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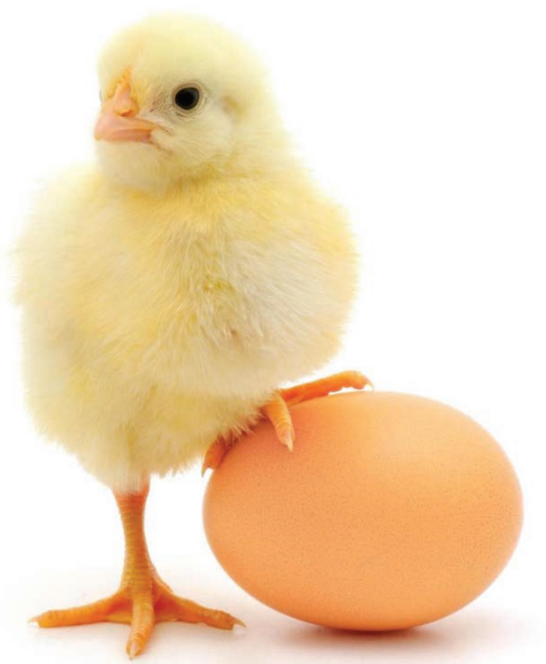

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Research Article
Combined Effect of Methionine, Lysine and Neem Leaf Meal Supplementation on Growth Performance, Blood Parameters and Oxidative Status of Finishing Broilers

[1]Tadondjou Tchingo Cyrille d'Alex, [2]Mube Kuietche Hervé, [1]Solefack Viviane Laure, [1]Ledang Narcisse, [2]Ngoula Ferdinand and [2]Teguia Alexis

[1]Department of Agriculture, Livestock and Derived Product, National Advanced School of Engineering, University of Maroua, Maroua, Cameroon
[2]Department of Animal Science, Faculty of Agriculture and Agricultural Sciences, University of Dschang, Dschang, Cameroon

Abstract
Objective: The objective of this study was to evaluate the effect of diet supplemented with methionine, lysine and neem leaves on growth parameters, carcass characteristics, blood parameters and oxidative status of the liver and the pectoralis muscle of broilers. **Materials and Methods:** A total of 180 broilers (21 days of age, weighing 431.21±50.62 g), were used in this study. Chicks were randomly assigned to 4 dietary treatments and 3 replicates of 15 chicks. Group 1 received a standard diet, group 2 received a diet supplemented with lysine (0.7%) and methionine (0.3%), group 3 received a diet supplemented with neem leaves (0.25%) and group 4 received a diet supplemented with lysine/methionine and neem leaves. Feed intake and live body weight were recorded weekly. At 42 days of age, three birds per replicate were randomly selected and fasted for 12 hrs, weighed and slaughtered and blood samples were collected for hematological and biochemical analyses. Liver and muscle samples were also collected for biochemical analyses. **Results:** Diet supplemented with methionine/lysine or neem leaves did not affect feed intake, body weight gain and feed conversion ratio. The diet supplemented with methionine/lysine and neem leaves significantly increased ($p<0.05$) the body weight proportion of abdominal fat, bursa and the serum level of globulin. The relative weight of the spleen was increased ($p<0.05$) with the dietary supplementation of methionine/lysine or neem leaves alone. The serum level of total cholesterol was decreased ($p<0.05$) in broilers fed on the diet supplemented only with neem leaves. The levels of total protein and the ferric reducing antioxidant power (FRAP) activities were increased ($p<0.05$) in the liver and muscle of chicken fed on the diet supplemented with neem leaves and lysine/methionine. The superoxide dismutase (SOD) activities were increased ($p<0.05$) in the liver with neem leaves and in the muscle of broilers with lysine/methionine. Irrespective of organs, broilers fed on the non-supplemented diet showed the highest levels ($p<0.05$) of malondialdehyde (MDA). Diet supplemented with lysine/methionine decreased ($p<0.05$) red blood cells count and pack cell volume. **Conclusion:** The combined supplementation of neem leaves (0.25%) and methionine/lysine (0.3%/0.7%) to the diet improved immunity and the antioxidant capacity in broilers without affecting growth performance.

