EFFECT OF THREE FODDER TREES ON *Haemonchus contortus* CONTROL AND WEIGHT VARIATIONS IN KIDS

Efecto de tres árboles forrajeros en el control de *Haemonchus contortus* y cambios de peso en cabritos

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ABSTRACT. The objective was to evaluate the activity against *Haemonchus contortus* by foliage taken from three tanniferous tree species and fed to Creole male kids experimentally infected with a dose of 350 larvae of *H. contortus* (L3) kg^{-1} body weight (BW). Twenty Creole kids weighing 12 ± 2.0 kg were randomly distributed in four treatments: T1 = control without foliage, and the addition of fresh foliage (10 % of the dry matter of the total diet) of *Guazuma ulmifolia* (T2), *Pithecellobium dulce* (T3) and *Acacia cochliacantha* (T4). For sixty days, the activity of the foliage against *H. contortus* was evaluated by quantifying the reduction in the excretion of eggs per gram of feces; also, the excretion of eggs was associated with the concentration of the blood cell pack, dry matter intake and weight change in animals. The data of the evaluated variables were analyzed in a completely randomized design and a Tukey test was carried out for comparison of means ($p < 0.05$). The secondary compounds of *P. dulce* and *A. cochliacanta* foliage were more effective ($p < 0.04$) in the control of *H. contortus* with minor excretion of eggs (1.48 and 1.18 Log^{10} g^{-1} of feces), respectively. The hematocrit was different ($p < 0.005$) mainly in hemoglobin, erythrocytes, lymphocytes and eosinophils; dry matter intake ($p < 0.05$) and changes in total body weight ($p < 0.01$) were higher in kids which received *P. dulce* foliage (T3) with 621.5 g d^{-1} and 2.4 kg, respectively. It was concluded that the secondary compounds of fresh *P. dulce* and *A. cochliacantha* foliage have the potential to control *H. contortus* without affecting the health and productive response in kids.
Key words: *H. contortus, Guazuma ulmifolia, Phitecellobium dulce, Acacia cochliacantha*.

RESUMEN. El objetivo fue evaluar la actividad contra *Haemonchus contortus* del follaje de tres árboles taniníferos en cabritos criollos infestados experimentalmente con una dosis de 350 larvas de *H. contortus* (L3) Kg^{-1} peso vivo. Veinte cabritos criollos de 12 ± 2.0 kg de peso vivo (PV), fueron distribuidos al azar en cuatro tratamientos: T1= testigos sin follaje, y la adición de follaje fresco (10 % de la materia seca de la dieta total) de *Guazuma ulmifolia* (T2), *Phitecellobium dulce* (T3) y *Acacia cochliacantha* (T4). Durante 60 d se evaluó la actividad de los follajes contra *H. contortus* mediante la reducción en la excreción de huevos por gramo de heces (hpgh); además, la excreción de huevecillos se asoció con la concentración del paquete celular sanguíneo y el cambio de peso de los animales. Los datos de las variables evaluadas se analizaron en un diseño completamente al azar y la prueba de Tukey para la comparación de medias ($p < 0.05$). Los compuestos secundarios del follaje de *P. dulce* y *A. cochliacanta* resultaron más eficaces ($p < 0.04$) en el control de *H. contortus*, con menor excreción de hpgh (1.48 y 1.18 Log^{10} g^{-1} de heces), respectivamente. El hematocrito fue diferente ($p < 0.005$) principalmente en hemoglobina, eritrocitos, linfocitos y eosinófilos; el consumo de materia seca ($p < 0.05$) y el cambio de peso vivo total ($p < 0.01$) fue mayor en los cabritos que recibieron el follaje de *P. dulce* con 621.5 g d^{-1} y 2.4 kg de peso, respectivamente. Se concluye que los follajes de *P. dulce* y

A. cochliacanta, por su contenido de taninos, tienen potencial para el control de *H. contortus*, lo que amerita mayor investigación.
Palabras clave: *H. contortus, Guazuma ulmifolia, Phitecellobium dulce, Acacia cochliacantha*.

INTRODUCTION

One of the biggest health problems faced by goats is their frequent exposure to parasites. Tropical studies have determined the presence of several genera of parasites, including the parasite *Haemonchus contortus*, which is one of the most important epidemiologically due to its abundance and severity of infestations (Olivares *et al.* 2012). The use of chemicals has played an important role in the control and treatment of severe parasite infestations in small ruminants; however, it is a costly and inappropriate method, and excessive use can result in the development of parasite resistance to the chemical formula of the product (Olivares-Pérez *et al.* 2011).

