Scientific Paper

Blood biochemistry indicators in cattle supplemented with *Cratylia argentea* and *Saccharomyces cerevisiae*

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Abstract

The objective of this study was to evaluate some indicators of blood biochemistry in fistulated cattle, supplemented with *Cratylia argentea* and *Saccharomyces cerevisiae* grazing *Brachiaria decumbens* cv. *Basilisk*. Four bulls fistulated in rumen were used (Creole x Zebu), distributed in a Latin square (4 x 4). The treatments were: T1: grazing without probiotic (NP), T2: grazing with probiotic (WP), T3: grazing supplemented with 3,5 kg of dry matter (DM) of *C. argentea* (NP), and T4: grazing supplemented with 3,5 kg DM of *C. argentea* (WP). The crude protein content of *C. argentea* was 2,6 times higher than that of *B. decumbens* (18,2 vs. 6,5 %). With regards to the values of creatinine (Cr), alanine aminotransferase (ALT), albumin and globulins, a similar performance was observed in all the treatments; while blood ureic nitrogen (BUN), glycemia, aspartate aminotransferase (AST), total protein, cholesterol and triglycerides increased ($p < 0,05$) due to the effect of the probiotic and of the supplementation with legume (T2, T3 and T4). A positive correlation was found for glycemia-triglycerides, glycemia-total protein, glycemia-cholesterol, albumin-cholesterol, triglycerides-protein, and a negative correlation for BUN-AST. It is concluded that the biochemical indicators were within the normal ranges for the species; nevertheless, total protein, glycemia, BUN, cholesterol and triglycerides were higher in the treatments to which *S. cerevisiae* and *C. argentea* were added.

Palabras clave: Fabaceae, yeast, metabolism.

Introduction

The flatland foothill of Colombia, like other tropical zones, shows the disadvantage of having low availability and nutritional quality of forage in certain seasons of the year, which brings about the instability in production when cattle are grazing. In view of this problem it is necessary to promote supplementation alternatives, such as the use of shrubby legumes available in the region (Mijares-López *et al.*, 2012), in order to supply the nutritional deficiencies in cattle throughout the year (Uribe *et al.*, 2011). It has been proven that the legume *Cratylia argentea* makes a higher protein contribution with regards to the grasses used in this zone (21,67 % *vs.* 6,52 %), and constitutes a use alternative because of its biomass production and resistance to environmental and climate factors (Plazas, 2015).

Regarding the use of probiotics to improve animal production, Cifuentes and González (2013) evaluated the weight gain in creole sheep grazing kikuyu (*Pennisetum clandestinum*) with addition of *Saccharomyces cerevisiae*, compared with a control; the weight gain of the experimental group was higher (220 *vs.* 100 g for the control), which allowed to determine that the addition of 15 g of yeast improved the feed conversion of the grazing sheep.

Other studies proved that the supplementation with *S. cerevisiae* in Holstein cows, subject to heat stress, increased in 1,57 kg the daily milk production, because the digestibility of nutrients in the rumen was favored, besides its intake was increased in 15 %, with an improvement of 22 % in the feed conversion (Betancourt *et al.*, 2011; Ponce, 2011).

In turn, the metabolic indicators at blood level have been used to evaluate the degree of effectiveness of feeding and the magnitude of the energy and protein unbalance. Studies conducted by Roa and Ladino (2015) indicated that the use of probiotics can modify aspects of rumen fermentation and, thus, its metabolites. Campos *et al.* (2007), in trials with heifers of seven different breeds which were fed with a similar diet, determined the influence of the breed on glucose, which was higher in Lucerna compared with the average of the other breeds (3,2 *vs.* 2,8 mmol L^{-1}), similar performance was observed for cholesterol.

