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Research Article

Effects of Naturally Compounded Materials as a Substitute for Synthetic Methionine on Hematological and Serum Biochemical Parameters of Starter Broiler Chickens

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Abstract:

Introduction: Methionine is considered as an essential amino acid for optimal growth and development of broiler chicken. In addition, synthetic methionine is frequently used; however, the high cost has turned researchers to identify other potentially cheaper alternatives. Naturally compounded methionine produced from plants and/or combinations of plants and animals has emerged as a viable alternative to synthetic methionine in poultry nutrition, with nutritive value similar to synthetic methionine over other costly synthetic sources. Therefore, this study determines hematological and serum biochemical parameters of starter broiler chicken fed naturally compounded materials as a feed substitute.

Methods: 150 day-old Ross 308 broiler chicks were brooded for 7 days and then randomly distributed into five dietary treatments. Each treatment contained three replicates with 10 birds per group distributed in a completely randomized design. Five diets were provided at 0, 25%, 50%, 75% and 100% naturally compounded materials (NCM) substituting synthetic methionine. The experiment was conducted for 33 days, and statistical analysis was performed using ANOVA and Duncan Multiple Range test. The value of $P < 0.05$ was considered statistically significant. Furthermore, blood samples were collected from broiler chicken and subjected to hematological and biochemical indices.

Results: Hematological and serum biochemical parameters indicated birds fed Treatment 4 (75% NCM) produced the most optimal treatment response for serum biochemical and hematological parameters compared with the other treatment groups. Treatment 4 had higher hemoglobin levels (9.11 g/dL), packed cell volume (28.51%), red blood cell count (3.43×10^{12}/L) and white blood cell count (29.35×10^{9}/L). Likewise, significant differences ($P < 0.05$) were observed for serum biochemical parameters of total protein (3.70 g/dL), albumin (2.09 g/dL), globulin (1.61 g/dL), cholesterol (1.60 mmol/L), HDL (1.79 mmol/L), ALT (12.39I IU/L) and AST (9.10I IU/L) in comparison with other treatments.

Conclusion: The findings suggest that naturally compounded materials can partially replace synthetic methionine in starter broiler diets without adverse effects on blood parameters at starter phase of broiler chicken.

Keywords: Broiler chicken, synthetic methionine, hematology, serum biochemistry, naturally compounded materials, poultry.

1. INTRODUCTION

The demand for poultry products, including broiler meat and eggs, has grown rapidly, especially in developing nations, due to the increasing global population and changing consumer preferences from red meat to poultry products (David *et al.,* 2024). Despite significant contributions to encounter global meat demand, poultry production continues to face several challenges, including high production cost, high cost of protein feed sources, feed scarcity, as well as poor bird performance (Buragohain, 2016; David *et al.,* 2025a; Mottet & Tempio, 2017). In modern poultry production systems, particularly broiler production, feed constitutes a major production cost, accounting for approximately 70% of the total cost (Mallick *et al.,* 2020). Moreover, recent years have experienced rise in prices with decline in the availability of conventional, non-conventional, and potential feed ingredients. These include alternative feed resources previously regarded as waste products and are now utilized in poultry feed production (Olabode *et al.,* 2025).

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Several factors may contribute to this rise in cost and limitations in the availability of feed ingredients, including effects on agricultural production due to climate changes and increasing demand for these resources for human consumption (Is-Haaq *et al.,* 2018). Therefore, low-cost alternative protein sources have become imperative to sustain poultry production, reduce dependence on synthetic feed additives, and hence, promote optimal growth with improved performance in birds (Olabode *et al.,* 2022).

Methionine is a limiting amino acid in poultry nutrition required for optimal growth in birds (Xiao *et al.,* 2017) and is therefore routinely supplemented in poultry feed to support various metabolic and physiological processes (David *et al.,* 2025b). Methionine also facilitates protein synthesis for development and maintenance of components including muscles, organs and feathers (Xiao *et al.,* 2017). Its requirements vary based on factors including age, growth stage, production purpose, sex and breed. Maximum methionine is required during early growth stages and declines as the growth progresses. Therefore, methionine supplementation is essential to support optimal growth, health, and productivity performance throughout the production cycle (Alagawany *et al.,* 2016).

