

Effects of the addition of probiotic (*Saccharomyces cerevisiae*) and the forage *Gliricidia sepium* on blood chemistry in chickens

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Abstract

This study assessed the combined effect of partial feed replacement with the forage *Gliricidia sepium* (GS) as a source of protein and supplementation with the yeast *Saccharomyces cerevisiae* (SC) on blood chemistry parameters in broilers. Six treatments were used: T1 (control), T2 (control; no SC + 10% GS), T3 (control; no SC + 15% GS), and T4 (SC + 0% GS), T5 (SC + 10% GS), and T6 (SC + 15% GS). T5 exhibited an increase in CK ($p = 0.0099$) as compared to T1 (5567 ± 733.2) IU/L. The use of SC and GS at 10% in broiler diet increased creatine kinase levels, which may be related to mild muscle or liver disturbances. Replacing part of the feed with GS increased protein availability, which correlated with increased availability of apoproteins for lipoprotein production.

1. INTRODUCTION

Today's poultry farming faces great challenges in terms of nutrition as agricultural production and consumption are expected to rise significantly in the near future. This will increase worldwide demand for meat, dairy products, eggs, fruit, and vegetables. For a long time, antibiotics have been used to promote animal growth, with the deleterious side effect of promoting bacterial resistance. Alternatively, probiotics are considered one of the most effective methods of microbial control and are not as harmful to the environment as antibiotics.

Probiotics have many advantages and few disadvantages; the prospect of using probiotics in poultry production is clearly positive. Pertaining to the family of probiotics, the yeast *Saccharomyces cerevisiae* (SC) is considered one of the most promising alternatives for supplanting the use of antibiotic growth promoters (APCs) in poultry production, since the former can have a positive effect on the intestinal microbial ecology and immune system of animals (Rodriguez, et al., 2017).

On the other hand, *Gliricidia sepium* (GS) – a perennial arboreal legume endemic in the tropics and used mainly in ruminant feed due to its high content of crude protein (22%) – seems to constitute promising feed material in poultry production as well. However, the separate and combined effects of feed replacement with GS and supplementation with SC on the blood biochemistry of monogastric birds such as chickens is not yet known. Therefore, this study aimed to assess the effects of feed replacement with the forage GS and supplementation with the probiotic SC on plasma concentrations of triglycerides, cholesterol (total, HDL, and LDL), glucose, insulin, ALT, AST, CK, and PPT in broilers.

2. MATERIALS AND METHODS

This study was carried out with the approval of the Institutional Ethics and Biosafety Committee on the Use of Animals, no. CO1-F01-013-2017.

2.1 Location

This study was conducted in the municipality of Villavicencio, Department of Meta, Colombia, latitude 4.15, longitude - 73.633, at an altitude of 420 meters above sea level, with average temperature of 28°C, annual rainfall of 4050 mm, and average relative humidity of 85%.

2.2 Experimental design

Tissue sampling was carried out with SC-treated broilers tended by the project “The effects of using tree forages: *Hibiscus rosa-sinensis*, *Trichanthera gigantea*, and *Gliricidia sepium* plus the probiotic *Saccharomyces cerevisiae* on productive parameters and digestibility in broilers”. This study included broilers treated to the probiotic SC and the forage GS and control animals. Fifty-nine animals were selected from a batch of chickens (200) admitted to Granja Barcelona in the municipality of Villavicencio.

The animals were divided into six treatments: T1 (control; commercial concentrate feed, no SC, no GS; n = 5); T2 (control; no SC + 10% GS; n = 5); T3 (control; no SC + 15% GS; n = 5); T4 (SC + 0% GS; n = 15); T5 (SC + 10% GS; n = 15); T6 (SC + 15% GS; n = 14). Soybean cake protein was replaced with GS flour at the aforementioned amounts and the probiotic SC was added at a concentration of 10 cfu/g to the experimental diet containing 21.5% total protein, with forage digestibility at 65%. Blood samples were collected in vacutainer tubes, taken to the physiology laboratory at Universidad de Los Llanos for centrifugation and sera separation, and later stored at -20°C until being processed.

The levels of triglycerides, cholesterol (total, HDL, and LDL), glucose, ALT, AST, CK, and PPT insulin were determined by spectrophotometry in blood serum on an A15 BioSystems Clinical Chemistry equipment (BioSystems; accessed in November 2021). Insulin levels were determined by ELISA tests using a 96-well Monobind Insulin AccuBind ELISA kit on a Multiskan ex Thermo Labsystems ELISA reader, following the manufacturer's instructions.

