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Original Research Article

Response of guinea fowls to dietary L-arginine supplementation

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Abstract

This experiment aimed at evaluating the influence of supplementing L-arginine in the diets fed to guinea fowls on growth response, haematological profile, serum biochemical indices and internal organs. A total of 300 one-day-old guinea fowl keets were allotted in a completely randomised design to three dietary treatments of basal diets (starter and grower) supplemented with 0, 0.5 and 1.0 g arginine/kg. Each treatment group was replicated four times consisting of 25 keets per replicate. At the starter phase, final weight, weight gain and feed conversion ratio ($p < 0.05$) improved as arginine level increased in the diet. However, feed intake was higher ($p < 0.05$) in the group fed 1.0 g/kg arginine supplemented diet when compared to other treatment groups. Final weight at the grower phase increased linearly ($p < 0.05$) with arginine supplementation without corresponding effect on weight gain in birds fed different arginine levels. Red blood cell counts, white blood cell counts and lymphocytes ($p < 0.05$) increased in the blood of guinea fowls fed diets supplemented with arginine. Creatinine and uric acid ($p < 0.05$) were reduced in guinea fowls fed arginine supplemented diets at the starter and grower phases, respectively. Liver weight linearly increased ($p < 0.05$) with arginine in the diets of the guinea fowls. This study indicates that supplementing guinea fowl diet with arginine at 1.0 g/kg at the starter phase and 0.5 g/kg at the finisher phase improved their growth and feed conversion ratio. Birds fed arginine supplemented diets had higher lymphocyte and reduced heterophil counts which may suggest a better immune response.

Keywords: *Numida meleagris*; feed intake; feed conversion; growth; body organs; blood profile; biochemistry

INTRODUCTION

To bridge animal protein shortage and ensure food security, poultry production has been referenced as a good choice to poverty alleviation (Bernacki et al., 2013). Guinea fowl (*Numida meleagris*) accounts for about 25 percent of domestic poultry population in Nigeria and second to chicken eggs and meat in terms of poultry product population and consumption (Ebegbulem and Asuquo, 2018). Guinea fowl is reported to have higher meat yield when compared to chickens due to its tiny bones (Musundire et al., 2017). The meat is very tender, lean, rich in protein and essential fatty acids (Laudadio et al., 2012). As a result of guinea fowl adaptability to the tropical region, Dahouda et al. (2007), suggested that guinea fowl production should be considered to increase meat production in the region. The high demand for guinea fowl is not always met due to slow growth rate of the bird and profitability achievements (Avornyo et al., 2016). Agbolosu et al. (2014) reported that intensive rearing of guinea fowl has the ability to be more profitable than extensive management system.

Poultry have been reported to have the highest arginine (Arg) requirement of all animals (Ball et al., 2007). This requirement is needed to create many molecules, which include glutamate, ornithine, creatinine and nitric oxide (Khajali and Wideman, 2010). L-arginine is an essential amino acid for poultry, because it cannot be synthesised within the body of the birds due to the incomplete urea cycle. Thus, arginine has to be provided in the diets to meet their needs for protein synthesis and other functions. Youssef et al. (2016) observed improvement in growth of broiler chickens fed diet supplemented with L-arginine. Several studies have shown that dietary arginine levels higher than National Research Council recommendations (NRC) (1994) can promote the growth of broilers (Fernandes et al., 2009; Jahanian, 2009), and improve the performance of laying hens (Youssef et al., 2015). Digestible arginine levels of 1.50, 1.20 and 0.91% were recommended for starter, grower and breeder guinea fowls, respectively (Leeson and Summers, 1997).

This study aimed to evaluate the effect of dietary L-arginine on the growth, blood variables and internal organs of guinea fowls. It can be hypothesed that guinea fowls grow bigger and healthier when fed diets supplemented with arginine.

MATERIALS AND METHODS

Experimental birds

This study was carried out according to the procedure of Ethics and Welfare Committee of the Federal University of Agriculture Abeokuta, Nigeria.

