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# Influence of Prebiotics, Probiotics, and Synbiotics on the Hematology and Serum - Biochemical Parameters of Broiler Chickens

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**Abstract**

This study investigated the effects of Prebiotics, Probiotics, and Synbiotics as alternatives to in-feed antimicrobials on the hematological and serum biochemical parameters of broiler

chickens. A total of 200 broilers were fed diets supplemented with these alternatives for 42 days. On days 14, 28, and 42, blood samples were collected for parameter analyses. R software, one-way analysis of variance followed by mean comparison using the Least Significant Difference post hoc test were used in data analyses. The results indicated these supplements significantly improved levels of aspartate aminotransferase, alkaline phosphatase, and blood urea nitrogen at different trial periods. The synbiotic-treated group exhibited the highest total protein concentrations and improved hematological profiles, including increased red blood cell count and hematocrit. Although red blood cell counts showed a slight increase across all groups, the differences were not statistically significant. However, hematocrit values were significantly higher in the synbiotic group on day 42 ($P = 0.037$). Conversely, alanine aminotransferase and creatinine levels did not differ significantly among the treatment groups. Interestingly, white blood cell counts were significantly elevated in the negative control group ($P < 0.05$), while hemoglobin levels showed a significant increase in the synbiotic group on day 28 ($P = 0.042$). Overall, the study findings suggest that these antimicrobial alternatives can positively influence physiological health markers in broiler chickens and may serve as viable alternatives to in-feed antimicrobials. Future studies are encouraged to explore the long-term effects of these alternatives.

**Keywords:** prebiotics, probiotics, synbiotics, broiler, hematology parameters, biochemical parameters

## 1. Introduction

In recent years, the poultry industry has seen a growing interest in antimicrobial alternatives, largely driven by the global ban on antimicrobial growth promoters (AGPs) and heightened concerns over antimicrobial resistance in both animals and humans (WHO, 2012). Consumer demand for poultry products free from antimicrobials has also increased across both developed and developing nations (Huang *et al.,* 2004; Panda *et al.,* 2006; Rashid *et al.,* 2022). As a result, poultry producers are shifting away from conventional in-feed antimicrobials towards alternative strategies that ensure food safety while maintaining animal health and welfare (Arif *et al.,* 2022).

Preliminary studies have identified a range of viable alternatives including plant extracts, phytobiotics, essential oils, enzymes, vitamins, antimicrobial peptides, organic acids, prebiotics, and probiotics that offer health benefits without the drawbacks of conventional antibiotics (Hussain *et al.,* 2021; Rani *et al.,* 2021; Rashid *et al.,* 2022; Yasmin *et al.,* 2020). These alternatives have shown promising effects in improving disease resistance, enhancing immune function, supporting intestinal health, and boosting performance in poultry (Alagawany *et al.,* 2021a). While each of these alternatives offers unique benefits, current research suggests that Prebiotics and Probiotics may have comparatively stronger and more consistent effects on poultry production (Abd El-Hack *et al.,* 2020; Hussein *et al.,* 2020a; Krysiak *et al.,* 2021; Popov *et al.,* 2021). Consequently, there is a growing interest in determining the most effective strains and combinations of these agents.

Prebiotics are defined as non-digestible feed ingredients that selectively stimulate the growth and activity of beneficial intestinal microbiota (Abd El-Hack *et al.,* 2017). Common examples

include pectin, inulin, mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), and xylo-oligosaccharides (Al-Sultan *et al.,* 2016; Bird *et al.,* 2010). In contrast, Probiotics are now formally defined by the Food and Agriculture Organization of the United Nations (FAO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO, 2007), are non-toxic, non-pathogenic microbes that positively influence the host's gut environment. Frequently used probiotic strains include *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Enterococcus faecium*, and *Bacillus subtilis* (Fuller, 1986). These Probiotics contribute to poultry health by preventing pathogen colonization, producing antimicrobial compounds, and modulating gut microbiota (Alam and Ferdaushi, 2019; Hashemipou *et al.,* 2019).

Limited studies have explored the effects of various Prebiotics and Probiotics on blood parameters, with findings highlighting their role in improving hematological and serum biochemical indices (Abd El-Hack *et al.,* 2022; Abudabos *et al.,* 2017; Saleh *et al.,* 2024). These parameters are critical indicators of the chickens' nutritional status and can help diagnose pathological conditions, particularly those affecting the liver and kidney (Prameela *et al.,* 2011; Seiser *et al.,* 2000). Hematological indices are often linked to feed quality and the effectiveness of dietary additives. However, existing studies have reported inconsistent results, which are thought to arise from variations in the strains and types of Prebiotics and Probiotics used, dosage, treatment duration, poultry breed, developmental stage, environmental factors, and interactions with other feed components (Du *et al.,* 2022; Elleithy *et al.,* 2023; Yosi and Metzler-Zebeli, 2023).

