Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Physiological behavior of broilers using diets with *Tithonia diversifolia* and probiotics

Roa, M.L.[@]; Corredor, J.R. and Hernández, M.C.

Grupo Investigación en Agroforestería. Universidad de los Llanos. Villavicencio.

SUMMARY

Probiotics allow establishing and controlling the beneficial microflora and gradually reduce the pathogenic microflora. *Tithonia diversifola* (TD) is an alternative to replace protein from other more expensive sources in diets for poultry; therefore, the treatments used TD replacements with 0, 5, 10 y 15% with probiotics (WP) and without probiotics (W/OP) addition: *Saccharomyces cerevisiae* (SC), *Lactobacillus Acidophilus* (LA) and *Bacillus subtilis* (BS) and the mix of these (MP) for estimating the in-vivo digestibility. 300 Cobb avian chickens were used, with an average weight of 453.4 ± 24 g randomly distributed with a factorial arrangement 4x5x3x5. Digestibility of dry matter (DM), crude protein (CP) and fiber (CF) was determined through the total feces collection method; additionally: Hemogram (CBC) and blood chemistry (BC) were analyzed. DM digestibility increased with the proportion of TD (15%) in the diet and reached 68% W/OP; while this variable rose to 70.67% with SC (P<0.05). Digestibility of CP and CF came up to 85.6 and 82.6% with 15% TD and LA respectively. In WP treatments the red blood cell count (RBC) increased by BS; medium corpuscular volume (MCV), concentration of mean corpuscular hemoglobin (MCHC), and platelet count with LA and MP; and eosinophils with SC; while the total count of white blood cells (WBC) decreased in all the treatments with regard to TD 0%, just like the monocyte differential (MD) with MP (P<0.05). The effect of TD was lower in hemoglobin (Hb) and the RBC with TD 5%, and MD with TD 10 and 15% (P<0.05). Cholesterol decreased with SC and MP, while triglycerides (TG) increased in all the treatments with regard to TD 0%. Aspartate aminotransferase (AST), total protein, sodium and potassium were modified by the probiotics, being this effect lower with TD; triglycerides, uric acid and sodium were affected with the three levels of TD replacement (P<0.05). We can conclude that the inclusion of TD from 10% in combination with probiotics in the diet of broilers, improves its digestibility, likewise that the CBC and BC parameters showed modifications, which allows assessing the nutritional behavior of the diet and providing guidance on an appropriate balance.

ADDITIONAL KEYWORDS
Blood chemistry.
Blood values.
Eficient microorganisms.
Hemogram (CBC).
Poultry.
Vegetable protein.

Comportamiento fisiológico de pollos de engorde usando dietas con Tithonia diversifolia y probióticos

RESUMEN

ELos probióticos permiten establecer y controlar la microflora beneficiosa y gradualmente reducen la microflora patógena. *Tithonia diversifolia* (TD) es una alternativa para reemplazar la proteína de otras fuentes más caras en las dietas para aves de corral; por lo tanto, los tratamientos usaron reemplazos de TD con 0, 5, 10 y 15% con (WP) y sin adición de probióticos (W/OP): *Saccharomyces cerevisiae* (SC), *Lactobacillus Acidophilus* (LA) and *Bacillus subtilis* (BS) y la mezcla de estos (MP) para estimar la digestibilidad in vivo. Se utilizaron 300 aves Cobb avian, con un peso promedio de 453.4 ± 24 g distribuidos aleatoriamente con un arreglo factorial 4x5x3x5. La digestibilidad de la materia seca (DM), proteína cruda (CP) y fibra (CF) se determinó mediante el método de recolección de heces totales; adicionalmente: se analizaron el hemograma (CBC) y la química sanguínea (BC). La digestibilidad de la DM aumentó con la proporción de TD (15%) en la dieta y alcanzó el 68% W/OP; mientras que esta variable aumentó a 70.67% con SC (P <0.05). La digestibilidad de CP y CF llegó a 85.6 y 82.6% con 15% TD y LA respectivamente. En los tratamientos con WP, el recuento de glóbulos rojos (RBC) aumentó por BS; el volumen corpuscular medio (MCV), la concentración de hemoglobina corpuscular media (MCHC) y el recuento de plaquetas con LA y MP; y eosinófilos con SC; mientras que el recuento total de glóbulos blancos (WBC) disminuyó en todos los tratamientos con respecto a TD 0%, al igual que el diferencial de monocitos (MD) con MP (P <0.05). El efecto de TD fue menor en la hemoglobina (Hb) y los glóbulos rojos con TD 5%, y la digestibilidad de MD con TD 10 y 15% (P <0.05). El efecto de TD fue menor en la hemoglobina (Hb) y los glóbulos rojos con TD 5%, y MD con TD 10 y 15% (P <0.05). El colesterol disminuyó con SC y MP, mientras que los triglicéridos (TG) aumentaron en todos los tratamientos con respecto al TD 0%. La aspartato aminotransferasa (AST), las proteína plasmaticas totales, el sodio y el potasio fueron modificados por los probióticos, siendo este efecto menor con TD; los triglicéridos, el ácido úrico y el sodio se vieron afectados con los tres niveles de reemplazo de TD (P <0.05). Se puede concluir que la inclusión de TD del 10% en combinación con probióticos en la dieta de los pollos de engorde, mejora su digestibilidad, asimismo, los parámetros CBC y BC mostraron modificaciones, lo que permite evaluar el comportamiento nutricional de la dieta y brindar orientación sobre un equilibrio adecuado.

PALABRAS CLAVE
Avicultura.
Hemograma.
Microorganismos eficientes.
Proteína vegetal.
Química sanguínea.
Valores sanguíneos.

INFORMATION
Cronología del artículo.
Recibido/Received: 18.08.2019
Aceptado/Accepted: 25.08.2020
On-line: 15.10.2020
Correspondencia a los autores/Contact e-mail:
mroa@unillanos.edu.co.

INTRODUCTION

Chicken meat is an ideal food in human nutrition being rich in B-complex vitamins, minerals, protein and micronutrients, essential for the formation of tissues, growth and development of the body. It is also one of the foods with the highest demands worldwide, especially from people living in extreme poverty, and even more today due to the population growth (MA Zapata 2017, pp. 51). Productivity of broilers depends on their management and environmental conditions, as well as on the supply of appropriate nutritional levels through an appropriate choice of raw materials (AVIAGEN-GROUP 2018, pp. 147). Chickens have the advantage that they can be produced in large quantities, but with a limiting factor which is the high cost of feed. This creates the necessity of using alternative feeds of plant origin, with natural potentialities and which are available in the region, so as to obtain economic and productive benefits.

Digestibility and absorption of nutrients in chickens is very similar to that of mammals, however this ability has an impact on the species by the anatomical variation of the digestive tract. In addition, it is important to highlight that the enzymes degrade food before bacteria and this happens mainly in broilers due to the low-fiber feed which does not allow them to develop the ceca (DC Church et al. 2002, pp. 636).

In trials in which fodder was used in broilers, MF Itzá et al. (2010, pp. 477-87) evaluated the digestibility of dry matter and crude protein using mulberry tree (*Morus alba*) leaf flour (MLF), obtaining a digestibility value of 32.18% and 17.88%, respectively. It was shown that MLF can be included up to 8% in chickens over the age of 35 days, without reducing the productive parameters. In this sense, JC González et al. (2014, pp. 45-58) state that when replacing feed with 20% of MLF, production costs were reduced without affecting consumption and weight gain.

Currently in poultry farming the use of probiotics is an alternative to improve the digestibility of nutrients without causing negative side effects, proving that its use can increase weight gain and feed conversion, on the grounds that these microorganisms balance the microbial activity of the digestive tract, inhibit the growth of pathogens and favor the homeostasis of the immune system (H Fernández et al. 2014, pp. 113-6; LA Gutiérrez et al. 2013, pp. 135-46), being *the Bacillus subtilis* the most used in poultry (MA Zapata 2017, pp. 51). Bacteria such as *Bacillus spp*, *Enterococcus*, *Lactobacillus* and yeast such as *Saccharomyces spp* are some of the microorganisms that enhance the digestive processes, which may be evidenced through physiological parameters (H Fernández et al. 2014, pp. 113-6). Likewise, there are frequent trials that use *Saccharomyces cerevisiae* and *Lactobacillus spp* in the diet to improve the productive indexes, humoral immunity, balance in gastrointestinal microflora, morphology of organs and the parameters of the hemogram and blood chemistry among other variables (D Arrieta et al. 2007, pp. 431-43; C Lara & Á Burgos 2012, pp. 31-40). In addition, there are other factors that cause variability in the composition of blood in chickens, such as the sex, age, nutritional and reproductive management, mainly, being that information little reported (L Cardoso et al. 2014, pp. 3450-64).