A total of three hundred (300) one-day-old unsexed pearl guinea fowl keets were assigned to the three dietary treatments in a completely randomised design. Each treatment group consisted of one hundred birds and was replicated four times with 25 keets per replicate. Each of the replicate pen was demarcated all round from the top with chicken net to prevent the birds from flying. The birds were brooded for the first four weeks, water and mash feed were supplied *ad libitum* throughout this phase.

Experimental diets

The test ingredient L-arginine was sourced from Shanghai TECH, Chemical Industry testing Co Ltd, Shanghai, People' Republic of China. Basal experimental diets were formulated according to NRC (1994) for the starting (0–7 weeks) and growing (8–14 weeks) phases as shown in Table 1. The basal diets at each of the phases were supplemented with L-arginine at 0, 0.5 and 1.0 g/kg resulting in three dietary treatments for each of the phases.

Data collection

Growth performance

The initial weights of the birds were taken at the start of the study and subsequently every week. Daily feed intake was recorded. Weight gain and feed conversion ratio (FCR) were calculated per replicate. Mortality was recorded as and when it occurred. Experimental diets were analysed for crude protein, crude fibre and crude fat using the official method of AOAC (2000). Phosphorus content of the diets was determined using the method of Motsara and Roy (2008) while the calcium content was determined using the titrimetry method as decribed by Lawani et al. (2014).

Blood indicators

At the end of the starter (day 49) and grower (day 98) phases of the experiment, blood samples were collected randomly from 3 guinea fowls in each replicate (n = 12 per treatment) through the wing vein. About 2.5 ml was emptied directly into ethylene diamine tetra-acetate (EDTA) containing bottles and the remaining blood in the syringe into a plain bottle for the determination of haematological indices and serum chemistry, respectively. Haematological indices measured included packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and WBC differential counts, which were determined according to Coles (1986). Serum was collected by allowing the blood to clot, then centrifuging at 1000 × *g* for 10 minutes and the resulting supernatant was collected as serum. Serum samples were analysed for total protein using the biuret method, albumin using bromocresol green method, uric acid according to Tinder's enzymatic method and creatinine using Basques-Lustosa's method. Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analysed using the Randox® commercial kit.

Organ collection

After the collection of blood samples, three birds per replicate were killed by exsanguination. The following organs – gizzard, heart, liver, spleen, lungs and kidneys were harvested and weighed. Adhering tissues were removed from each of the organs before weighing using the digital sensitive scale (Mettler, Mettler-Toledo, Leicester, UK) and expressed as relative weights (expressed as percentage of body weights).

Table 1. Composition of experimental basal diets (g/kg)

Ingredient	Starter (0–7 weeks)	Grower (7–14 weeks)
Maize	480.00	575.00
Fish meal (65%)	40.00	0.00
Wheat offal	87.00	159.00
Soybean meal	350.00	220.00
Bone meal	18.00	23.00
Limestone	15.00	15.00
L-Lysine	2.00	2.00
DL-Methionine	3.00	1.00
*Premix	2.50	2.50
Salt	2.50	2.50
Total	**1000.00**	**1000.00**
Determined values (%)		
Crude protein	24.20	18.20
Crude fiber	4.20	4.49
Crude fat	3.02	3.21
Calcium	1.30	1.25
Available Phosphorus	1.66	1.07
Calculated values (%)		
Lysine	1.67	1.20
Methionine	0.56	0.34
Digestible arginine	1.47	1.24
Lysine/dig. Arginine	1.14	0.97
Metabolisable energy (MJ/kg)	12.90	12.73

*Mineral and vitamin premix (per kg feed): Vit.A – 2,500,000 IU, Vit. D3 – 625,000 IU, Vit.E – 3750 mg. Vit. K3 – 500 mg. B1 – 500 mg, B2 – 1000 mg, B6 – 1000 mg, B12 – 3,750 mcg, Niacin – 7,500 mg, Pantothenic acid – 4,000 mg, Biotin – 15 mg, Folic acid – 125 mg, Choline – 75,000 mg, Selenium – 45 mg, Iodine – 175 mg Iron – 12,525 mg, Copper – 2,500 mg, Manganese – 19,500 mg, Zinc – 13,750 mg.