Among the Prebiotics and Probiotics studied, MOS and FOS have demonstrated synergistic potential when used alone or in combination with some Probiotics. MOS operates by binding to mannose-sensitive pathogens, thereby preventing their adherence to the gut lining and promoting their removal via the gastrointestinal tract (Benites *et al.,* 2008). Conversely, FOS is rapidly fermented by *Bifidobacteria* via β-fructokinase, promoting beneficial microbial growth (Jeurissen *et al.,* 2002). Likewise, *L. acidophilus* and *B. subtilis* have shown notable benefits to poultry health and performance, although studies examining their synergistic effects remain limited (Davis and Anderson, 2002; Park *et al.,* 2016). *L. acidophilus* produces lactic acid and other metabolites that create an acidic environment hostile to acid-sensitive pathogens, while *B. subtilis*, known for its robustness in the gastrointestinal tract, enhances gut microbiota balance (Hargis *et al.,* 2021; Hernandez-Patlan *et al.,* 2020). Together, these agents may help maintain gut homeostasis and prevent dysbiosis.

To date, there is limited research that has explored the combined use of these specific Prebiotics and Probiotics. We hypothesize that their synergistic use may produce additive or even multiplicative benefits in poultry. Therefore, the aim of this study was to evaluate the influence of MOS and FOS combinations, along with *L. acidophilus* and *B. subtilis*, on the Hematological and Serum biochemical profiles of Ross 308 broiler chickens.

## 2. Methods

### *2.1 Feed Composition*

The experimental diets were formulated according to the nutritional guidelines provided by Rostagno et al. (2005). Tables 1 and 2 present the ingredient composition and proximate analysis of the diets. The latter was conducted at the Central Veterinary Laboratory Agency (CVL) in Tanzania using Near Infrared Reflectance Spectroscopy (NIRS). All feed ingredients were sourced from the local market in Morogoro municipality. Their physical quality such as dryness and a fresh, pleasant odor were assessed prior to use. After thorough mixing of the ingredients, the dietary supplements under study were added based on the manufacturers' recommended dosages. The feed was then pelleted and stored in a dry environment.

Table 1. Composition of the formulated diet

| **Components (kg)** | **Starter (0 - 21 d)** | **Finisher (22- 42d)** |
|---|---|---|
| Corn | 45.4 | 52.5 |
| Corn bran | 12.5 | 11.0 |
| Soy bean meal | 37.0 | 32.0 |
| Salt | 0.5 | 0.5 |
| Dicalcium phosphate | 2.0 | 1.6 |
| Lysine | 0.3 | 0.2 |
| Methionine | 0.3 | 0.2 |
| Premix[1] | 2.0 | 2.0 |
| **Total** | **100** | **100** |

***Premix[1]*** *Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D3, 4000 I.U; Vitamin E, 12.50 mg; Vitamin K3, 3.60 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 5..050 mg; Vitamin B12, 18.0 mg; Niacin, 62.0 mg; D-Biotin, 198.0 mg; Calcium D-pantothenate, 20.43mg; Folic acid, 2.083 mg; manganese, 110.0 mg; iron, 70.0 mg; zinc, 75.0 mg; copper, 9.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 160.0 mg.*

Table 2. Proximate analysis of the diet

| Component | Starter (0-12d) | Finisher (22-2d) |
|---|---|---|
| Metabolizable energy (kcal/kgDM) | 2919 | 3172 |
| Crude Protein (%) | 25.4 | 20.8 |
| Methionine /Cystine (%) | 1.08 | 0.88 |
| Lysine (%) | 1.55 | 0.97 |
| Tryptophan (%) | 0.3 | 0.24 |
| Ash (%) | 7.8 | 7.3 |
| Crude fat (%) | 3.2 | 6.1 |
| Crude fiber (%) | 6.6 | 3.7 |
| Starch (%) | 37.8 | 42.8 |
| Total sugar (%) | 4.1 | 4.0 |
| Dry matter (%) | 89.8 | 89.3 |

