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ORIGINAL RESEARCH PAPER

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# EFFECTS OF PARTIAL REPLACEMENT OF DL-METHIONINE WITH NATU METHIONINE ON GROWTH PERFORMANCE, FEED EFFICIENCY, CARCASS TRAITS, AND SERUM BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

**KEY WORDS:** Broiler Chicken, DL-methionine, Herbal Methionine, Natu Methionine, Growth Performance, Feed Conversion Ratio, Liver Enzymes, Breast Meat Yield, Carcass Characteristics, Methionine Recycling.

**Nitin Singh** — M.V.Sc. Veterinary Pathology, Superintendent, Pullorum Disease Control, Department of Animal Husbandry, Moradabad, Uttar Pradesh, India.

**Durgesh Gupta** — Veterinary Assistant Surgeon, Department of Animal Husbandry, Government of Madhya Pradesh, India.

**Mujtaba Wani** — Technical Sales Manager, RIVANSH Animal Nutrition Pvt Ltd., Paonta Sahib, Himachal Pradesh – 173025, India.

**Dimple Kaparwan** — Ph.D. Research & Development, RIVANSH Animal Nutrition Pvt Ltd., Paonta Sahib, Himachal Pradesh – 173025, India.

**ABSTRACT**

Methionine is the first limiting essential amino acid in poultry diets and plays a pivotal role in protein synthesis, methyl group transfer, antioxidant defence, lipid metabolism, and overall growth performance. Increasing concerns regarding the dependence on synthetic amino acids have stimulated interest in natural alternatives capable of supporting methionine metabolism. Natu Methionine is a proprietary herbal formulation designed to promote endogenous methionine biosynthesis through multiple metabolic pathways involving S-adenosyl methionine (SAM), serine, folic acid, aspartic acid, phosphatidylcholine, and methionine adenosyl transferase (MAT)-mediated methionine recycling. The present study was designed to evaluate the efficacy of Natu Methionine as a replacement for 50% of supplemental DL-methionine in broiler chickens. A total of 480 day-old Ross 308 broiler chicks would be randomly allocated to four dietary treatments comprising a methionine-deficient basal diet, a diet supplemented with 100% DL-methionine, a diet containing 50% DL-methionine plus 50% Natu Methionine, and a diet supplemented with Natu Methionine according to the manufacturer's recommended inclusion level. Birds would be reared for 42 days under standard commercial management conditions. Growth performance parameters including body weight, body weight gain, feed intake, feed conversion ratio, and mortality would be recorded. Carcass characteristics, breast meat yield, giblet weights, serum biochemical indices, and liver function enzymes (AST and ALT) would also be evaluated. The study is intended to determine whether partial replacement of synthetic DL-methionine with Natu Methionine can maintain or improve broiler performance while supporting liver health and carcass quality through enhanced endogenous methionine metabolism and methyl donor recycling. The findings are expected to provide scientific evidence for the application of herbal methionine as a sustainable nutritional strategy in modern poultry production and to reduce dependence on synthetic methionine supplementation.

## INTRODUCTION

Methionine is recognized as the first limiting essential amino acid in practical corn–soybean meal-based poultry diets and is indispensable for optimum growth, feed efficiency, immune competence, and carcass development in broiler chickens. It serves as a fundamental building block for protein synthesis and plays a central role in methyl group transfer reactions, polyamine synthesis, antioxidant defence, lipid metabolism, and cellular proliferation. Adequate methionine nutrition is therefore essential for maximizing growth performance, improving feed conversion efficiency, enhancing breast muscle deposition, and maintaining overall health and productivity of modern fast-growing broiler strains.

Commercial poultry production has relied extensively on synthetic methionine sources, primarily DL-methionine and methionine hydroxy analogue (MHA), to meet dietary methionine requirements. Although these products effectively correct dietary deficiencies, increasing demand for sustainable poultry production, consumer preference for naturally derived feed additives, and the growing organic poultry sector have encouraged the search for natural alternatives capable of supporting methionine metabolism without compromising production performance. In addition, herbal and plant-derived feed additives have gained considerable attention owing to their multifunctional biological properties, including antioxidant, hepato-protective, immunomodulatory, and growth-promoting activities.

Unlike synthetic DL-methionine, herbal methionine products are designed to support endogenous methionine metabolism through multiple biochemical pathways rather than acting solely as a direct source of the amino acid. These formulations typically contain biologically active compounds involved in methionine synthesis and recycling, including S-adenosyl methionine (SAM), serine, folic acid, aspartic acid, phosphatidylcholine, and plant-derived bioactive molecules that facilitate one-carbon metabolism. SAM represents the biologically active form of methionine and functions as the principal methyl donor for numerous methylation reactions. Serine and aspartic acid serve as metabolic precursors in methionine biosynthesis, whereas folic acid and phosphatidylcholine participate in homocysteine remethylation, thereby recycling methionine and maintaining an adequate intracellular methionine pool. Furthermore, methionine adenosyl transferase (MAT) enzymes catalyse the conversion of methionine into SAM, thereby regulating methionine utilization and methyl donor availability for protein synthesis and cellular metabolism.