In contrast to the statements in which it is specified that probiotics are an alternative to improve the physiological and productive parameters in poultry, T Ayasan, et al., (2006 pp. 777-778). showed that the Inclusion of probiotic protexin on egg yield parameters of Japanese quails did not have any positive effect on live weight and age at sexual maturity, being 271.30, 291.48 and 300.00 g and 47-57, 51-53 and 51-57 days for groups of quails fed wiht 0, 0.5 or 1.0 kg / commercial concentrate, of 0 to 5 weeks of age

The hemogram parameters are indicators of the animal health condition (CF Gálvez *et al.* 2009, pp. 178-88). There are three types of cells that are evaluated in the hemogram: erythrocytes, leukocytes and platelets (AA Odunsi *et al.* 1999, pp. 317-25). In chickens, platelets are important cells for the non-specific immune response and resistance as they circulate in large numbers, having as its main feature being cells with the ability to adhere and engulf many foreign particles (ALSP Cardoso & ENC Tessari 2003, pp. 419-24).

On the other hand, blood chemistry values are indicators of the body metabolic conditions, which make it possible to assess how well organs such as the liver, kidney, heart, muscles, among others, are functioning. Glucose represents the most important sugar in the metabolism of carbohydrates in all vertebrates; in conventional diets for broiler chickens and laying hens, starches (amylose and amylopectin) present in maize, wheat, barley and soybeans, constitute the main source of circulating glucose. Albumin is synthesized in the liver, low concentrations of this plasma protein have been associated with liver and kidney diseases, nutritional deficiencies, and acute states of illnesses (S Miranda *et al.* 2007, pp. 150-60). By evaluating the balance of blood parameters (homeostasis) we can know the physiological status of the animal and infer the biochemical effects of the supplementation being studied (H Fernández *et al.* 2014, pp. 113-6).

It is assumed that if probiotics generate modifications in the digestive tract, which improves digestion and absorption of nutrients and modulate the immune response, the parameters related to digestibility of a diet with *Tithonia diversifolia* of hemogram and blood chemistry should be modified with regard to the controls. However, in an investigation carried out by T Ayaşan, (2013) pp 79-80, in Japanese quails, evaluating the effect of the use of protexin on their reproductive behavior, it was shown that this probiotic did not affect performance in relation to fertility, hatchability and mortality embryonic, which are important indices for producing chicks. In this study it was concluded that there were not positive effects of commercial probiotics. The average hatchability of the total number of eggs fixed was for the experimental groups (A: 0, B: 0.5 and C: 1.0, kg of protein supplement / t commercial concentrate), of: 66.75%, 67 , 17% and 68.25%, respectively.

Taking into account the above, the objective of this study was to estimate the *in vivo* digestibility of dry matter (DM) and crude protein (CP) and fiber (CF) and to establish the physiological response of broilers, measured through parameters in the hemogram and blood chemistry, in specimens supplemented with balanced diets by replacing the protein in four levels (0, 5, 10 and 15%) with *Tithonia diversifolia* flour and with or without the addition of probiotics (*Saccharomyces cerevisiae, Lactobacillus spp* and *Bacillus spp*).

MATERIAL AND METHODS

The project was carried out in the poultry unit of the University of Los Llanos, located on kilometer 4, via Puerto Lopez in the village Barcelona in the city of Villavicencio, Colombia, with a height of 420 meters above sea level, average temperature of 25.5 °C, average annual rainfall of 4383 mm, average relative humidity of 75%, and luminous intensity of approximately 5 hours/day (IDEAM 2015, pp. 48).

300 Broilers of the Cobb avian 45 line were used. They were enclosed in a shed with concrete floor and with husk bed, laterally opened with manual curtain and environment control, fed with a pre-initiation diet until 15 days of age; on the 16th day they were distributed into 60 metal cages, for an experimental period of 28 days, with supply of water and feed at will. The chickens entered into the experiment with an average weight of 453.4 ± 32.1 g and were distributed in a completely randomized experimental design with a factorial arrangement 4x5x3x5, four levels of replacement of soybean cake by *Tithonia diversifolia* (T1 0%, T2 5%, T3 10% and T4 15%), five ways of adding probiotics: P1 control, P2 *Saccharomyces cerevisiae* (SC), P3 *Lactobacillus acidophilus* (LA), P4 *Bacillus subtilis* (BS) and P5 mixture of the three probiotics SC+LA+BS, ensuring concentration of 107 cfu/g of experimental diet. The experimental design had three simultaneous repetitions with 5 chickens each. The diets used the following raw materials: soybean cake, TD flour, corn, rice flour, molasses, dicalcium phosphate, palm oil, vitamin and mineral premix (**Table I**), were isoproteic and isoenergetic to 21% protein and 3000 Kcal/K MS of metabolic energy (EM) of feed.

In order to assess the *in vivo* digestibility (INVD) of DM, crude fiber (CF) and protein, excreta was collected and subjected to bromatological analysis (AOAC 2005, pp. 2200). Digestibility of the abovementioned nutrients was determined with this method in order to establish the dynamics of balances between the ingested and

$$Digestibility\ coefficient\ (\%) = \left[\frac{(IN - NF)}{IN}\right] x\ 100$$

Where:

IN = Ingested nutrient

NF = Nutrient in feces or excreted

On the 43rd day blood samples were obtained through puncture of the brachial vein, prior 8-hour fasting. Three 3 ml of blood/chickens samples were extracted from each experimental unit with a replica of each treatment. The blood was stored in heparinized BD Vacutainer tubes with anticoagulant and in tubes without anticoagulant for the blood analysis and blood chemistry.

Variables of the hemogram included: Erythrocyte count (10^6/uL), hematocrit (CRIT) (%), hemoglobin (Hb) (g/dL), medium corpuscular volume (MCV) (fL), medium corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dL), platelets (10^3/uL) and leukocytes (10^3/uL). The immune response was assessed by the differential count (%) and recognition of heterophile, lymphocytes, basophils, eosinophils and monocytes. Blood chemistry variables evaluated were: Glucose (mg/dL), cholesterol (mg/dL), Triglycerides (mg/dL), uric acid (mg/dL), alanine aminotransferase (ALT) (U/L), aspartate aminotransferase (AST) (U/L), blood urea nitrogen (BUN) (mg/dL), total protein g/dL) albumin (g/dL), globulins (g/dL), Na^+ (mEq/L), K^+ (mEq/L) and Ca^{+2} (mg/dL).

Table I. Experimental diets for broilers made on a 100 kg basis for broilers during 30 days (Dietas experimentales elaboradas sobre 100 kg para los pollos de engorde durante 30 días).

Ingredients	Without *Tithonia diversifolia*	5% *Tithonia diversifolia*	10% *Tithonia diversifolia*	15% *Tithonia diversifolia*
Soybean cake	33.5	32.9	31.8	31.0
Maize	48.1	48.0	48.2	44.3
Rice Flour	8.7	8.5	8.5	12.6
Mexican Sunflower	0	0.8	1.6	2.4
Molasses	2	2.0	2.0	2.0
Dicalcium phosphate	2	2.0	2.0	2.0
Calcium Carbonate	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Additive and salt	0.8	0.9	0.9	0.7
Vitamin and mineral premix.	0.1	0.1	0.1	0.1
Oil	4.1	4.1	4.2	4.2
Percentage of protein	21.04	20.93	20.85	20.82
Metabolic Energy per kg MS Mcal	3.03	3.01	3.01	3.01

The information was recorded in a Microsoft Excel 2016 database. Analysis of Variance (ANOVA) was performed, subjecting the values to the Tukey comparison test with a significance level of *P<0.05*, using the SPSS statistical program version 19.

