Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora and lymphoid organs of growing turkeys

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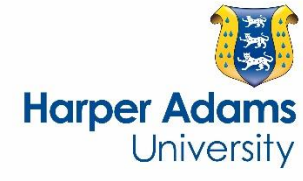

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Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora, and lymphoid organs of growing turkeys

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A B S T R A C T

A 8-wk feeding experiment was conducted to investigate the effect of dietary supplementation with Arg on haematological indices, serum chemistry, carcass yield, gut microflora, and lymphoid organ weights of growing turkeys. A total of one hundred and eighty 56-d-old male grower turkeys were weighed individually and randomly assigned to 1 of 3 dietary treatments with 6 replicate pens, and 10 turkeys per pen in a completely randomized design. Dietary treatments consisted of basal diets supplemented with 0, 0.5, and 1.0 g Arg/kg. Haematological indices and serum chemistry were measured at 84 and 112 d of study. Carcass yield, relative weights of retail cuts, organ weights, and gut microflora were measured at d 112. Except eosinophil, no effect of Arg supplementation was obtained on haematological indices at d 84. At d 112, finisher turkeys fed the diet supplemented with 0.5 g Arg/kg had the greatest red blood cell (quadratic, $P < 0.001$), lymphocyte (linear, $P = 0.011$; quadratic, $P < 0.001$), and basophil counts (quadratic, $P < 0.001$). In grower turkeys at d 84, total serum protein (quadratic, $P = 0.030$), and serum globulin concentrations (quadratic, $P = 0.043$) increased initially as Arg supplementation increased from 0 to 0.5 g/kg, but decreased with the 1.0 g Arg/kg. Uric acid concentration and alanine aminotransferase activity reduced as Arg supplementation increased from 0 to 0.5 g/kg, but increased with the 1.0 g Arg/kg (quadratic, $P = 0.002$). In finisher turkeys at d 112, total serum protein (linear, $P = 0.004$; quadratic, $P = 0.002$), serum globulin (linear, $P = 0.008$; quadratic, $P = 0.030$), serum albumin (linear, $P = 0.012$; quadratic, $P = 0.040$), and triodosterine concentrations (linear, $P = 0.025$; quadratic, $P = 0.033$) increased with increasing Arg supplementation. At d 112, spleen weights increased linearly ($P = 0.006$), while thymus weights increased quadratically ($P = 0.003$) with increasing dietary Arg supplementation.

Salmonella counts in the small intestinal content of turkeys at d 112 reduced quadratically as Arg supplementation increased from 0 to 1.0 g/kg (P = 0.029). In conclusion, Arg supplementation increased packed cell volume of finisher turkeys, improved serum chemistry of grower, and finisher turkeys as indicated by increased total serum protein, and reduced serum enzymes with appreciable improvement obtained when included at 0.5 g Arg/kg. Arginine supplementation enhanced the relative weights of thymus, spleen, and reduced Salmonella counts in small intestine of turkeys.

Keywords: Carcass yield, Gut microflora, Heamatological indices, Lymphoid organs, Serum chemistry

1. Introduction

Arginine is a functional amino acid needed as building blocks of proteins and polypeptides, functions in the regulation of key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity (Liu et al., 2012; Wu, et al., 2012). Arginine acts as substrate for biosynthesis of several molecules such as protein, nitric oxide (NO), proline, ornithine, polyamines, glutamate, and glutamine (Khajali and Wideman, 2010). Arginine regulated the expression of fat metabolic genes in porcine adipose tissues and skeletal muscles (Tan et al., 2011), increased muscle gain, and reduced body fat mass in growing-finishing pigs (Tan et al., 2009). Arginine has also been shown to ameliorate intestinal abnormalities and attenuate growth depression in pigs fed mold-contaminated diets (Yin et al., 2014). The relevance of Arg in the nutrition of poultry has been reported in literature. Arginine improved the growth performance of broiler chickens (Chen et al., 2011), improved carcass traits, and breast meat yield of meat-type ducks (Wu et al., 2011). Reduced carcass yield, breast meat yield

(Khajali et al., 2011), and leg muscle weight (Jiao et al., 2010) were also reported in broilers fed with Arg-deficient diets.

Arginine has been reported to improve the health status of humans and animals (Uni and Ferket 2003; Wu, 2009). Infected mice showed improved reproductive performance (Ren et al., 2012) and positive pregnancy outcomes (Ren et al., 2013) following dietary supplementation with Arg. Remarkable changes in serum profile of amino acids that alleviated damages caused by Dextran Sulphate Sodium Colitis were reported in mice following Arg supplementation (Ren et al., 2014). The white blood cell concentration and heterophil count in laying hens (Al-Hassani, 2011), as well as packed cell volume (PCV), red blood cell (RBC), and haemoglobin (Hb) concentrations of broiler chickens (Al-daraji and Salih, 2012) were influenced following dietary supplementation with Arg. Arginine plays a vital role in the development of lymphoid organs, which are crucial for effective immune system (Stechmiller et al., 2005). Dietary supplementation with Arg improved thymus functioning and spleen development (Bistrain, 2004). Relative weight of lymphoid organs reduced in chickens fed diet deficient in Arg when compared with chickens fed the Arg-supplemented diets (Kwak et al., 1999).