The development of resistance and the presence of chemical residues in animal products have prompted the search for substances that are less toxic to animals and man, but that are still effective in controlling parasites (Abbas *et al.* 2014). Fruit tree foliage and extracts have shown some activity against parasites (Calderón-Quintal *et al.* 2010, Patra and Saxena 2010, Zaman *et al.* 2012a) and positive effects on weight gain, dry matter intake, milk production and ruminal fermentation parameters (Salem 2011, Olivares *et al.* 2013a, Olivares *et al.* 2013b). They can also modify the acetate-propionate relationship and defaunation effects, as well as protect protein in the rumen and promote its absorption in the duodenum (Hart *et al.* 2008, Jiménez *et al.* 2011).

The use of tree foliage for feeding goats is most common in tropical regions where *P. dulce*, *A. cochliacantha* and *G. ulmifolia* have been identified as forage species (Rojas *et al.* 2010, Olivares *et al.* 2011). Studies have shown that tannins can improve goat resistance to gastrointestinal nematode infestations (Torres *et al.* 2008, Alemán *et al.* 2011). The aim of this study is to comparatively determine the chemical composition of *A. cochliacantha*, *P. dulce* and *G. ulmifolia* foliage, its total phenolic and condensed tannin contents, its effect against *H. contortus* in experimentally infected male Creole kids and its relationship to the animals' productive response.

MATERIALS AND METHODS

The study was conducted on the premises of the Faculty of Animal Husbandry and Veterinary Medicine at the Autonomous University of Guerrero, located 2.5 km from the Altamirano-Iguala road in Pungarabato Municipality, situated in the Tierra Caliente region of the State of Guerrero, Mexico.

Tree foliage used. Samples of fresh foliage (leaves, tender stems) of *A. cochliacantha*, *P. dulce* and *G. ulmifolia* of the same phenology were collected, and their chemical composition (AOAC 2000), condensed tannin and total phenolic content (Table 1) determined (Waterman and Mole 1994).

Animals and management

Twenty five-month-old Creole kids with body weights of 12.0 ± 2.0 kg were used. The animals were housed in metabolic cages, with a height of 110 cm from the floor (with free access to water and food for their adaptation), where they were subcutaneously impregnated with 0.2 mg kg^{-1} body weight of ivermectin (Sumano and Ocampo 2006). The excretion of eggs at days zero, seven, fourteen and twenty-one were monitored by McMaster tests to verify that the animals were at a very low or nil parasite load before initiating the study (MAFF 1986).

Subsequently the animals were experimentally infected with 350 L3 larvae of *H. contortus* per kilogram of body weight. Four weeks after infection, the animals were divided randomly into four groups of five animals, balanced according to the level of egg excretion and body weight, and fed with 10 % total dry matter with fresh foliage of the three tree

Table 1. Chemical composition of the foliage of *A. cochliacantha*, *P. dulce* and *G. ulmifolia*, used in the study.

Tabla 1. Composición química de los follajes de *A. cochliacantha*, *P. dulce* y *G. ulmifolia* usados en el estudio.

Nutrients (% DM basis)	*G. ulmifolia*	*P. dulce*	*A. cochliacantha*	SEM	*Significance*
Dry matter	90.1^b	92.4^b	96.9^a	0.44	0.001
Ash	06.7	08.2	6.9	1.43	ns
Organic matter	83.4^b	84.2b	90.0^a	1.20	0.010
Crude protein	16.7^b	25.7^a	18.9^{ab}	3.25	0.050
Crude fiber	26.1	44.0	30.4	9.06	ns
Neutral detergent fiber	39.2^c	72.7^b	78.3^a	0.77	0.001
Acid detergent fiber	23.8^c	40.7^b	50.3^a	5.11	0.050
Condensed tannins	4.1^c	8.8b	13.1^a	3.40	0.050
Total phenols	3.8^c	13.4^b	24.8^a	5.20	0.050

SEM: standard error of the mean, ^{abc}Values with a different letter in the same line are different, Tukey test ($p < 0.05$)

species in the following four treatments: T1= control without foliage, T2= *Guazuma ulmifolia*, T3= *Phitecellobium dulce*, T4= *Acacia cochliacantha*. All animals were fed a maintenance diet according to the requirements of CP and Mcal of ME with oat hay, wheat bran and mineral premix (Table 2) (NRC 2007) prepared for the growing stage.