### *2.2 Experimental Design*

A total of 200 one-day-old Ross 308 broiler chicks were sourced from Silverlands Hatcheries in Iringa, Tanzania. Upon arrival, the chicks were examined, weighed, and randomly assigned into five treatment groups following the completely randomized design outlined by Ali *et al.* (2023), with each group comprising 40 chicks. Each group was further subdivided into four replicates of 10 chicks each. Within each replicate, four birds were randomly selected and marked using wing tags for identification. Group one (negative control) received a basal diet alone. Group two (positive control) was fed a basal diet supplemented with Olaquindox 10% (antimicrobial growth promoters (AGPs) (Tan Veterina Co. Ltd. Dar es Salaam, Tanzania) at a dosage of 200 g/ton of feed. Group three received the basal diet supplemented with Prebiotics: Mannan-oligosaccharide (MOS) 99% and Fructo-oligosaccharide (FOS) 95% from Henan Showvet Industrial Co. Ltd (Henan, China) at 4 kg/ton and 1 kg/ton of feed, respectively. Group four was fed the basal diet with Probiotics: freeze-dried *Lactobacillus acidophilus* ($1\times10^{10}$CFU/g) and spore-forming *Bacillus subtilis* ($\geq1\times10^{11}$ CFU/g) from the same supplier in China, at 220 g/ton and 50 g/ton of feed, respectively. Group five received a combination of both Prebiotics and Probiotics at the same dosages.

### *2.3 Rearing Conditions*

The study was carried out at the Department of Animal, Aquaculture, and Range Sciences, specifically in the lower farm poultry units of Sokoine University of Agriculture, located in Morogoro, Tanzania (6.8520° S, 37.6576° E). Chicks were reared for 42 days under standard housing conditions, with ad libitum access to chlorine-free boiled water and feed. Lighting was provided using six-watt bulbs, and brooding temperatures were maintained with 250-watt infrared lamps starting at 34°C and gradually reduced to 24°C by day 28. Birds were

vaccinated against Newcastle disease virus (NDV) using Biovac VIR 116 vaccine (LaSota strain, BIOVAC Ltd, Israel) on days 7 and 21, and against Infectious Bursal Disease Virus (IBDV) with Gumboro 1-intermediate strain vaccine (Hester Biosciences Ltd, India) on days 14 and 28.

### *2.4 Sample Collection*

Blood samples were randomly collected from tagged chickens in each replicate across all treatment groups on days 14, 28, and 42. Birds were manually restrained, and feathers over the wing vein were gently plucked to expose the vessel. Approximately three milliliters of blood were drawn using a 23-gauge needle and syringe. The blood was immediately transferred into two types of vacutainer tubes: one containing ethylenediaminetetraacetic acid (EDTA) for hematological analysis, and a plain tube for serum biochemical analysis. All laboratory analyses were conducted at the Physiology and Biochemistry Laboratory, Sokoine University of Agriculture.

### *2.5 Hematological Indices*

Whole blood samples collected in EDTA vacutainer tubes were analyzed for hematological parameters using a semi-automatic hematology analyzer (MS4s, Melet Schloesing Laboratories, France). The parameters assessed included red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (Hb), hematocrit (HCT) percentage, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). The analyzer operated with specialized reagent kits provided by Melet Schloesing Laboratories, including EO-DIFF (eosinophil-specific lysing reagent), a reagent remaining alarm system, Isoflux (diluent), and Transflux (detergent). All procedures were carried out in accordance with the manufacturer's guidelines and accompanying reagent manuals.

### *2.6 Biochemical Indices*

Serum samples were analyzed using spectrophotometry with a Genesys 10 UV Scanning UV/Visible Spectrophotometer (Thermo Scientific™, USA). Biochemical parameters assessed included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), creatinine (CREA), and blood urea nitrogen (BUN). These analyses were conducted using specific diagnostic kits from ERBA Mannheim Biotechnology (Mannheim, Germany), following the manufacturer's protocol and instructions provided in the analytical kit manuals.

### *2.7 Analytical Statistics*

Data was first cleaned using Microsoft Excel 2010, and subsequently analyzed with R software version 4.4.1. A one-way analysis of variance (ANOVA) was employed to assess significant differences in the means of hematological and serum-biochemical parameters among the treatment groups. Means and standard errors were calculated, and where significant effects were detected at the 0.05 significance level ($P < 0.05$), post hoc comparisons were performed using the Least Significant Difference (LSD) method to validate the findings and enhance data interpretation.

## 3. Results

### *3.1 Hematological Indices*

Table 3 summarizes the hematological parameters of broiler chickens administered diets supplemented with antibiotic growth promoters, prebiotics, Probiotics, and Synbiotics over 14, 28, and 42 days.

