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Tithonia diversifolia forage meal: effect on physiological indicators and blood biochemistry of two poultry categories

Harina de forraje de *Tithonia diversifolia*: efecto en indicadores fisiológicos y de la bioquímica sanguínea de dos categorías avícolas

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The use and research of plant resources that are abundant in the tropic have great importance for the monogastric species feeding, above all the poultry due to the poor production of cereals and to the financial limitations for their acquisition. *Tithonia diversifolia* (tithonia) for its agronomic characteristics and its good biomass production is an attractive alternative to guarantee a sustainable production. In this paper are show the results of researchers that were performed to evaluate the effect of *T. diversifolia* plant material 10 forage meal intake on physiological indicators of digestive organs and accessories of the gastrointestinal tract, as well as blood biochemistry indicators of two poultry categories. For this the chemical composition and secondary metabolites present in the plant material were determined. A total of five level of the tree forage meal (0, 5, 10, 15 and 20 %) were studied and four levels (0, 10, 15 and 20 %) as partial substitution of the corn/soybean meal in diets for 42 days broilers and replacement pullets of laying hens of 18 weeks respectively. The impact of their intake was evaluated in: 1) macroscopic morphometric indicators of digestive organs: crop, proventriculus, gizzard, small intestine, caeca, large intestine and accessories (liver and pancreas) of the gastrointestinal tract; 2) microscopic morphometric indicators (histophatologic evaluation); 3) quantitative evaluation of the morphometric changes in the structure of the studied organs with the intake of *T. diversifolia* plant material 10 forage meal in laying hens replacement and 4) determination of the blood biochemistry indicators in both categories. The data were analyzed when considering the morphofunctional capacity of the digestive system and accessory organs in the mentioned poultry category. The results obtained contributed to the assessment of the physiological response and influence on the establishment of the adequate levels of *T. diversifolia* to achieve a sustainable animal production.

Key words: *birds, blood indicators, digestive organs and accessories of the gastrointestinal tract, morphometric indicators, Tithonia diversifolia forage meal*

La utilización e investigación de recursos vegetales que son abundantes en el trópico reviste gran importancia para la alimentación de especies monogástricas, sobre todo la avícola, debido a la deficiente producción de cereales y a las limitaciones financieras para su adquisición. *Tithonia diversifolia* (tithonia) por sus características agronómicas y su buena producción de biomasa es una alternativa atractiva para garantizar una producción sostenible. En este artículo se presentan los resultados de investigaciones que se condujeron para evaluar el efecto del consumo de harina de forraje de *T. diversifolia* material vegetal 10 en indicadores fisiológicos de órganos digestivos y accesorios del tracto gastrointestinal, así como indicadores de la bioquímica sanguínea de dos categorías avícolas. Para ello se determinó la composición química y los metabolitos secundarios presentes en el material vegetal Se estudiaron cinco niveles de harina de forraje de la arbórea (0, 5, 10, 15 y 20 %) y cuatro niveles (0, 10, 15 y 20 %) como sustituto parcial de la harina de maíz/soya en dietas para pollos de engorde de 42 días y pollitas de reemplazo de ponedoras de 18 semanas de edad, respectivamente. Se evaluó el impacto de su consumo en: 1) indicadores morfométricos macroscópicos de órganos digestivos: buche, proventrículo, molleja, intestino delgado, ciegos e intestino grueso y accesorios (hígado y páncreas) del tracto gastrointestinal; 2) indicadores morfométricos microscópicos (evaluación histopatológica); 3) evaluación cuantitativa de los cambios morfométricos en la estructura de los órganos estudiados con el consumo de harina de forraje de *T. diversifolia* material vegetal 10 en reemplazo de ponedoras y 4) determinación de indicadores de la bioquímica sanguínea en ambas categorías. Los datos se analizaron al considerar la capacidad morfofuncional del sistema digestivo y órganos accesorios en la categoría avícola referida. Los resultados obtenidos contribuyeron a la valoración de la respuesta fisiológica e influyeron en el establecimiento de los niveles adecuados de *T. diversifolia* para lograr una producción animal sostenible.

Palabras clave: *aves, harina de forraje de Tithonia diversifolia, indicadores morfométricos, indicadores sanguíneos, órganos digestivos y accesorios del tracto gastrointestinal*

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Introduction

Currently, according to FAO (2018), the use of tree and shrub plants to replace traditional sources such as soybean and corn in the diet of monogastric species, including poultry, constitutes a very attractive food alternative because it reduces the rations cost due to import substitution, and promotes a more diverse and sustainable feeding system. Among these plants *Tithonia diversifolia* plant material 10 highlighted, which is characterized by its availability and excellent nutritional value (Scull *et al.* 2019).

