken

INTRODUCTION

Fowl pox virus infections in chickens are caused by avipoxvirus belonging to the subfamily chordopoxvirinae in the family poxviridae affecting a wide range of vertebrate hosts (Quinn et al., 2011, Lawson et al., 2012, Meseko et al., 2012 and Bwala et al., 2015). The avipoxvirus genus contain fowl pox virus infecting fowls, turkey pox virus infecting turkeys and pigeon pox virus in pigeons which are closely related and are not strictly host-specific (Quinn et al., 2011). Avian pox disease affects both domestic and free living birds in nature which results in varying morbidity and mortality (Afonso et al., 2000). Avian pox virus infection is said to be characterized by Cutaneous (dry pox), diphtheritic (wet pox), systemic and oncogenic manifestation (Tsai et al., 1997, Lawson et al., 2012). Although only the cutaneous and diphtheritic forms have been documented in chickens to be caused by fowl pox virus (Tripathy and Reed, 2008). The systemic form has been reported in other avian species (Tripathy and Reed, 2008). Cutaneous form of pox in chicken is characterized by local epithelial hyperplasia that includes epidermis and underlying feather follicles (Tripathy and Reed, 2008), resulting in the formation of nodules, papules, vesicles and eventual formation of scabs (Tripathy and Reed, 2008). The diphtheritic form is reported to be more severe, causing significant mortality and economic losses in affected flocks (Singh et al., 2003), and it is characterized by the formation of white opaque nodules or yellowish patches which develop on the mucous membranes of the oral cavity, tongue, oesophagus or upper trachea (Tripathy and Reed, 2008). Nodules rapidly increase in size and often coalesce to become a yellow, cheesy, necrotic, pseudo diphtheritic, or diphtheritic membrane. A mixed cutaneous and diphtheritic forms are said to be common with development of lesions on the comb and wattles as well as diphtheritic lesions in the mouth and/or respiratory tract of the same bird. The systemic form of avian pox virus infection has been documented, whereby the liver had single to multiple soft white to yellow nodules ranging in size from 0.2- 0.5cm in diameter (Tripathy and Reed, 2008). In chickens, fowl

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pox is said to affects all ages, all sexes and all breeds (Weli and Tryland, 2011). Fowl pox infection is a slowly spreading disease and an economically important disease of chickens and turkeys as it can cause egg production losses and even mortality, especially in commercial poultry (Tripathy and Reed, 2003). The disease is mostly seen in poultry kept in free-range holdings (Bwala et al., 2015) in South Africa, although it is reported to be widespread in backyard and intensively reared poultry flocks in Nigeria (Adene and Fatumbi, 2004). The disease is reported to be spread by biting arthropods, which included mosquitoes and mites (Proctor and Owens, 2000), and through infective aerosols, contaminated feed or water, and skin trauma resulting from pecking by other birds (Bwala et al., 2015). The aim of this work is to report a mixed cutaneous and systemic form of fowl pox infection observed at the Nigerian National Veterinary Research Institute diagnostic laboratory.

MATERIALS AND METHODS

Over a 5-years period, 2011 to 2015, thirty-nine unvaccinated mixed sex backyard, commercial and indigenous chickens of different breeds and varying ages from 12 suspected cases of fowl pox infection (Table 1) presented to the Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom Nigeria were diagnosed by gross- and histo-pathology. Carcasses of the affected chickens were necropsied and the tissues including lung, trachea, oral mucosa, liver, facial and nasal scabs were removed and fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, mounted on clean glass slides, and stained with Hematoxylin and Eosin (H&E) stains for histopathological examination using low and high powered field of Carl Zeiss® binocular microscope.

Ethical Approval

This study was evaluated and followed the ethical guidelines of the Ethics Committee of the National Veterinary Research Institute, Vom, Nigeria.

RESULTS

Cases

Ten of the analyzed cases exhibited the cutaneous form of fowl pox virus infection, while in one case, both the Cutaneous and systemic forms were seen and the remaining one case showed the diphtheritic form (Table 1). The cutaneous form of the disease was found to be the most common, and affected all types (indigenous and commercial) and breed (pullet, broiler, cockerel and layers) of chicken examined cutting across all ages from 9-52 weeks old chickens. The cutaneous form also occurred at both the dry (October to January) and during the raining season (June-September) in the study area in Plateau and Bauchi states of Nigeria. The systemic form, which co-occurred with the cutaneous form of fowl pox, occurred in a young, 9 weeks old cockerel chicken and during the dry season of the year. The only case of the diphtheritic form occurred in 2 years old indigenous laying hen during the hamattan season of December.

Gross and histopathology

The cutaneous form of fowl pox infection in the majority of the cases in chickens are frequently characterized by pale to yellow, often times discolored combs with multifocal 0.05-0.5 cm in diameter nodule formation on the combs, face, peri-orbital and ocular areas, as previously described. Occasionally, the lesion is characterized by papule formation which thickens and coalesced to form large dark brown scabs which often occludes the nares (Figure 1a). The only case that exhibited the diphtheritic form (Figure 1b), occurred in an adult indigenous laying hen. In this hen, there was an extensive raised yellowish patchy, necrotic, diphtheritic membrane which covers the mucous membranes of the oro-pharynx and the proximal $1/8^{th}$ of the trachea. In one of the cases that showed the cutaneous form of fowl pox infection, a systemic form was also observed, wherein the liver had tiny multifocal soft white to yellow nodules ranging in size from 0.05-0.1cm in diameter (Figure 1c). At histopathology, a 9 weeks old pullet with large facial and nasal scabs (Figure 1a) showed hyperplasia of the stratified squamous cells exhibiting acanthosis with severe inflammatory exudation of heterophils, macrophages and lymphocytes (Figure 1c) and often times with haemorrhagic ulcers. Also, there were several small to medium sized eosinophilic intracytoplamic and intra-keratinocytic inclusions, identified as Bollinger bodies (Figure 1d) which at higher magnification, are central to eccentric eosinophilic intracytoplasmic bollinger bodies typical of fowl pox infection (Figure 1e).

DISCUSSION

During the study period, 2011-2015 only 12 cases were reported for the purpose of diagnosis at the Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom Nigeria. The cases involved 39 chickens cutting across different ages, sexes and breeds as previously been observed in fowl pox infections (Weli and Tryland, 2011), either in poultry kept in free-range holdings (Bwala et al., 2015) or in backyard (Akanbi et al., 2015) and to some extent commercially reared poultry flocks (Adene and

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Fatumbi, 2004). With eleven occurrences of these 12 cases (91%) in this study, the cutaneous form of fowl pox disease was found to be the most common as have been reported elsewhere (Lawson et al., 2012), and affected all types (indigenous and commercial) and breeds (pullet, broiler, cockerel and layers) of chickens. Only one case of the diphtheritic form was observed in this study and rather than a mixed cutaneous and diphtheritic form said to be common (Tripathy and Reed, 2008), only a mixed cutaneous and systemic form was observed in a young, 9 weeks old cockerel chicken (8%). The systemic form does not seem to have been documented in domestic chicken as far as our literature search was concerned. But we found this form to co-occur in this cockerel. The only diphtheritic form seen in this study occurred in a 2 years old indigenous laying hen. It is not clear whether, this case was as a result of virus reactivation due to stress or immunosuppression (Lawson et al., 2012) as there was a history of previous infection with pox virus in the flock. The pathology of the cutaneous and diphtheritic forms of the disease in these chickens are consistent with earlier findings (Tripathy and Reed, 2008), although our pathological findings in the systemic form in chicken vary slightly, as multifocal soft white to yellow nodules similar to the findings in the Andean condor (Tripathy and Reed, 2008) was only seen on the liver of the mixed cutaneous and systemic case. The only case of the diphtheritic form that occurred in the indigenous hen was severe and fatal, consistent with previous report (Singh et al., 2003). It was hypothesized that the poxvirus infection or its sequelae was the main contributory cause of death in these cases as no other pathology was observed. As it has been reported previously (Lawson et al., 2012) that susceptibility to Avipoxvirus infection varies among host species and in relation to host age with juveniles being most susceptible, the pox viral infection in this study were characterized by morbidity and severe mortality in mainly young birds and cocks. Therefore the effect of the disease on egg production was not observed as earlier reported in commercial poultry (Tripathy and Reed, 2003). As observed in this study, there seems to be no seasonal variation in the occurrence of fowl pox virus infections in the chicken flocks in this study over the 5-years period as the disease was recorded during the wet (Jun-Sept) and dry (Oct-Jan) seasons of the year. The notion of seasonal variation in the infection patterns of fowl pox has been responsible for fowl pox virus vaccine demands in Nigeria (O. Asala [Viral vaccine production, National Veterinary Research Institute, Vom, Nigeria], pers. comm., 18 January 2016). This may also be responsible for the unvaccinated status of all the cases in this study with the exception of the indigenous chickens which are rarely vaccinated against any infectious or contagious disease. In view of our findings, we therefore suggest a routine fowl pox vaccination program for susceptible chicken flocks

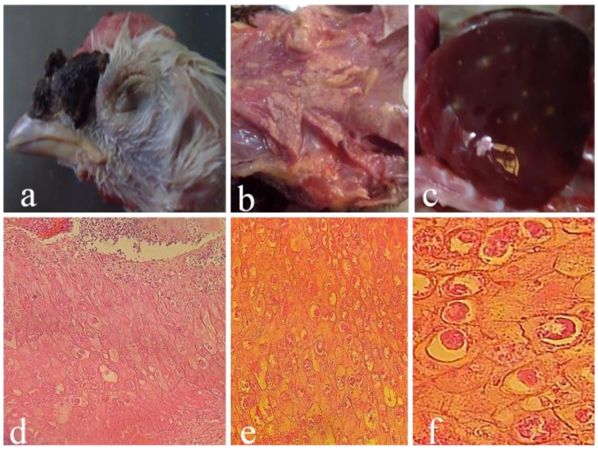

Figure 1. a: chicken, 9 weeks old pullet with large facial and nasal scabs; **b**: 2 years old indigenous layer chicken with large oro-pharyngeal and tracheal diphtherictic membrane; **c**: chicken, 9 weeks old pullet with multifocal tiny, up to 0.05cm in diameter nodules on the liver; **d**: scab tissue, hyperplasia of stratified squamous cells exhibiting acanthosis and hetero-lymphocytic infiltration X 10, H&E stain; **e:** facial tissue, showing small to medium sized eosinophilic intrakeratinocytic inclusions (Bollinger bodies) X10 H&E stain; **f:** higher magnification of eosinophilic intrakeratinocytic inclusions (Bollinger bodies) typical of fowl pox infection X40, H&E stain.

Table 1. Cases of different forms of fowl pox virus infection in unvaccinated mixed sex commercial and indigenous chickens in Bauchi and Plateau States of Nigeria during 2011-2015

Cases	Age in weeks (wks)/ sex	Onset	No. of Samples	Pox form
Jos 1	9 wks/ pullet	Jan	1 Carcass	Cutaneous
Toro 1	9 wks/ cockerel	Sept	7 Carcasses	Cutaneous
Toro 2	9 wks/ cockerel	Sept	5 Live	Cutaneous/ Systemic
Jos 2	9 wks /pullet	Oct	6 Carcasses	Cutaneous
Jos 3	9 wks/ pullet	Oct	13 Carcasses	Cutaneous
Jos 4	22 wks/ layer	June	1 Carcass	Cutaneous
Jos 5	52 wks /indigenous layer	July	1 Carcass	Cutaneous
Jos 6	Adult/ indigenous cock	July	1 Carcass	Cutaneous
Jos 7	9 wks broiler	Sept	1 Live	Cutaneous
Jos 8	17 wks/ cockerel	Nov	1 Carcass	Cutaneous
Jos 9	46 wks/ layer	June	1 Carcasses	Cutaneous
Langtang 1	104 wks/ indigenous hen	Dec	1 Carcass	Diphtheritic

CONCLUSION

It is concluded that routine vaccination of chicken flocks be instituted, especially small-holder flocks to prevent the losses due to morbidity and mortality associated with fowl pox infection.

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Competing interests

The authors declare that there are no significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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A Study on Food Safety Knowledge and Perceptions among Poultry Consumers in Mauritius

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ABSTRACT

Although previous research has been conducted to understand Mauritian consumers' knowledge of food safety risks, there is a lack of research on their knowledge, perception, and behavior towards risks associated with poultry sold in markets. Recently, there has been heightened concern regarding a particular market located in the capital of Mauritius. The market was previously sanctioned for malpractices due to unsafe trade of poultry. The target group identified in this study was therefore customers who regularly purchased poultry from the mentioned market who are thought to have inadequate knowledge in food hygiene, safety and microbiology. Therefore a study was carried out at the market to investigate the knowledge and perceptions of Mauritian consumers, on safe and hygienic handling of poultry, shed light on their domestic poultry preparation practices, and understand their attitudes and disposition towards poultry safety. A survey instrument was developed and administered, and data were collected during the period of June-November 2014. The results of this study showed that respondents often lacked knowledge of basic concepts in food safety, rendering them more prone to unsafe food practices. Moreover, poultry consumers, particularly the young demographic, were found to display unsafe food behaviors due to an optimistic bias, an illusion of control or habitual behavior. Poor regard to prevention of cross-contamination was noted. Lack of specific technical knowledge was estimated to be the central reason for unsafe behavior during poultry preparation. It was therefore recommended that education on food safety should start at an early age. Moreover, food labels should be designed to protect consumers from heath risks due to consumption of unsafe food and the media should wield a greater role in educating consumers on food safety.

Key words: Food safety, Knowledge, Perceptions, Poultry, Consumers

INTRODUCTION

Food safety is the degree of assurance that food will not present any adverse effects on the health of the consumer when it is handled, prepared, cooked and consumed according to its intended use (WHO, 2005). Potential hazards in foods cover a broad range, from natural (e.g. mycotoxins) and environmental contaminants (e.g. dioxins) to agrochemicals. Most cases of foodborne illness are preventable if food protection principles are adopted at all stages along the production to consumption continuum (Bucknavage and Cutter, 2011). Given that it is currently impossible for food manufacturers to ensure a pathogen-free food supply, the consumer is a critical link in the chain to prevent foodborne illness in the domestic setting. Thus, home food preparers need to know how to minimize the presence of hazards in their food.

Food can be mishandled at a number of places during food preparation, handling and storage, and studies show that consumers have inadequate knowledge of measures needed to prevent foodborne illnesses in the home (Mederios et al., 2001; Bearth et al., 2014). Indeed, contaminated raw foods, inadequate cooking, and consumption of food from an unsafe source were the factors most commonly associated with reported outbreaks of domestically acquired foodborne illnesses (Mederios et al., 2001). Studies have estimated that 50-87% of the reported food poisoning incidences have incriminated homemade food (Redmond and Griffith, 2002). Common malpractices noted included serving food products that were originally contaminated, cooking or heating food insufficiently, handling food by infected or carrier persons, having little consideration for food hygiene (WHO, 1997),

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engaging in food preparation practices that lead to cross-contamination (Sneed et al., 2015).

Since 1990, there have been an ascending number of food poisoning incidents and malpractices in Mauritius that have undermined the confidence of Mauritian consumers (Statistics Mauritius, 2013). Recent trends observed in Mauritius relate to particular concern about new foodborne pathogens that have resulted in major food poisoning outbreaks (Hotee, 2011).

Chicken is one of the most highly consumed meat in Mauritius and its growing popularity is a result of increasing prosperity. The preference for chicken will cause a rise in its production to 128 metric tons a year by 2020 and the proportion reaching global markets will grow too, from approximately 14% to 17% of total output (The Economist, 2013). In Mauritius, the Hindu community generally does not eat beef whereas the Muslim community does not consume pork, but both consume poultry meat (Heetun, 2014). The annual per capita of poultry consumption in Mauritius has increased from 14.3 kg in 1990 to 27 kg in 2006 coupled with an increase in poultry production (Statistics Mauritius, 2007). In the event of contamination of the fresh chicken meat supply, a considerable proportion of the population will be at risk of food poisoning. Indeed, a recent outbreak of salmonellosis in Mauritius incriminating raw chicken and eggs has been the cause of significant concern among regulatory authorities and more importantly Mauritian consumers (Le Defi, 2016). Therefore knowledge of food safety and safe food preparation is of paramount importance to minimize cases of food poisoning caused by consumption of contaminated poultry.

Therefore a study was carried out at a highly frequented market located in the Capital City of Mauritius to (i) investigate the knowledge and perceptions of Mauritian consumers, on safe and hygienic handling of poultry, (ii) shed light on their domestic poultry preparation practices, and (iii) understand their attitudes and disposition towards poultry safety. Knowing the baseline knowledge and behaviors in this target group will be essential for the development of effective health educational programs.

MATERIALS AND METHODS

For this study, data was collected at one of the main markets of the capital city of Mauritius, during the period spanning June-November 2014 via a self-administered questionnaire. A total of 150 customers from different geographical regions visiting the market were approached using a non-random convenience sampling method i.e. without any probability-based selection method (Price, 2013). Participants included adults, of all ages, who purchased poultry at the market fair. Participation was on a voluntary basis contingent on 1) attending the market fair, 2) choosing to respond to the survey and 3) limited to those who reported purchasing poultry that day at the market.

The survey instrument was developed after undertaking a desk review of prior research conducted on a similar target population. The draft instrument contained screener questions and the actual questionnaire. The questions aimed to assess the consumers' awareness of the following aspects: (i) safe temperature for refrigeration and freezing, (ii) safe temperature for cooking and cooling of chicken, (iii) knowledge of cross-contamination, (iv) knowledge of food safety hazards, (v) poultry preparation practices (vi) and their general attitudes vis a vis food safety. The survey comprised mostly of close-ended with some open-ended questions. The latter were preferably used when specific answers were not required and when it was important to know the opinion of the interviewed person.

A field test of the draft instrument was first conducted prior to the actual survey administration whereby participants (n = 12) reviewed the instrument for any sources of ambiguity or missing information. Several caveats noted were that (i) some questions led to biased answers, (ii) interviewed persons had little time to devote to answering all questions and (iii) some respondents were not able to answer open-ended questions, which required specific answers. Consequently, amendments were made to the initial version to enhance ease of survey taking and these included rephrasing the questions in plain English, shortening of the questionnaires to speed up the interview and converting open-ended questions, which required specific answers, to closed-ended questions.

For the actual survey, the majority of the participants were approached as they were leaving the market to ensure that they had made a purchase. Typically, customers carrying goods were approached and were more willing to participate in the research study. If a participant were within the targeted age range and indicated that they had purchased poultry from the market, they were directed into the survey questionnaire. The final instrument began with a consent form and consisted of two parts, screener questions and survey questions. To guarantee anonymity of responses, numbers were randomly assigned to each questionnaire. Items in the questionnaire were explained where necessary and administered at one sitting as far as possible. Each questionnaire took approximately 10 minutes to administer. Data was collected on weekday afternoons or during midday on weekends. Analysis of data was carried out using Excel and SPSS (Version 17.0) statistical package. Mean responses for the different questions were determined by computing the average number of responses for each category while percentages of responses for each category was calculated by dividing the number of responses of a certain category by the total number of responses obtained for that particular question and multiplying by 100, and presented in charts.

Ethical approval

The authors solemnly declare that publication ethics and good conduct were adhered to during preparation, reviewing, processing and proofreading of this article.

RESULTS AND DISCUSSION

Profile of respondents and disposition towards food safety

A total of 150 questionnaires were filled, out of which only 125 were analyzable. All survey respondents were customers who had the primary responsibility for food preparation in the home. None of them were professional food handlers. 76% of the respondents were females, 64% were married, and 24%, 48% and 28% were primary, secondary and tertiary school graduates respectively (Figure 1). All (100%) of the respondents mentioned having a positive inclination and disposition towards safe food practices.

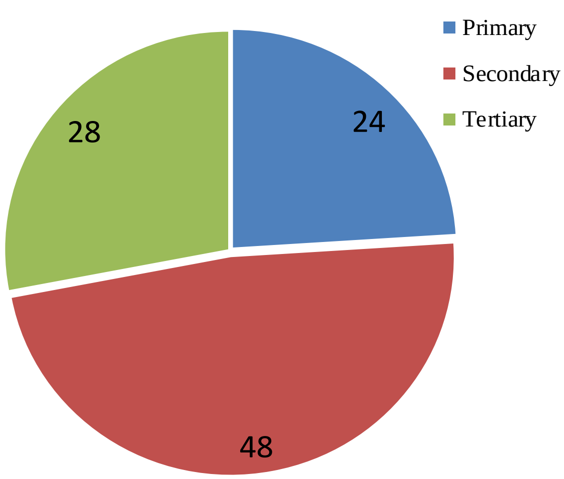

Figure 1. Percentage of survey participants with different level of education

Consumption rates of poultry

Eighty percent of the respondents stated that they consumed poultry two to three times per week compared with 12% of consumers who mentioned consuming poultry only once weekly. A minority (8%) of the respondents mentioned that they consumed poultry only occasionally. Most respondents reported that their diet regularly included chicken compared with other meat. This is in agreement with other studies, which revealed that the consumption of poultry worldwide is higher than other meats (The Economist, 2013). These findings can thus corroborate the significant rise in poultry consumption in Mauritius (Statistics Mauritius, 2013), Africa and Europe (Global Poultry Trends, 2012). A negative consequence of increased poultry consumption is that a larger population is at risk of contracting poultry-borne infections. Therefore poultry consumers should have a sound knowledge in food safety and should put this knowledge into practice.

Drivers for poultry consumption

The study revealed that the most important considerations for purchasing poultry were ‘taste’ (36%), ‘hygienic quality and safety’ (32%) followed by ‘high level of protein and low cholesterol’ (24%) and ‘culinary versatility’ (8%) (Figure 2).

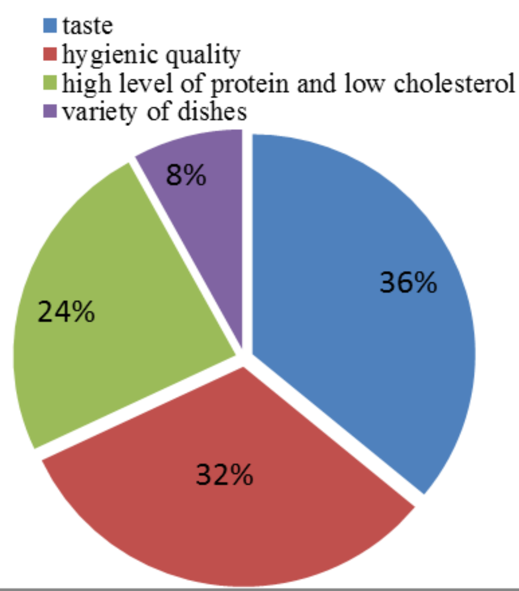

Figure 2. Drivers for consumption of poultry meat in Mauritius

Hence considerations such as ‘safety and hygienic quality’ of the products were actually secondary to ‘taste’ of the products. This reveals risky behavior on the part of consumers because unsafe foods may not exhibit any change in taste, flavor and color. In fact, it was expected that respondents would give more importance to safety rather than taste given their level of education, age and experience. As expected, most of the respondents who opted for reasons other than food safety were in the age group of 20-49, while most of the respondents who opted for safety considerations were of age higher than 49. This result is also congruent with those of Brennan et al. (2007) and Kennedy et al. (2005) where it was found that older people were more concerned about food safety practices and hazards. Therefore, our findings reveal that younger consumers have less food safety knowledge and therefore their food preparation practices call for improvement (Sanlier, 2009). Patil et al. (2005) further mentioned that young adults (18–29 years) are particularly vulnerable individuals to food poisoning although the propensity to adopt safe poultry practices is higher.

Confidence and food safety knowledge of consumers

Respondents were mostly (84%) confident in the safety of poultry purchased at the market and in their domestic preparation practices of poultry-based dishes. In addition, they showed little concern with regard to the safety of the food supply possibly due to a false sense of confidence and from a high internal locus of control (Green, 2004). The fact that survey participants perceived their food preparation practices as adequate suggests that they might be predisposed to an optimistic bias (Benkendorf et al., 1997). Indeed, Williamson et al. (1992) mentioned that survey participants generally perceive their homes to be locations at which the acquisition of food poisoning is improbable. Fein et al. (1996) indicated that the fact that consumers were not readily inclined to accept an association between home food-handling practices and foodborne illnesses is considered a serious impediment to convincing consumers to change inappropriate food-handling behaviors. Redmond and Griffith (2003) mentioned that members of focus groups expressed more concern about acquiring foodborne illnesses from locations away from the home, because they perceived themselves to have more control at home (Redmond and Griffith, 2001). This underestimation of personal risk posed by food poisoning may prevent consumers from taking the necessary steps to reduce their exposure to food hazards (Sammarco and Ripabelli, 1997). A large proportion (90%) of consumers from the United Kingdom perceive that there is a very low risk of getting food poisoning from food that they had prepared themselves (Redmond, 2002), and this finding corroborates the results obtained by Frewer et al. (1995) indicating that consumers associate the lowest personal risk of food poisoning with home-produced food. Hence consumers perceive themselves to have greater control over their own food safety than others, thus indicating judgments of optimistic bias (Redmond and Griffith, 2002).

Moreover, 76% of the participants were also confident of their knowledge in food safety. Redmond and Griffith (2003) similarly mentioned that the majority (80%) of consumers interviewed in their study thought themselves to be adequately informed regarding food safety. Almost everyone indicated familiarity with the term foodborne illness (97%). However, they also demonstrated a lack of awareness of other food safety concepts. The lack of familiarity with all food safety principles is in agreement with findings of Bruhn and Schultz (1999). The majority of the survey studies in the literature similarly concluded that consumer knowledge of food safety was generally inadequate and required improvement (Redmond and Griffith, 2003). Inadequate knowledge not only leads to implementation of common unsafe food preparation practices but also contributes to foodborne illnesses (Kerslake, 1995).

The highest level of education attained by consumers of poultry was secondary followed by tertiary and primary schooling (Figure 1). No respondents encountered were illiterate. This shows that the customers interviewed, who were recipient of a certain level of education but were still inclined to purchase their poultry products from market sellers in spite of their unsafe practices. While Kwon et al. (2008) have highlighted the importance of education in food safety knowledge and practices, other studies have reported that individuals with a higher level of education were less concerned about food risks and food safety (Fischer et al., 2008). Indeed, Bruhn and Schutz (1999) reported that many interviewees claimed they knew how to handle food safely, but their self-reported food-handling behaviors did not support this confidence. We also noted that consumers aged greater than 49 years had a greater disposition and inclination towards food safety than younger consumers (20-49 years old). Other studies have similarly found a correlation between ‘safe food practices’ and ‘knowledge in food safety’ with ‘age’ and ‘experience’. Taken together, it was observed that younger participants demonstrated the most pressing need for additional food safety education (Albretcht, 1995; Bruhn and Schutz, 1999 and Rimal et al., 2001).

Consumer awareness of food regulations

The result showed that a considerable proportion of respondents (68%) were aware of food regulations while 32% claimed to be unaware of food regulations. However, 100% of the respondents who claimed they knew the food regulations replied that they would not lodge complaints against illegal vendors. 30% of them were disinclined to report any illegal matters to the concerned authorities, while 70% mentioned being reluctant to do so as this could jeopardize their personal security. In the long run, this could encourage poultry vendors to perpetuate their unsafe practices with ensuing normalization of deviance. Indeed, it was revealed that normalization of deviance is induced by fear of retaliation (Maxfield et al., 2005). Therefore, although a certain proportion of the respondents claimed that they were aware of the pertinent regulations, they were not prepared to take any risks to enforce them. This finding is in agreement with Bruhn (1997) who showed that in spite of having a sound knowledge in food safety practices, consumers would not necessarily enforce them. On the other hand, Wiss (2012) emphasized that food safety knowledge is the sine qua non to enforcing food safety measures effectively. Hence, it can be inferred that if consumers show unwillingness to buy poultry displayed, sold or handled in an unhygienic or unsafe manner, this will deter retailers from perpetuating their malpractices. Therefore education of poultry consumers is important as it impacts their purchasing considerations.