On day 14**,** RBC counts did not differ significantly among treatment groups compared to both negative and positive controls (P = 0.342). However, numerically higher RBC counts were observed in the synbiotic-fed group (2.93±0.02 M/mm³), while the lowest was recorded in the prebiotic group (2.52±0.02 M/mm³). WBC counts, on the other hand, decreased significantly from 50.12±0.06 M/mm³ in the negative control to 29.98±0.03 M/mm³ in the synbiotic group (P = 0.030). Hb levels remained stable across all groups with no significant differences (P = 0.240), and HCT percentages also showed no significant variation (P = 0.140). MCV increased to 120.13±0.03 fL in the synbiotic group, approaching statistical significance (P = 0.051), while mean MCH values remained consistent across all treatments (P = 0.263).

By day 28**,** RBC counts showed a slight increase across all groups, with the AGP group recording the highest value (3.24±0.03 M/mm³), although differences remained non-significant (P = 0.324). WBC counts increased significantly again, reaching 55.13±0.05 M/mm³ in the negative control group (P = 0.024). Hb levels showed a significant rise, increasing from 11.30±0.03 g/dL in the negative control to 13.57±0.02 g/dL in the Probiotics group (P = 0.042). Although HCT percentages showed no significant difference (P = 0.231), the synbiotic group recorded the highest value (35.09±0.04%). MCV remained steady with no significant variation (P = 0.265), while MCH levels increased significantly to 35.49±0.04 pg in the synbiotic group (P = 0.014).

On day 42, RBC counts continued a slight upward trend with no significant differences among groups (P = 0.327), although the synbiotic group recorded the highest value (3.52±0.03 M/mm³). WBC counts dropped significantly once again, from 52.10±0.05 M/mm³ in the negative control to 38.21±0.04 M/mm³ in the synbiotic group (P = 0.013). Hb levels remained relatively stable with no significant differences across treatments (P = 0.536). However, HCT showed a significant increase, rising from 32.20±0.05% in the negative control to 38.13±0.04% in the synbiotic group (P = 0.037). Both MCV and MCH remained statistically unchanged (P = 0.439 and P = 0.537, respectively), though numerically higher values were observed in the synbiotic-fed group.

Table 3. Blood hematological indices of broiler chicken fed different supplements

| Day | Parameter | Control | AGP | Preb. | Prob. | Synbio. | P-value |
|---|---|---|---|---|---|---|---|
| **14** | RBC (M/mm³) | 2.68±0.03$^{a}$ | 2.89±0.03$^{a}$ | 2.52±0.02$^{a}$ | 2.67±0.03$^{a}$ | 2.93±0.02$^{a}$ | 0.342 |
| | WBC (m/mm³) | 50.12±0.06$^{a}$ | 41.20±0.05$^{ab}$ | 44.32±0.03$^{bc}$ | 35.17±0.03$^{bc}$ | 29.98±0.03$^{c}$ | 0.03 |
| | Hb (g/dL) | 10.17±0.05$^{a}$ | 12.94±0.03$^{a}$ | 11.0±0.03$^{a}$ | 10.52±0.02$^{a}$ | 11.51±0.03$^{a}$ | 0.24 |
| | HCT (%) | 30.24±0.05$^{a}$ | 34.81±0.04$^{a}$ | 32.67±0.04$^{a}$ | 31.10±0.04$^{a}$ | 33.90±0.04$^{a}$ | 0.14 |
| | MCV (fL) | 100.23±0.06$^{a}$ | 107.10±0.05$^{a}$ | 110.92±0.06$^{a}$ | 115.0±0.03$^{a}$ | 120.13±0.03$^{b}$ | 0.051 |
| | MCH (pg) | 30.60±0.05$^{a}$ | 33.42±0.04$^{a}$ | 32.17±0.05$^{a}$ | 31.68±0.03$^{a}$ | 34.32±0.04$^{a}$ | 0.263 |
| **28** | RBC (M/mm³) | 2.76±0.03$^{a}$ | 3.24±0.03$^{a}$ | 2.81±0.02$^{a}$ | 3.19±0.02$^{a}$ | 2.97±0.03$^{a}$ | 0.324 |
| | WBC (m/mm³) | 55.13±0.05$^{a}$ | 46.0±0.06$^{ab}$ | 40.27±0.04$^{bc}$ | 49.97±0.03$^{bc}$ | 35.09±0.04$^{c}$ | 0.024 |
| | Hb (g/dL) | 11.30±0.03$^{a}$ | 12.58±0.03$^{ab}$ | 12.10±0.03$^{ab}$ | 13.57±0.02$^{b}$ | 13.11±0.02$^{ab}$ | 0.042 |
| | HCT (%) | 31.60±0.05$^{a}$ | 33.14±0.03$^{a}$ | 32.93±0.03$^{a}$ | 34.11±0.03$^{a}$ | 35.09±0.04$^{a}$ | 0.231 |
| | MCV (fL) | 106.10±0.07$^{a}$ | 110.78±0.05$^{a}$ | 118.21±0.04$^{a}$ | 115.11±0.02$^{a}$ | 120.09±0.02$^{a}$ | 0.265 |
| | MCH (pg) | 31.84±0.06$^{a}$ | 34.62±0.04$^{a}$ | 32.27±0.04$^{a}$ | 33.81±0.04$^{a}$ | 35.49±0.04$^{b}$ | 0.014 |
| **42** | RBC (M/mm³) | 2.81±0.03$^{a}$ | 2.36±0.03$^{a}$ | 3.02±0.02$^{a}$ | 3.28±0.03$^{a}$ | 3.52±0.03$^{a}$ | 0.327 |
| | WBC (m/mm³) | 52.10±0.05$^{a}$ | 48.72±0.03$^{ab}$ | 41.0±0.04$^{bc}$ | 44.90±0.04$^{bc}$ | 38.21±0.04$^{c}$ | 0.013 |
| | Hb (g/dL) | 12.07±0.03$^{a}$ | 12.25±0.03$^{a}$ | 13.09±0.04$^{a}$ | 13.15±0.03$^{a}$ | 14.70±0.04$^{a}$ | 0.536 |
| | HCT (%) | 32.20±0.05$^{a}$ | 34.61±0.03$^{ab}$ | 35.17±0.04$^{ab}$ | 36.03±0.04$^{ab}$ | 38.13±0.04$^{b}$ | 0.037 |
| | MCV (fL) | 109.98±0.05$^{a}$ | 112.11±0.04$^{a}$ | 117.32±0.03$^{a}$ | 118.0±0.04$^{a}$ | 119.92±0.03$^{a}$ | 0.439 |
| | MCH (pg) | 32.12±0.05$^{a}$ | 35.20±0.04$^{a}$ | 34.10±0.04$^{a}$ | 33.98±0.03$^{a}$ | 36.13±0.05$^{a}$ | 0.537 |