2.3 Statistical Analysis

Excel databases were created and a parametric statistical analysis was conducted. D'Agostino and Pearson omnibus and normality test was performed. Subsequently, Bartlett's test was used to assess the equivalence of variances and ANOVA was performed. All statistical procedures were performed with GraphPad Instat, version 5.01 (1992–2007), $p < 0.05$. In addition, R-Studio was used to perform Pearson correlations between the blood chemistry data obtained for the animals treated to SC and GS and those for their controls.

3. RESULTS

The ANOVA test revealed statistical differences in blood chemistry between T5 (SC + 10% GS) and T1 (control; commercial concentrate feed, no SC, no GS), as shown in Table 1. T5 exhibited a statistically significant value for creatine kinase (CK) ($p = 0.0099$) as compared to T1 (no SC, no GS) (Fig. 1). No statistical differences were observed in cholesterol (total, HDL, and LDL), triglycerides, glucose, insulin, total serum protein, albumin, globulin, ALT, and AST in T4, T5, and T6, i.e., broilers treated to SC and GS at 0%, 10%, and 15%, respectively, as compared to their controls ($p > 0.05$).

Table 1

Effect of the addition of *Saccharomyces cerevisiae* and *Gliricidia sepium* flour at different proportions (0%, 10% and 15%) on total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, total protein, albumin, globulin, ALT, AST, and CK in the serum of Group 1 broilers (n = 59). ^{ab} within each line. Means whose index envelope does not have any letter in common are statistically different. Note: Data are expressed as means $\pm$ standard error. P < 0.05 unpaired t-test.

PARAMETERS	GROUPS					
	T1	T2	T3	T4	T5	T6
Total Cholesterol (mg/dL)	140.3 $\pm$ 8.13	117 $\pm$ 19.01	236 $\pm$ 95.84	177.3 $\pm$ 51.48	135.9 $\pm$ 2.96	133.2 $\pm$ 7.22
HDL Cholesterol (mg/dL)	15.95 $\pm$ 1.79	13.33 $\pm$ 1.36	32.68 $\pm$ 16.70	15.32 $\pm$ 0.93	15.14 $\pm$ 0.97	14.30 $\pm$ 0.70
LDL Cholesterol (mg/dL)	108.0 $\pm$ 5.81	92.30 $\pm$ 19.65	194.0 $\pm$ 83.76	140.3 $\pm$ 47.33	106.4 $\pm$ 3.24	104.1 $\pm$ 6.85
Triglycerides (mg/dL)	81.75 $\pm$ 5.13	83.67 $\pm$ 9.20	147.4 $\pm$ 56.72	94.21 $\pm$ 23.76	72.33 $\pm$ 1.44	74.00 $\pm$ 5.54
Glucose (mg/dL)	218.0 $\pm$ 7.94	200.7 $\pm$ 23.18	232.8 $\pm$ 10.27	200.3 $\pm$ 4.61	210.9 $\pm$ 4.55	222.5 $\pm$ 9.782
Insulin (ng/mL)	0.56 $\pm$ 0.14	0.42 $\pm$ 0.05	0.45 $\pm$ 0.039	0.49 $\pm$ 0.042	0.43 $\pm$ 0.021	1.31 $\pm$ 0.87
Total Protein (g/dL)	6.000 $\pm$ 2.540	3.750 $\pm$ 1.245	3.760 $\pm$ 0.8858	4.693 $\pm$ 1.145	3.107 $\pm$ 0.07956	3.221 $\pm$ 0.1651
Albumin (g/dL)	3.420 $\pm$ 1.351	1.650 $\pm$ 0.1555	2.70 $\pm$ 0.7991	2.693 $\pm$ 0.6804	1.973 $\pm$ 0.05893	2.050 $\pm$ 0.07468
Globulin (g/dL)	2.580 $\pm$ 1.190	2.100 $\pm$ 1.175	1.020 $\pm$ 0.1685	1.953 $\pm$ 0.5028	1.140 $\pm$ 0.04342	1.171 $\pm$ 0.09746
ALT (UI/L) ²	13.66 $\pm$ 0.9775	16.81 $\pm$ 5.549	17.20 $\pm$ 4.417	16.51 $\pm$ 2.564	19.66 $\pm$ 3.515	13.38 $\pm$ 1.910
AST (UI/L) ²	222.2 $\pm$ 15.73	252.5 $\pm$ 36.49	325.6 $\pm$ 89.06	360.8 $\pm$ 23.37	350.2 $\pm$ 25.65	298.2 $\pm$ 48.38
CK (UI/L)	1790 $\pm$ 272.8 ^a	3009 $\pm$ 770.1	2187 $\pm$ 541.2	3009 $\pm$ 544.2	5567 ^b $\pm$ 733.2	3586 $\pm$ 699.4