Key words: Antioxidant activity, broiler, growth, lysine, methionine, neem leaves

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Corresponding Author: Tadondjou Tchingo Cyrille d'Alex, Department of Agriculture, Livestock and Derived Product, National Advanced School of Engineering, University of Maroua, Maroua, Cameroon

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

During the past few decades, genetic selection and animal nutrition has made spectacular progress that has improved the growth performance of broilers. However, apart from genetics, growth rate and body composition are strongly influenced by management and environment. In fact, the animal deviates from its genetically predetermined growth curve under non-optimal conditions such as poor nutrition, disease, poor housing conditions and heat stress[1]. Animals often show compensatory or accelerated growth performance when favorable conditions are restored[1,2]. Breeders of 21-day-old chickens in Cameroon use the principle of compensatory growth to raise their chickens. Breeders often obtain 21-day-old broilers from the market, whose growth rate is well below the standard curve of the strain. Many breeders routinely use antibiotics to boost the growth performance of chickens during the grow-finish phase. The use of antibiotics as growth promoters have been prohibited in the poultry industry. In the present study, alternative solutions were examined to induce compensatory growth in 21-day-old broilers.

Several studies have been carried out on compensatory growth of broilers. Hadaeghi *et al.*[2] showed that feeding a nutrient-dense diet after a mild feed restriction can accelerate the growth rate of the broiler without affecting carcass traits or immune function. Other authors reported that, in broilers, a balanced diet rich in essential amino acids, especially lysine and methionine, promotes better growth[3]. Jankowski *et al.*[4] revealed that broiler health, survival, growth and immunity can be improved by supplementing corn and soybean diets with methionine and lysine. In fact, lysine is used exclusively in muscle protein synthesis and not involved in other metabolic routes, such as maintenance and feathering[5]. Additionally, lysine plays a role in cytokine synthesis and lymphocyte proliferation[6] and methionine constructively affects the immune system by improving both cellular and humoral responses[7]. Zhai *et al.*[8] reported that a high intake of methionine increased breast muscle growth. Moreover, essential amino acids and other feed additives, such as probiotics, prebiotics, symbiotics, organic acids, essentials oils, enzymes or phytobiotics have been widely used as growth promoters[9].

Neem leaf meal is one of the most promising phytobiotics candidates for the poultry industry for making their production economically viable and environmentally sustainable[10-12]. Ansari *et al.*[13] reported that diet supplemented with neem had significantly improved body weight and feed efficiency of broilers at 28 and 42 days of age. Neem leaves infusion act as a growth promoter[14], improves performance and haematological parameters[15] and immune response[16] in broilers. *Azadirachta indica* possesses antimicrobial and antioxidant properties due to its rich source of antioxidants and other active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin and quercetin. By activating antioxidative enzyme, rupturing the cell wall of bacteria and regulating cellular pathways, these active constitutes may act as chemopreventive agents[11]. The aim of this study was to evaluate the combined effect of methionine, lysine and neem leaf meal supplementation on the growth performance of 21-day-old broilers.

MATERIALS AND METHODS

Study area: The present study was carried out at a farm located in Maroua division of the far-north region (latitude: 10E35'N, longitude: 14E19'E, altitude 384 m), from November to December 2022. During the experiment, the mean temperature registered at the study area was 32°C, while the relative humidity was around 38%.

Chemical composition of local ingredients: The ingredients used for experimental ration preparation were purchased from the market. The synthetic amino acids used were purchased from a veterinary pharmacy in Ngaoundéré. Ingredients such as soybean meal, premix, lysine and methionine were directly used on the basis of the nutritional characteristics recorded on the packaging (Table 1). On the other hand, the chemical composition of maize and neem leaves was determined by analyses (Table 2) at the lab of nutrition and animal feed of the Faculty of Agronomy and Agronomic Sciences, University of Dschang[17].