In the countries of the Latin American and Caribbean region, such as Mexico, Colombia and Cuba, researches to establish the level of tithonia intake, as leave meal or foliage in broilers were carried out (Gutiérrez-Castro and Hurtado 2019). Tithonia was also used as forage meal (leaves +young stems) in broilers (Rodríguez *et al.* 2020) or in laying hens and their replacements (Rodríguez *et al.* 2018, Fuentes-Martínez *et al.* 2019 and Vázquez *et al.* 2023). Most of the studies refer to nutritional aspects, such as productive performance, carcass yield, very few deals with the physiological response of the digestive system and its accessory organs as well as the analysis of the blood biochemistry profile (Gutiérrez-Castro and Corredor Mateus 2019) in each of these categories.

The objectives of this study were to show the researches performed in the Instituto de Ciencia Animal to evaluate the effect on physiological indicators and the blood biochemistry when including different levels of *T. diversifolia* plant material 10 forage meal as partial substitution of corn/soybean meal in diets of broilers and replacement pullets of laying hens of 42 days and 18 weeks of age, respectively.

For this the fallowing researches were performed:

- To determine the chemical composition and secondary metabolites of *T. diversifolia* forage meal
- To determine the physiological indicators (macroscopic morphometric) of digestive organs and accessories of the gastrointestinal tract, in broilers and replacement pullets of laying hens that intake five levels (0, 5, 10, 15 and 20 %) and four levels (0, 10, 15 and 20 %) of *T. diversifolia* forage meal, respectively
- To evaluate microscopic morphometric indicators (histophatologic evaluation) with the intake of four levels (0, 10, 15 and 20 %) of *T. diversifolia* forage meal in replacement pullets of laying hens
- To perform a quantitative structural evaluation of the morphometric changes in the different studied organs of replacement pullets of laying hens which intake the mentioned levels of *T. diversifolia* forage meal
- To determine indicators of the blood biochemistry in broilers and replacement pullets of laying hens that intake levels of 0, 5, 10, 15 and 20 % and 0, 10, 15 and 20 % of *T. diversifolia* forage meal, respectively

Materials and Methods

In all the experiments the tree species *Tithonia diversifolia* plant material 10 was used which was establish in red ferralitic soil of rapid drainage and uniform profile (Hernández *et al*. 2019). The plant material was harvested in Unidad de Pastos y Forrajes Miguel Sistachs Naya from Instituto de Ciencia Animal. The leaves and young stems of the plant were used with cutting ages between 60 and 70 days at a height of 15cm (Ruiz *et al*. 2017). The tithonia forage meal was made according to Savón *et al*. (2020).

Chemical characterization of Tithonia diversifolia forage meal: For the study of the chemical composition a total of 1 kg of forage meal at a 1mm particle size was milled and stored in amber bottles until the moment of the analysis. The analysis of the chemical composition indicators was performed by sextuplicate.

The bromatological analysis was performed to determine the dry matter (DM), crude protein (CP) and ashes (A) according to AOAC (2019). The Ca was quantified in atomic absorption equipment. The phosphorous determination (P) was performed according to Amaral (1972) methodology. The cell wall components were also analyzed. The neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (Lig) and cellulose (Cel) were determined according to the procedures described by Göering and VanSoest (1970). Also, the phytochemical sieves tests were performed to the plant material 10 forage meal according to Miranda and Cuellar (2000). The presence of 12 functional groups was researched: alkaloids, saponins, triterpenes/steroids, tannins, proantocianidines, coumarines, quinines, reducing sugars, amine groups, resins and cardenolides. The total condensed tannins (TCT) were quantified according to the procedures of Porter *et al.* (1986) test, modified by Makkar (2003).

Statistical analysis: A descriptive statistical analysis was carried out (mean, standard deviation and variation coefficient (%). For the analysis of results the statistical system Infostat (Di Rienzo *et al*. 2012) was used.

Animals and diets: A total of 40 male broilers of the Cuban hybrid EB_{24} of 42 days old and 32 replacement pullets of laying hens White Leghorn L_{33} of 18 weeks were used to evaluate the effect of tithonia intake on macroscopic morphometric indicators of digestive organs and accessories of the gastrointestinal tract of both. In the replacement pullets it was also performed a qualitative histopathologic evaluation and microscopic structural changes in digestive organs and accessories of the gastrointestinal tract. All the animals were from a productive performance experiment. The broilers were distributed in five inclusion treatments (0, 5, 10, 15 and 20 %) of plant material, while for the pullets were included four levels (0, 10, 15 and 20 %). The basic diet was soybean meal as protein source and corn as energy source. In all cases a total of eight repetitions per treatment were performed. During all the experimentation time the animals had free access to water and food.