Hygiene assessment of the market

With respect to the prevailing level of hygiene in the market, 88% of the respondents thought that the level of hygiene prevailing at the market was satisfactory while 12% said that the hygienic state of the market was fair. In addition, 56% of the respondents thought that the market did not need any improvement as far as hygiene was concerned. In fact, an objective assessment of the market would be ‘fair’ because although the market/fair was equipped with facilities such as availability of water, electricity and regular cleaning by manual workers, there were no chilling cabinets in the poultry section. The consumers’ assessment of the hygienic status of the market revealed that those who were mostly educated were not able to give a correct assessment in spite of the fact that education is important for understanding the basis of food safety. This observation is in agreement with that reported by Fischer et al. (2008), who pointed out that those who have a higher education were in fact less worried about food safety measures. It is also to be pointed out that the respondents who gave the correct rating of ‘fair’ were more than 50 years old.

Knowledge of cooking temperatures

Only 15 (12%) correctly replied that the safe cooking temperature for poultry should be ≥ 74°C. 70 of the respondents (56%) replied that they did not know the answer and 40 respondents (32%) incorrectly answered that the safe cooking temperature should be ≤ 65°C. Snyder (1998) reported that 15% to 20% of consumers did not know what the temperature should be inside a piece of meat for it to be considered safe to eat. It was noted that a few respondents who correctly answered fell in the age group of >50. However, a large majority of interviewed persons especially those who were less than 50 years of age were not able to give the right answer. Therefore older people are more knowledgeable about food safety practices. This inference aligns with observations of Brennan et al. (2007) and Sanlier (2009) who reported that younger consumers have less food safety knowledge.

Redmond and Griffith (2003) noted that adequate heating of food products by consumers tended to differ widely depending on the cooking method employed. In a study carried out by Redmond et al. (2001), the author noted that most consumers cooked poultry adequately and only 3% of consumers failed to fry chicken pieces for the recommended time. Similarly, Griffith et al. (1999) reported that all consumers cooked chicken curry to a sufficiently safe level. However, 83% failed to cook a roast chicken for the recommended time (Griffith et al., 1999). In fact, Anderson et al. (2000) noted that the majority of consumers (93%) had a tendency to rely on visual indicators to determine the doneness of roasted meat products, as opposed to using a meat thermometer (Snyder, 1998). Undercooking has thus been acknowledged as a significant risk factor associated with foodborne diseases (Mathias, 1999).

Knowledge of food storage temperatures

A large majority of survey respondents (81%) were aware that keeping poultry in the refrigerator will reduce the risks of food poisoning. This is very much in agreement with findings of Redmond (2001) who reported that 84% of consumers surveyed agreed that it is unacceptable to store meats at room temperature. Moreover, Mathias (1999) observed in their study that 72% of consumers were not inclined to store food at room temperature, hence showing an overall positive attitude. A minority of respondents (19%) replied that they were not aware that refrigeration slows down bacterial multiplication and hence enhances the safety and quality of the poultry products. With regard to consumers’ knowledge of chilling temperatures, 40 respondents (32%) incorrectly answered that the refrigeration temperature should be above 5°C. Only 25 consumers (20%) rightly stated that the safety range should be between 0 and 5°C while 60 (48 %) respondents admitted not knowing the answer of this question (Figure 3). In fact, studies have demonstrated that large proportions of consumers (46 to 60%) lack knowledge of adequate refrigeration temperatures (Redmond, 2002). Moreover, investigations of consumers' refrigerators have revealed that a large proportion (~ 70%) of consumers' refrigerators exceeded the recommended temperatures (Daniels, 2001; Johnson et al., 1998; Weinstein and Klein, 1996), giving rise to conditions that encourage the proliferation of bacterial cells to potentially dangerous levels and increasing the risk of illness.

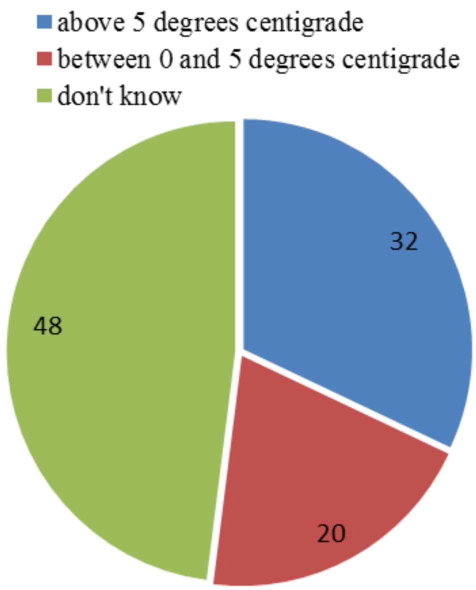

Figure 3. Perception of poultry consumers about correct chilling temperatures in Mauritius

Regarding the knowledge of freezing temperature, 15 respondents (12%) knew that the ideal freezing temperature should be at -18°C or lower. 65 poultry consumers (52%) replied that they did not know the correct answer while the rest (36%) wrongly thought that the best freezing temperature should be 0°C.

With regard to hot holding temperatures, 19 respondents (15%) correctly stated that the hot holding temperature for poultry should be >63°C. 60 of the respondents (48%) did not know the answer while 46 (37%) yielded an incorrect answer. With regard to the statement that "hot food should be cooled as quickly as possible and then refrigerated", 50 (40%) respondents disagreed, 67 (54%) neither disagreed nor agreed and only 7 (6%) persons agreed. That is in sharp contrast with findings of Mathias (1999) and Redmond (2002) who reported that a greater percentage (~ 50%) of consumers, agreed that there is a need to cool hot food quickly after cooking. However, Redmond (2002) also demonstrated that 84% of consumers unknowingly thought that it is also acceptable to cool foods at room temperature. As Redmond and Griffith (2013) rightly said, there is confusion among consumers as to what constitutes acceptable and safe cooling practices.

Knowledge of food hazards and safe food practices

Regarding the nature of hazards in poultry, the study showed that 18 respondents (14%) incorrectly assumed that food safety hazards were limited to physical hazards. Only 14 respondents (11%) knew that food safety hazards include physical, chemical and biological hazards while 93 respondents (75 %) were unaware of the food hazards and their different types. Survey participants who incorrectly answered this question spanned all age groups. Many surveys have identified a lack of notion of food hazards (Albrecht, 1995; Bloomfield and Neal, 1997; Sammarco and Ripabelli, 1997) among respondents. Redmond and Griffith (2013) showed that a lack of awareness of possible hazards generally leads to a failure in implementing safe food preparation behaviors.

We also noted that knowledge of food safety is not necessarily a guarantor for correct implementation of safe food behaviors and at the same time, a notion of food safety may not be the sole driver for implementing safe food practices. For instance, for several questions pertaining to safe food practices, respondents (56-78%) gave correct answers out of experiential learning rather than from theoretical knowledge. A comparatively large number of respondents (84%) correctly answered that poultry should be kept in a refrigerator to prevent food poisoning since a large majority of Mauritians customarily keep food in the refrigerator to increase its shelf life and to prevent quality deterioration. Redmond (2002) similarly found that a significant proportion of consumers surveyed in the United Kingdom reported mechanically practicing basic food hygiene precautions without knowing the underlying rationale (Redmond, 2002).

With regard to specific questions in food safety such as questions addressing the 'meaning and importance of HACCP', only 14 respondents (11%) gave the correct answer, implying that only a minority had a sound knowledge of food safety. Therefore the majority of consumers were not aware of the importance of HACCP, which is considered to be an effective tool for controlling pathogens in most food establishments. Consequently it will not be intuitive for most of the poultry consumers to choose poultry products from suppliers who are HACCP certified. In the absence of adequate knowledge of HACCP and its underlying rationale, most consumers will not be willing to pay a premium for HACCP-certified products, which are in fact safer.

Knowledge of good hygienic practices and good manufacturing practices

All respondents (100%) agreed that water used for cleaning the market should be clean and chlorinated. However, correct answers given for adherence to Good Manufacturing Practices (GMPs) were relatively low. For instance, few people (11-14%) who were interviewed were aware of safe thawing procedures, importance of display of poultry in chilling cabinets and the safe length of time for exposing cooked food. Hence the results mostly reveal that poultry consumers lack specific knowledge of GMPs and this finding is in agreement with the study of Bearth (2014).

Consumer awareness of bacterial cross-contamination prevention

Our observations pertaining to consumer practices to avoid cross-contamination are presented in Figure 4. Only 13 respondents (10%) knew that cross-contamination is the transfer of harmful bacteria from one food to another directly or indirectly via hands, chopping board, utensils and other means of contact. However, 99 respondents (79%) knew that plates and utensils that held raw chicken should be properly washed before using them again. Redmond and Griffith (2013) also mentioned that a large majority of consumers (75%) lacked familiarity with the term 'cross-contamination' and principles associated with cross-contamination although 72 (58%) respondents were aware that plates and utensils for cooked and raw meats should be separated. We further noted that 50 respondents (40%) knew that cutting boards used for animal and plant-derived foods should be separated and 28 (22%) respondents were aware that these commodities should be separated even after washing. This percentage is relatively low compared to those reported by other authors. For instance, Griffith et al. (1999), Mathias (1999) and Redmond (2002) found that as high as 81% to 90% of consumers agreed that it is better to use separate chopping boards for cutting of raw and cooked meats. Similarly, 90% of consumers believed that the use of different utensils or washed utensils for the preparation of raw and Ready-to-eat (RTE) foods will help to prevent food poisoning (Griffith et al., 2001).

The home has been described in the literature as a common point of origin for poultry-borne infections. Since our study revealed that only a relatively small percentage of consumers had a sound knowledge of bacterial cross-contamination including cross-contamination events by poultry-borne pathogens *Salmonella* and *Campylobacter*, the risks of food contamination in the domestic environment remain alarming. Usha et al. (2010) indicated that bacterial cross-contamination occurs during food preparation and bacterial residues on food contact surfaces can eventually cause illnesses. The same author further demonstrated that utensils harbored a higher level of *Campylobacter* spp. (1.4-223.3 MPN/ml rinse) than hands (0.7-43.4 MPN/ml rinse) and transference rates of *Campylobacter* spp. from utensils to food varied from 0% to more than 100%. Rusin et al. (1998) observed kitchen environments to be heavily contaminated with coliforms, suggesting a high risk of spreading infections in the home. It is therefore recommended that food safety initiatives include explanation of terms such as cross-contamination to ensure that messages are effectively communicated and to circumvent microbiological risks associated with the contamination of RTE foods.

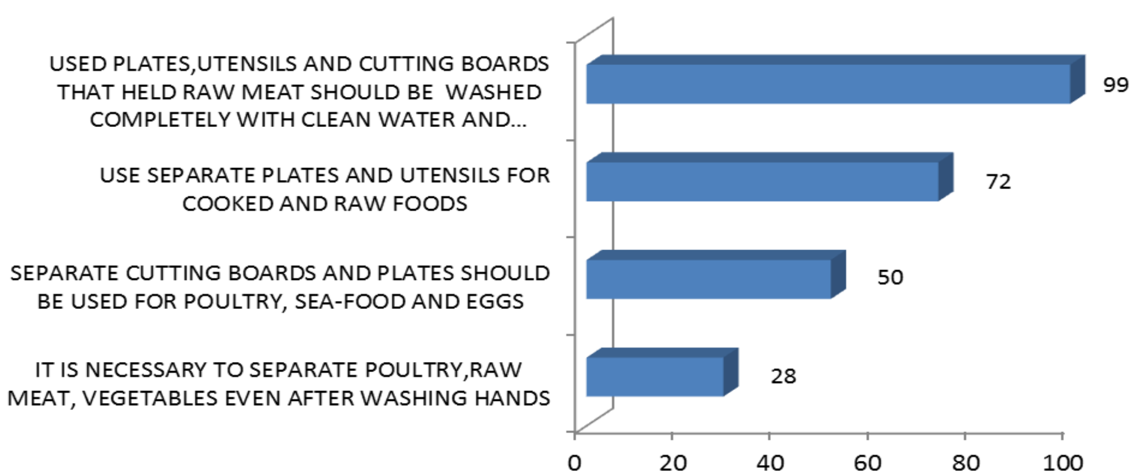

Figure 4. Practices of poultry consumers to prevent cross-contamination in Mauritius

Washing of poultry before cooking

Washing of poultry prior to cooking is a practice that is heavily discouraged as it leads to contamination in the kitchen (USDA, 2013 and USDA, 2014). The survey revealed that 89 persons (71%) thought that poultry should be washed before cooking. There was a prevailing misconception among a majority of respondents that washing poultry could physically remove the pathogens that may adversely affect their health. However, this is a misconception as rinsing is ineffective at destroying pathogens and only cooking is the ultimate killing step (USDA, 2014). Moreover, poultry washing can cause poultry juices to spread to other foods in the process. The relatively high number of Mauritian consumers who wash poultry before cooking as noted in our study tallies well with findings of Bruhn (2014) who reported that almost half of survey participants washed poultry before cooking. Henley et al. (2012) also reported that African-American, Asian-American and Hispanic consumers washed chicken prior to cooking. Hence, food safety educators should remind consumers not to wash poultry. An animated video illustrating cross-contamination could be an effective tool to dissuade consumers from washing raw poultry (Godoy, 2013). Those reportedly washing poultry in our survey mentioned doing it out of a habit or following a practice handed down to them by their elders. Indeed food preparation can be described as a habitual behavior because it is a frequently repetitive (Fisher and De Vries, 2008). In this particular situation habit did induce the respondents to potentially unsafe practices.

In the light of this study, we noted that Mauritian customers purchasing poultry were generally aware of several safe food-handling practices although they were found to lack knowledge of others. It was also observed that those who fell in the age group of 20-49 years old were less knowledgeable about poultry safety and that is probably caused by the lack of experience. The fact that not all the survey participants falling in the age group of >49 years old provided satisfactory answers in relation to safe storage and cooking may be because older persons may not necessarily put their food safety knowledge into application.

Taken together, older people were relatively more concerned about food hazards and safe practices. The generally high confidence of respondents of their knowledge in food safety and their adherence to safe food practices suggest an optimistic bias and 'illusion of control'. Effective ways to prevent poultry-borne illnesses rely on early consumer education as well as proper sensitization.

It is worth acknowledging one limitation of the survey design: the demographics might have been skewed due to the location of the market as well as possible selection-bias due to volunteering to participate. Due to restricted location and small size, the results of this study are not intended to generalize, rather to serve as a reference point for future studies.

Competing interests

The authors have declared that no competing interest exists.

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Determination of Crude Protein and Metabolisable Energy of Japanese Quail (*Coturnix coturnix* japonica) during Laying Period

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ABSTRACT

This study was carried out to determine the energy and protein requirements of laying Japanese quails. A completely randomized design of treatments comprising four dietary protein levels (18, 20, 22 and 24%) and three levels of metabolisable energy (3000. 3100, 3200 kcal/kg) in a 4 × 3 factorial arrangement was used. 144 Japanese quails aged 7 weeks were randomly divided into 12 dietary treatments with 3 replicates per treatment and each replicate with 4 birds. The experiment lasted for five weeks. The results of the study showed that there was no significant (P>0.05) effect of protein, energy or their interaction on feed intake, feed conversion ratio, hen day production, egg weight and egg number. However, protein as a single variable had a significant effect (P<0.05) on feed intake. There was also no significant (P>0.05) effect of protein, energy or their interaction on egg quality traits (yolk colour, yolk weight, albumen weight, shape index, shell thickness, shell weight, and haugh unit). However, birds fed 20% crude protein and 3000 kcal/kg metabolisable energy had better hen day production, number of eggs per bird and egg quality traits compared with birds on the other groups. Dietary protein increased egg production and egg weight, augmented by energy. The yolk colour was increased with increasing energy level. Therefore, the results of the experiment revealed that 20% crude protein and 3000 kcal/kg metabolisable energy could be used to obtain the best production performance and good egg quality traits of Japanese quails at the laying phase.

Key words: Japanese quail, Production performance, Egg quality traits, Metabolisable energy, Protein.

INTRODUCTION

One of the major sectors of the livestock industry in Nigeria is poultry production. This sector has hitherto been dominated by the rearing of chickens. However in recent years, Japanese quail has gained worldwide importance as a laboratory animal (Baumgartner, 1994) and productive bird because of its advantageous attributes such as small body size, rapid growth rate, and early sexual maturity (Siyadati, 2011) in 5 to 6 weeks of age, high rate of reproduction, ability to produce 3 to 4 generations in a year, relative ease of maintaining the colony (Shim and Vohra, 1984), lesser space and feed requirements compared with the domestic chickens, cheaper cost of production, hardiness and ability to strive in small cages. Therefore they are suited for commercial rearing, egg and meat production under intensive management.

Despite these attributes, the major constraint of quail production in Nigeria is the continually review of the nutritional requirements of Japanese quail for production over time by Beane and Howes (1966), Vohra (1971) and NRC (1994), the non availability of economical and efficient rations (Barque et al., 1994), poor documentation of energy and protein requirements, the efficiency of feed utilization for quails (Monica et al., 2010) and lower productivity of quail fed based on nutritional requirements data obtained in other countries with different climatic conditions (Soares et al., 2003). However Alaganawy et al. (2014) reported that adequate amino acid balance is the most important nutrient for Japanese quails. Other research reported energy and crude protein levels of 2900 kcal ME/kg and 22%, respectively (Reda et al., 2015) and levels of 3000 kcal ME/kg and 26% respectively (Jahanian and Edriss 2015).

For optimum productivity of Japanese quail in Nigeria, the energy and crude protein requirements are important. The objective of this study was to determine the energy and protein requirement of Japanese quail birds in the laying period and also investigate the effects of

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different dietary levels of protein and energy on egg production.

MATERIALS AND METHODS

Experimental diets and management of birds

The experiment was carried out at the poultry unit of the teaching and research farm, university of Ibadan, Ibadan, Nigeria. The experiment lasted for five weeks between August and September 2014. Birds were acclimatized to the experimental diets by the first week, while the remaining 4 weeks were used for egg quality data collection, feed intake data collection, hen day production and for measurement of internal egg quality parameters. The experimental diets as shown on table 1, were corn-soybean based with four dietary protein levels (18, 20, 22 and 24%) and three levels of metabolisable energy (3000. 3100, 3200 kcal/kg). A total number of 144 Japanese quails aged 7 weeks were used for the experiment. The birds were housed in a 36 compartments cage with 4 birds in each compartment. These contained the twelve experimental units with three replicates each. The birds were allocated to 12 diets in a completely randomized design. The hens received the diets from 7 weeks of age till 12 weeks of age and were provided water *ad libitum*.

Data and sample collection

Performance parameters (feed intake, egg production, egg weight, and feed conversion ratio) were calculated during the course of the trial. To determine the cholesterol and fatty acid profile of egg yolk, three eggs were randomly sampled at week six of the experiment from each treatment respectively. Egg quality parameters (yolk weight, yolk index, albumin weight, haugh unit, shell weight, and shell thickness) were measured at week six, using five eggs from each treatment.

Performance evaluation

Daily egg production per replicate was recorded and number of eggs per hen per week was calculated. Eggs laid per replicate were weighed daily and average weight for that particular week was calculated. The data thus generated (egg production and egg weight) was used to calculate egg mass/bird/week (weekly egg no. in replicate x average egg weight). Weekly feed intake was determined (total feed offered during a week - Feed refused at the end of week). Data on feed intake and egg mass were used to calculate feed conversion (feed intake/egg mass; g/g).

Egg quality evaluation

External qualities: Egg weight was measured using Mettler top-loading weigh balance. The length and width (cm) of each egg was measured using Vernier caliper. The width was measured as the distance between two ends of the egg at the widest cross sectional region using Vernier caliper. The length was measured as the distance between the broad and narrow ends of the eggs.

Egg shape index (ESI) was calculated as the percentage of the egg breadth (width) to the egg length (Panda, 1996). The formula that was used is as follows:

$$\text{Egg shape index} = \frac{\text{Width of egg (mm)}}{\text{Length of egg (mm)}} \times 100$$

The thickness of individual air-dried shells is measured to the nearest 0.01mm using micrometer screw gauge (Chowdhury, 1987). Eggshells were air-dried in the crates. The relative shell weight was calculated by relating the shell weight to the weight of the egg. Shell thickness was measured using a micrometer gauge (in mm).

Internal qualities: Yolk height, yolk width and yolk diameter (cm) were measured using a Vernier caliper. Albumen height**:** The egg was gently broken and the maximum albumen height was measured with tripod micrometre (Doyon *et al*., 1986) Albumen weight is the difference between the egg weight and the sum of weight of yolk and dry eggshell expressed as a percentage of the whole egg. Percentage of Albumen weight was calculated as the percentage of the albumen weight to other egg weight. Yolk weight was measured using Melttler top-loading weighing balance. Percentage Yolk weight was calculated as the percentage of the yolk weight to the egg weight.

Yolk index was estimated from ratio of yolk height to yolk width. Visual yolk colour was determined with a yolk colour fan (scale 1 to 15).

Haugh unit (HU**)** is a relationship between egg weight and height of thick albumen surrounding yolk. This was calculated using the values obtained from the egg weight and albumen height as expressed by Haugh (1937) in the formula shown below:

$$HU = 100 \log [H + 7.57 – 1.7 W^{0.37}]$$

Where, H = Albumen Height (mm) and W = Weight of the egg (g).

Data collection

Data of feed offered and body weight were recorded weekly and used to calculate feed intake, weight gain, feed conversion ratio and protein efficiency ratio.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS statistical package (SAS, 2003) as a 4×3 factorial arrangement in a completely randomized design. Significant means were separated using Duncan multiple range test at $P < 0.05$.

Table 1. Composition of experimental diet fed to Japanese quails during laying for the determination of crude protein and metabolisable energy

Ingredients, g/kg	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
Corn	654.7	665.7	657.7	635.7	635.7	623.7	578.7	578.7	558.7	513.7	508.7	488.7
Soybean meal	262.0	262.0	262.0	300.0	300.0	300.0	352.0	352.0	352.0	410.0	410.0	410.0
Soybean oil	12.0	23.0	39.0	8.0	19.0	35.0	18.0	28.0	48.0	25.0	40.0	60.0
Wheat bran	30.0	8.0	0	15.0	4.0	0	10.0	0	0	10.0	0	0
Dicalcium phosphate	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5
Limestone (38% Ca)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
L-Lysine.HCl	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Threonine	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Nutrient content												
Protein, g/kg	185.6	183.0	181.0	201.9	200.2	198.5	221.5	219.9	218.2	242.5	242.1	240.1
ME, kcal/kg	3007.5	3109.5	3205.7	3024.6	3102.7	3190.2	3031.2	3102.2	3200.8	2992.9	3101.6	3200.2

RESULTS AND DISCUSSION

Table 2 shows the overall effects of protein, energy and their interaction on production performance of laying Japanese quails. The result obtained shows that there were no significant effects ($P>0.05$) of protein, energy or their interaction on the performance of laying quails, except the feed intake, that was significantly affected ($P<0.05$) by protein.

Feed intake

Different levels of dietary energy did not affect performance parameters significantly ($P>0.05$). For different levels of protein, there was a significant difference ($P<0.05$) in feed intake. 20% and 22% crude protein (CP) inclusion levels differ significantly from 18% and 24% inclusion level. However, 20% inclusion level had the highest value of feed intake.

Feed conversion ratio

The FCR was not significantly affected by different dietary levels of protein as shown in table 3. However, inclusions at 20% CP led to the highest value of FCR while inclusion at 18% had the lowest value. It shows that inclusion at 18% had the best FCR. Also different levels of energy did not affect FCR significantly, although birds fed with the dietary energy of 3,000 kcal/ kg had the lowest and the best value of FCR. This result agreed with the findings of Jahanian and Edriss (2015) which reported 26% Crude Protein and energy levels of 3000 kcal ME/kg as adequate for Japanese quails.

The combination of 24% CP and 3200kcal had the highest value and significantly different ($P<0.05$) from 18% CP and 3000, 3200kcal/kg metabolisable energy (ME) and 24% CP and 3000, 3200 kcal ME, but significantly different from other combinations. However, the combination of 24% CP and 3100kcal/kg ME had the lowest and the best value of FCR.

Hen-day production

Protein as a variable did not affect hen-day production (HDP) at different inclusion levels. Also different energy levels did not significantly affect hen day production. However, birds fed with 3000 inclusion level had the highest value while birds on 3200kcal inclusion level had the lowest value.

The combined effect of 20% CP and 3000kcal had the highest value of hen-day production and significantly different (P<0.05) from inclusion level at 18% CP and 3100kcal/kg ME but not significantly different from other combinations.

Number of eggs/bird

Both energy and protein at different levels of inclusion had no significant (P>0.05) difference on the number of eggs laid per bird respectively. The combination of 20% CP and 3000kcal ME had the highest number of egg/bird and is significantly different from the combination of 18%CP and 3100kcal ME. However, the combined effect of 20% CP and 3000 ME did not differ significantly from other combinations though it has the highest value.

Average egg weight

24% CP had a significant difference from other levels of CP on the average weight of egg though the weight are similar in value with difference not greater than 0.22. The effects of different levels of energy on the average weight of egg were not significantly different. The combined effects of 18% and 24% CP each with 3000kcal ME is significantly different from the combined effects of groups that had 3100 and 3200 kcal ME each with 18% CP and 3000 and 3100 kcal ME each with 20% CP and 22% CP with 3000 kcal ME.

The results shown in table 3 indicated that there were no significant effects (P>0.05) of protein, energy or their interaction on the performance of laying quails, except feed intake, that was significantly affected (P<0.05) by protein. However, the mean effect of protein and energy on feed intake of experimental quail birds fed on 20% and 3000kcal had the highest feed intake, 34.28g/bird/day, while quails on 18% CP and 3200kcal had the lowest feed intake. These results agree with Tuleun et al. (2013) who reported a similar result.

Feed conversion was lowest in birds fed with 24% CP and 3000 kcal, though it was not significantly different (P>0.005) from birds fed with 22%, 20% and 18% CP. This support the findings of Murakami et al. (1993) who reported that dietary protein had no significant influence on feed efficiency when laying Japanese quails were fed on diets with different protein levels. The groups fed with 3000 kcal ME had a better feed conversion ratio. This shows that the dietary energy level at 3000kcal helped improved production, directing the use of crude protein in the diet, instead of energy generation.

Hen day production was higher in quails fed 18% and 20% CP and 3000 kcal/kg ME. This result agrees with the result of Khosro et al. (2011) who reported higher egg production in birds fed 20% CP but a lower energy level (2950kcal/kg). They are also similar to the 20% CP suggested by NRC (1994) and Garcia et al. (2005) with 2,850 and 2,950kcal/kg ME respectively. The lowest hen day production was obtained in quails fed 24% CP and 3200kcal/kg ME. This also is in line with the results of Khosro et al. (2011). Egg production is costly in terms of energy and protein. The required energy for egg formation may be derived from daily feed intake or from the body reserve. Daily feed intake is a more important source of nutrient for small birds like quail than body reserve. If energy or protein is limiting, birds can compensate by reducing egg size or the number of eggs laid or by increasing the laying interval and spreading the loss of egg formation over a longer period (Brand et al., 2003).