The statistical design used was: $Y_{ijk} = \mu + \alpha j + \beta \kappa\ \tau\iota + \varepsilon\iota j\kappa$, where:

Y_{ijk} = Response variable score of the subject under the combination of the value of the factor and the value of the factor

μ = Effect of the general mean

αj = effect of j level of the treatment variable (four levels of protein replacement)

βκ = value of the treatment variable (five treatments with and without probiotics)

(αβ) jκ = effect of the interaction between the value of and the value of and = experimental error or random effect of sampling.

RESULTS

Bromatological analysis made to the diets fed to the chickens in the fattening stage, show that when incorporating the TD, neither the protein content nor the CF was affected (**Table II**), due to the fact that the average of diets was in 20.93% and 4.09% respectively, which is consistent with the values contained in the commercial concentrate (CC) that vary between 19% to 21% protein and maximum 5% of CF. Following with this comparison, something different was observed with grease and ashes, nutrients that were higher in the experimental diets 7.65% and 9.05% respectively, with respect to the percentages of the CC (2.5% and 5% maximum).

The level of TD included did not affect significantly the digestibility of DM of the diets in any of the three proportions are used, but it is noted that as you increase the percentage of this forage plant, increases the utilization of the diet, going from a 75.44% to 78.21% (**Figure 1**).

In addition, it was demonstrated that the use of the different probiotics in the diet, along with a replacement of 10% TD presented a DM digestibility higher than 76% in all cases, highlighting the result obtained with SC, where digestibility was 78.83% (**Figure 1**).

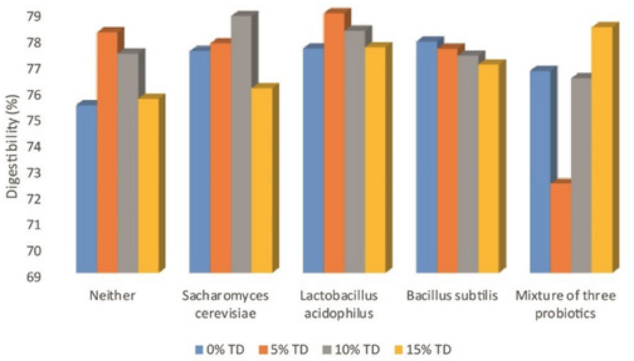

Figure 1. Dry matter digestibility of the diets with inclusión of Tithonia diversifolia and probiotics (Digestibilidad de la materia seca de las dietas con la inclusión de Tithonia diversifolia y probióticos**).**

The digestibility of the protein was associated with the percentage of TD substitution. In this experiment, the maximum percentage of replacement was 15%, with results of increasing digestibility, which were always above 79%. The digestibility came up to 85% when incorporating the probiotic SC, LA and the mixture of SC+LA+BS into this proportion of TD, and without the inclusion of TD, digestibility was 79%. The results also show that the lowest value of digestibility with probiotic occurred when using 5% TD and the probiotic LA with 79.3% (**Table III**).

Digestibility of crude fiber (CF) varied between 74.3% and 81.6% with the use of the TD flour, which contrasts with the values obtained without its inclusion, which was 69.7% (**Table IV**). When adding the probiotic digestibility was higher than 80%, when including levels of TD from 10%, with maximum results that came up to 82.6% when using the probiotic LA and the SC+LA+BS mix. It is worth noting that the result of DM digestibility without TD was the lowest (69.7%),

Table II. Nutritional composition (%) of Tithonia diversifolia and diets fed to broilers during 30 days on average* (Composición nutricional (%) del botón de oro y dietas suministradas a los pollos de engorde durante 30 días en promedio*).

Item	Tithonia diversifolia	Without Tithonia diversifolia	5% Tithonia diversifolia	10% Tithonia diversifolia	15% Tithonia diversifolia
Protein	18.5	21.0	20.93	20.9	20.9
Fat	1.1	7.6	7.6	7.7	7.7
Crude Fiber	12.1	3.7	4.17	4.3	4.2
ENN	44.5	49.2	49.6	49.6	48.9
Ashes	12.9	8.4	8.7	9.7	9.4
EM Mcal/kg	2.53	3.03	3.01	3.01	3.01

*Tests performed in the Animal Nutrition Laboratory at UNILLANOS, average of the analysis of three samples.

Table III. **Digestibility coefficient of protein in broiler chickens fed diets with *Tithonia diversifolia* and probiotics during a 28-day period** (Coeficiente de digestibilidad de la proteína en pollos de engorde alimentados con dietas con botón de oro y probióticos durante un periodo de 28 días).

Probiotic	*Tithonia diversifolia* 0%	*Tithonia diversifolia* 5%	*Tithonia diversifolia* 10%	*Tithonia diversifolia* 15%	Average
Without probiotic	0.790^{aA}	0.826^{bAB}	0.843^{bB}	0.850^{bB}	0.827
Saccharomyces cerevisiae	0.800^{aA}	0.836^{bB}	0.843^{bB}	0.850^{bB}	0.833
Lactobacillus acidophilus	0.806^{aA}	0.793^{aA}	0.840^{bB}	0.856^{bB}	0.823
Bacillus subtilis	0.760^{aA}	0.833^{bAB}	0.830^{bAB}	0.830^{bAB}	0.813
Mixture of the three probiotics	0.796^{aA}	0.833^{bAB}	0.836^{bB}	0.850^{bB}	0.828
Average	0.790	0.824	0.838	0.847	

Different small letters in the same row indicate the differences in levels of Tithonia diversifolia and different capital letters in the same row indicate differences in probiotics (P<0.05).

but when using the SC+LA+BS probiotics mix, it rose to 80.5%, similar to the digestibilities obtained by using TD in the higher proportions in this study.

The results obtained from the digestibility studies can be extrapolated with the corresponding hemogram and blood chemistry tests. The averages of blood parameters obtained in the study groups supplemented with probiotics are shown in **Table V**. The erythrocyte count, CRIT, Hb, MCV and MCH showed no significant differences (*P>0.05*).

The MCHC varied significantly (*P<0.05*) with the use of *Lactobacillus* and the mix with respect to the control, which had slightly lower values, a result which coincides with the higher digestibility of protein (85.6%) and CF (82.6%) when using the probiotic LB and TD at 15%, for the first and LB mix and TD at 15% for the second.

The use of probiotics in the diet showed platelet counts lower than the control. Particularly, when using a mixture of them, values significantly more reduced (*P<0.05*) were observed, which could result in possible risk of hemorrhage in these specimens. The total leukocyte count was lower in all the treatments with probiotics than in the control, showing significantly low (*P<0.05*) values when using *Bacillus* and the mixture of probiotics. The differential count of lymphocytes was higher in all the treatments using probiotics although no significant differences were found.

The mean values of blood parameters related to the use of *Tithonia diversifolia* as a substitute of protein are shown in **Table VI**. These values had less variability than those found with the use of probiotics. CRIT showed a significantly lower (*P<0.05*) value when using the substitute at 5%, a situation that was identical when measuring the concentration of Hb.

In the same way, digestibility of DM, the lowest values of protein and CF digestibility were found with the use of TD at 5%. With regard to the leukocyte count, values significantly lower (*P<0.05*) were found when using the substitute at 15%.

When observing the differential count, there was a significantly lower proportion of monocytes (*P<0.05*) by using the substitute at 10%, situation that is not clear given that the use of the substitute at 15%, showed the highest proportion of these cells, although it is not significant (*P>0.05*). The other haematological parameters did not show significant differences with respect to the

Table IV. **Digestibility coefficient of protein in broiler chickens fed diets with Tithonia diversifolia and probiotics during a 30-day period** (Coeficiente de digestibilidad de la fibra cruda en de pollos de engorde alimentados con dietas con botón de oro y probióticos durante un periodo de 30 días).

Probiotic	Tithonia diversifolia 0%	Tithonia diversifolia 5%	Tithonia diversifolia 10%	Tithonia diversifolia 15%	Average
Without probiotic	0.697^{aA}	0.743^{aA}	0.813^{bB}	0.816^{bB}	0.767
Saccharomyces cerevisiae	0.713^{aA}	0.796^{bB}	0.826^{bB}	0.806^{bB}	0.785
Lactobacillus acidophilus	0.733^{aA}	0.743^{aA}	0.813^{bB}	0.826^{bB}	0.778
Bacillus subtilis	0.733^{aA}	0.790^{bB}	0.803^{bB}	0.800^{bB}	0.781
Mixture of the three probiotics	0.805^{bB}	0.793^{bB}	0.803^{bB}	0.826^{bB}	0.80
Average	0.736	0.773	0.811	0.814	

Different small letters in the same row indicate the differences in levels of Tithonia diversifolia and different capital letters in the same row indicate differences in probiotics (P<0.05).