In studies with grazing Brahman cows, it was determined that the glycemia, triglycerides and cholesterol were similar in pre-partum and post-partum with the same diet (Villa *et al.*, 2011). However, the same did not occur with Rubia Gallega cows (Quintela *et al.*, 2011) in post-partum and pre-partum, in which the glycemia and blood ureic nitrogen were increased; while the alanine aminotransferase, aspartate aminotransferase, total protein, albumin, cholesterol and triglycerides decreased.

The objective of this study was to evaluate some blood biochemistry indicators in fistulated cattle, supplemented with *C. argentea* and *S. cerevisiae* under grazing of *Brachiaria decumbens* cv. *Basilisk*.

Experimental methodology

Location. The trial was conducted in the rainy season, in the cattle production unit of the Barcelona farm, animal nutrition and veterinary clinical laboratories of the University of the Llanos, in Villavicencio –Meta, Colombia–, the area is located at an altitude of 465 masl and its climate characteristics are the following: temperature between 20 and 32 ºC, annual rainfall of 1 830-3 568 mm and relative humidity from 69 to 85 % (IDEAM, 2013).

Treatments and design. Four rumen-fistulated bulls (Creole x Zebu), with an average weight of 350 ± 20 kg, were used, distributed in a Latin square (4 x 4). The treatments were: T1: grazing without probiotic (NP), T2: grazing with probiotic (WP), T3: grazing supplemented with 3,5 kg of dry matter (DM) of *C. argentea* (NP), and T4: grazing supplemented with 3,5 kg DM of *C. argentea* (WP), with a duration of 20 days each (15 of adaptation and five for sampling). All the animals were under continuous grazing of *B. decumbens* cv. Basilisk, mineralized salt and water *ad libitum*. The probiotic was used at a rate of 10 mL animal^{-1}day^{-1} orally supplied –each milliliter contains *S. cerevisiae* 2,0 x 10^4 CFU– (Lila *et al*., 2004).

Samples to evaluate the biochemical profile. In each period, blood was extracted at 7:00 a.m. from the jugular of the four animals which had not eaten for 12 h. The bulls were quiet because they were used to daily handling; vacutainer tubes with and without anticoagulant were used. The samples were analyzed in a Mindray BA-88® analyzer and glucose (glycemia), blood ureic nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), cholesterol (Cl), total plasmatic protein (TP), albumin and globulin were determined.

Total digestible nutrients (TDN) were calculated using the mathematic prediction formula (NRC, 2001):

$$\% \, TDN = CP^* \, 1{,}15 + EE^*1{,}75 + CF^*0{,}45 + ELN^2 \, {}^*0{,}00085 + ELN^*0{,}25\text{-}3{,}4$$

Where:

CP: Crude protein
EE: Ethereal extract
CF: Crude fiber
ELN: Non-nitrogen extract

Statistical analysis. A variance analysis was applied with the statistical program SPSS® version 19, and the comparisons among treatments were detected through Tukey's comparison test. Pearson's correlations (rxy) were also made among the glycemia, triglycerides and cholesterol; among the total protein, glycemia and cholesterol; and among the albumin, glycemia and cholesterol, through the formula:

$$rxy = \frac{\sum Zx * Zy}{N}$$

Results and Discussion

The dry matter of *C. argentea* (31,0 % ± 3,2) was higher than that of *B. decumbens* (table 1); these values were lower than the ones obtained by Roa and Ladino (2015) in the winter season (33,3 %).

The crude protein of *C. argentea* was approximately 2,8 times higher than that of *B. decumbens* and is within the interval described by Plazas (2015), although the regrowth age influences this nutrient.

In *B. decumbens* the dry matter (26,3 %) and crude protein content (6,6 %) were lower than the ones reported by Sánchez *et al*. (2009) –DM: 30 % and CP: 10 %–, which means that these forages vary their composition according to the planting site and the agronomic management (table 1).

The digestible nutrient content in *C. argentea* (67,5 %) was higher than in *B. decumbens* (51,3 %), for which this legume constitutes a feeding alternative to supply the protein and energy contents in the animals which grazed the grass (Plazas, 2015).