Egg number per bird was higher in birds fed 20% CP and 3000kcal/kg ME, with the value, 6.06g compared with 5.17g from birds fed on 18% CP and 3200kcal/kg ME.

Average egg weight was higher in birds fed 24% CP and 3000 kcal/kg ME. This result is similar to the findings of Garcia *et al*. (2005) who reported a quadratic effect of protein level on the produced egg weight and maximum egg production with 23.1% CP in the diet. However, Khosro *et al*. (2011) reported a higher egg mass with 20% CP and 2,900 kcal ME in the diet. The result of this study shows that egg size and weight depend greatly on daily crude protein intake augmented by adequate dietary energy (3000 kcal/kg) since layers do not store a large amount of protein.

Egg quality traits

Table 4 shows the overall effects of protein, energy and their interaction on egg quality traits of laying Japanese quails. The result obtained shows that there were no significant effects (P>0.05) of protein, energy or their interaction on egg quality traits of laying birds

Table 5 shows the mean effects of protein and energy on egg quality traits. Data obtained indicated that there were no significant (P>0.05) differences among the treatment groups for most of the egg quality traits except haugh unit and shell weight for protein effect and yolk weight for energy effect.

Egg weight, shape index, shell thickness and shell weight

The combination of 22% CP and 3000kcal ME has the highest value and is significantly different (P<0.05) from all other combinations of different protein and energy levels. The combination of 18% CP and 3000 kcal ME had the highest significant value but not significantly different from other combinations except the combinations of protein and energy levels of 18%, 24% CP and 3200, 3000kcal ME respectively. The combination of 18% CP and 3200kcal ME is significantly different (P<0.05) from the combination of 20% CP and 3100kcal ME and the combinations of 22% CP with the three levels of energy. The combination of 24% CP and 3200kcal ME had the highest value but not significantly different from other combinations except for the combinations of 22% CP and 3000kcal ME and 20% CP and 3200kcal ME, which are in turn not significantly different from other combinations.

Yolk colour, yolk weight and albumen weight

The combined effect of 20% CP and 3200 kcal ME was significantly different. However, this was not significantly different from the combination of 22, 24, 24% CP and 3000, 3100, 3200kcal ME respectively, which in turn were not significantly different from other combinations. The combination of 18% CP and 3200kcal ME had the highest yolk weight. Statistically, it is significantly (P<0.05) different from the combination of 22% CP and 3000kcal ME, but not significantly different from other combinations except the combinations of protein and energy. There is no significant effect of different combinations of energy and protein on albumen weight across the treatments. However, the combination of 20% CP and 3000kcal ME had the highest value of albumen.

Table 2. Effect of protein, energy and their interaction on performance of laying Japanese quails at 12 weeks of age

	Factors		
Parameters	**Protein**	**Energy**	**Protein×Energy**
Feed intake (g)	0.0094	0.6801	0.7294
Feed conversion ration (g/g)	0.8980	0.2836	0.2642
Hen day production (%)	0.7333	0.2179	0.7548
Egg/bird	0.6784	0.3104	0.7780
Egg weight (g)	0.1012	0.9619	0.0848

Table 3. The effects of protein and energy on production performance of laying Japanese quails at 12 weeks of age

	Parameters					
Protein level (%)	**HDP (%)**	**Egg/bird**	**AEW (g)**	**FI (g)**	**FI/bird**	**FCR**
18	81.05	5.43	10.77 [b]	867.36 [b]	30.98 [b]	3.80
20	80.56	5.69	10.76 [b]	943.14 [a]	33.68 [a]	3.96
22	78.67	5.61	10.88 [ab]	941.36 [a]	33.62 [a]	3.95
24	77.78	5.50	10.99 [a]	884.83 [b]	31.60 [b]	3.89
SEM	2.36	0.16	0.07	19.47	0.69	0.17
Energy level (kcal/kg)						
3000	82.29	5.73	10.84	909.60	32.49	3.72
3100	78.94	5.52	10.84	919.44	32.84	3.92
3200	77.31	5.43	10.86	898.48	32.09	4.05
SEM	2.05	0.14	0.06	16.86	0.61	0.14

*Means with the different superscript on the same column are significantly different (P < 0.05); HDP: hen day production, AEW: average egg weight, FI: feed intake, FCR: feed conversion ratio. SEM: Standard error of mean

Egg quality traits

Egg yolk and albumen weight: There was a significant effect (P<0.05) of protein and energy on egg yolk weight (g) index (%). Improved yolk weight and index were obtained from birds fed CP levels of 24% and 3200kcal/kg ME and 18% CP and 3200kcal/kg ME, with values 4.04±0.32 and 3.84±0.26 (P>0.05) respectively. Garcia et al. (2005) reported that protein levels had an effect on yolk percentage, which is consistent with the current study. Also the result of this study is in line with Khosro et al. (2011) who reported similar result.

For albumen weight, there were no significant differences (P>0.05) with the levels of energy and protein across the group. However, the three energy levels with 20% CP had the highest value of albumen weight.

Yolk color

Dietary protein significantly affected yolk color (P < 0.05). These results suggest a relationship between dietary protein and egg yolk color. Increasing dietary energy increased egg yolk color, this is because of an increased in the inclusion of corn in the diet. This result is in agreement with the report of Khosro et al. (2011). It is known that egg yolk color is a result of dietary carotenoids transferred to the egg yolk, and mostly the color is from xanthophylls, and partly carotene and cryptoxanthin. Yellow corn, corn gluten meal, etc., are dietary sources of xanthophylls. Corn gluten meal contains 5-8 times the xanthophylls of yellow corn. Therefore, in this study, it is presumed that corn intake contributed greatly to the lightening of the egg yolk color at 20% CP.

Haugh unit: The combination of 20% CP and 3200kcal/kg ME had the highest statistically significant value (88.96), with similar values to other levels of 20% CP, which are 88.59 and 87.44 combined with 3000, 3100 kcal/kg ME respectively. Haugh unit is used to determine the freshness and protein content of egg. Higher haugh unit value denotes better quality of the egg (fresher, higher quality eggs have thicker whites). The result obtained in this experiment shows that inclusion of 20% CP with any of the three levels of energy is suitable for good quality eggs and will be better in storage of egg.

Shell weight: Eggshell was significantly ($P < 0.05$) affected by different levels of protein. Birds fed 18% CP at 3200 kcal/kg ME showed higher egg shell weight than other birds. Yakout et al. (2000) suggested that eggshell percentage may be reduced by higher lysine levels. In chickens, Gardner and Young (1972) reported that increasing the dietary protein level from 12 to 18% produced a significant increase in the relative proportion of egg yolk, and a subsequent significant decrease in the proportion of eggshell. However, when comparisons were made among dietary protein levels from 9.3 to 20.5% (Fisher, 1969) and from 14 to 20% (Yamagami and Kobayashi, 1983), no significant differences were found in egg composition. The findings in present study are in consonance with the report of Fisher (1969) and Yamagami and Kobayashi (1983) in chicken and also it is in agree with Khosro et al. (2011) in quails.

Table 4. Mean effect of protein, energy and their interaction on egg quality of laying Japanese quails at 12 weeks of age

	P value		
Parameters	**Protein**	**Energy**	**Protein×Energy**
Egg weight (g)	0.4879	0.4353	0.3372
Yolk colour	0.7759	0.411	0.055
Yolk weight(g)	0.4212	0.3876	0.4522
Albumen weight (g)	0.2952	0.623	0.9615
Shape index (%)	0.3828	0.6552	0.3278
Shell thickness (mm)	0.421	0.3671	0.4368
Shell weight (g)	0.1959	0.2238	0.2237
Haugh unit	0.1034	0.2866	0.626

Means in the same column with different superscripts are significantly different ($P<0.05$). Egg wt- Egg weight, ST- Shell thickness, HU- Haugh unit, Yolk wt- Yolk weight; Yolk wt- Yolk weight, Alb.wt- Albumen weight

Table 5. Effects of protein and energy on egg quality traits of laying Japanese quails at 12 weeks of age

	Parameters						
Protein level (%)	**Egg weight (g)**	**Shape Index (%)**	**Shell thickness (mm)**	**Yolk weight (g)**	**Yolk colour**	**Albumen weight (g)**	**Haugh unit**
18	11.8^{ab}	77.73^{a}	0.40^{a}	3.66^{a}	1.15^{a}	6.85^{a}	85.57^{b}
20	11.11^{b}	78.79^{a}	0.30^{a}	3.49^{a}	1.19^{a}	7.25^{a}	88.33^{a}
24	12.06^{a}	77.19^{a}	0.40^{a}	3.65^{a}	1.19^{a}	6.98^{a}	86.17^{ab}
SEM*	0.27	0.68	0.03	0.17	0.05	0.17	0.88
Energy							
3000	11.38^{a}	78.40^{a}	0.30^{a}	3.30^{b}	1.12^{a}	7.09^{a}	87.06^{a}
3100	11.50^{a}	77.83^{a}	0.30^{a}	3.52^{ab}	1.14^{a}	6.97^{a}	85.48^{a}
3200	11.67^{a}	77.68^{a}	0.30^{a}	3.75^{a}	1.19^{a}	6.87^{a}	86.85^{a}
SEM	0.21	0.58	0.20	0.14	0.15	0.15	0.77

*SEM: Standard error of mean

CONCLUSION

This study was done to know the best energy and protein level suitable for production of laying Japanese quail. From the results obtained in this research, it was observed that neither protein nor energy had a significant effect on both performance and egg quality traits of laying Japanese quails. However, it was observed that quails fed 20% CP with 3000 kcal/kg metabolisable energy had a better production performance and egg characteristics apart from yolk index and yolk colour than birds on other dietary treatments.

Thus, it can therefore be concluded that laying Japanese quail, *Coturnix coturnix japonica* requires 20% CP with 3000kcal/kg ME for optimum egg production and good egg quality characteristics in Nigeria.

Competing Interests

Authors have declared that there is no competing interest.

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The Effects of Housing and Equipment Status on Egg Yearly Monitored Production Rates in Open Poultry Houses in Gezira State, Sudan

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ABSTRACT

This study was carried out in Gezira state, Sudan to investigate the effects of housing and equipment status on egg production in open layer houses. Data were collected through individual interviews (questionnaire) of 97 randomly selected among poultry farm owners. The height of 80% of north and south sides of wall were 50-100 cm in Almanagil, 76.5% in Alkamleen and 57%in south of the Gezira localities, while the height of the wall side at the east and west were (3-3.5m) in all (100%) houses in east of the Gezira, 77.8% in Alhasahesa, 60% in Almanagil and 47.1% in Alkamleen. The width was 5-8m in most poultry houses in Gezira State's localities surveyed. In Greater Medani, all the houses were at the width mentioned above while 76.4% and 73.5% in of those building were 5-8 cm in Almanagil, and Alkamleen localities respectively. The most of wall houses were not painted where 50% of those houses were with painted walls in east of the Gezira and 76% in Alkamleen locality. The most floor types were made of bricks. Flours with that type were 55.6% in Alhasahesa and 76.5% in Alkamleen locality. The layer of sand was thin in the major litter type of poultry houses surveyed in Gezira state localities though some houses were without litter, which affect birds' performance by low ventilation and insulation. Round feeders of 40 - 50 cm length were the majority feeders' type observed. In Alhasahesa 55.6% houses had that type of feeders while all houses surveyed had round feeders in east of the Gezira and Greater Medani localities. Oil containers were used as drinkers in most poultry houses surveyed. The troughs were with unsuitable height for hens to drink conveniently. The percent of house with that type of drinkers were 58.8% in Alkamleen and south of the Gezira localities. Birds/feeder and birds/drinker capacity varied between 50 and 75 birds. Clay pots were the mostly used egg nest type in the state. That type of nests were used by 88.2% of farm owners in Greater Medani to 100% in east of the Gezira, Alhasahesa and Almanagil localities. In average one egg nest was allotted to 15 hens. Yearly monitored egg production ranged between 60-70%.

Keywords: Layer, Production constraints, Housing, Equipment

INTRODUCTION

Poultry production is an ancient activity and it has been practiced traditionally in different parts of Sudan. The growing demand of poultry meat and eggs motivated private sector to start commercial production of poultry. Concomitant with that, the government initiated modern poultry farming first in Khartoum and later in many city centers of the country. During the last decades both broiler and layer production gained momentum in Sudan. The fact that poultry is a good source of income and cheap provider of valuable protein products that are needed to secure human body with the essential amino acids encouraged its expansion. Improved methods of poultry management were introduced to Sudan in the mid 1950s in Khartoum province, since then poultry industry started to grow gradually around Khartoum and other cities in the country (Habani, 2008). The establishment of the research and extension center in early 1960s, led to more development in poultry industry in Sudan (Osman, 1988). There were significant increase in numbers of modern farms established in Sudan, largely concentrated in big cities and communities in urban areas. There are numerous small private farms which are widely distributed in the urban areas and some rural

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areas (Elzaki et al., 2011). Although feeding is the main challenges for commercial and small producers**,** housing has a crucial influence in poultry production. Itshould provide good protection from predators (Fanatico, 2007). Open sided poultry houses are constructed with its length extending from east to west direction to minimize the entry of direct sunlight, to exploit the direction of the prevailing wind and to minimize solar heat radiation (Viswanathan, 2001 and Winchell, 2001). A good location, with a good water source and a well-insulated building, equipped with proper ventilation, heating, lighting, feeding and watering system are important in all types of poultry housing(Winchell, 2001). Wall characteristics are important in good bird's welfare. So the house walls should be without cracks to prevent parasites availability. Walls must be painted with light colored paint such as white color, to reduce heat stress inside bird's house. The height of the sidewall in East-West directions will be around 3-4 meters. North-south direction sidewalls height will be about 0.5 meter to one meter and the remaining space must be covered with chain link mesh. Wall building materials include bricks, concrete bricks and other local materials (Jacob et al, 2014).Poultry house should have elevated ceiling to keep heat radiation away from the birds. Roof height must be about 3-4 meters. The width of the open sided deep litter house is around 8 to 10 m**,** length of the house determined by flock size and it could be up to 50 m (ElBeeli, 2009).Concrete floor is recommended to ensure all-weather operation, and to secure the birds against rodents and dogs. About 5–10cm thick layer of wood shavings, peanut hulls or other bedding materials, as sand, straw are used as an absorptive base (Grisso, 2009 and Sadkhan, 2011).Egg nests are very important in poultry house equipment; it is made of many different materials, the nest must be safe to the birds and to the eggs; it must distributed in the dark part of the house (Al-Haaisha, 2008).There are many feeder types made of plastic or metal, and get circular or longitudinal shapes. Birds spacing in circular feeder is about 2cm for a bird, and for the longitudinal feeder is about 10 cm for a bird (Merck Veterinary Manual, 2012).This spacing provides suitable area for birds and prevents birds crowding round feeders (Jacob et la., 2014). Minimum floor area per bird in square inches requirements for white leghorn egg-strain birds was reported by Brown (2015) being 60 square inches per bird at18 months of age onwards. Payne (1990) reported that a suitable feeding space for 100 layer birds is about 7.6 to 10.7 m (Payne, 1990).

MATERIALS AND METHODS

The present study was conducted in Gezira state, Sudan which lies between latitudes 13 - 15.2^0 N and longitudes 32.5 - 34^0 E. The total area about is 23373 km^2. The state is bounded by Khartoum in the north, Gedarif state in the east, White Nile State in the west and Sennar state in the south. The state is located within the semi arid climate which is characterized by seasonal and limited raining in the summer months (July-September).The Blue Nile River is the most important feature of the surface and is characterized by its course and its water though being low in salinity of high percentage turbidity during the rainy season (Sudan Metrological Services, 2005).The Gezira state was an approximately ranked second in poultry production in Sudan. Despite of that there was no enough information about birds housing and flocks management (Idris and Ahmed, 1997). A random sample was taken among layer farms owners for data collection of this study using questionnaires from the Gezira state localities (south of the Gezira, east of the Gezira, Alhasahesa, Almanagil, Alkamleen and Greater Medani) from April 5th to June 10th/ 2010. The questionnaire was consisted of poultry farms operation subjects, including questions in poultry housing, equipments, egg production. The data were then analyzed using Statistical Packaged for Social Sciences (SPSS version 20) at $P<0.05$ and Microsoft excel was used to analyze the collected data.

Ethical approval

Not applicable. This research did not involve the introduction of any intervention in/on birds, or direct collection of cells, tissues or any material from birds.

RESULTS

Poultry house status

Result showed that birds' house direction was from east to west in all localities (100%).The height of poultry houses at north and south sides were about 50-100 cm in most of Gezira State localities studied. The heights incited were reported by 80% in Almanagil, 76.5% in Alkamleen localities, by57% in South of the Gezira locality. Wall of 40 cm height was construed by44.5 %, 42.9 % and 41.2 % in Alhasahesa, South of the Gezira and Greater Medani municipality respectively. The height of eastern and western sidesof wall wasabout 3-3.5 m in the localities surveyed. All respondents (100%) reported having walls of that height in East of the Gezira, 77.8% in Alhasahesalocality,60% in Almanagil locality and 47.1% in Alkamleen locality. The width of the majority of house was about (5-8m) in most localities. It was about 100%, 76.4% and 73.5% in Almanagil, Greater Wad Medani and Alkamleen localities, respectively. It was observed that walls were not cracked though were mostly unpainted; status of walls indicated were 76%, 60% and 51% in Almanagil and South of the Gezira locality, respectively (Table 1).The most dominant floor types in poultry houses reported was made of bricks in Gezira State localities covered by this survey. This type was about 76.5%, 75%, 64.71%, 60% and 55.6% in Alkamleen, South and East of the Gezira localities, Greater Medani, Almanagil and Alhasahesa localities, respectively (Figure 1). Most houses were bedded with sand in localities. Such type of bed was followed by86.7% in Alkamleen, 82.4% in Grade Medani and 80% in Almanagil locality (Figure 2). Litter depth was less than 5 cm in most localities; the depth indicated was reported by100%, 88.3%, 82.6% and 80% in Almanagil, Grade Medani, Alkamleen and Alhasahesa localities, respectively (Figure 3).

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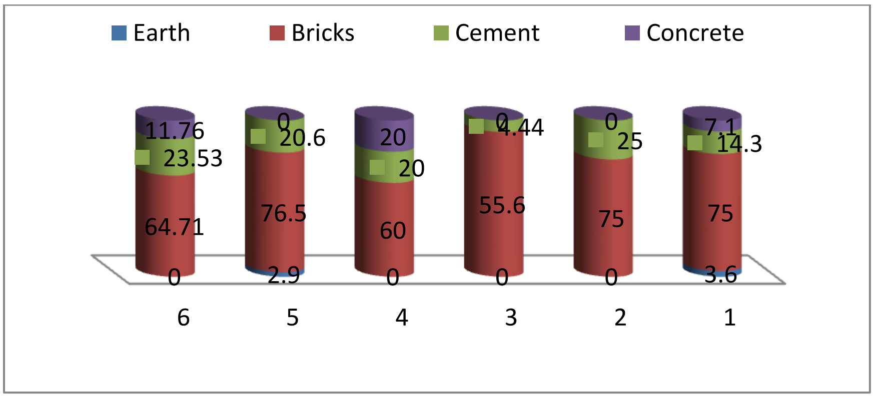
Table1. Birds house longwise, width and status in poultry farms in different localities in Gezira state in Sudan

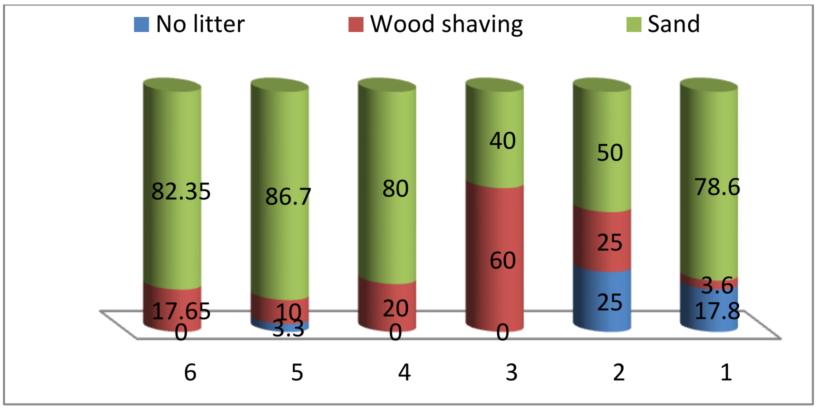
Locality	House direction%	House width (m)%				House status%					
		5 – 8	8 - 10	10 - 12	12 – 15	c.n.p	c.p	g.n.p	g.p	v.g.n.p	v.g.p
South of the Gezira	100	67.9	10.7	14.3	7.1	21.0	7.0	5.0	14.0	-	7.0
East of the Gezira	100	25.0	50.0	25.0	-	25.0	25	50.0	0.0	-	0.0
Alhasahesa	100	55.6	44.4	-	-	11.0	11.0	45.0	33.0	-	0.0
Almanagil	100	100.0	-	-	-	20.0	-	0.0	20.0	20.0	0.0
Alkamleen	100	73.5	23.6	2.9	-	9.0	3.0	76.0	6.0	6.0	0.0
Greater Medani	100	76.4	11.8	11.8	-	18.0	11.0	11.0	31.0	18.0	11.0

c.n.p: cracked and not painted; c.p: cracked and painted; g.n.p: with limited racks not painted; g.p: without cracks and painted; vgnp: very good not painted; vgp: very good painted



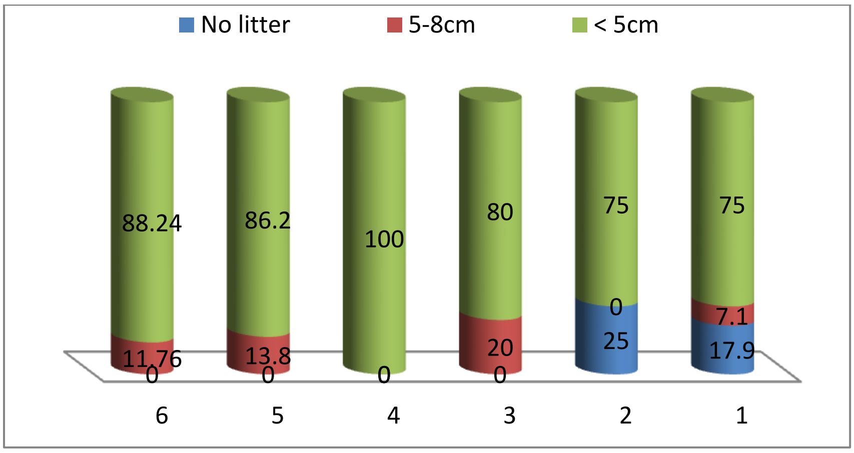
Floor type (%)

Figure 1. Floor type of poultry farms in different localities in Gezira State, Sudan (Localities: 1: South of the Gezira. 2: East of the Gezira.3: Alhasahesa. 4: Almanagil.5: Alkamleen. 6: Greater Medani)



Litter type (%)

Figure 2. Litter type of poultry farms in different localities of Gezira state in Sudan (Localities 1: South of the Gezira. 2: East of the Gezira. 3: Alhasahesa. 4: Almanagil.5: Alkamleen. 6: Greater Medani)



Litter depth (%)

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Figure 3. litter depth in the poultry farms in different localities in Gezira state in Sudan (Localities: 1: South of the Gezira. 2: East of the Gezira. 3: Alhasahesa. 4: Almanagil.5: Alkamleen. 6: Greater Medani)

Poultry house equipments

Feeder types and size were shown in (Table 2).Circular feeders were the main feeder type used in the Gezira State localities surveyed; This type was reported being used by all (100 %) of respondents in east of the Gezira and Almanagil localities, 97 % in Alkamleen, 89.3% in south of the Gezira and 88.24%Greater Wad Medani locality. Round feeders diameter was around (40-50 cm) in most Gezira State localities; This type was reported being owned by 100% 87.9% and 80% in east of the Gezira and Greater Wad Medani localities, Alkamleenlocality,Alhasahesa and Almanagil localities, respectively. Longitudinal feeders Size was about 100-150 cmin poultry houses in south of the Gezira, Alkamleen and Greater Wad Medani localities (Table 2). Birds/feeder varied in Gezira State localities; it was less than 50 birds in east of the Gezira locality (100%) and was about50 birds in Almanagil locality (60%)while it was about 75 birds in Greater Medani locality (82.4%) (Figure 4). Oil containers (jerricans) of 18 liter size was the main drinkers used in GeziraState localities; it was about 96.4%, 77.8%, 76.5%, 75%, 60% and 58.8% in south of the Gezira, Alhasahesa, greater Medani, east of the Gezira, Almanagil and Alkamleen.

Greater Medani, East of the Gezira, Almanagil and Alkamleen localities, respectively. Manual plastic drinkers were around 40% in Almanagil, 25% in East of the Gezira and 22.2% in Alhasahesa localities. Birds/drinker varied between 50 and 75 birds in most localities. Fifty birds/drinker represented 53.6% in South of the Gezira and 53.1%in Alkamleen localities while 75 birds/drinker represents 82.35% in Greater Wad Medani and 55.6% in Alhasahesa localities (Table 3). Pots were the major egg nest type used in thelocalities; it was used by 100 % in East of the Gezira, Alhasahesa and Almanagil localities, 93.8% in Alkamleen and 92.9%in South of the Gezira locality (Figure 5). There were more than 15 birds per nest in most localities. This ratio represented 86.58%, 65.6% and 66%in Alhasahesa, Alkamleen and Almanagil localities, respectively (Table 4).

Birds egg production

Egg production rate was about (60-70%) in most of localities. That rate was reported by 83%, 75% and 60% in Alhasahesa, East of the Gezira and Almanagil localities, respectively.

The relationship between housing status and equipment and average egg production

Table 5, 6, 7, 8, 9 and 10 showed a positive relationship between well building house with suitable equipments and average egg production. When the house was well constructed and the equipments were as recommended the production rate was higher.

