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INFLUENCE OF HYPO AND HYPER METHIONINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNOLOGICAL RESPONSES TO NEWCASTLE DISEASE VACCINATIONS OF BROILER CHICKENS

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ABSTRACT

This study was carried out to determine the effect of varying levels of methionine supplementation in diet of broiler chickens on their immunological response to Newcastle disease, growth performance, organ characteristics and haematological parameters. A total of two hundred birds were raised from day-old to six (6) weeks. They were distributed into four treatments A, B, C and D with five replicates per treatment, each replicate having 10 birds. At both starter and finisher phases of the experiment, there were four experimental diets A, B, C and D. Diet A was the control diet and contained a standard of 0.38% methionine, diet B was the diet containing 0.5% methionine as permitted by National Research Council (NRC) standards while diets C and D contained 0.63% and 0.75% methionine supplementary levels, respectively. By the third week of the experiment, some of the general observations made included leg weakness which later progressed to complete paralysis in about 40% of the birds placed on diet C and D. Mortality occurred in 70% of the paralysed birds. The immunological responses after vaccination with Newcastle disease vaccine intra-ocular (NDV i/o) revealed that chickens fed with 0.5% methionine (treatment B) recorded the highest antibody titre value ($\log_2 7$) while treatment D (fed with 0.75% methionine) had the lowest mean titre value ($\log_2 5$). Thereafter, administration of NDV LaSota elicited immune responses with antibody titre values being highest in treatment B ($\log_2 9$) and least in both treatments A and D ($\log_2 7$). The performance characteristics such as the final body weight (FBW), weight gain (TWG), feed intake (TFI) and feed conversion ratio (FCR) showed significant ($p<0.05$) differences among the various dietary treatments, with birds on NRC requirement methionine based diet having significantly ($p<0.05$) higher FBW, TWG, FCR and TFI than those fed the rest test diets throughout the experimental period. Relative weight of the organs liver and gizzard were also significantly ($P< 0.05$) different between treatment groups. Among the haematological variables only the red blood cell count (RBC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) values were significantly different ($p<0.05$) among treatment groups in the starter phase of the experiment and erythrocyte sedimentation rate (ESR), basophil and eosinophil in the finisher phase. It was concluded that methionine supplementation at this high inclusion levels is detrimental to the immune response, growth performance, nutrient utilization and organ characteristics of broiler chickens.

Key words: Methionine, immunity, Newcastle disease, performance, vaccinations, antibody titre, growth, supplementation

INTRODUCTION

Poultry need to be supplied enough dietary nutrients and energy to allow the birds to express desired growth and feed efficiency. Since the possibility of disease outbreaks is always present in today's poultry operations, the bird's metabolism and immune system are constantly adjusting to the disease condition or stress of the environment and thus nutrient requirements may need to be increased at certain times such as during disease outbreaks and poor weather conditions.

The immune system benefits greatly from adequate nutrition, which is feeding on a balanced diet and supplementation of some essential nutrients. Not only does the immune system benefit directly from adequate nutrition, it indirectly prepares the body for periods of stress by reducing the adverse effects and enhancing speedy recovery from such stressful periods. Therefore, in many instances, proper nutrition lessens the immune suppression associated with the stress response in the body. In the case of domestic chickens, these facts are equally true [1].

Although most nutrients required by the immune system are present in the diet in sufficient concentrations, there is evidence that increased dietary supplementation of certain nutrients such as methionine above the optimal level for growth is of benefit to immune response and maximum performance/productivity [1]. Methionine is a sulphur containing amino acid that cannot be synthesized but where synthesized it is usually at a very low rate incapable of meeting the basic metabolic requirement of the birds [1]. It is an essential amino acid and primarily serves as a methyl donor for transmethylation reactions, especially in the biosynthesis of lipids and other compounds, hence the need to supplement methionine where crude protein cannot provide the required level.

