

**Supplementation of broiler chicken diets with *Talinum triangulare* leaf meal, *Saccharomyces cerevisiae* or combination of both: Comparison of the effects on their haematology and serum lipid profile**

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**Abstract**

This study evaluated the comparative effects of dietary supplementation with *Talinum triangulare* leaf meal (TTLM), *Saccharomyces cerevisiae* (SC) or their combination on haematology and serum lipid profile of broiler chickens. A total of 120 day-old broiler chicks were randomly assigned to four treatment groups in a completely randomized design with three replicates per treatment. Group A received basal diet only (control), while Groups B, C and D received the basal diet supplemented with 60 g/kg TTLM, 0.8 g/kg SC or a combination of 60 g TTLM + 0.8 g SC per kg of feed, respectively. The feeding trial lasted six weeks. Blood samples were collected at weeks 3, 4 and 5 for haematology and serum lipid profile assay. Data were analyzed using one-way analysis of variance and Duncan's multiple range test, and significance was accepted at  $p < 0.05$ . The mean packed cell volume and haemoglobin concentration of all the supplemented groups (Groups B, C and D) were significantly higher than that of the unsupplemented control (Group A) at week 5. Red blood cell counts were significantly lower ( $p < 0.05$ ) in Group C birds at week 4. Total white blood cell counts were significantly lower ( $p < 0.05$ ) in Group D birds at weeks 3 and 4. Heterophil counts and heterophil: lymphocyte ratio were significantly lower ( $p < 0.05$ ) in Groups A, B and C at week 3, but lymphocyte counts were significantly lower ( $p < 0.05$ ) in Group D at week 3. Mean serum total cholesterol level was significantly lower ( $p < 0.05$ ) in group D birds at week 5, while mean serum HDL level was significantly lower ( $p < 0.05$ ) in Group C birds at week 3. Serum triglyceride and VLDL levels were significantly lower in Groups B, C and D birds at week 5, while mean serum LDL level was significantly higher in Group C birds at week 3. Taken together, the findings suggest that individual supplementation with TTLM or SC as used in Groups B and C were beneficial to the birds but the combination of the two (as used in Group D) did not produce superior results.

**Keywords:** Supplementation; Broiler chicken diets; *Talinum triangulare* leaf meal; *Saccharomyces cerevisiae*; Combination; Haematology and Serum lipid profile.

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**Article History:** Initial manuscript submission received – March 18, 2026; Final revised form received – May 23, 2026; Accepted for publication – May 28, 2026; Published – June 01, 2026.

## Introduction

The poultry industry has remained one of the fastest-growing livestock sectors globally, contributing significantly to food security and animal protein supply. Productivity in broiler production depends largely on feed efficiency, disease resistance and metabolic stability. For decades, antibiotic growth promoters were widely used to enhance growth performance and reduce disease burden; however, increasing concerns regarding antimicrobial resistance, drug residues and consumer health have resulted in global restrictions on their use (Castanon, 2017). This has stimulated research into alternative feed additives capable of improving productivity without compromising food safety. Substances classified as natural growth promoters (NGP), such as phytobiotics, prebiotics, probiotics, organic acids, enzymes and silicates are being investigated for use as potential substitutes to antibiotics and hormones in broiler production. Phytobiotics and probiotics are prominent among others and are considered as environmentally safe feed additives in the poultry industry (Patterson and Burkholder, 2003, Grashorn, 2010).

Plant-derived compounds have received wide attention because they have been reported to possess antimicrobial, antioxidant and immune-modulatory properties, and also due to their ability to improve gut micro-biota balance, enhance nutrient utilization and support immune responses in poultry (Grashorn 2010; Prabakar *et al.* 2016; Gheisar and Kim, 2017). *Talinum triangulare* is a nutrient-dense tropical vegetable rich in flavonoids, essential fatty acids and bioactive compounds which may contribute to improved metabolic and physiological functions in animals (Ezekwe *et al.*, 2013; Swarna and Ravindhran, 2013). Probiotics such as *Saccharomyces cerevisiae* improve gut microbial balance, enhance digestive enzyme activity and stimulate immune competence (Elghandour *et al.*, 2020; Attia *et al.*, 2023;

Ting *et al.*, 2026) Reports of earlier studies have shown that probiotic supplementation can improve growth performance, blood profile and lipid metabolism in broilers (Krysiak *et al.*, 2021; Khan *et al.*, 2024).