The experimental diets were formulated according to the nutritional recommendations for broilers of Rostagno *et al.* (2017), while for the pullets were fallow the UECAN (2013) recommendations. For the formulation of broilers diets, look Savón *et al.* (2022) and in the case of pullets look Vázquez *et al*. (2018).

Experimental procedure: At 42 days of age a total of 8 birds per treatment were weighed (total of animals 40), and at 18 weeks of age the replacement pullets of laying hens were weighed (total of animals 32), whose weights were corresponded with the average value of each treatment. The selection was made based on the average weight of each group of birds on a range of 10 % and the indicators that the following experimental procedure shows were determined.

Morphometric indicators of digestive organs and accessories: The birds were sacrificed two hours and thirty minutes after food ingestion, by the method of jugular vein bleeding, described by Sánchez (1990). Subsequently, the procedure described by Savón *et al*. (2021) was fallowed. The data of organs were expressed as absolute weight (g) and as relative weight (g g live weight^{-1} 100). The lengths of the small intestine, caeca and colon - rectum were measured with a tape measure.

Histopathologic evaluation: A total of 32 replacement pullets of laying hens were used and sacrificed according to that described by Savón *et al*. (2021). For the microscopic test four 1cm^2 samples of proventriculus, gizzard, small intestine (ileum), caeca and liver were taken. The histopathologic evaluation was performed according to POT (05.22.016) in Pathology Laboratory of Toxicology Experimental Center (CETEX) from Centro para la Producción de Animales de Laboratorio (CENPALAB). A microscope Motic, BA410E was used for this.

Histological evaluation (quantitative): The process start performing the observation of histological sheets with the optical microscope MoticBA410E.Then the corresponding images were taken with a camera for instant digital microscopy Motican 1080 BMH, with a resolution of 3 megapixels, in magnification of 40× and those images were analyzed with the software for images treatment Motic® Images plus 3.0 (Motic China Group Co.)

For the morphometric study of the histological cuts of the digestive system seven variables per animal were determined which were measured with the help of the software processing and analysis of images: in the proventriculus mucus height (µm), diameter of the glandular buds (µm); in the gizzard: mucus height (µm); in the small intestine: villi height (µm), villi width (µm), depth of Lieberkühn crypts (µm) and in the caeca: mucus height (µm).

Later, the images were filed in JPG format to keep all details observed in the microscope and after the variables were measured with the help of the software processing and analysis of images Motic. An area per variable and animal was counted.

Blood biochemistry indicators: The blood sample was directly taken from the jugular vein when the animals were slaughtered. A total of 40 male broilers and 32 replacement pullets were used (8 per treatment for both categories). In broilers were determined: cholesterol, glucose, triglycerides, total proteins, albumin and the enzymes alanine amino transferase, aspartate amino transferase and the uric acid. In the case of the replacement pullets were analyzed: total proteins, cholesterol, triglycerides and glucose. All determinations were performed on blood serum using a Cobas integra 400PLUS automatic analyzer (Roche Diagnostic System).

Experimental design and statistical analysis: The results of the morphometric experiment of broilers were analyzed according to a completely random design, the initial weight of animals was fitted as covariable. The data was processed with the statistical package InfoStat 2012 (Di Rienzo *et al.* 2012). For means comparison the Duncan (1955) test was used, for $p<0.05$, in the necessary cases.

For the experiment of macroscopic morphometric indicators of digestive organs and accessories of the gastrointestinal tract of replacement pullets of laying hens, the design and statistical analysis were similar to those used in broilers.

In the case of the microscopic morphometric evaluation of the structural changes in the digestive system organs the data obtained from the performed measurements were introduce in the statistical package Graphpad, version 8.1. From them the descriptive statistics for each analyzed indicator were determined. The normality was determined by the Kolmogorov-Smirnov test. corrected by Lilliefors (1967) The homogeneity of variances was determined by the Levene test (1960). When these premises were fulfilled, simple factorial Analysis of Variance (ANOVA) was performed taking into account the variable experimental group; using to determine the differences the Student test. When it was not fulfilled one or both premises (normality or homogeneity of variance), a non -parametric analysis was carried through the Kruskal-Wallis (1952) test using to determine the differences the non parametric Dunn (1964) test. It was work for a signification level of $p<0.05$.

Results and Discussion

Chemical characterization and secondary metabolites of T. diversifolia plant material 10 forage meal: The thitonia plant material 10 forage meal showed a contribution of 24.6 % of crude protein, 20.3 % of ashes, 2.38 % of Ca, 36.07 % of neutral detergent fiber, 32.48 % of acid detergent fiber and 25.87 % of cellulose, which characterized it as an alternative protein source high in fiber.