Methionine has been shown to benefit immune function when added to diets of poultry at higher concentration than is required to maximize growth and feed efficiency [2]. However, too much dietary methionine was observed to suppress the immune system [3].
In some previous studies, methionine had been supplemented to overcome growth depression in poultry [4, 5, 6, 7]. Also it has been shown that supplementing methionine in a bird's diet correlated with the tendency to have less total body fat [8]. Methionine supplementation has also been seen to increase egg size and numbers by moulted hens [9].

Optimal supplementation of methionine is envisaged to improve growth performance and carcass quality of broilers [7]. It is therefore important to ensure that poultry diets contain enough methionine to ensure the desired growth, feed conversion and immune response, but not too much so as to be toxic to the birds.

Newcastle disease is one of the most rampant viral diseases of poultry with a prevalence rate of 28.9% in Nigeria [10]. Two of the major tools that can be used to provoke immunity in birds for both the prevention and control of the spread of the disease are vaccination and good nutrition [10]. Information as to the effect of hypo and hyper methionine supplementation on growth and immunological responses to Newcastle disease in broiler chickens are rare.

Hence, this work studied the effect of supplementing methionine above the minimum recommended NRC (1994) requirement in the diets of broiler chickens on the growth performance, haematological parameters, relative organ weight and immunological response to Newcastle disease vaccinations.

MATERIALS AND METHODS

Experimental Diets

Four diets were formulated and used for the trial with the basal composition shown in Table 1.

Diet A- control diet containing a standard of 0.38% methionine

Diet B- the control diet containing 0.5% of methionine supplementation (NRC requirement standard)

Diet C- diet containing 0.63% of methionine supplementation (25% increase over NRC requirement)

Diet D- diet containing 0.75% of methionine supplementation (50% increase over NRC requirement)

Experimental Design

A total of 200 day old broiler chicks (Ross 308) were used for the trial. The experiment was carried out at the Teaching and Research farm, Federal University of Technology Akure. They were reared on deep litter and brooding was done in a conventional way. A completely randomized design was used for the trial. Birds were randomly chosen and divided into 4 groups of 50 birds each, with each treatment having 5 replicates of 10 birds each. The chicks in the various groups were fed with their experimental diets and given water *ad libitum*.

Vaccination

The birds were vaccinated with Newcastle disease vaccines using a standard vaccination regime- NDV intra-occular (Hithner B1 strain) at 3 days old and NDV LaSota at 21 days old.

Performance Characteristics

The birds were fed their respective experimental diets for 6 weeks during which records for daily feed intake, weekly weight gain, final body weight and feed conversion ratio were taken.

Sample Collection

Blood and serum samples were collected from birds in each treatment group at day old via the heart to determine baseline maternal antibody against Newcastle disease (ND) virus. Thereafter bleeding was done 10 days after each vaccination process for haematological and serological analysis where the birds were bled through the jugular vein.

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Relative Organ Measurements

At the end of the feeding trial 3 birds per replicate in each treatment were slaughtered, defeathered and eviscerated. The organs were dissected out and measured. The organs measured were liver, heart, spleen, lungs and gizzard, expressed in g/kg body weight.

Haematological Parameters

The erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed. The Mean corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were calculated accordingly [11].

Haemagglutination and haemagglutination inhibition test (HA/HI test)

Serum samples were analysed using beta (β) micro haemagglutination inhibition technique to determine the antibody titre levels to Newcastle disease vaccinations as a measure of the immunological response elicited in the experimental birds.

Haemagglutination (HA) titration

The micro-haemagglutination technique was used [12]. The aim of the HA titration was to determine the viability or the potency of the vaccine used. The Newcastle disease vaccine (LaSota strain) used as the antigen for the HA titration was locally produced by the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. Clean, dry, micro-titre plates used were labelled as required, and 0.2ml of normal saline was dispensed into each of a pair of wells using a micro-pipette. A drop of the antigen was added into the first pair of wells and mixed thoroughly using a pair of inoculating loops and serial dilution was carried out. Finally 0.02ml of the guinea pig RBC indicator previously diluted with normal saline was added to each well. The plates were then incubated on the laboratory bench for about 30 minutes at room temperature. After precisely 30 minutes, the end point of the titre was determined as the pair of wells where haemagglutination was clearly observed.