Table 2. Feeder type and size of poultry farms in different localities in Gezira state, Sudan

Locality	Feeder type (%)		Feeder size (%of owners)						
	Circular	Longitudinal	Circular (cm)			Longitudinal (cm)			
			<40	40-50	>50	80-100	100-150	150-200	>200
South of the Gezira	89.3	10.7	28.0	72.0	-	-	100.0	-	-
East of the Gezira	100.0	-	-	100.0	-	-	-	-	-
Alhasahesa	55.6	44.4	20.0	80.0	-	50.0	50.0	-	-
Almanagil	100.0	-	20.0	80.0	-	-	-	-	-
Alkamleen	97.1	2.9	12.1	87.9	-	-	100.0	-	-
Greater Medani	88.24	11.76	-	100.0	-	-	100.0	-	-

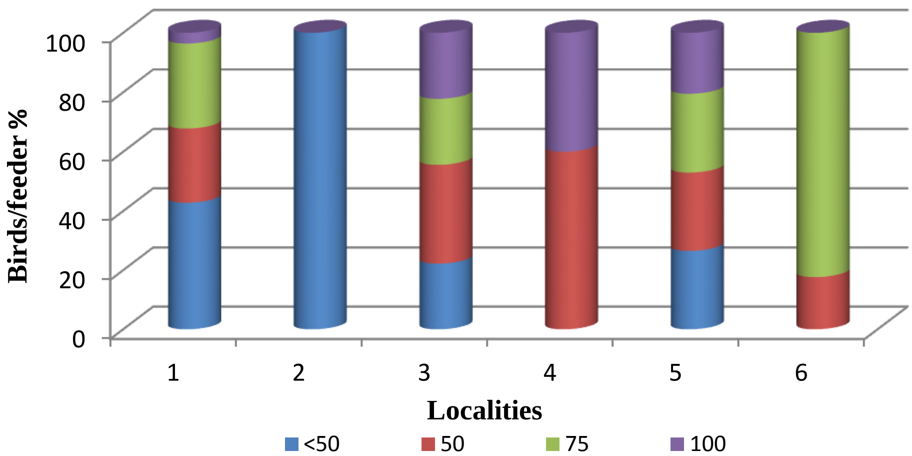

Figure 4. Bird's feeder in poultry farms in different localities in Gezira state, Sudan (Localities: 1: South of the Gezira, 2: East of the Gezira, 3: Alhasahesa, 4: Almanagil, 5: Alkamleen 6: Greater Medani)

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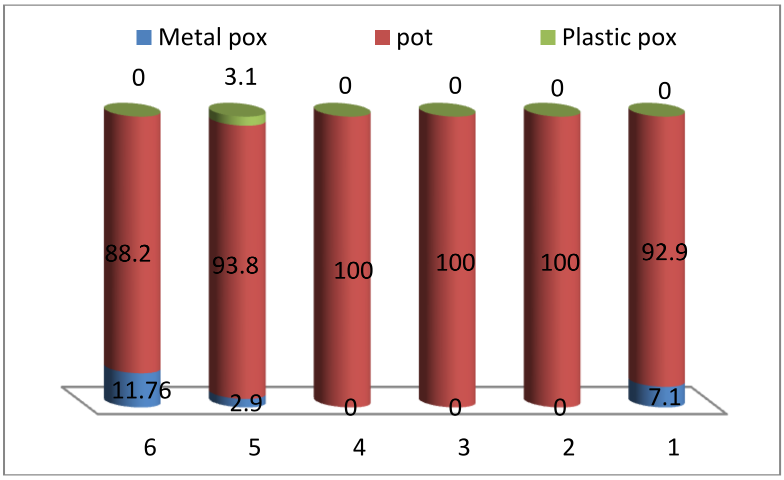
Table 3. Characteristics of drinkers and number of birds per drinker in poultry farms in different localities in Gezira state, Sudan

Locality	Drinker types (%)			Drinker (litter)				Bird/drinker				
	Manual plastic	Jerricans	Automatic	8	10	12	18	< 50.0	50.0	75.0	100.0	125.0
South of the Gezira	3.6	96.4	-	3.6	-	3.6	92.8	14.3	53.6	21.4	10.7	0.0
East of the Gezira	25.0	75.0	-	25.0	-	-	75.0	-	50.0	25.0	25.0	0.0
Alhasahesa	22.2	77.8	-	22.2	-	-	77.8	1.11	22.2	55.6	0.0	1.11
Almanagil	40.0	60.0	-	40.0	-	-	60.0	20.0	20.0	40.0	20.0	0.0
Alkamleen	20.6	58.8	20.6	29.5	2.9	8.8	58.8	-	53.1	34.3	6.3	6.3
Greater Medani	17.6	76.5	5.9	17.65	0.0	0.0	82.35	-	-	82.35	11.65	0.0



Egg nest type %

Figure 5. Egg nest type in poultry farms in different localities in Gezira State, Sudan (**Localities:** 1: South of the Gezira. 2: East of the Gezira. 3: Alhasahesa. 4: Almanagil. 5: Alkamleen. 6: Greater deMedani)

Table 4. Egg production /year and birds per egg nest in poultry farms in different localities in Gezira state, Sudan

Locality	**Egg production /year %**					**Birds/egg nest %**			
	<60%	**60-70%**	**70-80%**	**80- 90%**	**>90**	**5.0**	**6 -10**	**11-15**	**> 15**
South of the Gezira	4.0	39.0	25.0	18.0	14.0	3.6	-	39.3	57.1
East of the Gezira	-	75.0	25.0	-	-	25.0	-	50.0	25.0
Alhasahesa	-	80.0	20.0	-	-	-	6.7	6.7	86.6
Almanagil	40.0	60.0	-	-	-	-	-	40.0	60.0
Alkamleen	6.0	31.0	22.0	41.0	-	-	-	34.4	65.6
Greater Medani	11.0	39.0	25.0	18.0	7.0	-	17.7	29.4	52.9

Table 5. Correlation between wall status and average egg production (%) in poultry farms in Gezira State, Sudan

Parameters studies	**Percent respondents**	**Pearson correlation**
Status of wall		
Cracked and not painted	15.5	
Cracked and painted	7.2	
Good and painted	52.5	
Very good and not painted	6.2	0.819**
Very good and painted2.1	2.1	
Daily egg production percentage		
< 60 %	36.1	
60-70 %	52.6	
70-80 %	11.3	

** Correlation is significant at 0.01 level

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Table 6. Correlation between floor types and average egg production in poultry farms in Gezira state, Sudan

Parameters studied	Percent respondents (%)	Pearson correlation
Type of floor		
concrete	5.2	
Cement	21.6	0.696**
Bricks	71.1	
Earthen	2.1	
Average egg production (%)		
< 60 %	36.1	
60-70 %	52.6	
70-80 %	11.3	

** Correlation is significant at 0.01 level

Table 7. Correlation between litter depth and average egg production in poultry farms in Gezira state, Sudan

Parameters studied	Percent respondents (%)	Pearson correlation
Litter depth (cm)		
< 5cm	82.5	
5-8 cm	16.5	0.637**
No litter	1%	
Average egg production (%)		
< 60 %	36.1	
60-70 %	52.6	
7 0-80 %	11.3	

** Correlation is significant at 0.01 level**.**

Table 8. Correlation between birds/feeder and average egg production in poultry farms in Gezira state, Sudan

Parameters studied	Percent respondents (%)	Pearson correlation
Birds/feeder		
>50	24.7	
50	23.7	0.874**
75	35.1	
100	16.5	
Average egg production (%)		
< 60 %	36.1	
60-70 %	52.6	
70-80 %	11.3	

** Correlation is significant at 0.01 level.

Table 9. Correlation between drinker types and average egg production in poultry farms in Gezira state, Sudan

Parameter studied	Percent respondents (%)	Pearson correlation
Drinker types (%)		
Manual plastic	16.5	
Jerricans	75.3	0.720**
Automatic	8.2	
Average egg production (%)		
< 60	36.1	
60-70	52.6	
70-80	11.3	

** Correlation is significant at 0.01 level

Table 10. Correlation between birds/drinker and average egg production in poultry farms in Gezira state, Sudan

Parameters studied	Percent respondents	Pearson correlation
Birds/drinker		
<50	6.2	
50	39.2	0.867**
75	41.2	
100	10.3	
125	3.1	
Average egg production (%)		
< 60 %	36.1	
60-70 %	52.6	
70-80 %	11.3	

** Correlation is significant at 0.01 level**.**

DISCUSSION

Feeder space allocation/hen, stocking density, and other aspects of the housing environment can affect the productivity of laying hens housed in cages (Bell and Weaver, 2002).

Bird's house direction that had been found in this study was in line with the findings of Viswanathan (2001) and Winchell (2001) to provide good ventilation in birds' house and to avoid heat stress. The most house width in the present study disagrees with ElBeeli (2009) who mentioned that a width of8-10 m was best for keeping the birds in good ventilation. The floor and litter types were in line with the findings of Fanatico (2006), Grisso (2009) and Sadkhan (2011). Litter depth of 2-5 cm was in disagreement with the previous findings Grisso (2009) who mentioned 5-12cm depth and Sadkhan (2011) who reported (5-10 cm) for well moisture absorption. The results of this study might be attributed to the desire of farm owners in reducing production cost by using very thin litter layers. Use of plastic containers previously used for oil jerricans as drinkers may be due to their availability at low price. Such a trough did not provide adequate drinking space for the birds. Birds/feeders found in the present study was different from those reported by Payne (1990) and Jacob et al. (2010)who mentioned about 10 cm for a single bird per longitudinal feeder and 2 cm in round one as efficient space. This spacing allowed 7.6 -10.7 m for hundred layer birds, respectively and was important to provide adequate feeding space and prevent birds crowding around feeders. Birds/drinker was different from Payne (1990), Ward and McKague (2007) findings who reported 2.5m for 100 birds, and about 10 cm for a single bird to provide well drinking space and to prevent birds crowding around drinkers. Pots were the main egg nest type in the Gezira State localities and might be due to its availability, safety for birds and cheaper price. Birds/nest disagreed with the previous observations of Sonyia (2000) who mentioned (8-10 birds) to provide adequate egg laying space for each bird. .

Englmaierová et al. (2014) studied the effects of laying hens housing system on laying performance and found that the housing system significantly (P< 0.001) influenced the performance characteristics. The authors observed that the highest egg production, lowest daily feed consumption, and feed conversion ratio were measured in conventional cages compared to litter and aviaries. Holt et al. (2011) studied and found that a move from conventional cages to either an enriched cage or a non cage system might have affected the safety or quality or both of the eggs laid by hens raised in that new environment.

CONCLUSION

The result indicated that most poultry houses; length were from east to west, and most of the houses wall were not painted. Bricks were the most floor type and sand in a very thin layer was the most houses litter, beside some of the houses were not bedded. Most feeder type was the round one of 40to50 cm diameter and oil containers were the most drinkers' type. Birds/feeder and drinker were varied. Pots were the most egg nest type and birds/egg nest were very high in most houses. It was concluded that housing and equipment provided were not all in agreement with standards of layer hens housing and equipment provision of optimal environment. That is why production rates were low.

Competing interests

Authors have declared that there is no competing interest.

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The Effect of Highly Pathogenic Avian Influenza (HPAI) H5N1 Outbreaks on Mixed Species Poultry Farms in Nigeria

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ABSTRACT

The first outbreak of highly pathogenic avian influenza (HPAI) H5N1 virus in Nigeria was in 2006 and it involved different poultry species, mostly chickens in different ages, reared and bred on the same premises with some numbers of ducks, geese, turkeys and ostriches. To determine the effect of HPAI on mixed species poultry farming in the face of the ongoing 2015-2016 resurgent HPAI in Nigeria, data of confirmed 2006-2008 HPAI H5N1 outbreak in poultry were expressed as percentage proportions and used to produce spatial map using ArcGIS10.3 (ESRI®, USA) against some ecological features of the country. The outbreaks were more clustered in poultry farm dense areas especially in the northern states while very few clustering were observed around Important Bird Area and wetlands. A total of 177,996 (25.9%) on farm bird mortality was recorded from the selected outbreaks. From the backyard flock, the total mortality was 25, 915 birds (14.6%) and from the commercial flock, total mortality was 152, 081 birds (85.4%). The commercial flocks recorded higher mortality rate ($P<0.0001$). In the single species flock, total mortality recorded was 173, 425 (25.5%) while in the mixed species flock, total mortality was 4, 571 (52.9%). Mortality rate was much higher in the mixed species flock ($P<0.0001$) and ranged from 4.92 – 73.15% with the chicken-duck-turkey mixed flock farms having the highest rate (73.15%). Results show a higher risk of HPAI disease occurrence in multiple, mixed species poultry than in single species poultry production.

Key words: HPAI, Mixed species, Nigeria, Poultry

INTRODUCTION

Poultry production is a major economic activity in Nigeria and poultry population is reported to be the largest in Africa (Durosinlorun, et al., 2010; Nawathe and Abegunde, 1980 and Mohan et al., 1981). Poultry production is said to contributes significantly to the family income, particularly in the sub-urban and less privileged rural communities (CBN, 2004) and it is a major protein source for rural villages (Joannis et al., 2006). Since the evolution of H5N1 in Hong Kong in 1996 and its spread in Asia, Europe, and Africa with interspecies transmission, many human deaths have been recorded (Swayne, 2000; WHO, 2006) and several millions of poultry have been affected. Interspecies transmission usually occurs especially between closely related host species in the same taxonomic family (Mohan et al., 1981; Swayne, 2008). In Nigeria, evidence has emerged on the circulation of HPAI in apparently healthy waterfowls (Meseko et al., 2010), signifying the importance of these species in the maintenance and transmission of the virus. Waterfowls, ducks and geese are known natural reservoir of influenza viruses, although ducks have higher virus isolation rates (Shortridge, 1992). Waterfowls have been reported to be less susceptible to HPAI infection than chickens (Keawcharoen et al., 2008; Stallknecht and Shane, 1992), thereby being able to shed the virus as healthy carriers in backyard farms and in live bird markets (Meseko et al., 2010). Waterfowls are also seen to be a linkage between wild birds and domestic poultry population in farms and live bird market (LBM) (Meseko et al., 2010). The first outbreak of highly pathogenic avian influenza (HPAI) H5N1 virus in Nigeria in 2006 involved about 47,000 birds of different species, mostly chickens in different age category, reared and bred on the same premises (Joannis et al., 2008). A small number of geese, turkeys

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and ostriches, raised in the open, were also affected (Adene et al., 2006; Joannis et al., 2008). Due to the first H5N1 avian influenza infection in Nigeria, more than a million poultry were affected with one confirmed human death (Joannis, et al., 2008). At the end of the 2006-2008 outbreak, it is reported that 1654 cases were officially documented in 97 local government areas in 32 states and the federal capital territory (FCT) (Ekong et al., 2011), out of which 299 cases (Akanbi, 2014, Ekong et al., 2011, Joannis, et al., 2008) were positive in 25 states and the FCT (Akanbi, 2014 and Joannis et al., 2008). The country experienced severe losses in poultry accounting for approximately one million birds by June 2006, which stood at a cost of US$ 4.82 million (Metras et al., 2013 and Otte, 2008). By 2008, about 1,264,191 birds had been depopulated, and the compensation paid to farmers was about N631 million (US$5.43million) (Maina, 2008). In the face of the ongoing 2015 resurgent HPAI in Nigeria (Monne et al., 2015), this study is aimed at determining the possible factors influencing the mortality of different bird species and their association; also, to analyze the effect of the Nigeria HPAI H5N1 on mixed species poultry farms during 2006-2008. This will contribute to effective control measures for the ongoing 2015-2016 resurgent HPAI in Nigerian poultry.

MATERIALS AND METHODS

Data from the HPAI H5N1 outbreaks in Nigeria from 2006-2007 were used for this study and were sourced from the database of the National Veterinary Research Institute (NVRI), Vom, Nigeria, the national diagnostic laboratory for the HPAI H5N1 in Nigeria. The data included H5N1confirmed cases mainly from the backyard, small scale commercial and free range poultry, excluding positive sera and samples from LBMs which lacks flock history. The data included date of outbreak, farm location, flock size, morbidity and mortality records supplied directly by the clients who submitted poultry mortalities to the laboratory. These carcasses were confirmed positive for HPAI H5N1 using virus isolation (VI) in 9-11 days embryonating eggs and/or reverse transcriptase polymerase chain reaction (PCR). Of the 299 HPAI H5N1 confirmed cases available in the database (Akanbi, 2014; Joannis et al., 2008 and NVRI, 2008), 170 (56.9%) cases with full history (flock size and number dead inclusive) were selected for this study. All the spatial data were added to Geographical Information System (GIS) using Environmental Systems Research Institute (ESRI) ArcGIS 10.3 (ESRI®, California, USA) and Quantum GIS (QGIS) 2.8.2 Desktop (OSGeo, Oregon, USA) to generate maps against some ecological features of the country(important bird areas (IBA), urban areas, water bodies and wetlands), national poultry population and farm locations. The spatial data was visualized using QGIS. The outbreaks were categorized into two groups: Group 1: outbreak with single species flock, comprising of 156 cases of the total 170 cases (91.76%) and group 2: outbreak with mixed species flock, comprising of 14 cases of the total 170 cases (8.23%). Single species flocks included: chicken only; duck only; turkey only and guinea fowl only flocks. Mixed species flocks included: chicken and duck only; chicken and turkey only; chicken, duck and turkey only; chicken, duck and guinea fowl only flocks. Data sorting and descriptive analysis were conducted in Excel (Microsoft® Office Excel 2003) and statistical tests were undertaken in MedCalc® software version 11.1 (MedCalc, 2011). The proportions of dead birds were calculated for each flock type. The Chi-square (χ^2) test for comparisons of two proportions (with Yates' correction for continuity) was used to test for differences in proportions between the backyard and commercial flocks and between the single species and mixed species flocks. The ratio of the odds of mortality among the different categories was also compared. In all the analyses, confidence interval for this difference was held at 95% and values of $P \leq 0.05$ were considered significant.

Ethical Approval

This study was evaluated and followed the ethical guideline of the Ethics Committee of the National Veterinary Research Institute, Vom, Nigeria.

RESULTS

The 170 HPAI H5N1 cases with a full history used for this study were distributed across 20 States (Jigawa, Kaduna, Bauchi, Kano, Edo, Nasarawa, Adamawa, Katsina, Taraba, Plateau, Niger, Bornu, Enugu, Lagos, Anambra, Rivers, Kwara, Oyo, Ogun and Benue) and the FCT, Abuja. The spatial relationship of the coordinates of the outbreaks against the background of ecological features (important bird areas, urban areas, water bodies and wetlands), national poultry population and farm locations are depicted in figure 1. The outbreaks were more clustered in poultry farm dense areas especially in the northern states of Plateau, Kano and Kaduna. Very few clustering were observed around IBA and wetlands. Although there are more farms in Lagos and Ogun state in the south, less cases of HPAI were observed with less clustering. Bauchi state, with highest poultry density in the country (Adene and Oguntade, 2006), especially subsistence had several cases around dispersed farm settlements. Out of the 170 outbreak cases selected for this analysis, 131 outbreak cases (77.1%) represents backyard flock while 39 (22.9%) represents commercial poultry flocks (Table 1).

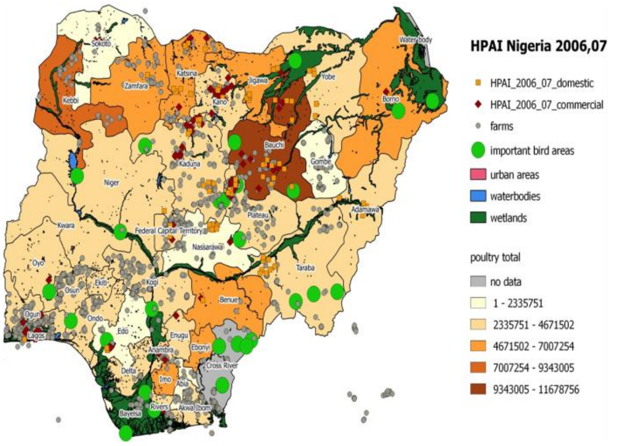

Figure 1. Spatial relationship of HPAI outbreaks against the background of ecological features, national poultry population and farm locations in Nigeria during 2006-2007

A total of 177,996 (100%) on farm poultry bird mortality was recorded from the 170 outbreak cases selected. From the backyard flock, total mortality was 25, 915 poultry birds (14.6%) and from the commercial flock, total mortality was 152, 081 poultry birds (85.4%), (Table 1). A statistically significant proportional difference of 2.2% (P <0.0001) was found between the percentage mortality in the commercial flocks compared to backyard flock (Table 2).

The commercial flocks recorded a slightly higher percentage mortality (P <0.0001). In the single species flock, the total poultry mortality recorded was 173, 425 (97.4%) while in the mixed species flock, total mortality was 4, 571 (2.6%). A statistically significant proportional difference of 27.4% (P <0.0001) was found for this group. Percentage mortality was much higher in the mixed species flock (P <0.0001), while the percentage mortality in the single species flock type farms ranged from 10.4 – 92.08% with turkey-only -farm having the highest rate (92.08%). This is followed by duck-only-farm (58.62%) the lowest was in guinea fowl –only-farm (10.40%), (Table 1). Mortality rate was much higher in the turkey only flocks (P <0.0001) and duck only flocks (P <0.0001), compared to chicken only flocks (Table 2). Higher percentage mortality was recorded among local chickens only flocks (P <0.0001), compared to exotic chicken only flocks. The percentage mortality in the mixed species flock type farms ranged from 4.92 – 73.15% with the chicken-duck-turkey mixed flock farms had the highest rate (73.15%), the lowest was in chicken-geese mixed farm (4.92 %), (Table 1). The mixed species flocks that included chickens had a higher mortality rate (P <0.0001), compared to mixed species flocks that did not include chickens. Mixed species flocks that included ducks had a higher percentage mortality (P <0.0001), compared to mixed species flocks that did not include ducks. Mixed species flocks that included chicken and ducks had a higher percentage mortality (P <0.0001), compared to mixed species flocks that did not include chickens and ducks. Mixed species flocks that included turkeys had a higher percentage mortality (P <0.0001), compared to mixed species flocks that did not include turkeys. Mixed species flocks that included turkeys and ducks had a higher percentage mortality (P <0.0001), compared to mixed species flocks that did not include turkeys and ducks.

Table 1. Flock size and mortalities rates of different bird species and flock types during the 2006-2008 HPAI in Nigeria. Analysis from MedCalc® software.

Categories	Flock number	Total flock size	No dead	No alive	Mortality rates
Flock Type					
Single Flock	156	679291	173425	505866	25.53
Mixed Flock	14	8637	4571	4066	52.92
Husbandy					
Backyard	131	107732	25915	81817	24.06
Commercial	39	580196	152081	428115	26.21
Single Flock					
Chicken (All)	147	678931	173243	505688	25.52
Duck	4	116	68	48	58.62
Guinea fowl	1	125	13	112	10.4
Ostrich	1	18	8	10	44.44
Turkey	3	101	93	8	92.08
Chicken Type					
Chicken	132	673267	170846	502421	25.38
Local Chicken	14	2838	1029	1809	36.26
Mixed Flock Type					
Chicken, Duck	1	41	17	24	41.46
Chicken,Duck, Turkey	2	5587	4087	1500	73.15
Chicken, Geese	1	508	25	483	4.92
Chicken,Geese, Turkey	1	364	29	335	7.97
Chicken, Guinea fowl	1	250	135	115	54
Chicken, Guinea fowl, Duck	1	17	6	11	35.29
Chicken, Guinea fowl, Duck, Turkey	1	393	25	368	6.36
Chicken, Pigeon, Turkey	1	370	142	228	38.38
Chicken, Turkey	2	690	61	629	8.84
Geese, Duck	2	371	28	343	7.55
Ostrich, Geese, Peasant	1	46	16	30	34.78
Mixed Flock Group					
Mixed with Chicken	9	8220	4527	3693	55.07
Mixed without Chicken	2	417	44	373	10.55
Mixed with Duck	5	6409	4163	2246	64.96
Mixed without Duck	6	2228	408	1820	18.31
Mixed with Chicken and Duck	4	6038	4135	1903	68.48
Mixed without Chicken and Duck	7	2599	436	2163	16.78
Mixed with Turkey	5	7404	4344	3060	58.67
Mixed without Turkey	6	1233	227	1006	18.41
Mixed with Turkey and Duck	2	5980	4112	1868	68.76
Mixed without Turkey and Duck	9	2657	459	2198	17.28

Table 2. Odds ratio analysis from MedCalc® software of the association between different bird flock types (single or mixed) during the 2006-2007 HPAI H5N1 outbreaks in Nigeria.

Categories	Odds ratio	95% Confidence interval	p-value
Mixed Flock	3.28	3.14-3.42	<0.0001
Single Flock	1		
Husbandry			
Commercial	1.12	1.11-1.14	<0.0001
Backyard	1		
Single Chicken Flock			
Local Chicken	1.67	1.55-1.81	<0.0001
Chicken	1		
Single Flock			
Duck	4.32	2.97-6.27	<0.0001
Turkey	33.93	16.48-69.87	<0.0001
Ostrich	2.34	0.92-5.92	0.0738
Guinea Fowl	0.34	0.19-0.60	0.0002
Chicken (All)	1		
Mixed Flock Group			
Mixed with Chicken	10.39	7.58-14.25	<0.0001
Mixed without Chicken	1		
Mixed with Duck	8.27	7.34-9.31	<0.0001
Mixed without Duck	1		
Mixed with Chicken and Duck	10.78	9.60-12.11	<0.0001
Mixed without Chicken and Duck	1		
Mixed with Turkey	6.29	5.41-7.32	<0.0001
Mixed without Turkey	1		
Mixed with Turkey and Duck	10.54	9.40-11.82	<0.0001
Mixed without Turkey and Duck	1		

DISCUSSION

The majority of the cases included in this study were distributed across 20 States of Nigeria which included Jigawa, Kaduna, Bauchi, Kano, Nasarawa, Adamawa, Katsina, Taraba, Plateau, Niger, Bornu, Kwara, Edo, Enugu, Anambra, Rivers, Lagos, Oyo, Ogun and Benue and the FCT, Abuja. Predominantly backyard poultry flocks most of which were from the northern part (north central, north east and northwest) of the country were mostly involved.

It is reported that the northern part of Nigeria sustains a large backyard poultry population and the highest concentration of domestic ducks, reared under free-range conditions (Cecchi et al., 2008), it cannot be ascertained whether this result is a reflection of the finding of Cecchi et al. (2008) or perhaps, the reported HPAI cases were more from this part of the country. Although our findings highlighted the significant role, the north of Nigeria and backyard poultry play in the transmission of highly pathogenic avian influenza infections in Nigeria, cases were also recorded from the southern part of the country; and also in commercial poultry. Our spatial analysis also suggests that poultry farm-dense areas had an influence on the occurrence and frequency of HPAI outbreaks especially in the northern states of Plateau, Kano and Kaduna. This is logical, as higher biosecurity would be needed in such areas in other to reduce HPAI transmission from one poultry farm to the other. Also, ecological features such as important bird areas, wet lands, water bodies and urban areas seem to have clustering of HPAI cases in this study. This may suggest under reporting of HPAI cases from this areas, less poultry farming activities or that these features may not have played significant role in the transmission of HPAI in Nigeria. The backyard poultry system (Adene and Oguntade, 2006) contributes significantly to the family income, especially in sub-urban and less privileged rural communities (CBN, 2004) and plays a major role in protein supply (Joannis et al., 2006). Of the two poultry production systems practiced in Nigeria (Adene and Oguntade, 2006), the backyard poultry recorded a higher HPAI cases than the commercial poultry. In Nigeria, backyard poultry has been identified as one of the two major source populations for the HPAI H5N1 virus in northern Nigeria for its frequent contact with wild birds (Fusaro et al., 2010). This is evident in our result which showed that of the 170 HPAI H5N1 cases, most of which are from the north of Nigeria used for this study, the backyard flock represents 77.1% (131) while commercial poultry flock represents 22.9% (39) and the chicken cases and farms were most infected. This is similar to the development in south-east Asia, whereby, the backyard poultry have been found to be an important source and persistence of HPAI H5N1 (Tiensin et al., 2005). Other factors found to be responsible for the dissemination of avian influenza virus in poultry, included rearing of multiple species in backyard poultry (Bavinck et al., 2009). Our results showed that mortality rate was twice as high in mixed species flocks as in single species flock, a statistically significant proportional difference of 27.4% (P <0.0001) and with highest odds ratio (P <0.0001) was found for this group. Percentage mortality was much higher in the mixed species flock (P <0.0001). Highest HPAI mortality rate of 73.15% was found for chicken, duck and turkey mixed species flocks, higher than any other species combinations involved in this study, suggesting a higher risk of HPAI disease occurrence in this combination of mixed poultry farming. It is reported that interspecies transmission usually occurs especially between closely related host species in the same taxonomic family (Mohan et al., 1981 and Swayne, 2000). In Nigeria, evidence has emerged on the circulation of HPAI in apparently healthy waterfowls (Meseko et al., 2010), signifying the importance of these species in the maintenance and transmission of the virus. Waterfowls, ducks and geese are known natural reservoir of influenza viruses, although ducks have higher virus isolation rates (Shortridge, 1992). Waterfowls have been reported to be less susceptible to HPAI than chickens (Stallknecht and Shane, 1988) thereby being able to shed the virus as healthy carriers in backyard farms and in live bird markets (Meseko et al., 2010). Waterfowls are also seen to be a linkage between wild birds and domestic poultry population (Meseko et al., 2010) in farms and Live Bird Market (LBM).