The achievement of improved gut health is dependent on intestinal environment and gut microflora, which protect the host from oral pathogens (Ziegler et al., 2003). The influence of nitric oxide (a metabolic molecule produced by Arg) on innate immunity (Ren et al., 2014) and the proliferation of intestinal pathogenic microbes (Allen, 1999; Li et al., 2007) has been reported in literatures. Nitric oxide (NO) played a vital role in the destruction of some pathogenic microbes by neutrophils and macrophages (Li et al., 2007). Daily oral administration of 500 mg Arg/kg alleviated unhealthy effects and negative impact of *Eimeria tenella* in chickens (Allen, 1999). Dietary inclusion of Arg improved the proliferation of intestinal intra-epithelial

92 lymphocytes and increased its toxicity against infectious bursal disease virus in chickens (Tayade
93 et al., 2006). This study investigated the effect of supplemental Arg on haematological indices,
94 serum chemistry, carcass yield, gut microflora, and lymphoid organs of growing turkeys.

95

96 **2. Materials and methods**

97 *2.1. Management of turkeys*

98 This study was conducted at the turkey unit of the Teaching and Research Farms,
99 University of Agriculture, Abeokuta, Nigeria during the late dry season. Experimental
100 procedures complied with the guidelines of the Animal Care Committee of the Federal
101 University of Agriculture (Abeokuta, Nigeria). A total of 200 one-day-old, male turkey poults
102 obtained from a commercial hatchery (British United Turkeys; Obasanjo Farms Ltd, Ibadan,
103 Nigeria) were reared together under a deep litter housing system for a 56-d pre-experimental
104 period. Dried wood shavings were used as litter material. The pre-experimental period lasted for
105 the pre-starter (d 0 to 28) and starter (d 28 to 56) phases of the turkeys. Brooding of turkeys was
106 done for 0 to 28 d of age, while normal ambient temperature prevailed after the brooding period.
107 Turkeys were fed with commercial maize-soybean meal based pre-starter (metabolizable energy
108 (ME) = 11.79 MJ/kg, crude protein (CP) = 278 g/kg, Met = 5.1 g/kg, and Lys = 16 g/kg) and
109 starter diets (ME = 12.13 MJ/kg, CP = 259 g/kg, Met = 4.6 g/kg, and Lys = 15 g/kg), which met
110 the NRC (1994) nutritional requirements of the various age groups. During the pre-experimental
111 period, feed and clean water were supplied ad libitum, while no mortality occurred. After the 56-
112 d pre-experimental period, the feeding study was initiated, which lasted for 8 wk.

113 *2.2. Dietary treatments and composition*

114 At d 56, 180 male turkeys of similar weights were selected, weighed individually, and
115 allotted to 1 of 3 treatments with 6 pens (dimension, 2.5 × 1.8 m) per treatment, and 10 turkeys
116 per pen in a completely randomized design. A total of 18 similar floor pens were used in this
117 study. The pens were furnished with wood shavings as beddings. A maize-soybean meal diet
118 (basal diet), which met the NRC (1994) nutritional requirement was formulated for grower (d 56
119 to 84), and finisher (d 84 to 112) phases of turkeys (Table 1). Two additional experimental diets
120 were subsequently formulated for the grower and finisher phases by supplementing the basal diet
121 with 0.5, and 1 g Arg/kg (Shanghai TECH Chemical Industry, Shanghai, China). Turkeys had ad
122 libitum access to feed and water. Feed samples were analyzed for dry matter (Method 934.01),
123 crude fibre (Method 978.01), ether extract (Method 920.39), ash (Method 942.05), and crude
124 protein (N × 6.25; Method 990.03) using standard methods of AOAC (2000). Amino acids
125 contents of the feed samples were determined (Harper Adams University Laboratory, Newport,
126 UK) using HPLC (SSNIFF Spezialdiäten GmbH, Soest, Germany) and following standard
127 methods (European Commission, 1998).

128 *2.3. Measurement of haematological indices and serum chemistry*

129 *2.3.1. Blood sample collection*

130 At 84 and 112 d of study, blood sample (3 mL each) was collected from the brachial wing
131 vein of one turkey per pen (selected at random) into vials containing ethylene diamine tetra-
132 acetate for the determination of haematological indices. Another set of blood was collected into
133 plain bottles (without ethylene diamine tetra-acetate), centrifuged (2,500 × *g* for 15 min at 8^{o}C),
134 and used for serum chemistry analysis.