Haemagglutination inhibition (HI) titration

The beta haemagglutination inhibition technique was used and the stock antigen was diluted according to the HA titre obtained; thus for an antigen with a titre 1:256, the 4HAµ will be equal to 1:64 dilution of test stock. The micro-titre plates were labelled as required and 0.2ml of the test stock was then dropped into each pair of the wells on a row of the micro-titre plates. After this, a drop of the serum sample was added into the first pair of wells, thoroughly mixed and serially diluted. Lastly a drop of the prepared guinea pig RBC indicator was added to each well. The micro-titre plates were incubated at room temperature on the bench for 30 minutes. The end point of the titre was determined as the pair of wells where haemagglutination inhibition is clearly observed.

Statistical analysis

The data collected on immunological response to NDV, performance characteristics, haematological parameters and relative organ measurements were subjected to analysis of variance [13]. Where significant differences were found, the means were separated using Duncan multiple range test with the SPSS version 12 statistical package.

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RESULTS

General Observations: In the first week, the birds were healthy and eating well. Thereafter, at the end of second week, the birds fed on excess methionine diets (diets C and D) became anorexic, that is, lost their appetite, became dull, unthrifty and started showing signs of leg weakness. By the 3rd week, complete paralysis was noticed in 40% of the birds placed on diets C and D. At this time mortality was recorded in treatments C and D which continued till the 5th week. A total of 15 birds died in treatment C while 20 birds in treatment D. No mortality was recorded in treatment B but 3 birds died in treatment A. Majority (70%) of the paralysed birds died during the course of the study. It was observed that birds in treatment B thrived well during the study.

Performance of birds

Table 2 shows the final body weight (FBW), total weight gain (TWG), total feed intake (TFI) and feed conversion ratio (FCR) of birds fed with diets containing varying levels of methionine over the 6 weeks experimental period. It was observed that all the performance characteristics measured were significantly ($p<0.05$) influenced by the dietary treatments at different ages of the birds. The FBW of the birds at 14 days old was highest in birds fed NRC requirement (324.14g). While this was significantly different ($p<0.05$) from those fed 25% and 50% higher methionine than the NRC requirement, it was similar ($p>0.05$) to those fed the control diet A (300.43g). Birds fed on diets other than those on NRC requirement significantly ($p<0.05$) had lower TWG than those on the NRC requirement. While the TFI was not significantly ($p>0.05$) affected, birds on NRC requirement and 50% methionine above the NRC requirement utilized their diets better than the birds on the remaining test diets. At 0-28 days, the FBW, TWG and TFI followed similar trends. The birds on NRC requirement methionine-based diet had significantly ($p<0.05$) higher FBW, TWG, FCR and TFI than those fed the rest test diets with birds fed on 25%-50% higher methionine diets (C and D) having the lowest values. At 0-42 days, the FBW, TWG, TFI and FCR of birds fed on diet containing the NRC requirement were significantly ($p<0.05$) higher than those fed on the other test diets.

HA/HI Tests

The results of the HA and HI tests are presented in Table 3. A random sampling of birds for maternal baseline antibody titre level gave a mean value of $\log_2 4$. The antibody titre values of the experimental birds after the Newcastle disease vaccinations were significantly ($p<0.05$) different among the varying dietary treatments. After vaccination with NDV (Hitchner B1 strain) intra-ocular, chickens fed on diet B which contained 0.5% methionine recorded the highest antibody titre value of $\log_2 7$ while those fed diet D containing 0.75% methionine had the lowest mean titre value of $\log_2 5$. Thereafter, administration of NDV LaSota elicited immune responses with antibody titre values being highest in birds fed diet B with titre value of $\log_2 9$ and least in birds on diets A and D having an average titre of $\log_2 7$.

