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Administering Aqueous Pepper Extract Influence the Growth Performance, Nutrient Utilization and Selected Haematological and Serum Characteristics of Three Breeds of Broiler Chickens

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Abstract

The aim of the study is to evaluate the effect of giving an aqueous extract of pepper on the growth performance, nutrient digestibility, and selected haematological and serum characteristics of three breeds of broiler chickens. With this in mind, 216-day-old broiler chickens of the Arbor acre, Ross308, and Cobb500 were used for the research. The birds were administered four levels of aqueous pepper extract: 0, 3, 3.5, and 4mL, respectively. Parameters measured were body weight changes, feed intake, feed conversion ratio, degree of apparent nutrient digestibility, and some haematological and serum characteristics. The results showed that, Cobb500 birds had significantly ($p<0.05$) better feed conversion ratio, final body weight and body weight gain than Arbor acre and Ross308 birds. Also, birds on the aqueous pepper extract had better ($p<0.05$) feed conversion ratio, and body weight changes than those in the control (antibiotic). Final body weight (2784.93 g), body weight gain (2748.90 g), and total feed intake (4902.58 g) were observed to be better ($p<0.05$) in Cobb500 birds compared to the other birds at 0-56 d. No level of administration effect ($p>0.05$) was observed at 0-56 d. However, breed x level interaction led to better body weight changes and total feed intake. Significant ($p<0.05$; 0.01) effect of breed was observed in the apparent nutrient digestibility of the birds. Packed cell volume, white blood cells count, eosinophil and blood cholesterol levels were affected ($p<0.05$) by the breed of the birds while white blood cell count, eosinophil and blood triglyceride of the birds were also affected ($p<0.05$) by the level of pepper extract administered. Conclusively, the aqueous pepper extract had positive impact on the performance of the birds used in the study, mediated by the breed, and level of administration of the pepper extract.

Keywords: Genetic differences, Antibiotic growth promoters, Nutrient digestibility, Feed additives, Phytogenic

Introduction

Antibiotics were introduced into animal husbandry practices mainly for therapeutic purposes in order to improve the health and well-being of animals. It was discovered that its usage led to a desirable improvement in the growth rate and feed conversion efficiency of chickens (Puvaca et al., 2015a). With time, however, its continued usage was discouraged due to the accumulation of remnants in milk, meat of slaughtered animals, and eggs of poultry. There is also the problem of antibiotic resistance occasioned by the consumption of animal products caused by food-borne bacteria. There are many antibiotics in use in the poultry sector (Landers *et al.,* 2012; Boamah *et al.,* 2016). Although most of them are considered very essential in human medicine (WHO, 2010; WHO, 2017), their indiscriminate usage in the production of animals is likely to accelerate the development of antibiotic resistant pathogens. The resultant effect of this resistance will likely be felt in economic losses, treatment failures, and even in the resistant microbes becoming a genetic pool for transmission to human beings. This occurs when an antibiotic eliminates susceptible bacterial strains in the gastrointestinal tracts of birds, leaving only those that can resist the drug. The resistant bacteria then increase in number, becoming the dominant population thereby acquiring the propensity to transfer their resistance genes to other bacteria (Laxminakayan *et al.,* 2013; Madigan *et al.,* 2014). It is such resistant bacteria that are transferred from poultry products to human beings when consumed, or via handling of contaminated meat and other poultry products (Mehdizadeh *et al.,* 2010); Goetting *et al.,* 2011; Addo *et al.,* 2011; Mirlohi *et al.,* 2013). Once established in the human system, the resistant bacteria colonize the intestinal tract and their resistant genes could be shared or transferred to the intestinal flora; this can greatly jeopardize future treatments of infections caused by such organisms (Marshall and Levy, 2011; van den Bogaard and *Stobberingh,* 2011). Because of pathogens like Salmonella and Campylobacter, which are becoming a public health concern to consumers (Kumar et al., 2018), the European Commission (EC) banned its usage as a growth promoter in animal husbandry within its union and also banned the importation of antibiotic-raised animal products in the Union's territory. This consumer pressure is, therefore, pushing producers of animal products, particularly the poultry industry into rearing poultry without the use of antibiotics (Puvaca et al., 2015b).