Experimental animals: A total of 180 broilers (21 days of age), weighing 431.21±50.62 g, obtained from the same producer were used in this experiment. Each chick was identified by a ring bearing his number in one of its paws. Chicks were kept under similar environmental and managerial conditions during the experiment (21 days). Feed and water were given *ad libitum* in adapted equipment.

The research was conducted according to the guidelines of the Cameroonian Bioethics Committee (Reg N° FWA-IRB00001945) and following HIN-care and use of laboratory animals manual (8th Edition).

Experimental design: Chicks were randomly assigned to 4 dietary treatment groups. Each group was divided into 3 replicates of 15 chicks. Group 1 (control) received a standard

Table 1: Some nutritional value of ingredients

	Soybean meal	Premix	Lysine	Methionine
Metabolizable energy (kcal kg^{-1})	339	2100	3615	3643
Crude protein (DM%)	44.2	40	95.60	58.40

DM: Dry matter

Table 2: Chemical composition of maize and neem leaf

	Dry matter	Organic matter (DM%)	Fibre (DM%)	Fat (DM%)	Crude protein (DM%)	Metabolizable energy (kcal kg^{-1})
Maize	95.05	98.49	3.41	3.68	7.98	3787.72
Neem leaves	93.87	88.87	16.11	2.98	17.14	2230.30

DM: Dry matter

Table 3: Ingredients and nutrient composition of experimental diets

Ingredients	R1	R2	R3	R4
Maize (g)	68.00	67.50	67.75	67.25
Soybean meal (g)	20.00	19.50	19.50	19.50
Premix 10% (g)	10.00	10.00	10.00	10.00
Bone meal (g)	2.00	2.00	2.00	2.00
Lysine (g)	-	0.70	-	0.70
Methionine (g)	-	0.30	-	0.30
Neem leaf (g)	-	-	0.25	0.25
Total	100.00	100.00	100.00	100.00
ME (kcal kg^{-1})	3463.65	3463.99	3459.75	3460.09
CP (%)	18.26	18.85	18.28	18.87
E/P	189.68	183.76	189.26	183.36

diet, group 2 received a standard diet supplemented with lysine (0.7%) and methionine (0.3%), group 3 received a standard diet supplemented with neem leaves (0.25%) and group 4 received a standard diet supplemented with lysine/methionine and neem leaves (Table 3).

Data collection: Feed intake and live body weight were recorded weekly, with a scale of 5 kg and 1 g precision. At 42 days of age, three birds per pen replicate (nine per treatment) were randomly selected and fasted for 12 hrs, weighed and slaughtered and blood samples were collected for hematological and biochemical analyses. Liver and muscle samples were also collected for biochemical analyses. Chicks were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Growth parameters: After measuring the feed intake and live body weight, the energy intake, protein intake, body weight gain and feed conversion ratio were calculated in accordance with the following equations:

$$\text{Energy intake} = \text{Energy concentration of diet} \times \text{Feed intake}$$

$$\text{Protein intake} = \text{Crude Protein content of diet} \times \text{Feed intake}$$

$$\text{Body weight gain} = \text{Live body weight of week}_{(n+1)} - \text{Live body weight of week}_{(n)}$$

$$\text{Feed conversion ratio} = \frac{\text{Weekly feed intake (g)}}{\text{Weekly body weight gain (g)}}$$

Carcass characteristics: The carcass and inner organs (heart, liver, gizzard, abdominal fat, intestine, pancreas and spleen) of sacrificed chicks were weighed and expressed as a percentage of live body weight. A scale of 5 kg and 1 g precision was used for carcass and intestine weight measurement. Other organs were weighted individually with a scale of 200 g and 10^{-3} precision.

