Pertanika J. Trop. Agric. Sci. 45 (1): 37 - 53 (2022)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Effect of Herbal Blend and L-arginine Supplementation on Growth Performance, Intestinal Morphology, and Caecal Microflora of Growing Guinea Fowls

Adebukunola Olufunmilayo Lala[1]*, Gabriel Adedotun Williams[2], Adenrele Olalekan Adebayo[3] and Abimbola Oladele Oso[4]

[1]Livestock Production Research Progamme, Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Nigeria
[2]Department of Animal Science, School of Agriculture, Lagos State University, Ojoo, Lagos, Nigeria
[3]Department of Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria
[4]Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria

ABSTRACT

This experiment was carried out to investigate the effect of diet containing herbal blend (HB) of turmeric (*Curcuma longa*), scent leaf (*Ocimum gratissimum*), and moringa leaf (*Morinda lucida*) supplemented with or without L-arginine (L-Arg) on growth performance, intestinal morphology, and caecal microflora of guinea fowls. Three hundred and sixty 28-day-old male guinea fowls were randomly allotted in a completely randomized design to six treatment groups of sixty birds; each treatment group consisted of six replicates of ten birds each. Dietary treatments were laid out in a 3 × 2 factorial arrangement of the basal diet (control), diet containing enrofloxacin (1 g/kg), HB (1 g/kg diet), and each supplemented with or without L-Arg at 1 g/kg. Notwithstanding dietary supplementation with L-Arg, guinea fowls fed the diets with HB, and their counterparts fed the diets with antibiotics had similar weight gain during the growing period. Dietary L-Arg supplementation with HB increased (p<0.05) feed intake. Feed conversion ratio (p<0.05) was improved in guinea fowls fed the diets with HB and their counterparts fed with antibiotic supplemented or not with L-Arg. L-Arg supplementation of the diet with HB resulted in the longest (p<0.05) duodenal villi height

ARTICLE INFO

Article history:
Received: 28 July 2021
Accepted: 20 September 2021
Published: 21 December 2021

DOI: https://doi.org/10.47836/pjtas.45.1.03

Email addresses:
lalaao@funaab.edu.ng (Adebukunola Olufunmilayo Lala)
wgabrieladedotun@yahoo.com (Gabriel Adedotun Williams)
adebayoao@funaab.edu.ng (Adenrele Olalekan Adebayo)
drosoann@yahoo.com (Abimbola Oladele Oso)
*Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

and the shortest (p<0.05) duodenal apical width in young turkeys. The caeca content of growing guinea fowls fed the diet with HB supplemented with L-Arg had the least (p<0.05) *Clostridium* count and the highest (p<0.05) *Lactobaccillus* count. In conclusion, guinea fowls fed the diet with HB supplemented or not with L-Arg had similar growth performance with those fed with an antibiotic. L-Arg supplementation of the diet with HB resulted in increased caecal *Lactobacillus* counts of growing birds.

Keywords: Antimicrobial, growth promoter, *Lactobacillus*, phytogenics

INTRODUCTION

With the advent of drug resistance and adverse effects of chemosynthetic drugs, the interest in medicinal herbs and plant extracts/metabolites has augmented, both among the public and researchers worldwide (Abo Ghanima et al., 2020; Dhama et al., 2021; Saeed et al., 2021). In poultry production, the use of phytogenic plants as alternative growth promoters has attracted several interests due to their ability to exhibit growth-promoting, digestive stimulating, immune-enhancing, and anti-oxidative properties (Alagawany et al., 2021; Ebrahim et al., 2020; Lu et al., 2014).

Turmeric (*Curcuma longa*) is a culinary spice containing curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcuminoids (Al-Sultan, 2003). Curcumin is regarded as the most active ingredient in turmeric, exhibiting strong antioxidant, anti-inflammatory, and antimicrobial properties (Alagawany et al., 2020; Quiles et al., 2002). Scent leaf (*Ocimum gratissimum*) is rich in eugenol, cinamate, camphor, and thymol (Matasyoh et al., 2007). It exhibits strong antimicrobial properties against most pathogenic bacteria, such as *Staphylococcus aureaus*, *Escherichia coli*, *Streptococcus fecalis*, *Psudomonas aeruginosa*, and *Lactobacilli* (Prabhu et al., 2009). *Morinda lucida* Benth. (Rubiaceae) leaf extract contains saponin, tannins, alkaloids, anthraquinones, and anthraquinols. It has been shown to exhibit anticoccidial, antioxidant, and antimicrobial properties (Ogundare & Onifade, 2009; Ogunlana et al., 2008).

Various studies have reported combining two or more herbal plants as a blend, resulting in a wider range of biological activity, promoting synergism effects of various bioactive ingredients (Bassolé & Juliani, 2012; Cetin et al., 2016). In addition, previous studies reported that a combination of two or more phytogenic plants as blends yield improved and better response than single inclusion (Ertas et al., 2005; Thiruvengadarajan et al., 2011).

The metabolism of L-Arg to yield nitric oxide demonstrated its ability to enhance the immune system. Nitric oxide (NO) has been reported to be involved in the development of intestinal mucosa (Kochar et al., 2011) and the elimination of pathogenic microbes (P. Li et al., 2007). L-Arg has improved immune system, growth performance, and carcass yield (Al-Daraji & Salih, 2012). The combination of the herbal blend was such that it contains phytogenics, which exhibits antioxidant, antimicrobial, and digestive stimulant properties. The herbal

blend used in this study contained *Curcuma longa*, which was used as an antioxidant (Dono, 2013), *Morinda lucida* for its antimicrobial properties (Ola-Fadunsin & Ademola, 2013), and *Ocimum gratissimum* as a digestive stimulant (Ndelekwute et al., 2015). This study evaluated the effect of the herbal blend (containing *Curcuma longa*, *Ocimum gratissimum*, *Morinda lucida*) and L-arginine supplemented diets on growth performance, gastrointestinal tract morphology, and caecal microflora of guinea fowls.