Different superscripts in the same row indicate statistically significant differences ($P < 0.05$) between treatments in the same trial period.

*3.2 Serum-biochemical Indices*

Table 4 summarizes the serum biochemical parameters of broiler chickens fed diets supplemented with antibiotic growth promoters, prebiotics (Preb.), Probiotics (Prob.), and Synbiotics (Synb.).

On day 14, AST levels were significantly lower in the Probiotic (29.43±0.08 U/L) and Synbiotic (25.31±0.13 U/L) groups compared to both the negative (54.26±1.31 U/L) and positive control (44.99±0.14 U/L) groups ($P = 0.027$). The AGP-fed group showed numerically

higher AST levels throughout the trial compared to other supplemented groups. ALT levels decreased in all supplemented groups, though the reduction was not statistically significant (P = 0.051). The lowest ALT was observed in the basal diet group (11.11±0.36 U/L), followed by Synbiotics (11.98±0.06 U/L). ALP levels significantly decreased in the Probiotic (409.30±0.34 U/L) and Synbiotic (369.92±0.2 U/L) groups compared to the negative (651.38±0.42 U/L) and AGP (651.84 U/L) controls (P = 0.046). CREA levels showed no significant differences among treatments (P = 0.103). BUN levels were significantly reduced in all supplemented groups and AGPs compared to the negative control (P = 0.035), with the lowest values in Synbiotics (9.53±0.05 mg/dL) and Prebiotics (9.93±0.04 mg/dL). TP levels were significantly increased in all AGP alternatives (P = 0.037), highest in Probiotics (2.72±0.04 g/dL) and lowest in Synbiotics (2.68±0.04 g/dL), while the AGP group showed no statistical improvement over controls.

By day 28, the Synbiotic group maintained significantly reduced AST levels (53.63±0.08 U/L) compared to the negative control (67.91±0.33 U/L, P = 0.035). AGP-fed birds continued to show higher AST. ALT levels remained numerically lower in supplemented groups, especially Synbiotics (11.66±0.05 U/L), though not statistically significant (P = 0.206). ALP levels dropped significantly in the Synbiotic group (271.33±0.13 U/L vs. 515.88±0.82 U/L in control, P = 0.004). CREA remained non-significant (P = 0.153) with lower values in Prebiotics (0.20±0.004 mg/dL) and Synbiotics (0.21±0.004 mg/dL). BUN levels were significantly lower in all alternatives, particularly Synbiotics (8.71±0.03 mg/dL) and Probiotics (9.03±0.05 mg/dL), compared to controls (P = 0.001). TP levels were significantly elevated in the Synbiotic group (4.88±0.04 g/dL) compared to both controls (3.76±0.06 and 3.86±0.05 g/dL, P = 0.032).