Hematological profile: Blood was collected into tubes containing the anticoagulant Ethylene Diamine Tetra-acetate (EDTA) to prevent clotting. Each blood sample collected was subjected to hematological analysis of White and Red Blood Cells (RBC), hemoglobin (Hb), Packed Cell Volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in the laboratory. White and Red blood cells count were determined using Natt and Herrick's method[18]. Hemoglobin and Packed Cell Volume determination were performed using an automatic analyzer, SNFS BC30S. The erythrocytes indexes (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were calculated according to the following equations:

$$MCHC\ (g\ L^{-1}) = \frac{Hb}{PCV}$$

$$MCV\ (fl) = \frac{PCV}{RBC}$$

$$MCH\ (pg) = \frac{Hb}{RBC}$$

Biochemical analysis: Blood samples of each slaughtered bird were collected from the jugular vein into dry tubes. After clotting, the serum was separated by centrifugation at 3000 rpm for 15 min and the aliquots were stored at -20°C for biochemical analysis. Serum contents of total protein, albumin, total cholesterol, Gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and glucose were measured using a spectrophotometer (URIT-810) and according to the protocol described by the manufacturer's kits (HUMAN commercial kit). Globulins were determined using the following equation:

$$\text{Globulins} = \text{Total protein-Albumin}$$

The levels of total protein[19] and the oxidative status in liver and muscle samples were also measured. The activity of superoxide dismutase was evaluated by the colorimetric method of Misra et Fridovish[20]. The level of reduced glutathione was determined as described by Ellman[21]. The homogenate content of malondialdehyde was evaluated according to Wilbur.[22]. The DPPH antioxidant assay was carried out as recommended by Sun *et al.*[23], meanwhile, the FRAP antioxidant activity was performed as described by Benzie and Strain[24].

Data analyses: Data collected were expressed as Mean±standard deviation and then subjected to one way analysis of variance (ANOVA) using a general linear model procedure of Graph Pad Prism 8. Statistical significance was considered at p<0.05. To separate the means, the Tukey multiple test was used as a post-test.

RESULTS

Growth parameters: The impact of lysine/methionine and neem leaves on the growth performance of broilers is summarized in Table 4. Between 21 and 42 days, feed intake, energy intake and protein intake were not significantly different between the dietary treatments (p>0.05), although, broilers fed diet supplemented with lysine/methionine and neem leaves showed lower values than those of the other groups. As compared to non-supplemented group, the body weight gain of broilers was not affected (p>0.05) by the diet supplementation with methionine/lysine or neem leaves. However, incorporation of neem leaves and lysine/methionine in the diet significantly decreased (p<0.05) the body weight gain of broilers, as compared to the value obtained in broilers treated with a diet supplemented with lysine/methionine only, or with neem leaves alone. At the end of the experiment, it was observed that the feed conversion ratio of broilers was not affected (p>0.05) by the treatments.

Carcass characteristics: Table 5 shows the carcass characteristics of chickens fed control and supplemented diets. Irrespective of treatment, carcass yield and the relative weight of the legs, the pancreas, the intestine and the caeca were statistically similar (p>0.05). At 42 days of age, as compared to control, the relative weight of the gizzard and the liver were not affected (p>0.05) by diet supplementation. However, broilers fed diet supplemented with lysine/methionine only had lower value (p<0.05) as compared to birds fed diets supplemented with neem leaves alone. Diet supplemented with methionine/lysine and neem leaves significantly increased (p<0.05) the body weight proportion of abdominal fat and bursa. When compared to control, diet supplemented with methionine/lysine or neem leaves alone significantly increased (p<0.05) the relative weight of the spleen.