Blood indicators allow to characterize the metabolic pathways, as well as to have elements about the particularities of the consumed ration and the biotransformation of the ingredients. In this sense, it was observed that the probiotic *S. cerevisiae* and *C. argentea* did not affect the concentrations of ALT, creatinine, albumin and globulins, and their performance was similar in all the analyzed samples (table 2). When comparing average creatinine and albumin of the four treatments (1,76 mg dL^{-1} and 2,9 g dL^{-1}) with the values obtained by Campos *et al*. (2007), the latter were slightly higher (1,4 mg dL^{-1} and 2,6 g dL^{-1}); while the globulin values were

Table 1. Nutritional composition of the forages (%).

Nutrient	*Brachiaria decumbens*	*Cratylia argentea*
Dry matter	26,3 ± 4,2	31,0 ± 3,2
Crude protein	6,5 ± 1,1	18,2 ± 1,9
Fat	2,1 ± 0,66	2,4 ± 0,71
Crude fiber	30,0 ± 3,8	16,5 ± 2,5
Ash	11,6 ± 2,6	8,3 ± 2,3
Neutral detergent fiber	61,0 ± 4,2	47,0 ± 3,8
Acid detergent fiber	48,0 ± 3,7	37,4 ± 3,2
Total digestible nutrients	51,3 ± 2,5	67,5 ± 2,6

Table 2. Metabolic indicators of the fistulated bulls.

Indicator	Unit	Reference value	*B. decumbens*		*C. argentea*	
			T1	T2	T3	T4
Glycemia	mg dL^{-1}	45-75	60,4 ± 3,9^{a}	68,6 ± 2,7^{bc}	65,3 ± 2,6^{b}	72,4 ± 2,9^{c}
BUN	mg dL^{-1}	8-24	15,9 ± 1,9^{a}	22,2 ± 0,9^{b}	22,8 ± 0,9^{b}	23,6 ± 0,9^{b}
Creatinine	mg dL^{-1}	0,8-1,4	1,83 ± 0,43^{a}	1,71 ± 0,1^{a}	1,54 ± 0,1^{a}	1,96 ± 0,1^{a}
ALT	U L^{-1}	7-35	35,8 ± 2,1^{a}	36,7 ± 1,5^{a}	35,0 ± 1,4^{a}	35,4 ± 1,4^{a}
AST	U L^{-1}	45-132	67,3 ± 4,2^{a}	82,9 ± 3,3^{b}	66,6 ± 2,7^{a}	84,7 ± 3,4^{b}
Cholesterol	mg dL^{-1}	80-180	90,3 ± 7,6^{a}	97,9 ± 3,9^{b}	98,3 ± 3,9^{bc}	105,8 ± 4,2^{c}
Triglycerides	mg dL^{-1}	0-140	53,9 ± 5,8^{a}	77,8 ± 3,1^{b}	59,6 ± 2,4^{a}	79,4 ± 3,2^{b}
TP	g dL^{-1}	6,3-8,9	7,01 ± 0,71^{a}	7,78 ± 0,3^{b}	7,93 ± 0,3^{b}	8,44 ± 0,3^{b}
Albumin	g dL^{-1}	2,8-3,8	2,84 ± 0,1^{a}	2,91 ± 0,2^{a}	2,87 ± 0,1^{a}	2,97 ± 0,1^{a}
Globulin	g dL^{-1}	3,0-3,4	2,75 ± 0,3^{a}	3,01 ± 0,2^{a}	2,87 ± 0,1^{a}	2,55 ± 0,1^{a}

Different letters in the same row are statistically different ($p > 0,05$).
BUN: blood ureic nitrogen, ALT: alanine transaminase, AST: aspartate aminotransferase, TP: total protein.
Samples processed in the clinical laboratory of UNILLANOS and confirmed by Biomédica, Colombia.

lower. Noro *et al*. (2011) observed similar performance of albumin in dairy cows, and there were no differences in this indicator when the animals were supplemented with a concentrate feed, compared with a control (4,36 *vs*. 4,15 g dL^{-1}).