135 *2.3.2. Haematological indices*

Hemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958). Packed cell volume (PCV), red blood cell (RBC), and white blood cell counts (WBC) were determined with Wintrobe haematocrit tube according to the method of Schalm et al. (1975). Differential leucocyte counts (heterophils, lymphocytes, basophils, eosinophils, monocytes) were carried out on blood smears stained with May-Grunwald-Giemsa stain and further calculated.

2.3.3. Serum chemistry

Total serum protein (Varley et al., 1980) and serum uric acid concentrations (Wootton, 1964) were measured according to standard procedures. Serum enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Bergmeyer (1983) with the aid of commercial kits (Roche COBAS testing Kits, Roche, Basel, Switzerland). Total thyroxine (T_4) concentration (Kozwich et al., 2000) and triodotyronine (T_3) (Kozdag et al., 2005) were measured according to standard procedures.

2.4. Carcass yield

At d 112, 1 turkey per pen whose weight is a representative of the average weight of turkeys in each pen was selected, slaughtered, defeathered, and eviscerated following standard commercial procedures (Jensen, 1984). The body weight and dressed weights, were measured, while the dressing percentage was calculated. Cut parts, which include head, neck, breast, back, thighs, drumsticks, and shanks, were weighed, and recorded as relative weights (percentage of body weight). The organs, which include kidney, lungs, gizzard, liver, heart, caecum, bursa, and spleen, were collected, weighed, and calculated as percentages of respective body weights

2.5. Gut microflora

158 At d 112, 1 turkey per replicate was selected and slaughtered for the collection of two
159 sets of intestinal contents. Fresh digesta from the small intestine (from the distal end of the
160 duodenum to the ileo-caecal junction) were collected and emptied into labeled sterile bottles.
161 Fresh cecal content collected from a pair of ceca of the selected turkey was also collected in
162 different labeled sterile bottle. All samples collected were used for the estimation of gut
163 microbiota according to the methods of Xia et al. (2004). One gram of sample was dispersed in a
164 9 mL phosphate-buffered saline solution with 0.5 g/L of Cys.HCl, and further diluted to a factor
165 of 10^{-8}. For the enumeration of bacteria, 0.1 mL of diluted sample was spread onto Petri dish
166 containing selective media. The small intestinal and cecal content samples were incubated with
167 Wilkins-Chalgren agar (Merck GmbH, Darmstadt, Germany) + novobiocin (8 mg/L) + colistin
168 sulphate (8 mg/ L) at 37°C for 72 h for estimation of clostridium counts, ES agar (Merck GmbH,
169 Darmstadt, Germany) at 37°C for 24 h for estimation of coliform counts, de Man Rogosa Sharpe
170 agar (Merck KGaA, Foster City, California, United States) at 37°C for 72 h for estimation of
171 lactobacilli count, and brilliant green agar (Merck Ltd, Mumbai, India) at 37°C for 24 h for
172 estimation of salmonella count. Microbial counts were expressed as colony-forming units (cfu)
173 of microorganism per gram of fresh sample.

174

175 *2.6. Statistical analysis*

176 Colony-forming units calculated per gram of sample obtained for gut microflora were
177 transformed as log10 of viable bacteria per gram of fresh matter. Data obtained from this study
178 was subjected to one-way analysis of variance as a completely randomized design. The pen was
179 used as the experimental unit for the statistical analysis. The data were analyzed using the

ANOVA procedure of SAS (1999). Linear and quadratic polynomial contrasts were applied to evaluate the effect of varying supplemental levels of Arg.

3. Results

3.1. Haematological indices

Table 2 shows the effect of Arg supplementation on haematological indices of grower and finisher turkeys measured at d 84 and 112, respectively. Except eosinophil, no effect of Arg supplementation was obtained on haematological indices measured at d 84. Dietary supplementation with 1 g Arg/kg showed a linear reduction ($P = 0.017$) in eosinophil value.

In finisher turkeys at 112 d, PCV increased (linear, $P = 0.019$; quadratic, $P = 0.017$) with Arg supplementation. Lymphocytes increased linearly and quadratically (linear, $P = 0.011$; quadratic, $P < 0.001$), RBC and basophil counts increased quadratically ($P < 0.001$) initially as Arg supplementation increased from 0 to 0.5 g/kg, but decreased with the 1.0 g Arg/kg. Heterophil (linear, $P = 0.032$; quadratic, $P < 0.001$), monocyte (linear, $P = 0.019$; quadratic, $P < 0.001$), and WBC counts (quadratic, $P = 0.004$) reduced initially as Arg supplementation increased from 0 to 0.5 g/kg, but increased with the 1.0 g Arg/kg.