With all the problems enumerated above, the need arises for natural alternatives that will confer all the health and productivity benefits of synthetic antibiotics in poultry, while at the same time being readily available, accessible and cheap. Some of these alternatives include probiotics, prebiotics, organic acids, essential oils, medicinal plants, and parts of aromatic plants like oregano, ginger, pepper, garlic, thyme, and basil (Egena *et al.*, 2010; Stanacev *et al.*, 2011; Alabi *et al.*, 2012; Puvaca *et al.*, 2013). Red pepper (*Capsicum annuum* L) is one of the most used spices in the world. Apart from its richness in flavonoids, it is very rich in vitamin C which impacts positively in reducing heat stress in birds (Al-Kassie *et al.,* 2012). Red pepper has anti-oxidative, anti-inflammatory, anti-allergenic, and anti-carcinogenic properties (Lee *et al.,* 2005) and has also been reported to reduce the incidence of cancer (Nashino *et al.,* 2009). It has a higher inhibiting ability against lipid peroxidation (Conforti *et al.,* 2007) compared to vitamin E (Luqman and Razvi, 2006).

The addition of red pepper to broiler chickens' diet was reported to influence feed intake, and body weight gain (Puvaca *et al.,* 2014; 2015b). Al-Kassie *et al.* (2011) observed improved body weight gain and feed efficiency in birds fed 0.50, and 0.75% *Capsicum annuum*; they also observed a decrease in blood cholesterol concentration in birds fed 0.25, 0.50, 0.75, and 1.00% *Capsicum annuum.* Al-Kassie *et al.* (2012) reported a significant increase in body weight gain, feed intake, feed conversion ratio, and dressing percentage of chickens fed red chili pepper. Red and black pepper in broiler chickens' diet were also implicated in the reduction of triglyceride, low-density lipoprotein, and high-density lipoprotein (Puvaca *et al.,* 2015b). A careful search of the literature revealed that most of the work carried out using red pepper in broiler chickens' nutrition, involved including it in-feed. Abdulrasheed (2021) however, included it in water up to 2.00% with promising results observed in the production parameters. Studies carried out to evaluate the tolerance and utility of red pepper by different breeds of broiler chickens to determine which ones will perform better under >2.00% inclusion levels are somewhat scarce. Hence, our study aims to evaluate the effects of administering >2.00% levels of aqueous red pepper (*Capsicum annuum* L.) extract via drinking water on the performance and haematology of three breeds of broiler chickens.

Materials and methods

The study was carried out at the poultry section of the Department of Animal Production, Gidan Kwano campus. The Gidan Kwano campus lies between latitude 9°32' and 9°42' N, and longitude 6°30' and 6°40' E. The daylight temperature fluctuates between 24°C in the middle of the wet season, and 35°C at the pinnacle of the dry season; the annual rainfall is between 1200-1300mm (Ojimaduka *et al.,* 2020). The red pepper used for the study was washed, and placed in an electric blender (Kenwood, BL440 500W, China) and water was added at the ratio of 1:1 (1kg of pepper: 1 liter of water). This was then blended until a smooth consistency was achieved. The blended pepper was decanted and sieved using a muslin sheet. The supernatant was collected and stored refrigerated at 4^{o} C until when needed for use.

The single-phase diet fed to the birds was compounded from maize, 30% broiler starter concentrate, and maize bran to supply 21% crude protein and 2975kcal/kg Metabolizable Energy (ME). This diet was fed throughout the experimental period and its effects were evaluated at 1-28 days, and 0-56 days, respectively. The study involved the use of 216-day-old broiler chicks using a 3 x 4 x 3 factorial arrangement in a randomized complete block experimental design; this had 3 breeds of chicken (Arbor acre, Cobb500, Ross308), 4 levels of inclusion of the aqueous pepper extract (control- 0 pepper, 3.00, 3.50, and 4.00mL), and 3 replications of 18 birds each. The chickens on arrival were allocated to the various treatments after weighing to obtain the initial weight. They were brooded using charcoal pots and lighting was provided for night feeding. Water and feed were supplied *ad libitum.* The chickens were vaccinated against Newcastle and infectious bursal diseases following the recommended veterinary standard of the area. They were also treated against coccidiosis with Amprolium®. Other routine management practices were observed throughout the duration of the trial.

During the experiment, weekly feed intake, individual live body weight, body weight gain, and Feed Conversion Ratio (FCR), which is a feed consumption index, were measured and recorded. The apparent nutrient digestibility of the birds was evaluated on days 49-56 using a total of 30 birds. The apparent nutrient digestibility coefficient of dry matter, crude protein, crude fibre, ether extract, ash, and nitrogen-free extract were calculated for each nutrient using the formula;

Apparent nutrient digestibility = $\frac{NI-NVF}{NI} x\ 100$

Where NI is the nutrient in feed consumed, and NVF is the nutrient voided in the faeces

Five (5ml) of blood samples were collected from 30 chickens at the wing vein using a 21 gauge syringe on day 56. The blood samples were divided into two tubes: one with EDTA, and the other without it, respectively, to be used for haematological and biochemical analysis. The following biochemical parameters were analyzed: blood glucose and cholesterol levels; blood glucose and cholesterol were determined according to the method described by Aslam *et al*. (2010). The cholesterol was assayed by the enzymatic method. Red blood cells count, white blood cells count, haemoglobin concentration, and haematocrit were all determined using a haematology analyzer (Nicros-ABX).