Table V. Hemogram values in chickens fed with diets including different species of probiotics (Valores de hemograma en aves alimentadas con inclusión de diferentes especies de probióticos).

Variable	Without probiotic	*Saccharomyces cerevisiae*	*Lactobacillus acidophilus*	*Bacillus subtilis*	S+L+B
Erythrocytes (10^6/uL)	2.2 ± 0.6^{ab}	2.0 ± 0.4^{ab}	2.4 ± 0.4^{ab}	2.2 ± 0.5^{b}	1.8 ± 0.3^{a}
CRIT (%)	32.0 ± 1.7	34.9 ± 4.1	33.3 ± 2.6	34.8 ± 4.1	32.8 ± 1.6
Hb (g/dL)	10.6 ± 0.6	11.6 ± 1.4	11.1 ± 0.8	11.6 ± 1.4	10.9 ± 0.5
VCM (fl)	148.2 ± 27.2^{ab}	172.8 ± 23.6^{ab}	144.0 ± 31.4^{a}	160.2 ± 32.6^{ab}	178.6 ± 29.7^{b}
HCM (pg)	63.2 ± 50.7	57.5 ± 7.8	48.0 ± 10.4	53.5 ± 11.0	59.4 ± 9.8
MCHC (g/dL)	33.2 ± 0.08^{ab}	33.2 ± 0.09^{ab}	33.3 ± 0.07^{b}	33.3 ± 0.07^{ab}	33.2 ± 0.1^{a}
Platelets (10^3/uL)	361.8 ± 162.3^{b}	234.9 ± 99.1^{ab}	341.2 ± 153.9^{b}	219.9 ± 165.2^{ab}	135.4 ± 50.6^{a}
Leukocytes (10^3/uL)	34.8 ± 11.8^{c}	23.0 ± 6.1^{ab}	25.1 ± 4.8^{ab}	19.3 ± 6.9^{a}	27.5 ± 3.2^{b}
Heterophiles (%)	47.2 ± 12.9	40.6 ± 9.8	43.0 ± 13.8	45.8 ± 7.1	40.7 ± 9.3
Lymphocytes (%)	45.3 ± 11.5	50.0 ± 11.7	44.1 ± 10.7	47.0 ± 10.4	51.6 ± 12.2
Monocytes (%)	4.9 ± 1.4^{b}	3.5 ± 2.7^{ab}	4.2 ± 2.1^{b}	3.5 ± 2.1^{ab}	2.0 ± 1.8^{a}
Eosinophils (%)	2.5 ± 2.8^{a}	5.7 ± 5.2^{b}	3.7 ± 2.6^{ab}	2.25 ± 2.7^{a}	3.1 ± 2.0^{ab}
Basophils (%)	0	0	0	0.12	0.25

S+L+B: basal diet with a mixture of the three probiotics. Different letters indicate significant differences (P<0.05). Hb= Hemoglobin. CRIT= Hematocrit. MCV= Mean corpuscular volume. MCH= Mean corpuscular hemoglobin. CHCM= Mean corpuscular hemoglobin concentration.

use of the *Tithonia diversifolia* as a substitute for protein in the diet of chickens in this experiment (*P<0.05*).

Blood chemistry parameters in chickens supplemented with different species of probiotics are presented on **Table VII**. Blood glucose levels showed no significant differences amongst the groups (*P<0.05*). The plasma cholesterol showed the highest values with the use of the *Bacillus*, although there were not significant differences with the control, on the contrary, the mixture of probiotics generated a significant drop (*P<0.05*) of this variable, with respect to other treatments and the control. Triglycerides behaved with concentrations significantly lower (*P<0.05*) with the use of the *Saccharomyces*, while with the use of the *Bacillus* they showed the highest value (*P<0.05*).

ALT concentrations were significantly lower and higher with the use of *Lactobacillus* and *Bacillus* respectively (*P<0.05*), while the AST showed significantly higher values in all treatments other than the control, but highlighting the effect of *Bacillus*, which presented the highest value of this enzyme (*P<0.05*).

Total protein levels were significantly higher in all the treatments that used probiotics (*P<0.05*), highlight-

Table VI. Values of hemogram in birds fed with different levels of inclusion of *Tithonia diversifolia* flour (Valores de hemograma en aves alimentadas con diferentes niveles de inclusión de harina de botón de oro).

Variable	Without Tithonia diversifolia	Tithonia diversifolia 5%	Tithonia diversifolia 10%	Tithonia diversifolia 15%
Erythrocytes (10^6/uL)	2.16 ± 0.5	2.02 ± 0.6	2.23 ± 0.5	2.27 ± 0.3
Hematocrit (%)	34.4 ± 1.9^{b}	31.3 ± 2.1^{a}	34.7 ± 2.9^{b}	33.9 ± 3.1^{b}
Hemoglobin (g/dL)	11.47 ± 0.7^{b}	10.44 ± 0.7^{a}	11.5 ± 0.9^{b}	11.3 ± 1.0^{b}
VCM (fl)	163.2 ± 27.0	167.4 ± 44.4	161.9 ± 30.3	150.5 ± 17.5
HCM (pg)	54.5 ± 8.8	67.8 ± 45.1	52.9 ± 9.9	50.1 ± 5.8
CHCM (g/dL)	33.2 ± 0.06	33.3 ± 0.1	33.2 ± 0.1	33.2 ± 0.1
Platelets (10^3/uL)	217.9 ± 148.7	285.4 ± 138.4	261.0 ± 148.9	270.3 ± 194.0
Leukocytes (10^3/uL)	27.2 ± 5.5^{ab}	26.3 ± 7.7^{ab}	28.8 ± 11.9^{b}	21.4 ± 5.7^{a}
Heterophiles (%)	44.4 ± 9.0	41.7 ± 12.6	48.2 ± 12.0	38.7 ± 10.1
Lymphocytes (%)	45.3 ± 10.5	51.1 ± 13.6	43.3 ± 10.9	50.7 ± 10.4
Monocytes (%)	4.03 ± 2.6^{ab}	3.4 ± 1.8^{ab}	2.6 ± 1.6^{a}	4.5 ± 2.4^{b}
Eosinophils (%)	4.1 ± 1.6	4.0 ± 4.6	2.7 ± 2.1	3.1 ± 2.8
Basophils (%)	0.1	0.0	0.2	0.0

Different letters indicate significant differences (P<0.05) ing the use of the *Bacillus spp*, which showed the most significant concentration (3.7 ± 0.8 g/dl, *P<0.05*).

The values of albumins found did not differ significantly amongst the groups by using probiotics (*P>0.05*). However the effect of the mixture showed the highest average for this variable (5.7 ± 0.4 g/dl).

In terms of ions, the concentrations of sodium and potassium were significantly higher than the control with the use of probiotics (*P<0.05*), being the highest values of sodium (143.9 ± 2.9 mEq/L) and potassium (5.5 ± 1.1 mEq/L) with the probiotic BS. With regard to the calcium, their concentrations did not show significant differences (*P>0.05*).

The effects on the blood chemistry parameters (**Table VIII**) in broiler chickens fed with different percentages of replacement of protein with TD flour were lower than those obtained by the effect of probiotics. Triglycerides and uric acid presented a significantly high concentration (70.8 ± 2.9; 26.0 ± 0.8 mg/dl; *P<0.05*, respectively) when using the protein substitute at 15%. However, the concentrations of TG and uric acid using the TD at 5 and 10% were also higher than the control, but not significant (*P>0.05*).

With respect to the ions, sodium showed a higher plasma concentration by using the substitute at 10%, which was significant (*P<0.05*), the other two ions (K^+ and Ca^{+2}), did not show significant differences between the treatments related to the substitute (*P>0.05*).

DISCUSSION

Diets designed for this experiment were balanced in such a way that the metabolizable energy was similar in each of them, so that the proportion of proteins, fats, CF, nitrogen-free extract NFE and ashes, had very close values, as noted in **Table II**.