3.2. Serum chemistry

The effect of Arg supplementation on serum chemistry of grower and finisher turkeys measured at 84 and 112 d of study is shown in Table 3. At d 84, total serum protein (quadratic, $P = 0.030$) and serum globulin concentrations (quadratic, $P = 0.043$) increased initially as Arg supplementation increased from 0 to 0.5 g/kg, but decreased with the 1.0 g Arg/kg. Uric acid concentration and ALT activity reduced quadratically (P = 0.002) as Arg supplementation

202 increased from 0 to 0.5 g/kg, but increased with the 1.0 g Arg/kg. Similarly, ALP activity
203 reduced linearly and quadratically with increasing Arg supplementation (linear and quadratic, *P*
204 < 0.001).

205 At d 112, total serum protein (linear, *P* = 0.004; quadratic, *P* = 0.002), serum globulin
206 (linear, *P* = 0.008; quadratic, *P* = 0.030), serum albumin (linear, *P* = 0.012; quadratic, *P* =
207 0.040), and T_3 concentrations (linear, *P* = 0.025; quadratic, *P* = 0.033) increased with increasing
208 Arg supplementation. Alanine aminotransferase and AST concentrations reduced linearly and
209 quadratically with increasing Arg supplementation (linear and quadratic, *P* < 0.001). Serum uric
210 acid concentration reduced linearly and quadratically as Arg supplementation increased from 0 to
211 0.5 g/kg, but increased with the 1.0 g Arg/kg (linear and quadratic, *P* < 0.002).

212 *3.3. Carcass yield and lymphoid organs*

213 The effect of Arg supplementation on carcass yield and relative organ weights of
214 finisher turkeys at d 112 is shown in Table 4. Highest body weight (quadratic, P = 0.040),
215 defeathered weight (quadratic, P = 0.040), and dressing percentage (linear, P = 0.042; quadratic,
216 P = 0.022) were recorded with turkeys fed the diet supplemented with 1 g Arg/kg. Dietary
217 supplementation with Arg had no effect on the relative weights of retail cut parts. The spleen
218 weights increased linearly (*P* = 0.006), while thymus weights increased quadratically (*P* = 0.003)
219 with increasing dietary Arg supplementation. The relative weight of the heart reduced
220 quadratically (*P* < 0.003) as Arg supplementation increased from 0 to 0.5 g/kg, but later
221 increased with the 1.0 g Arg/kg.

222 *3.4. Gut microflora*

223 Table 5 shows the effect of Arg supplementation on gut microflora of turkeys at d
224 112. Clostridium and Coliform counts of the small intestinal content increased linearly, and

quadratically ($P < 0.001$), while Salmonella counts reduced quadratically ($P = 0.029$) as Arg supplementation increased from 0 to 1.0 g/kg. Lactobacillus counts of the small intestinal content reduced quadratically as Arg supplementation increased from 0 to 0.5 g/kg, but showed a quadratic increase with the 1.0 g Arg/kg ($P = 0.002$).

Cecal content of turkeys at d 112 showed a quadratic increase in Lactobacillus counts as Arg supplementation increased from 0 to 1.0 g/kg ($P = 0.030$). Dietary supplementation with Arg had no effect on cecal Clostridium, Coliform, and Salmonella counts

4. Discussion

4.1. Haematological indices

The findings of the present study which showed no effect of Arg on PCV, Hb, RBC, WBC, heterophil, lymphocyte, basophil, and monocyte counts of grower turkeys at d 94 suggested that Arg supplementation posed no adverse effect on the health status of growing turkeys. This corroborates the findings of Emadi et al. (2010) and Atakisi et al. (2009) who reported that Arg supplementation posed no adverse effect on health status but rather improved some blood traits of broilers. Al-Hassani, (2011) also reported no significant effect of Arg supplementation on lymphocyte, heterophil, basophil nor monocyte of laying hens.

A linear and quadratic increase in PCV of finisher turkeys at 112 d with Arg supplementation obtained in the current study implied improved protein intake and tissue synthesis. Reduced PCV in poultry birds have been linked with inhibition of protein synthesis, immune suppression, and anaemic condition (Denli et al., 2009). Quadratic increase in RBC of finisher turkeys at d 112 as Arg supplementation increased from 0 to 0.5 g/kg implied higher oxygen carrying capacity of the blood and improved health status of finisher turkeys at 0.5 g Arg/kg. The findings of this study

agreed with Al-Daraji and Salih (2012) who reported that Arg supplementation improved erythrocyte count, PCV, and haemoglobin concentration of broiler chickens. The mechanism behind improved PCV and RBC concentrations obtained with Arg supplementation could be linked with the secretion of insulin-like growth factor (IGF) following supplemental Arg (Le Roith et al., 2001), which in turn fosters the proliferation, and differentiation of burst and colony forming units erythroid, myeloid progenitor, and peripheral blood cells (Deicher and Walter, 2005). The proliferation, differentiation, and maturation of RBC stimulated by Arg is as a result of erythropoietin, which is the hematopoietic growth factor produced by the kidney. This factor acts directly on certain RBC progenitors and precursors in the bone marrow (Westenfelder, 2002).