3.1 Correlations

Table 2 shows the correlations for T1 (control; no SC, no GS) with significant differences ($p < 0.05$) in blood parameters (total, HDL, VLDL, and LDL cholesterol, triglycerides, glucose, insulin, ALT, AST, CK, total proteins, albumin, and globulin). A significant positive correlation ($r = 0.99$) was found between glucose, cholesterol (total, VLDL, and LDL), and triglycerides whereas significant negative correlations ($r = -0.98$) were found for glucose with proteins and globulin. Cholesterols showed a strong significant correlation

with total and VLDL cholesterol ($r = 1$). That is, an increase in triglycerides ($r = 1$) caused VLDL and, consequently, total cholesterol levels to rise. As expected, significant positive correlations ($r = 1$) were found between protein, albumin, and globulin.

Table 2
Correlations of blood chemistry parameters with control group (without probiotic; without *Gliricidia sepium*) with significant statistical difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Glucose	Total Cholesterol	0.99	0.009**
Glucose	Cholesterol VLDL	0.99	0.011*
Glucose	Cholesterol LDL	0.99	0.011*
Glucose	Triglycerides	0.99	0.011*
Glucose	Protein	-0.98	0.038*
Glucose	Globulin	-0.98	0.029*
Total Cholesterol	Cholesterol VLDL	1	< 0.001***
Total Cholesterol	Cholesterol LDL	1	< 0.001***
Total Cholesterol	Triglycerides	1	< 0.001***
Cholesterol VLDL	Cholesterol LDL	0.99	0.005**
Cholesterol VLDL	Triglycerides	1	< 0.001***
Cholesterol LDL	Triglycerides	0.99	0.005**
Protein	Albumin	1	< 0.001***
Protein	Globulin	1	< 0.001***
Albumin	Globulin	1	< 0.001**

Significant differences ($p < 0.05$) were obtained by correlating blood parameter data (total cholesterol, HDL cholesterol, VLDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, ALT, AST, CK, total proteins, albumin, and globulin) from T2 broilers (control; no SC + 10% GS) to those from T1 broilers (control; commercial concentrate feed, no SC, no GS), as shown in Table 3. The presence of 10% GS in the control group T2 is associated to a significant rise in triglyceride mobilization, which in turn increased VLDL cholesterol, and to the highly significant total positive correlation ($r = 1$).

Table 3
Correlations among blood chemistry parameters in T2
(without probiotic; *Gliricidia sepium* 10%) with
statistically significant difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Cholesterol VLDL	Triglycerides	1	< 0.001***

In T3 (control; no SC + 15% GS), a significant correlation was found between its levels of cholesterol (total, HDL, VLDL, and LDL), triglycerides, glucose, total proteins, albumin, and globulin (Table 4) when correlated with each other. GS at 15% led to a rise in cholesterol, protein, and triglyceride levels, with highly positive correlations between VLDL ($r = 1$), HDL ($r = 0.99$), LDL ($r = 1$), triglycerides ($r = 1$), proteins ($r = 1$), and albumin ($r = 0.99$), which contributed to increasing total cholesterol, triglycerides, and cholesterol levels. High levels of triglycerides caused high-density cholesterol VLDL ($r = 0.99$), LDL ($r = 1$), and total cholesterol levels ($r = 1$) to rise. Elevated LDL cholesterol, in turn, increased the amount of proteins ($r = 1$), albumin ($r = 0.99$), and total cholesterol ($r = 1$).

Table 4
Correlations among blood chemistry parameters in T3 (without probiotic; *Gliricidia sepium* 15%) with statistically significant difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Total Cholesterol	Cholesterol HDL	0.99	0.038*
Total Cholesterol	Cholesterol VLDL	1	0.002**
Total Cholesterol	Cholesterol LDL	1	< 0.001**
Total Cholesterol	Triglycerides	1	0.002**
Total Cholesterol	Protein	1	0.009**
Total Cholesterol	Albumin	0.99	0.048*
Cholesterol HDL	Cholesterol VLDL	0.99	0.037*
Cholesterol HDL	Triglycerides	0.99	0.037*
Cholesterol HDL	Protein	1	0.005**
Cholesterol VLDL	Cholesterol LDL	1	0.006**
Cholesterol VLDL	Triglycerides	1	< 0.001***
Cholesterol VLDL	Protein	1	0.007**
Cholesterol VLDL	Albumin	0.99	0.048*
Cholesterol LDL	Triglycerides	1	0.006**
Cholesterol LDL	Protein	1	0.026*
Cholesterol LDL	Albumin	1	0.030*
Triglycerides	Protein	1	0.007**
Triglycerides	Albumin	0.99	0.048*