Haematological variables

Results of haematological variables of experimental birds are presented in Tables 4 and 5. Table 4 shows that RBC, MCH and MCV values were significantly different ($p<0.05$) among treatment groups in the starter phase of the experiment. The RBC values of birds fed Diets A ($2.07 \times 10^6 mm^3$) and B ($2.02 \times 10^6 mm^3$) were similar ($p>0.05$) but were significantly ($p<0.05$) different from those fed on diets C ($1.70 \times 10^6 mm^3$) and D ($1.94 \times 10^6 mm^3$), which were not

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significantly different from each other. The MCH value (5.05 pg) and MCV value (1.51 μ^3) of birds fed diet C were significantly ($p<0.05$) higher than those fed other test diets.

Table 5 shows that the ESR, MCV, Basophil and Eosinophil values were significantly ($p<0.05$) influenced by the dietary treatments at the finisher phase. The ESR of birds on diet A (2.11mm/hr) was significantly ($p < 0.05$) different from those fed on diet D (1.44mm/hr) but similar to those fed diets B and C. The MCV of birds on diets C and B were similar ($p>0.05$) but these were significantly ($p < 0.05$) higher than those fed diets A and D. For basophil value birds on diet A (2.44%) had significantly ($p < 0.05$) higher value than those fed on diet B (2.00%) but similar to those fed on diets C and D. The values of eosinophil for birds on diets with hyper methionine supplementation, diets C and D (1.00%) were significantly ($p < 0.05$) higher than those on diets B and A.

Carcass and relative Organ Measurement

Table 6 shows that all the measured parameters were significantly different ($p < 0.05$) among the various dietary treatment groups. The varying methionine supplementary rates had significant effect on the carcass and organ development of the experimental birds. The dressed and eviscerated weight of birds fed diet B (88.7%, 79.6%) while significantly different from birds on diets C and D, was similar to that of birds on diet A. The weight of heart (6.11g) of birds fed diet C was significantly different from that of birds on the other test diets. The liver and spleen of birds fed diets C and D were similar but significantly different ($p < 0.05$) from birds on diets A and B. The weight of the gizzard of birds fed diet D (32.57g) was the highest, though significantly different ($p < 0.05$) from birds fed diets A and C but similar to birds on diet B. The weight of lungs of birds fed diet D was not significantly different ($p>0.05$) from birds on diets B and C but significantly different from birds on diet A.

DISCUSSION

The immense benefits of adequate nutrition on the immune system cannot be over emphasized and supplementing certain nutrients above required levels have proven to increase immune responses during periods of stress and disease outbreaks in livestock animals. In this study, the hyper supplementation of methionine in diets of broiler chickens led to significant adverse effects on general wellbeing - the immune status and growth rate of the birds. The result of this study has supported the reports of several works [14, 15, 16 and 17]. At the second week, the birds fed with excess methionine diets (diets C and D) were observed to be anorexic (lost their appetite), dull, unthrifty and started showing signs of weakness in their legs. This is in line with previous findings that observed leg weakness in chicks fed with excess methionine [18, 19]. In the 3rd week, complete paralysis was noticed in 40% of the birds fed with methionine higher than the NRC concentration levels. This study tends to suggest that excess methionine could be responsible for the leg weakness and later complete paralysis since these clinical signs were observed only in broiler chickens fed with diets supplemented with 25% and 50% methionine above the NRC requirement. This study tends to suggest that methionine has effect on calcium and phosphorus mineralization and thus adverse skeletal development in broiler chickens.

The excess supplementation of methionine at the rate of 25% and 50% over NRC requirement in the diets accounted for a significant reduction in weight gain of birds in these 2 groups within the first few weeks of the study. Feed intake of birds in these 2 groups followed similar trend and was lower than those on NRC methionine requirement level. This corroborates previous findings which reported growth depression in broiler chickens and turkeys fed hyper levels of methionine [14, 17, 18]. Another report stated that at different age ranges of 4-11 days and 11-14 days, chicks fed with excess methionine showed a great depression of appetite and greater exponential growth rate that later led to compensatory growth [18]. This is in accordance with the findings of this study which showed that birds fed with 50% methionine above the NRC requirement had depressed appetite and lower weight gain at about 2-3 weeks of age but later had slight improvement in feed intake which culminated in improved weight gain. A study reported that an amino acid surplus stimulates the synthesis of protein and represses the breakdown of protein in the liver which leads to an altered plasma amino acid pattern and subsequently a depressed feed intake and growth depression [16]. This was ascertained in this study by the fact that depressed feed intake which is characteristic of an amino acid imbalance was evident in birds fed with diets containing hyper methionine levels.