In all species it was found that the inclusion of fats in the ration provides the following benefits: it reduces the energy cost of the diet, it provides essential fatty acids and fat-soluble, vitamins and it improves the taste of the ration and animal products for human consumption. Fats and oils are the most concentrated source of energy, and chickens, especially broilers, have a great capacity to use them (Quishpe 2006, pp. 27). In the formulation of rations, unsaturated oils or fats of vegetable origin are used because they have better digestibility that saturated forms such as fattening fed (tallow). The presence of unsaturated fat in the basal ration, favors the formation of micelles in the intestinal lumen which facilitates the absorption of fatty acids provided. RP Ruiz et al. (2015 pp. 38-46) used the fruit of oil palm to feed broilers in the final phase and concluded that this fruit is a viable alternative to be used in low proportions. They determined that the 7% of replacement seems to be within optimum ranges, which is in line with the percentage to be used in the experimental diets.

The different TD levels did not significantly affect the digestion of DM. L Mahecha & M Rosales (2005, pp. Art. 100) calim that the TD in flour has a DM *in vitro* digestibility (DMIVD) of 63.3%, values close to those obtained in this study. However (DM Verdecia et al. 2011, pp. 1-13) after evaluating the TD quality reported an DMIVD of 75.28% in the rainy season and 78.59% in the low rain season. (R Murgueitio & SD Ospina 2002, pp. 302) conducted an inclusion of TD up to 20 % in the diet for broilers without affecting consumption and weight gain. D Nieves et al. (2011 pp. 309-314) evaluated the TD nutrients digestibility of in broiler rabbits, finding a value of 51.25% for DM. It is probable that the conditions of the soil, fertility, season of the year, rainfall and species, among other variables, affect the quality of TD foliage and therefore its digestibility.

The inclusion of probiotics in the diet substantially improved digestibility of the DM. This is due to the fact that when these microorganisms develop changes in the gastrointestinal mucosa they modify the digest-

Table VII. **Blood chemistry values in birds fed with diets including different species of probiotics** (Valores de química sanguínea en aves alimentadas con diferentes especies de probióticos).

Variable	Without probiotic	*Saccharomyces cerevisiae*	*Lactobacillus acidophilus*	*Bacillus subtilis*	Mixture of the three probiotics
Glucose (mg/dl)	149.6 ± 45.8	160.2 ± 35.2	157.0 ± 45.6	144.7 ± 31.1	159.3 ± 44.8
Cholesterol (mg/dl)	144.5 ± 19.1^{c}	128.9 ± 11.3^{ab}	142.6 ± 13.2	147.8 ± 19.9^{c}	125.1 ± 9.6^{a}
Triglycerides (mg/dl)	58.5 ± 11.6^{ab}	47.4 ± 7.6^{a}	59.2 ± 15.5^{ab}	79.6 ± 35.8^{b}	58.6 ± 23.2^{ab}
Uric acid (mg/dl)	2.6 ± 0.7	2.6 ± 0.7	2.3 ± 0.4	2.2 ± 0.4	2.9 ± 0.8
ALT (U/L)	16.8 ± 3.1^{ab}	17.6 ± 2.8^{ab}	15.7 ± 1.9^{a}	20.3 ± 7.7^{b}	17.8 ± 2.7^{ab}
AST (U/L)	226.1 ± 39.1^{a}	232.6 ± 43.1^{ab}	227.1 ± 48.9^{ab}	265.7 ± 45.5^{b}	247.5 ± 35.4^{ab}
BUN (mg/dl)	3.6 ± 2.2	3.3 ± 0.7	3.2 ± 0.8	3.8 ± 0.8	3.2 ± 0.4
Protein (g/dl)	3.1 ± 0.3^{a}	3.3 ± 0.3^{ab}	3.4 ± 0.5^{ab}	3.7 ± 0.8^{b}	3.3 ± 0.4^{ab}
Albumin (g/dl)	1.2 ± 0.2	1.4 ± 0.3	1.7 ± 0.2	1.5 ± 0.3	5.7 ± 0.4
Globulin (g/dl)	1.8 ± 0.3	1.9 ± 0.3	1.6 ± 0.5	2.2 ± 0.7	1.9 ± 0.3
Na^+ (mEq/L)	140.4 ± 1.8^{a}	143.3 ± 1.9^{b}	141.8 ± 2.1^{ab}	143.9 ± 2.9^{b}	143.2 ± 1.3^{b}
K^+ (mEq/L)	4.33 ± 0.2^{a}	4.9 ± 0.7^{ab}	5.2 ± 0.7^{b}	5.5 ± 1.1^{b}	5.1 ± 0.4^{b}
Ca^{+2} (mg/dl)	8.77 ± 1.9	8.33 ± 1.4	7.8 ± 0.8	8.5 ± 1.5	7.8 ± 0.6

Different letters indicate significant differences (P<0.05)

Table VIII. Values of blood chemistry in birds fed with different levels of inclusion of Tithonia diversifolia flour (Valores de química sanguínea en aves alimentadas con diferentes niveles de inclusión de harina de botón de oro).

Variable	Without *Tithonia diversifolia*	*Tithonia diversifolia* 5%	*Tithonia diversifolia* 10%	*Tithonia diversifolia* 15%
Glucose (mg/dl)	145.9 ± 43.4	145.9 ± 49.2	165.9 ± 20.0	160.7 ± 38.0
Cholesterol (mg/dl)	138.7 ± 18.2	138.3 ± 10.2	134.2 ± 12.0	140.0 ± 25.9
Triglycerides (mg/dl)	50.7 ± 9,1[a]	56.0 ± 17.7[ab]	65.2 ± 32.9[ab]	70.8 ± 26.0[b]
Uric acid (mg/dl)	2.2 ± 0,4[a]	2.3 ± 0.4[a]	2.7 ± 0.8[ab]	2.9 ± 0.8[b]
ALT (U/L)	17.8 ± 2.9	17.5 ± 5.1	17.6 ± 2.4	17.8 ± 6.1
AST (U/L)	223.3 ± 45.9	245.2 ± 49.9	239.0 ± 40.6	251.7 ± 36.2
BUN (mg/dl)	3.4 ± 2.0	3.2 ± 0.6	3.3 ± 0.8	2.9 ± 0.7
Protein (g/dl)	3.2 ± 0.4	3.4 ± 0.6	3.4 ± 0.3	3.5 ± 0.7
Albumin (g/dl)	4.9 ± 0.4	1.5 ± 0.3	1.4 ± 0.2	1.4 ± 0.3
Globulin (g/dl)	1.7 ± 0.5	1.8 ± 0.5	2.0 ± 0.4	2.0 ± 0.5
Na^+ (mEq/L)	141.5 ± 2,6[a]	142.3 ± 1.9[ab]	143.9 ± 2.0[b]	142.2 ± 2.4[ab]
K^+ (mEq/L)	4.9 ± 0.6	4.9 ± 0.8	5.0 ± 0.6	5.1 ± 1.1
Ca^{+2} (mg/dl)	7.8 ± 0.9	8.5 ± 1.5	8.5 ± 1.8	8.0 ± 1.0

Different letters indicate significant differences (P<0.05)

ibility of nutrients by increasing their absorption (M Peralta et al. 2008, pp. 1-11). These modifications to the digestive mucosa are favored by the cell walls of some of the microorganisms used, as is the case of the yeast SC, which is mainly made up of polysaccharides (glucans and mannans), which contribute to this action. These effects are similar to those obtained in production systems using antibiotics as growth promoters (B Aguilar & J Francois 2003, pp. 268-74).

Digestibility of the protein improved substantially, although not significantly, with the increase in the proportion of TD and the addition of probiotics, reaching values higher than 85%, as mentioned above. M Freitas *et al.* (2003 pp. 503-6) found that with the use of a commercial probiotic containing *Lactobacillus acidophilus*, *Enterococcus faecium* and *Saccharomyces cerevisiae*, gain in live weight improved by 5% as well as the food conversion trend, which they attributed to better digestion and absorption of protein which is favored by the probiotics, as was evidenced in this study. It has been demonstrated that with the use of probiotics as growth promoters will have better outcomes than those additives using a single probiotic. In addition, E Veizaj et al. (2010, pp. 249-51) and H Timmerman et al. (2004, pp. 219-33) proved in trials that the final body weight and daily weight in broiler chickens increased significantly by using combined probiotics.