On day 42, AST levels were significantly reduced in all AGP alternatives versus the negative control (P = 0.022), with Synbiotics showing the lowest value (52.99±0.07 U/L). The AGP group maintained elevated AST levels. ALT differences were not statistically significant (P = 0.255), but Synbiotics (12.94±0.05 U/L) and Probiotics (13.17±0.06 U/L) showed numerically lower levels. ALP levels declined significantly in Synbiotics (304.66±0.09 U/L) versus the negative control (502.29±0.81 U/L, P = 0.041). AGPs had significantly higher ALP levels compared to Synbiotics. BUN was significantly reduced in Probiotics (10.26±0.05 mg/dL) and Synbiotics (10.10±0.05 mg/dL) compared to controls (P = 0.008). CREA levels were statistically non-significant (P = 0.309), although Synbiotics showed the lowest numerical value (0.61±0.01 mg/dL). Finally, TP levels were significantly increased in Synbiotic-fed chickens (4.36±0.03 g/dL), outperforming both control groups (P = 0.006).

Table 4. Serum-biochemical indices of Broiler chickens fed different supplements

| Parameter | | Control | AGP | Preb. | Prob. | Synb. | P-value |
|---|---|---|---|---|---|---|---|
| 14 | AST (U/L) | 54.26±1.31$^{a}$ | 44.99±0.14$^{b}$ | 40.78±0.04$^{b}$ | 29.43±0.08$^{c}$ | 25.31±0.13$^{c}$ | 0.027 |
| | ALT (U/L) | 11.11±0.36$^{a}$ | 16.19±0.07$^{a}$ | 14.05±0.04$^{a}$ | 12.78±0.05$^{a}$ | 11.98±0.06$^{a}$ | 0.051 |
| | ALP (U/L) | 651.38±0.42$^{a}$ | 651.84±1.04$^{a}$ | 530.92±0.07$^{b}$ | 409.30±0.34$^{c}$ | 369.92±0.2$^{c}$ | 0.046 |
| | TP (g/dL) | 1.79±0.05$^{a}$ | 2.24±0.04$^{b}$ | 2.69±0.02$^{b}$ | 2.72±0.04$^{b}$ | 2.68±0.04$^{b}$ | 0.037 |
| | CREA (mg/dL) | 0.59±0.03$^{a}$ | 0.51±0.01$^{a}$ | 0.37±0.004$^{a}$ | 0.62±0.01$^{a}$ | 0.44±0.004$^{a}$ | 0.103 |
| | BUN (mg/dL) | 10.7±0.13$^{a}$ | 10.88±0.07$^{b}$ | 9.93±0.04$^{b}$ | 10.49±0.06$^{b}$ | 9.53±0.05$^{b}$ | 0.035 |
| 28 | AST (U/L) | 67.91±0.33$^{a}$ | 61.72±0.16$^{b}$ | 55.14±0.06$^{b}$ | 64.74±0.17$^{b}$ | 53.63±0.08$^{c}$ | 0.035 |
| | ALT (U/L) | 12.94±0.11$^{a}$ | 13.33±0.07$^{a}$ | 12.54±0.04$^{a}$ | 11.90±0.07$^{a}$ | 11.66±0.05$^{a}$ | 0.206 |
| | ALP (U/L) | 515.88±0.82$^{a}$ | 514.33±0.57$^{a}$ | 489±0.23$^{b}$ | 326.61±0.23$^{c}$ | 271.33±0.13$^{c}$ | 0.004 |
| | TP (g/dL) | 3.76±0.06$^{a}$ | 3.86±0.05$^{a}$ | 3.96±0.03$^{a}$ | 4.08±0.04$^{a}$ | 4.88±0.04$^{b}$ | 0.032 |
| | CREA (mg/dL) | 0.32±0.01$^{a}$ | 0.25±0.01$^{a}$ | 0.20±0.004$^{a}$ | 0.24±0.004$^{a}$ | 0.21±0.004$^{a}$ | 0.153 |
| | BUN (mg/dL) | 10.16±0.06$^{a}$ | 10.68±0.07$^{b}$ | 10.13±0.03$^{b}$ | 9.03±0.05$^{c}$ | 8.71±0.03$^{c}$ | 0.001 |
| 42 | AST (U/L) | 148.93±0.34$^{a}$ | 101.07±0.24$^{b}$ | 67.67±0.05$^{c}$ | 61.31±0.15$^{c}$ | 52.99±0.07$^{c}$ | 0.022 |
| | ALT (U/L) | 13.81±0.06$^{a}$ | 13.54±0.07$^{a}$ | 13.40±0.04$^{a}$ | 13.17±0.06$^{a}$ | 12.94±0.05$^{a}$ | 0.255 |
| | ALP (U/L) | 502.29±0.81$^{a}$ | 461.59±0.52$^{b}$ | 437.63±0.05$^{b}$ | 430.91±0.27$^{b}$ | 304.66±0.09$^{c}$ | 0.041 |
| | TP (g/dL) | 3.42±0.06$^{a}$ | 3.66±0.03$^{a}$ | 3.98±0.05$^{a}$ | 4.23±0.05$^{a}$ | 4.36±0.03$^{b}$ | 0.006 |
| | CREA (mg/dL) | 0.87±0.01$^{a}$ | 0.63±0.003$^{a}$ | 0.63±0.01$^{a}$ | 0.62±0.01$^{a}$ | 0.61±0.01$^{a}$ | 0.309 |
| | BUN (mg/dL) | 10.69±0.08$^{a}$ | 10.68±0.03$^{a}$ | 10.57±0.06$^{a}$ | 10.26±0.05$^{b}$ | 10.10±0.05$^{b}$ | 0.008 |