Although individual effects of phytobiotics and probiotics have been widely studied, limited information exists regarding their combined effects. Interactions between plant metabolites and microbial supplements may produce either synergistic or antagonistic physiological responses. The present study evaluated the effects of *Talinum triangulare* leaf meal and *Saccharomyces cerevisiae* individually, and their combination on the haematological and serum biochemical parameters of broiler chickens.

## Materials and Methods

The study was conducted at the Poultry Research Unit of the Department of Animal Health and Production, University of Nigeria, Nsukka.

The *Talinum triangulare* leaf meal (TTLM) was prepared from fresh waterleaf plants that were harvested in Nsukka, Nigeria, identified and authenticated. The leaves were washed, shade-dried for four weeks and milled into powder. The plant material was stored at 4°C until use. The level of TTLM supplementation adopted for the study was 60 g per kilogramme of the basal feed, based on earlier reports of the effectiveness and safety of this level of supplementation (Akubueze and Aronu, 2023; Aronu *et al.*, 2023).

The *Saccharomyces cerevisiae* (SC) that was used for the study was sourced commercially from B. F. P. Dock Road, Felixstowe, United Kingdom. The yeast was cultured on Sabouraud dextrose agar for 48 hours to confirm viability, absence of contamination and to determine the numbers of colony forming units. 0.8 g of SC per kg of broiler basal feed was the supplementation level

adopted for the study, based on earlier reports of the effectiveness and safety of this level of supplementation (Ezema *et al.*, 2012; Ahiwe *et al.*, 2021; Akubueze and Aronu, 2023).

One hundred and twenty day-old Agriated™ broiler chicks were obtained from a commercial hatchery. Housing facilities were cleaned, disinfected and fumigated prior to chick placement. They were acclimatized for one week before the experiment commenced. Routine vaccinations and prophylactic treatments were administered according to standard poultry health schedules. Feed and water were administered *ad libitum*. All procedures on the birds complied with the University of Nigeria institutional guidelines for the care and use of experimental animals.

The birds were randomly assigned into four treatment groups (Groups A, B, C and D), consisting of 30 birds each with three replicates of ten birds per replicate. Group A birds were the Control group, fed the basal diet. Group B birds were fed the basal diet plus 60 g TTLM/kg. Group C birds were fed the basal diet plus 0.8 g SC/kg, while Group D birds were fed the basal diet plus a combination of 60 g TTLM/kg and 0.8 g SC/kg. The feeding experiment lasted for six weeks.

Blood samples were collected from the jugular vein of three birds per replicate at weeks 3, 4 and 5 for haematology and serum lipid profile assay. Samples for haematology were collected into EDTA bottles while serum samples were collected into plain glass test tubes. The blood for serum biochemistry was allowed to stand at room temperature to clot and was afterwards centrifuged at 3,000 revolutions per minute using a table centrifuge, and the supernatant was aspirated and used for the serum lipid profile assay.

For the haematology, the packed cell volume was determined by the microhaematocrit method (Thrall *et al.*, 2002). Haemoglobin (Hb.) concentration was determined by the

cyanomethaemoglobin method (Higgins *et al.*, 2008). Red blood cell (RBC) and total white blood cell (WBC) counts were done using a haemocytometer (Campbell, 1994). Differential leukocyte counts were done on thin blood smears stained by the Leishman technique (Schalm *et al.*, 1975).

In the serum lipid profile assay, the total cholesterol and triglycerides were determined by the enzymatic colorimetric methods (Rifai *et al.*, 2008). High density lipid was determined using precipitation technique (Naito, 1984). The LDL was calculated using Friedewald equation while VLDL was estimated from triglyceride values (Friedewald *et al.* 1972).

Data obtained were subjected to one way analysis of variance, using SPSS version 23. Differences among means were separated using Duncan's multiple range test. Significance was accepted at  $p < 0.05$  (Daniel, 1995). Results were expressed as mean  $\pm$  SD.