The linear reduction in WBC counts, linear and quadratic reduction in heterophil, and monocyte counts of finisher turkeys at d 112 as Arg supplementation increased from 0 to 0.5 g/kg obtained in this study implied improved health status with finisher turkeys fed the diet supplemented with 0.5 g Arg/kg. Elevated WBC counts have been recorded under diseased condition, infection or immune system disorder (Maroufyan et al., 2010). Heterophils have phagocytic action in the inflammatory response against infectious agents (Montalli, 1988) and are essential in fighting infection in poultry (Swaggerty et al., 2005). Reduced heterophil counts were also obtained in broiler chickens fed diets supplemented with Arg (Al-Daraji and Salih, 2012).

Linear and quadratic increase in lymphocyte counts of finisher turkeys at d 112 obtained in the present study as Arg supplementation increased from 0 to 0.5 g/kg could be related to the positive effect of Arg on thymus size which stimulates the production of lymphocytes by the thymus, and restores the production of thymic hormones to higher levels (Dean, 1999). This

271 could be connected with the increased thymus weights obtained with turkeys fed diet
272 supplemented with L-arg. The health status and lymphocyte counts of animals have been
273 associated with the development and size of lymphoid organs. Chickens fed diet deficient in Arg
274 has been reported to show reduced lymphocyte counts, poor thymus, and spleen development
275 (Kwak et al., 1999).
276
277 *4.2. Serum chemistry*
278 The quadratic increase in total serum protein and serum globulin concentrations of
279 grower turkeys as Arg supplementation increased from 0 to 0.5 g/kg, linear and quadratic
280 increase in total serum protein, serum albumin, and globulin of finisher turkeys with increasing
281 Arg supplementation suggested improved health status, and efficient dietary protein utilization
282 following Arg supplementation. Reduced total serum protein concentration has been implicated
283 as indications of low dietary protein utilisation (Schalm et al., 1975). The trend observed in the
284 present study agreed with results of Emadi et al. (2011) who reported increased total serum
285 protein and serum albumin following Arg supplementation. Increased serum protein
286 concentration obtained following dietary supplementation with Arg could be due to the
287 stimulating effect of Arg on pituitary and pancreatic hormones. Arginine has been reported to
288 stimulate the release of pituitary and pancreatic hormones, including glucagon, and growth
289 hormone which in turn increase protein synthesis (Davila et al., 1987).
290 The linear and quadratic increase in T_3 concentration of finisher turkeys at d 112 with
291 increasing Arg supplementation obtained in the current study may be related to an increased
292 metabolic rate, especially related to energy production, as well as improved growth, and
293 development of the turkeys. Thyroid hormones (T_3 and T_4) are involved in a wide range of

metabolic activities influencing energy production, growth, and development. Klandorf et al. (1981) confirmed that T_3 was metabolically more active during energy production. Bobek et al. (1976) showed that T_3 played far greater roles in bio-oxidation processes in cells, regulating oxygen consumption in growing chickens than T_4. Linear and quadratic reduction in ALP concentration of grower turkeys at d 84, ALT, and reduction in AST concentrations of finisher turkeys at d 112 with increasing Arg supplementation in the present study indicated good health status, and lack of abnormalities in liver functioning. Serum enzyme concentrations were reported to exist at low concentration in a normal healthy animal but increased under stressful conditions, hepatotoxic situation, and inhibition of protein synthesis (Grunwaldt et al., 2005). Increased concentration of liver enzymes has been reported in situations of liver abnormalities, stress, and disease condition (Ewuola et al., 2008). Rosa et al. (2001) reported impaired carbohydrate, and lipid metabolism in animals with increased liver enzymes. The least serum uric acid obtained with grower and finisher turkeys fed the diet supplemented with 0.5 g Arg/kg showed indications of improved, efficient protein utilization, and reduced deamination following Arg supplementation in a dose-dependent manner. Oduguwa and Ogunmodede (1995) reported high serum uric acid concentrations due to inefficient protein utilization. High serum uric acid concentration has been reported to be typical of animals fed with nutritionally imbalanced dietary amino acids (Szabo et al., 2005).