The ashes content is an indicator of the proportion of inorganic compounds present in the plant. In this study the ashes levels were high, aspect that is in correspondence with the Ca high concentrations which were find and are related with previous studies (Fuentes-Martínez *et al.* 2019). Despite, the insoluble fiber constituted the majority component of the meal, the values are within the specified for animal food and are lower to those reported by Savón (2010) for a group of tropical plants.

The nutritional value of a food depends among other factors, of the secondary metabolites presence. These compounds can have beneficial or anti-nutritional effects on the animal, depending on their concentration, chemical structure and bioavailability, as well as, the characteristics of the animal species (Scull 2018). From the functional groups looked through the qualitative tests of the phytochemical sieve there were found low levels of alkaloids, phenolic compounds and triterpenes, metabolites that can provoke a marked biological activity in the animals.

González *et al*. (2019) reported the presence of other secondary metabolites in the plant which there were not find in this study as: saponins, coumarines and quinines. This could be related with the insufficient concentration of these compounds in the meal for their effective detection with the used procedures. The above could be determined by changes in the metabolism of these compounds due to genetic differences and phenological state. The absence of saponins, coumarines and the low amounts of alkaloids and terpenes point to the phytochemical quality of the meal from this material as animal food. The secondary compounds essentially, can cause nutritional disorder, weight loose and gastrointestinal difficulties, when they are in high concentrations or are use in high proportions in the diet (Scull *et al.* 2017).

The quantification of the proportions of phenolic compounds showed values of 9.83 and 3.85 g/kg of DM for total tannins (TT) and condensed tannins (CT), respectively refer to figures of total protein (TP) of 19.45 g/kg DM. The low concentration of CT, TT and TP corroborate the results of the phytochemical sieve and it is comparable with the values reported by García *et al.* (2008) in trees and forage scrubs of use in animal feeding. Taking into account the above, this group of compounds should caused beneficial effects in the animal and influence positively in their biological activity. According to González-Sierra *et al.* (2019), the antioxidant activity that *T. diversifolia* has is associated with the presence of flavonoids and other phenolic compounds present in the plant.

Conclusion: The results of this experiment showed that the plant material 10 forage meal has small amounts of phytochemical compounds of great biological importance, that can has a beneficial impact for the health and nutrition of monogastric animals.

Macroscopic morphometric indicators of digestive organs and accessories of the gastrointestinal tract of broilers and replacement pullets of laying hens: Once the chemical composition and secondary metabolites presents in the *Tithonia diversifolia* plant material 10 forage meal were determined, the effect of this tree intake was evaluated on morphometric indicators of the gastrointestinal tract and accessories organs of the gastrointestinal tract of broilers and replacement pullets of laying hens.

In broilers, the relative weights of the empty gastrointestinal tract, full gizzard, empty small intestine and full colon-rectum were similar for all the experimental treatments (table 1). The above means that the intake of tithonia forage meal did not influence on the relative weights of these organs. In comparison, it was observed that the 20 % of this fibrous food caused an increase of the empty gizzard weight, which is associated with a great activity of the organ as result of the increasing amounts of insoluble fiber provide by the inclusion of tithonia in the diets (Jiménez-Moreno *et al.* 2010).

The relative length of the small intestine of birds was similar for all the experimental treatments, with the exception of treatment 10% of tithonia forage meal that decrease with respect to levels of 15 and 20 % of substitution. Respect to the caeca, the relative length of the left caecum of the birds that intake the control diet, 5 and 10 % of inclusion of tithonia forage meal did not differ among them, but it increase with the levels 15 and 20 %. This is due to physiological response that is caused by the increase of the stay time of the fibrous particles in this organ, the action of the microbial mass and final products of fermentation. These sections inside the digestive tract are the only place where the fiber digestion takes place due to the fermentative activity the bacteria and cellulolytic fungi presents there has (Rodríguez *et al.* 2012). The magnitude of the response can be affected by the physiological composition of the fiber present in the diet (Savón 2010).

The intake of 20 % of tithonia forage meal caused an increase of the relative weight of the crop full and empty (table 2). The high intake of insoluble fiber provided by the tithonia forage meal increased the organ volume and alters their morphology.

The intake of tithonia forage meal did not influence on the relative weights of the full and empty proventriculus, the empty colon-rectum and the abdominal fat (tabla 2). Therefore, it was observed a significant increase of the relative weight of the full caeca, in relation with the control treatment with increasing fiber levels.

The above, it was not due to fermentative processes that occur in this section of the gastrointestinal tract, since the empty caecum did not showed differences among treatments. It is possible that the fiber contained in the digest, draw water in its organ and this cause the caeca contents increase. The relative length of the colon-rectum was similar. The analysis of the accessory organs of the gastrointestinal tract (liver and pancreas) did not showed differences among treatments.