The SPSS software version 16 (SPSS, 2006) was used to analyze all the data collected during the study. A two-way analysis of variance was used to analyze the data and significant differences were separated using Duncan multiple range test. The statistical model used for the study was;

$Y_{ij} = \mu + \alpha_i + \beta_j + C_{ij} + e_{ijk}$

Where Y_{ij} is the observed response of the chickens to the treatments, μ is the overall mean, α_i is the effect of the $_i{}^{th}$ breed ($_i$ = 3; Arbor acre, Ross308, Cobb500), β_j is the effect of the $_j{}^{th}$ level of inclusion of the aqueous pepper extract ($_j$ = 0.00, 3.00, 3.50, and 4.00mL), C_{ij} is the interaction between the breed of chickens and the level of inclusion of the aqueous pepper extract, and e_{ijk} is the random error.

Results

The main effects of the breed of broiler chickens as affected by the administering of varying doses of aqueous pepper (days 0-28) are reflected in Figures 1a, 1b, and 1c, respectively. Results revealed significant (p<0.05) differences in the final body weight, body weight gain, and FCR, while the total feed consumption was not affected (p>0.05). Final body weight, body weight gain, and FCR were observed to be better in Cobb500 birds, followed by Ross308, and Arbor acre in that order.

The effect of the level of aqueous pepper administration on the broiler chickens (days 0-28) is displayed in Figure 1d. The results revealed a significant (p<0.05) influence of the level of administration on the final body weight, body weight gain, and FCR, while the total feed intake was not affected (p>0.05). Final body weight and body weight gain were similar (p>0.05) in birds dosed with 3 and 3.5ml of aqueous pepper, but they were significantly (p<0.05) better compared with those on the control (0 pepper); those on 4ml aqueous pepper were somewhat similar (p>0.05) with those on the control. In terms of FCR however, birds on the aqueous pepper extract were observed to be better than those in the control group (Figure 1e).

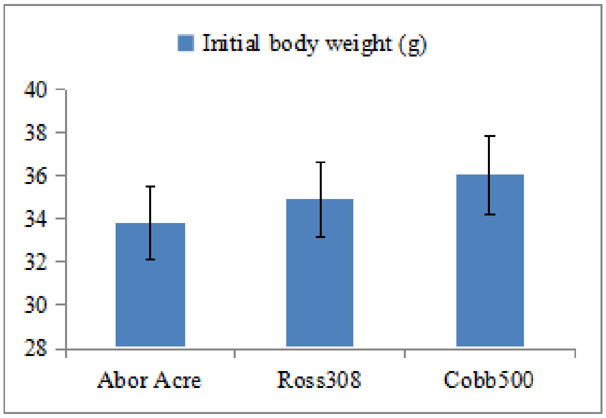

Figure 1a: Initial body weight of broiler chickens used for the experiment.

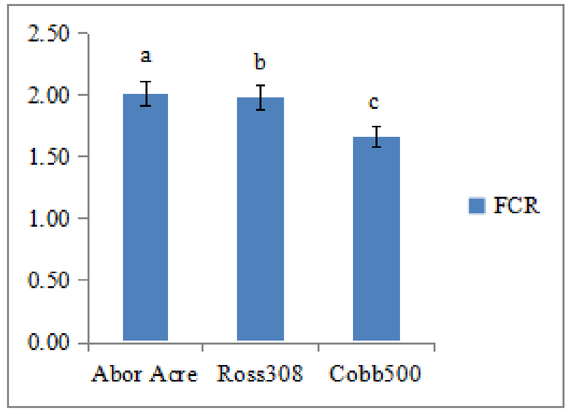

Figure 1b: Breed effect of FCR of broiler chickens on varying aqueous pepper extract doses

The main effects of the breed of broiler chickens as affected by the administering of varying doses of aqueous pepper extract (day 0-56) are shown in Figures 2a and 2b, respectively. Results revealed significant ($p<0.05$) differences in the final body weight, body weight gain, and total feed intake, while the FCR was not affected ($p>0.05$). Final body weight, body weight gain, and total feed intake were observed to be better in Cobb500 birds.