Statistical analysis

Data generated were subjected to one way analysis of variance using SAS (2012). Orthogonal contrast was done using SPSS 16.0.

RESULTS

Growth performance

The growth response of growing guinea fowls fed diets supplemented with varying concentrations of arginine is shown in Table 2. Final weight, weight gain, feed intake, and FCR were linearly affected with arginine supplementation at the starting phase. These growth variables with the exception of percentage mortality were affected ($p < 0.05$) as the level of Arg supplementation increased from 0.5 to 1.0 g/kg in the starter diet. Final weight, weight gain and FCR improved with arginine supplementation in the diet while, birds fed 1.0 g/kg Arg-supplemented diet consumed ($p < 0.05$) more feed when compared with birds in other treatment groups lower and similar feed intake. Percentage mortality of the birds ($p < 0.05$) decreased with arginine supplementation, which further decreased as the level of arginine increased in the diet.

At the growing phase, final weight and weight gain ($p < 0.05$) improved with Arg supplementation in the diet of the birds. There was quadratic increase in final weight of birds fed 0.05 g/kg and 1.0 g/kg Arg-supplemented diet. However, similar weight gain was observed for the two Arg-supplemented diets. Feed intake was ($p < 0.05$) highest in birds fed 1.0 g/kg Arg diet and lowest in birds fed diet supplemented with 0.5 g/kg Arg. Feed conversion ratio was ($p < 0.05$) higher in birds fed the control diet and 1.0 g/kg Arg diet than in those birds fed 0.05 g/kg Arg supplemented diet.

Haematological indices

The haematological indices of guinea fowl fed diets supplemented with Arg are shown in Table 3. Haemoglobin, RBC, WBC and lymphocyte counts linearly increased ($p < 0.05$) in birds fed

Table 2. Growth performance of guinea fowls fed diets supplemented with varying levels of L-Arginine

Parameter	Levels of L-arginine (g/kg)				*P*-values		
	0.00	0.50	1.00	SEM[1]	T[2]	L[3]	Q[4]
Starter phase (0–7 wks)							
Initial weight (g/bird)	25.58	25.57	25.57	0.01	0.105	0.582	0.870
Final weight (g/bird)	470.27[c]	480.62[b]	528.93[a]	7.74	0.001	0.009	0.000
Weight gain (g/bird)	444.68[c]	455.05[b]	503.26[a]	7.74	0.001	0.009	0.000
Total feed Intake (g/bird)	1285.78[b]	1296.74[b]	1403.62[a]	16.06	0.001	0.012	0.000
Feed conversion ratio	2.89[a]	2.85[b]	2.78[c]	0.01	0.001	0.050	0.000
Mortality (%)	5.33[a]	4.00[b]	2.67[c]	0.87	0.007	0.002	0.579
Finisher phase(8–14wks)							
Initial weight (g/bird)	470.35[c]	480.62[b]	530.13[a]	7.74	0.001	0.009	0.000
Final weight (g/bird)	1310.00[c]	1330.50[b]	1380.50[a]	8.65	0.001	0.000	0.001
Weight gain (g/bird)	839.65[b]	849.88[a]	850.37[a]	3.81	0.001	0.019	0.932
Total feed Intake (g/bird)	3213.16[b]	3160.94[c]	3262.09[a]	12.78	0.001	0.030	0.002
Feed conversion ratio	3.82[a]	3.72[b]	3.83[a]	0.01	0.001	0.044	0.016
Mortality (%)	2.90	0.00	1.39	0.77	0.564	0.581	0.574
Number of observations	100	100	100				

1 – Sum of error of means, 2 – Treatment, 3 – Linear, 4 – Quadratic

Table 3. Haematological indices of guinea fowls fed diets supplemented with L-arginine