In T4 (SC + 0% GS), significant differences were found when its parameters of total, HDL, VLDL, and LDL cholesterol, triglycerides, glucose, insulin, ALT, AST, CK, total proteins, albumin, and globulin were correlated with each other (Table 5). T4 exhibited significant and negative correlations between glucose and proteins ($r = -0.93$), albumin ($r = -0.79$), and globulin ($r = -0.97$). However, due to the effect caused by the yeast, LDL ($r = 1$), VLDL ($r = 0.99$), and triglycerides ($r = 0.99$) showed significant and positive correlations with total cholesterol.

Table 5
Correlations among blood chemistry parameters in T4 (with probiotic - without *Gliricidia sepium*) with significant statistical difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Glucose	protein	-0.93	< 0.001***
Glucose	Albumin	-0.79	0.031*
Glucose	Globulin	-0.97	< 0.001***
Total Cholesterol	Cholesterol VLDL	0.99	< 0.001***
Total Cholesterol	Cholesterol LDL	1	< 0.001***
Total Cholesterol	Triglycerides	0.99	< 0.001***
Total Cholesterol	Protein	0.78	0.048*
Total Cholesterol	Albumin	0.92	< 0.001***
Cholesterol VLDL	Cholesterol LDL	0.98	< 0.001***
Cholesterol VLDL	Triglycerides	0.99	< 0.001***
Cholesterol VLDL	Albumin	0.89	< 0.001***
Cholesterol LDL	Triglycerides	0.99	< 0.001***
Cholesterol LDL	Protein	0.79	0.031*
Cholesterol LDL	Albumin	0.92	< 0.001***
Triglycerides	Albumin	0.90	< 0.001***
Protein	Albumin	0.96	< 0.001***
Protein	Globulin	0.9	< 0.001***

Also due to the effect of SC, there was a positive and significant correlation between total cholesterol and protein ($r = 0.78$) and albumin ($r = 0.92$) in T4. Likewise, significant and positive correlations were found between VLDL and triglycerides and between VLDL and albumin, $r = 0.99$ and $r = 0.89$, respectively. For its part, the LDL fraction of cholesterol presented highly significant correlations with triglycerides ($r = 0.99$), proteins ($r = 0.79$), and albumin ($r = 0.90$), attributable to the action of *Saccharomyces cerevisiae*.

T5 (SC + 10% GS) exhibited significant differences when its parameters of cholesterol (total, HDL, VLDL, and LDL), triglycerides, glucose, insulin, ALT, AST, CK, total proteins, albumin, and globulins were correlated with each other (Table 6). In this treatment, a highly significant and positive correlation was observed between total cholesterol and low-density cholesterol ($r = 0.96$) and between total proteins and albumin ($r = 0.87$).

Table 6
Correlations among blood chemistry parameters in T5 (with probiotic; with *Gliricidia sepium* at 10%) with significant statistical difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Total Cholesterol	Cholesterol LDL	0.96	< 0.001***
Protein	Albumin	0.87	0.003***

T6 (SC + 15% GS) exhibited significant differences when its results for cholesterol (total, HDL, VLDL, and LDL), triglycerides, glucose, insulin, ALT, AST, CK, total proteins, albumin, and globulin were correlated with each other (Table 7). This treatment allowed clear observation of the effect of GS on serum glucose: four highly significant and positive correlations were found with total ($r = 0.91$), VLDL ($r = 0.92$), and LDL cholesterol ($r = 0.87$) and triglycerides ($r = 0.92$), pointing to a relationship between the increase in glycemia and characteristics of different cholesterol and triglycerides. A similar result was obtained for LDL and VLDL cholesterol and triglycerides, which contributed to an increase in total cholesterol. Triglycerides showed strong and positive correlations with VLDL ($r = 1$), LDL ($r = 0.86$), and total cholesterol ($r = 0.90$).

Table 7
Correlations among blood chemistry parameters in T6 (with probiotic; with *Gliricidia sepium* at 15%) with significant statistical difference ($p < 0.05$).