## Results

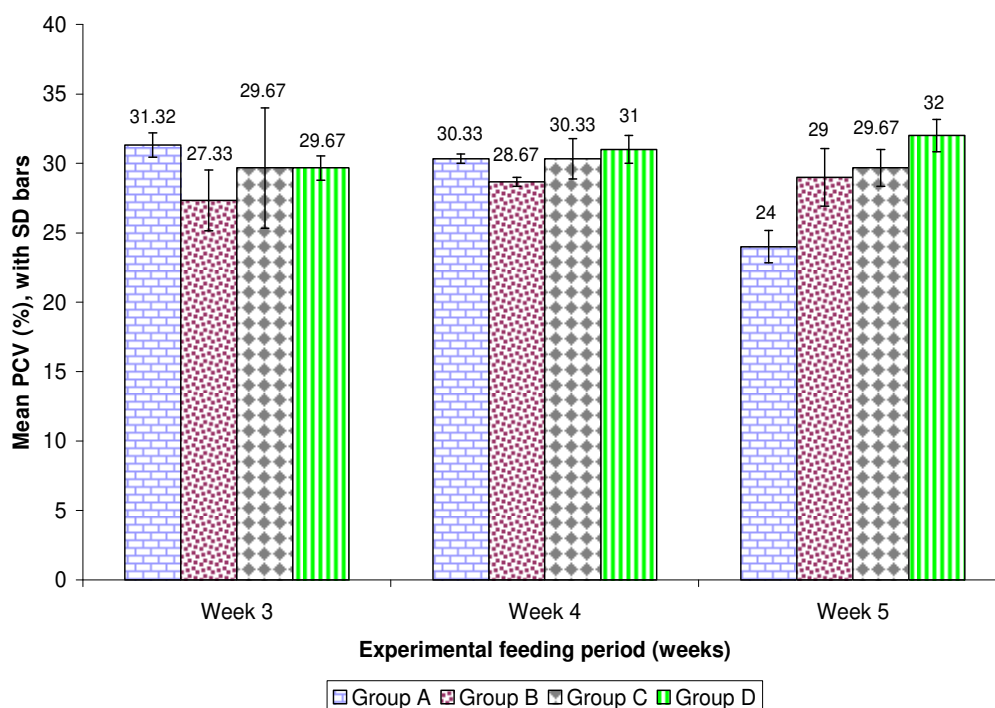
There were no significant differences ( $p > 0.05$ ) between the groups in their mean PCV and Hb. concentrations on weeks 3 and 4, but on week 5, the mean PCV and Hb. concentration of birds in Groups B, C and D that were fed supplemented diets was significantly ( $p < 0.05$ ) higher than that of unsupplemented control Group A (Figures 1 and 2). The RBC counts of the Group B birds was significantly higher ( $p < 0.05$ ) than that of Group C at week 4, but there were no significant ( $p > 0.05$ ) differences between the RBC counts of the groups on week 3 and 5 (Figure 3).

The total WBC counts of Groups A and B birds were significantly ( $p < 0.05$ ) higher than those of Groups C and D on weeks 3 and 4, but there were no significant ( $p > 0.05$ ) differences between the groups in their mean total WBC counts on week 5 (Table 1). The heterophil counts and the heterophil: lymphocyte (H/L)

ratio of the Group D birds was significantly higher than those of all other groups at week 3, but the lymphocyte counts of birds in Group A, B and C were significantly ( $p < 0.05$ ) higher than that of birds in Group A at week 3 (Tables 2, 3 and 4). There were no significant ( $p > 0.05$ ) differences between the groups in their heterophil and lymphocyte counts and the H/L ratio at weeks 4 and 5 (Tables 2, 3, and 4).

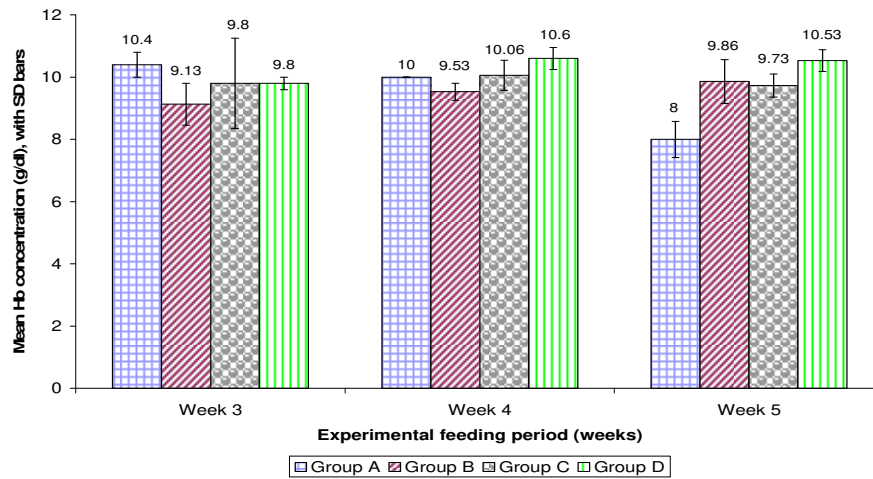
There were no significant differences in the mean serum total cholesterol levels at weeks 3 and 4, but at week 5, the serum total cholesterol levels of the Group D birds was significantly ( $p < 0.05$ ) lower than those of other groups (Table 5). The serum HDL levels of Groups A and B birds were significantly higher than that of Group C birds at week 3,