It has been reported that the mortality due to Avian influenza may be low in the ducks and geese (Compitelli et al., 2004), this is contrary to our findings in single flock ducks where mortality was 58.62%, only second to that of turkey which has the highest mortality rate (92.08%) amongst single flocks. Also, contrary to field observations that highest mortalities have been recorded mostly in chickens and turkeys (Aly et al., 2008), our study found the highest mortalities in duck and turkey single flocks in Nigeria. Earlier report of cases in four northern states in Nigeria by Saidu et al. (2008) found higher mortality rates for both geese and ducks than the mortality rates for turkeys, chickens, pigeons and guinea fowls. In this national study, it is observed that turkey had the highest mortality rate among single flock type while mortality rate was not as high in chicken as it is in duck. Mortality rates in the single species flock type farms ranged from 10.4 – 92.08%, with guinea fowl being the lowest (10.4%).

This study showed that HPAI occurrence was reported more in backyard poultry, and mixed species poultry farming especially combination of duck, turkey and chicken with increases in mortality rate and odds of infection by HPAI outbreak in Nigeria. Also, it reveals that, among single flock, mortality rate was highest in turkeys. The findings emphasizes the role played by poultry farming practices in the dissemination of avian influenza in Nigeria, shedding insights towards better HPAI control measures which can be beneficial to controlling the ongoing 2015 HPAI H5N1 outbreak in Nigeria.

CONCLUSION

The findings from this study showed that there is a higher risk of HPAI H5N1 infection in mixed species poultry farming in Nigeria. Therefore to militate against the effect of the ongoing 2015-2016 resurgent HPAI H5N1 in poultry in Nigeria, farmers in particular, small holders should be discouraged from mixed poultry farming as one of the species could be more susceptible to HPAI and thereby be the source of introduction.

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Competing interests

The authors declare that there are no significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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Variations in Morphometric Traits of Local Chicken in Gomoa West District, Southern Ghana

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ABSTRACT

The study was undertaken to identify the variations among morphometric traits of local chicken in the Gomoa West district of Ghana. Thirteen body measurements namely Weight (WT), Body Length (BDL), Chest Circumference (CC), Thigh Circumference (TC), Shank Length (SL), Neck Length (NL), Wing Length (WGL), Head Length (HDL), Hip Length (HL), Wattle Length (WAL), Beak Length (BKL), Drumstick Length (DL) and Comb Length (CL) were taken on 500 birds and analyzed under general linear model to determine the fixed effects of sex, comb type, feather distribution and skin colour on variabilities in the traits. The male birds had significantly ($P<0.001$) larger heads (NL=9.11 cm, HDL=6.59 cm, CL=5.23 cm etc.) and bodies (WT=1.19 kg, BDL=24.64 cm, CC=14.32 cm etc.) than their female counterparts. Cushion comb-type chickens were significantly ($P<0.001$) superior to all other comb-type chickens in all head and body measurements. Feather distribution had significant ($P<0.05$) influence on WT and BDL, as naked neck birds appeared superior. Birds with grey skin colour had significantly ($P<0.001$) larger chest circumference than all other birds. These findings could be useful as selection criterion, thereby providing a basis for genetic manipulation and improvements of the local chicken in Ghana.

Key words: Comb type, Measurement, Naked neck, Poultry, Skin colour

INTRODUCTION

The domestic local chicken (*Gallus gallus domesticus*) is the populous genetic resources among poultry that can be found in virtually every community in Ghana. These local breeds of domestic chicken are kept mainly by smallholder farmers under traditional management practices, and have adapted to a wide range of ecological settings. They are characterized by nondescript and hyper-variable phenotypic landscape (Dana et al., 2010; Egahi et al., 2010; Melesse and Negesse, 2011). Local chicken show striking morphological variations in plumage colour and pattern, comb shape, ear lobe colour, shank colour, etc. Other characteristics such as naked neck, frizzled feathers, single, pea, rose and cushion combs are common within the flock of local chicken (Hassabellah et al., 2014; Negesa et al., 2014 and Liyanage et al., 2015). It has been verified that single comb (the wild type) is recessive to all comb types, except the comb-less variant whiles the causative genetic variants for some morphological traits were mapped to their respective genomic region (Wragg et al., 2012). Smallholder farmers usually have broad breeding objectives to fulfill their versatile needs (Moges et al., 2010), hence they keep flock of diverse phenotypes. However, there is some sort of selection on visual traits by smallholder farmers who keep chickens not only for eggs and meat production but also to satisfy their visual appeal and to meet their cultural and religious needs (Dana et al., 2010; Melesse and Negesse, 2011).

The future improvement and sustainability of local chicken production systems is dependent on the availability of genetic variation (Benitez, 2002), which can be ascertained through characterization studies. Since morphological traits constitute major components of phenotypes in animal genetic resources, knowing the variations of morphological traits is fundamental to characterization of local genetic resources. Morphometric measurements have been found useful in contrasting size and shape of animals (Latshaw and Bishop, 2001; Ajayi et al., 2008). Phenotypic characteristics are very important in describing the uniqueness of animal genetic resources, and providing data for conservation of poultry genetic resources. However, not much is known about the morphometric description of the local chicken in Gomoa West district in Ghana. Hence this study was undertaken with the aim of describing variabilities in the body dimensions and size of local chicken in the district.

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MATERIALS AND METHODS

Study area

This study was carried out in the Gomoa West district of Central Region of Ghana. The district lies within latitudes 5^0 14^1N and 5^0 35^1N and longitudes 0^0 22^1 W and 0^0 54^1W (Getamap.net, 2016). The area experiences two rainfall patterns thus major rainy season (April to July) and minor rainy season (September to November) with mean annual rainfall ranging between 700 and 900 mm in the southern coastal belt and 900 to 1100 mm in the northern and northwestern semi-deciduous forest areas. The mean annual temperature ranges between 26^0C and 29^0C.

Ethical approval

Not applicable. This research did not involve the introduction of any intervention in/on birds, or direct collection of cells, tissues or any material from birds.

Sources of study birds and data collection

Within the period of July to August 2014, simple random sampling was used to select 10 communities within the district, and six farmers each from each district. A total of 500 (370 females and 130 males) local chickens aged four months or above were selected from the flock of smallholder farmers for measurements. Ages of birds were obtained from farmers' records or estimates (where records were unavailable) (Figure 1).The comb type (cushion, pea, rose and single) (Figure 2), skin colour (brown, grey, red, violet, white and yellow) and feather distribution (frizzled, naked neck and normal) (Figure 3) of each bird were noted. The following morphometric traits were measured on each bird. Weight (WT): The overall mass of a live bird measured with a kitchen scale; Body Length (BDL): The distance between the tip of the rostrum maxillare (beak) and the tip of the caudal (tail, without feathers) end; Chest Circumference (CC): The distance around the chest, taken behind the wings, through the anterior border of breast-bone crest and the central thoracic vertebra; Thigh Circumference (TC): It is the distance around the widest point of the thigh; Shank Length (SL): The distance along the metatarsus, measured from the shank joint to the extremity of the digituspedis; Neck Length (NL): The distance between the occipital condyle and the cephalic borders of the coracoids; Wing Length (WGL): It is the distance measured from the shoulder joint to the extremity of the terminal phalanx; Head Length (HDL): It is the distance measured between the occipital bone to the point of insertion of the beak to the skull; Hip Length (HL): This measurement was taken as the distance from the right to the left pelvic bone; Wattle Length (WAL): Vertical distance from the beginning to the end of the wattle; Beak Length (BKL): The distance from the rectal apterium to the maxillary nail; Drumstick Length (DL): The distance from the hip joint to the attachment of the shank; Comb Length (CL): It is the distance from the base to the tip end of the comb. Body weight was measured in kg while all linear traits were measured in cm.

Data analysis

The Statistical Package for Social Science (SPSS, version 17) was used to analyze the data. The multivariate command under general linear model was used to analyze the fixed effects of sex, age, comb type, skin colour and feather distribution on the quantitative traits and mean differences separated using LSD pair-wise comparison under the post hoc multiple comparisons at 5% level of significance.

RESULTS

Morphometric traits of the head of local chicken

The results clearly indicate a highly significant ($P<0.001$) difference on all traits of the head across sex (Table 1). Males were generally superior to females in all measurements. For wattles in particular, males had much longer wattles than their female counterparts. Age had significant ($P<0.001$) influence on all the head measurements and as expected, the sizes of the traits increased with increasing age (Table 1). Birds aged 25 months or more had the longest neck, head, wattle, comb and beak while birds within ages of 4 to 12 months had similar comb and beak lengths (Figure 1).

All the measurements on the head were significantly ($P<0.05$) affected by comb type (Table 2). Birds with cushion combs were generally superior in all measurements even though their neck length, head length, comb length and beak length were similar to those of birds with rose type combs. Skin colour had no significant ($P>0.05$) effect on all traits except for head length ($P<0.001$). In numerical values, local chickens with red skin colour had the longest neck whiles those with white skin colour had the longest head, comb and wattle (Table 2).

Morphometric traits of the general body of local chicken

There were highly significant ($P<0.001$) variations on all traits of the general body across sex (Table 3). The males were notably superior, recording the highest weight, longest body, drumstick, wing, shank and biggest chest, thigh and hip as compared to the females. Age was also a highly significant ($P<0.001$) source of variation on all traits of the general body (Table 3). Expectedly, local fowls with the ages ranging from 25 or more months had the highest weight and all body measurements. Clearly, feather distribution had no significant ($P>0.05$) effect on all traits of the body except for weight, body length and thigh circumference (Table 4). Naked neck chickens though appearing superior, had similar weight with frizzled feathered and similar body length with normal feathered chicken. The comb type had significant ($P<0.01$) effects on all traits on the body of local chickens (Table 4). Birds with cushion type combs were particularly superior to all other birds in all the body traits measured. Body measurements were generally similar among all other comb types. Skin colour had significant effect on only chest circumference and hip width. Chicken having grey skin were significantly ($P<0.001$) larger at the chest and had significantly ($P=0.001$) longer hips

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Table 1. Effects of sex and age on quantitative traits (Means ± SE) of the head of local chicken in Gomoa West district, Ghana

Variable	**Trait**				
Sex	**NL**	**HDL**	**CL**	**WAL**	**BKL**
Female	8.37±0.05^{b}	5.90±0.05^{b}	2.52±0.07^{b}	0.84±0.06^{b}	3.00±0.02^{b}
Male	9.11±0.10^{a}	6.59±0.08^{a}	5.23±0.13^{a}	2.71±0.10^{a}	3.23±0.03^{a}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Age (months)					
4 – 6 (n=72)	8.03±0.10^{e}	5.71±0.09^{c}	2.33±0.17^{b}	0.55±0.13^{e}	3.00±0.03^{d}
7 – 12 (n=277)	8.53±0.05^{d}	5.97±0.04^{b}	2.98±0.09^{b}	1.24±0.07^{d}	3.02±0.02^{d}
13 – 18 (n=107)	8.76±0.08^{c}	6.08±0.07^{b}	3.65±0.14^{a}	1.80±0.11^{c}	3.11±0.02^{c}
19 – 24 (n=42)	9.12±0.13^{b}	6.60±0.11^{a}	4.62±0.22^{a}	2.34±0.17^{b}	3.24±0.04^{b}
25 – 30 (n=2)	12.50±0.59^{a}	7.50±0.51^{a}	5.00±1.02^{a}	5.50±0.78^{a}	4.00±0.17^{a}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c,d,e} Means with different superscript in a column are significantly different by sex and age; SE: Standard Error; NL: Neck Length; HDL: Head Length; CL: Comb Length; WAL: Wattle Length; BKL: Beak Length; n: number of birds

Table 2. Effects of comb type and skin colour on quantitative traits (Means ± SE) of the head of local chicken in Gomoa West district, Ghana

Traits	**Comb types**					**Skin colour**						
	Cushion	**Pea**	**Rose**	**Single**	**P-values**	**Brown**	**Grey**	**Red**	**Violet**	**White**	**Yellow**	**P-values**
NL	9.58±0.25^{ab}	8.55±0.28^{b}	8.63±0.48^{ab}	8.41±0.12^{b}	0.007	8.20±0.45	8.50±0.61	9.35±0.22	8.74±0.18	8.00±0,86	8.62±0.23	0.217
HDL	6.89±0.20^{a}	6.04±0.23^{bc}	5.63±0.39^{c}	6.13±0.10^{b}	0.006	7.60±0.37^{a}	7.00±0.49^{ab}	6.09±0.17^{bc}	6.27±0.14^{a}	8.00±0.70^{a}	5.81±0.19^{c}	<0.001
CL	4.18±0.43^{a}	2.20±0.50^{c}	4.00±0.85^{a}	3.17±0.22^{b}	0.018	3.00±0.80	5.00±1.07	3.28±0.38	3.23±0.31	5.00±1.52	3.08±0.40	0.637
WAL	4.23±0.29^{a}	0.67±0.33^{c}	1.63±0.57^{b}	1.25±0.15^{bc}	<0.001	1.23±0.53	2.00±0.72	2.63±0.25	1.91±0.21	4.00±1.02	1.46±0.27	0.896
BKL	3.40±0.07^{a}	3.00±0.08^{b}	3.13±0.13^{ab}	3.06±0.03^{b}	<0.001	2.98±0.13	3.50±0.17	3.31±0.06	3.07±0.05	3.00±0.24	3.11±0.06	0.163

^{a,b,c} Means with different superscript in a row are significantly different by comb types and skin colour; SE: Standard Error; NL: Neck Length; HDL: Head Length; CL: Comb Length; WAL: Wattle Length; BKL: Beak Length

Table 3. Effects of sex and age on quantitative traits (Means ± SE) of the general body of local chicken in Gomoa West district of Ghana

Traits	Sex			Age (months)					
	Female	**Male**	**P-values**	**4-6**	**7-12**	**13-18**	**19-24**	**25-30**	**P-value**
WT	0.94±0.01^{b}	1.19±0.02^{a}	<0.001	0.69±0.03^{e}	0.95±0.02^{d}	1.08±0.02^{c}	1.30±0.03^{b}	2.10±0.11^{a}	<0.001
BDL	22.31±0.13^{b}	24.64±0.23^{a}	<0.001	21.17±0.29^{e}	23.07±0.16^{d}	23.69±0.22^{c}	24.88±0.31^{b}	29.00±1.11^{a}	<0.001
CC	13.27±0.11^{b}	14.32±0.19^{a}	<0.001	12.32±0.25^{e}	13.67±0.14^{d}	13.76±0.19^{c}	14.52±0.26^{b}	18.00±0.94^{a}	<0.001
DL	12.23±0.07^{b}	13.75±0.12^{a}	<0.001	12.49±0.15^{d}	12.75±0.08^{d}	12.82±0.11^{c}	13.48±0.16^{b}	15.50±0.57^{a}	<0.001
WGL	17.30±0.09^{b}	19.46±0.16^{a}	<0.001	17.47±0.20^{d}	18.05±0.11^{d}	18.18±0.15^{c}	19.051±0.21^{b}	22.50±0.78^{a}	<0.001
TC	7.28±0.06^{b}	8.02±0.11^{a}	<0.001	6.72±0.14^{e}	7.41±0.08^{d}	7.81±0.11^{c}	8.15±0.15^{b}	10.00±0.55^{a}	<0.001
SL	8.55±0.07^{b}	10.04±0.13^{a}	<0.001	8.57±0.17^{e}	9.18±0.09^{d}	9.32±0.12^{c}	9.75±0.17^{b}	11.50±0.63^{a}	<0.001
HL	9.29±0.06^{b}	10.25±0.10^{a}	<0.001	8.98±0.13^{e}	9.51±0.07^{d}	9.66±0.10^{c}	10.25±0.13^{b}	13.00±0.49^{a}	<0.001

$^{a, b c, d, e}$ Means with different superscript in a row are significantly different by sex and age; WT: Weight; BDL: Body length; CC: Chest Circumference; DL: Drumstick Length; WGL: Wing Length; TC: Thigh Circumference; SL: Shank Length; HL: Hip length

Table 4. Effects of feather distribution and comb type on quantitative traits (Means ± SE) of the general body of local chicken in Gomoa West district of Ghana

Variable	Traits							
Feather distribution	**WT**	**BDL**	**CC**	**DL**	**WGL**	**TC**	**SL**	**HL**
Frizzled	0.97±0.05^{ab}	21.79±0.43^{b}	13.63±0.31	12.08±0.21	17.25±0.30	7.42±0.19^{ab}	8.50±0.23	9.33±0.17
Naked neck	1.10±0.0 ^{a}	23.55±0.65^{a}	14.09±0.45	12.91±0.31	18.36±0.44	7.91±0.28^{a}	9.36±0.34	9.64±0.25
Normal	0.92±0.07 ^{b}	23.02±0.10^{a}	13.37±0.07	12.36±0.05	17.44±0.07	7.26±0.04^{b}	8.84±0.05	9.34±0.04
P-values	0.035	0.013	0.222	0.094	0.097	0.054	0.106	0.501
Comb type								
Cushion	1.34±0.06 ^{a}	26.13±0.55 ^{a}	14.79±0.40 ^{a}	13.62±0.28 ^{a}	19.44±0.39 ^{a}	8.83±0.24 ^{a}	10.56±0.30^{a}	10.16±0.22^{a}
Pea	0.89±0.07 ^{b}	22.30±0.64 ^{b}	13.77±0.46 ^{b}	11.98±0.32 ^{b}	17.20±0.45 ^{b}	6.86±0.28 ^{b}	8.91±0.35 ^{b}	8.95±0.26 ^{b}
Rose	1.08±0.12 ^{b}	23.25±1.09 ^{b}	13.38±0.79^{b}	12.25±0.54 ^{b}	17.75±0.77 ^{b}	7.75±0.48 ^{b}	8.88±0.60^{b}	9.63±0.44 ^{b}
Single	0.94±0.03 ^{b}	22.49±0.28 ^{b}	13.93±0.20 ^{b}	12.07±0.14 ^{b}	17.09±0.20 ^{b}	7.26±0.12 ^{b}	8.64±0.15 ^{b}	9.26±0.11 ^{b}
P-values	<0.001	<0.001	0.008	<0.001	<0.001	<0.001	<0.001	<0.001
Skin colour								
Brown	1.06±0.12	23.15±1.02	15.50±0.74 ^{b}	11.50±0.51	16.25±0.72	6.70±0.45	8.70±0.56	8.40±0.41^{c}
Grey	1.20±0.16	26.00±1.37	19.00±1.00^{a}	12.00±0.69	16.50±0.97	7.50±0.60	9.50±0.76	10.50±0.56^{a}
Red	1.11±0.06	23.80±0.49	14.09±0.35^{c}	12.79±0.24	18.25±0.34	7.87±0.21	9.50±0.27	9.57±0.20 ^{a}
Violet	0.96±0.05	23.55±0.39	13.51±0.29^{c}	12.35±0.20	17.61±0.28	7.46±0.17	9.25±0.22	9.40±0.16 ^{a}
White	1.30±0.22	27.00±1.94	15.00±1.41^{bc}	13.00±0.97	19.00±1.37	9.00±0.85	11.00±1.07	9.00±0.79 ^{a}
Yellow	1.07±0.06	22.55±0.52	13.73±0.37^{c}	12.75±0.26	18.20±0.36	7.84±0.23	8.94±0.28	9.73±0.21^{ab}
P-values	0.047	0.211	<0.001	0.249	0.190	0.127	0.888	0.001

^{a,b,c} Means with different superscript in a column differ significantly; WT: Weight; BDL: Body length; CC: Chest Circumference; DL: Drumstick Length; WGL: Wing Length; TC: Thigh Circumference; SL: Shank Length; HL: Hip length

Figure 1. Ages (in months) of local chicken in Gomoa West district of Ghana: (a) cock: 26, (b) cock: 17; hens: 19, (c) cock: 20, (d) hen: 11, (e) Pullet: 7 and (f) Pullet: 4

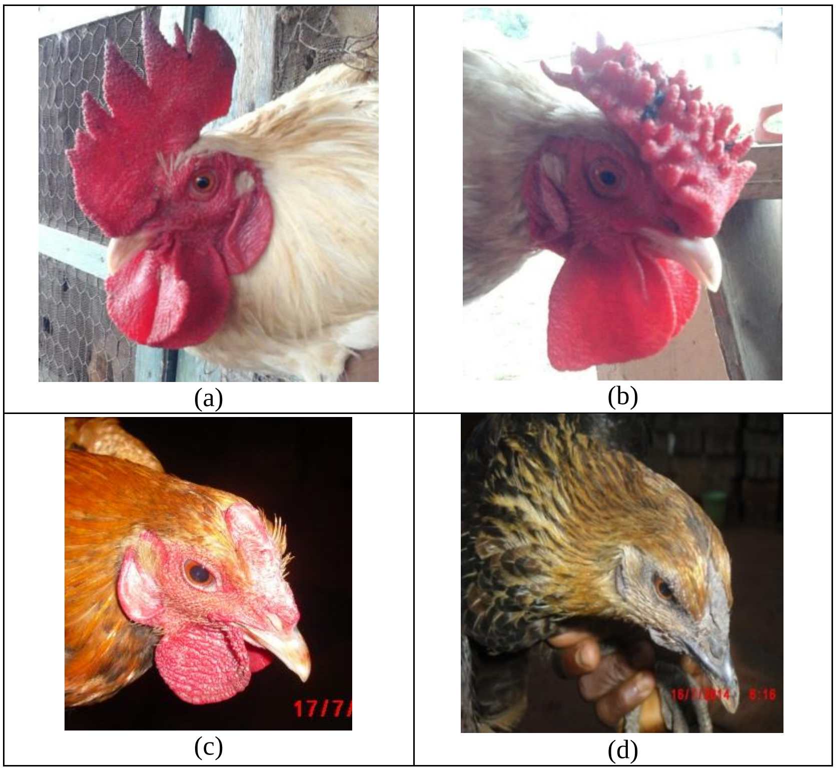

Figure 2. Comb types in local chicken in Gomoa West district of Ghana: (a) single, (b) rose, (c) cushion and (d) pea comb

(a) (b) (c)

Figure 3. Feather distribution in local chicken in Gomoa West district of Ghana: (a) Frizzled feathers, (b) Naked neck and (c) Normal feathers.

DISCUSSION

Morphometric traits of the head of local chicken

This current study showed that the males were generally superior to females in all measurements of the head. For wattles in particular, males had much longer wattles than their female counterparts. The larger combs and wattles of the males suggest that indigenous chicken exhibit sexual dimorphism in the expression of those traits. This finding is in agreement with the findings of Banerjee et al. (2013) who reported that the male chickens were superior to females in all measurements of the head in Harro and Jarso districts of Ethiopia. Though the comb lengths of male and female chicken in this study were much similar to those of male and female local chicken respectively in Southeastern Oromia Regional State of Ethiopia, the present birds had shorter wattles but much longer beaks than those Ethiopian chicken (Negesa et al., 2014). The superiority of cushion comb-type birds to other comb types in all measurements of the head could be attributed to the fact that, the cushion comb may be controlled by or associated with productive genes whose expression is favoured in the tropical climate. The comb type may be useful as selection criterion, thereby providing a basis for the genetic manipulation and improvement of local chicken in Ghana.

The larger heads, combs as well as wattles of white skin birds probably suggests that, such birds have advantage over birds with other skin colours in the growth and developments of head traits. It may also mean that the underlying carotenoid pigmentation for white skin colouration is possibly associated with genes which favour the development of head, comb and wattle. The significant effects of skin colour observed in this study disagreed with the findings of Tabassum et al. (2014) in Bangladesh where body measurements were not affected by skin colour in indigenous chicken. White skin coloured birds are expected to reflect light very effectively and so will experience less heat stress, thereby adapting and growing well in a tropical environment.

Morphometric traits of the general body of local chicken

The present local chickens were generally smaller than village chicken of Sri Lanka (Liyanage et al., 2015). The superiority of males in this study was similar to reports of earlier works in chicken (Petrus et al., 2011; Guni and Katule, 2013). The birds in the present study weighed less when compared to local chicken of Oman which weighed 1.65kg for cocks and 1.24kg for hens (Petrus et al., 2011). The body weights of the present birds were comparable to indigenous chicken of Sherpur district in Bangladesh (Tabassum et al., 2014), but less than indigenous chicken of Ethiopia (Negesa et al., 2014). Al-Qamashoui et al. (2014) reported that cocks were significantly heavier (1.33±0.65kg) than hens (1.17±0.86 kg) and Guni and Katule (2013) also reported that male chickens were heavier (2.86kg) than female (1.03kg) chickens.

The longer bodies and shanks of cocks in this study agreed with the findings of Al-Qamashoui et al. (2014) who reported that cocks had higher values for body length (18.4±0.14cm) and shank length (8.1±0.11cm) than hens (17.3±0.13cm; 7.1±0.14cm) in Oman. Guni and Katule (2013) reported that males were superior to females in all measurements of the body. The birds in this study were longer than 20.2cm and 18.1cm reported for male and female chickens respectively in Botswana (Badubi et al., 2006). Shank length of males from Horro and Jarso districts were 11.32cm and 9.99cm respectively and among the local hens were 9.22 cm and 8.51cm respectively (Dessie et al., 2013). In small ruminants, male superiority has been reported in sheep (Birteeb et al., 2014) and goats (Birteeb and Lomo, 2015), which suggests occurrence of differential growth and development due to sex in domesticated livestock species. The general superiority of males to females suggests that body size is a clear exhibition of sexual dimorphism in local chicken. The variability of measurements of the present chicken from their counterparts in other localities might be attributed to a wider variation in the genetic resource of chicken as well as differential response to different environmental conditions.

The superior weight of the naked neck birds may mean that weight related genes are expressed better in these birds than the normal and frizzled feathered birds. It has been suggested that the naked neck chickens have greater weight in the hot season due to genes that cause a reduction in the number of feathers from four to seven weeks than their counterpart chickens (Abdul-Rahman, 2000). Perhaps the naked neck birds dissipate heat easier than their counterparts during hot conditions. This could result in increased performance on growth and productivity.

The significant effects of comb type on body measurements were earlier reported in indigenous chicken in Bangladesh (Tabassum et al., 2014). The higher measurements of cushion comb-type birds than other comb-types may mean that birds with cushion combs have the ability to grow and develop well and faster in the study area. It means that such birds have potential of being developed into broilers. Such birds could be favoured for breed improvement in the local chicken genetic resources in Ghana.

CONCLUSION

The age, sex and comb type of birds affected the variabilities in all morphological traits of local chicken. Male birds were superior in all measurements of the head and body than their female counterparts. Cushion comb type chickens were also generally superior to all birds of other comb types in all head and body measurements. Feather distribution also affected body weight and thigh circumference while skin colour affected chest circumference and hip length. The knowledge of these morphological variabilities could be useful as selection criterion to poultry breeders, researchers and farmers, thereby providing a basis for genetic manipulation and improvements of the genetic resources of local chicken in Ghana.