PARAMETER 1	PARAMETER 2	r	P-VALUE
Glucose	Total Cholesterol	0.91	< 0.001***
Glucose	Cholesterol VLDL	0.92	< 0.001***
Glucose	Cholesterol LDL	0.87	0.004*
Glucose	Triglycerides	0.92	< 0.001*
Total Cholesterol	Cholesterol VLDL	0.90	0.001**
Total Cholesterol	Cholesterol LDL	0.99	< 0.001***
Total Cholesterol	Triglycerides	0.90	< 0.001***
Cholesterol HDL	Protein	-0.89	0.002**
Cholesterol HDL	Globulin	-0.90	< 0.001***
Cholesterol VLDL	Cholesterol LDL	0.86	0.007**
Cholesterol VLDL	Triglycerides	1	< 0.001***
Cholesterol LDL	Triglycerides	0.86	0.007**
Protein	Albumin	0.95	< .001***
Protein	Globulin	0.97	< .001***
Albumin	Globulin	0.84	0.014*

Triglycerides are the major constituent of VLDL, LDL, and total cholesterol and, thus, exhibit high and significant correlations with these parameters. However, HDL cholesterol was only negatively correlated with serum total protein ($r = -0.89$) and globulin ($r = -0.90$) in T6 (SC + 15% GS). Synthesized in the liver, apoproteins are needed for transporting lipoproteins (LPs), thus being linked to VLDL and HDL. In the bloodstream, endothelial cells contain LP-lipase, which metabolizes VLDL to produce LDL, as evidenced by the strong and positive correlation found between VLDL and LDL ($r = 0.86$). Albumin and total protein exhibited significant and positive correlations: $r = 0.95$ and 0.84 , respectively, due to globulins and albumin contributing to total protein.

4. DISCUSSION

This study showed that birds treated to SC at a concentration of 10^6 cfu/g and GS at 10% (T5) presented significantly higher levels of creatine kinase (CK) as compared to the control groups (T1, T2, and T3). CK concentration in plasma is known to increase when muscle and liver damage occurs, mainly in striated

skeletal muscles, in situations such as necrosis or reversible cell injury (Latimer, 2011). In addition, the SC-supplemented specimens exhibited higher weight as compared to those fed the control diet. Probiotics stimulate appetite and improve the intestinal environment, thus improving production parameters such as higher breast weight and better body-to-legs ratio in broilers (Barros Barrios et al., 2021). These changes, which imply an increase in CK activity, could be related to the effect of the yeast on the metabolism of the animals with regard to acceleration of morphological development and growth stimulation (Çalışlar & Kanat, 2021). On the other hand, GS supplementation is likely to have contributed a higher percentage of crude protein to the basal diet fed to the broilers in this experiment. In animals, increased bioavailability of proteins promotes muscle growth, which increases serum CK levels (Ogungbesan, et al., 2013).

This study found that T1 (control; commercial concentrate feed, no SC, no GS) exhibited significant and positive correlations of glucose with triglycerides and cholesterol (total, VLDL, and LDL) ($r = 0.99$). In animals, carbohydrates are stored in the form of large polymers called glycogens. Carbohydrates constitute an excellent source of instant energy and stored energy (as polysaccharides). Carbohydrates conjugated to glycoproteins or glycolipids are part of a variety of structural components of cells and organs (Hernández & Puig, 2010). In the digestive tract, hydrolysis of carbohydrates to monosaccharides allows their intestinal absorption and subsequent transport through the portal blood to the liver, where they are metabolized for energy, stored as glycogen or converted to amino acids or fats (Latimer, 2011). It is a well-known fact in poultry farming that lipid absorption is altered when changes in diet composition affect the intestinal microflora in chickens, disturbing the conversion of primary bile salts (chenodeoxycholic and cholic acids) to secondary ones (lithocholic and deoxycholic acids) needed to digest fat (Osorio & Flórez, 2011)

Broilers from the control group (T1 exhibited significant and negative correlations ($r = -0.98$) of glucose with protein and globulin. Gluconeogenesis employs amino acids or fatty acids as raw material (Arneson & Brickell, 2007), which can explain this negative correlation by indicating that amino acids – fundamental components of proteogenesis – may have been used for glucose synthesis. Glucose binds strongly and steadily to proteins throughout the lifespan of a particular protein. Therefore, levels of glucose-bound proteins reflect average blood glucose concentrations during the half-life of the protein. When the protein in the complex is albumin or total serum protein, the product is called fructosamine (FrAm) (Kaneko et al., 1997).

In the control groups (T1 and T3), cholesterol exhibited a significant correlation ($r = 0.99$) with total cholesterol and VLDL ($r = 1$). Lipids are transported by lipoproteins in the bloodstream. There are four classes of lipoproteins in the blood: chylomicrons, VLDL, LDL, and HDL. Chylomicrons transport triglycerides (TAG) to vital tissues (heart, skeletal muscles, and adipose tissues). Synthesized by the liver, VLDL redistributes TAG to adipose tissues, heart, and skeletal muscles. LDL transports cholesterol into cells whereas HDL transports cholesterol from cells back to the liver. TAG-rich lipoproteins and their remnants are atherogenic and attributed to other lipid risk factors (Carvajal, 2014).