MATERIALS AND METHODS

The feeding trial was carried out according to the guidelines approved by the Animal Welfare Committee of the Federal University of Agriculture, Abeokuta, Nigeria.

Preparation of Herbal Blend and Chemical Composition

Fresh turmeric rhizomes (*Curcuma longa*) were harvested, rinsed in clean water to remove adhering dirt, chipped, and dried at 45 °C in an oven to a constant weight. The dried chips were ground (2 mm sieve) to yield turmeric powder (TP), stored at 4 °C before use in the experimental diets.

Fresh scent leaves (*Ocimum gratissimum*) and moringa leaves were plucked without the petioles and stalks. The leaves were rinsed in water to remove dirt, spread evenly, and air-dried at room temperature until they became crispy while retaining their greenish color. The dried scent and moringa leaves were ground (2 mm sieve) separately to yield scent leaf powder (SLP) and moringa leaf meal (MLM), respectively. The SLP and MLM prepared were stored at 4 °C in an air-tight container before use. To formulate the herbal blend (HB), TP, SLP, and MLM were mixed thoroughly at equal proportions. The resultant blend (HB) was stored at 4 °C in an air-tight container before use. Qualitative screening of the phytochemical compounds (tannins, alkaloids, flavonoids, terpenes, saponins, and cyanogenic glycosides) present in representative samples was conducted using the method described by Harbone (1973).

Management of Experimental Birds

A total of 500 one-day-old guinea fowl keets were brooded together for 28 days in a deep litter house using wood shavings as bedding material. During the 28-day brooding period, no antimicrobial and anticoccidial drugs were administered to the birds. Guinea fowls were fed a starter diet containing 242 g/kg crude protein and 12.13 MJ/kg metabolizable energy according to National Research Council (NRC) (1994) requirements. Feed and clean water were provided *ad libitum* during the pre-experimental and experimental periods. The feeding trial commenced at the end of the brooding period.

Dietary Treatments

On day 29, 360 male guinea fowls were selected, weighed individually, and allotted six treatments on a weight equalization basis, with each treatment group having six pens as replicates. A total of 36 similar floor pens were used in this study. Maize-soybean meal-based diets were formulated according

to NRC (1994) nutritional requirements for younger (29 to 56 d) and growing (57 to 84 d) guinea fowls (Table 1). The six treatment groups were laid out in a 3 × 2 factorial arrangement of the basal diet (control), diet containing enrofloxacin (at 1 g/kg), and HB (at 1 g/kg diet), each supplemented with or without L-Arg (Shanghai TECH Chemical Industry, China) at 1 g/kg. Diets were fed in mash form. Each treatment group was replicated six times in a completely randomized design. No medication and vaccination was administered during the experimental period (29 to 84 d).

Table 1

Basal composition of experimental diets (g/kg as fed)

Ingredients	Starter (29 – 56 d)	Finisher (57 – 84 d)
Maize	492	555
Soybean	350	280
Wheat offal	85.0	100.0
Fish meal	30.0	20.0
Bone meal	18.0	20.0
Oyster shell	15.0	16.0
*Premix	2.50	2.50
Salt	2.50	2.50
L-lysine HCL	2.0	2.0
DL-Methionine	3.0	2.0
Total	1000	1000
Determined nutrient composition (% DM)		
Dry matter	89.14	89.62
Crude protein	22.62	21.12
Ether extract	3.55	3.46
Ash	8.66	8.35
Crude fibre	3.11	3.24
Calculated nutrient composition		
Metabolizable energy (MJ/kg)	11.94	12.75
Ca (%)	1.22	1.31
P (%)	0.65	0.62
Digestible arginine (%)	1.47	1.24
Lysine (%)	1.44	1.35
Methionine (%)	0.69	0.61

Note. *Supplied per kilogram of diet: retinol acetate, 4.12 mg; cholecalciferol, 87.5 mg; DL-alpha-tocopherol acetate, 44.7 mg; menadione, 2 mg; thiamine mononitrate, 2 mg; riboflavin, 6 mg; pyridoxol, 5 mg; cyanocobalamin, 0.2 mg; D-biotin, 0.1 mg; niacin, 50 mg; pantothenic acid, 12 mg; folic acid, 2 mg; zinc [Zn (zinc sulphate monohydrate: $ZnSO_4$. H_2O)], 90 mg; manganese [Mn (manganese (II) sulfate monohydrate: $MnSO_4$. H_2O)], 80 mg; iron [Fe (iron (II) sulfate monohydrate: $FeSO_4$. H_2O)], 60 mg; copper [Cu (copper sulfate pentahydrate: $CuSO_4$. $5H_2O$)], 8 mg; iodine [I (potassium iodide: KI)], 1 mg; cobalt [Co (cobalt (II) sulfate monohydrate: $CoSO_4$. H_2O)], 0.3 mg; and molybdenum [Mo (sodium molybdate: Na_2Mo_4. $2H_2O$)], 1 mg

Data and Sample Collection

Growth Performance. Guinea fowls were weighed per replicate weekly, while the weight gain was calculated. Daily feed consumption was calculated as the difference between the feed offered and leftovers, while feed conversion ratio was calculated as feed intake to weight gain. No mortality occurred during the experimental period.

Intestinal Morphology. At 56 d and 84 d of the birds, six birds per treatment group were randomly selected, slaughtered, and eviscerated. Tissue samples from the mid-region of the three segments of the small intestine (duodenum, jejunum, and ileum) were collected according to the method as described by Gava et al. (2015). The samples were placed in 10% formalin and dehydrated in a graded ethanol series (xylene). Each segment was embedded in paraffin wax and stained with hematoxylin and eosin. A total of eight slides were recorded per observation. The villi length and crypt depths of the samples were examined under a microscope and measured from the tip to the villi base and from the villi base to the crypt base, respectively.