Biochemical parameters: Some biochemical parameters of broilers fed control and supplemented diets are presented in Table 6 and 7. Table 6 shows that the serum content

Table 4: Growth parameters of broilers fed on a diet supplemented with neem leaves and lysine/methionine

	Control	Lys/Met	Neem leaves	Lys/Met+neem leaves	p-value
DFI (g)	115.400±23.23	111.000±23.79	116.100±25.19	106.00±21.10	0.4561
DEI (kcal)	403.200±81.63	387.600±83.39	404.900±87.95	370.30±74.27	0.6738
DPI (g)	21.250±4.30	21.050±4.59	21.390±4.64	20.19±4.05	0.8073
DBWG (g)	65.050±7.499[ab]	67.280±4.211[a]	68.390±7.923[a]	59.00±6.806[b]	0.0013
FCR	1.737±0.212	1.631±0.202	1.701±0.304	1.75±0.214	0.7441

Values in the same line not sharing a common superscript differ significantly (p<0.05). Lys: Lysine, Met: Methionine, DFI: Daily feed intake, DEI: Daily energy intake, DPI: Daily protein intake, DBWG: Daily body weight gain and FCR: Feed conversion ratio

Table 5: Carcass characteristics of broilers fed on different diets supplemented or not with neem leaves and lysine/methionine

Carcass characteristics (LW%)	Control	Lys/Met	Neem leaves	Lys/Met+neem leaves	p-value
Carcass yield	72.1200±2.205	70.150±2.761	73.160±1.807	71.300±1.422	0.2140
Legs	3.6730±0.340	3.263±0.339	3.597±0.351	3.811±0.274	0.0700
Heart	0.4200±0.036	0.425±0.054	0.438±0.065	0.472±0.041	0.1492
Gizzard	1.6210±0.121[ab]	1.464±0.082[b]	1.690±0.131[a]	1.690±0.112[a]	0.0039
Liver	2.0140±0.125[ab]	1.879±0.456[b]	2.003±0.108[ab]	2.306±0.293[a]	0.0424
Abdominal fat	1.4740±0.242[b]	1.344±0.066[b]	1.689±0.330[ab]	1.893±0.324[a]	0.0021
Pancreas	0.2300±0.045	0.215±0.039	0.251±0.020	0.218±0.016	0.2894
Spleen	0.0800±0.015[b]	0.107±0.011[a]	0.108±0.018[a]	0.095±0.004[ab]	0.0007
Bursa	0.0247±0.006[b]	0.030±0.002[ab]	0.026±0.002[ab]	0.035±0.0073[a]	0.0205
Intestine	3.8510±0.848	4.213±0.589	3.720±0.535	3.857±0.467	0.4973
Caeca	0.4870±0.111	0.514±0.061	0.497±0.087	0.607±0.104	0.1116

Values in the same line not sharing a common superscript differ significantly (P<0.05). Lys: lysine; Met: methionine

Table 6: Biochemical parameters of serum of broilers fed on different diets supplemented or not with neem leaves and lysine/methionine

Parameters	Control	Lys/Met	Neem leaves	Lys/Met+neem leaves	p-value
Total proteins (g L^{-1})	90.570±3.780	91.710±4.990	88.710±0.755	90.290±3.861	0.4770
Albumin (g L^{-1})	42.570±4.077	41.710±5.345	43.000±3.958	38.570±4.077	0.2708
Globulins (g L^{-1})	46.710±4.112[b]	51.710±3.729[ab]	45.710±3.450[b]	55.140±3.579[a]	0.0002
Glucose (g L^{-1})	0.867±0.062	0.801±0.077	0.828±0.099	0.835±0.079	0.7293
Total cholesterol (g L^{-1})	2.143±0.489[a]	2.214±0.186[a]	1.643±0.399[b]	2.300±0.230[a]	0.0037
GGT (U L^{-1})	38.570±5.563	34.000±8.103	27.570±5.442	30.290±12.67	0,1117
GOT (U L^{-1})	41.430±4.756	44.140±4.634	42.140±3.237	41.000±2.082	0.5547
GPT (U L^{-1})	51.290±2.059	50.860±4.018	48.000±4.203	50.140±0.690	0.1497