Respect to the replacement pullets of laying hens, the inclusion of 15 and 20 % of tithonia forage meal increased the relative weight of the empty gastrointestinal tract of birds, which is associated with the increase of their digestive capacity when intake this bulky food (Savón *et al.* 2019). These levels also increased the relative weight of the gizzard, similar effect which was previously observed in broilers.

An interesting result was obtained in the relative length of the small intestine (cm/g LW x100) which was increased with the levels of tithonia forage meal (8.89, 9.20, 9.59 and 10.43 for 0, 10, 15 and 20 %, respectively) ($p<0.0515$), very close of $p<0.05$. This show that there are differences from the biological point of view.

Table 1. Relative weight (g g^{-1} LWx100) and relative length (cm $organ^{-1}$ LWx100) of digestive organs of broilers that intake different levels of tithonia forage meal. (Significant covariable)

Treatments/Indicators	Levels of tithonia forage meal (%)					Signif. Covariables	Signif.
	0	5	10	15	20		
Live weight	2160[a]	2204[a]	2113[a]	2091[a]	1933[b]		
Empty gastrointestinal tract	6.60 (±0.21)	6.50 (±0.22)	6.44 (±0.20)	7.02 (±0.20)	6.94 (±0.24)	p=0.0055	p=0.2510
Full gizzard	3.24 (±0.17)	3.50 (±0.18)	2.87 (±0.17)	3.42 (±0.17)	3.25 (±0.20)	p=0.0172	p=0.0969
Empty gizzard	1.95[b] (±0.08)	2.13[b] (±0.09)	2.11[b] (±0.08)	2.42[a] (±0.08)	2.40[a] (±0.10)	p=0.0251	p=0.0032
Empty small intestine	3.04 (±0.12)	2.87 (±0.13)	2.73 (±0.12)	3.04 (±0.12)	2.74 (±0.15)	p=0.0014	p=0.2247
Full colon-rectum	0.35 (±0.04)	0.41 (±0.05)	0.43 (±0.04)	0.47 (±0.04)	0.39 (±0.05)	p=0.0107	p=0.4562
Relative length of small intestine	9.72[ab] (±0.28)	9.24[ab] (0.30)	8.93[b] (±0.27)	10.39[a] (±0.27)	10.09[a] (±0.33)	p=0.0014	p=0.0070
Left cecum length	0.99[b] (±0.03)	1.05[ab] (±0.04)	1.04[ab] (±0.03)	1.13[a] (±0.03)	1.13[a] (0.03)	p=0.0101	p=0.0453
Rigth cecum length	0.92 (±0.03)	0.97 (±0.03)	0.98 (±0.03)	1.06 (±0.04)	1.05 (±0.04)	p=0.0413	p=0.0762

[ab] Means with different letters in the row significantly differs (Duncan 1955)

Table 2. Relative weights (g g LW^{-1}) of full and empty digestive organs of broilers that intake different levels of tithonia forage meal (NS covariable)

Treatments Indicators	Levels of tithonia forage meal (%)					EE (±), Signif.
	0	5	10	15	20	
Live weight	2160[a]	2204[a]	2113[a]	2091[a]	1933[b]	42.06, p=0.0009
Full GIT	10.42[b]	11.00[b]	9.64[b]	11.87[b]	14.47[a]	0.73, p= 0.0005
Full Crop	0.93[b]	1.95[b]	1.48[b]	1.96[b]	4.05[a]	0.48, p=0.0007
Empty Crop	0.47[b]	0.45[b]	0.56[b]	0.52[b]	0.79[a]	0.05, p=0.0004
Full proventriculus	0.56	0.52	0.50	0.49	0.49	0.04, p=0.7316
Empty Proventriculus	0.48	0.42	0.39	0.41	0.42	0.03, p=0.2803
Full small intestine	4.76[a]	4.03[b]	3.56[c]	4.51[ab]	4.81[a]	0.23, p=0.0020
Empty Colon-rectum	0.21	0.21	0.25	0.23	0.23	0.02, p=0.4377
Full caeca	0.70[c]	0.81[b]	0.84[b]	1.01[ab]	1.13[a]	0.08, p=0.0033,
Empty caeca	0.44	0.39	0.40	0.41	0.41	0.02, p=0.7267
Colon-rectum length	0.48	0.46	0.57	0.1	0.57	0.04, p=0.0517
Abdominal fat	1.46	1.41	1.39	1.58	1.70	0.14, p=0.4609

[abc] Means with different letters in the row differ to $P<0.05$ (Duncan 1955)

The length of the small intestine could be relate with surface of the intestinal mucus and an increase of this one involve a high nutrients absorption, which lead to good body development of the future laying hen (Savón *et al.* 2017). This necessarily influence on the reproductive and productive response of the pullets. The length of the colon-rectum did not alter with the different levels of tithonia forage meal in the diet. The meal did not influence on the rest of the evaluated physiological indicators.