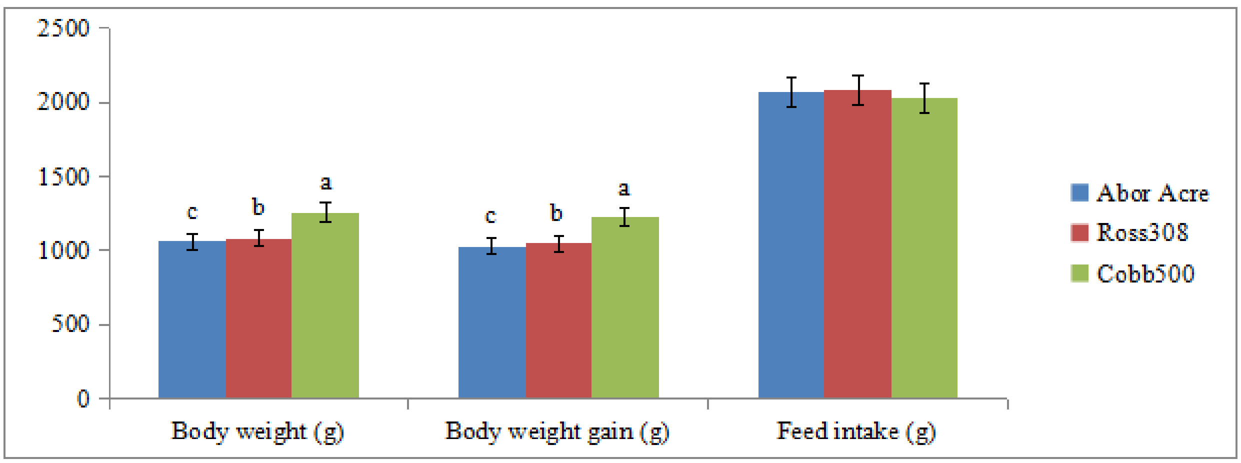

Figure 1c: Main effect of body weight, body weight gain, and feed intake of broiler chickens administered varying doses of aqueous pepper extract

The effect of the level of aqueous pepper administration on the broiler chickens (days 0-56) are displayed in Figures 2c and 2d, respectively. The results revealed no significant ($p>0.05$) effect of the level of administration of the pepper extract on the final body weight, body weight gain, and total feed intake. Breed x level interaction was felt on final body weight, body weight gain, and total feed intake only.

The apparent nutrient digestibility of the birds administered aqueous pepper extract is shown in Table 1. The ability of the birds to digest the nutrients was affected ($p<0.05$) by the breed of the chickens. Cobb500 and Ross308 birds had similar DM, CP and CF digestibility. The level of administration also affected ($p<0.05$; 0.01) the DM, CP, ash, and NFE digestibility. Breed x level interaction significantly ($p<0.05$; 0.01) affected the DM, CP, ash, and NFE digestibility of the birds.

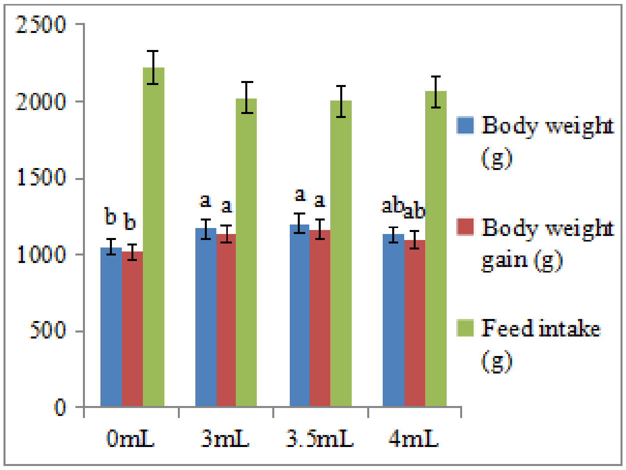

Figure1d: Effect of level of administration of aqueous pepper extract on body weight, body weight gain and feed intake of broiler chickens

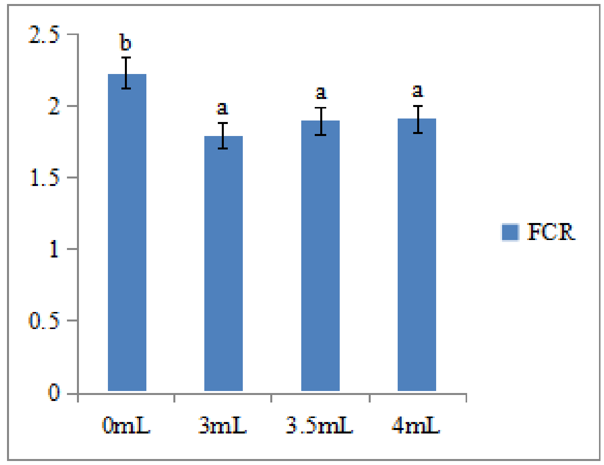

Figure 1e: Effect of level of administration of aqueous pepper extract on FCR of broiler chickens