Values in the same line not sharing a common superscript differ significantly (p<0.05), Lys: Lysine, Met: Methionine, GGT: Gamma glutamyl transferase, GOT: Glutamic oxaloacetic transaminase and GPT: Glutamic pyruvic transaminase

Table 7: Total protein content and oxidative status of the liver and muscle of broilers fed on different diets supplemented with neem leaves and lysine/methionine

Parameters	Organs	Control	Lys/Met	Neem leaves	Lys/Met+neem leaves	p-value
TP (mg/100 mL hom.)	Liver	34.2100±7.868[b]	35.6100±7.255[b]	41.9400±7.701[ab]	47.3500±4.330[a]	0.0186
FRAP (mgET/100 mL)	Liver	43.5200±7.346[b]	38.0700±6.131[b]	36.9500±2.446[b]	64.4700±3.169[a]	<0.0001
DPPH (mgET/100 mL)	Liver	70.7800±6.555	68.6300±11.04	71.9300±8.212	58.8600±13.03	0.1215
SOD (U mg^{-1} protein)	Liver	49.5900±6.712[b]	47.9900±9.375[b]	66.3400±10.33[a]	62.0300±10.19[a]	0.0007
MDA (µmole g^{-1} of organ)	Liver	0.0722±0.0276[a]	0.0428±0.0066[b]	0.0576±0.0154[ab]	0.0594±0.0222[ab]	0.0130
GSH (µmole mg^{-1} protein)	Liver	36.3100±6.398	45.0100±6.693	37.2200±12.04	49.4200±13.08	0.1159
TP (mg/100 mL hom.)	Muscle	27.2400±9.555[b]	33.0400±6.230[ab]	35.5100±5.478[ab]	43.4100±3.981[a]	0.0025
FRAP (mgET/100 mL)	Muscle	35.6400±9.464[b]	32.5100±4.222[b]	31.2300±3.144[b]	61.8400±2.484[a]	<0.0001
DPPH (mgET/100 mL)	Muscle	67.2200±8.501	68.9400±9.100	56.0500±9.362	68.8800±11.03	0.1008
SOD (U mg^{-1} protein)	Muscle	41.7000±6.978[b]	63.7200±12.06[a]	51.9300±14.56[ab]	51.7000±11.45[ab]	0.0203
MDA (µmole g^{-1} of organ)	Muscle	0.0663±0.0126[a]	0.0464±0.0112[ab]	0.0529±0.0094[ab]	0.0400±0.0122[b]	0.0078
GSH (µmole mg^{-1} Protein)	Muscle	30.1500±9.303	33.2300±4.810	33.4400±3.842	36.1900±5.350	0.5772

Values in the same line not sharing a common superscript differ significantly (p<0.05). Lys: lysine, Met: methionine, L: liver, M: Muscle; GSH: reduced glutathione , MDA: Malondialdehyde, SOD: Superoxide dismutase, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power, TP: Total protein, mgET/100 mL: mg equivalent trolox/100 mL of homogenate and hom.: Homogenate

of total protein, albumin, glucose, gamma glutamyl transferase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were not statistically affected by lysine/methionine or neem leaves supplementation (p>0.05). The serum level of globulin was significantly higher (p<0.05) in chickens fed diets supplemented with lysine/methionine and neem leaves, while the levels of total cholesterol were statistically decreased (p<0.05) in broilers that received the diet supplemented only with neem leaves.

Table 7 shows that, as compare to control, the levels of total protein and the FRAP activities were increased (p<0.05) in the liver and muscle of chicken fed diet supplemented with neem leaves and lysine/methionine. At the end of the experiment, the analyses of the oxidative status of the liver and the muscle revealed that irrespective of organs, the DPPH antioxidant capacity and the level of reduced glutathione were not statistically different (p>0.05) between treatments. As compared to the non-supplemented group, the SOD activities significantly increased (p<0.05) in the liver with neem leaves and with lysine/methionine in the muscle of broilers. Irrespective of organs, broilers fed on the non-supplemented diet showed the highest levels of MDA (p<0.05).