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Competing Interests

The authors declare that they have no competing interests.

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Dietary Modelling of Nutrient Densities: Effect and Response in Different Growing Phases on Growth Performance, Nutrient Digestibility, Litter Quality and Leg Health in Turkey Production

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ABSTRACT

An experiment was conducted to explore the time bound (different growth phases) effect of different dietary nutrient densities i.e., different energy and protein concentration while maintaining the ratio between the two, all with the same ideal amino acid profile, on litter quality and leg health (footpad dermatitis (FPD) and hock burn (HB)), when fed to growing turkeys. The effects of dietary nutrient modelling on growth performance parameters, water intake and excretion, dry matter (DMD), organic matter (OMD), crude protein (CPD) digestibility coefficients and apparent metabolisable energy (AME) were also examined, when fed to growing turkeys in varying growth phases. At twenty-eight days of age one hundred and seventy five male turkeys (BUT 8) were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds in a pen, all pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consisting of 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases). Each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered *ad libitum* throughout the experiment. Five dietary treatments, containing either 77, 85, 100, 110 or 120% of the crude protein (CP) and metabolisable energy (ME) content recommended by the breed standard. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finishing at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). Nutrient density had a positive and linear effect ($P<0.001$) on weight gain, feed efficiency and dry matter digestibility (DMD) whereas the effect of nutrient density on dietary protein digestibility (CPD) only approached significance ($P=0.081$). As might be expected increasing nutrient density had a negative and linear effect on feed ($P<0.001$) and water ($P<0.01$) intake and did not affect the ratio between these two parameters. Increasing nutrient density had a positive effect on litter quality (linear ($P<0.001$)), with both the litter moisture ($P<0.01$) and the litter score decreasing ($P<0.001$). Conversely litter ammonia concentration increased ($P<0.001$) as nutrient density increased, similarly as nutrient density increased so did the prevalence of hock burn ($P<0.01$). Notably there was no effect ($P>0.05$) of treatment on FPD. The results suggest that an increase in nutrient concentration can reduce the moisture content of the litter and so improve overall litter quality. However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health problems in turkey production.

Key words: Nutrient density, Digestibility, Performance, Wet litter, Ammonia, Footpad dermatitis, Hock burn.

INTRODUCTION

Litter quality is an important component of many poultry production systems but especially for broilers and meat producing turkeys as these birds stay in contact with the litter throughout their life (Ekstrand *et al.*, 1997). High litter moisture and ammonia (NH_3), content and quality are correlated with dirty footpads, footpad dermatitis (FPD) and hock burn (HB) lesions in poultry (Ekstrand *et al.*, 1997; Dawkins *et al.*, 2004; Haslam *et al.*, 2006 and Mayne *et al.*, 2007). Therefore, the three most important aspects of litter quality are the moisture content, stickiness and nitrogen or NH_3 content in the litter (Lister, 2009). A good quality litter should satisfy the bird's welfare requirements by absorbing moisture, providing a warm and dry surface to rest on, providing a substrate that allows microbial activity to degrade excreta and should encourage dust bathing and litter directed activity.

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The effect of dietary energy on feed intake is emphasised in literature which is correlated with water intake. Some reports (Collin *et al.*, 2003) suggest that achieving a higher AME to CP ratio by using a lower CP concentration might encourage birds to increase feed intake to meet their amino acid requirements, which may also increase water intake (WI) and have an impact on the litter quality. However, it is not clear whether the absolute protein concentration itself or the ratio between the dietary protein and energy was the reason for the deterioration of the litter quality or to the changes in the CP to AME ratio. Therefore, the aim of this experiment was to compare the effect on WI and litter quality (e.g. moisture content, pH and NH_3 content) of different nutrient density diets formulated to give a constant CP to AME ratio in all diets and to establish how these dietary modifications can affect litter characteristics and the correlation of these characteristics with the FPD and HB in turkeys.

Materials and Methods

Animal ethics

The study was approved by The Animal Experimental Committee of Scottish agricultural college.

House preparation

Prior to the reception of poults the house was vacant and thoroughly cleaned. This included proper washing and disinfection of the room. A foot dipping tank was in place at all times on the door step of the house to maintain biosecurity.

Feed preparation

In the pre-study period, from 0 to 4 weeks of age, the birds were fed a standard crumb starter turkey feed (table 1). The starter diet consisted of major feed ingredients such as wheat, soybean meal, and fish meal containing crude protein 263 g/kg and ME 12.15 MJ/kg.

Five experimental diets in total were used for each growth phase (4 weeks each and starting at 4 weeks of age until 20 weeks) in the study. The wheat-soybean based diets in pelleted form was prepared according to the formulation for BUT 8 (Aviagen Turkeys Ltd., UK) as presented in table 3 to table 6. Diet T3 served as control with 100% of crude protein and energy according to BUT 8 requirement for each growth phase, while diets T1, T2, T4 and T5 contained 77, 85, 110 and 120% concentration of crude protein and energy, respectively. All the diets were formulated according to the respective growth phase nutrient recommendation of BUT 8 other than protein and energy content. Digestible amino acid profile was similar during a growth phase of 4 weeks for all the diets according to BUT 8 recommendations with some missing data values for amino acids being obtained from Firman and Boling (1998) and upgraded according to commercial values (table 2). Amino acids like lysine, methionine and threonine were included where deficient, to meet the requirement. Each experimental diet for the respective growth phase was fed randomly to selected seven replicates for the period from 4 to 20 weeks. All feed was pelleted. The diets used for experiment were analysed for their dry matter (DM), crude protein (CP) minerals, crude fat (EE), Neutral detergent fibre (NDF), ash, ME and amino acid content.

Dry matter (DM) in feed and excreta was determined by drying at 100°C for 24 hours in a force draft oven (AOAC 925.10, 1990). The fat content was determined with AOAC 920.39 method using a Soxtec 1043 extraction unit (Foss Ltd, Wigan, UK). The dietary neutral detergent fibre (NDF) fraction was determined according to procedure described by Holst (1973).

Feed conversion efficiency, organic matter efficiency and protein efficiency ratios calculations

The Feed Conversion Efficiency (FCE) was calculated by dividing weight gain by feed intake. The same applied for the Organic Matter Efficiency (OME), and for the protein efficiency ratio (PER)-by calculating by dividing body weight gain with total protein intake. Whereas Energy Efficiency Ratio (EER) was calculated as weight gain (kg/d) / AME intake (MJ/d).

Nutrient digestibility coefficients calculations

To determine dietary nutrient digestibility and AME at 7 weeks of age, all the birds from each pen were transferred to one of the 35 raised floor pens for 24 hours. The excreta voided were collected on trays placed beneath each raised floor pen and the feed intake for the same period was determined. Then excreta samples were freeze dried, weighed and milled to pass through a 0.75 mm mesh.

Dietary N – corrected apparent metabolisable energy (AMEn) was determined as previously described (Hill and Anderson, 1958). The coefficients of apparent digestibility of dietary dry matter (DMD), organic matter (OMD) and crude protein (CPD) as well as amino acid digestibility coefficients were also determined by the difference between nutrient intake (feed intake multiplied by the nutrient content in feed) and nutrient output (excreta voided for 24 hours multiplied by the nutrient content in excreta) divided by the nutrient intake.

Comparison of turkey growth performance

One hundred and eighty five day old male turkeys (BUT 8) were weighed and placed in a controlled environment building. For the pre-study period (first 4 weeks of age) birds were placed in the floor pen containing 10 cm thick bedding material of wood shaving. During the pre-study period all birds were offered the same standard turkey starter crumb diet and had *ad libitum* access to feed and water. Birds were wing tagged at day 10 for identification. The average air temperature of the house was recorded every day and was maintained at 30°C for 7 days and gradually reduced to 22°C at 4 weeks of age. For the first day 24 hour light was provided which then changed to a lighting schedule of 16 hour light and 8 hour dark period throughout the trial.

At twenty-eight days of age one hundred and seventy five turkeys were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds in a pen (1.01 x 0.35 m/pen floor area) within a controlled environment room. All the pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consisting of 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases). Each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered *ad libitum* throughout the experiment. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finish at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). The same house environment as for the end of the pre-study period was provided until the end of the study. The experiment ended when the birds were 20 weeks of age.

Table 1. Ingredient composition (g/kg) of the starter diet fed to the turkeys during the pre-study period from 0 to 4 weeks of age.

Ingredients	g/kg
Fish meal - (72%-CP)	30
Soybean meal - (48%-CP)	275
Wheat	575
Soy oil	17.4
Corn gluten - (60%-CP)	20
Casein	30
Lysine HCl	1.9
DL Methionine	2.8
L-Threonine	3.9
Salt	2.2
Limestone	7
Dicalcium phosphate	21.5
Vit./min. premix[1]	2.8
Coccidiostat	0.5
Pellet binder	10
Calculated nutrient analysis	
Metabolisable energy (ME), MJ/kg[2]	12.15
Crude protein (CP) (g/kg)	263.1
Crude fibre (g/kg)	29
Ca (g/kg)	10
Available Phosphorus (g/kg)	5
Na (g/kg)	1.5
Cl (g/kg)	2.3
K (g/kg)	8.2
Indispensable amino acids	
Arginine (g/kg)[3]	12.2
Cystine (g/kg)[3]	4.2
Isoleucine (g/kg)[3]	9.6
Lysine (g/kg)[3]	13.1
Methionine (g/kg)[3]	5.1
Phenylalanine (g/kg)[3]	10.5
Threonine (g/kg)[3]	8.1
Tryptophan (g/kg)[3]	3.1
Valine (g/kg)[3]	10.4
Dispensable	
Tyrosine (g/kg)[3]	9.4

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diet): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).[3]Concentration of amino acid on digestible basis.

Table 2. Ideal protein ratios for different growth phases of turkeys.

Amino acids[3]	Ideal protein ratios expressed as % relative to lysine for different growth phases			
	week 4-8	week 8-12	week 12-16	week 16-20
Arginine[1]	97.5	91.1	90.4	90.3
Cystine[1]	31.6	34.8	34.9	38.7
Isoleucine[2]	71.5	71.1	74.3	78.5
Lysine[1]	100.0	100.0	100.0	100.0
Methionine[1]	38.6	40.7	44.4	45.2
Phenylalanine[2]	78.5	77.8	76.6	74.9
Threonine[1]	61.4	60.0	60.1	60.2
Valine[2]	77.8	77.8	72.2	70.1
Tryptophan[1]	24.1	23.0	22.8	22.6
Tyrosine[2]	70.3	69.6	68.7	66.3

[1]From Aviagen Turkeys Ltd., UK; [2]From Firman and Boling (1998); [3]The ratios between amino acids were calculated on the basis of digestible concentration of each amino acid.

Table 3. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 4-8 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	193.0	229.7	297.3	341.8	386.2
Wheat, White	449.6	426.8	384.8	357.2	329.6
Wheat Middlings	150.00	121.50	69.00	34.50	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Corn gluten meal - (60%-CP)	0.00	1.90	5.40	7.70	10.00
Casein	0.00	9.50	27.00	38.50	50.00
Soybean OiL	0.00	23.85	67.77	96.64	125.50
L-Lysine HCl	3.40	2.75	1.56	0.78	0.00
DL-Methionine	2.50	2.75	3.20	3.50	3.80
L-Threonine	3.30	3.64	4.27	4.69	5.10
Common Salt	2.30	2.28	2.25	2.22	2.20
Limestone	12.20	10.72	7.99	6.19	4.40
Dicalcium phosphate	20.00	19.91	19.73	19.62	19.50
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	9.72	10.61	12.26	13.35	14.43
Crude protein (g/kg)	201.4	222.4	261.1	286.6	312.0
Crude fibre (g/kg)	54.30	48.92	39.02	32.51	26.00
Ca (g/kg)	10.00	9.98	9.95	9.92	9.90
Available Phosphorus (g/kg)	5.00	5.00	5.00	5.00	5.00
Na (g/kg)	1.50	1.50	1.50	1.50	1.50
Cl (g/kg)	2.50	2.41	2.23	2.12	2.00
K (g/kg)	8.90	9.01	9.22	9.36	9.50
Mn (mg/kg)	105.7	100.4	90.5	84.0	77.5
Zn (mg/kg)	105.0	99.9	90.5	84.3	78.1
Indispensable amino acids					
Arginine (g/kg)[3]	10.10	11.13	13.02	14.26	15.50
Cystine (g/kg)[3]	3.20	3.54	4.17	4.59	5.00
Isoleucine (g/kg)[3]	6.70	7.65	9.40	10.55	11.70
Lysine (g/kg)[3]	10.20	11.28	13.28	14.59	15.90
Methionine (g/kg)[3]	3.90	4.32	5.09	5.59	6.10
Phenylalanine (g/kg)[3]	7.10	8.13	10.02	11.26	12.50
Threonine (g/kg)[3]	6.20	6.87	8.09	8.90	9.70
Tryptophan (g/kg)[3]	2.50	2.75	3.20	3.50	3.80
Valine (g/kg)[3]	7.30	8.38	10.38	11.69	13.00
Dispensable					
Tyrosine (g/kg)[3]	6.20	7.17	8.95	10.13	11.30

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).[3]Concentration of amino acid on digestible basis.

Table 4. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 8-12 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	5.70	16.20	23.10	30.00
Soybean Meal - (48%-CP)	80.0	124.7	206.9	261.0	315.0
Wheat, White	510.6	491.8	457.1	434.4	411.6
Wheat Middlings	200.00	162.00	92.00	46.00	0.00
Wheat Bran	150.0	121.5	69.0	34.5	0.00
Corn gluten meal - (60%-CP)	0.00	3.80	10.80	15.40	20.00
Casein	10.00	13.80	20.80	25.40	30.00
Soybean OiL	0.00	27.65	78.57	112.04	145.50
L-Lysine HCl	3.50	3.18	2.58	2.19	1.80
DL-Methionine	2.40	2.69	3.21	3.56	3.90
L-Threonine	1.80	2.31	3.26	3.88	4.50
Common Salt	1.30	1.34	1.41	1.45	1.50
Limestone	10.70	9.71	7.89	6.70	5.50
Dicalcium phosphate	16.00	16.19	16.54	16.77	17.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.04	11.00	12.77	13.94	15.10
Crude protein (g/kg)	169.0	187.2	220.7	242.8	264.8
Crude fibre (g/kg)	50.30	45.63	37.02	31.36	25.70
Ca (g/kg)	8.50	8.50	8.50	8.50	8.50
Available Phosphorus (g/kg)	4.20	4.20	4.20	4.20	4.20
Na (g/kg)	1.20	1.18	1.15	1.12	1.10
Cl (g/kg)	1.90	1.88	1.85	1.82	1.80
K (g/kg)	7.60	7.73	7.98	8.14	8.30
Mn (mg/kg)	106.3	100.4	89.4	82.2	75.0
Zn (mg/kg)	106.9	100.5	88.6	80.8	73.1
Indispensable amino acids					
Arginine (g/kg)[3]	8.10	8.97	10.58	11.64	12.70
Cystine (g/kg)[3]	3.00	3.32	3.92	4.31	4.70
Isoleucine (g/kg)[3]	5.80	6.52	7.85	8.73	9.60
Lysine (g/kg)[3]	8.70	9.63	11.35	12.47	13.60
Methionine (g/kg)[3]	3.60	3.94	4.57	4.99	5.40
Phenylalanine (g/kg)[3]	6.10	6.96	8.53	9.57	10.60
Threonine (g/kg)[3]	5.30	5.87	6.92	7.61	8.30
Tryptophan (g/kg)[3]	2.10	2.31	2.69	2.95	3.20
Valine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Dispensable					
Tyrosine (g/kg)[3]	5.20	6.00	7.47	8.43	9.40

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg. [2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994). [3]Concentration of amino acid on digestible basis.

Table 5. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 12-16 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	41.70	70.83	124.48	159.74	195.00
Wheat, White	614.7	598.5	568.8	549.2	529.6
Wheat Middlings	144.2	116.8	66.3	33.2	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	7.60	21.60	30.80	40.00
Soybean OiL	0.00	27.1	77.1	109.9	142.7
L-Lysine HCl	4.90	4.37	3.39	2.74	2.10
DL-Methionine	2.80	3.10	3.66	4.03	4.40
L-Threonine	2.10	2.42	3.02	3.41	3.80
Common Salt	1.40	1.38	1.35	1.32	1.30
Limestone	9.00	7.56	4.90	3.15	1.40
Dicalcium phosphate	15.50	15.60	15.77	15.89	16.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.44	11.38	13.12	14.27	15.41
Crude protein (g/kg)	146.5	162.2	191.1	210.0	229.0
Crude fibre (g/kg)	47.70	43.24	35.01	29.61	24.20
Ca (g/kg)	7.50	7.50	7.50	7.50	7.50
Available Phosphorus (g/kg)	3.80	3.80	3.80	3.80	3.80
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.30	2.22	2.08	1.99	1.90
K (g/kg)	6.70	6.66	6.59	6.55	6.50
Mn (mg/kg)	100.4	95.2	85.6	79.3	73.0
Zn (mg/kg)	98.93	93.84	84.45	78.29	72.12
Indispensable amino acids					
Arginine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Cystine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Isoleucine (g/kg)[3]	4.70	5.40	6.70	7.55	8.40
Lysine (g/kg)[3]	8.10	8.96	10.53	11.57	12.60
Methionine (g/kg)[3]	3.60	3.98	4.68	5.14	5.60
Phenylalanine (g/kg)[3]	5.00	5.74	7.11	8.00	8.90
Threonine (g/kg)[3]	5.20	6.02	7.52	8.51	9.50
Tryptophan (g/kg)[3]	1.70	1.87	2.19	2.39	2.60
Valine (g/kg)[3]	5.20	5.77	6.82	7.51	8.20
Dispensable					
Tyrosine (g/kg)[3]	4.30	5.00	6.30	7.15	8.00

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg. [2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994). [3]Concentration of amino acid on digestible basis.

Table 6. Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 16-20 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
	g/kg				
Fish meal - (72%-CP)	0.00	11.31	32.13	45.82	59.50
Soybean Meal - (48%-CP)	0.00	25.3	71.9	102.6	133.2
Wheat, White	639.6	630.0	612.2	600.5	588.8
Wheat Middlings	169.60	137.38	78.02	39.01	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	5.70	16.20	23.10	30.00
Soybean OiL	0.00	29.83	84.78	120.89	157.00
L-Lysine HCl	3.20	2.59	1.47	0.74	0.00
DL-Methionine	1.60	1.83	2.25	2.52	2.80
L-Threonine	0.20	0.39	0.74	0.97	1.20
Common Salt	1.40	1.34	1.24	1.17	1.10
Limestone	8.20	6.64	3.77	1.89	0.00
Dicalcium phosphate	12.50	12.54	12.61	12.65	12.70
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.48	11.52	13.43	14.69	15.95
Crude protein (g/kg)	129.5	142.5	166.5	182.3	198.0
Crude fibre (g/kg)	48.70	43.93	35.15	29.37	23.60
Ca (g/kg)	6.50	6.52	6.55	6.58	6.60
Available Phosphorus (g/kg)	3.20	3.16	3.09	3.05	3.00
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	1.90	1.81	1.63	1.52	1.40
K (g/kg)	6.20	6.09	5.88	5.74	5.60
Mn (mg/kg)	101.3	95.6	84.9	78.0	71.0
Zn (mg/kg)	100.8	95.2	84.8	78.0	71.1
Indispensable amino acids					
Arginine (g/kg)[3]	5.70	6.33	7.48	8.24	9.00
Cystine (g/kg)[3]	2.30	2.55	3.00	3.30	3.60
Isoleucine (g/kg)[3]	4.20	4.75	5.77	6.43	7.10
Lysine (g/kg)[3]	6.00	6.65	7.84	8.62	9.40
Methionine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Phenylalanine (g/kg)[3]	4.50	5.11	6.23	6.96	7.70
Threonine (g/kg)[3]	3.50	3.90	4.63	5.12	5.60
Tryptophan (g/kg)[3]	1.50	1.63	1.88	2.04	2.20
Valine (g/kg)[3]	4.70	5.37	6.59	7.40	8.20
Dispensable					
Tyrosine (g/kg)[3]	3.80	4.39	5.47	6.19	6.90

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg. [2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994). [3]Concentration of amino acid on digestible basis.

Water intake

A plastic header tank with a recorded weight of water was placed on the corner of each pen for water intake determination each week for a period of 24hours. On the day of water intake determination a turkey bell drinker was attached to the header tank and after 24hours the water intake was recorded as the difference between the water offered and the water remained in the header tank at both occasions. To get the measurements of evaporative losses five bell drinker with identical volume of water were placed each day at bird height and at different points within the experimental room but out of the reach of birds. The water measurements then were recorded as kg/bird/day after correcting the evaporative losses.

Feed intake

To determine the feed intake, the feed offered at the beginning of each growth phase was recorded and the weigh back was done at the end of each phase. During the digestibility trial (on 49th day of the trial), feed intake was determined separately to get the feed intake for 24hours. The values of daily feed intake were recorded in kg/day/bird.

Body weight (BW)

Birds were weighed individually before placing them in pens to get the initial weight and then on a 4 weekly basis birds in each pen were weighed individually to get the measurements for body weight gain. This was then converted to body weight gain in kg/day/bird.

Excreta collection

For the determination of dietary nutrient digestibility coefficients (i.e. DM, CP, amino acids, minerals, organic matter, ash and metabolisable energy) excreta were collected for a period of 24hours at 7 weeks of age. Excreta were freeze-dried, weighed and milled to pass through a 0.75mm mesh.

Litter quality, Footpad and Hock score determination

A visual assessment for litter score of the entire pen was done at the end of each feeding phase (at 8, 12, 16 and 20 weeks of age). The total area of the pen was scored by attributing a percentage value to the litter which scored 1 to 5 (Da Costa *et al*., 2014). A score 1 was given to a litter that was friable, and there was no capping or compaction; score 2 was given when there was a light capping, under a friable crumb surface; when the surface was capped and compacted the score was 3; score 4 was given when the surface was wet and sticky; when the litter depth was wet and dough-like the score was 5. A percentage of each pen was allotted the appropriate score, to the nearest 5%, in the relevant score category.

Litter score were calculated and recorded as follows:

[(1 x %) + (2 x %) + (3 x %) + (4 x %) + (5 x %)]/100

A lower score will be associated with better litter quality.

Litter NH_3, temperature (T°) and pH were determined at 8, 12, 16 and 20 weeks of age by using the pH probe placed directly in to the litter and in the center of each pen (Hanna HI 99163 meter, Hanna Instruments Ltd, Bedfordshire, UK). Atmospheric ammonia was measured using a handheld Dräger meter tube (Ammonia 2/a) attached to a Dräger Multi Gas Detector pump (Draeger Safety AG and Co. KGaA, Luebeck, Germany). Ammonia concentrations were recorded from each pen, almost 3 cm above litter surface and from the central point of the pen by stroking the pump five times (approximate one minute/pen). The Dräger tubes change from yellow to blue for a positive value for ammonia.

The principle of the reaction was:

NH? + pH indicator → blue reaction product.

Litter samples were taken from the centre and mid-way between centre and four corners of each pen at the end of each growth phase. The litter samples collected were combined and homogenized in plastic bags and the moisture contents were determined by placing in an oven at 80°C for 48 hours.

Footpad and hock lesions were scored for both the left and right leg, including all birds, and classified according to a scale from Hocking *et al*. (2008) from 0 (no lesion) to 4 (very severe lesions). All birds were scored at the end of week 8, 12, 16 and 20. A composite mean of the pen was used for statistical analysis.

Amino acid determination

The amino acid content of feed and excreta was determined by High performance liquid chromatography following oxygen-free hydrochloric acid digestion (Jones *et al.*, 1981). The system comprised a Dionex ASI-100 autosampler fitted with a Dionex P580 pump and a Dionex RF-2000 detector (Sunnyrale, California, USA). The flow rate used was 1 mL min^{-1} and the column used was a Spherisorb ODS2 (150x4.6mm fitted with a Waters guard cartridge). Since this method of hydrolysis destroys methionine, cystine and tryptophan, data on these amino acids are not reported. Metabolisability coefficient for glycine is not presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al.*, 1971).

Mineral determination

The procedure followed for mineral analyses (Na, Ca, P, K, Mg, Zn and Mn) in samples of feed and excreta was the same; the digestion of samples was carried out by using Microwave Accelerated Reaction System (MARS) as used for the rapid preparation of sample for atomic absorption and the optical plasma emission spectrometry (Optima 4300 DV Dual View ICPOE spectrometer, Perkin Elmer, Beaconsfield, UK), (Tanner *et al.*, 2002).

Statistical procedure

Seven replicates per treatment were used for the experiment with a total of one hundred and seventy five turkeys. For the analysis of data, statistical measurements, average, and standard errors of differences of means were obtained for all numeric variables analysed (descriptive statistical techniques).

Randomised complete block analysis of variance (ANOVA) model, with two factors (treatment and time) for repeated measures, including the Greenhouse–Geiser degrees of freedom corrections and ANOVA for two factors, when the analysis was performed between treatments and times (inferential statistical techniques) (Zar, 1999). The model included dietary nutrient density (5 levels of dietary nutrient concentration), time (weeks ending the growth phase i.e. 8, 12, 16 and 20),and the interaction between dietary density and weeks ending the growth phases. The pens were treated as experimental units. Orthogonal polynomials were also used for average values of all numeric variables (e.g. litter moisture, litter NH_3, litter pH etc.) to compare treatment differences for linear and quadratic relationships with increasing dietary nutrient concentration. Comparison contrast test was used on the average values of all numeric variables analysed (above mentioned) to compare low nutrient density diets (i.e. 77 and 85% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation) as well as high nutrient density diets (i.e. 110 and 120% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation).

However, for data i.e. Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), neutral detergent fibre intake (NDF I), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) and amino acid intake, excretion, retention and digestibility values determined after 7th weeks of birds age (at 49th day of birds age). The data entered on an Excel spreadsheet and Genstat software, release 11 (IACR Rothamstead, Harpenden, Hertfordshire) was used to perform ANOVA for the comparison of different treatments for litter quality parameters i.e. moisture, NH_3, pH and temperature and other parameters such as water intake, feed intake, body weight gain, feed conversion efficiency and nutrient digestibility. Correlation coefficients were also generated on average values to test for a possible relationship between different variables. Differences were reported as significant at P<0.05 and trends were noted when the P value was near to 0.1.

The data obtained for FPS and HBS were compared using the values (weighted means for each pen for TFPS and THS) for each pen for good hock (GHS), bad hock (BHS), total hock (THS) scores and for good footpad (GFPS), bad footpad (BFPS) and total footpad (TFPS) scores, by using ANOVA for the comparison of different treatments. There were not enough different non zero scores to make a multinomial analyses (or chi-squared) possible for FPS and HBS data (real values) and also, it was not possible to incorporate the random structure in the data using Chi-squared, however, since the residual plot were unacceptable after running Residual maximum likelihood (REML). Therefore, generalized linear mixed models (GLMM), were fitted using residual maximum likelihood (REML) to binary data: FPD>0, or not, and HB>0, or not (binomial, link logit transformed) and fixed effects time+treatment and random effects bird weight category, block and pen with dispersion fixed at 1. There was not enough information in the data to include the interaction term (i.e. time x treatment). The P-values, estimated means, SEMs and back transformed means are reported in the result tables. Since no FP lesions appeared at the end of week 8 the data for FPS, this time point was not included in analysis.