but on weeks 4 and 5, there were no significant differences between all the groups in their mean serum HDL levels (Table 6). There were no significant differences ( $p > 0.05$ ) between the groups in their mean serum levels of triglycerides and VLDL at weeks 3 and 4, but at week 5, the serum levels of triglycerides and VLDL of the Group A birds was significantly higher ( $p < 0.05$ ) than those of Groups B, C and D (Table 7 and 8). The mean serum LDL level of Group C birds was significantly ( $p < 0.05$ ) higher than those of Groups A, B and D at week 3, but at weeks 4 and 5, there were no significant ( $p > 0.05$ ) differences between the groups in their serum LDL levels (Table 9).



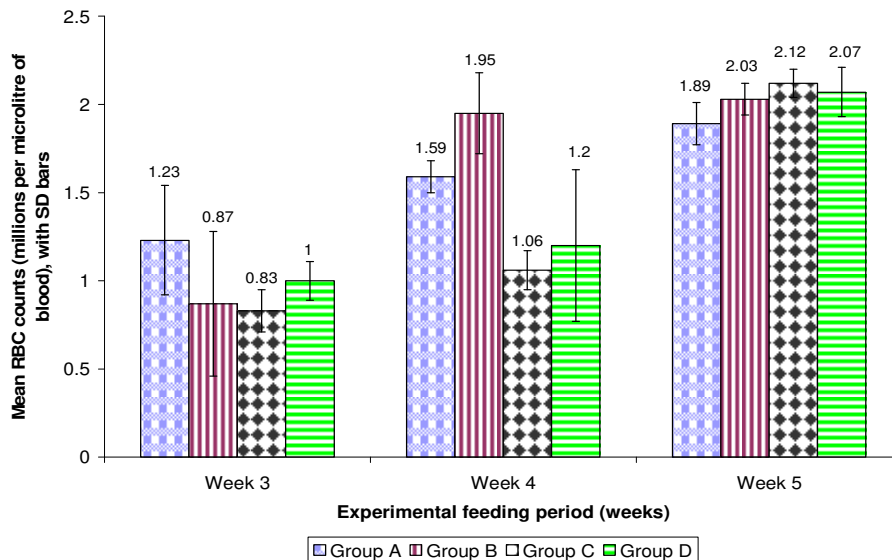
**Figure 1.** Packed cell volume (PCV) of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.



**Figure 2.** Haemoglobin (Hb) concentration of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.



**Figure 3.** Red blood cell (RBC) counts of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 1.** Total white blood cell (TWBC) counts of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean TWBC counts ( $10^3/\mu\text{l}$ ) $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	22.67 $\pm$ 3.76 <sup>a</sup>	21.32 $\pm$ 1.54 <sup>a</sup>	28.13 $\pm$ 3.91 <sup>a</sup>
Group B	23.48 $\pm$ 0.88 <sup>a</sup>	21.00 $\pm$ 3.46 <sup>a</sup>	35.40 $\pm$ 1.30 <sup>a</sup>
Group C	16.00 $\pm$ 2.65 <sup>bc</sup>	17.00 $\pm$ 0.58 <sup>ab</sup>	32.00 $\pm$ 1.53 <sup>a</sup>
Group D	12.33 $\pm$ 1.45 <sup>c</sup>	11.67 $\pm$ 1.86 <sup>b</sup>	34.00 $\pm$ 2.59 <sup>a</sup>