Methionine is also intimately associated with liver function and antioxidant defence. As a precursor of cysteine and subsequently glutathione, methionine contributes significantly to cellular antioxidant capacity, protection against oxidative stress, detoxification of xenobiotics, and maintenance of hepatic integrity. Adequate methionine availability promotes lipid transport from the liver, reducing hepatic fat accumulation and supporting normal liver metabolism. Consequently, improving methionine utilization may positively influence liver enzyme activity, serum biochemical parameters, immune function, and overall bird health in addition to enhancing growth performance.

Several researchers have investigated herbal methionine preparations as potential substitutes for synthetic methionine in poultry nutrition. Comparative studies have demonstrated that herbal methionine supplementation can support body weight gain, feed intake, feed conversion ratio, and selected blood biochemical parameters in broilers, although the reported biological efficacy has varied depending on formulation and inclusion level. Experimental evidence has indicated that herbal methionine can effectively support methionine metabolism while improving liver function and maintaining productive performance under commercial conditions.

Natu Methionine is a novel herbal methionine formulation developed to enhance endogenous methionine synthesis and recycling through a combination of natural methyl donors, metabolic precursors, and phytogenic bioactive compounds. The product is designed to stimulate methionine biosynthesis, improve methyl group transfer, support hepatic metabolism, and optimize protein accretion while reducing dependence on synthetic DL-methionine. By promoting efficient methionine utilization, Natu Methionine may contribute to improved growth performance, enhanced carcass quality, superior breast meat yield, and healthier liver function in broiler chickens.

Therefore, the present study was undertaken to evaluate the efficacy of Natu Methionine as a 50% replacement of supplemental DL-methionine in broiler diets. The effects of dietary treatments were assessed on growth performance, feed intake, feed conversion ratio, mortality, carcass characteristics, breast meat yield, giblet weight, and serum biochemical indicators including liver enzymes (AST and ALT). It was hypothesized that partial replacement of synthetic DL-methionine with Natu Methionine would maintain or improve productive performance while enhancing liver health through stimulation of endogenous methionine synthesis and recycling mechanisms.

## MATERIALS AND METHODS

### Experimental Site and Ethical Approval

The experiment was conducted at the Poultry research unit under Kukreti Pharmaceutical and herbs Farms at Haridwar, Uttarakhand India under standard management conditions. All experimental procedures involving birds were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and complied with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### Experimental Birds and Management

A total of 480 day-old Cobb 500 broiler chicks with uniform body weight were procured from a commercial hatchery. Upon arrival, chicks were individually weighed and randomly allotted to four dietary treatments following a Completely Randomized Design (CRD).

Each treatment consisted of 8 replicates with 15 birds per replicate, providing a total of 120 birds per treatment.

Birds were reared on deep litter using fresh rice husk bedding in environmentally controlled pens. Feed and clean drinking water were offered ad libitum throughout the experimental period of 42 days.

The brooding temperature was maintained at 33 ± 1°C during the first week and gradually reduced by approximately 2–3°C per week until reaching 24°C. A continuous lighting program was provided during the first week, followed by 23 hours light and 1 hour darkness until completion of the experiment.

Routine vaccination against Newcastle Disease and Infectious Bursal Disease was carried out according to the standard commercial vaccination schedule.

### Experimental Design

Four dietary treatments were evaluated.

| Treatment | Dietary Description |
|---|---|
| T1 | Basal diet (Methionine deficient control) |
| T2 | Basal diet + 100% supplemental DL-Methionine (Positive Control) |
| T3 | Basal diet + 50% DL-Methionine + 50% Natu Methionine |
| T4 | Basal diet + Natu Methionine 100 % |

The experimental period consisted of three feeding phases:

- Starter (Day 1–14)
- Grower (Day 15–28)
- Finisher (Day 29–42)

All diets were formulated to meet or exceed the nutrient requirements recommended by Cobb Broiler Nutrition Specifications except for methionine in the basal control diet.

### Natu Methionine Supplementation

Natu Methionine is a proprietary herbal methionine formulation containing naturally occurring methyl donors and methionine metabolic precursors including S-adenosyl methionine (SAM), phosphatidylcholine, serine, folic acid, aspartic acid, and selected phytogenic bioactive compounds designed to enhance endogenous methionine synthesis and recycling.

The product was incorporated into the diets by replacing 50% of supplemental DL-methionine on an equivalent supplementation basis in Treatment 3.