Variables measured

Egg excretion: Fecal samples were collected every seven days to measure the excretion of nematode eggs. Egg counts were performed according to the modified McMaster technique (MAFF 1986).

Red blood cell count: Blood samples were collected in vacutainer tubes with EDTA added at days 0, 15, 30, 45 and 60 of the study by puncturing the jugular vein. Blood samples were analyzed for hematocrit, hemoglobin, erythrocytes and leucocytes (neutrophils, lymphocytes, eosinophils and monocytes). Hemoglobin concentration was determined using the cyanmethaemoglobin method. Hematocrit was determined by the micro hematocrit technique. Erythrocyte and differential leucocyte counts were determined using the haemocytometer method (Archer and Jeffcott 1977).

Productive response of male kids: Dry matter intake was measured in kids by the difference between the dry matter offered daily and the amount rejected. Also, secondary compound intake was calculated with the total phenol and condensed tannin content in the foliage multiplied by the dry matter intake of male kids fed with the foliage (Table 1), and it was associated with the excretion of eggs in the feces and the blood cell concentration in the animals. Body weight was measured at 0, 15, 30, 45 and 60 d of the experiment. With body weight data, the total weight change was calculated by the difference in final body weight minus the initial body weight, and feed conversion was calculated by dividing the dry matter intake between the weight changes observed in the animals (Olivares *et al.* 2013a).

Statistical analysis

The chemical composition and content of phenols and condensed tannins in foliage and excretion in feces of *H. contortus* eggs, the hematocrit, hemoglobin, erythrocytes, neutrophils, lymphocytes, eosinophils and monocytes, as well as the variables of animal response (dry matter intake, weight change and secondary compound intake) in male kids, were analyzed in a completely randomized design (SAS 2002). Statistical model: ; where, = is the response variable due to the treatment (= 1, 2, 3 *G. ulmifolia*, *P. dulce* and *A. cochliacantha* foliage in chemical composition and phenolic and condensed tannin content; and = treatment diets 1: control without foliage, 2: *G. ulmifolia*, 3: *P. dulce* and 4: *A. cochliacantha* foliage in hematological and productive response of male kid variables); μ

Table 2. Ingredients and chemical composition of different treatments, including (%) dry matter basis.

Tabla 2. Ingredientes y composición química de los diferentes tratamientos, inclusión (%) en base a la material seca.

Ingredients (%)	T1	T2	T3	T4
Oat hay	38.0	38.0	41.0	40.0
Foliage of *G. ulmifolia*	0.0	10.0	0.0	0.0
Foliage of *P. dulce*	0.0	0.0	10.0	0.0
Foliage of *A. cochliacantha*	0.0	0.0	0.0	10.0
Wheat bran	61.0	51.0	48.0	49.0
Mineral premix	1.0	1.0	1.0	1.0
Total dry matter (%)	100.0	100.0	100.0	100.0
Chemical composition (%)				
Dry matter	96.3	95.1	96.2	96.5
Crude protein	10.8	10.8	10.8	10.9
Metabolizable energy (Mcal)	2.1	2.0	2.3	2.2

T1: control group without foliage, T2: foliage of *G. ulmifolia*, T3: foliage of *P. dulce*, T4: foliage of *A. cochliacantha*.

= is the overall mean and = random error. The significance between means was compared by the Tukey test (alpha, $p < 0.05$) (SAS 2002).

RESULTS

Chemical composition of foliage

In the chemical composition of the three types of foliage used, it is observed that the dry and organic matter content was higher in *A. cochilacantha* foliage ($p < 0.001$) with 96.6 and 90.0 % respectively (Table 1). The crude protein content was higher in the *P. dulce* foliage ($p < 0.05$) with 25.7 %. The contents of neutral ($p < 0.001$) and acid ($p < 0.01$) detergent fibers, condensed tannins ($p < 0.01$) and total phenols ($p < 0.01$) were higher in the *A. cochliacantha* foliage with 78.3, 50.3, 13.1 and 24.8 %, respectively, compared to *P. dulce* and *G. ulmifolia* foliage (Table 1).

Fecal egg counts

It was observed that the *P. dulce* and *A. cochliacantha* foliage decreased ($p < 0.01$) the excretion of *H. contortus* eggs in the feces of male kids, compared to control animals (T1) and those fed with *G. ulmifolia* foliage (T2) (Figure 1). The excretion of *H. contortus* eggs in the feces of male kids of T3 and T4 was of 1.18 Log^{10} g^{-1} and 1.48 Log^{10} g^{-1}, respectively, and in male kids of T1 and T2 was of 2.18 Log^{10} g^{-1} and 3.08 Log^{10} g^{-1}, respectively (Figure 1).