TP and BUN were lower ($p < 0,05$) in T1 with regards to the other treatments: 7,01 g/dL and 15,9 mg dL^{-1} (table 2); the concentrations are within the normal range, which indicates active protein metabolism that benefits the animals from the nutritional and reproductive points of view.

A similar performance was reported by Cattaneo *et al*. (2013) in cows which suffered ovarian cystic disease, and when comparing them with the healthy ones they showed higher concentration of TP (7,58 *vs*. 6,5 g dL^{-1}) and of BUN (22,1 *vs*. 13,8 mg dL^{-1}).

Oquendo *et al*. (2013) obtained, in dairy cows, the lowest TP values in February (6,58 g dL^{-1}) with regards to December (7,67 g dL^{-1}), results that can be explained by the lower forage offer and quality in the former; this proves that TP is an important indicator to evaluate the utilization of forages.

Glycemia and cholesterol increased ($p < 0,05$) due to the probiotic and the legume. Specifically the cholesterol increased from 90,3 to 105,8 mg dL^{-1} (table 2); nevertheless, these values are lower than the ones reported by Sánchez *et al*. (2014).

The BUN and TP values also increased, similarly to the report by Plazas (2015), who stated that the supplementation with *C. argentea* and a probiotic of the yeast family (*S. cerevisiae*) resulted in a higher concentration of rumen ammonium (3,0 *vs*. 7,5 mg dL^{-1}), higher bacterial protein flow to the duodenum

(3,3 *vs*. 5,5 g day^{-1}) and total nitrogen (8,4 *vs*. 14,2 g day^{-1}) and higher apparent nitrogen absorption (4,7 *vs*. 8,2 g day^{-1}), compared with the diet of the grass alone (*B. decumbens*), which implies higher nitrogen absorption towards the blood stream.

The glycemia content was higher ($p < 0,05$) in the samples of cattle supplemented with T4 (72,4 mg dL^{-1}) with regards to T3 (65,3 mg dL^{-1}); such values are in contradiction with the ones found by Ceballos *et al*. (2002), who determined that the addition of *S. cerevisiae* has a hyperglycemic effect, because the probiotic stimulates insulin production in the body.

Rúgeles *et al*. (2012), in studies with Brahman bulls grazing *B. decumbens* and *Brachiaria humidicola*, found that the glycemia and cholesterol levels were higher (66,5 and 133,9 mg dL^{-1}) with regards to T1 in this trial. If these values are compared with those of T4, glucose was higher (72,4 mg dL^{-1}); however, the same did not occur with cholesterol, which was 105,8 mg/dL for T4. This indicates that supplying the legume and the probiotic influenced more the glucose concentration than the cholesterol concentration (table 2).

With regards to the cholesterol levels, Crespi *et al*. (2014) observed that this indicator did not significantly change in Holando heifers to which protein was supplied, compared with a control; while in this study there was variation between T1 and the other treatments; the concentrations found by the above-mentioned authors were higher (276 mg dL^{-1}) because the animals were supplied protein.

The blood activity of AST and the triglycerides were higher ($p < 0,05$) in the cattle supplemented with probiotic (T2 and T4). These values indicate that the liver, heart, muscle, kidney and brain tissues were working adequately. If any of these organs or tissues were affected by a lesion or disease, high quantities of AST out of the normal range would be released into the blood stream.

With regards to triglycerides, the addition of the probiotic to the diet generated increases ($p < 0,05$) compared with the diets without *S. cerevisiae*, although there was no variation in the range established for cattle. Such information does not coincide with that reported by Ayala *et al*. (2001), who expressed that tryglicerides in the cattle group supplemented with yeast had decreased with the passing of days and that those values were similar ($p < 0,05$) to the ones of the control group.