Indicator	Levels of L-arginine (g/kg)				*P*-values		
	0.00	0.50	1.00	SEM[1]	T[2]	L[3]	Q[4]
Starter phase (day 49)							
Packed cell volume (%)	30.00	30.54	30.67	0.33	0.084	0.062	0.083
Haemoglobin (mg/L)	126.31[b]	130.34[a]	133.06[a]	1.12	0.016	0.021	0.015
Red blood cell (10^{12}/L)	3.10[c]	3.27[b]	4.00[a]	0.06	0.001	0.027	0.015
White blood cell (10^9/L)	9.27[c]	10.63[b]	12.03[a]	0.62	0.001	0.025	0.018
Heterophils (%)	33.67[a]	31.67[b]	30.00[b]	1.05	0.023	0.054	0.063
Lymphocytes (%)	64.33[b]	67.00[a]	67.67[a]	1.25	0.001	0.034	0.091
Eosinophils (%)	0.67	0.33	0.67	0.12	0.454	0.075	0.082
Basophils (%)	0.00	0.33	0.33	0.10	0.327	0.062	0.129
Monocytes (%)	1.33	0.67	1.33	0.14	0.061	0.061	0.070
Grower phase (day 98)							
Packed cell volume (%)	30.33[c]	32.54[b]	34.00[a]	0.65	0.048	0.039	0.012
Haemoglobin (mg/L)	130.00	138.26	144.72	2.48	0.516	0.031	0.048
Red blood cell (10^{12}/L)	3.13[c]	3.53[b]	3.91[a]	0.05	0.024	0.048	0.045
White blood cell (10^9/L)	12.63[b]	12.90[b]	13.43[a]	0.21	0.014	0.020	0.016
Heterophils (%)	36.33[a]	34.33[b]	30.00[c]	0.78	0.002	0.010	0.035
Lymphocytes (%)	62.00[c]	64.33[b]	66.67[a]	0.82	0.007	0.040	0.038
Eosinophils (%)	0.33	0.33	1.00	0.18	0.224	0.080	0.162
Basophils (%)	0.33	1.00	1.67	0.37	0.391	0.263	0.092
Monocytes (%)	1.00	0.00	0.67	0.24	0.258	0.063	0.071
Number of observations	12	12	12				

1 – Sum of error of means
2 – Treatment, 3 – Linear, 4 – Quadratic

Arg-supplemented diet at the starter phase. There were quadratic increases ($p < 0.05$) in Hb, RBC and WBC values as the level of arginine supplementation increased. Heterophil counts decreased linearly ($p < 0.05$) with arginine supplementation in the diet. Packed cell volume, eosinophil, basophils and monocytes were not affected by dietary supplementation of Arg.

During the growing phase, Hb, PVC, RBC, WBC and lymphocyte values increased ($p < 0.05$) linearly with Arg supplementation, which further increased ($p < 0.05$) as Arg supplementation in the diet increased. However, heterophil counts ($p < 0.05$) decreased in birds fed Arg-supplemented diets at both the starting and growing phases.

Serum Biochemistry

Serum biochemistry of guinea fowls fed diets supplemented with Arg (Table 4) showed that supplementing Arg resulted in increased ($p < 0.05$) total protein and albumin whereas creatinine concentration declined linearly ($p < 0.05$) with Arg in the diet of the guinea fowls at the starter phase. On the other hand, dietary Arg had no effect on uric acid, ALP, AST and ALT activities.

Table 4. Serum biochemistry of guinea fowls fed diets supplemented with L-arginine