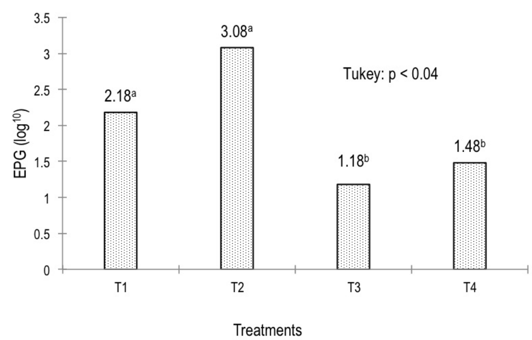

Figure 1. Excretion of eggs per gram (EPG) of feces in male Creole kids experimentally infected with *H. contortus* and fed with fresh tree foliage (T1: Control without foliage, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Figura 1. Excreción de huevos por gramo (HPG) de heces en cabritos criollos machos experimentalmente infectados con *H. contortus* y alimentados con follaje fresco de los árboles (T1: Control sin follaje, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Red blood cell count

The male kids of T1 and T2 had low hematocrit ($p < 0.005$) with 19.60 and 19.48 %, hemoglobin ($p < 0.001$) with 6.53 and 6.49 g / 100 mL, erythrocytes ($p < 0.001$) with 3.26 x 1012 L^{-1} and 3.24 x 10^{12} L^{-1} and lymphocytes ($p < 0.001$) with 71.36 and 64.56 %, respectively, compared to animals of T3 and T4 (Table 3). The eosinophils showed the highest concentration ($p < 0.01$) in kids of T1 (1.84 %) and T2 (2.08 %), compared to animals of T3 and T4 (Table 3). The level of neutrophils and monocytes was not different among animals under treatments ($p > 0.05$) (Table 3).

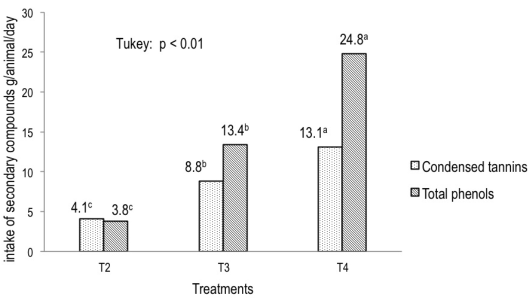

Figure 2. Intake of secondary compounds derived of the consumption of 100 g d^{-1} animal of dry matter of each foliage in treatment diets (T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Figura 2. Consumo de compuestos secundarios derivado del consumo de 100 g d^{-1} animal de materia seca de cada follaje en las dietas tratamiento (T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Productive response of male kids

Higher dry matter intake ($p < 0.05$) was observed in male kids which received T3 (621.5 g d^{-1} animal) and T4 (634.4 g d^{-1} animal) compared to animals of T2 (Figure 3). The total weight gain was higher ($p < 0.01$) in kids which received T3 with 2.4 kg animal compared to animals of other treatments (T1, T2 and T4) (Figure 4). Also, differences in total weight gain between male kids of T4 with 0.6 kg and those of T2 with -0.4 kg were observed ($p < 0.01$) (Figure 4).

DISCUSSION

Fecal egg counts

The results in fecal egg counts can be attributed to the consumption of secondary compounds (condensed tannins between 8.8 to 13.1 g d^{-1} animal and total phenols between 13.4 to 24.8 g d^{-1} animal) by kids of T3 and T4 (Figure 2), which received the foliages of *P. dulce* and *A. cochliacantha* that affected the reproductive cycle of the parasite and the consequent excretion of eggs in the feces of animals (Lange *et al.* 2006, Villalba *et al.* 2010, Hamad *et al.* 2013). Reports indicate that the use of plants with a certain content of condensed tannins decreases the viability of larvae and adult worms of *H. contortus* (Terrill *et al.* 2007, Ademola and Eloff 2011, Zaman *et al.* 2012b).