4.3. Carcass yield and lymphoid organs

The role of Arg in protein synthesis, tissue accretion, and subsequently on carcass yield has been documented (Kwak et al., 1999; Kidd et al., 2001). Highest body weight, defeathered weight, and dressing percentage obtained in the current study with turkeys fed the diet

supplemented with 1 g Arg/kg agreed with the study of Al-Daraji and Salih (2012) who reported that carcass yield, and breast meat yield of broilers increased with increasing dietary inclusion of Arg. Jiao et al. (2010) also reported that Arg supplementation improved carcass yield of broilers. Corzo et al. (2003) also reported significant improvement in carcass yield, and reduction in abdominal fat of heavy broiler chickens fed diets containing increased level of Arg from 42 to 56 d. Fernandes et al. (2009) noted enhancement in breast weight and breast fillet weight of broilers fed diets supplemented with Arg. Improved dressing percentage obtained with turkeys fed diet supplemented with 1 g Arg/kg could be related to the functions of Arg, acting as substrate for biosynthesis of several molecules (such as protein, creatine, proline, ornithine, and polyamine), which are essential for growth, and tissue development (Chen et al., 2011). However, relative weights of retail cut parts were not affected in this study.

The development of lymphoid organ has been known to correlate directly with the health status of animals. Thymus and spleen weights are often times measured as indicators of health, and immunological stress (Kwak et al., 1999). The linear increase in thymus weights and quadratic increase in spleen weights obtained in the current study with increasing Arg supplementation corroborated the work of Munir et al. (2009) who reported that supplemental Arg enhanced thymus and spleen weights of broilers. Poor development of thymus and spleen has been associated with Arg deficiency (Kwak et al., 1999). Arginine supplementation was reported to improve thymus weight, function (Bristrain, 2004), and acted as a sensitive indicator of health, acute, and chronic stress responses (Shelat et al., 1997). Thymus weight is related to the magnitude of developing T cells, while spleen weight is related to the proliferation of immune cells within the secondary lymphoid tissue during periods of infection (Elmore, 2006; Pozo et al., 2009).

Various reports also established the efficacy of Arg on the development of lymphoid organs. Abdukalykova and Ruiz-Feria (2006) demonstrated that high level of Arg accelerated antibody production in broiler chickens. Broilers fed diet supplemented with Arg and challenged with infectious bursal disease virus achieved higher thymus weight (Ruiz-Feria and Abdukalykova, 2009). Experimental models have also established the fact that Arg played an important role as a potent immunological modulator through production of nitric oxide, which has a direct influence on the immune system of birds (Friedman et al., 1998; Kidd et al., 2001).

4.4. Gut microbiota

Nutrition can be used to manipulate immune responsiveness to pathogens by providing substrate for immune cells or pathogens, protecting animal against immunopathology, influencing gut microbial populations, and the hormonal environment (Humphrey, 2004; Klasing, 2007). The gut microflora played a vital role in improving gut health of animals by protecting the host from oral pathogens (Ziegler et al., 2003). Greatest Lactobacillus count obtained in small intestinal content of turkeys fed the diet supplemented with 1 g/kg of L-arg, and quadratic increase in cecal Lactobacillus counts as Arg supplementation increased from 0 to 1.0 g/kg were indicative of improved gut health. Lactic acid bacteria happen to be the normal flora of gastrointestinal tract, which ferment carbohydrates or starch to produce lactic acid, and hydrogen peroxide as an end product (Harley and Prescott, 1993). Lactic acid reduced the pH of the gut, and thereby inhibited the growth of other bacteria including the enteropathogens; hence it had positive association with animal health (Rowland, 1992). The linear and quadratic increase in Clostridium and Coliform counts of the small intestinal content recorded with increasing dietary supplementation levels of Arg may not necessary suggest serious illness but

363 may indicate the possible presence of other pathogenic organisms of faecal origin (Todar,
364 2007). High coliform count particularly *E. coli* species might be due to changes in gut profile to
365 a population of coliform bacteria potentially beneficial to growth (Ravindran et al., 2006).
366 Quadratic reduction in Salmonella count obtained in the small intestinal content of 112 d
367 turkeys as Arg supplementation increased from 0 to 1.0 g/kg suggested a reduction effect on the
368 concentration of Salmonella organisms following Arg supplementation. Eriksson et al. (2003)
369 earlier noted that Arg supplementation reduced intestinal salmonella counts of poultry. The
370 reduction effect of Arg on intestinal salmonella counts has been linked with nitric oxide
371 produced following Arg supplementation. Nitric oxide has been described as a potent agent
372 capable of limiting the growth of not only *Salmonella Typhimurium* but also that of other
373 intracellular parasites (Eriksson et al., 2003). Identification of the Arg pathway which produces
374 nitric oxide has led to the research demonstrating that macrophages produced by nitric oxide was
375 increased by a local concentration of Arg, and could function as a defence mechanism against
376 infection (Sung et al., 1991).

377

378 **5. Conclusion**

379 Arginine supplementation improved the haematology of finisher turkeys as indicated by
380 increased PCV, improved serum chemistry of grower and finisher turkeys as indicated by
381 increased total serum protein, and reduced serum enzymes with appreciable improvement
382 obtained at 0.5 g Arg/kg. Arginine supplementation further enhanced the relative weights of
383 thymus, spleen, and reduced Salmonella counts in small intestine of finisher turkeys.