Conclusions: The relative weights of the digestive organs and accessories of the gastrointestinal tract of broilers EB_{34}, with the exception of the relative length of the left caecum, are not modified up to the 10 % of inclusion of tithonia forage meal in the diet. In replacement pullets of laying hens L_{33} of 18 weeks, the inclusion up to 20 % of tithonia forage meal did not modify the relative weights of the digestive organs and accessories of the gastrointestinal tract. Therefore, increase the small intestine length, which suggest higher nutrients absorption and a beneficial effect by their intake.

Effect of the tithonia forage meal intake on the histology of digestive organs and accessories of the gastrointestinal tract of replacement pullets of laying hens: In the case of replacement pullets due to the importance of this category, an histopathology study was performed with the purpose of research if the tithonia effect was able to cause a lesion at level of tissues of the digestive organs and accessories of this category.

The proventriculus, gizzard, caeca and liver were analyzed. It was observed that the changes find at level of tissues in the proventriculus, gizzard and liver were not attributable to the tithonia intake, since there were found also in the control animals

The most important find was identified in the caeca, where there was observed a moderate hypertrophy of the caecal tonsils which was in the treated animals and with an incidence of the 50, 25 and 12.5 % for the groups 2, 3 and 4, respectively.

The caecal tonsils are a specialized lymphoid tissue and their hypertrophy could be related with the supply of the plant material under study. These structures are exposing directly to the bolus during the digestion process, which has food, pathogen agents or vaccine. This allows the recognition of antigens with the production of effective immune responses in this level. The fact that the incidence of this change will be higher in the group supplied with the lower level of tithonia can be related with the individuality of the immune response of each individual. The above has great importance in the study of the immune response.

Quantitative structural evaluation of the morphometric changes in digestive organs of replacement pullets of laying hens, that intake T. diversifolia forage meal: The diet is one of the most important factors in the modulation of the structural and functional development of the intestine. The change of diet which the animal is faced is accompanied of modifications in the digestive and transportation functions with the characteristics of the mature organ are acquired. That is why it is important to perform a morphometric study of structures from the digestive system with the aim of quantify the changes associated to the administration of the diets with the inclusion of different levels of tithonia forage meal.

Table 3 shows the morphometric study of the values of the mean and standard deviation of each evaluated variable, per experimental group. There was only significant statistical differences in the width of the intestinal villi, between the control group and the supplied with the 20 % of tithonia meal (p=0.0153). This result possibly is associated to the supply of the higher concentration (20 %) of tithonia forage meal and it confirms the increase found in the length of the small intestine in the study of the macroscopic morphometric indicators.

Table 3. Means values and standar desviation (SD) of the studied variables per experimental group

Morfhometrics variable analised	Control	10 % HTD	15 % HTD	20 % HTD
Diameter of the proventriculus buds	1745, SD=269.7	1828, SD=262.8	1835, SD=259.2	1789, SD=372.3
Height of the proventriculus mucus membrane (µm)	828.4, SD=125.4	969.8, SD=125.4	849.0, SD=115.8	958.8, SD=155.1
Height of the gizzard mucus membrane (µm)	975.5, SD=210.5	951.4, SD=501.0	1034, SD=382.5	853.0, SD=55.25
Height of the intestinal villi (µm)	1402, SD=543.9	1649, SD=319.4	1566, SD=591.3	2014, SD=288.5
Width of the intestinal villi (µm)	161.9^{c}, SD=131.1	363.9^{b}, SD=92.22	345.0^{b}, SD=179.5	411.9^{a}, SD=45.84
Depth of the intestinal crypts (µm)	138.6, SD=62.44	170.9, SD=39.50	173.3, SD=66.80	194.3, SD=37.11
Heigth of ceacum mucus membrane (µm)	1150, SD=421.6	1633, SD=438.0	1349, SD=373.1	1427, SD=296.1

^{a,b,c} Means with different letters differs P< 0.05 Dunn (1964)

SD: Standard deviation

Figure 1 shows the difference in ileum vili width between birds that received a control diet (A) based on maize/soya and those that include 20 % of tithonia forage meal in the diet (B).