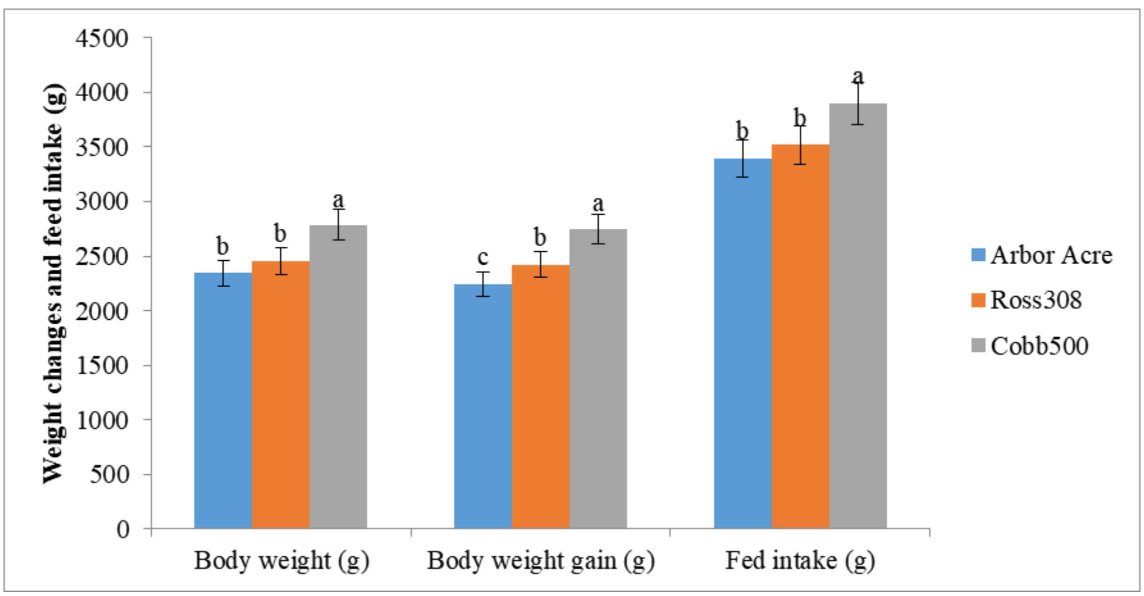

Figure 2a: Main effect of body weight, body weight gain, and feed intake of broiler chickens administered varying doses of aqueous pepper extract (day 0-56)

The results of haematological and serum characteristics of the birds administered aqueous pepper extract are shown in Table 2. The packed cell volume, white blood cells count, eosinophil and blood cholesterol of the birds were significantly affected ($p<0.05$) by the breed of the chickens. The eosinophil count was observed to be higher in Arbor acre chickens compared to the Cobb500 and Ross308 birds. Blood cholesterol was also observed to be lower in Cobb500 chickens when compared to the other breeds. The level of administration was observed to have affected ($p<0.05$; 0.01) the white blood cells count, eosinophil, and blood triglyceride while the other parameters were not affected ($p>0.05$). Significant ($p<0.05$) breed x-level interaction was observed only for white blood cell count.

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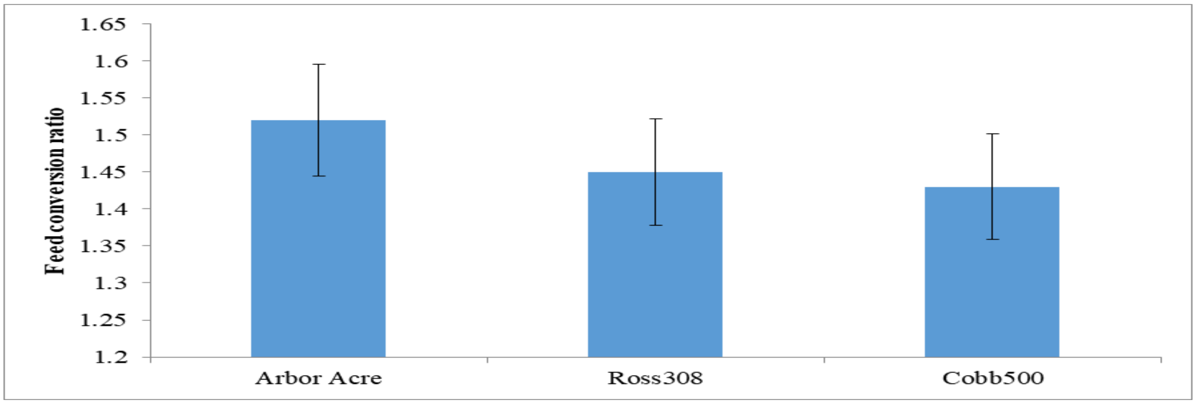