The degree of correlation among the different metabolites analyzed in the biochemical profile of the treated cattle was positive or proportional (r_{xy} = 0,93; 0,885) for the glycemia levels, compared with cholesterol and tryglicerides, respectively (fig. 1), which can be ascribed to the effect exerted by insulin on those components. In the case of glycemia, insulin favors the utilization of glucose (oxidation and deposit), inhibits gluconeogenesis; in tryglicerides and cholesterol, it improves their synthesis and inhibits their hydrolysis and liver cytogenesis, and also facilitates their peripheral utilization. Berrio *et al*. (2003) concluded that a high blood concentration of these three metabolites (glycemia, cholesterol and TG) is considered a good predictor of the nutritional status and body reserve of cattle; which is corroborated with the results obtained by Weber *et al*. (2013) in cows that were

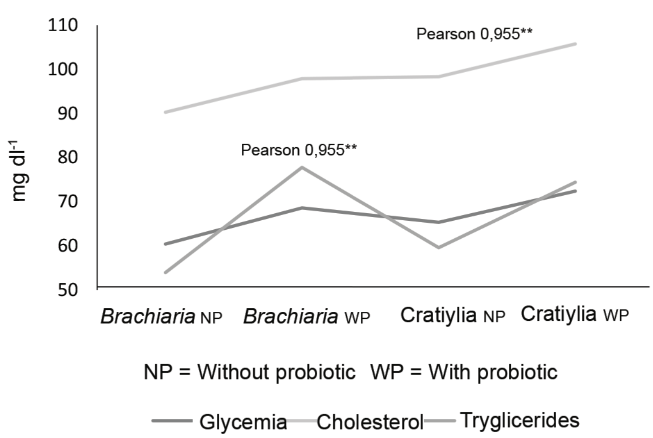

Figure 1. Pearson's correlation for glycemia with regards to triglyerides and cholesterol.

in postpartum, which mobilized high quantities of free fats from the liver towards the blood and decreased more their body weight with regards to the others, whose plasmatic TG values were lower.

A positive correlation (r_{xy} = 0,97) was observed between glycemia and TP (fig. 2), caused by the similar action exerted by insulin on nitrogen compounds. Shresta (1987) found a similar direct correlation among the levels of serum glucose, albumin and protein, and qualifies them as estimators of body condition and production degree of cattle. A positive correlation was also observed between cholesterol and TP (r_{xy} = 0,93), due to a better effect on the animals subject to treatments with legume and probiotic, which promoted the formation of lipid compounds and the protein synthesis at cell level.

Total Proteins

In the case of BUN and the enzyme AST an inverse correlation (r_{xy} = -0,542) was found, which indicates that the addition of the probiotic and the legume decreased the AST and increased the BUN; this coincides with the correlation obtained (r_{xy} = -0,47) by Restrepo *et al.* (2010), who stated that the activity of AST has been negatively associated to the liver health and functionality; and it is to be expected that the animals that have the capacity to detoxify large quantities of ammonia and, thus, to generate high BUN levels, have good liver functionality and show lower AST values.

Finally, a positive correlation was found (fig. 3) between cholesterol and albumin (r_{xy} = 0,995); it is considered a direct association (r_{xy} = 0,13), according to Restrepo *et al.* (2010), ascribed to the fact that albumin is responsible for transporting at plasmatic level some compounds derived from cholesterol, among them hormones. It is possible that the treatments with probiotic and *C. argentea* benefit all these processes for a better utilization of forages in tropical climates.

It is concluded that the biochemical indicators were within the normal ranges for the species. Total protein, glycemia, BUN, cholesterol and triglycerides were higher in the treatments to which *S. cerevisiae* and *C. argentea* were added. Likewise, a positive correlation was found for the biochemical indicators glycemia-triglycerides, glycemia-total protein, glycemia-cholesterol, albumin-cholesterol, triglycerides protein, and a negative correlation was observed for BUN-AST.