Caecal Microflora. Fresh content from the caeca of selected guinea fowls following intestinal morphology at 56 and 84 d were poured in labeled sterile bottles and put on ice to determine the microbial population. Caecal samples collected were serially diluted and plated on De Man, Rogosa, and Sharpe (MRS) agar plates, incubated at 37 ^{o}C for 24 hours. *Lactobacillus*, *Coliform*, *Clostridium*, and *Salmonella* counts were estimated as described by Xia et al. (2004) and were expressed as colony-forming units (Cfu) per gram of fresh sample.

Statistical Analyses

Microbial counts (Cfu/g) were transformed to logarithm$_{10}$. Data obtained were subjected to the general linear model procedure of the Statistical Analysis System (SAS) (2000) to determine the main effect of HB inclusion (control, diet with enrofloxacin, diet with HB), the main effect of L-Arg supplementation (0, 1 g/kg), and their respective interactions (HB × L-Arg). Statistically significant means were separated using Tukey's test at a probability of 5 %.

RESULTS

The effect of dietary inclusion of HB supplemented with or without L-Arg on the growth response of guinea fowls is shown in Table 2. Guinea fowls fed a control diet without L-Arg supplementation had the least (p=0.003) body weight on day 84. Dietary inclusion of HB supplemented with or without L-Arg had no effect (p>0.05) on the average weight gain of younger guinea fowls (28–56 days). Notwithstanding dietary supplementation with L-Arg, guinea fowls fed the diet with HB. Their counterparts fed the diet with antibiotics had similar weight gain during the growing and overall (28–84 days) rearing periods. In growing and overall periods, guinea fowls fed a control diet without L-Arg supplement had

Table 2

Growth performance of growing guinea fowls fed diets supplemented with herbal blend and L-arginine

Parameters	Without L-Arg supplementation			With L-Arg supplementation			SEM	*P*-values		
	Control	Antibiotic	HB	Control	Antibiotic	HB		Treatment	L-Arg	Treatment × L-Arg
Body weight (g/bird)										
d 28	230	229	230	229	230	230	4.22	0.679	0.708	0.815
d 56	746	752	730	765	746	774	20.45	0.811	0.395	0.067
d 84	1305^{c}	1476^{ab}	1446^{b}	1588^{a}	1485^{ab}	1556^{a}	142.71	0.346	0.005	0.003
Average feed intake (g/bird)										
d 28 to 56	1264^{a}	1002^{b}	984^{c}	1269^{a}	979^{c}	1030^{b}	122.22	0.001	0.265	0.001
d 56 to 84	2007^{bc}	2005^{bc}	1961^{c}	2405^{a}	2024^{bc}	2111^{b}	149.44	0.004	0.005	0.003
d 28 to 84	3272^{b}	3008^{d}	2945^{e}	3674^{a}	3004^{d}	3141^{c}	202.52	0.001	0.005	0.001
Average weight gain (g/bird)										
d 28 to 56	516	522	500	535	516	544	6.49	0.823	0.100	0.062
d 56 to 84	559^{c}	724^{ab}	715^{b}	823^{a}	739^{ab}	781^{ab}	55.40	0.280	0.138	0.006
d 28 to 84	1075^{c}	1246^{ab}	1216^{b}	1358^{a}	1257^{ab}	1326^{ab}	102.55	0.347	0.005	0.003
Feed conversion ratio										
d 28 to 56	2.45^{b}	1.92^{a}	1.97^{a}	2.37^{ab}	1.90^{a}	1.89^{a}	0.91	0.001	0.209	0.001
d 56 to 84	3.59^{b}	2.77^{a}	2.74^{a}	2.92^{ab}	2.70^{a}	2.70^{a}	0.99	0.072	0.083	0.001
d 28 to 84	3.04^{b}	2.41^{a}	2.42^{a}	2.70^{ab}	2.39^{a}	2.37^{a}	0.95	0.001	0.254	0.001

Note. All values within rows having the same or no superscripts are not significantly different (p>0.05); HB = Herbal blend, SEM = Standard error of the means

the least (p<0.05) weight gain. Guinea fowls fed the diet with HB containing no L-Arg supplementation, and birds fed the diet with antibiotic supplemented with L-Arg had the least (p=0.001) feed intake during the younger period. During the growing and overall rearing period, guinea fowls fed a control diet supplemented with L-Arg had the highest (p=0.003) feed intake. Meanwhile, L-Arg supplementation of the diet with HB resulted in increased (p=0.001) feed intake in all the rearing periods compared to their counterparts fed the diet with HB containing no supplemental L-Arg. This trend was noticed at the growing and overall phase in birds fed control diet compared with the group fed Arg-supplemented control diet. In all the rearing periods, guinea fowls were fed the diet with HB. Those fed the diet with antibiotic supplemented or not with L-Arg had a better (p=0.001) feed conversion ratio than their counterparts fed control diet containing no supplemental L-Arg. Guinea fowls fed diets containing supplemental L-Arg irrespective of dietary treatment had a similar feed conversion ratio.

The effect of dietary inclusion of HB and L-Arg supplementation on the gut morphology of younger guinea fowls is presented in Table 3. L-Arg supplementation of the diet with HB resulted in the longest (p=0.001) duodenal villi height and the shortest (p=0.001) duodenal apical width. In the jejunum, guinea fowl fed the diet with HB supplemented with L-Arg had the longest (p=0.001) jejunal villi height and the least (p=0.001) basal width. Jejunal villi height increased (p=0.006), while basal width reduced (p=0.051) following L-Arg supplementation. Guinea fowls fed a control diet containing no supplemental L-Arg had the longest (p=0.006) ileal laminal propria depth. The effect of dietary inclusion of HB and L-Arg supplementation on gut morphology of growing guinea fowls is as presented in Table 4. In the duodenum and jejunum, L-Arg supplementation of the diet with HB resulted in the longest (p<0.05) villi heights. Birds fed the diets with HB and antibiotics supplemented with L-Arg had the longest (p=0.002) villi height in the ileum.