RESULTS

The birds remained healthy and overall mortality was less than 1% throughout the experiment, with no significant difference between treatment groups (data not shown).

The Analysed chemical composition of the basal diets is presented in tables (table 7 to 10). The analysed values for the concentration of CP content were lower than the calculated values in table 3 to 6, however, the analysed values for K, Ca and Na concentration were higher than the calculated values. Digestible amino acid data taken from the literature was derived from studies on the birds of varying breed, sex and age as well as method of digestibility determination (ileal and total tract). In contrast the data collected during the course of this study has been obtained from controlled groups of birds of same breed, sex and age as well as using total tract method for digestibility determination, so no comparison is made here.

Water intake measurements

Increased nutrient density had a negative effect on water intake (WI) and feed intake used for water:feed determination (feed intake measured for 24 hours time period to determine water:feed, FI W:F) which decreased linearly (P<0.01 and 0.001, respectively) as the density increased (table 16). However there was no effect (P>0.05) of the dietary nutrient density recorded on water:feed (W:F). The WI, FI W:F linearly increased (P<0.001) with the increase of the age of the birds, the WI and FI W:F values were observed during the last feeding phase of the study. The increase of the birds age had a negative effect (P<0.01) on W:F and the lowest values were recorded in the last two feeding phases of the study (table 16). The results for WI, FI W:F and W:F were subject to a dietary density x time interaction (P<0.001 for WI and P<0.05 for the rest), showing that the responses to feed density were different during growing periods. For example, an increase in nutrient density during the first feeding phase led to an increase in WI, although the response during the rest of the feeding phases was the opposite and the WI decreased when nutrient density increased. An increase in dietary density did not have significant effect on the FI W:F during the first two feeding phases, but led to a decrease FI during the last two feeding phases. Dietary density increased W:F during the first feeding phase, although the responses of W:F were inconsistent for the rest of the study.

Table 7. Analysed composition of experimental diets for 4-8 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	868.8	868.9	869.2	869.3	869.5
Crude protein (g/kg)	193.2	215.7	257.2	284.4	312.1
Gross energy (MJ/kg)	16.27	16.77	17.70	18.31	18.94
Ash (g/kg)	64.74	64.92	65.26	65.48	65.77
Crude fat (g/kg)	30.24	46.95	77.73	97.96	118.32
Neutral detergent fibre (g/kg)	99.94	89.10	69.15	56.04	42.98
Ca (g/kg)	11.64	11.36	10.85	10.51	10.18
Total Phosphorous (g/kg)	8.64	8.68	8.76	8.81	8.87
Na (g/kg)	1.13	1.26	1.51	1.67	1.83
K (g/kg)	9.56	9.89	10.50	10.90	11.31
Cu (mg/kg)	19.55	19.68	19.93	20.09	20.27
Mg (g/kg)	2.00	1.97	1.90	1.86	1.83
Mn (mg/kg)	139.0	135.2	128.3	123.7	119.2
Zn (mg/kg)	125.1	128.3	134.1	137.9	141.8
Indispensable amino acids					
Arginine (g/kg)	9.84	11.01	13.16	14.57	16.01
Histidine (g/kg)	3.56	4.03	4.90	5.48	6.06
Isoleucine (g/kg)	8.32	9.49	11.63	13.04	14.47
Leucine (g/kg)	13.59	15.43	18.83	21.06	23.32
Lysine (g/kg)	10.62	12.06	14.71	16.45	18.21
Methionine (g/kg)	3.14	3.59	4.41	4.96	5.51
Phenylalanine (g/kg)	8.98	10.04	11.99	13.27	14.56
Threonine (g/kg)	7.02	8.19	10.34	11.75	13.18
Valine (g/kg)	8.80	9.93	12.01	13.37	14.76
Dispensable					
Alanine (g/kg)	6.95	7.93	9.73	10.91	12.11
Aspartic acid (g/kg)	16.85	19.20	23.52	26.36	29.23
Glutamic acid (g/kg)	39.98	43.55	50.13	54.46	58.85
Glycine (g/kg)	5.96	6.84	8.47	9.55	10.63
Serine (g/kg)	6.01	6.88	8.49	9.55	10.62
Tyrosine (g/kg)	5.01	5.72	7.03	7.89	8.76

Table 8. Analysed composition of experimental diets for 8-12 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	850.9	849.7	847.3	845.8	844.3
Crude protein (g/kg)	156.3	176.8	214.1	238.7	263.0
Gross energy (MJ/kg)	15.87	16.51	17.67	18.44	19.19
Ash (g/kg)	59.57	59.08	58.10	57.53	56.89
Crude fat (g/kg)	23.83	45.60	85.46	111.63	137.57
Ca (g/kg)	9.62	9.49	9.25	9.10	8.95
Total Phosphorous (g/kg)	7.98	7.88	7.68	7.56	7.44
Na (g/kg)	0.60	0.74	1.00	1.18	1.35
K (g/kg)	7.74	7.99	8.44	8.74	9.03
Cu (mg/kg)	16.08	16.50	17.24	17.75	18.23
Mg (g/kg)	1.96	1.91	1.81	1.75	1.69
Mn (mg/kg)	120.8	118.8	114.8	112.3	109.7
Zn (mg/kg)	124.3	128.5	136.0	141.1	146.0
Indispensable amino acids					
Arginine (g/kg)	6.73	7.93	10.11	11.55	12.97
Histidine (g/kg)	2.57	3.08	4.02	4.64	5.25
Isoleucine (g/kg)	5.96	7.18	9.41	10.89	12.34
Leucine (g/kg)	10.31	12.34	16.03	18.47	20.87
Lysine (g/kg)	8.60	9.78	11.92	13.33	14.73
Methionine (g/kg)	3.11	3.59	4.46	5.04	5.60
Phenylalanine (g/kg)	6.60	7.84	10.10	11.59	13.07
Threonine (g/kg)	4.77	5.94	8.06	9.46	10.85
Valine (g/kg)	6.83	7.89	9.82	11.09	12.35
Dispensable					
Alanine (g/kg)	5.17	6.06	7.68	8.75	9.80
Aspartic acid (g/kg)	11.52	14.08	18.76	21.84	24.89
Glutamic acid (g/kg)	30.74	34.65	41.77	46.47	51.10
Glycine (g/kg)	5.12	6.05	7.75	8.86	9.97
Serine (g/kg)	4.37	5.21	6.74	7.75	8.75
Tyrosine (g/kg)	3.53	4.26	5.58	6.45	7.31

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Table 9. Analysed composition of experimental diets for 12-16 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.3	849.8	850.6	851.2	851.7
Crude protein (g/kg)	138.1	156.8	191.1	213.6	236.3
Gross energy (MJ/kg)	15.75	16.38	17.51	18.25	19.01
Ash (g/kg)	51.45	51.87	52.58	53.01	53.51
Crude fat (g/kg)	20.12	40.87	79.13	104.2	129.5
Ca (g/kg)	8.66	8.75	8.91	9.01	9.12
Total Phosphorous (g/kg)	7.37	7.39	7.43	7.45	7.48
Na (g/kg)	0.68	0.76	0.91	1.01	1.11
K (g/kg)	6.79	6.93	7.18	7.33	7.50
Cu (mg/kg)	18.08	19.49	22.08	23.76	25.47
Mg (g/kg)	1.70	1.64	1.52	1.44	1.36
Mn (mg/kg)	124.8	126.6	129.7	131.7	133.8
Zn (mg/kg)	114.6	116.7	120.4	122.8	125.2
Indispensable amino acids					
Arginine (g/kg)	5.90	6.92	8.79	10.01	11.25
Histidine (g/kg)	2.42	2.85	3.64	4.16	4.69
Isoleucine (g/kg)	5.31	6.28	8.05	9.21	10.38
Leucine (g/kg)	9.20	10.66	13.35	15.10	16.88
Lysine (g/kg)	8.57	9.68	11.73	13.08	14.43
Methionine (g/kg)	3.89	4.44	5.44	6.10	6.76
Phenylalanine (g/kg)	6.16	7.01	8.58	9.61	10.65
Threonine (g/kg)	4.56	5.58	7.47	8.70	9.95
Valine (g/kg)	6.65	7.62	9.41	10.58	11.77
Dispensable					
Alanine (g/kg)	4.71	5.53	7.04	8.03	9.03
Aspartic acid (g/kg)	9.64	11.62	15.27	17.66	20.07
Glutamic acid (g/kg)	32.21	35.43	41.34	45.20	49.12
Glycine (g/kg)	4.80	5.72	7.41	8.52	9.64
Serine (g/kg)	3.98	4.73	6.10	7.00	7.91
Tyrosine (g/kg)	2.90	3.41	4.36	4.99	5.61

Table 10. Analysed composition of experimental diets for 16-20 weeks growth phase of turkeys

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.7	851.3	854.2	856.2	858.1
Crude protein (g/kg)	120.0	133.7	159.3	176.1	193.1
Gross energy (MJ/kg)	15.77	16.42	17.64	18.45	19.27
Ash (g/kg)	46.41	45.85	44.88	44.23	43.59
Crude fat (g/kg)	20.06	44.73	90.44	120.65	151.01
Ca (g/kg)	8.50	8.40	8.22	8.10	7.98
Total Phosphorous (g/kg)	6.72	6.79	6.91	7.00	7.08
Na (g/kg)	0.77	0.83	0.95	1.03	1.12
K (g/kg)	6.04	6.04	6.06	6.08	6.09
Cu (mg/kg)	17.68	17.28	16.56	16.09	15.62
Mg (g/kg)	1.62	1.54	1.39	1.30	1.20
Mn (mg/kg)	123.3	121.9	119.7	118.2	116.7
Zn (mg/kg)	122.4	124.8	129.4	132.5	135.6
Indispensable amino acids					
Arginine (g/kg)	4.65	5.32	6.58	7.41	8.25
Histidine (g/kg)	2.04	2.27	2.70	2.99	3.28
Isoleucine (g/kg)	4.30	5.10	6.59	7.57	8.55
Leucine (g/kg)	7.76	8.95	11.15	12.61	14.07
Lysine (g/kg)	5.96	6.59	7.77	8.55	9.34
Methionine (g/kg)	1.92	2.40	3.29	3.88	4.47
Phenylalanine (g/kg)	5.29	5.98	7.26	8.11	8.97
Threonine (g/kg)	2.55	3.12	4.19	4.89	5.60
Valine (g/kg)	5.12	5.91	7.38	8.35	9.33
Dispensable					
Alanine (g/kg)	3.74	4.30	5.33	6.01	6.70
Aspartic acid (g/kg)	7.34	8.92	11.87	13.81	15.77
Glutamic acid (g/kg)	29.39	31.68	35.94	38.76	41.60
Glycine (g/kg)	4.15	4.89	6.27	7.18	8.09
Serine (g/kg)	3.21	3.66	4.51	5.06	5.62
Tyrosine (g/kg)	2.08	2.50	3.26	3.77	4.28

Table 11. Effect of dietary nutrient concentration and time on litter moisture (LM), litter ammonia (NH_3, ppm), litter pH (pH), litter temperature (T°) and litter score (LS) parameters.

	Treatments		LM	NH_3	pH	T°	LS
	Diets						
	T1		362.5	6.57	7.74	20.74	2.08
	T2		328.9	6.81	7.85	20.45	1.88
	T3		328.2	8.53	8.21	20.37	1.75
	T4		297.8	8.87	8.15	20.61	1.70
	T5		280.5	9.50	8.12	20.69	1.59
SEM			29.05	0.371	0.069	0.119	0.129
	Time (wks)						
	4-8		225.6	3.21	7.63	21.02	1.43
	8-12		318.0	14.42	8.58	19.83	1.80
	12-16		358.5	9.69	8.13	20.52	2.03
	16-20		376.2	4.90	7.71	20.92	1.94
SEM			9.52	0.268	0.070	0.121	0.044
	Diets	**Time (wks)**					
	T1	4-8	244.0	2.91	7.69	20.98	1.50
	T2	4-8	236.2	3.16	7.49	21.21	1.47
	T3	4-8	232.1	3.73	8.01	20.80	1.44
	T4	4-8	208.7	2.63	7.49	21.11	1.40
	T5	4-8	207.1	3.59	7.47	21.00	1.36
	T1	8-12	348.4	12.50	8.37	20.26	2.07
	T2	8-12	335.1	13.14	8.42	19.61	2.06
	T3	8-12	318.0	14.84	8.64	19.69	1.70
	T4	8-12	302.5	15.07	8.76	19.51	1.69
	T5	8-12	286.0	16.54	8.71	20.06	1.49
	T1	12-16	422.2	7.07	7.53	20.66	2.27
	T2	12-16	355.4	7.07	7.94	20.31	2.15
	T3	12-16	377.8	10.81	8.39	20.19	2.11
	T4	12-16	323.3	10.79	8.40	20.74	1.85
	T5	12-16	313.6	12.71	8.40	20.69	1.76
	T1	16-20	435.5	3.79	7.37	21.06	2.49
	T2	16-20	388.7	3.86	7.55	20.64	1.83
	T3	16-20	384.8	4.71	7.79	20.79	1.76
	T4	16-20	356.7	7.00	7.97	21.09	1.84
	T5	16-20	315.4	5.14	7.88	21.03	1.75
SEM			27.60	0.638	0.152	0.263	0.129
			Probabilities of statistical differences				
Diets			P=0.08	<0.001	<0.001	NS	<0.05
Linear			<0.01	<0.001	NS	NS	<0.001
Quadratic			NS	NS	P=0.06	NS	NS
Contrast 1			NS	<0.001	NS	NS	P=0.07
Contrast 2			NS	NS	NS	NS	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			NS	<0.01	NS	NS	<0.05

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 12. Effect of dietary nutrient concentration and time on leg health parameters i.e. good hock score (GHS), bad hock score (BHS) and total hock score (THS).

	Treatments		GHS	BHS	THS
	Diets				
	T1		0.721	0.279	0.329
	T2		0.829	0.171	0.302
	T3		0.657	0.343	0.491
	T4		0.670	0.330	0.462
	T5		0.559	0.441	0.868
SEM			0.0607	0.0607	0.1150
	Time (wks)				
	4-8		0.456	0.544	0.726
	8-12		0.696	0.304	0.501
	12-16		0.811	0.189	0.333
	16-20		0.559	0.214	0.401
SEM			0.0324	0.0324	0.0493
	Diets	**Time (wks)**			
	T1	4-8	0.543	0.457	0.543
	T2	4-8	0.600	0.400	0.571
	T3	4-8	0.500	0.500	0.621
	T4	4-8	0.314	0.686	0.800
	T5	4-8	0.321	0.679	1.093
	T1	8-12	0.757	0.243	0.300
	T2	8-12	0.807	0.193	0.371
	T3	8-12	0.664	0.336	0.486
	T4	8-12	0.771	0.229	0.286
	T5	8-12	0.479	0.521	1.064
	T1	12-16	0.779	0.221	0.250
	T2	12-16	0.936	0.064	0.150
	T3	12-16	0.814	0.186	0.314
	T4	12-16	0.800	0.200	0.371
	T5	12-16	0.729	0.271	0.579
	T1	16-20	0.807	0.193	0.221
	T2	16-20	0.971	0.029	0.114
	T3	16-20	0.650	0.350	0.543
	T4	16-20	0.793	0.207	0.393
	T5	16-20	0.707	0.293	0.736
SEM			0.0873	0.0873	0.1495
	Probabilities of statistical differences				
Diets			P=0.06	P=0.06	<0.05
Linear			<0.05	<0.05	<0.01
Quadratic			Ns	NS	NS
Contrast 1			NS	NS	NS
Contrast 2			NS	NS	NS
Time			<0.001	<0.001	<0.001
Diets x Time			NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

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Table 13. Effect of dietary nutrient concentration and time on leg health parameters i.e. good footpad score (GFPS), bad footpad score (BFPS) and total footpad score (TFPS).

	Treatments		GFPS	BFPS	TFPS
	Diets				
	T1		0.876	0.124	0.167
	T2		0.879	0.121	0.160
	T3		0.867	0.133	0.117
	T4		0.857	0.143	0.226
	T5		0.905	0.095	0.105
SEM			0.0471	0.0471	0.0805
	Time (wks)				
	4-8		--	--	--
	8-12		0.721	0.279	0.350
	12-16		0.970	0.030	0.036
	16-20		0.939	0.061	0.079
SEM			0.0308	0.0308	0.0405
	Diets	**Time (wks)**			
	T1	4-8	--	--	--
	T2	4-8	--	--	--
	T3	4-8	--	--	--
	T4	4-8	--	--	--
	T5	4-8	--	--	--
	T1	8-12	0.750	0.250	0.350
	T2	8-12	0.729	0.271	0.357
	T3	8-12	0.664	0.336	0.286
	T4	8-12	0.714	0.286	0.479
	T5	8-12	0.750	0.250	0.279
	T1	12-16	1.000	0.000	0.000
	T2	12-16	0.971	0.029	0.029
	T3	12-16	0.971	0.029	0.029
	T4	12-16	0.943	0.057	0.086
	T5	12-16	0.964	0.036	0.036
	T1	16-20	0.879	0.121	0.150
	T2	16-20	0.936	0.064	0.093
	T3	16-20	0.964	0.036	0.036
	T4	16-20	0.914	0.086	0.114
	T5	16-20	1.000	0.000	0.000
SEM			0.0734	0.0734	0.1090
Probabilities of statistical differences					
Diets			NS	NS	NS
Linear			NS	NS	NS
Quadratic			NS	NS	NS
Contrast 1			NS	NS	NS
Contrast 2			NS	NS	NS
Time			<0.001	<0.001	<0.001
Diets x Time			NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 14. Effect of dietary nutrient concentration and time on leg health parameters i.e. incidences of hock burn (HB) and incidences of footpad dermatitis (FPD), from generalized linear mixed models (GLMM) on logit scale and back transformed on proportion scale (i.e. % of birds with HB>0, FPD>0).

	Treatments	Logit of HB Incidence	Incidence of HB>0	Logit of FPD Incidence	Incidence of FPD>0
	Diets				
	T1	-1.317	21.13	-2.632	6.71
	T2	-2.057	11.33	-2.527	7.40
	T3	-0.799	31.03	-2.856	5.44
	T4	-0.970	27.49	-2.408	8.25
	T5	-0.308	42.37	-2.828	5.58
Min and max SEM		0.5121-0.5510		0.5528-0.5915	
	Time (wks)				
	4-8	0.225	55.59	--	--
	8-12	-1.104	24.89	-1.200	23.15
	12-16	-1.830	13.83	-3.758	2.28
	16-20	-1.651	16.10	-2.993	4.77
Min and max SEM		0.4231-0.4458		0.2772-0.5117	
Probabilities of statistical differences					
Diets		<0.05		NS	
Time		<0.001		<0.001	

There is a statistical significant difference when P<0.05; SEM- standard errors of means (min= Minimum and max= Maximum). The p-values and SEMs are associated with the estimated means on the logit scale of the analysis.

Table 15. Effect of dietary nutrient concentration, time (growth phases) and their interaction on total weight gain ((TWG) kg/b/4 weeks), weight gain ((WG) kg/b/d), feed intake ((FI) kg/b/d), feed conversion efficiency ((FCE) wt gain kg/kg FI) and protein efficiency ratio (PER, wt gain kg/CP intake g).

	Treatments		TWG	WG	FI	FCE	PER
	Diets						
	T1		4.12	0.147	0.479	0.354	1.84
	T2		4.45	0.159	0.519	0.359	1.96
	T3		4.57	0.163	0.462	0.401	2.03
	T4		4.49	0.160	0.433	0.417	2.13
	T5		4.66	0.166	0.410	0.453	2.12
SEM			0.078	0.0028	0.0146	0.0072	0.105
	Time (wks)						
	4-8		3.34	0.119	0.201	0.597	2.49
	8-12		5.00	0.179	0.429	0.419	2.14
	12-16		5.15	0.184	0.600	0.311	1.78
	16-20		4.34	0.155	0.613	0.259	1.66
SEM			0.051	0.0018	0.0069	0.0045	0.033
	Diets	**Time (wks)**					
	T1	4-8	3.18	0.114	0.208	0.551	2.34
	T2	4-8	3.25	0.116	0.211	0.554	2.42
	T3	4-8	3.32	0.119	0.201	0.592	2.40
	T4	4-8	3.41	0.122	0.194	0.629	2.62
	T5	4-8	3.53	0.126	0.192	0.659	2.68
	T1	8-12	4.62	0.165	0.446	0.372	1.96
	T2	8-12	4.92	0.176	0.456	0.387	2.05
	T3	8-12	5.09	0.182	0.425	0.428	2.08
	T4	8-12	5.10	0.182	0.420	0.434	2.30
	T5	8-12	5.26	0.188	0.396	0.477	2.29
	T1	12-16	5.02	0.179	0.632	0.287	1.65
	T2	12-16	5.12	0.183	0.663	0.277	1.69
	T3	12-16	5.09	0.182	0.583	0.314	1.87
	T4	12-16	5.20	0.186	0.582	0.321	1.87
	T5	12-16	5.30	0.189	0.541	0.356	1.81
	T1	16-20	3.65	0.130	0.632	0.207	1.42
	T2	16-20	4.52	0.161	0.747	0.217	1.66
	T3	16-20	4.75	0.170	0.640	0.268	1.78
	T4	16-20	4.24	0.152	0.534	0.285	1.73
	T5	16-20	4.55	0.163	0.512	0.319	1.71
SEM			0.126	0.0045	0.0198	0.0113	0.123
Probabilities of statistical differences							
Diets			<0.001	<0.001	<0.001	<0.001	NS
Linear			<0.001	<0.001	<0.001	<0.001	<0.05
Quadratic			NS	NS	NS	NS	NS
Contrast 1			<0.01	<0.01	<0.05	<0.001	NS
Contrast 2			NS	NS	<0.05	<0.001	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			<0.01	<0.01	<0.001	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 16. Effect of dietary nutrient concentration, time (growth phases) and their interaction on water intake ((WI) kg/b/d), feed intake for water ratio feed (FI W:F) kg/b/d) and water ratio feed ((W:F) kg/kg).

	Treatments		WI	FI W:F	W:F
	Diets				
	T1		0.843	0.500	1.73
	T2		0.823	0.518	1.69
	T3		0.791	0.479	1.75
	T4		0.738	0.458	1.72
	T5		0.684	0.402	1.81
SEM			0.0381	0.0191	0.050
	Time (wks)				
	4-8		0.471	0.219	2.15
	8-12		0.788	0.449	1.76
	12-16		0.855	0.581	1.48
	16-20		0.989	0.635	1.57
SEM			0.0180	0.0101	0.029
	Diets	**Time (wks)**			
	T1	4-8	0.439	0.227	1.93
	T2	4-8	0.459	0.222	2.07
	T3	4-8	0.452	0.209	2.15
	T4	4-8	0.501	0.224	2.24
	T5	4-8	0.506	0.214	2.36
	T1	8-12	0.792	0.471	1.69
	T2	8-12	0.841	0.478	1.77
	T3	8-12	0.858	0.459	1.86
	T4	8-12	0.736	0.432	1.71
	T5	8-12	0.711	0.402	1.77
	T1	12-16	1.004	0.640	1.58
	T2	12-16	0.922	0.629	1.48
	T3	12-16	0.832	0.581	1.44
	T4	12-16	0.767	0.551	1.40
	T5	12-16	0.752	0.505	1.50
	T1	16-20	1.136	0.660	1.73
	T2	16-20	1.070	0.742	1.45
	T3	16-20	1.023	0.665	1.53
	T4	16-20	0.946	0.624	1.52
	T5	16-20	0.768	0.486	1.61
SEM			0.0516	0.0279	0.075
Probabilities of statistical differences					
Diets			<0.05	<0.01	NS
Linear			<0.01	<0.001	NS
Quadratic			NS	P=0.09	NS
Contrast 1			NS	NS	NS
Contrast 2			NS	<0.05	NS
Time			<0.001	<0.001	<0.001
Diets x Time			<0.001	<0.01	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 17. The effect of dietary protein and energy on growth performance, water intake, litter quality and nutrient utilisation parameters of turkeys

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Energy efficiency ratio (EER, kg/MJ)	0.054	0.036	0.032	0.034	0.028	0.0056	<0.05	<0.01	NS	P=0.06	NS
N Excreted (g/b/d)	3.810	3.867	4.775	5.184	5.945	0.3170	<0.001	<0.001	NS	<0.05	P=0.05
AAN (g/b/d)	0.935	1.406	1.586	1.599	2.170	0.1586	<0.001	<0.001	NS	<0.05	NS
UAN (g/b/d)	1.521	2.461	3.189	3.585	3.775	0.1934	<0.001	<0.001	<0.05	<0.001	<0.05
NDF I (g/b/d)	18.03	16.29	12.08	9.47	7.17	0.366	<0.001	<0.001	NS	<0.001	<0.001
AME (MJ/kg)	11.53	13.43	15.17	16.04	17.44	0.422	<0.001	<0.001	NS	<0.001	<0.01
AMEn (MJ/kg)	10.92	12.62	14.20	15.04	16.24	0.542	<0.001	<0.001	NS	<0.001	<0.01
AME I (MJ/b/d)	2.07	2.46	2.65	2.71	2.91	0.084	<0.001	<0.001	NS	<0.001	NS
CPD	0.499	0.595	0.597	0.554	0.609	0.0293	P=0.081	P=0.08	NS	NS	NS
DMD	0.587	0.664	0.701	0.709	0.746	0.0241	<0.001	<0.001	NS	<0.05	NS
OMD	0.622	0.690	0.724	0.731	0.766	0.0221	<0.001	<0.001	NS	<0.05	NS

Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) were determined at 49th days of age. However, AME I values represents for growth phase 4-8 weeks were obtained on dry matter basis. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

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Table 18. The effect of dietary protein and energy on total tract amino acid digestibility coefficients by turkeys at 8 weeks of age.

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Alanine	0.730	0.782	0.821	0.843	0.871	0.0133	<0.001	<0.001	NS	<0.001	<0.05
Arginine	0.856	0.873	0.903	0.910	0.921	0.0080	<0.001	<0.001	NS	<0.001	NS
Aspartic acid	0.766	0.818	0.842	0.866	0.872	0.0164	<0.001	<0.001	NS	<0.05	NS
Glutamic acid	0.864	0.888	0.895	0.895	0.911	0.0083	<0.01	<0.001	NS	P=0.06	NS
Histidine	0.838	0.867	0.887	0.900	0.894	0.0136	<0.05	<0.01	NS	<0.05	NS
Isoleucine	0.782	0.825	0.856	0.859	0.883	0.0135	<0.001	<0.001	NS	<0.01	NS
Leucine	0.781	0.827	0.858	0.859	0.905	0.0147	<0.001	<0.001	NS	<0.01	NS
Lysine	0.834	0.864	0.896	0.900	0.917	0.0093	<0.001	<0.001	NS	<0.001	NS
Phenylalanine	0.783	0.826	0.852	0.840	0.870	0.0118	<0.001	<0.001	NS	<0.01	NS
Serine	0.819	0.849	0.877	0.879	0.895	0.0102	<0.001	<0.001	NS	<0.01	NS
Threonine	0.805	0.845	0.871	0.874	0.892	0.0099	<0.001	<0.001	NS	<0.001	NS
Tyrosine	0.816	0.857	0.881	0.889	0.905	0.0104	<0.001	<0.001	NS	<0.01	NS
Valine	0.731	0.787	0.822	0.831	0.868	0.0163	<0.001	<0.001	NS	<0.01	NS

Amino acids digestibilities were determined at 49th days of age. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 19. Correlation matrix for bird performance, litter quality, dietary nutrient digestibility, and leg health in response changes in nutrient density.