Indicators	Levels of L-arginine (g/kg)				*P*-values		
	0.00	0.50	1.00	SEM[1]	T[2]	L[3]	Q[4]
Starter phase (day 49)							
Total protein (g/L)	47.32[b]	64.31[a]	65.68[a]	3.62	0.035	0.028	0.162
Albumin (g/L)	35.82[b]	53.04[a]	51.02[a]	3.32	0.046	0.019	0.095
Uric acid (mg/L)	67.66	62.73	62.69	4.22	0.087	0.092	0.247
Creatinine (mg/L)	14.31[a]	11.18[b]	8.70[c]	1.18	0.011	0.018	0.036
Alkaline phosphatase (μkat /L)	0.40	0.38	0.37	0.01	0.083	0.072	0.810
Aspartate aminotransferase (μkat /L)	0.80	0.77	0.75	0.04	0.904	0.061	0.247
Alanine aminotransferase (μkat /L)	0.40	0.42	0.43	0.02	0.752	0.140	0.223
Grower phase (day 98)							
Total protein (g/L)	49.74[b]	53.03[a]	55.39[a]	1.23	0.050	0.055	0.084
Albumin (g/L)	37.26[c]	41.01[b]	42.65[a]	0.87	0.048	0.041	0.062
Uric acid (mg/L)	75.00[a]	69.73[b]	65.02[b]	4.75	0.057	0.048	0.072
Creatinine (mg/L)	18.69[a]	15.00[b]	13.28[c]	2.23	0.052	0.035	0.081
Alkaline phosphatase (μkat /L)	0.50	0.48	0.48	0.02	0.930	0.085	0.982
Aspartate aminotransferase (μkat /L)	0.93	0.86	0.80	0.04	0.463	0.078	0.069
Alanine aminotransferase (μkat /L)	0.42	0.41	0.37	0.02	0.217	0.194	0.063
Number of observations	12	12	12				

1 – Sum of error of means, 2 – Treatment, 3 – Linear, 4 – Quadratic

Table 5. Relative weight of organs of guinea fowls fed diets supplemented with L-arginine at day 98

Indicator (% live weight)	Levels of L-arginine (g/kg)				P-values		
	0.00	0.50	1.00	SEM[1]	T[2]	L[3]	Q[4]
Gizzard	2.52	2.69	2.78	0.06	0.734	0.134	0.146
Liver	1.47[c]	1.57[b]	1.62[a]	0.03	0.045	0.043	0.071
Kidneys	0.35	0.36	0.36	0.01	0.138	0.280	0.974
Lungs	0.48	0.47	0.47	0.01	0.143	0.158	0.928
Heart	0.40	0.40	0.41	0.01	0.711	0.927	0.783
Spleen	0.05	0.06	0.06	0.00	0.988	0.874	0.986
Number of observations	12	12	12				

1 – Sum of error of means, 2 – Treatment, 3 – Linear, 4 – Quadratic

Guinea fowls in their growing phase showed similar trend in total protein, albumin and creatinine as observed in their starter phase. However, uric acid was higher ($p < 0.05$) in the serum of birds fed the control diet when compared with those fed Arg-supplemented diet.

Organs

Supplementing Arg in the diets of guinea fowls did not have any effect on gizzard, kidneys, lungs, heart and spleen as shown in Table 5. However, the relative liver weight linearly increased ($p < 0.05$) with Arg in the diets. Also a quadratic weight increase ($p < 0.05$) was observed in the liver of birds as the level of Arg supplementation increased from 0.05 to 1.0 g/kg. Although not significantly affected by arginine supplementation, the gizzard, kidneys and spleen grew marginally bigger with arginine supplementation whereas the lungs were bigger in birds fed control diet.

DISCUSSION

The increase in final weight and weight gain in birds fed arginine supplemented diet at the starting phase could be attributed to the digestive and absorptive capacity of arginine. The growth-regulating properties of arginine is as a result of it's primary component of body protein and creatinine which is a precursor of connective tissue forming proline and hydroxy-proline (Khajali and Wideman, 2010). According to Silva et al. (2012), arginine improves growth by increasing the release of insulin, GH and IGF-I into the blood stream. Corzo and Kidd (2003) observed a significant requirement for dietary arginine in chicks, connected with immune system development and early microbial challenges. Yu et al. (2018) reported a higher body weight in chickens fed a diet supplemented with 2% or 4% arginine above the NRC (1994) requirements than the control chickens during the starter period.

The improvement in final weight of birds fed Arg-supplemented diet at the finisher phase across the treatments was a result of better arginine activity. Various studies have reported arginine as a protein constituent that is involved in the secretion of insulin by pancreas, and arginine, a nutritional additive having a positive effect on the growth of chickens when included in their diet (Fernandes et al., 2009; Jahanian, 2009; Youssef et al., 2016).