There is vast evidence of metabolic differences between birds and mammals, including lipid metabolism (Osorio & Flórez, 2011). In birds, lipoproteins called portomicrons (PMs), which are equivalent to chylomicrons in mammals, are transported to the liver via the bloodstream instead of the lymphatic route, through the endothelial intracytoplasmic vesicles, reaching the liver directly through the portal vein. However, due to their large size, PMs are not metabolized in said organ (Fraser et al., 1986 cited by Osorio et al., 2016). After overnight fasting, endogenous triglycerides are synthesized in the liver, together with apolipoprotein B and cholesterol, and released into the bloodstream as very low-density lipoprotein particles (VLDL). Upon contact with fat cells and muscle capillary beds, VLDL triglycerides are hydrolyzed by LP-lipases. Hence, VLDL particles are catabolized into intermediate forms within hours, which are in turn further catabolized into low-density lipoproteins (LDL) (Wahl et al., 1984).

In T2 (control; no SC + 10% GS), the presence of 10% GS is associated to a significant correlation between VLDL cholesterol and triglycerides ($r = 1$). In VLDL lipoproteins, the lipid component consists of triglycerides, cholesterol, and phospholipids at an approximate ratio of 4:1:1, which are mainly synthesized in the liver. VLDLs export triglycerides and cholesterol from the liver and distribute triglycerides to adipose tissues and skeletal muscles (Latimer, 2011).

Lipogenesis in adipose tissues of birds is limited, which implies that fat deposition depends on lipids present in the diet and hepatic lipogenesis (Griffin et al., 1989). Therefore, accumulation of triglycerides in adipocytes is related to the metabolism of VLDL and other substances. However, it can equally be affected by substances that do not directly alter VLDL (Xu et al., 2003).

T3 broilers (control; no SC + 15% GS) exhibited a close and significant correlation of total proteins and albumin with all cholesterol (total, LDL, HDL, VLDL) and triglycerides. A typical broiler starter feed contains 21–24% protein, with grains and milling by-products meeting about half of the protein needs in most poultry feeds. Additional protein is provided by protein-rich concentrates, which can be of animal or vegetable origin (Saavedra, 2020).

In this study, GS contributed 22% more crude protein to the control diet, indicating that raising the amount of GS in the diet increases protein availability. This, in turn, increases the bioavailability of proteins and amino acids in the bloodstream for later use in the formation of apoproteins needed to transport lipids. Likewise, an increase in apoproteins causes HDL, LDL, and VLDL levels to rise because both triglycerides and cholesterol in plasma are contained in lipoproteins. Therefore, increases in serum cholesterol concentrations reflect higher concentrations of cholesterol-rich lipoproteins (e.g., LDL and HDL) (Latimer, 2011).

In T4 (SC + 0% GS), significant and negative correlations were observed between glucose and protein ($r = -0.93$), albumin ($r = -0.79$), and globulin ($r = -0.97$), which can be attributed to the action of the yeast. SC lowers glucose levels in a very significant way as shown in the correlation between the SC-supplemented group and the control group, which could explain this inverse correlation. That is, since blood glucose did not increase, the combined effect of glycosylation and protein oxidation did not occur.

Due to the action of SC, a highly significant correlation of total protein and albumin with total cholesterol was also found. Although T4 did not have GS as an additional source of protein, the yeast generated an increase in plasma protein, attributable to the abovementioned effect (in regard to glucose), which produced bioavailability of proteins and amino acids in the bloodstream to participate in the formation of apoproteins needed for transporting lipids. This effect was reflected in the high correlation found between total cholesterol and serum proteins, which in turn explains the correlation between total cholesterol and VLDL and LDL lipoproteins and triglycerides. This is also consistent with the highly significant correlations of VLDL, LDL, and triglycerides with albumin and total proteins, since maturation of these particles requires numerous protein factors mediating the binding of lipids with apoproteins (Carvajal, 2014).

T5 broilers (SC + 10% GS) exhibited a highly significant correlation between total cholesterol and low-density cholesterol (LDL), which comprises 60–70% of serum total cholesterol. That is, SC supplementation and GS replacement at 10% increased protein availability, which in turn promoted production of apoproteins needed for transport of total cholesterol by low-density lipoproteins.