Different superscripts in the same row indicate statistically significant differences ($P < 0.05$) between treatments in the same trial period.

## 4. Discussion

Hematological and serum biochemical indices serve as important indicators of the nutritional and clinical health status of chickens (Orawan and Aengwanich, 2007). Overall, supplementation with MOS, FOS, *L. acidophilus*, and *B. subtilis*, either individually or in combination, resulted in notable improvements in AST, ALP, and BUN levels. Conversely, birds receiving synbiotic supplements exhibited the highest levels of TP and better hematological profiles, such as elevated RBC count, HCT, and Hb, which suggest enhanced

erythropoiesis and improved oxygen transport capacity.

The hematological analysis findings underscored the health benefits of these AGP alternatives. All supplemented groups exhibited WBC counts above the normal range of 1–9.5 m/mm³ (Barde *et al.,* 2022). However, our results differ from those of Saleh *et al.* (2024) and Sunu *et al.* (2021), who reported normal WBC levels in synbiotic-fed broilers. Correspondingly, a study by Saleh *et al.* (2024) found no statistical difference in the level of WBC when the synbiotic (Poultrystar®) which contains *Enterococcus faecium, Lactobacillus reuteri, Bifidobacterium animalis*, and *Pediococcus acidilactici* and fructo-oligosaccharides (prebiotic) fed groups were compared to the control. The numerical low levels in supplemented groups suggest the potential of these supplements in their protection role against invading enteric pathogens. Higher WBC counts in the control group suggest an increased stress response, potentially linked to exposure to pathogens.

Although no statistically significant differences in RBC counts were observed across the study period, the synbiotic-fed group demonstrated a numerical increase, with AGP-fed birds showing similar trends. All RBC values remained within the normal range (2.0 to 3.2 M/mm$^3$) for broilers aged 5–6 weeks (Barde *et al.,* 2022). This increase is likely due to improved nutrient absorption and digestion facilitated by a balanced gut microbiota in the supplemented groups. HCT and Hb values also remained within their respective normal ranges of 22–35% and 7.0–13.0 g/dL respectively (Ajay *et al.,* 2015; Sugiharto *et al.,* 2018). Our findings concur with those of Sunu *et al.* (2021) but contrast with Saleh *et al.* (2024), who found no significant differences. The numerically higher HCT and Hb in the synbiotic group further suggest enhanced erythropoiesis and oxygen delivery. Additionally, MCV and MCH were within the normal ranges of 95 to 187 fL and 25 to 59 pg respectively, indicating normal blood viscosity and oxygen-carrying capacity (Saripinar Aksu *et al.,* 2010).

The study also revealed a significant reduction in liver enzymes, including ALP and AST, throughout the trial period. ALT levels decreased significantly from week four onwards. Since elevated liver enzyme levels are commonly associated with hepatic injury, the observed reductions are linked to the hepato-protective effect of the supplements. These results are consistent with previous studies by Abd El-Hack *et al.* (2022) and Abramowicz *et al.* (2019), who reported improved liver enzyme profiles following synbiotic or probiotic supplementation. Similarly, Żbikowski *et al.* (2020) observed reduced ALP levels by day 7 and significantly lower AST by day 42 in synbiotic-fed groups. Further corroborating this, Abudabos *et al.* (2016) highlighted improved organ function in supplemented birds when low AST levels were observed. However, Abdel-Fattah and Farah (2009) found no significant influence of such supplements on liver enzyme activity, suggesting that outcomes may vary depending on several factors.