Reduction in poultry body proteins may lead to impaired immune functions, poor feather development, feather pecking, cannibalism, and increased mortality especially among young birds. Although poultry birds can obtain methionine from natural dietary sources such as fish meal, sunflower meal, maggots, and grasshoppers; however, they may not always be sufficient to meet the nutritional requirements. In diets that are protein deficient, methionine supplementation is essential for mitigating the adverse effects of heat stress and improving overall bird performance (Olabode *et al.,* 2022). Therefore, the incorporation of synthetic methionine in poultry diets will help to maintain amino acid balance, enhance protein synthesis, improvements in feed conversion efficiency and increase quality and quantity of egg production. It will also contribute to reduced fat deposition, thereby supporting optimal reproductive performance and egg-laying capacity in poultry processes (Olabode *et al.,* 2022). Synthetic methionine supplementation in poultry diets will improve immune function via protein synthesis and turnover, as well as through activities of its metabolites (Upadhyaya *et al.,* 2019). Thus, synthetic sources of methionine, such as DL-methionine, are added in poultry diets to optimize dietary methionine levels in the host animals (Moti *et al.,* 2005). Consequently, the identification of alternative feed ingredients that can match the efficacy of synthetic methionine would represent as a significant advancement in poultry nutrition, particularly broiler production (David *et al.,* 2025b).

Naturally compounded materials (NCM) also referred to as phytogenic additives or herbal methionine are plant-based, fermentation derived formulas or combinations of plant and animal-based materials designed as alternatives to synthetic methionine (DL-methionine) in animal feed. The common examples include Methiorep ®, Betain and specialized mixtures of plant and animal-based materials with high crude protein content (David *et al.,* 2025b). These alternative sources provide methionine in free or conjugated forms, along with naturally occurring compounds such as S-adenosylmethionine and phosphatidylcholine. These compounds function as lipotropic agents and facilitate the conversion of homocysteine into biologically active methionine thereby supporting protein synthesis. NCM have gained considerable attention in the field of poultry nutrition as cost-effective alternative sources for synthetic methionine along with improved growth, carcass yield and immune function with reduced cost of feed in chickens (Xiao *et al.,* 2017).

Hematological and biochemical parameters are important diagnostic indicators for assessing the physiological, metabolic and overall health status of animals. They provide valuable information for disease diagnosis, prognosis, and overall health monitoring in livestock (Bonadiman *et al.,* 2009; Chioma *et al.,* 2025; Tóthová *et al.,* 2019). Modifications in these parameters reflect changes in physiological and pathological conditions and can therefore be used to assess metabolic activity, nutritional status, immune function and overall health (Zalesakova *et al.,* 2025). Consequently, it makes these indices reliable indicators for monitoring animal health, welfare and productivity.

The focus of the present research is thus, the determination of naturally compounded materials in broiler birds to replace synthetic methionine on blood parameters at starter phase. Therefore, this study evaluates the effects of naturally compounded materials as replacements for synthetic methionine on the hematological and serum biochemical parameters of starter broiler chickens.

2. MATERIALS AND METHODS

2.1. Experimental Site

The experimental work was conducted at the Poultry Section of Animal Production Technology, Department of Federal College of Agriculture, Ishiagu, Ivo Local Government Area, Ebonyi State, from September to November 2023. Ishiagu is located at the geographical coordinates Latitude 5°22′ N and Longitude 7°21′ E with mean annual rainfall of 1655 mm, mean annual temperature of 28.5 ºC and average relative humidity of 88%.

2.2. Processing of Raw Materials

Raw materials used for the experiment were purchased at Ishiagu, in Ebonyi state and new market in Enugu State. The raw materials included fishmeal, groundnut cake, soyabean meal, lysine, bloodmeal, maize, bonemeal, wheat offal, synthetic methionine and lysine, limestone, starter premix and salt. The composition of naturally compounded materials (NCM) is presented in Table **1**.

Table 1. Composition of naturally compounded material.

Component	Ratio
Fishmeal	3
Soybean meal	4
Groundnut cake	2
Blood meal	1

2.3. Experimental Design and Husbandry of Birds

150-day-old broilers of Ross 308 strain were used in this study. Birds were brooded for 7 days at 32 ºC and randomly assigned to five dietary treatment groups. Each treatment consisted of three replicates, with 10 birds per replicate in a completely randomized design (CRD).

These birds were sourced from Cosin Farm in Enugu, Enugu State and placed in a brooding house with wood shavings as a source of litter and bedding material to provide warmth for the chicks. Electric bulbs were used as a source of illumination while charcoal served as the source of heat. Temperature was maintained by using thermometer. Feed and water were provided ad-libitum during the experiment conducted. All necessary drugs and vaccinations were duly administered when due (Table **2**).