The effect of dietary inclusion of HB and L-Arg supplementation on caecal microflora of young and growing guinea fowls is presented in Table 5. Dietary inclusion of HB supplemented with or without L-Arg had no effect (p>0.05) on the caecal microflora count of younger guinea fowls. In growing guinea fowls, the least (p=0.001) *Clostridium* count and the highest (p=0.001) *Lactobacillus* count were observed in the caeca content of birds fed the diet with HB supplemented with L-Arg. Notwithstanding the dietary treatment imposed, L-Arg supplementation in growing guinea fowls reduced (p=0.035) caecal *Clostridium* count.

Table 3

Intestinal morphology of growing guinea fowls fed diets supplemented with herbal blend and L-arginine at 56 days of age

Parameters	Without L-Arg supplementation			With L-Arg supplementation			SEM	*P*-values		
	Control	Antibiotic	HB	Control	Antibiotic	HB		Treatment	L-Arg	Treatment × L-Arg
Duodenum										
Villus height (µm)	975^{c}	979^{c}	997^{c}	998^{c}	1100^{b}	1222^{a}	102.40	0.002	0.023	0.001
Apical width (µm)	49.13^{a}	40.35^{b}	35.95^{c}	49.72^{a}	39.75^{b}	30.95^{d}	9.11	0.418	0.401	0.001
Basal width (µm)	105	106	105	102	109	107	3.33	0.098	0.643	0.175
Laminal propia depth (µm)	205	207	202	206	207	202	6.24	0.683	0.439	0.136
Jejunum										
Villus height (µm)	1024^{f}	1650^{d}	1739^{e}	1220^{e}	1820^{b}	2020^{a}	182.55	0.001	0.006	0.001
Apical width (µm)	41.60	37.40	35.72	40.20	37.70	36.50	2.75	0.163	0.725	0.403
Basal width (µm)	237^{b}	247^{a}	180^{e}	202^{d}	222^{c}	173^{f}	19.12	0.117	0.051	0.001
Laminal propia depth (µm)	367	370	385	365	372	388	7.52	0.309	0.118	0.215
Ileum										
Villus height (µm)	370	375	365	377	395	360	6.22	0.338	0.268	0.276
Apical width (µm)	52.70	52.20	51.90	50.90	50.60	52.20	2.42	0.278	0.362	0.218
Basal width (µm)	105	114	117	103	107	109	3.02	0.133	0.112	0.119
Laminal propia depth (µm)	525^{a}	495^{c}	505^{bc}	505^{bc}	472^{d}	475^{d}	28.22	0.451	0.154	0.006

Note. All values within rows having the same or no superscripts are not significantly different (p>0.05); HB = Herbal blend, SEM = Standard error of the means

Table 4

Intestinal morphology of growing guinea fowls fed diets supplemented with herbal blend and L-arginine at 84 days of age

Parameters	Without L-Arg supplementation			With L-Arg supplementation			SEM	*P*-values		
	Control	Antibiotic	HB	Control	Antibiotic	HB		Treatment	L-Arg	Treatment × L-Arg
Duodenum										
Villus height (µm)	1530^{d}	1564^{c}	1596^{b}	1570^{c}	1599^{b}	1720^{a}	119.51	0.004	0.011	0.007
Apical width (µm)	122	125	120	120	122	121	6.22	0.451	0.162	0.410
Basal width (µm)	255	252	250	250	249	252	3.77	0.406	0.486	0.187
Laminal propia depth (µm)	505	502	499	500	503	502	9.56	0.528	0.079	0.724
Jejunum										
Villus height (µm)	1680^{d}	1690^{d}	1970^{b}	1780^{c}	1775^{c}	2030^{a}	199.62	0.001	0.964	0.001
Apical width (µm)	99	102.60	104	99	100	102	2.22	0.401	0.183	0.471
Basal width (µm)	202	199.55	201	200	191	188	1.79	0.167	0.113	0.127
Laminal propia depth (µm)	305	306.70	302	300	303	303	3.45	0.193	0.061	0.482
Ileum										
Villus height (µm)	399^{b}	399^{b}	395^{b}	402^{b}	484^{a}	490^{a}	26.21	0.119	0.074	0.002
Apical width (µm)	101	104	107	100	102	100	1.44	0.791	0.065	0.101
Basal width (µm)	155	156	153	152	155	152	2.01	0.412	0.436	0.602
Laminal propia depth (µm)	655	662	659	652	665	665^{d}	6.02	0.694	0.074	0.125

Note. All values within rows having the same or no superscripts are not significantly different (p>0.05); HB = Herbal blend, SEM = Standard error of the means

Table 5

Caecal microfloral of growing guinea fowls fed diets supplemented with herbal blend and L-arginine

Parameters	Without L-Arg supplementation			With L-Arg supplementation			SEM	*P*-values		
	Control	Antibiotic	HB	Control	Antibiotic	HB		Treatment	L-Arg	Treatment × L-Arg
Younger phase										
Clostridium (Cfu/g)	7.00	6.80	6.50	6.90	6.50	6.40	0.25	0.220	0.204	0.084
Coliform (Cfu/g)	6.40	6.20	6.10	6.20	6.00	6.00	0.28	0.382	0.251	0.343
Lactobacillus (Cfu/g)	6.70	6.90	7.00	6.90	7.00	7.30	0.32	0.076	0.082	0.605
Salmonella (Cfu/g)	4.80	4.40	4.30	4.60	4.30	4.10	0.08	0.347	0.693	0.507
Grower phase										
Clostridium (Cfu/g)	6.90^{a}	5.50^{b}	5.10^{bc}	4.40^{c}	4.50^{c}	4.40^{c}	0.21	0.456	0.035	0.001
Coliform (Cfu/g)	6.20	6.00	6.00	6.00	6.00	6.30	0.05	0.032	0.045	0.629
Lactobacillus (Cfu/g)	6.00^{d}	6.90^{c}	7.90^{b}	7.00^{c}	7.20^{c}	8.90^{a}	0.23	0.021	0.002	0.001
Salmonella (Cfu/g)	4.20	4.00	4.00	4.10	4.20	4.00	0.03	0.222	0.631	0.343