Synbiotic supplementation also significantly increased total protein levels at different phases of the study, particularly compared to the control groups. These findings align with those of Ismail *et al.* (2011), and Sardar *et al.* (2024), who documented similar enhancements in TP levels with prebiotic-probiotic combinations. In contrast, Żbikowski *et al.* (2020) reported no change in TP levels when Ross 308 broilers were fed Synbiotics containing multiple

*Lactobacillus* strains, *S. cerevisiae*, and inulin. Elevated TP levels generally reflect improved digestion, absorption, and feed conversion efficiency, which are often linked to a balanced gut microbiota. Enhanced protein metabolism may also contribute to better muscle development and meat quality, critical attributes in poultry production (Acharya *et al.,* 2024).

With respect to creatinine, no statistically significant differences were observed across treatment groups throughout the trial period. Similar findings were reported by El Sayed *et al.* (2024) using various probiotic preparations. However, during the first two weeks, CREA levels were slightly above the normal range in the control, AGP, and probiotic groups. By day 28, values normalized in all supplemented groups, suggesting a potential improvement in kidney health. Conversely, CREA levels on day 42 exceeded the normal range (0.10–0.40 mg/dL), indicating possible renal function concerns. This observation underscores the need for further investigation into the long-term effects of these supplements on renal health. Our findings are contrary to a study by Latipudin *et al.* (2024) when dried Probiotics were supplemented to broiler chickens. Although statistically non-significant, BUN levels were numerically lower in the supplemented groups compared to controls aligning with findings by Latipudin *et al.* (2024). Despite exceeding the normal range, the relative reduction in BUN in supplemented birds suggests improved renal function, potentially attributed to the AGPs alternative supplements.

The inconsistencies observed when comparing our results with previous studies may stem from variations in supplement strains, methodological setting, chicken breed and feed formulations. This has been supported by previous studies as those of Yang *et al.* (2007). Other studies observed that, differences in dosage, method of administration (e.g., feed vs. water), and environmental conditions may also contribute to contrasting outcomes (Mountzouris et al., 2010; Du et al., 2022; Elleithy et al., 2023). Moreover, genetic differences, age, and developmental stages of the broilers may influence results, as reported by Bogusławska-Tryk *et al.* (2021). Therefore, these disparities hinder the generalization of the current findings and necessities the regulatory authorities in the country (Tanzania) to undertake a rigorous and double-check the efficacy and other properties of these or similar additives before their approval in the poultry industry application.

## 5. Conclusion

The study found that incorporating Prebiotics, Probiotics, and Synbiotics into broiler diets may offer a viable strategy to enhance bird health and productivity while promoting sustainable farming practices. The observed improvements in hematological and serum biochemical indices point to enhanced liver, kidney, and immune functions. Reductions in AST, ALP, and BUN levels, along with elevated TP and RBC parameters, underscore the potential of Synbiotics to promote growth, improve meat quality, and mitigate organ stress. Thus, the study confirms the influence of Prebiotics, Probiotics, and Synbiotics as promising alternatives to in-feed antimicrobial on the Hematology and Serum-biochemical indices of Ross 308 broiler chickens. However, the study didn't consider the long-term impacts of different combinations, dosages, and delivery methods across various chicken breeds and farming systems, and the cost-effectiveness of these alternatives on a commercial scale.

Therefore, future studies are encouraged to address these limitations and furthermore explore the effects of these additives on hepatic and renal health, mainly focusing on ALT and CREA levels respectively, using similar methodological settings.

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**Authors contributions**

Dr. Tumwesige M. Katto and Prof. Gaymary G. Bakari were responsible for the study design, data collection, and initial drafting of the manuscript. Prof. Amandus P. Muhairwa and Prof. Zhang Ziping contributed to the critical revision of the manuscript. All authors read and approved the final version prior to its submission for publication. We affirm that all authors contributed equally to the successful completion of this study.

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**Competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Informed consent**

Not applicable

**Ethics approval**

This study was carried out in accordance with the ethical guidelines of the Sokoine University of Agriculture (SUA) Research Ethics Committee, and the research permit was granted by the Directorate of Research, Technology Transfer and Consultancy (DPRTC) of SUA under approval reference number DPRTC/R/186/32.

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## Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Data sharing statement

No additional data are available.

## Open access



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