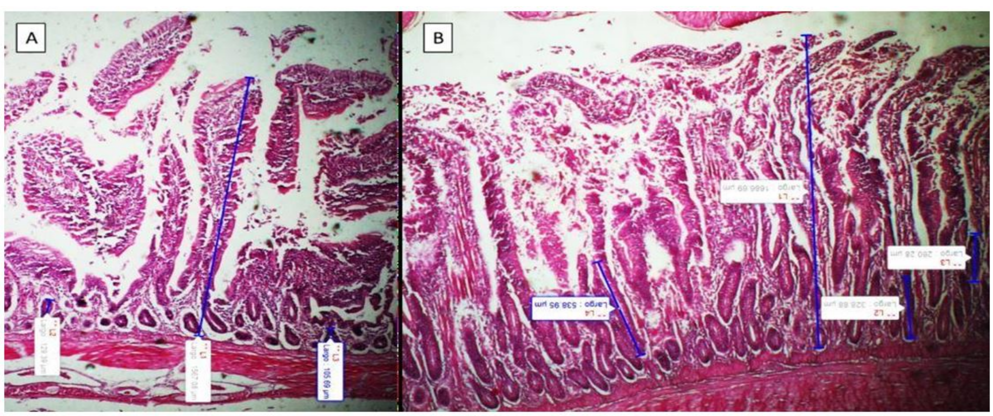
The rest of the studied variables did not differ between treatments. It is important to highlight that although there was not significant statistical difference as to villi height and the dep of crypts between the experimental groups, the results show increase of the values as the concentration of food in the diet increase.

Conclusion: The results of the morphometric experiments of the replacement pullets allow recommending the inclusion 20 % of *Tithonia diversifolia* forage meal in the formulation of diets for this poultry category.

Indicators of the blood biochemistry in broilers and replacement pullets of laying hens that intake different level of T. diversifolia forage meal: The indicators of the blood biochemistry are resources which are use to evaluate the physiological state and consider the nutritional performance of the animal once it provide a knowledge of the metabolism and functioning of organs as the kidney and liver of birds (Gutiérrez-Castro y Corredor-Mateus 2019) and its determination is an important tool in the monitoring of the health of all species.

Table 4 shows indicators of blood biochemistry of 42-days-old broilers fed with different levels of tithonia forage meal.

The serum indicators of the protein metabolism: total proteins, albumin and albumin/globulin ratio did not differ between treatments, with the exception of the uric acid. Related to the total proteins are reported as normal values 30-50 g/L (Miranda *et al.* 2007) and 35.6 ± 0.34 g/L (Nunes *et al.* 2018), while Café *et al.* (2012) show figures of 26.5 g/L which are close to those obtained in this experiment. In this regard, the differences may be due to the influence of various factors such as the diets, breed and



Control Treatment of 20 %

Figure 1. Histological analysis of ileum villi width

Table 4. Indicators of the blood biochemistry of broilers that intake tithonia forage meal

Treatments Variables	Levels of tithonia forage meal (%)					EE (±), Signif.
	0	5	10	15	20	
A/G	0.80	0.79	0.88	0.86	0.84	0.07, p=0.8574
TP (g/L)	25.26	28.30	25.36	26.19	28.61	1.76, p=0.5122
ALB (g/L)	10.78	11.96	11.08	12.45	12.39	0.56, p=0.1421
ALAT (U/L)	2.63	2.13	2.00	3.00	2.38	0.42, p=0.4596
ASAT (U/L)	224.38	258.88	243.88	251.13	235.50	12.36, p=0.3360
CHOL (mmol/L)	3.42	3.53	3.45	4.03	3.80	0.18, p=0.0936
GLU (mmol/L)	12.30	12.68	12.43	13.40	12.87	0.75, p=0.8551
TG (mmol/L)	1,39	1.50	1.56	1.69	1.68	0.17, p=0.7126
UA (mmol/L)	278.50^{b}	305.38^{b}	275.00^{b}	396.63^{a}	330.50^{ab}	29.91, p=0.0424

ALB: albumin, A/G: relation albumin/globulin, GLU: glucose, TP: total proteins, TG: triglycerides; UA: uric acid, CHOL: cholesterol, ASAT: Aspartate amino tansferase, ALAT: alanine amino transferase.

^{a,b} Means in the same row with different letters differ to P<0.05 Duncan (1955) environmental conditions in which the experiments were carried out.

Albumin values were within the known range for this indicator (10.8-16 g/L), according to Gálvez *et al.* (2009), which suggests that the studied tithonia forage meal levels did not affect the liver protein synthesis. Regarding globulins (taking into account that they are calculated as the difference between total proteins and albumin) were lower compared to those obtained by Gutiérrez-Castro and Corredor-Mateus (2019) (17-20 g/L) with tithonia forage meal (does not specified if it is leaf or forage). Despite this, the albumin/globulin ratio is considered quite favorable and indicative that the birds were fine from a nutritional point of view, since it is close to which is the optimum ratio.

The serum uric acid depends on the quality as well as the quantity of protein supplied in the diet. The highest figure was find with the level of 15 % of tithonia forage meal, which differed (P<0.0424) in 118.13 µmoles/L from the control, although it was similar to the 20 % inclusion of this food that, in turn, did not differ from the control.