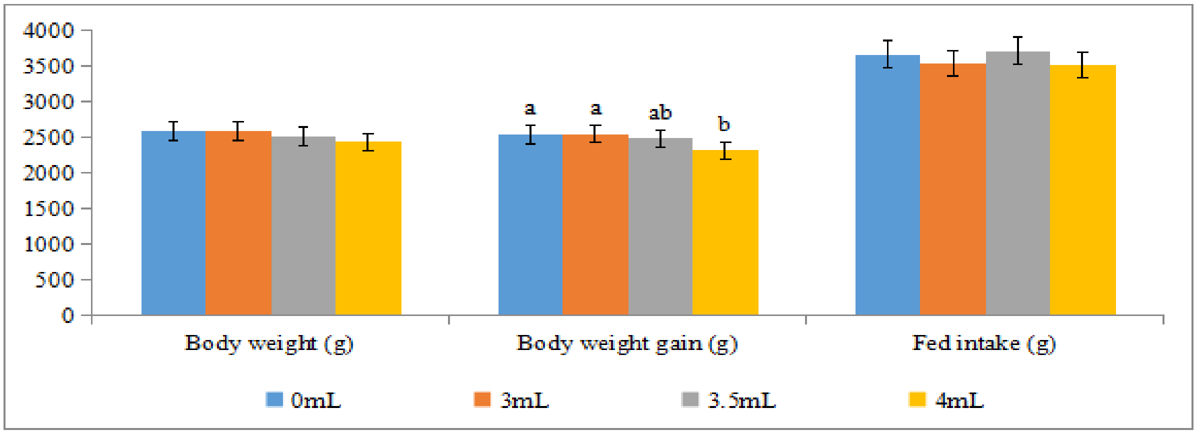


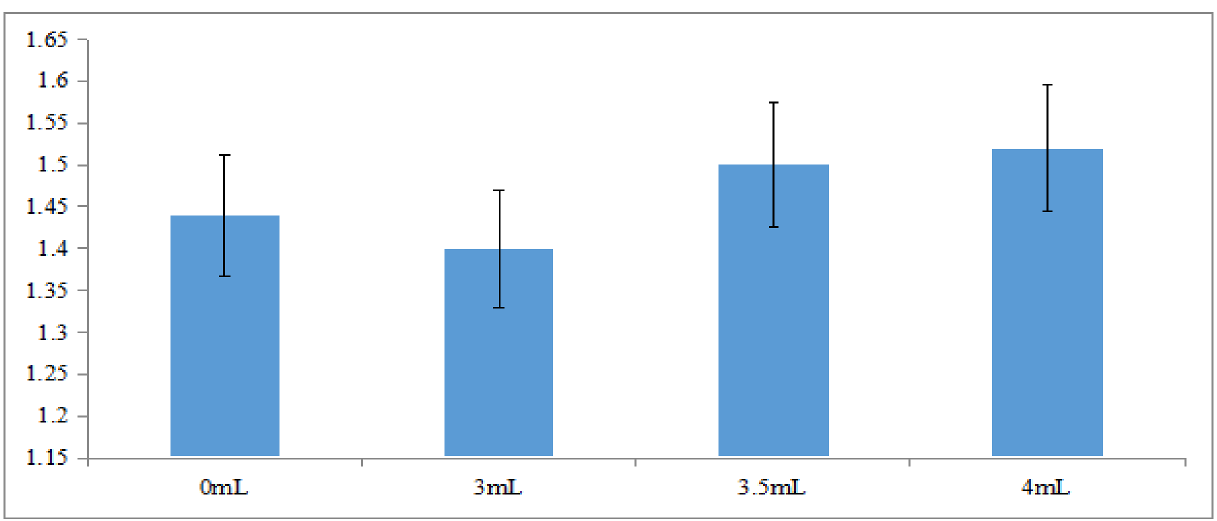
Figure 2b: Breed effect of FCR of broiler chickens on varying aqueous pepper extract doses (day 0-56)

Figure 2c: Effect of level of administration of aqueous pepper extract on body weight, body weight gain, and feed intake of broiler chickens (day 0-56)

Figure 2d: Effect of level of administration of pepper extract on FCR of broiler chickens (day 0-56)

Discussion

The administration of pepper indicated a significant impact of breed effect as regards some of the performance parameters evaluated in the study. This means that the chicken breeds reacted differently to the varying doses of aqueous pepper given and this was reflected in how they performed. Fathi *et al.* (2018) observed a similar trend when three different breeds of chickens (White Leghorn, Saudi Black, and Saudi Brown) were administered dietary probiotics. Results also showed aqueous pepper extract to be an important additive that could be used to improve body weight changes (0-4 weeks; 0-8 weeks) and feed intake of broiler chickens (0-8 weeks). The level of inclusion of the pepper extract also impacted the performance of the birds. This is because birds on 3mL pepper extract showed the best body weight changes indicating that it is better compared with other levels of aqueous pepper used. The better FCR



observed in the birds on the aqueous pepper extract compared to those in the control is a pointer that its usage in broiler chickens' nutrition is advisable. Liu *et al.* (2021) reported improved performance of chickens in terms of body weight and body weight gain and feed intake broiler chickens.