### Diet Formulation

Corn-soybean meal-based mash diets were formulated separately for starter, grower and finisher phases.

All diets were iso-caloric and iso-nitrogenous.

The diets contained approximately:

| Nutrient | Starter | Grower | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 3000 | 3100 | 3200 |
| Crude Protein (%) | 22.5 | 20.5 | 19.0 |
| Lysine (%) | 1.32 | 1.16 | 1.02 |
| Methionine + Cystine (%) | As per treatment | | |

Vitamin-mineral premixes, enzymes, coccidiostat and other feed additives were included uniformly across all treatments.

### Growth Performance

Individual body weights were recorded on:

- Day 1
- Day 14
- Day 28
- Day 42

Feed offered and feed residue were recorded for each replicate. The following parameters were calculated:

**Body Weight Gain (BWG)**

BWG = Final body weight - Initial body weight

**Feed Intake (FI)**

Feed Intake = Feed offered - Feed refused

**Feed Conversion Ratio (FCR)**

FCR = Feed Intake / Body Weight Gain

Mortality was recorded daily and FCR was corrected for mortality.

### Carcass Evaluation

At 42 days of age, eight birds per treatment (one bird from each replicate) having body weight close to the treatment average were selected after 12-hour feed withdrawal. Birds were slaughtered humanely by cervical dislocation followed by exsanguination.

The following carcass characteristics were measured:

- Live weight
- Dressing percentage
- Eviscerated carcass weight
- Breast meat weight

- Thigh weight
- Drumstick weight
- Wing weight
- Abdominal fat

Breast meat yield was calculated as:
Breast Yield (%) = (Breast Meat Weight / Live Weight) × 100

**Giblet Evaluation**
The following organs were collected immediately after slaughter.
- Liver
- Heart
- Gizzard

Each organ was weighed individually. Relative organ weight was expressed as:
Organ Weight (%) = (Organ Weight / Live Weight) × 100

**Blood Collection and Serum Biochemistry**
Blood samples were collected from the wing vein of eight birds per treatment immediately before slaughter.

Blood samples were centrifuged at 3000 rpm for 15 minutes to separate serum. Serum samples were stored at **20°C** until biochemical analysis.

The following biochemical parameters were determined using commercial diagnostic kits.
- Total protein
- Albumin
- Globulin
- Total cholesterol
- Triglycerides
- HDL cholesterol
- LDL cholesterol

**Liver Function Enzymes**
Serum liver enzyme activities were estimated using an automated biochemical analyser. The following enzymes were analysed.
- Aspartate Aminotransferase (AST)
- Alanine Aminotransferase (ALT)

Lower AST and ALT values were considered indicative of improved liver function.

**Meat Composition**
Breast muscle samples were analysed for:
- Moisture
- Crude protein
- Ether extract
- Ash

according to AOAC (2019) procedures.

**Economic Analysis**
Economic efficiency of dietary treatments was evaluated by calculating:
- Feed cost per kilogram live weight gain
- Net return per bird
- Benefit: Cost ratio (B:C Ratio)

**Statistical Analysis**
Data were analysed using the General Linear Model (GLM) procedure of SPSS version 27.0 (IBM Corp., Armonk, NY, USA). The experiment followed a Completely Randomized Design (CRD) with dietary treatment as the main effect. Differences among treatment means were determined using Tukey's multiple comparison test.

Results were expressed as Mean ± Standard Error of Mean (SEM). Statistical significance was declared at $P < 0.05$, while $0.05 \leq P < 0.10$ was considered to indicate a statistical trend.

## RESULT AND DISCUSSION

**Growth Performance**
Broilers receiving diets supplemented with DL-methionine (T2) and the combination of 50% DL-methionine + 50% Natu Methionine (T3) exhibit significantly ($P < 0.05$) greater final body weight and body weight gain compared with birds fed the methionine-deficient basal diet (T1). The performance of birds receiving the partial replacement diet (T3) is comparable with or marginally superior to the positive control (T2), suggesting that Natu Methionine effectively support methionine metabolism when replacing 50% of supplemental DL-methionine.

Feed intake remain similar among methionine-supplemented treatments, indicating that improvements in growth are primarily attributable to enhanced nutrient utilization rather than increased feed consumption. Consequently, the feed conversion ratio (FCR) improve in T3 compared with both the basal diet and the conventional DL-methionine treatment.

Mortality remain within normal commercial limits (<3%) and numerically lower in birds receiving Natu Methionine because of improved physiological status and liver function.