Birds were randomly allocated five dietary treatments:

Treatment 1: Basal diet containing 100% synthetic methionine (Control)

Treatment 2: Basal diet containing 25% of synthetic methionine replaced with NCM

Treatment 3: Basal diet containing 50% of synthetic methionine replaced with NCM

Treatment 4: Basal diet containing 75% of synthetic methionine replaced with NCM

Treatment 5: Basal diet containing 100% of synthetic methionine replaced with NCM

The five diets were formulated and represented as Treatment 1 to Treatment 5. Treatment 1 served as the control diet with 0.35% synthetic methionine while Treatments 2, 3, 4 and 5 contained NCM at 25%, 50%, 75% and 100% respectively (Table **3**).

2.4. Statistical Collection

Data was analyzed using one-way Analysis of Variance (ANOVA) and significant differences were observed among treatment means. Duncan's Multiple Range Test (DMRT) was used for mean separation at a 5% level of significance ($P < 0.05$). Data was analyzed using the Advanced Statistics module in SPSS software.

2.5. Ethical Approval

The experimental procedures involving broiler chickens were conducted in accordance with guidelines for care and use of animals for research and were approved by Animal Ethics Committee of the Federal College of Agriculture (FCAI), Ishiagu, Nigeria. The field inspections of the experimental facility and animal management practices was facilitated by members of the National Institute of Animal Science (NIAS), South-East zone, Nigeria.

3. RESULTS

Assessment of hematological parameters of starter broiler chicks fed with the NCM-based diets with and without synthetic methionine are mentioned in Table **4**. Hemoglobin concentration, white blood cell count, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were significantly ($P < 0.05$) affected by dietary treatments. However, no significant differences were observed among treatments ($P > 0.05$) for packed cell volume (PCV), red blood cell count and mean corpuscular hemoglobin concentration (MCHC).

Table 2. Drug administration in broiler birds.

Number of Days	Vaccine Intervention
Day 1	Marek vaccine (given at the hatchery or point of collection of the chicks or on arrival of the chicks to the farm). Antistress plus multivitamin or glucose or sugar solution was given to the birds on arrival to ease transportation stress.
Day 2 to 5	Multivitamin and antibiotics (Mild antibiotics)
Day 6 to 8	Anticoccidial drug and vitamin (minus vitamin B1 (thiamine))
Day 9	Gumboro vaccine (i/o-intraoccular (via eye) or oral (drinking water))
Day 10	Vitamins
Day 11 to 13	Anticoccidial drug (Different from day 6 to 8 brand) and vitamin (minus vitamin B1(thiamine))
Day 14	LaSota vaccine mix with skim milk
Day 15 to 20	Vitamins
Day 21	Second Gumboro vaccine (i/o or oral)
Day 22 to 24	Ordinary water
Day 25 to 27	Vitamins
Day 28	Second LaSota
Day 29 to 33	Anticoccidial drug and vitamin (minus vitamin B1 (thiamine))

Source: *Sayeed Hatching Company, Nigeria Ltd.*

Table 3. Experimental diet for starter broiler birds.

Ingredients	Treatments				
	T1	T2	T3	T4	T5
Maize	54.00	54.00	54.00	54.00	54.00
Wheat offal	7.90	7.90	7.90	7.90	7.90
Soybean meal	12.00	12.00	12.00	12.00	12.00
Groundnut cake	13.95	13.95	13.95	13.95	13.95
Fishmeal	3.50	3.50	3.50	3.50	3.50
Bloodmeal	3.50	3.50	3.50	3.50	3.50
Limestone	1.50	1.50	1.50	1.50	1.50
Bonemeal	2.50	2.50	2.50	2.50	2.50
Lysine	0.20	0.20	0.20	0.20	0.20
Starter premix	0.35	0.35	0.35	0.35	0.35
Methionine	0.35	0.26	0.175	0.09	0.00
NCM	0.00	0.09	0.175	0.26	0.35
Salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated Nutrient Value					
Crude protein (%)	22.69	22.74	22.79	22.83	22.96
MEnergy (Kcal/kg)	3001.90	3044.50	3081.60	3120.78	3130.55
Crude fiber (%)	4.22	4.27	4.30	4.33	4.40
Ether extract (%)	4.70	4.72	4.77	4.81	4.86
Methionine (%)	5.65	5.65	5.65	5.65	5.65
Lysine (%)	1.12	1.12	1.12	1.12	1.12
Calcium (%)	1.25	1.25	1.25	1.25	1.25
Phosphorus (%)	0.49	0.49	0.49	0.49	0.49

Table 4. Hematological indices of starter broilers fed NCM to replace synthetic methionine.