The addition of *Saccharomyces cerevisiae* (36×10^7/g), *Lactobacillus* (30×10^4/g) or Mannan-oligosaccharides (1 g/kg of food) improved feed conversion of the chickens that received this additive, achieving better live weight of chickens on the 14, 28 and 48 days of life (H Upendra & S Yathiraj 2003, pp. 1075-7). NH Mansoub (2010, pp. 184-6) administered *L. acidophilus* and *L. casei* as a supplement in the diet of broilers and found an increase in weight gains and a reduction in the conversion, attributing this to the fact that these bacteria improve digestion, absorption and availability of nutrients in the chickens intestines. On the other hand, A Alkhalf et al. (2010, pp. 219-225) found that the use of lactic acid bacteria improves the daily weight gain of broiler chickens and therefore the conversion, mainly due to the increase in the processes of digestion and absorption of nutrients furthermore the use of probiotics and prebiotics as growth promoters in broilers, does generate risk to the consumers´ health by eliminating the use of antibiotics.

Digestibility of crude fiber yielded results that ranged from 74.3 to 81.6%. The inclusion of adequate levels of fiber in the diets of monogastric animals can modify their nutritional value through various mechanisms, such as higher anatomical development of the intestinal tract, regulation of the gastrointestinal transit speed, improving the capacity of ion exchange and potential use as a substrate for microbial fermentation (J Gargallo 1979, pp. 288-92).

The inclusion of fiber in diets for chickens can have benefits at the productive level or at least not be harmful within certain limits (G Mateos et al. 2002, pp. 15-37). H Hetland y B Svihus (2001, pp. 354-61) observed that the inclusion of 4% of oat husks in diets based on barley increased consumption, but did not affect the weight gain of chicks from 7 to 21 days of age.

Relationship with hematologic findings

There is great variability in the reports found for the hematological values of chickens in general. The CRIT, Hb, and the count of red and white cells in chickens fluctuate around levels that are characteristic for each individual, and according to the method used for the estimate (F Perozo et al. 2003, pp. 59-64). The results obtained in this study show that the use of probiotics in the diet induced alterations in some hematological parameters that generated significant differences. The erythrocyte count showed average values lower than those found in the literature, (BL Avilez et al. 2015, pp. 33-9) reporting that the average number of erythrocytes in chickens at the sixth week of age was 5.79×10^6 µl. Likewise, an opposite trend is observed in one of the experimental groups in the count of red blood cells which was significantly lower (*P<0.05*), corresponding to the T4 when using the mixture of probiotics (SC+LA+BS), as if this inclusion would have limited the contribution of nutrients necessary for erythropoiesis. However, the result of this variable seems to be offset by the value found for the same group of MCV, which was the highest (*P<0.05*) compared with the other treatments. The above matches the values found of digestibility of DM, where the lowest result is found by using the mixture of probiotics, which could infer that a lower digestibility of DM, generates lower nutrient inputs, a fact that is reflected in the findings of count of RBC, but that thanks to compensatory mechanisms of the animal organs, this reduction was corrected with the MCV values.

The findings of the CRIT are consistent with the ranges reported by F Perozo et al. (2003, pp. 59-64) and L Cardoso et al. (2014, pp. 3450-64), but differ from the result obtained by C Marchini et al. (2011) sp. in commercial chickens (CRIT: 26.8%), where the specimens were subjected to the test at different temperatures, which may explain in part the result, given the additional stress that the specimens were exposed to. Y Haile and M Chanie (2014, pp. 88-95) cite that chickens tend to have a lower hematocrit (as low as 24%) and that this value increases with age.

With regard to the MCHC, the values found differ with what is reported by L Cardoso et al. (2014, pp. 3450-64) who found an average value of MCHC in chickens fed with probiotics and a control group (22.56 and 23,115 g/dL respectively), values lower than those reported in this study which were always above 33 g/dL, a difference that is possibly related to the diet used.

The use of probiotics showed platelet counts lower than the control, in particular when using the mixture of these, which could result in possible risk of hemorrhage in these specimens. There is no clear explanation to this result.

The total leukocyte count was lower with the use of probiotics than the control, especially with the use of *Bacillus* and the mixture of probiotics, aspect that relates to the same behavior of erythrocyte and platelet count. Again, this result is consistent with the higher values of digestibility of protein and CF when using the LB and the mixture of probiotics. This situation requires to be deepened in subsequent studies. The lymphocyte count was higher in treatments than in controls, which may represent a greater capacity of specific immunity for these specimens. These results were very close to those reported by EN Castiglioni et al. (2006), pp. 924-9 in the control groups of chickens with mean values of 49.5%.

The lactobacilli used as probiotics are able to stimulate the immune system through two channels: The first one, migration and multiplication of the probiotic microorganisms through the intestinal wall by stimulating the farthest parts, and the second one, the recognition of death probiotic organisms as antigens that may directly stimulate the immune system (D Lázaro et al. 2005, pp. 97-102).

The hematologic findings related to the use of *Tithonia diversifolia*, showed fewer changes than the same relationship with probiotics. When using this forage plant at a rate of 5%, the parameters of digestibility were the lowest, as well as the CRIT value. This could take us to infer that this percentage of the substitute does not meet the nutritional requirements necessary to maintain an adequate erythropoiesis in these specimens.

The leukocyte count was significantly lower when using the TD at 15%, indicating that this percentage could generate some degree of depression of the Leucopoyesis. It is likely that unidentified alkaloids of this fodder tree could be affecting this physiological process, however specific studies are needed to clarify this behavior. In the same way the differential monocyte count was lower with the use of the TD at 10%, situation that is not clear given that when using it at 15%, it showed the highest proportion of these cells, so that the effect found when using it at 10%, must be related to another variable. Remember that the total leukocyte count was lower in treatments in which probiotics were used.

Relationship with the blood chemistry findings

Given that the levels of blood glucose showed no differences between groups by the use of different probiotics, this indicates that they did not influence its absorption. All the chickens of the evaluated treatments showed blood glucose concentrations that were within the range of 152-182 mg/dL, which is considered to be normal, according to what is reported by S Miranda et al. (2007, pp. 150-60). Plasma cholesterol presented the highest value with the use of the *Bacillus*, while the mix generated the lowest value. Cholesterol is the raw material for the synthesis of steroid hormones in nature, such as corticosteroids and sex hormones, which have anabolic effects by generating greater development of muscle mass, an aspect that is paramount in broilers, and therefore it is reasonable to think that this variable has higher concentrations with the use of probiotics, which we have already discussed, favor the absorption of nutrients.

The triglycerides had erratic behavior when using the lowest concentrations with *Saccharomyces*, and the highest with the *Bacillus*, these two probiotics generated the most extreme behavior for this variable. When confronting these probiotics with the digestibility variables, it was found that the use of the SC had the highest digestibility of DM and the protein and the second one on the digestibility of the DM. On the other hand, the use of the BS, presented the lowest average value of the digestibility of the protein. It is evident that the levels of cholesterol and triglycerides are associated with the use of Bacillus. The results show that as the digestibility of the DM and the protein related to the use of probiotics increase, lipid concentrations decrease and vice versa. It is important to note that elevations of triglycerides and cholesterol may be related to ischaemic heart disease, a situation that needs to be clarified in specific studies.

ALT and AST concentrations had significant changes with the use of probiotics, highlighting the effect of *Bacillus*, which presented the highest AST value. The effect of the BS probiotic on these variables is consistent with the report on digestibility of protein, which presented the lowest average value with the use of this probiotic; in any case, the digestibility of protein was always higher than the one obtained by the treatment without the use of probiotic.

However, the results do not differ from those reported by S Miranda et al. (2007, pp. 150-60) who suggest that an average value of ALT plasma concentration in broilers is 25.0 IU/L, (normal range: 9.50-37.2 U/L).

The ALT and AST are synthesized mainly in the liver, but also in the kidneys, heart, and muscles. When there is an injury to these tissues, it is released into the bloodstream, then their levels increase; so that significantly elevated levels of these enzymes with the use of probiotic *Bacillus spp* could be associated with a higher metabolic activity of the liver and/or muscle activity.