A further report also inferred that when amino acids are supplied in excess, deamination occurs and highly toxic uric acid and also sulphuric acid is formed in the blood leading to toxicity [15]. In reaction to these contaminants one sees depression in feed intake and growth rate as initial signs of toxicity. Also feed conversion ratio was negatively affected by hyper methionine supplementation since the birds fed with diet supplemented with 50% methionine above the NRC requirement had the highest FCR values throughout the trial and therefore did not effectively utilize their feed as compared to birds fed with NRC requirement methionine supplemented diets that had lower FCR values. This is also in accordance with the findings in a previous study that reported less efficient use of feed by chickens fed excess methionine in their diets [18]. It could also be deduced that birds on diet A had the least relative weight for all the organs measured. This points to the fact that methionine is essential or is needed for adequate development of organs and growth in chickens as earlier reported by some researchers, that supplemented methionine to overcome growth depression in poultry [4,5,6,7].

The blood indices mostly affected by dietary treatments were the RBC, ESR, HB, PCV, plasma protein and glucose [20]. In the finisher phase of this study ESR, MCV, basophil and eosinophil were influenced by the dietary treatment as seen in Table 5. The influence of the dietary treatments on these haematological indices could be due to the methionine supplementation as excess methionine has been observed as a threat to normal haematological parameters in broiler chickens [21]. In general, the values of the haematological indices of the experimental birds fell within the normal range for chickens [22]. It was however observed at the finisher phase that values of all the haematological variables except for eosinophil were consistently lower in birds on diet A which had 0.38% methionine in their feed and higher in birds on diet B with NRC methionine requirement level. This fact reiterates and emphasizes the importance of methionine since blood represents a means of assessing health status in clinical and nutritional feeding trials [20]. Therefore, the practice of methionine supplementation in diets of poultry for maximum productivity cannot be discarded.

In an earlier study, it was reported that protection against ND is measured by the presence of specific antibodies to ND virus [23]. Therefore, the level of immunity to ND following vaccination as illustrated by the average antibody titre values showed that birds fed on diet B (diets with NRC methionine requirement level) had the highest immune response. Chickens fed with 0.38% and 0.75% methionine supplementation elicited the least immunological response after the ND vaccination regime. This study is in accordance with the work of various researchers that excess dietary methionine will suppress the immune system [3,19]. It has also been reported that high methionine supplementation caused depression in both humoral and cell mediated immunity in broiler chickens [24]. The low immune response in birds fed with 0.38% methionine based diets also implies that lack or inadequate methionine supplementation in diets of broiler chickens is detrimental to immune response to ND vaccinations.

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CONCLUSION

The study showed that methionine supplementation levels higher than the NRC requirement in broiler diets caused adverse effects in the immune response to locally produced Newcastle disease vaccines (by NVRI), growth performance (up to complete paralysis), organ characteristics and blood variables of birds. This was especially noted at the starter phase of the study (in birds between the age of 0-21 days). Therefore, it is clear from this study that hyper methionine supplementation above NRC requirement is detrimental to overall/general performance of broiler chickens.