Table 1: Apparent nutrient digestibility of broiler chickens administered aqueous pepper extract

Parameter	DM	CP	CF	Ash	EE	NFE
			Breed			
Arbor Acre	76.06^{b}	71.41^{b}	50.68^{b}	23.87^{c}	82.81^{b}	83.93^{c}
Ross308	79.85^{a}	77.49^{a}	58.18^{a}	46.61^{a}	86.85^{a}	84.81^{b}
Cobb500	80.50^{a}	75.57^{a}	56.05^{a}	36.02^{b}	85.14^{ab}	88.34^{a}
SEM	0.28	0.49	0.67	1.43	0.61	0.15
Sig.	*	*	**	*	*	**
			Level			
0mL	79.70^{a}	71.34^{b}	54.79	25.47^{b}	84.84	87.37^{a}
3mL	79.43^{ab}	76.72^{a}	55.06	40.19^{a}	86.02	86.88^{a}
3.5mL	77.85^{b}	74.02^{ab}	52.88	36.11^{a}	83.74	84.81^{b}
4mL	78.83^{ab}	74.90^{a}	57.03	33.54^{ab}	85.08	84.82^{b}
Sig.	*	*	NS	*	NS	*
Breed x Level	*	*	NS	*	NS	**

^{ab}: means separated by different superscripts within the same column differ significantly (p<0.05; 0.01), DM = dry matter, CP = crude protein, CF = crude fibre, EE = ether extract, NFE = nitrogen free extract, SEM = standard error of mean, Sig. = level of significance.

This improved performance the authors observed, was over control birds given a commercial antibiotic (chlortetracycline). The good results of the current study were also achieved over control birds dosed with the antibiotic oxytetracycline. The improvement observed in the current study could be attributed to the presence of capsicidins a component of red pepper which affects afferent nerve fibre and appetite (Galib *et al.,* 2011). Other authors have reported on the performance of broiler chickens when various forms of pepper were used in broiler nutrition; some of these studies include the reports of Moradi *et al.* (2016) where hot red pepper was used at 200g/ton of the feed, Afolabi *et al.* (2017) who used hot red pepper at 0.1, 0.2, and 0.3 percent of feed as compared to the control group, Younis and Abdel-Latif (2017) using water supplemented with 1 and 2 percent hot red pepper compared to the control diet, and Adegoke *et al.* (2018) who observed that the final weight and total body weight gain of broiler chickens supplemented with hot red pepper at 100g/100kg of feed as compared to the control group, were significantly higher compared with the non-supplemented ones. The interaction between the breed of the chickens and the level of administration impacted positively on the body weight, body weight gain, and feed intake of the chickens.

The apparent nutrient digestibility of the birds on the aqueous pepper extract was better for Cobb500 and Ross308 birds for all the nutrients compared to the Arbor acre birds. This could be because these birds were better able to tolerate the pepper extract given. This led to better digestion and utilization of the feed nutrients. The recorded differences in the nutrient utilization and growth performances of the chickens based on breed differences cannot be unconnected with the possible impact of the bioactive components of the aqueous pepper extract. This could also be connected with the immune-competence of the chickens, and since the immune-competence of livestock plays an important role in farm profitability and directly affects health maintenance, this is a positive outcome for farmers. Hence, genetic differences of the chickens could significantly influence the immune system, and genotypic structure since modern fast-growing chickens have been changed, particularly after decades of breeding for higher production (Emam *et al.,* 2014).

Overall, the results of this study indicate that aqueous pepper extract and the level of its administration led to improved nutrient utilization which was reflected in the better performance observed in the breeds that better performed, and in those administered the pepper extract. The results obtained for CP and EE digestibility are similar to the works of Liu *et al.* (2021), which evaluated the effect of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broiler chickens. According to the reports of Kishawy *et al.* (2022), there is a positive effect of using pepper on nutrient utilization by chickens. Munglang and Vidyarthi (2019) stated in a review of the use of hot red pepper powder-supplemented diets in broiler chickens' nutrition that, it can potentiate the activities of pancreatic and intestinal enzymes, increase bile acid secretion, and increase body weight gain in broiler chickens while at the same time reducing heat stress, and improving feed digestibility in broiler chickens.