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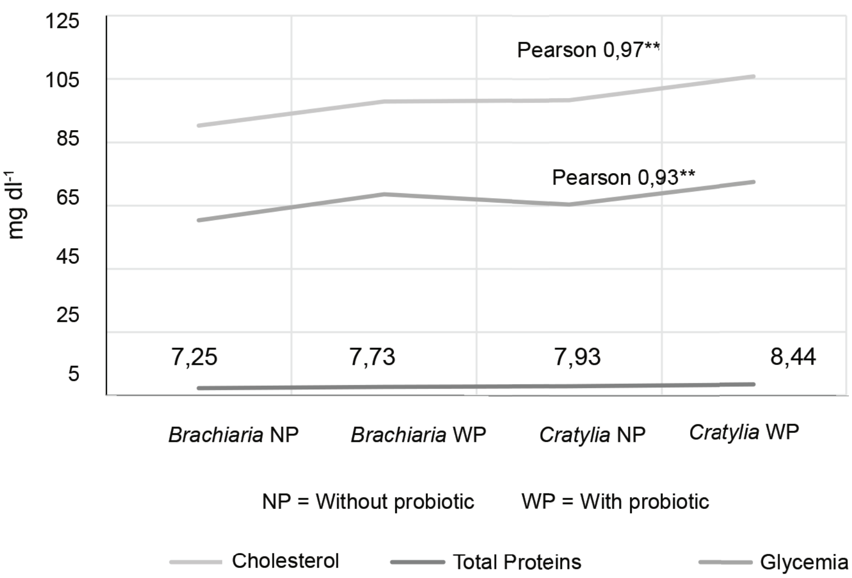

Figure 2. Pearson's correlation for total protein with regards to glycemia and cholesterol.

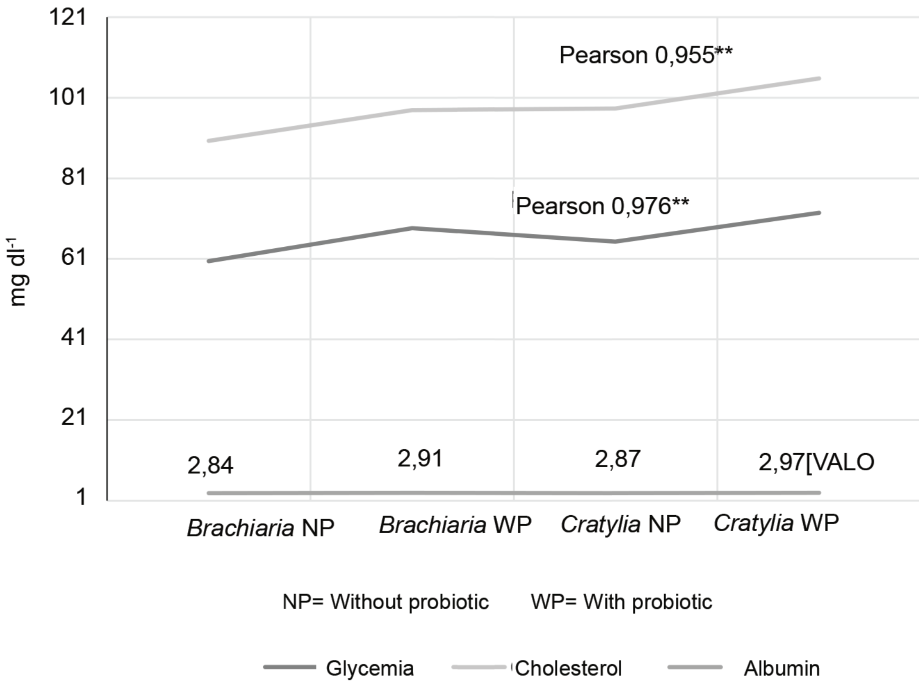

Figure 3. Pearson's correlation for albumin with regards to glycemia and cholesterol.

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