Hematological parameters: Statistical analyses of hematological parameters (Table 8) revealed that white blood cells and hemoglobin content were not significantly affected (p>0.05) by diet supplementation with lysine/methionine and

Table 8: Haematological characteristics of broilers fed on a diet supplemented with neem leaves and lysine/methionine

	Control	Lys/Met	Neem leaves	Lys/Met+neem leaves	p-value
WBC (10^3 L^{-1})	104.400±4.401	104.600±7.004	103.400±2.149	104.100±2.610	0.9420
RBC (10^{12} L^{-1})	1.419±0.183[a]	0.878±0.268[b]	1.327±0.154[a]	1.549±0.070[a]	<0.0001
Hemoglobin (g L^{-1})	10.470±0.930	10.070±1.183	10.470±1.023	9.929±1.186	0.6884
PCV (%)	20.600±2.195[a]	13.790±3.758[b]	19.510±2.310[a]	21.430±1.005[a]	<0.0001
MCV (fl)	145.700±5.559[ab]	153.000±5.657[a]	147.000±2.828[ab]	141.600±4.117[b]	0.0026
MCH (pg)	74.540±9.803[ab]	84.310±1.806[a]	79.040±3.824[a]	68.240±1.364[b]	0.0004
MCHC (g L^{-1})	50.990±5.068[b]	71.400±13.84[a]	53.740±2.435[b]	49.130±1.024[b]	<0.0001

Values in the same line not sharing a common superscript differ significantly (p<0.05). Lys: Lysine, Met: Methionine, WBC: White blood cell, RBC: Red blood cell, PCV: Pack cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin and MCHC: Mean corpuscular hemoglobin concentration

neem leaves. Diet supplementation with lysine/methionine decreased (p<0.05) red blood cells count and pack cell volume; meanwhile mean corpuscular hemoglobin concentration (MCHC) increased significantly (p<0.05).

DISCUSSION

In the poultry industry, it has always been a challenge to maintain a high rate of growth to achieve market weight at an early age and at a low production cost. In this study, we evaluated the combined effect of methionine/lysine and neem leaves supplementation on the growth performance, haematological and biochemical parameters of broilers. Our results showed that between 21 and 42 days of age, diet supplementation with methionine/lysine or neem leaves didn't affect the growth parameters as compare to the non-supplemented group. These findings are in accordance with previous studies which reported no effects of methionine and lysine supplementation or neem leaves supplementation on feed intake, body weight gain and feed conversion ratio[3,10,12]. Our results are different from those of Anseri *et al.*[13] who reported that broilers fed diets supplemented with 2.5 g kg^{-1} of neem leaf meal had significantly greater body weight, improved feed efficiency at 28 and 42 days of age. The variation in the findings may be due to the duration and the developmental phase of the supplementation. Our experiment lasted 3 weeks, during growth-finisher phase, while theirs lasted from starter to finisher phase.

Diet supplemented with methionine/lysine and neem leaves significantly increased the relative weight of bursa, suggesting higher immune activity of the primary lymphoid organ[25]. The bursa of Fabricius is necessary for the development of the postbursal B cell population and B cell maturation between the first 3-4 weeks after hatching. After 4 weeks, the bursa may primarily produce immunoglobulins until involution occurs[26]. In this study, broilers with higher relative weight of the bursa showed higher serum level of globulins. Moreover, the biochemical analyses revealed that broilers with the highest relative weight of liver showed the highest total protein in liver and the higher serum content of globulins. As part of the immune response, the liver contains antigen-presenting cells and lymphocytes. It synthesizes a number of immune effector proteins[27]. As compared to control, the relative weight of the spleen increased with the supplementation of methionine/lysine or neem leaves. Spleen weight gain is an indicator of immunomodulatory activity[28]. In fact, the spleen represents the main organ of the macrophage-phagocyte system. Spleen enlargement in mice treated with neem leaves extract has been reported[28].