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Table 1: Gross and calculated composition of experimental diets

Ingredients%	Diets A	B	C	D
Maize	58.62	58.50	58.37	58.25
Soybean meal	18.50	18.50	18.50	18.50
Fish meal	4.00	4.00	4.00	4.00
Groundnut cake	12.50	12.50	12.50	12.50
Vegetable oil	2.00	2.00	2.00	2.00
Bone meal	2.75	2.75	2.75	2.75
Oyster shell	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30
Premix	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10
Methionine	0.03	0.15	0.28	0.40
Total	100	100	100	100
Calculated composition				
Crude protein (%)	23.09	23.08	23.07	23.05
ME (MJ/kg)	13.14	13.13	13.11	13.09
Calcium (%)	1.73	1.73	1.73	1.73
Avg. phosphorus (%)	0.77	0.77	0.77	0.77
Lysine (%)	1.14	1.14	1.14	1.14
Methionine (%)	0.38	0.50	0.63	0.75

Metabolizable energy

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Table 2: Performance characteristics of broiler chickens (0 – 42 days) fed diets with varying levels of methionine

Days	Parameters	DIETS A	B	C	D	Pooled SEM
	Initial body Wt	33.67	34.00	35.33	34.00	34.25
0-14	FBW (g)	300.43^{a}	324.14^{a}	291.53^{b}	287.76^{b}	3.67
	TWG (g)	266.76^{b}	290.14^{a}	256.2^{b}	253.86^{b}	4.10
	TFI (g)	357.8	353.47	341.73	311.47	10.47
	FCR	1.34^{b}	1.18^{a}	1.33^{b}	1.23^{a}	0.04
	Initial body Wt	33.67	34.00	35.33	34.00	34.25
0-28	FBW(g)	446.53^{b}	542.76^{a}	390.78^{c}	375.45^{c}	13.81
	TWG(g)	412.86^{b}	508.76^{a}	355.45^{c}	341.45^{c}	19.74
	TFI (g)	619.29^{b}	722.43^{a}	572.27^{c}	559.97^{c}	39.93
	FCR	1.50^{b}	1.42^{a}	1.61^{b}	1.64^{b}	0.05
	Initial body Wt	33.67	34.00	35.33	34.00	34.25
0-42	FBW (kg)	1.41^{b}	1.62^{a}	1.36^{b}	1.24^{c}	0.04
	TWG (kg)	1.38^{b}	1.59^{a}	1.32^{b}	1.20^{c}	0.04
	TFI (kg)	2.37^{b}	2.57^{a}	2.28^{b}	2.14^{c}	0.10
	FCR	1.72^{b}	1.63^{a}	1.73^{b}	1.80^{b}	0.05

Means with different superscripts within the same row are significant ($p<0.05$)

SEM- Standard error of mean
FBW- Final body weight
TWG- Total weight gain
TFI-total feed intake
FCR- Feed conversion ratio
Diet A- 0.38% methionine
Diet B- 0.50% methionine
Diet C- 0.63% methionine
Diet D- 0.75% methionine

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Table 3: Average antibody titre values of broiler chickens fed low or high Methionine diets in response to Newcastle disease vaccinations

	Base line antibody titre	**Anti body titre for the 1st NDV**	**Anti body titre for the 2nd NDV**
Diet A	$Log_2 4$	$Log_2 6^{ab}$	$Log_2 7^{b}$
Diet B	$Log_2 4$	$Log_2 7^{a}$	$Log_2 9^{a}$
Diet C	$Log_2 4$	$Log_2 6^{ab}$	$Log_2 8^{ab}$
Diet D	$Log_2 4$	$Log_2 5^{b}$	$Log_2 7^{b}$
Pooled mean	$Log_2 4$	$Log_2 5.75$	$Log_2 7.75$
Significance	NS	*	*

NS- not significant; *- significant at $p < 0.05$; ND- Newcastle disease

Diet A- 0.38% methionine

Diet B- 0.50% methionine

Diet C- 0.63% methionine

Diet D- 0.75% methionine

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Table 4: Haematological variables of broilers at starter phase (0-28 days) fed diets with varying levels of methionine