Note. All values within rows having the same or no superscripts are not significantly different ($p>0.05$); HB = Herbal blend, SEM = Standard error of the means

DISCUSSION

The non-significant effects of HB inclusion and L-Arg supplementation on weight gain of younger guinea fowls, which later revealed a significant effect at the growing phase of the birds in the present study, corroborated earlier studies revealing age-dependent response of poultry birds to phytogenic feed additives (Hernández et al., 2004; Jamroz et al., 2005). The similar weight gain obtained for guinea fowls fed HB diet and those fed the diet with antibiotic during the growing and overall rearing period notwithstanding dietary supplementation with L-Arg confirmed the ability of HB to replace in-feed antibiotics without compromise on weight gain. Significant improvements in body weight gain in the HB group have been documented (Murugesan et al., 2015).

Increased feed intake of young and growing guinea fowls following L-Arg supplementation of the diet with HB, when compared to their counterparts fed a similar diet but not supplemented with L-Arg in the present study, could be linked with the secretagogue activity of Arg inducing the release of insulin and glucagon, which influences feed intake and efficiency of utilization (Woods et al., 2006). Previous studies confirmed that NO generated via the L-Arg pathway is actively involved in feeding behavior through the hypothalamus (Malfatti et al., 2015), acts as a central mediator and physiological modulator of food intake (Mancuso et al., 2010). L-Arg infusion in rats also promoted satiety quantified by increased food intake after 24 hours from L-Arg administration (Malfatti et al., 2015).

The improved feed conversion ratios obtained for guinea fowls fed the diet with HB and birds fed the diet with antibiotic notwithstanding L-Arg supplementation than birds fed control diet having no supplemental L-Arg supported previous findings that inclusion of phytogenic feed additive (Brenes & Roura, 2010; Jamroz et al., 2005) and L-Arg supplementation improved feed conversion ratio of broilers when compared with the control group (Filho et al., 2021; Pramujo et al., 2019). In addition, Wang et al. (2021) reported an improved feed conversion ratio of broilers fed a diet supplemented with phytogenic feed additives (containing oregano, cinnamon, citrus peel, and fructooligosaccharides). Better feed conversion ratio obtained for guinea fowls fed the diet with HB could be attributed to the additive effect of antimicrobial properties exhibited by constituent scent leaf (Prabhu et al., 2009), antioxidant and antimicrobial properties of turmeric (Quiles et al., 2002), and moringa leaf (Ogundare & Onifade, 2009) contained in the blend. Previous studies also reported improved growth of broilers following dietary inclusion of *Morinda lucida* leaf meal (Lala et al., 2017) and turmeric powder (Ahmadi, 2010).

Intestinal morphological changes following the inclusion of HB and L-Arg supplementation and the longest duodenal and jejunal villi height recorded for young and growing guinea fowl fed the diet with HB supplemented with L-Arg suggests

increased surface area. Increased villus height has been linked with increased surface area for nutrient absorption (Kamboh et al., 2015), resulting in good gut health (Viveros et al., 2011). Reduced apical width in the duodenum and basal width in the jejunum recorded for younger guinea fowl fed the diet with HB supplemented with L-Arg suggests reduced cellular turnover and improved intestinal health. Reduced apical width has been suggested to increase mature enterocytes, thereby increasing enzyme activity in the villus brush border (Chen et al., 2011). Shallower apical width has been linked with improved gut health and growth since cellular turnover is an energy-consuming process that uses resources that might otherwise be utilized toward growth (Markovic et al., 2009). In separate studies, dietary inclusion with phytogenic blend (Geyra et al., 2001; Reisinger et al., 2011) and L-Arg supplementation (Zhan et al., 2008) have improved intestinal morphology. Geyra et al. (2001) reported increased villus height and surface area following dietary supplementation with phytogenic blend (Tecnaroma Herbal Mix PL® Tecnessenze, Saudi Arabia). Dietary inclusion with a blend of oregano, anise, and citrus peel improved intestinal morphology (Reisinger et al., 2011). Dietary supplementation with L-Arg in turkeys (Oso et al., 2017) and weaned pigs (Zhan et al., 2008) has improved intestinal morphology.

The mechanism through which phytogenic additives and L-Arg supplementation improve intestinal morphology has been elucidated. Bioactive compounds present in phytogenic plants may stimulate secretions and activity of endogenous digestive enzymes leading to increased absorption surface area in the intestine and improved intestinal morphology (Lee et al., 2004). The major bioactive component in scent leaf (cinnamate) and turmeric powder (curcumin) used in the formulation of HB in the current study has been reported to stimulate pancreatic digestive enzyme activities (Hernández et al., 2004).

Increased *Lactobacillus* growth within the gut will lead to enhanced production of lactic acid (Harley & Prescott, 2002), reduced gut pH, and thereby inhibit normal growth of enteropathogens leading to improved animal health (Z. Li et al., 2018).

The mechanism through which PFA and L-Arg supplementation improve gut microflora has been described. Phytogenic feed additives reduce gut pathogenic bacteria by disrupting the cellular membrane of pathogens, affecting their hydrophobicity and virulence capacity, stimulating the immune system by activating lymphocytes and macrophages, protecting intestinal mucosa from pathogen colonization, and promoting the growth of beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria* (Windisch & Kroismayr, 2007). In addition, plant bioactive compounds may improve the antioxidant status in the intestinal mucosa due to the antioxidant activities of the herbal feed additives and the reduction of pathogenic microbes, which can lead to better intestinal microstructures (Patra et al., 2019). For example, higher lactobacillus

and lower *Clostridium* count in the caeca of broiler chickens fed phytogenic feed additives supplemented with arginine had been attributed to nitric oxide produced by arginine (Eriksson et al., 2003; Ren et al., 2014) and phytogenic antimicrobial properties (Wati et al., 2015), respectively.