Total protein levels were higher in all treatments where probiotics were used, while the albumins did not differ amongst them. However, when using the mix, the highest average for albumin was found, a fact which coincides with the higher digestibility of the CF, which is also reported with the use of the mixture and the TD at 15%. The average reference for the concentration of plasma albumin in broilers at 21 days of age is: 1.92 mg/dL, with the normal range being: 1.10-2.74 mg/dL (S Miranda et al. 2007, pp. 150-60). The total proteins obtained in this study agree with no doubt with those reported by F Perozo et al. (2003, pp. 59-64) and S Miranda et al. (2007, pp. 150-60), with average values of 3.18 ± 0.4 and 3.16 ± 0.2 g/dL, respectively. Probiotics, by improving and promoting a microenvironment in the intestinal lumen, generate greater growth of the intestinal villi and counteract the effect of undesirable microorganisms, facilitating in great extent better absorption of nutrients from the intestinal mucosa to the liver, which increases the metabolic activity (ALT and AST) and therefore increasing the synthesis of plasma proteins.

The concentrations of sodium and potassium were higher than in the control when using probiotics, especially BS, which in turn presented the lowest digestibility for protein when using TD at 10 and 15%. After comparing the values of the natremia with the parameters reported by EA Díaz et al. (2014, pp. 31-42) of 140.50 to 149.50 mmol/L, for broilers with 42 days, the results obtained were consistent with those in the different treatments. It is worth noting that these electrolytes are regulated by aldosterone, a steroid hormone of the adrenal cortex which produces sodium retention and potassium depletion. The increased metabolic activity of broilers requires higher bioelectric activity, being these two ions (Na^+ and K^+) the fundamental ones for this process.

The effects on the blood chemistry parameters in broiler chickens fed with different percentages of protein replacement with TD flour were lower than those obtained by the effect of probiotics. The triglycerides and uric acid concentrations showed high concentrations with the protein substitute at 15%, probably due to the fatty acid content, which is consistent with the DM digestibility recorded, which was higher at this level of TD replacement, as well as with the digestibility of protein and CF which reached the highest average with this percentage of replacement. Concentrations of TG and uric acid using TD at 5 and 10% were also higher than the control, which means that the fodder used to replace part of the protein fed to broilers complies with the requirements of the species, taking into account that the uric acid is associated with the consumption of protein in the diet and the results showed that as proportion of the TD increases in the ration the levels of this variable were higher.

With respect to the ions, sodium showed higher plasma concentrations by using the substitute at 10%, but not with the other levels of replacement, so we can establish a dose-response relationship. However it must be remembered that when associating the electrolytes with the use of probiotics, their values were higher in all cases, which indicates that the effect on the electrolyte is more dependent on the number of the latter than the former.

CONCLUSIONS

The inclusion of *Tithonia diversifolia* from 10% on in diets for broilers improves their digestibility parameters, reaching values above 80 % in regard to protein and crude fiber and 67.8% for dry matter.

The combination or individual inclusion of probiotics (*Saccharomyces cerevisiae* and *Bacillus subtilis*) improved digestibility parameters above 75%, a synergy that reaches up to 82 and 85% when incorporating *Tithonia diversifolia* into the diet in proportions of 10 and 15%, respectively.

The values in the hemogram and blood chemistry had significant changes when using TD as a substitute of protein and different probiotics, isolated or in mixture. The hematological variables with the most notorious changes were: RBC, MCV and MCHC, platelet count, leukocyte, monocytes and eosinophils; in addition, the use of probiotics significantly modified blood chemistry variables: Cholesterol, TG, ALT, AST, total protein, Na^+ and K^+.

The use of TD flour generated lower effect in the hemogram, showing differences in: CRIT, Hb, leukocyte count and monocytes; on the other hand, the use of TD significantly modified blood chemistry variables to a lesser extent: TG, uric acid and Na^+.

The blood chemistry and hemogram parameters allow for tracing in order to estimate the nutritional behavior of the ration, by comparing them with digestibility variables and providing guidance on an adequately balanced diet.

ACKOWLEGMENTS

The authors express our gratitude to the General-Directorate of Research at Universidad de los Llanos, entity that financed the implementation of this project (Code: FCARN- 15-2014).

BIBLIOGRAFÍA

Aguilar, B & Francois, J 2003, A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation, *Letters in Applied Microbiology*, vol. 37, no. 3, pp. 268-74. doi: 10.1046/j.1472-765X.2003.01394.x

Alkhalf, A, Alhaj, M & Al-Homidan, I 2010, Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens, *Saudi Journal of Biological Sciences*, vol. 17, no. 3, pp. 219-25. doi: 10.1016/j.sjbs.2010.04.005

AOAC 2005, *Official methods of analysis of AOAC International*, Gaithersburg, United States of America: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, pp. 2200.

Arrieta, D, Perez, ML, Luengo, A, Hernández, JP, Lista, D & Mosquera, J 2007, Alteraciones histológicas hepáticas e incremento de proteínas séricas en pollos de engorde alimentados con dietas suplementadas con Saccharomyces cerevisiae, *Investigación Clínica,* vol. 48, no. 4, pp. 431-43.

AVIAGEN-GROUP 2018, *Pollo de engorde: Manual de manejo*: Retrieved from http://es.aviagen.com/assets/Tech_Center/BB_Foreign_Language_Docs/Spanish_TechDocs/Ross-BroilerHandbook2018-ES.pdf, pp. 147.

Avilez, BL, Rugeles, C, Jabib, L & Herrera, YM 2015, Parámetros hematológicos en pollos de engorde criados en una granja de producción cerrada en el trópico bajo, *Revista de Medicina Veterinaria,* vol., no. 29, pp. 33-9. doi: 10.19052/mv.3444

Ayaşan T, 2013. Effects of dietary inclusion of protexin (probiotic) on hatchability of Japanese quails. Indian J Anim Sci, 83(1): 78-81

https://www.researchgate.net/publication/287696519_Effects_of_dietary_inclusion_of_protexin_probiotic_on_hatchability_of_Japanese_quails

ayasan, T., Ozcan, B.D., Baylan, M., Canogullari., S., 2006. The Effects of Dietary Inclusion of Probiotic Protexin on Egg Yield Parameters of Japanese Quails (Coturnix coturnix Japonica). International Journal of Poultry Science. 5(8):776-779.

http://free-journal.umm.ac.id/files/file/The%20Effects%20of%20Dietary%20Inclusion%20of%20Probiotic%20Protexin%20on%20Egg%20Yield%20Parameters.pdf

Cardoso, ALSP & Tessari, ENC 2003, Estudo dos parâmetros hematológicos em frangos de corte, *Arquivos do Instituto Biológico,* vol. 70, no. 4, pp. 419-24.

Cardoso, L, Cassimira da Silva, C, Silva, P, da Silva, CS, de Albuquerque, R, Araújo, LF, et al. 2014, Effects of the use of probiotics on the immune response and blood parameters of broilers *Revista Eletrônica Nutritime, 11*(3), 3450-64, Retrieved from http://www.nutritime.com.br/arquivos_internos/artigos/ARTIGO250.pdf

Castiglioni, EN, Fernandes, CA, Cardoso, ALSP, Ledoux, DR & Rottinghaus, G 2006, Parâmetros hematológicos de frangos de corte alimentados com ração contendo aflatoxina B1 e fumonisina B1, *Ciência Rural,* vol. 36, no. 3, pp. 924-9. doi: 10.1590/S0103-84782006000300030

Church, DC, Pond, WG & Pond, KR 2002, *Fundamentos de nutrición y alimentación de animales* (2ª Ed ed.), México: Ed Limusa.

Díaz, EA, Uribe, LF & Narváez, WV 2014, Bioquímica sanguínea y concentración plasmática de corticosterona en pollo de engorde bajo estrés calórico, *Revista de Medicina Veterinaria,* vol., no. 28, pp. 31-42. doi: 10.19052/mv.3179

Fernández, H, Morales, M, Amela, M, Salerno, C, Ganduglia, HR, Arenaz, F, et al. 2014, Efectos de la adición de probiótico (Bacillus subtilis) y omega 3 (Salvia hispanica L.) sobre los parámetros sanguíneos en pollos parrilleros, *Revista Agronómica del Noroeste Argentino,* vol. 34, no. 2, pp. 113-6.