	FI	WG	FCE	WI	W:F	LS	LM	NH_3	CPD	DMD	HBS
WG	-0.490										
FCE	-0.918	0.787									
WI	0.890	-0.757	-0.980								
W:F	-0.808	0.486	0.796	-0.733							
LS	0.732	-0.941	-0.933	0.920	-0.595						
LM	0.737	-0.846	-0.915	0.959	-0.549	0.955					
NH_3	-0.882	0.817	0.972	-0.935	0.671	-0.953	-0.900				
CPD	-0.176	0.929	0.545	-0.522	0.344	-0.760	-0.657	0.552			
DMD	-0.666	0.968	0.899	-0.885	0.555	-0.996	-0.940	0.924	0.814		
HBS	-0.831	0.709	0.922	-0.906	0.930	-0.810	-0.806	0.813	0.561	0.781	
FPS	0.128	-0.415	-0.283	0.185	-0.663	0.252	0.106	-0.167	-0.560	-0.280	-0.557

d.f. = 33 Correlation coefficients greater than 0.349 and 0.449 are statistically significant at 5% ($P<0.05$) and 1% level ($P<0.001$), respectively.
Key:FI (feed intake), WG (weight gain), FCE (feed conversion efficiency), WI (water intake), W:F (water to feed ratio), LS (litter score), LM (litter moisture content), NH_3 (ammonia in litter), CPD (crude protein digestibility), DMD (dry matter digestibility), HBS (hock burn scores) and FPS (footpad dermatitis scores).

Litter quality associated parameters

Increased nutrient density had a negative effect on litter moisture (LM), and litter score (LS) which decreased in a linear way (P<0.01 and 0.001, respectively) as the density increased (table 11). However, the LM and LS linearly increased (P<0.001) with the increase of the age of the birds, the highest LM and LS values were observed during the last feeding phases of the study. Increased nutrient density had a positive effect on litter ammonia (NH_3) which increased in a linear way (P<0.001) as the density increased (table 11). The time response of litter NH_3 concentration was also quadratic (P<0.01) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases. Litter pH tended (P=0.06) to have a quadratic response to dietary density. The time response of litter pH was also quadratic (P<0.001) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases. Litter temperature (T°) was not affected by dietary density (P>0.05) but responded in a quadratic manner to time as the lowest T° was observed between 8-12 weeks of age. The results for litter ammonia and litter score (NH_3 and LS, respectively) were subject to a dietary density x time interaction (P<0.05), showing that there were different patterns of response during different growing phases. For example, the response of the LS to diets T4 and T5 seems not to be influenced by the feeding phase although the response of feeding the rest of the diets tended to follow a quadratic pattern. The response of litter NH_3 to dietary density during different feeding phases was also inconsistent. The comparison contrast test did not find a difference in LM, pH, T° and LS between diet T3 and low nutrient density group (T1 and T2) as well as diet T3 and higher nutrient density group (T4 and T5). However, significantly higher litter $NH_3$was recorded in groups fed the control diet when compared with groups fed lower nutrient density diets, whereas, no difference (P>0.05) was recorded when the control diet fed group was compared with higher nutrient density fed groups.

Leg health parameters

As nutrient density increased so did the prevalence of hock burn (P<0.05). Increasing nutrient density had a negative linear effect (P<0.05) on good hock scores (GHS). It, however, resulted in a linear increase in bad hock scores (BHS) and total hock scores (THS) (P<0.05 and P<0.01, respectively) (table 12). The growth phases had significant effect (P<0.001) on all hock score parameters, where GHS increased with growth phases, conversely BHS and THS decreased as the bird aged. There was no time and diets interaction noted (P>0.05) for hock burn parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05). There was no effect of nutrient densities observed (P>0.05) for the footpad quality score (table 13). However, growth phase had a significant effect (P<0.001) on all foot score parameters, where good footpad scores (GFPS) increased with growth phases, conversely bad footpad scores (BFPS) and total footpad scores (TFPS) decreased (P<0.001) as the birds aged. There was no time by diets interaction noted (P>0.05) for footpad quality parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05) (table 13).

As for hock burn (HB) the results obtained showed an increase in HB incidence in birds fed diet containing higher nutrient density (P<0.05). However, there was a significant decrease (P<0.001) in the incidence of HB as birds grew older 56% vs. 16% birds with HB>0 at the end of week 8 and 20, respectively. The incidence of footpad dermatitis (FPD) however, was not affected by treatment (P>0.05). However, the effect of time period was significant (P<0.001) for both HB and FPD as there were higher incidences recorded at the end of weeks 8 and 12, respectively which fell at the end of week 16 with an increase at week 20.

Correlations between variables are shown in (table 19). Hock burn score (HBS) was associated with many of the parameters and in particular water to feed ratio (r = 0.930; P<0.001), feed conversion efficiency (r = 0.922; P<0.001), water intake (r = -0.906; P<0.001) and ammonia in litter (r = 0.813; P<0.001). Interestingly, footpad score (FPS) was only associated with the water to feed ratio (r = - 0.663; P<0.001).

Growth performance, dietary nutrient intake and utilisation

Overall body weight (BW) was higher than the breed standards at 20 weeks of age, i.e. 18.81 kg vs. target of 15.18 kg (data not included in tables). Increased nutrient density had a positive effect on total weight gain (TWG), weight gain (WG) and feed conversion efficiency (FCE) which increased following a linear pattern (P<0.001) when density increased (table 15). Increasing nutrient density had a negative linear effect (P<0.001) on feed intake (FI). TWG and WG increase (P<0.001) with the increase in the age of the birds whereas FCE decreased linearly (P<0.001) with the increase in the age of the birds. The protein efficiency ratio (PER) response to feed density was also linear (P<0.05) and as expected, the PER decreased (P<0.001) with age. The FCE value for the control diet was higher (P<0.001) than the lower nutrient density fed group, and lower (P<0.001) than the higher nutrient density fed group, respectively (table 15). The results for TWG, WG and FI were subject to a dietary density x time interaction (P<0.001), showing that the responses to feed density differed with age. The response of TWG and WG to nutrient density was linear (P<0.001) during the growth phases consist of 4-8 and 8-12 weeks. While a non-significant (P>0.05) effect of dietary nutrient density on these parameters were recorded during 12-16 weeks time period, whereas, the response of these parameters to dietary nutrient density was quadratic (P<0.05) during time period 16-20 weeks. The response of FI to nutrient density was linear (P<0.001) during growth phases consisting of 4-8, 8-12 and 12-16 weeks. Whereas, the response of FI to dietary nutrient density was quadratic (P<0.05) from 16-20 weeks.

Nutrient density had a positive and linear effect (P<0.001) on dry matter digestibility (DMD) and organic matter digestibility (OMD), whereas the effect of nutrient density on dietary crude protein digestibility (CPD) only approached significance (P=0.081) (table 17). No difference (P>0.05) existed for the CPD when the comparison was made between birds fed control diet (T3-100% of standard breed recommendation) and lower nutrient density (T1and T2, 77 and 85% of standard breed recommendation, respectively), and control diet fed vs. higher nutrient density diets (T4 and T5, 110 and 120% of standard breed recommendation, respectively) fed birds. Control diet fed birds had higher (P<0.01) DMD and OMD almost 12 and 10%, in comparison to birds offered the lower nutrient concentration diets. However, no difference (P>0.05) in DMD and OMD amongst birds existed when the comparison was made between the control diet and higher nutrient density diets.

Increasing dietary nutrient concentration led to a linear (P<0.001) improvement in apparent metabolisable energy (AME) and apparent metabolisable energy corrected to nitrogen (AMEn) values of the diets, as AME and AMEn values were reduced for diets T1, T2, T3 and T4 ranged from 34 to 8% lower as compared to T5 diet. Birds fed control diet had higher (P<0.001) dietary AME and AMEn values in comparison to birds offered the lower nutrient concentration diets. However, AME and AMEn values were 9% lower (P<0.01) for the control diet, compared with higher nutrient density fed birds (table 17). The response of AME intake (AME I) to dietary nutrient concentration was a linear function (P<0.01), where AME I increased with higher dietary nutrient concentration. Birds fed control diet had higher (P<0.001) AME I values in comparison to birds offered the lower nutrient concentration diets, however, no difference (P>0.05) in AME I amongst birds existed when the comparison was made between the control diet and higher nutrient density diets (table 17).

There was a linear increase (P<0.001) in nitrogen excretion (NEx), nitrogen excretion as part of amino acids (AAN) and nitrogen excretion as uric acid (UAN) as nutrient density increased. On the contrary energy efficiency ratio (EER) positively increased (P<0.001) with lower dietary nutrient concentration, similarly intake of neutral detergent fibre (NDF) increased with a decrease in dietary nutrient density (table 17). Birds fed diet T1 had significantly higher intake of NDF (P<0.001), almost 134% higher, when compared with the birds fed diet T5. There was a significantly higher (P<0.05) NEx, AAN and UAN was noted when control diet fed birds were compared with lower and higher nutrient density diets fed birds, however, the difference was not significant (P>0.05) for the AAN when comparisons were made between control diet and higher nutrient density diets fed birds. There was no difference in EER between the control diet and lower and higher nutrient density diets fed birds. The intake of NDF was significantly higher (P<0.05) when comparisons were made between the control diet and lower nutrient density diets, however, there was a significantly (P<0.001) lower intake of NDF when the control diet was compared with high nutrient density diet.

Overall the response of amino acid digestibility (during digestibility measurements after 7th week at 49 days of birds age) i.e. for Ala, Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was best described as positive linear function (P<0.001) to dietary nutrient concentration (table 18). Birds fed the control diet had higher (P<0.001) amino acid digestibility in comparison to birds offered the lower nutrient concentration diets. However, amino acid digestibility was either lower or there was a trend of lower (P<0.05 to P=0.09) values when control birds were compared to birds offered the high nutrient concentration diets, and comparative difference of Val and Met digestibility did not differ (P>0.05) between control and lower nutrient density diet fed birds. No difference (P>0.05) in digestibility of Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was noted when control birds were compared to birds offered the high nutrient concentration diets.

Discussion

The analysed dietary concentration of crude protein (CP) were slightly lower and the values for K, Ca and Na concentration were higher than the calculated values, which was probably due to differences between the composition of the actual ingredients that were used in the present study and the NRC (1994) values for the same ingredients. The relatively higher final body weight of the birds, when compared to breed standards, may be explained by the 'small pen' effect, e.g. a reduction in competition for, and closer proximity to, drinkers and feeders.

Water intake measurements

At moderate temperatures feed intake, or more specifically dry matter intake, is the main determinant of the daily water requirement of poultry (Pond *et al*., 1995). However water intake and the ratio of water to food intake are increased by high dietary mineral and protein concentrations (Fuller *et al*., 2004). In order to maintain water balance, water intake must exactly counterbalance the water lost from the body as well as water stored in new growth therefore any over consumption from the requirement can lead to higher than normal water excretion. Since the dietary concentration of nutrients other than CP and AME were kept similar in all dietary treatments, however, NDF content changed significantly due to feed formulation constraints in the lower nutrient density diets, therefore, higher feed intake resulted in a higher mineral and NDF intake, which are known to increase water intake and excretion in poultry (Van der Klis *et al*., 1995). Therefore as expected higher feed intake (FI) in the present study in birds fed on lower nutrient density diets resulted in higher water intake (WI) which then resulted in poor litter quality.

Feed intake and feed composition can affect metabolism and utilisation of individual amino acids which then can affect normal gut functioning and can impair absorption of other nutrients. Certain dietary factors such as fibre, lignins, tannins and lectins can influence threonine availability to the animal. It has been shown in the literature that threonine deficiency caused by either inadequate dietary supply or due to factors mentioned above can result in increased excretion of mucins and abrasion leading to severe diarrhoea in pigs (Law *et al.*, 2007). Higher level of dietary NDF in poor nutrient density fed birds of present study could have resulted in poor absorption of nutrients across GIT, hence resulted in higher retention within digesta. In the present study lower amino acid digestibility in diets where nutrient density was lowest therefore, indicates that the dietary NDF content in diets formulated with lower nutrient density might have been the cause of lower amino acid digestibility and imbalance. An amino acid imbalance is highly likely to make things worse when compared with a well-balanced amino acid profile (D'Mello, 1993; D'Mello, 1994; Moran and Stilborn, 1996).Symptoms of imbalance or deficiency of linoleic acid in the domestic fowl include retarded growth, increased water consumption (Stevens, 2004). Higher NDF intake in birds fed with lower nutrient density diets in the present study created a severe imbalance of amino acids causing a reduction in protein utilisation and a lower FCE. Fibre itself is responsible for decreased protein digestibility in pigs, with water retention capacity being shown to increase ileal protein losses (Larsen *et al.*, 1993). It has been reported by Fairclough*et al*. (1980) that free amino acids exert more osmotic pressure than peptides, and free amino acids may in some cases be utilized even less efficiently than protein-bound amino acids (Boisen, 2003). Therefore, this situation could lead to excretion of water more than normal through excreta as reported in the present study. Diarrhoea can affect the availability of other amino acids (e.g. methionine) required for gut function and metabolism. For example, threonine is regarded as crucial for normal gut structure and function so its requirement is quite high. Pigs can use almost 60% of their threonine intake for gut development and functioning (Stoll *et al.*, 1998). Since threonine is required for gastrointestinal secretions (mucin) that protect mucosa from digestive proteases, dehydration, microbial and parasitic invasion and therefore, believed to play an important role in development and normal functioning of the gut (Bertolo *et al.*, 1998; Stoll *et al.*, 1998). Likewise any imbalance or improper supply of other amino acids such as leucine can affect gut functioning and structure. Adequate arginine intake is crucial for normal metabolic function in pigs and any deficiency can result in increased plasma ammonia concentration leading to metabolic disturbance (hyperammonemia) (Urschel *et al.*, 2007). These problems can be addressed by dietary supplementation of arginine (Zhan *et al.*, 2008). As it is required for the synthesis of protein, urea, nitric oxide and other metabolites and any inadequate supply for one or the other reasons can change the priority of its usage. This can result in higher concentration of ammonia in the plasma which is toxic and required more water for excretion. It is also documented in the literature that higher feed and mineral intake can depress DMD (Koreleski *et al.*, 2010) and amino acid absorption.

Further to amino acid imbalance and digestibility association with litter quality problems, undigested starch and protein favour proliferation of coliform bacteria in pigs (Jeaurond *et al.*, 2008). However, fibre can reverse the ratio of coliform bacteria to other beneficial bacteria (lactobacilli) and can reduce ammonia contents in GIT (Bikker *et al.*, 2006). But it is worth noting that source of fibre can produce different affects as fibre from wheat bran provides intermediate results.

Goldstein and Skadhauge (2000) highlighted that lower protein fed birds when had limited dietary energy available can have relatively higher quantity of nitrogen excreted in forms other than uric acid it is just to conserve energy. These forms e.g. urea and ammonia are osmotically active and require alot of water to be excreted. The lower dietary energy and its relationship with higher amino acids being oxidsed to be used as energy source were explained (Church, 1991; Pfeiffer, 1995; Musharaf and Latshaw, 1999) highlighting the fact that it is not the absolute dietary CP but the ratio between ME and CP is perhaps more important when a control on litter moisture and nitrogen is to be ensured. Caution is therefore necessary in reaching any conclusions when evaluating studies referring to relationship of dietary CP with litter moisture contents.

Litter quality associated parameters

An increase in nutrient density resulted in a reduction in the litter moisture (LM) content and this relationship suggested that the optimum dietary nutrient density for reduced LM does not match with the determined optimal density for bird growth. Therefore, the higher LM content reported in this study could have been the reflection of higher nutrient retention in digesta possibly due to poor DMD, OMD, amino acid digestibilities and presence of higher NDF content, when birds were fed lowest level of dietary energy and protein concentrations. However, present findings differ to some extent from findings reported by Khajali and Moghaddam, (2006) that there was no effect of lower dietary crude protein concentration on litter moisture content. However, they are in agreement with present findings of reduction in nitrogen excretion when birds were fed lower dietary protein concentration.

In terms of nitrogen excretion by the bird and a reduction in the litter NH_3 concentration these results are in line with previous findings of different studies which reported that a reduction in dietary protein content can help control nitrogen excretion and NH_3 emission from poultry litter (Jacob *et al.*, 1994; Moran and Stilborn, 1996; Ferguson *et al.*, 1998; Hussein *et al.*, 2001; Bregendahl *et al.*, 2002; Rezaei *et al.*, 2004; Si *et al.*, 2004). Uric acid is the end product of protein degradation in avian species and is a direct measure of protein catabolism in birds. Some researchers reported a decrease in uric acid concentration in the blood when lower protein diets were fed to broilers (Rosebrough *et al.*, 1996; Collin *et al.*, 2003). Different researches (Cheng *et al.*, 1997; Aletor *et al.*, 2000; Swennen *et al.*, 2004; Swennen *et al.*, 2005; Swennen*et al.* 2006) have reported that birds have mechanism to reduce amino acid oxidation as a sparing mechanism which therefore, is the reason of lower plasma uric acid level. Therefore, probable reason of this lower litter NH_3 content was due to the lower uric acid excretion by the birds fed on lower nutrient density diets.

Leg health parameters

Increasing litter score (reflecting deterioration in litter quality) had a positive correlation with WI however, the negative correlation of WI with hock burn scores (HBS) may appear contrary to previous findings (Mayne *et al.*, 2007), because it might be expected that high water intake would result in poor litter quality or high LM with a resulting increase in contact dermatitis. The reduced litter moisture and lower litter scores were achieved with an increase in nutrient density which is in agreement with the findings of Kenny *et al.* (2010). However this improvement in litter quality did not correspond with the incidence of HB or FPD. The higher incidences of HB were associated with birds fed the higher nutrient density diet, in agreement with the findings of Bilgili*et al.* (2006). The positive correlation of HB with litter NH_3 indicates that perhaps litter chemical properties are important contributor in skin damage and litter moisture may only aggravate the damage by making skin more prone to these damages. Therefore, present findings suggested that it may be the litter NH_3 and pH which has a much greater effect on incidence of hock burn than litter moisture content alone. Therefore, in terms of HBS it was notable that increases in litter moisture were not associated with increased HBS. It is likely that the cause of the higher HBS in groups fed higher nutrient density diets was primarily litter NH_3. Unlike Ekstrand*et al.* (1997) and (1998) litter moisture was the main cause of footpad dermatitis (FPD). However, Dawkins *et al.* (2004) reported that a combination of litter moisture and ammonia was associated with poor health and correlated with ‘dirty foot pads’. Berg (2004) also noted that HB lesions are commonly caused by a combination of moisture, high ammonia content, and other unspecified chemical factors in the litter. There is another possible reason for higher incidences of HB in birds fed the higher nutrient density diets. These birds may spend less time standing for feed and therefore, spend more time sitting on the litter. Haslam*et al.* (2007) reported that factors which increase bird weight or which are related to reduced litter quality, tend to increase hock burn.

Although litter moisture increased with age in this study there was a reduction in the HBS as well as FPDS which highlights that it is not litter moisture alone that can cause skin damage. These findings agree with the findings of Bilgili*et al.* (2006) who reported that the proportion of birds with footpad dermatitis tended to increase until 49 days of age after which they started to decline. So it is possible that older birds may become less susceptible to litter moisture damage (Mayne*et al.*, 2007).

The findings in this study contrast with those of Mayne*et al.* (2007), who reported that litter moisture was the cause of FPD in turkeys. Increased litter moisture not associated with more incidences of FPD although these findings may be consistent with those of Dawkins *et al.* (2004) who concluded that both litter moisture and NH_3 are required to predispose birds to FPD rather than litter moisture alone.

Growth performance, dietary nutrient intake and utilisation

It is well documented that dietary composition and the ratios between macronutrients have a major impact on performance and body composition of chickens (Macleod, 1990; Macleod, 1992; Nieto *et al.*, 1997; Collin *et al.*, 2003). In the present study birds fed on lower nutrient density had lower crude protein digestibility (CPD) as well as lower feed conversion efficiency (FCE) and protein efficiency ratio (PER) which are consistent with previous reports. For example, some studies have reported a negative effect on feed conversion ratio of lower crude protein concentration even when supplemented with synthetic amino acids (Moran and Stilborn, 1996; Ferguson *et al.*, 1998; Neto *et al.*, 2000). Layer birds eat to meet their energy requirement, so physical capacity and energy content can affect both feed intake (Morris, 1968; Golian and Maurice, 1992; Leeson *et al.*, 1993). Study of Huang *et al.* (2009), the present findings suggest that meat producing birds also try to compensate for any energy deficiency by increasing their feed intake when fed a lower nutrient density diet however, in this study, they were not able to match the similar weight gain as recore recorded in birds fed with higher nutrient density diets. The lower weight gain and poor feed conversion efficiency in the present study in birds fed on lower nutrient density was consistent with Hidalgo *et al.* (2004) who reported the same when broilers were fed diets with suboptimal levels of energy and crude protein while maintaining ME:CP. Farrell *et al.* (1973) and Farrell (1974) suggested that there is an optimum energy concentration in the diet beyond which the performance of birds does not appear to improve and that in some cases, it may actually deteriorate. The present findings agree with this conclusion only during the last growth phase (16-20 weeks) where maximum weight gain was recorded when birds fed with diet contain 100% nutrient density compared to either of the lower or higher nutrient density diet fed birds.

Others reported a reduced growth performance with a reduction of as little as 30g/kg dietary crude protein concentration even when the diet was supplemented with synthetic amino acids (Fancher and Jensen, 1989a; Fancher and Jensen, 1989b; Fancher and Jensen, 1989c; Pinchasov *et al.*, 1990; Colnago *et al.*, 1991; Kerr and Kidd, 1999; Aletor *et al.*, 2000; Waldroup, 2000; Bregendahl *et al.*, 2002). Whereas Aletor*et al.* (2000) reported improved protein efficiency ratio with lower dietary crude protein concentration because dietary protein is preferentially used for protein deposition. However, other studies also indicated the importance of dietary energy concentration along with CP as they reported poor protein deposition in the carcass in case the energy availability becomes limiting (Macleod, 1990; Musharaf and Latshaw, 1999).

Overall decrease in FCE, PER and an increase in feed intake (FI) with age in the present findings can be best explained by the fact that birds are able to retain more protein at younger age and with the age this ability decrease and they retain more fat. Fat contains more energy than protein and gaining body fat require more feed intake to be converted to less body growth compared to protein.

The experimental diets were formulated to contain graded levels of dietary energy and protein concentrations, because, it was hypothesised, would affect feed and water intake and hence litter quality and would allow test of their response to different dietary concentrations. However, the overall changes in growth performance parameters were expected, i.e. most of the dietary energy and protein concentrations were beyond those used in commercial practice, therefore, they are not further discussed here.

The higher energy efficiency ratio (EER) in birds fed lower nutrient density diets seems to be at variance from the FCE and PER results. However, this can be explained by the uric acid excretion values of birds fed lower nutrient density diets being lower than for those birds fed on higher nutrient density diets. Uric acid formation and excretion is a process that requires significant energy. Therefore, birds fed on higher nutrient density diets use energy on uric acid excretion, hence had lower EER values. The present findings agree with the findings of Skinner *et al*. (1992) who reported that an increase in dietary nutrient density resulted in depressed energy efficiency.

Poor nutrient utilisation i.e. CPD, dry matter (DM), organic matter (OM) and amino acid digestibilities in birds fed lower nutrient density diets in the present study could be explained by the presence of higher concentration of neutral detergent fibre (NDF) in the diets formulated to present lower nutrient concentrations. The proportion of cellulose and lignin in the crude fibre fraction also determines the digestibility of crude fibre or its solubility in the intestine. AWT (2005) report by-products of cereal processing such as wheat bran to be particularly high in fibre while soybean meal (especially high protein grades) bring little fibre into the formulation (e.g. pentosans i.e. arbinose and xylose etc. wheat bran 250 g vs. 35 g/kg DM in soybean meal). Since fibre has no direct nutritive benefit in poultry nutrition the high cellulose and lignin concentrations as result of formulation constraint to add wheat bran could have resulted in reduced nutrient digestibility.

Conclusion

The present experiment has shown that an increase in the concentration of dietary crude protein (CP) and apparent metabolisable energy (AME) can reduce water intake (WI), decreasing moisture content in the litter and thereby reduce the litter score (indicating improved overall litter quality).However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health (defined in this study as FPD and HB) problems in turkey production.

The incidence of hock burn (HB) was associated with litter NH_3. Since CP intake was related to litter NH_3 concentration, then modifying the CP intake by altering the calorie to CP ratio may be one way of controlling HB by dietary manipulation.

It is perhaps important to report that good litter score (based on physical appearance) was not related to litter NH_3 and pH therefore litter score per se is of limited or no value in terms of lowering HB incidences in turkey production.

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Competing Interests

The authors declare that they have no competing interests.

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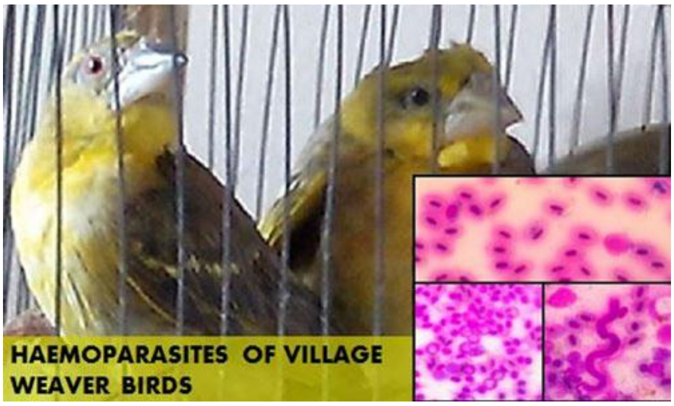

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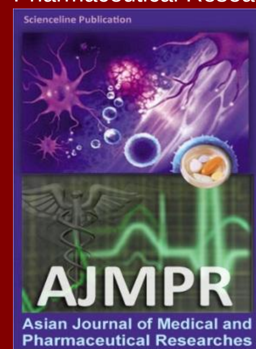

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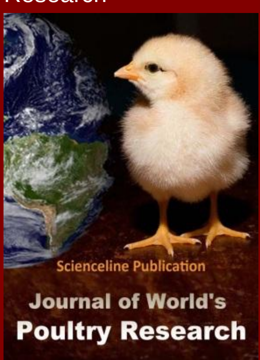

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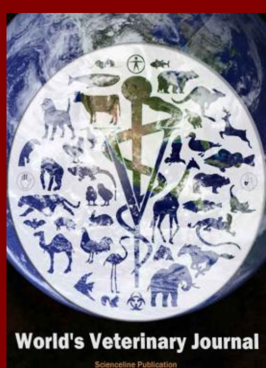

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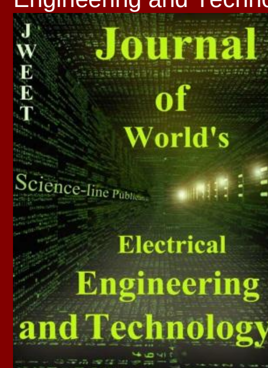

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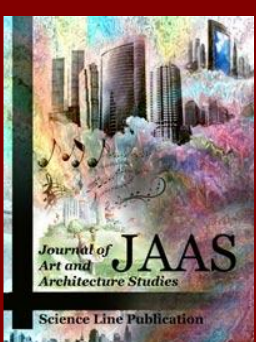

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