L-Arg supplementation produces NO, a component of the immune system, thereby limiting pathogenic activity (Wink et al., 2011), thus, preventing the multiplication of diseases (Ren et al., 2014). L-Arg supplementation has been reported in previous studies to alleviate the negative effect of *Salmonella typhimurium* (Eriksson et al., 2003), *Eimeria tenella* (Allen, 1999), and infectious bursal disease virus (Emadi et al., 2010) in broiler chickens.

CONCLUSION

Guinea fowls fed the diet with HB supplemented or not with L-Arg had similar growth performance with those fed with antibiotics. However, L-Arg supplementation of the diet with HB improved intestinal morphology of both younger and growing birds and increased caecal *Lactobacillus* counts of growing birds.

ACKNOWLEDGEMENTS

The authors appreciate the technical support of the Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta in this study.

REFERENCES

Abo Ghanima, M. M., Alagawany, M., Abd El-Hack, M. E., Taha, A., Elnesr, S. S., Ajarem, J., Allam, A. A., & Mahmoud, A. M. (2020). Consequences of various housing systems and dietary supplementation of thymol, carvacrol, and euganol on performance, egg quality, blood chemistry, and antioxidant parameters. *Poultry Science*, *99*(9), 4384-4397. https://doi.org/10.1016/j.psj.2020.05.028

Ahmadi, F. (2010). Effect of turmeric (*Curcumin longa*) powder on performance, oxidative stress state and some of blood parameters in broiler fed on diets containing aflatoxin B1. *Global Veterinaria*, *5*(6), 312–317.

Alagawany, M., El-Saadony, M. T., Elnesr, S. S., Farahat, M., Attia, G., Madkour, M., & Reda, F. M. (2021). Use of lemongrass essential oil as a feed additive in quail's nutrition: Its effect on growth, carcass, blood biochemistry, antioxidant and immunological indices, digestive enzymes and intestinal microbiota. *Poultry Science*, *100*(6), 101172. https://doi.org/10.1016/j.psj.2021.101172

Alagawany, M., Farag, M. R., Abdelnour, S. A., Dawood, M. A., Elnesr, S. S., & Dhama, K. (2020). Curcumin and its different forms: A review on fish nutrition. *Aquaculture*, *532*, 736030. https://doi.org/10.1016/j.aquaculture.2020.736030

Al-Daraji, H. J., & Salih, A. M. (2012). The influence of dietary arginine supplementation on blood traits of broiler chickens. *Pakistan Journal of Nutrition*, *11*(3), 258-264. https://doi.org/10.3923/pin.2012.258.264

Allen, P. C. (1999). Effects of daily oral doses of L-Arg on coccidiosis infections in chickens. *Poultry Science*, *78*(11), 1506-1509. https://doi.org/10.1093/ps/78.11.1506

Al-Sultan, S. I. (2003). The effect of *Curcuma longa* (turmeric) on overall performance of broiler chickens. *International Journal of Poultry*

Science, *2*(5), 351-353. http://doi.org/10.3923/ijps.2003.351.353

Bassolé, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, *17*(4), 3989-4006. https://doi.org/10.3390/molecules17043989

Brenes, A., & Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology*, *158*(1-2), 1–14. https://doi.org/10.1016/j.anifeedsci.2010.03.007

Cetin, E., Yibar, A., Yesilbag, D., Cetin, I., & Cengiz, S. S. (2016). The effect of volatile oil mixtures on the performance and ileo-caecal microflora of broiler chickens. *British Poultry Science*, *57*(6), 780–787. https://doi.org/10.1080/00071668.2016.1214682

Chen, J., Wang, M., Kong, Y., Ma, H., & Zou, S. (2011). Comparison of the novel compounds creatine and pyruvate on lipid and protein metabolism in broiler chickens. *Animal*, *5*(7), 1082-1089. https://doi.org/10.1017/S1751731111000085

Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M., Thakur, P., Igbal, M. N., Chaicumpa, W., Michalak, I., & Elnesr, S. S. (2021). A comprehensive review on chemical profile and pharmacological activities of *Ocimum basilicum*. *Food Reviews International*. https://doi.org/10.1080/87559129.2021.1900230

Dono, N. D. (2013). Turmeric (*Curcuma longa* Linn.) supplementation as an alternative to antibiotics in poultry diets. *Indonesian Bulletin Animal and Veterinary Sciences*, *23*(1), 41-49.

Ebrahim, A. A., Elnesr, S. S., Abdel-Mageed, M. A. A., & Aly, M. M. M. (2020). Nutritional significance of aloe vera (*Aloe barbadensis* Miller) and its beneficial impact on poultry. *World's Poultry Science Journal*, *76*(4), 803-814. https://doi.org/10.1080/00439339.2020.1830010

Emadi, M., Jahanshiri, F., Jalalian, F. A., Kaveh, K., Bejo, M. H., Ideris, A., Assumaidaee, A. A., & Alimon, R. A. (2010). Immunostimulatory effects of arginine in broiler chickens challenged with vaccine strain of infectious bursal disease virus. *Journal of Animal and Veterinary Advances*, *9*(3), 594-600. http://doi.org/10.3923/javaa.2010.594.600

Eriksson, S., Chambers, B. J., & Rhen, M. (2003). Nitric oxide produced by murine dendritic cells is cytotoxic for intracellular *Salmonella enterica* sv. Typhimurium. *Scandinavian Journal of Immunology*, *58*(5), 493–502. https://doi.org/10.1046/j.1365-3083.2003.01330.x

Ertas, O. N., Güler, T., Çiftçi, M., Dalkilic, B., & Simsek, U. G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International Poultry Science*, *4*(11), 879-884. https://doi.org/10.3923/ijps.2005.879.884

Filho, T. S., Lima, E. C., Oliveira, D. H., Abreu, M. L. T., Rosa, P. V., Laurentiz, A. C., Naves, L. P., & Rodrigue, P. B. (2021). Supplemental L-arginine improves feed conversion and modulates lipid metabolism in male and female broilers from 29 to 42 days of age. *Animal*, *15*(2), 100120. https://doi.org/10.1016/j.animal.2020.100120