Some of the haematological and serum characteristics evaluated in the chickens were affected by the breed, as well as the level of administration of the aqueous pepper extract. The increase in the WBC count of the birds is evidence of the immune-boosting capacity of the pepper extract. This was more so in the Arbor acre and Ross308 breeds compared to the Cobb500. This conferred a greater capacity to withstand allergic reactions in the birds (Arbor acre and Ross308) as evidenced by the elevated amount of eosinophils. The lower amount of eosinophil observed in birds on 4mL of aqueous pepper means they were exposed to a lower allergenic reaction. This reaction evidently is not linked to the pepper itself since even birds on the antibiotic regime (0 pepper extract) had higher levels of eosinophil than those on the 4mL. Cobb500 birds had lower levels of blood cholesterol compared to the others. This is a pointer that they were better able to utilize the pepper extract to burn off the cholesterol in the blood. This implies abdominal fat deposition, meaning the Cobb500 chickens are more likely to be leaner.

The cholesterol-reducing ability of pepper and other herbs has been reported by Puvaca *et al.* (2015a), Puvaca *et al.* (2015b), and Kishawy *et al.* (2022). The triglyceride level was least in chickens administered 4mL of aqueous pepper extract. This clearly is a positive effect of the pepper extract. Meat from birds fed at this level is expected to be leaner. Several authors (Shahverdi *et al.,* 2013; Puvaca *et al.,* 2015b; Moradi *et al.,* 2016) have reported on the triglyceride-reducing propensity of pepper when used in the nutrition of broiler chickens.

Conclusions

Based on the obtained results from this study, it can be concluded that aqueous extract of pepper in broiler chickens had a positive effect on productive performances. The performance of the broiler chickens was observed to be breed and level of administration dependent. Also deduced was a significant lowering effect of the pepper extract administration on plasma cholesterol and triglycerides of the broiler chickens; this is an indication that the extract could enhance the health and well-being of the chickens. The aqueous pepper extract was effective in regulating lipid metabolism favourably and the result obtained suggests that it is possible to prevent atherosclerosis or other coronary heart diseases in humans through the consumption of chicken orally administered aqueous pepper extract since they could be capable of producing safer meat for human health.

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Table 2: Haematological and serum characteristics of broiler chickens administered aqueous pepper extract

Parameter	**RBC (l)**	**PCV (%)**	**Hb (g/dl)**	**WBC (l)**	**Neu. (%)**	**Lym. (%)**	**Eosi. (%)**	**Mono. (%)**	**MCV (Fi)**	**MCH (pg)**	**MCHC (g/dl)**	**Chol. (mmol)**	**Trigly. (mmol/l)**
							Breed						
Arbor Acre	3.13	26.20[a]	8.93	232.50[a]	32.20	62.10	3.55[a]	2.15	83.05	28.25	29.90	2.27[a]	0.65
Ross308	2.93	23.30[b]	8.09	225.30[a]	30.85	63.80	3.20[b]	2.30	73.29	27.70	28.45	1.89[ab]	0.69
Cobb500	2.89	23.95[ab]	8.23	195.20[b]	31.55	63.35	3.05[b]	2.05	81.80	27.55	28.85	1.74[c]	0.65
SEM	0.06	0.52	0.19	4.78	0.60	0.60	0.14	0.16	3.04	0.32	0.49	0.07	0.03
Sig.	NS	*	NS	*	NS	NS	*	NS	NS	NS	NS	*	NS
							Level						
0mL	2.93	24.33	8.27	217.22[ab]	29.33	65.33	3.33[ab]	2.00	83.00	28.33	29.67	2.20	0.63[c]
3mL	2.89	23.50	8.17	200.67[b]	31.83	62.67	3.67[a]	2.00	78.67	27.17	29.50	1.77	0.68[ab]
3.5mL	3.11	25.17	8.72	237.44[a]	31.67	62.17	3.44[ab]	2.72	74.77	27.83	27.83	1.86	0.78[a]
4mL	2.97	24.83	8.39	215.00[ab]	31.83	63.67	2.67[b]	1.83	83.50	28.33	29.67	2.20	0.53[c]
Sig.	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	**
Breed x Level	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b,c]: means separated by different superscripts within the same column differ significantly (p<0.05; 0.01), RBC = red blood cell count, PCV = packed cell volume, Hb = haemoglobin concentration, WBC = white blood cell count, Neu. = neutrophil, Lym. = lymphocyte, Eosi. =eosinophil, Mono. = monocyte, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin count, Chol. = cholesterol, Trigly. = triglyceride, SEM = standard error of mean, Sig. = level of significance.

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