It is worth noting that GS contains secondary compounds with anti-nutritional properties. One of the most important secondary metabolites are condensed tannins, which can have positive and negative effects on digestibility of proteins, carbohydrates, and fibers in animal feeds (Romero, et al., 2000). However, some authors have suggested that adding exogenous enzymes to feeds can help minimize the harmful effects of these secondary metabolites in non-ruminant animals (Oloruntola et al., 2018).

In this research, the various types of enzymes found in SC cells – namely, vacuolar protease (e.g., serine, aspartyl, and metalloprotease), pectinase (Ferreira, et al., 2010), amylase, maltase, sucrose, lactate dehydrogenase, proteinase, polypeptide, dipeptidase, deaminase, transaminase, lipase, phospholipase, phosphatase, and phytase (Çalışlar and Kanat, 2021) – may have counterbalanced the anti-nutritional effects of GS and, as a result, promoted the development of the specimens under investigation.

T6 (SC + 15% GS) showed effects of GS on serum glucose as there were four highly significant and positive correlations with total cholesterol ($r = 0.91$), including the fractions VLDL ($r = 0.92$), LDL ($r = 0.87$), and triglycerides ($r = 0.92$), indicating that an increase in serum glucose causes the different fractions of lipoproteins to increase as well.

A similar result was found for LDL, VLDL, and triglycerides, which contribute to increasing total cholesterol, with positive and highly significant correlations. Triglycerides exhibited strong and positive correlations with VLDL ($r = 1$), LDL ($r = 0.86$), and total cholesterol ($r = 0.90$). This result can be attributed to GS percentage in the diet, which despite possible degradative effects of *Saccharomyces* enzymes, may not have been sufficient to counterbalance the total anti-nutritional effects of GS present in the diet. As this forage contains coumarols, which confer a bitter flavor to it, cyanogenic glycoside, tannin, saponin, and cell wall content that makes it less useful for monogastric animals (Ogungbesan et al., 2013). Tannins are heat-stable and reduce protein digestibility in animals and humans, probably by making protein partially unavailable or by inhibiting digestive enzymes and increasing fecal nitrogen.

Present in food products, tannins inhibit the action of trypsin, chymotrypsin, amylase, and lipase, lower the quality of food proteins, and interfere with iron absorption. In addition, tannins are responsible for reducing feed intake, growth rate, feed efficiency, and protein digestibility in experimental animals. For their part, saponins reduce intestinal absorption of certain nutrients (e.g. glucose and cholesterol) through intraluminal physicochemical interaction, which is why hypocholesterolemic effects have been reported (Tadele, 2015).

Oloruntola and colleagues (2018) supplemented a 15%-GS rabbit diet and found that the anti-nutritional properties of this forage can be counterbalanced by using exogenous enzymes. They also showed that triglyceride levels drop when the amount of GS in the diet is raised. They attributed this finding to the presence of bioactive compounds in this forage, which reduce fat absorption and, consequently, plasma depletion. Likewise, Agbede and Aletor (2003), who found a decrease in cholesterol and LDL levels in rabbits fed a 50%-GS diet, consider the effect of *Gliricidia sepium* on triglyceride reduction beneficial to consumers, especially people predisposed to heart diseases.

T6 also exhibited negative correlations of HDL with protein and globulin, possibly due to the increase in Apo A-I apoproteins, whose concentration can rise in response to the increase in protein availability promoted by GS, thus increasing HDL and decreasing serum protein and globulins. Needed for transporting lipoproteins, apoproteins bind to VLDL and HDL and are released from the liver into the bloodstream. Endothelial cells release LP-lipase, which metabolizes VLDL into LDL. In this study, this function of lipoproteins was evidenced by the strong and positive correlation found between VLDL and LDL ($r = 0.86$).

5. CONCLUSIONS

Partial feed replacement with the forage *Gliricidia sepium* at 10% and supplementation with the probiotic *Saccharomyces cerevisiae* increased the level of the enzyme creatine kinase in broilers, a finding that may be related to mild liver or muscle disorders.

Partial diet replacement with GS increased protein availability, which correlated with an increase in availability of apoproteins for lipoprotein production.

Declarations

Funding

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Ethics approval and consent to participate and the authors declare no competing interests.

The study was carried out in the Histopathology laboratory of the Universidad de los Llanos (Villavicencio, Colombia), and the protocol was approved by the Bioethics Committee of the Universidad de los Llanos in session 008, act 008 of September 17, 2020, which authorized the use of histological tissues deposited in the histopathology laboratory. All methods and procedures were carried out in accordance with the standards established by the Bioethics Committee of the Universidad de los Llanos, which comply with national and international regulations for use in scientific research.