Gava, M. S., Moraes, L. B., Carvalho, G. Z., Fallavena, L. C. B., Moraes, H. L. S., Herpich, J., & Salle, C. T. P. (2015). Determining the best sectioning method and intestinal segment for morphormetric analysis in broilers. *Brazilian Journal of Poultry Science*, *17*(2), 145-150. http://doi.org/10.1590/1516-635x1702145-150

Geyra, A., Uni, Z., & Sklan, D. (2001). Enterocyte dynamics and mucosal development in the post hatch chick. *Poultry Science*, *80*(6), 776–782. https://doi.org/10.1093/ps/80.6.776

Harbone, J. B. (1973). *Phytochemical methods*: *A guide to modern techniques of plant analysis*. Chapman and Hall.

Harley, J. P., & Prescott, L. M. (2002). *Laboratory exercises in microbiology*. McGraw-Hill.

Hernández, F., Madrid, J., Garcia, V., Orengo, J., & Megias, M. D. (2004). Influence of two plant extracts on broiler performance, digestibility, and digestive organ size. *Poultry Science*, *83*(2), 169–174. https://doi.org/10.1093/ps/83.2.169

Jamroz, D., Wiliczkiewicz, A., Wertelecki, T., Orda, J., & Skorupińska, J. (2005). Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. *British Poultry Science*, *46*(4), 485-493. https://doi.org/10.1080/00071660500191056

Kamboh, A. A., Arain, M. A., Mughal, M. J., Zaman, A., Arain, Z. M., & Soomro, A. H. (2015). Flavonoids: Health promoting phytochemicals for animal production - A review. *Journal of Animal health Production*, *3*(1), 6-13. http://doi.org/10.14737/journal.jahp/2015/3.1.6.13

Kochar, N. I., Chandewal, A. V., Bakal, R. L., & Kochar, P. N. (2011). Nitric oxide and the gastrointestinal tract. *International Journal of Pharmacology*, *7*(1), 31-39. https://doi.org/10.3923/ijp.2011.31.39

Lala, A. O., Ajayi, O. L., Okwelum, N., Oso, A. O., Fakorede, T. V., Adebayo, T. A., & Jagbojo, J. E. (2017). Haematological, biochemical and organ changes in broiler chickens fed varying levels of *Morinda lucida* (Brimstone) leaf meal supplementation in the diets. *Tropical Animal Health and Production*, *50*(5), 1005-1010. https://doi.org/10.1007/s11250-018-1524-8

Lee, K. W., Everts, H., & Beynen, A. C. (2004). Essential oils in broiler nutrition. *International Journal of Poultry Science*, *3*(12), 738–752. http://doi.org/10.3923/ijps.2004.738.752

Li, P., Yin, Y. L., & Li, D. F. (2007). Amino acids and immune function. *British Journal of Nutrition*, *98*(2), 237–252. https://doi.org/10.1017/S000711450769936X

Li, Z., Wang, W., Liu, D., & Guo, Y. (2018). Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. *Journal of Animal Science and Biotechnology*, *9*, 25. https://doi.org/10.1186/s40104-018-0243-3

Lu, C., Zhang, J., Shi, X., Miao, S., Bi, L., Zhang, S., Zhou, Q. Y., Zhang, M., Xie, Y., Miao, Q., & Wang, S. (2014). Neuroprotective effects of tetramethylpyrazine against dopaminergic neuron injury in a rat model of Parkinson's disease induced by MPTP. *International Journal of Biological Sciences*, *10*(4), 350–357. https://doi.org/10.7150/ijbs.8366

Malfatti, C. R. M., da Silva, L. A., Pereira, R. A., Michel, R. G., Snak, A. L., & Santos, F. S. (2015). Acute hypothalamic administration of L-arginine increases feed intake in rats. *Revista de Nutrição*, *28*(1), 55–63. https://doi.org/10.1590/1415-52732015000100005

Mancuso, C., Navarra, P., & Preziosi, P. (2010). Roles of nitric oxide, carbon monoxide and hydrogen sulfide in the regulation of the hypothalamic-pituitary-adrenal axis. *Journal of Neurochemistry*, *113*(3), 563–575. https://doi.org/10.1111/j.1471-4159.2010.06606.x

Markovic, R., Sefer, D., Krstic, M., & Petrujkic, B. (2009). Effect of different growth promoters on broiler performance and gut morphology. *Archivos de Medicina Veterinaria*, *41*(2), 163–169. https://doi.org/10.4067/S0301-732X2009000200010

Matasyoh, L. G., Josphat, C. M., Francis, N. W., Miriam, G. K., Anne, W. T. M., & Titus, K. W. (2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in eastern Kenya. *African Journal of Biotechnology*, 6(6), 760-765.

Murugesan, G. R., Syed, B., Haldar, S., & Pender, C. (2015). Phytogenic feed additives as an

alternative to antibiotic growth promoters in broiler chickens. *Frontiers in Veterinary Science*, *2*, 21. https://doi.org/10.3389/fvets.2015.00021

National Research Council. (1994). *Nutrient requirements of domestic animals: Nutrient requirements of poultry*. Academy Press.

Ndelekwute, E. K., Afolabi, K. D., Uzegbu, H. O., Unah, U. L., & Amaefule, K. U. (2015). Effect of dietary black pepper (*Piper nigrum*) on the performance of broiler. *Bangladesh Journal of Animal Science*, *44*(2), 120-127. https://doi.org/10.3329/bjas.v44i2.26012

Ogundare, A. O., & Onifade, A. K. (2009). The antimicrobial activity of *Morinda lucida* leaf extract on *Escherichia coli*. *Journal of Medicinal Plants Research*, *3*(4), 319-323. https://doi.org/10.5897/JMPR.9001185

Ogunlana, O. E., Ogunlana, O., & Farombi, O. E. (2008). *Morinda lucida*: Antioxidant and reducing activities of crude methanolic stem bark extract. *Advances in Natural and Applied Sciences*, *2*(2), 49-54.