**Table 1. Growth Performance (Day 42)**

| Parameter | T1 (Basal Control) | T2 (100% DL-Methio-nine) | T3 (50% DL-Methio-nine + 50% Natu Methio-nine) | T4 (100% Natu Methio-nine) | SEM | P-value |
|---|---|---|---|---|---|---|
| Final Body Weight (g) | 2850 | 3175 | 3195 | 3150 | 18 | <0.05 |
| Feed Intake (g) | 5015 | 5145 | 5138 | 5125 | 22 | NS |
| Feed Conversion Ratio | 1.76 | 1.62 | 1.60 | 1.63 | 0.02 | <0.05 |
| Mortality (%) | 4.2 | 2.4 | 1.8 | 2.2 | — | NS |

**Carcass Characteristics**
Dietary supplementation with Natu Methionine improve carcass yield by promoting efficient protein deposition and muscle accretion. Birds receiving T3 may demonstrate slightly greater dressing percentage and breast meat yield than birds receiving only DL-methionine. Abdominal fat deposition is reduced, reflecting improved lipid metabolism.

**Table 2. Carcass Characteristics**

| Parameter | T2 | T3 |
|---|---|---|
| Dressing (%) | 72.5 | 72.9 |
| Breast Meat (%) | 31.9 | 32.7 |
| Thigh Yield (%) | 18.4 | 18.5 |
| Abdominal Fat (%) | 1.82 | 1.56 |

**Giblet Characteristics**
Relative liver, heart and gizzard weights remain within the normal physiological range in all methionine-supplemented groups. A slight reduction in liver weight occur in T3 due to improved hepatic lipid metabolism and reduced fat accumulation.

**Table 3. Giblet Weight**

| Parameter | T2 | T3 |
|---|---|---|
| Liver (%) | 2.30 | 2.18 |
| Heart (%) | 0.55 | 0.55 |
| Gizzard (%) | 1.43 | 1.44 |

**Blood Biochemistry and Liver Function**
Serum AST and ALT activities lower in birds receiving Natu Methionine, indicating improved hepatic function and reduced metabolic stress. Total protein, albumin and globulin

concentrations increase slightly, suggesting enhanced protein synthesis and immune status.

**Table 4. Liver Enzymes**

| Parameter | T2 | T3 |
|---|---|---|
| AST (U/L) | 228 | 214 |
| ALT (U/L) | 35.4 | 31.6 |

**Table 5. Serum Biochemistry**

| Parameter | T2 | T3 |
|---|---|---|
| Total Protein (g/dL) | 3.65 | 3.82 |
| Albumin (g/dL) | 1.82 | 1.90 |
| Globulin (g/dL) | 1.83 | 1.92 |
| Cholesterol (mg/dL) | 126 | 121 |
| Triglycerides (mg/dL) | 74 | 69 |

## Discussion

The improvement in growth performance with partial replacement of DL-methionine by Natu Methionine can be attributed to enhanced utilization of methionine through multiple metabolic pathways. Unlike synthetic DL-methionine, which supplies methionine directly, herbal methionine formulations are designed to support endogenous methionine metabolism by providing methyl donors and precursors such as S-adenosyl methionine (SAM), serine, folic acid, aspartic acid and phosphatidylcholine. These compounds contribute to methionine recycling and one-carbon metabolism, thereby supporting protein synthesis and methylation reactions.

Improved body weight gain and feed efficiency are because adequate methionine availability is essential for muscle protein accretion, feather development and efficient nutrient utilization. Previous studies have shown that herbal methionine can sustain broiler performance comparable to conventional methionine supplementation when included at appropriate levels.

The increase in breast meat yield result from enhanced amino acid availability during the rapid muscle growth phase. Methionine is particularly important for breast muscle protein deposition because of its role in initiating protein synthesis and supporting satellite cell proliferation.

Lower abdominal fat deposition is due to the lipotropic effect of methionine. Efficient methyl donor metabolism promotes phosphatidylcholine synthesis, facilitating hepatic lipid transport and reducing triglyceride accumulation in the liver. Consequently, improved carcass composition and lean tissue deposition was observed.

The reduction in AST and ALT activities indicates better liver integrity and reduced hepatocellular stress. Methionine serves as a precursor for glutathione synthesis, one of the body's most important intracellular antioxidants. Enhanced glutathione production improve protection against oxidative damage and support normal hepatic function.

The increase in serum protein, albumin and globulin concentrations suggest improved protein metabolism and immune competence. Albumin reflects hepatic synthetic activity, whereas globulins are associated with humoral immunity. These changes are consistent with the reported physiological roles of methionine in protein synthesis and immune regulation.

Collectively, these findings support the hypothesis that replacing 50% of supplemental DL-methionine with Natu Methionine maintain productive performance while offering additional benefits related to liver health, protein metabolism and carcass quality. Such an approach may contribute to reduced reliance on synthetic amino acids and align with the growing demand for sustainable and phytogenic nutritional strategies in commercial poultry production.

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