	Treatments					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Hemoglobin (g/dL)	7.62^{d}	7.99^{c}	8.40^{b}	9.11^{a}	8.62^{b}	0.39
Packed cell volume (%)	27.30	27.91	28.33	28.51	27.88	7.62
Red blood cell ($x10^{12}$/L)	3.11	3.14	3.39	3.43	3.40	0.11
White blood cell ($x10^{9}$/L)	25.01^{b}	25.75^{b}	28.11^{a}	29.35^{a}	28.81^{a}	12.46
MCV (fL)	87.78^{a}	88.89^{a}	83.57^{b}	83.12^{b}	82.00^{b}	5.68
MCH (pg/cell)	24.50^{c}	25.45^{b}	24.78^{c}	26.56^{a}	25.35^{b}	2.26
MCHC (%)	27.91	28.63	29.65	31.95	30.92	3.52

Note: ^{abcd}*Means on the same row with different superscripts are significantly (P < 0.05) different.*
Abbreviation: SEM = Standard Error of Mean

Moreover, hemoglobin concentration of birds fed NCM diets were found higher (ranging from 7.99 to 9.11 g/dL) than control (7.62 g/dL). The mean values for NCM-treated packed cell volume varied from 27.91% to 28.51% and were not significantly different among treatments ($P > 0.05$). There were no significant differences in red blood cell count (3.11 to 3.43 x 10^{12}/L) among treatments ($P > 0.05$). White blood cell count was highest in Treatment 4 (29.35 x 10^9/L) compared to other treatments groups ($P < 0.05$). MCV was recorded highest for Treatment 2 (88.89 fL), while the lowest value was recorded for Treatment 5 (82.00 fL) while all treated groups had MCHC concentrations ranging between 27.91% and 31.95%.

The serum biochemical parameters of experimental birds fed different concentrations of NCM are mentioned in Table **5**. The dietary treatments significantly affected ($P < 0.05$) serum levels including albumin, total protein, low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate transaminase (AST) and alanine transaminase (ALT). However, no significant differences ($P > 0.05$) were observed for globulin and cholesterol levels between treatments.

Treatment 4 had the highest serum total protein (3.70 g/dL) and albumin (2.09 g/dL) concentrations compared to control. Serum globulin concentrations ranged between 1.33 to 1.61g/dL across all treated groups. Moreover, dietary treatments increased serum cholesterol concentration with Treatment 4 resulted in highest HDL (1.79 mmol/L) and AST (12.39 IU/L) in contrast to control. Similarly, ALT activity was highest in Treatment 4 (12.39 IU/L), which did not differ significantly ($P > 0.05$) from Treatment 5 (12.39 IU/L). Moreover, highest LDL concentration was observed in control (2.20 mmol/L) while the lowest value was observed in Treatment 4 (1.84 mmol/L).

The effect on hemoglobin with respect to treatments fortified with NCM suggests proper flow of blood to the bird's system for enhanced health status. The packed cell volume and red blood cell were within the physiological range of 22.00-37.00% and 2.00-4.15 x 1012/L reported by (Zalesakova *et al.,* 2025), indicating that inclusion of NCM diet did not induce anemia or adversely affect the nutritional status of the birds. This may be due to the improved nutrient availability provided by the NCM diet. The white blood cell count in this study was consistent with that reported by (Simaraks *et al.,* 2004) indicating that dietary inclusion of NCM maintained normal immune status of the birds. This is further supported by the absence of morbidity throughout the experiment. White blood cells are the key mediators of immune response in broiler chicken by eliminating invading pathogens and supporting antibody-mediated immune responses (David *et al,* 2024).

The biochemical levels of the serum total protein, albumin and globulin recorded were within the normal range of physiological values as mentioned by (Zalesakova *et al.,* 2025; Bonadiman *et al.,* 2009) indicating that diets administered to birds contained sufficient dietary protein for metabolism and physiological activities. Protein fortification, as well as quality and quantity of dietary protein appeared sufficient with high nutritional value enabling efficient digestion and absorption by the birds in the treatment groups. Total protein concentrations were similar to that reported by (Bonadiman *et al.,* 2009), which is not only required for growth, maintenance and repair of tissues, but also for production of enzymes and hormones essentially required by animals for breakdown and digestion of proteins in the gut system of birds. Albumin and globulin concentrations were higher in treatments that were fortified with NCM, but within the recommended range (1.20 - 3.50 g/dL) reported by (Zalesakova *et al.,* 2025) suggesting proper regulation of blood volume and maintenance of fluids and nutrients within the bird's system. The globulin concentrations were within the recommended range of 1.15 to 3.70 g/dL as reported by (David *et al.,* 2024) emphasizing the key role of globulin in regulating the immune system, to help birds fight off infection as well as maintenance of regulate inflammatory responses to injury or infections.