The determination of uric acid is carried out to evaluate the renal function of animals. Is the main product of protein catabolism in birds, constituting between the 60 and 80 % of the total nitrogen excreted in the urine, so serum concentrations higher than 308.57 µmoles/L, suggest alteration of renal function. Rodríguez (2017) and Gutiérrez-Castro and Corredor-Mateus (2019) find that the diets with higher substitutions of tithonia forage meal showed the highest uric acid values, so it is infer that when the birds intake a higher protein percentage , the uric acid excretion increase.

The alanine amino transferase and aspartate amino transferase enzymes showed normal values for all treatments, so the intake of levels of up to 20 % of tithonia forage meal did not cause health disorders in the animals. These enzymes refer the state of liver function. From these results it can be inferred that, since there were no significant differences between treatments, the birds did not have liver problems that could be attributed to the supplied diets.

The indicators of energy metabolism: glucose, cholesterol and triglycerides did not differ between treatments. The first two were in the normal range for the species: glucose (11-21 mmol/L) and cholesterol (2.58-5.17 mmol/L), according to Gálvez *et al.* (2009) and Holguin *et al.* (2009), respectively. For triglycerides, the figures were higher than 0.45- 1.35 µmol/L, reported by Nunes *et al.* (2018).

In the case of the replacement pullets, the blood biochemistry indicators: total proteins, uric acid and triglycerides (table 5) did not differ with the increase of the tithonia forage meal levels. The total proteins were in the normal intervals of 30-50 g/L (Miranda *et al*. 2007). It was observed that the highest figure of the uric acid was find with 20 % of tithonia forage meal and showed an increase of 83.00, 90.50 and 150.32 µmol/L respect to 0, 10 and 15 % of tithonia forage meal, respectively. Noticed that 15 % represent the half of the serum concentration of the inclusion of 20 % of tithonia forage meal (338.00 µmol/L). This last result should be analyzed, since it was refer in the above experiment, the uric acid, final product of the protein catabolism in birds, is increase with the protein levels (Gutiérrez-Castro and Corredor-Matheus 2019).

Table 5. Blood indicators of replacement pullets of laying hens (1-18 weeks) that intake different levels of tithonia forage meal

Teatment Indicators	Levels of tithonia meal forage (%)				EE(±), Signif.
	Control	10	15	20	
TP (g/L)	41.03	43.80	47.05	43.15	±2.32, p=0.3649
UA, µmol/L	255.00	244.50	187.68	338.00	±46.77, p=0.2088
Chol, mmol/L	3.11	3.06	3.53	3.38	±0.23, p=0.4701
TG, mmol/L	0.95	1.08	1.26	0.98	±0.17, p=05761

Chol: cholesterol; TP: total proteins; TG triglycerides; UA: uric acid

The lipid metabolism indicators cholesterol and triglycerides either differ between treatments. The cholesterol was in the normal range of (2.58-5.12 mmol/L) stipulated for birds (Holguín *et al.* 2009).

It is of interest that the cholesterol levels are similar to those obtained with the broilers. According to Martínez (2004) the lipid fractions, are affected by the physiological and nutritive state of the bird.

From the physiological point of view, this type of birds show an increase in the lipid metabolism compared with broilers (Osorio and Florez 2011 and Osorio and Florez 2018). Unfortunately, the total lipids which could clarify the differences in the lipid accumulation between males and females were not determined.

Conclusions

The serum indicators of the protein metabolism (total proteins, albumin, and A/G ratio as well as the energy metabolism indicators (glucose, cholesterol and triglycerides) did not differ with the inclusion of up to 20 % of tithonia forage meal in the diet of broilers of 42 days, except the uric acid that was increased.

The alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) enzymes did not differ between treatments and were in the normal ranges with the intake of up to 20 % of tithonia forage meal in the diet of broilers of 42 days, which guarantee the good liver function of the animals.

The blood biochemistry indicators: total proteins, uric acid, cholesterol and triglycerides, did not differ with the increase of up to 20 % of tithonia forage meal in substitution of corn/soybean of the diet of replacement pullets of laying hens.

Future perspectives

As result of the general analysis of these researches it can state:

- It is necessary to quantify the majority secondary of the metabolites which are in the tithonia plant material 10 forage meal and to assess their biological activity.
- In broiler is essential to perform a histophatologic study of the digestive organs and accessories of the gastrointestinal tract to assess if there are damages caused by the intake of tithonia plant material 10 forage meal.
- To analyze the microscopic structural changes of the digestive organs of the gastrointestinal tract of broilers that intake tithonia plant material 10 forage meal.
- In replacement pullets of laying hens to perform studies of apparent retention of nutrients with a view to prove the results obtained in the morphometric microscopic studies.
- To extend these studies to other productive poultry species.

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