384 **Conflict of interest statement**

There is no conflict of interest with any individual or organization regarding the materials discussed in the manuscript.

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590 **Table 1**

591 Composition of basal diet for grower (d 56 to 84) and finisher (d 84 to 112) turkeys[a]

Item	Grower	Finisher
Ingredient (g/kg)		
Maize	575	622
Fish meal (720 g/kg)	89	46
Soybean meal	264	240
Soybean oil	-	2
Wheat offal	47	65
Bone meal	6	6
Limestone	8	8
Vitamin/mineral premix	5	5
L-Lys	1	1
DL-Met	2	2
Salt	3	3
Total	1,000	1,000
Calculated composition		
Ca (g/kg)	10.5	9.5
P (g/kg)	6.5	5.4
Metabolizable energy (kcal/kg)	3,196	3,275
Determined composition (g/kg DM basis)		
Crude protein	230.3	195.5
Crude fiber	33.2	39.0
Ether extract	32.1	30.7
Indispensable amino acids		
Arg	16.3	14.3
His	6.6	5.9
Ile	11.0	9.3
Leu	21.6	20.0
Lys	14.3	11.3
Met	5.5	4.6
Phe	11.7	10.5
Thr	11.5	8.1
Val	12.4	10.3
Dispensable amino acids		
Asp	24.8	21.4
Cys	36.0	33.0
Glu	43.4	39.9
Gly	11.2	9.4
Ala	13.7	11.9
Pro	14.8	14.0
Ser	11.5	10.3
Tyr	5.2	4.8

[a]Provided vitamin-mineral premix per kilogram of diet: 1,200 IU vitamin A; 300 IU vitamin D_3; 4.2 mg vitamin E; 0.2 mg vitamin K_3; 0.2 mg vitamin B_1; 0.66 mg vitamin B_3; 0.5 mg vitamin B_6; 2 μg vitamin B_{12}; 0.1 mg folic acid; 0.02 mg biotin; 1.5 mg Ca pantothenate, 0.07 g choline chloride; 12 mg antioxidant (butylhydroxytoluene); 0.23 g Ca; 0.5 mg Cu; 5.1 mg Zn; 6 mg Fe; 7.1 mg Mn; 0.06 mg I; and 0.02 mg Se.

598 **Table 2**
599 **E**ffect of Arg supplementation on haematological indices of grower (d 56 to 84) and finisher (d
600 84 to 112) turkeys[a]

Item	Arg (g/kg)			Pooled	*P*-value	
	0	0.5	1.0	SEM	Linear	Ouadratic
d 84						
Packed cell volume (%)	35.33	35.83	36.33	1.64	0.555	0.190
Hemoglobin (g/dL)	11.57	11.77	12.22	0.54	0.468	0.429
Red blood cell ($\times 10^{12}$/ L)	2.33	2.34	2.40	0.05	0.609	0.790
White blood cell ($\times 10^{9}$/ L)	8.13	11.22	15.32	1.51	0.254	0.107
Heterophil (%)	36.2	39.5	43.5	1.9	0.403	0.205
Lymphocyte (%)	55.0	60.5	56.0	2.6	0.797	0.152
Eosinophil (%)	3.5	3.5	2.7	0.5	0.017	0.142
Basophil (%)	0	0	0	0	-	-
Monocyte (%)	0.5	0.5	0.0	0.1	0.072	0.281
d 112						
Packed cell volume (%)	29.17	33.17	33.00	5.69	0.019	0.017
Hemoglobin (g/dL)	9.97	10.60	9.89	0.17	0.908	0.097
Red blood cell ($\times 10^{12}$/ L)	1.95	2.77	1.85	0.12	0.751	<0.001
White blood cell($\times 10^{9}$/ L)	13.03	11.25	12.41	2.25	0.319	0.004
Heterophil (%)	32.50	23.67	27.50	3.98	0.032	<0.001
Lymphocyte (%)	65.7	72.5	70.5	9.8	0.011	<0.001
Eosinophil (%)	0	0.3	0	0.1	0.070	0.116
Basophil (%)	0.5	2.5	1.0	0.3	0.441	<0.001
Monocyte (%)	0.5	0.0	1.5	0.2	0.019	<0.001

601 [a] Based on 6 pens/treatment; and SEM = standard error of the mean.
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Table 3

Effect of Arg supplementation on serum chemistry of grower (d 56 to 84) and finisher (d 84 to 112) turkeys