Authors contributions:

Silvana J. Peña Baena and Kelly A. Torres Murcia wrote the paper and performed research; Nicolas Torres and José R. Corredor-Matus designed research; Julieta E Ochoa-Amaya analyzed data and wrote the paper.

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.

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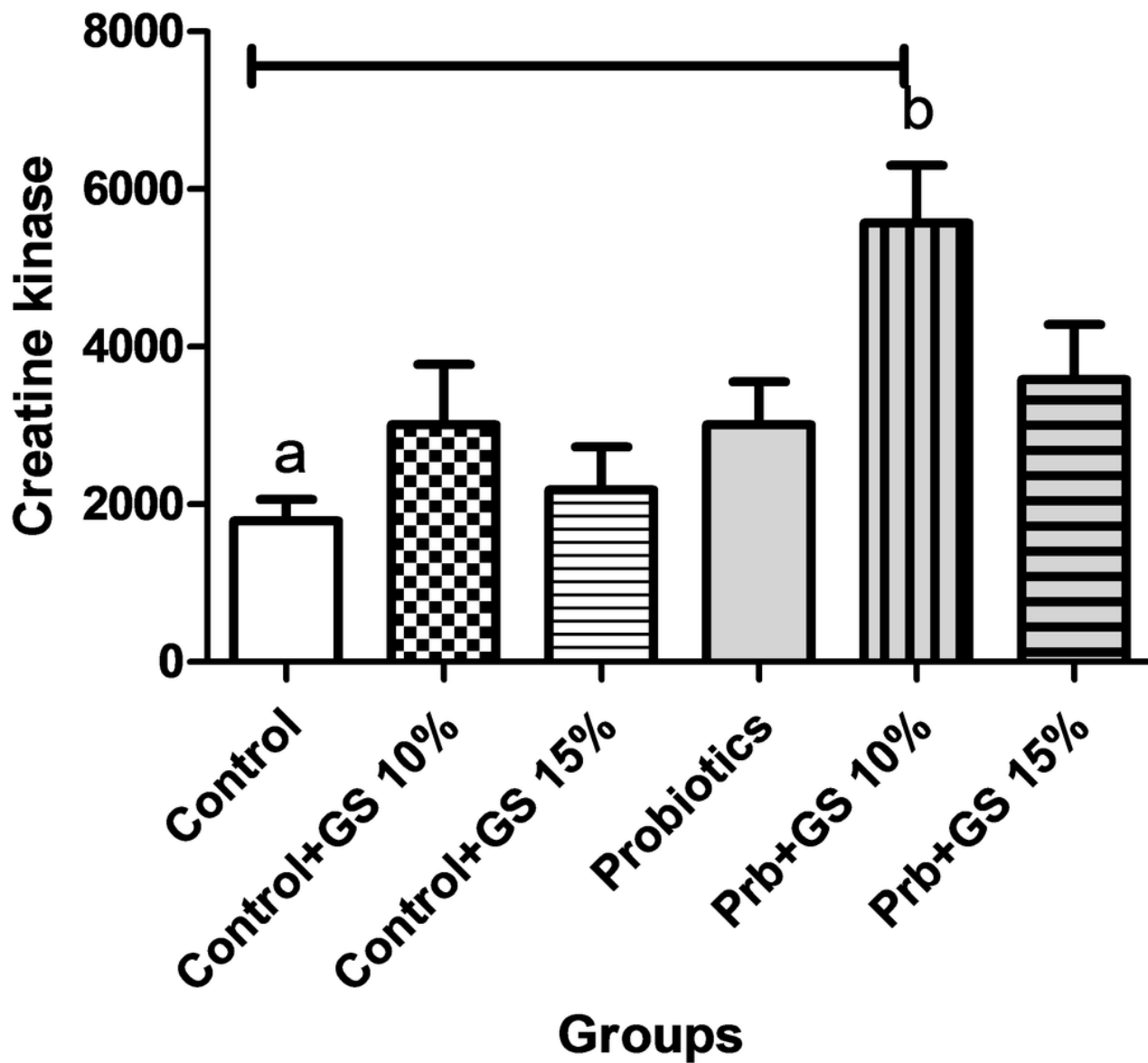
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Figures



C=5; C+GS 10%=4; C+GS 15%=5;
 Prb=15; Prb+GS 10%=15; Prb+GS 15%=14

Figure 1

Effect of the addition of *Saccharomyces cerevisiae* and *Gliricidia sepium* meal at 10% on Creatine kinase, in the serum of broilers (n = 59).