Broilers fed diet supplemented with lysine/methionine and neem leaves had significantly higher abdominal fat proportion. This result suggested that neem leaves might reduce the bioavailability of methionine/lysine, which has been shown to lower fat level[29]. In line with the findings of Lal and Panda[12], our results showed that the diet supplementation with neem leaves significantly decreased the total cholesterol in broilers. Neem leaves have hypocholesterolemic properties due to their defatted parts which are rich in fibrous content and may block intestinal cholesterol absorption[12].

The serum content of gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were not statistically affected by treatments, suggesting non-significant variation in the metabolic activity of the liver. However, the serum levels of GGT tend to decrease in the presence of neem leaves. GGT catalyzes the transfer of gamma glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water, forming glutamate[30]. Lee *et al.*[31] indicated that GGT can also exert a pro-oxidant role. The GGT decreased in broilers fed diet supplemented with neem leaves, it might be related to the hepato-protective effect of neem leaves due to its anti-oxidant activities.

The lipid peroxidation and oxidative status of the liver and the pectoralis muscle were evaluated. In the pectoralis muscle, methionine/lysine and neem leaves supplementation decreased the level of MDA, suggesting that those supplements reduced lipid peroxidation. These findings are in line with some previous studies that reported such effects with methionine[32] or neem leaves[33]. Reduced glutathione levels (GSH) supported the reduction of lipid peroxidation which tended to increase in the liver and the muscle of broilers fed diets supplemented with neem leaves and lysine/methionine. GSH play a vital role in the cellular oxidant defence system and is essential for preventing lipid peroxidation by free radicals and oxygen[34]. Other studies have shown that methionine[35] and neem leaves[34] supplements increased the GSH synthesis in broilers. In the cells, GSH activity is highly supported by glutathione and antioxidant enzymes such as superoxide dismutase (SOD) or catalase[36]. The SOD degrades the superoxide anion radical to water and oxygen. Diet supplemented with neem leaves increased the SOD activity in the liver of broilers. In fact, neem leaves are rich in phenolic compounds such as gallic acid and ferulic acid which are reported to have high antioxidant activities[33]. Moreover, neem leaves contained quercetin, a flavonoid compound known to be a more powerful anti-oxidant than the other antioxidant-nutrients such as vitamin C, vitamin E and ß carotene[12]. In the muscle, the SOD activity was increased in broilers fed diet supplemented with lysine/methionine only. According to some previous studies, the diet supplementation with methionine improved the antioxidant activities[35,37].

Irrespective of the organs, the combined supplemented group showed a significant increase in the FRAP activities without any change in the DPPH anti-oxidant capacity. FRAP is one of the widely used assay to measure the general antioxidant power based on the ability of a sample to reduce iron[38]. FRAP increases suggested that neem leaves and methionine/lysine supplementation could increase liver and muscle antioxidant levels reacting with chromogenic reagents.

The decreased red blood cells count and pack cell volume, suggest a lower metabolic activity of broilers fed diet supplemented with methionine/lysine solely. According to Luger *et al.*[39] increased pack cell volume may be associated with increased metabolic activity as a result of the higher energy demands for maintenance and growth under stressful conditions.

CONCLUSION

The aim of this study was to evaluate the combined effect of methionine, lysine and neem leaves meal supplementation on the growth performance, carcass traits, biochemical and haematological parameters of 21-day-old broilers. The findings of this study showed that during the grower-finisher phase, the dietary supplementation of methionine/lysine or neem leaves did not affect the growth performance of broilers. Neem leaves increased the carcass quality and the antioxidant capacity. The combined supplementation of neem leaves (0.25%) and methionine/lysine (0.3%/0.7%) to the diet improved immunity and the antioxidant capacity in broilers without affecting growth performance. However, based on the results of this study, the combined supplementation of lysine/methionine and neem leaves to boost growth performance in 21-day-old broilers can't be recommended.

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