	DIETS				
PARAMETERS	**A**	**B**	**C**	**D**	**±SEM**
ESR (mm/hr)	2.17	2.33	2.83	2.33	0.12
PCV (%)	27.83	28.33	25.83	27.17	0.48
RBC ($x10^6mm^3$)	2.07^a	2.02^a	1.70^b	1.94^b	0.06
HB (g/100ml)	9.28	9.42	8.60	9.05	0.16
MCHC (%)	33.34	33.25	33.29	33.30	0.80
MCH (pg)	4.48^b	4.66^b	5.05^a	4.66^b	2.96
MCV (μ^3)	1.34^b	1.40^b	1.51^a	1.40^b	1.93
Lymphocyte (%)	56.83	55.83	58.83	59.00	0.98
Heterophil (%)	24.83	25.17	24.33	25.17	0.40
Monocyte (%)	11.33	14.50	13.67	12.83	0.79
Basophil (%)	2.00	2.00	2.17	2.00	0.04
Eosinophil (%)	1.00	0.83	1.00	1.00	0.04

ESR = Erythrocyte sedimentation Rate, PCV = Packed cell volume, RBC=Red Blood cell Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell Haemoglobin, MCV = Mean cell volume. Means with different superscripts within the same row are significant ($P < 0.05$) SEM – Standard Error of Mean

Diet A- 0.38% methionine

Diet B- 0.50% methionine

Diet C- 0.63% methionine

Diet D- 0.75% methionine

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Table 5: Haematological variables of broilers at finisher phase (0-42 days) fed diets with varying levels of methionine

	DIETS				
PARAMETERS	**A**	**B**	**C**	**D**	**± SEM**
ESR (mm/hr)	2.11^{a}	1.78^{ab}	1.89^{ab}	1.44^{b}	0.09
PCV (%)	32.67	33.33	32.33	34.89	0.57
RBC ($x10^6mm^3$)	2.47	2.30	2.23	2.67	0.08
HB (g/100ml)	10.87	11.11	10.78	11.64	0.19
MCHC (%)	33.27	33.33	33.34	33.36	0.00
MCH (pg)	4.40	4.43	4.43	4.35	1.02
MCV (μ^3)	1.32^{b}	1.44^{a}	1.44^{a}	1.30^{b}	3.03
Lymphocyte (%)	56.44	59.22	58.89	57.56	0.60
Heterophil (%)	24.44	25.22	24.67	24.56	0.35
Monocyte (%)	14.67	12.89	13.33	14.89	0.52
Basophil (%)	2.44^{a}	2.00^{b}	2.22^{ab}	2.11^{ab}	0.07
Eosinophil (%)	0.89^{ab}	0.67^{b}	1.00^{a}	1.00^{a}	0.05

ESR = Erythrocyte sedimentation Rate, PCV = Packed cell volume, RBC=Red Blood cell Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCH = Mean cell Haemoglobin, MCV = Mean cell volume. Means with different superscripts within the same row are significant ($P < 0.05$) SEM – Standard Error of Mean

Diet A- 0.38% methionine

Diet B- 0.50% methionine

Diet C- 0.63% methionine

DietD-0.75%methionine

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Table 6: Effect of Varying Levels of Methionine Supplementation on Organ Characteristic of Broiler Chickens

PARAMETERS	Diet A	Diet B	Diet C	Diet D	± SEM
Dressed weight%	88.27[a]	88.76[a]	85.90[b]	84.98[b]	0.69
Eviscerated wt%	79.24[a]	79.62[a]	76.37[b]	75.34[b]	0.78
Heart(g)	4.70[c]	5.23[b]	6.11[a]	5.73[b]	0.19
Liver(g)	18.82[b]	19.73[b]	22.20[a]	23.01[a]	0.62
Spleen(g)	0.96[c]	1.20[b]	1.67[a]	1.76[a]	0.12
Gizzards(g)	28.56[b]	30.98[a]	26.68[c]	32.57[a]	0.76
Lungs(g)	4.76[b]	5.16[a]	4.98[ab]	5.34[a]	0.24

SEM – Standard Error of Mean

Diet A- 0.38% methionine

Diet B- 0.50% methionine

Diet C- 0.63% methionine

Diet D- 0.75% methionine

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