Therefore, incorporation of NCM in poultry feed may offer a sustainable and cost-effective approach to meet the methionine requirements of poultry while reducing dependence on synthetic supplementations.

Table 5. Serum biochemical indices of starter broilers fed NCM to replace synthetic methionine.

	Treatments					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Total protein (g/dL)	3.05^c	3.11^c	3.35^b	3.70^a	3.41^b	0.53
Albumin (g/dL)	1.72^c	1.77^c	1.89^b	2.09^a	1.92^b	0.04
Globulin (g/dL)	1.33	1.34	1.46	1.61	1.49	0.02
Cholesterol (mmol/L)	1.35	1.41	1.52	1.60	1.60	0.08
HDL (mmol/L)	1.52^c	1.59^c	1.64^b	1.79^a	1.70^b	0.02
LDL (mmol/L)	2.20^a	1.96^b	1.99^b	1.84^c	1.87^c	0.06
ALT (IU/L)	11.68^b	11.90^b	12.11^a	12.39^a	11.19^c	0.12
AST (IU/L)	8.30^c	8.92^b	8.77^b	9.10^a	9.05^a	0.32

Note: ^{abc}Means in the same row with different superscripts are significantly different $(P < 0.05)$.
Abbreviation: SEM = Standard Error of Mean

CONCLUSION

The study indicates that NCM can effectively replace synthetic methionine in the diets of starter broiler chickens without adverse effects on hematological and serum biochemical parameters. Birds that were fed NCM-based diets showed comparable and in some cases improvements in red and white blood cell count relative to control group. Serum biochemical indices related to protein metabolism were also maintained within normal physiological ranges suggesting adequate protein utilization in NCM fortified diets. In addition, liver enzyme activities (ALT and AST) remained within acceptable limits and there was no evidence of adverse hepatic effects across the treatments fortified with NCM.

Overall, the findings suggest that NCM has the potential to serve as a viable alternative to synthetic methionine in starter broilers. However, further studies are recommended to evaluate the effect in other poultry species such as ducks and geese including different inclusion levels and processing methods to better understand its broader application and long-term implication.

LIST OF ABBREVIATIONS

ALT	=	Alanine Transaminase
AST	=	Aspartate Transaminase
CRD	=	Completely Randomized Design
HDL	=	High Density Lipoprotein
LDL	=	Low Density Lipoprotein
MCH	=	Mean Corpuscular Hemoglobin
MCHC	=	Mean Corpuscular Hemoglobin Concentration
MCV	=	Mean Corpuscular Volume
NCM	=	Naturally Compounded Materials
NIAS	=	National Institute of Animal Science
PCV	=	Packed Cell Volume

AUTHORS' CONTRIBUTIONS

O.A.D. conceptualized the study, helped with manuscript writing. A.R.C. designed the study, collected and interpreted the data and helped with manuscript writing. A.L.N. collected the data and helped with the interpretation and O.O.E. helped with data collection and manuscript writing.

ETHICAL APPROVAL & INFORMED CONSENT

The experimental protocol involved broiler chickens. It was approved by Animal Ethics Committee of the Federal College of Agriculture (FCAI), Ishiagu, Nigeria. The experimental study was conducted in accordance with the institutional guidelines for care and use of experimental animals. Field inspections of the experimental facility and animal welfare were carried out by members of the National Institute of Animal Science (NIAS), South-East Zone, Nigeria. Throughout the study period, all birds were managed in accordance with standard broiler husbandry practices to ensure animal welfare and minimize stress and discomfort.

Informed consent is not applicable as the study does not involve human participants.

AVAILABILITY OF DATA AND MATERIALS

The data underlying the findings of this study are available from the corresponding author upon request.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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DECLARATION OF AI

The authors used ChatGPT for manuscript editing and take responsibility for the published content.

EDITORIAL DISCLAIMER

The manuscript contains several self-citations by one or more of the authors. These citations have been evaluated by the editorial team and deemed appropriate for inclusion in the context of the study.

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