Item	Arg (g/kg)			Pooled	*P*-value	
	0	0.5	1.0	SEM	Linear	Quadratic
d 84						
Total protein (g/dL)	3.52	3.91	3.70	0.85	0.169	0.030
Globulin (g/dL)	2.02	2.33	2.22	0.55	0.136	0.043
Albumin (g/dL)	1.50	1.58	1.48	0.03	0.838	0.415
Creatinine (mg/dL)	0.49	0.55	0.55	0.02	0.051	0.054
Uric acid (mg/dL)	4.35	3.55	5.42	0.91	0.129	0.002
ALT (U/L)	12.9	10.1	15.3	1.1	0.094	0.002
AST (U/L)	158	169	158	5	0.984	0.641
ALP (U/L)	73.8	69.5	66.0	8	<0.001	<0.001
T_3 (nmol/L)	42.0	40.4	40.2	0.8	0.075	0.090
T_4 (ng/mL)	20.2	20.1	19.5	1.1	0.079	0.065
d 112						
Total protein (g/dL)	3.98	4.68	4.96	0.92	0.004	0.002
Globulin (g/dL)	1.73	2.03	2.18	0.47	0.008	0.030
Albumin (g/dL)	2.25	2.65	2.78	0.44	0.012	0.040
Creatinine (mg/dL)	0.49	0.54	0.52	0.02	0.050	0.054
Uric acid (mg/dL)	2.9	2.1	3.2	0.5	<0.001	<0.001
ALT (U/L)	30.8	20.0	19.5	1.6	<0.001	<0.001
AST (U/L)	65.2	49.3	48.0	4.0	<0.001	<0.001
ALP (U/L)	29.3	32.8	28.7	1.0	0.795	0.196
T_3 (nmol/L)	40.2	48.1	49.0	3.5	0.025	0.033
T_4 (ng/mL)	20.5	22.5	21.9	0.9	0.077	0.095

[a] Based on 6 pens/treatment, SEM = pooled standard error of means, PCV = packed cell volume, RBC = red blood cell, WBC = white blood cell, ALT = alanine amino transferase, AST = aspartate amino transferase, ALP = alkaline phosphate, T_3 = triodosterine, and T_4 = total thyroxine.

Table 4

Effect of Arg supplementation on carcass yield and relative organ weights of turkeys[a]

Item	Arg (g/kg)			Pooled	*P*-value	
	0	0.5	1.0	SEM	Linear	Quadratic
Body weight (g/bird)	9,027	8,660	9,480	164	0.125	0.040
Defeathered weight (g/bird)	8,167	7,833	8,867	158	0.062	0.020
Dressing percentage (%)	75.92	74.67	79.70	7.98	0.042	0.022
Retail cut parts (percentage body weight)						
Shank	3.99	4.01	3.63	0.34	0.709	0.805
Breast	26.56	26.71	27.15	0.42	0.626	0.889
Thigh	9.50	9.51	10.28	0.65	0.072	0.104
Drum stick	10.32	9.27	9.97	0.38	0.738	0.348
Back	15.11	10.78	16.42	1.20	0.603	0.053
Wings	11.1	10.7	10.0	0.5	0.055	0.060
Organs and offals (percentage body weight)						
Liver	1.14	1.26	1.07	0.04	0.482	0.098
Kidney	0.43	0.43	0.44	0.01	0.669	0.804
Lungs	0.55	0.45	0.33	0.09	0.007	0.882
Whole gizzard	2.33	2.22	2.15	0.07	0.341	0.902
Empty gizzard	1.60	1.56	1.52	0.05	0.559	0.984
Proventriculus	0.17	0.14	0.13	0.01	0.062	0.409
Pancreas	0.13	0.12	0.11	0.01	0.085	0.584
Bursa	0.05	0.06	0.04	0.01	0.284	0.146
Spleen	0.05	0.07	0.08	0.01	0.006	0.131
Gastrointestinal weight	2.96	2.97	2.67	0.12	0.380	0.605
Crop	0.68	0.62	0.36	0.09	0.202	0.622
Thymus	0.03	0.06	0.06	0.01	0.791	0.030
Heart	0.42	0.34	0.43	0.02	0.884	0.003

[a] Based on 6 pens/treatment, and SEM = pooled standard error of means.

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628 **Table 5**

629 Effect of Arg supplementation on gut microflora (Log10 cfu microorganism/g) in turkeys[a]

Item	Arg (g/kg)			Pooled	*P*-value	
	0	0.5	1.0	SEM	Linear	Quadratic
Small intestine						
Clostridium	5.6	6.2	6.3	1.0	<0.001	<0.001
Coliform	5.4	5.8	6.0	0.9	<0.001	<0.001
Lactobacillus	5.2	5.0	5.6	1.0	0.092	0.002
Salmonella	5.6	5.1	5.0	1.0	0.060	0.029
Caecum						
Clostridium	7.6	7.5	7.7	0.1	0.085	0.090
Coliform	6.9	6.9	6.6	0.1	0.077	0.095
Lactobacillus	6.4	6.9	6.9	1.0	0.066	0.030
Salmonella	6.2	6.4	6.0	0.1	0.072	0.065

630 [a] Based on 6 pens/treatment, and SEM = pooled standard error of means.

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