<sup>a, b, c</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups. Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 2.** Heterophil counts of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean heterophil counts (%) $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	4.67 $\pm$ 3.28 <sup>a</sup>	34.33 $\pm$ 4.41 <sup>a</sup>	33.33 $\pm$ 3.18 <sup>a</sup>
Group B	6.00 $\pm$ 3.06 <sup>a</sup>	31.33 $\pm$ 8.37 <sup>a</sup>	29.67 $\pm$ 10.87 <sup>a</sup>
Group C	13.00 $\pm$ 6.11 <sup>a</sup>	35.33 $\pm$ 5.21 <sup>a</sup>	25.67 $\pm$ 4.91 <sup>a</sup>
Group D	34.67 $\pm$ 5.46 <sup>b</sup>	32.00 $\pm$ 3.21 <sup>a</sup>	29.00 $\pm$ 0.58 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups. Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 3.** Lymphocyte counts of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean lymphocyte counts (%) $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	94.36 $\pm$ 2.84 <sup>a</sup>	65.37 $\pm$ 4.67 <sup>a</sup>	66.00 $\pm$ 3.79 <sup>a</sup>
Group B	94.00 $\pm$ 2.98 <sup>a</sup>	68.67 $\pm$ 8.36 <sup>a</sup>	70.00 $\pm$ 11.02 <sup>a</sup>
Group C	87.00 $\pm$ 5.92 <sup>a</sup>	64.33 $\pm$ 4.91 <sup>a</sup>	73.33 $\pm$ 5.78 <sup>a</sup>
Group D	65.33 $\pm$ 5.45 <sup>b</sup>	68.00 $\pm$ 3.24 <sup>a</sup>	78.00 $\pm$ 1.15 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups. Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 4.** Heterophil: Lymphocyte (H/L) ratio of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean H/L ratio $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	0.05 $\pm$ 0.04 <sup>a</sup>	0.54 $\pm$ 0.10 <sup>a</sup>	0.51 $\pm$ 0.08 <sup>a</sup>
Group B	0.07 $\pm$ 0.04 <sup>a</sup>	0.50 $\pm$ 0.19 <sup>a</sup>	0.49 $\pm$ 0.20 <sup>a</sup>
Group C	0.16 $\pm$ 0.09 <sup>a</sup>	0.57 $\pm$ 0.12 <sup>a</sup>	0.36 $\pm$ 0.10 <sup>a</sup>
Group D	0.55 $\pm$ 0.12 <sup>b</sup>	0.48 $\pm$ 0.07 <sup>a</sup>	0.37 $\pm$ 0.09 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 5.** Serum total cholesterol levels of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean serum total cholesterol (mg/dl) $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	111.60 $\pm$ 9.80 <sup>a</sup>	146.90 $\pm$ 22.85 <sup>a</sup>	115.10 $\pm$ 22.50 <sup>ab</sup>
Group B	108.50 $\pm$ 5.14 <sup>a</sup>	104.00 $\pm$ 15.61 <sup>a</sup>	136.00 $\pm$ 19.59 <sup>ab</sup>
Group C	114.90 $\pm$ 5.60 <sup>a</sup>	109.90 $\pm$ 9.18 <sup>a</sup>	177.36 $\pm$ 29.98 <sup>b</sup>
Group D	104.20 $\pm$ 6.23 <sup>a</sup>	135.90 $\pm$ 27.46 <sup>a</sup>	85.21 $\pm$ 4.66 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 6.** Serum high density lipoprotein (HDL) levels of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean serum HDL (mg/dl) $\pm$ standard deviation		
	Week 3	Week 4	Week 5
Group A	50.50 $\pm$ 9.28 <sup>a</sup>	41.90 $\pm$ 15.04 <sup>a</sup>	53.10 $\pm$ 11.64 <sup>a</sup>
Group B	53.60 $\pm$ 6.27 <sup>a</sup>	53.50 $\pm$ 6.88 <sup>a</sup>	72.10 $\pm$ 16.93 <sup>a</sup>
Group C	12.90 $\pm$ 2.39 <sup>b</sup>	40.00 $\pm$ 11.14 <sup>a</sup>	93.90 $\pm$ 3.41 <sup>a</sup>
Group D	39.50 $\pm$ 12.46 <sup>ab</sup>	60.10 $\pm$ 13.11 <sup>a</sup>	51.80 $\pm$ 18.15 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 7.** Serum triglyceride (TAG) levels of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean serum TAG (mg/dl) ± standard deviation		
	Week 3	Week 4	Week 5
Group A	54.06 ± 3.18 <sup>a</sup>	95.86 ± 22.10 <sup>a</sup>	125.90 ± 14.02 <sup>a</sup>
Group B	66.98 ± 15.25 <sup>a</sup>	69.70 ± 11.78 <sup>a</sup>	74.10 ± 28.52 <sup>b</sup>
Group C	52.47 ± 7.16 <sup>a</sup>	64.60 ± 11.36 <sup>a</sup>	73.77 ± 13.58 <sup>b</sup>
Group D	46.94 ± 18.05 <sup>a</sup>	51.50 ± 15.48 <sup>a</sup>	46.51 ± 13.27 <sup>b</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 8.** Serum very low density lipoprotein (VLDL) levels of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean serum VLDL (mg/dl) ± standard deviation		
	Week 3	Week 4	Week 5
Group A	10.82 ± 0.64 <sup>a</sup>	19.17 ± 4.42 <sup>a</sup>	25.18 ± 2.80 <sup>a</sup>
Group B	13.40 ± 3.05 <sup>a</sup>	13.94 ± 2.36 <sup>a</sup>	11.49 ± 4.68 <sup>b</sup>
Group C	10.49 ± 1.43 <sup>a</sup>	12.91 ± 2.27 <sup>a</sup>	14.75 ± 2.72 <sup>b</sup>
Group D	9.39 ± 3.61 <sup>a</sup>	10.30 ± 3.10 <sup>a</sup>	9.31 ± 2.65 <sup>b</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