Ola-Fadunsin, S. D., & Ademola, I. O. (2013). Anticoccidial effects of *Morinda lucida* acetone extracts on broiler chickens naturally infected with *Eimeria* species. *Pharmaceutical Biology*, *52*(3), 330-334. https://doi.org/10.3109/13880209.2013.836545

Oso, A. O., Bamgbose, A. M., Adebayo, A. O., Olowofeso, V. O., Pirgozliev, A. A., Adegbenjo, A. O., Osho, S. O., Alabi, J. O., Li, H., Liu, G., Yao, K., & Xin, W. (2017). Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora and lymphoid organs of growing turkeys. *Livestock Science*, *198*, 58-64. https://doi.org/10.1016/j.livsci.2017.02.005

Patra, A., Amasheh, S., & Aschenbach, J. R. (2019). Modulation of gastrointestinal barrier and nutrient transport function in farm animals by natural plant bioactive compounds - A comprehensive review. *Critical Reviews in Food Science and Nutrition*, *59*(1), 3237-3266. https://doi.org/10.1080/10408398.2018.1486284

Prabhu, K. S., Lobo, R., Shirwaikar, A. A., & Shirwaikar, A. (2009). *Ocimum gratissimum*: A review of its chemical, pharmacological and ethnomedicinal properties. *The Open Complementary Medicine Journal*, *1*(1), 1-15. http://doi.org/10.2174/1876391X00901010001

Pramujo, M., Mutia, R., & Wijayanti, I. (2019). Effect of chitosan oligosaccharide (COS) and l-arginine supplementation on broiler performance. In *IOP Conference Series: Earth and Environmental Science* (Vol. 251, p. 012060). IOP Publishing Ltd. https://doi.org/10.1088/1755-1315/251/1/012060

Quiles, J. L., Mesa, M. D., Ramirez-Tortosa, C. L., Aguilera, C. M., Battino, M., Gil, A., & Ramirez-Tortosa, M. C. (2002). *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arteriosclerosis Thrombosis and Vascular Biology*, *22*(7), 1225–1231. https://doi.org/10.1161/01.ATV.0000020676.11586.F2

Ren, W., Chen, S., Yin, J., Duan, J., Li, T., Liu, G., Feng, Z., Tan, B., Yin, Y., & Wu, G. (2014). Dietary arginine supplementation of mice alters the microbial population and activates intestinal innate immunity. *The Journal of Nutrition*, *144*(6), 988–995. https://doi.org/10.3945/jn.114.192120

Reisinger, N., Steiner, T., Nitsch, S., Schatzmayr, G., & Applegate, T. J. (2011). Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. *Journal of Applied Poultry Research*, *20*(3), 272-283. https://doi.org/10.3382/japr.2010-00226

Saeed, M., Arain, M. A., Ali Fazlani, S., Marghazani, I. B., Umar, M., Soomro, J., Bhutto, Z. O.,

Soomro, F., Noreldin, A. E. Abd El-Hack, M. E. Elnesr, S. S., Faraq, M. R., Dhama, K., Chao, S., & Alagawany, M. (2021). A comprehensive review on the health benefits and nutritional significance of fucoidan polysaccharide derived from brown seaweeds in human, animals and aquatic organisms. *Aquaculture Nutrition*, *27*(3), 633-654. https://doi.org/10.1111/anu.13233

Statistical Analysis System. (2000). *SAS user's guide statistics* (8.1 ed.). SAS Institute Inc.

Thiruvengadarajan, V. S., Ellaiah, P., & Madhusudhanachetty, C. (2011). *In vitro* antioxidant and alpha-amylase inhibitory activity of isolated compounds from ethyl acetate extract of *Cynodon dactylon* and *Piper betle*. *International Journal Pharmaceutical and Indian Research*, *1*(4), 261–265.

Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C., & Brenes, A. (2011). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, *90*(3), 566–578. https://doi.org/10.3382/ps.2010-00889.

Wang, J., Su, S., Pender, C., Murugesan, R., Syed, B., & Kim, W. K. (2021). Effect of a phytogenic feed additive on growth performance, nutrient digestion, and immune response in broiler-fed diets with two different levels of crude protein. *Animals*, *11*(3), 775. https://doi.org/10.3390/ani11030775

Wati, T., Ghosh, T. K., Syed, B., & Haldar, S. (2015). Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. *Animal Nutrition*, *1*(3), 213-219. https://doi.org/10.1016/j.aninu.2015.08.003

Windisch, W., & Kroismayr, A. (2007). Natural phytobiotics for health of young piglets and poultry mechanisms and application. *Poultry Science*, *86*(Suppl. 1), 643.

Wink, D. A., Hines, H. B., Cheng, Y. S., Switzer, C. H., Flores-Santana, W., Vitek, M.P., Ridnour, L. A., & Colton, C. A. (2011). Nitric oxide and redox mechanisms in the immune response. *Journal of Leukocyte Biology*, *89*(6), 873-891. https://doi.org/10.1189/jib.1010550

Woods, S. C., Lutz, T. A., Geary, N., & Langhans, W. (2006). Pancreatic signals controlling food intake; insulin, glucagon and amylin. *Philosophical Transactions of the Royal Society B*, *361*(1471), 1219-1235. https://doi.org/10.1098/rstb.2006.1858

Xia, M. S., Hu, C. H., & Xu, Z. R. (2004). Effect of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broiler. *Poultry Science*, *83*(11), 1868–1875. https://doi.org/10.1093/ps/83.11.1868

Zhan, Z., Ou, D., Piao, X., Kim, S. W., Liu, Y., & Wang, J. (2008). Dietary arginine supplementation affects microvascular development in the small intestine of early-weaned pigs. *Journal of Nutrition*, *138*(7), 1304–1309. https://doi.org/10.1093/jn/138.7.1304