Addition of L-arginine to the broiler chickens diet has been reported to enhance immune system function by inhibiting muscle loss (Atakisi et al., 2009) and reduced nitrogen excretion (Beski et al., 2015).

Higher feed consumption by birds fed 1.0 g/kg Arg diet is as a result of higher inclusion of Arg which increases feed intake due to the release of pituitary and pancreatic hormones (Davila et al., 1987; Kwak et al., 1999). High Arg in breeder diets has been reported to increase feed intake (Basiouni et al., 2006).

Better feed conversion in starting guinea fowls fed 1.0 g/kg arginine in the diet can be due to higher feed intake and efficient utilisation of feed. Arginine has been observed to stimulate the release of pituitary and pancreatic hormones including glucagon and insulin resulting in better feed conversion and protein utilisation efficiency (Abbasi et al., 2014). Lower mortality recorded in birds fed Arg diets is indicative of its ability to act as an immune system booster. Feeding Arg at levels higher than those recommended by the NRC could play a complementary role in the innate and humoral immune responses, thereby enhancing the immune response to field infections (Perez-Carbajal et al., 2010).

The improvements in haemoglobin, RBC, WBC and lymphocytes observed in guinea fowls fed diets supplemented with arginine at the starter and grower phases is a reflection of Arg in the diet. Blood transfusion in the blood vessels has been identified to be influenced by Arg which is a source of nitric oxide synthase released to form nitric oxide (Hassani, 2011).

According to Al-Daraji and Salih (2012), increasing the Arg level in the diet of chickens has a beneficial effect on the red blood cell system and haemoglobin concentrations. Jankowski et al. (2020) observed lower Hb concentration in the blood of turkeys fed a diet with the lowest Arg level than in the turkeys receiving a diet with higher Arg level.

The elevated lymphocyte counts as the Arg level increases could be a physiological adjustment against negative antigenic effects. This study has shown the stimulatory effects of Arg on immune system to fight infections with the increased WBC and lymphocyte numbers of the birds. Elevated WBC is suggestive of an improvement in immune system to destroy an infection. Fouad et al. (2013) demonstrated an improvement in immune system as a result of stress in broiler chickens fed Arg-supplemented diets. High level of arginine subsequently resulting in increased protein in the diet have been reported to positively affected the cellular immunity by increasing lymphocyte number and overall white blood cell numbers (Hindi et al., 2012)

The reduced heterophil values in birds fed arginine are in agreement with the study of Kidd et al. (2001), who reported a reduction in the proportion of heterophils in peripheral blood of broiler chicks fed an Arg-supplemented diet.

Arginine has been shown to promote protein synthesis through the high total protein and albumin in this study. Emadi et al. (2011) reported that increasing arginine levels in the diet of broiler chickens increased albumin and total protein contents.

The reduced creatinine and uric acid concentrations in bird fed Arg-supplemented diets at the starter and grower phase, respectively, is suggestive of increase in protein utilisation by birds fed Arg diets. Crystalline amino acids are known to be more digestible and bioavailable thereby increasing the efficient utilisation of dietary nitrogen and produce less uric acid (Hilliar et al., 2019). According to Chamruspollert et al. (2002), high Arg availability for protein synthesis reduces creatinine excretion.

The significant increase in relative liver weight of birds fed Arg-supplemented diet indicates that the metabolic capacity of the liver was greatly enhanced. Individual organ sensitivities have been reported to be mediated through the organ-specific expression of arginine uptake transporters (Humphrey and Klasing, 2005).

The levels of dietary arginine supplementation in this study could be responsible for its non_significant effect on most of the organ weights. Studies have shown that higher levels of arginine increase relative weight of lymphoid organs in broilers (Munir et al., 2009; Adibmoradi et al., 2015).

CONCLUSION

Supplementing guinea fowl diet with 1.0 g/kg L-arginine improved growth response at the starter phase whereas 0.5 g/kg can be supplemented at the finisher phase for a better feed conversion. Supplementing arginine in the diets of guinea fowls improved the immune status of the birds.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest on this manuscript.

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