**Table 9.** Serum low density lipoprotein (LDL) levels of broiler chickens fed diets supplements with 60 g *Talinum triangulare* leaf meal per kg of feed, 0.8 g *Saccharomyces cerevisiae* per kg of feed, or a combination of the two, compared with a Control that was fed unsupplemented feed.

Groups	Mean serum LDL (mg/dl) ± standard deviation		
	Week 3	Week 4	Week 5
Group A	50.31 ± 15.53 <sup>a</sup>	83.80 ± 36.67 <sup>a</sup>	33.23 ± 17.68 <sup>a</sup>
Group B	41.44 ± 9.44 <sup>a</sup>	36.48 ± 20.72 <sup>a</sup>	49.05 ± 18.46 <sup>a</sup>
Group C	91.51 ± 6.44 <sup>b</sup>	56.95 ± 13.59 <sup>a</sup>	68.62 ± 25.48 <sup>a</sup>
Group D	55.31 ± 8.04 <sup>a</sup>	65.48 ± 15.27 <sup>a</sup>	32.11 ± 10.49 <sup>a</sup>

<sup>a, b</sup> Different superscripts in a column indicate significant difference ( $p < 0.05$ ) between the groups.

Group A – Unsupplemented control; Group B – Supplemented with 60 g TTLM/kg of feed; Group C – Supplemented with 0.8 g *S. cerevisiae* per kg of feed; Group D - Supplemented with a combination of 60 g TTLM + 0.8 g *S. cerevisiae* per kg of feed.

## Discussion

Haematological indices generally are important indicators of physiological status, nutritional adequacy, immune competence and stress response in animals and poultry in particular. Variations in these parameters will often reflect the ability of dietary interventions to support metabolic functions and health status of individuals.

The significant improvement recorded in packed cell volume and haemoglobin concentration in the supplemented groups at week 5 suggests enhanced erythropoietic activity. This improvement may be attributed to the rich nutrient composition of *Talinum triangulare*, particularly its content of iron, essential amino acids and antioxidant phytochemicals which may support haematopoiesis. Improved erythropoiesis is known to enhance oxygen transport capacity and tissue metabolism which may translate to improved growth and productivity. Similar improvements in haematological parameters following phytogenic supplementation or supplementation with *Saccharomyces cerevisiae* have been reported by Ezema *et al.* (2012), Adeyemi *et al.* (2021), Dhama *et al.* (2022) and Ogunmoyole *et al.* (2021), who attributed such effects to improved nutrient bioavailability and antioxidant protection of erythrocytes.