Freitas, M, Tavan, E, Cayuela, C, Diop, L, Sapin, C & Trugnan, G 2003, Host-pathogens cross-talk. Indigenous bacteria and probiotics also play the game, *Biology of the Cell,* vol. 95, no. 8, pp. 503-6. doi: 10.1016/j.biolcel.2003.08.004

Gálvez, CF, Ramírez, GF & Osorio, JH 2009, El laboratorio clínico en hematología de aves exóticas, *Biosalud,* vol. 8, no. 1, pp. 178-88.

Gargallo, J 1979, Efectos nutricionales de la utilización de fibra en las dietas de monogástricos, *XVII Symposium de la Sección Española de la WPSA, Barcelona, 2*, 288-92, Retrieved from https://ddd.uab.cat/pub/selavi/selavi_a1980m8v22n8/selavi_a1980m8v22n8p288.pdf

González, JC, Hahn von-Hessberg, C & Narváez, W 2014, Características botánicas de Tithonia diversifolia (Asterales: Asteraceae) y su uso en la alimentación animal, *Boletín Científico Museo de Historia Natural,* vol. 18, no. 2, pp. 45-58.

Gutiérrez, LA, Montoya, OI & Vélez, JM 2013, Probióticos: una alternativa de producción limpia y de remplazo a los antibióticos promotores de crecimiento en la alimentación animal, *Producción+ Limpia,* vol. 8, no. 1, pp. 135-46.

Haile, Y & Chanie, M 2014, Comparative aspects of the clinical hematology of birds: a review, *British Journal of Poultry Sciences,* vol. 3, no. 3, pp. 88-95. doi: 10.5829/idosi.bjps.2014.3.3.83263

Hetland, H & Svihus, B 2001, Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens, *British Poultry Science,* vol. 42, no. 3, pp. 354-61. doi: 10.1080/00071660120055331

IDEAM 2015, *Características climatológicas de ciudades principales y municipios turísticos*: INSTITUTO DE HIDROLOGÍA, METEOROLOGÍA Y ESTUDIOS AMBIENTALES Retrieved from http://www.ideam.gov.co/documents/21021/21789/1Sitios+turisticos2.pdf/cd4106e9-d608-4c29-91cc-16bee9151ddd, pp. 48.

Itzá, MF, Magaña, MÁ & Sanginés, JR 2010, Evaluación de la harina de hoja de morera (Morus alba) en la alimentación de pollos de engorda, *Zootecnia Tropical,* vol. 28, no. 4, pp. 477-87.

Lara, C & Burgos, Á 2012, Potencial probiótico de cepas nativas para uso como aditivos en la alimentación avícola, *Revista Colombiana de Biotecnología,* vol. 14, no. 1, pp. 31-40.

Lázaro, D, Carcelén, C, Torres, A & Ara, G 2005, Efecto de probióticos en el alimento de marranas sobre los parámetros productivos de lechones, *Revista de Investigaciones Veterinarias del Perú,* vol. 16, no. 2, pp. 97-102. doi: 10.15381/rivep.v16i2.1546

Mahecha, L & Rosales, M 2005, Valor nutricional del follaje de botón de oro Tithonia diversifolia (Hemsl.) Gray, en la producción animal en el trópico, *Livestock Research for Rural Development,* vol. 17, no. 9, pp. Art. 100.

Mansoub, NH 2010, Effect of probiotic bacteria utilization on serum cholesterol and triglycerides contents and performance of broiler chickens, *Global Veterinaria,* vol. 5, no. 3, pp. 184-6.

Marchini, C, Nascimento, M, Silva, P & Guimarães, E Parámetros hematológicos de pollos de engorde sometidos a temperatura ambiental cíclica elevada, *XXII Congreso Latinoamericano de Avicultura 2011*, Retrieved from https://www.engormix.com/avicultura/articulos/parametros-hematologicos-pollos-engorde-t29072.htm

Mateos, G, Lázaro, R & Gracia, M 2002, Modificaciones nutricionales y problemática digestiva en aves, *XVIII Curso de especialización FEDNA.* , 15-37, Retrieved from https://www.researchgate.net/profile/Gonzalo_Mateos/publication/28179834_Modificaciones_nutricionales_y_problematica_digestiva_en_aves/links/0fcfd51421a8197bea000000.pdf

Miranda, S, Rincón, H, Muñoz, R, Higuera, A, Arzálluz, AM & Urdaneta, H 2007, Parámetros productivos y química sanguínea en pollos de engorde alimentados con tres niveles diéticos de harina de granos de fríjol (vigna unguiculata (l.) Walp.) Durante la fase de crecimiento, *Revista Científica,* vol. 17, no. 2, pp. 150-60.

Murgueitio, R & Ospina, SD 2002, *Tres especies vegetales promisorias: Nacedero Trichanthea gigantea (H. &B.) Nees; Botón de Oro Tithonia diversifolia (Hemsl.) Gray; y Bore Alocasia macrorrhiza (Linneo.) Schott*, Cali, Colombia: COLCIENCIAS-CAB-CIPAV.

Nieves, D, Terán, O, Cruz, L, Mena, M, Gutiérrez, F & Ly, J 2011, Digestibilidad de nutrientes en follaje de árnica (Tithonia diversifolia) en conejos de engorde, *Tropical and subtropical agroecosystems,* vol. 14, no. 1, pp. 309-14.

Odunsi, AA, Onifade, AA & Babatunde, GM 1999, Response of broiler-chicks to virginiamycin and dietary protein concentrations in the humid tropics, *Archivos de Zootecnia,* vol. 48, no. 183, pp. 317-25.

Peralta, M, Miazzo, R & Nilson, A 2008, Levadura de cerveza (Saccharomyces cerevisiae) en la alimentación de pollos de carne, *REDVET. Revista Electrónica de Veterinaria,* vol. 9, no. 10, pp. 1-11.

Perozo, F, Ferrer, J, Alvarado, M, Rincon, H, Mavarez, Y & Gil, M 2003, Haematological values in broiler chicks during long time-Low level exposure to Aflatoxin B-1 in Zulia State, Venezuela, *Revista Científica FCV-LUZ,* vol. 13, no. 1, pp. 59-64.

Quishpe, GJ 2006, *Factores que afectan el consumo de alimento en pollos de engorde y postura,* Agricultural Engineer Thesis, Zamorano University, Zamorano, Honduras, Retrieved from https://bdigital.zamorano.edu/bitstream/11036/930/1/T2297.pdf

Ruiz, RP, Romero, E, Hernández, H & Salinas, I 2015, Evaluación del fruto entero de palma de aceite ofrecido a voluntad en la alimentación de pollos de engorde, *Revista CITECSA,* vol. 5, no. 9, pp. 38-46.

Timmerman, H, Koning, C, Mulder, L, Rombouts, F & Beynen, A 2004, Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy, *International Journal of Food Microbiology,* vol. 96, no. 3, pp. 219-33. doi: 10.1016/j.ijfoodmicro.2004.05.012

Upendra, H & Yathiraj, S 2003, Effect of supplementing probiotics and Mannan Oligosaccharide on body weight, feed conversion ratio and livability in broiler chicks, *Indian Veterinary Journal,* vol. 80, no. 10, pp. 1075-7.

Veizaj, E, Piu, T, Lekaj, P & Tafaj, M 2010, Using combined probiotic to improve growth performance of weaned piglets on extensive farm conditions, *Livestock Science,* vol. 134, no. 1-3, pp. 249-51. doi: 10.1016/j.livsci.2010.06.155

Verdecia, DM, Ramírez, JL, Leonard, I, Álvarez, Y, Bazán, Y, Bodas, R, et al. 2011, Calidad de la Tithonia diversifolia en una zona del Valle del Cauto, *REDVET. Revista electrónica de Veterinaria,* vol. 12, no. 5, pp. 1-13. doi: http://www.veterinaria.org/revistas/redvet/n050511/051113.pdf

Zapata, MA 2017, *Efecto de la infusión de lippia alba en los parámetros bioquímicos en pollos de engorde,* Veterinarian Zootechnist Thesis, Universidad Técnica de Machala, Machala, Ecuador, Retrieved from http://repositorio.utmachala.edu.ec/bitstream/48000/10539/1/DE00007_TRABAJODETITULACION.pdf