The significantly lower RBC counts recorded for Group C supplemented with *S. cerevisiae* at week 4 may indicate that supplementation with SC primarily improved erythrocyte quality rather than absolute numbers as the PCV and Hb. concentrations were not significantly lower. Improved PCV and haemoglobin concentration without concurrent changes in RBC numbers may suggest improved haemoglobin synthesis efficiency rather than erythrocyte proliferation in actual sense.

Higher total WBC counts are often associated with stress or infection, hence moderate reduction in WBC as recorded in Groups C and

D at week 3 and 4 may indicate reduced physiological stress and improved gut health. Probiotics such as *Saccharomyces cerevisiae* have been shown to improve immune homeostasis by enhancing beneficial gut microbiota and reducing inflammatory responses (Sugiharto, 2018; Al-Mansur *et al.*, 2011; Ashayerizadeh *et al.*, 2011).

The significantly higher heterophil counts and lower lymphocyte counts recorded for Group D that was given the combined TTLM and SC supplements may indicate transient physiological stress or immune modulation. The heterophil to lymphocyte ratio is widely recognized as an indicator of stress response in poultry. Therefore, elevated heterophils may reflect activation of innate immune mechanisms while reduced lymphocytes may indicate redistribution of immune resources during physiological adaptation. This response may suggest that the combined supplementation initially imposed mild metabolic adjustment before physiological stabilization occurred.

The significantly lower serum total cholesterol levels recorded for the Group D birds at week 5 suggests that the combined supplementation led to improved lipid profile of the group. The lower serum triglyceride and VLDL levels of all the supplemented groups suggests improved lipid metabolism possibly through reduced hepatic lipogenesis and improved lipid utilization. Phytobiotics are known to modulate lipid metabolism through inhibition of key enzymes such as HMG-CoA reductase involved in cholesterol synthesis (Yang *et al.*, 2019). Similarly, probiotics have been reported to influence lipid metabolism through bile salt hydrolase activity, leading to increased cholesterol excretion and reduced circulating lipid concentrations (Cho and Kim, 2015; Wu *et al.*, 2022). The lower serum triglyceride and VLDL levels recorded in the supplemented groups may therefore indicate improved lipid transport efficiency and reduced risk of excessive fat deposition. The

higher serum HDL levels in the Group C birds supplemented with *S. cerevisiae* suggest enhanced reverse cholesterol transport mechanisms: higher serum HDL level is generally considered beneficial as it facilitates removal of excess cholesterol from tissues. The higher serum LDL level recorded for the Group C birds at week 3 may reflect an adaptive metabolic response to dietary supplementation before homeostatic regulation was achieved. Such transient responses have been reported in probiotic supplementation studies and may reflect metabolic adjustment rather than adverse effects.

The absence of clear synergistic advantage in the combined supplementation group is particularly noteworthy. While phytobiotics and probiotics often show complementary effects, their combined use may sometimes produce neutral or antagonistic interactions depending on dosage, bioavailability and microbial compatibility. It is possible that certain phytochemicals present in *Talinum triangulare* may have influenced yeast metabolic activity or gut colonization efficiency, thereby reducing expected synergistic effects. Similar neutral interactions have been reported where combined feed additives did not out-perform individual supplementation (Elghandour *et al.*, 2020). Another possible explanation is competition for nutrient substrates between probiotic organisms and host metabolic processes. Additionally, phytochemical antimicrobial properties may have partially influenced probiotic activity. Further microbiome and metabolomic studies would be required to clarify these interaction mechanisms.

Overall, the results of this study demonstrate that both *Talinum triangulare* and *Saccharomyces cerevisiae*, individually improved physiological stability of broiler chickens through enhancement of the erythrocytic and leukocytic profile and lipid metabolism. These individual effects support

their potential as natural growth promoters capable of supporting sustainable poultry production.

**Conclusion:** The findings of this study demonstrate that individual dietary supplementation with *Talinum triangulare* leaf meal or *Saccharomyces cerevisiae* positively influenced key haematological indices and lipid metabolism of broiler chickens without inducing adverse physiological effects. Overall, the results indicate that *Talinum triangulare* leaf meal or *Saccharomyces cerevisiae* can be safely incorporated into broiler diets as natural growth promoters capable of improving physiological stability and metabolic health. Their use may contribute to reducing dependence on antibiotic growth promoters in poultry production systems. However, combined supplementation did not demonstrate any superior benefits over single supplementation. Further studies are recommended.

#### Conflict of Interest

The authors declare no conflict of interest.

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