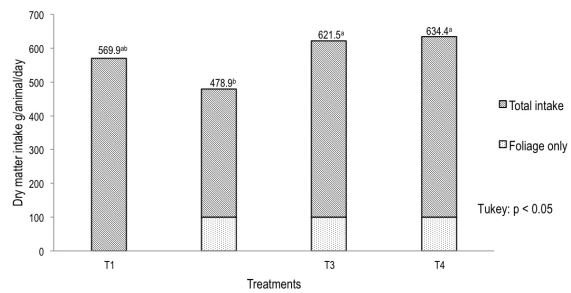

Figure 3. Dry matter intake of male Creole kids experimentally infected with *H. contortus* and fed with fresh tree foliage (T1: Control without foliage, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Figura 3. Consumo de material seca de cabritos criollos machos experimentalmente infectados con *H. contortus* y alimentados con follaje fresco de los árboles (T1: Control sin follaje, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

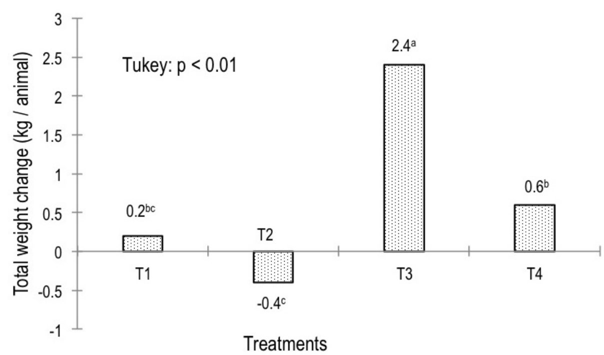

Figure 4. Total weight change of male Creole kids experimentally infected with *H. contortus* and fed with fresh tree foliage (T1: Control without foliage, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Figura 4. Cambio de peso total de cabritos criollos machos experimentalmente infectados con *H. contortus* y alimentados con follaje fresco de los árboles (T1: Control sin follaje, T2: *G. ulmifolia*, T3: *P. dulce*, T4: *A. cochliacantha*).

Red blood cell count

Differences in the blood cell count in animals were attributed to the degree of parasitic infestation of the male kids (Figure 1). The decrease in

Table 3. Hematocrit, hemoglobin, erythrocytes and leucocytes in male Creole kids experimentally infected with *Haemonchus contortus*.

Tabla 3. Hematocrito, hemoglobina, eritrocitos y leucocitos en cabritos criollos machos experimentalmente infectados con *Haemonchus contortus*.

Treatments	Hematocrit (%)	hemoglobin (g / 100 mL)	Erythrocytes (x 10^{12} L^{-1})	Lymphocytes (%)	Eosinophils (%)	Neutrophils (%)	Monocytes (%)
T1	19.60^b ± 1.9	6.53^b ± 0.4	3.26^b ± 0.2	71.36^b ± 0.8	1.84^{ab} ± 0.1	26.08^a ± 9.1	0.40a ± 0.4
T2	19.48^b ± 3.7	6.49^b ± 0.4	3.24^b ± 0.1	64.56^c ± 1.8	2.08^a ± 0.5	26.64^a ± 3.9	0.28a ±4 0.4
T3	25.44^a ± 1.3	8.48^a ± 0.3	4.24^a ± 0.2	79.12a ± 0.6	0.44^c ± 0.8	19.64^a ± 6.7	0.48a ± 0.3
T4	24.00^{ab} ± 2.4	8.00^a ± 0.6	3.99^{ab} ± 0.2	75.04^{ab} ± 6.1	0.84^{bc} ± 0.4	24.84^a ± 6.0	0.68^a ± 0.3
Pr>F	0.005	0.001	0.001	0.001	0.002	0.46	0.54

T1: Control without foliage, T2: *Guazuma ulmifolia*, T3: *Pithecellobium dulce*, T4: *Acacia cochliacantha*. ^{abc}Values with a different letter in the same column are different, Tukey test ($p < 0.05$). ± Standard deviation.

hematocrit, hemoglobin, erythrocytes and lymphocytes in male kids of T1 and T2 can be attributed to gastritis caused by injury to the mucosa of the digestive tract and blood suction caused by *H. contortus* as a hematophagous parasite (Table 3) (Schwarz *et al.* 2013, Blackie 2014). Moreover, the effect of intake of secondary compounds (condensed tannins and total phenols) of fresh *P. dulce* (T3) and *A. cochliacantha* (T4) foliage (Figure 2) for reducing the excretion of *H. contortus* eggs (Figure 1) favored the concentration of hematocrit, hemoglobin, erythrocytes and lymphocytes in male kids under treatments T3 and T4 (Table 3). The results indicate that *H. contortus* affects the concentration of white blood cells responsible for the innate immune response in the animals (Provenza and Villalba 2010). However, it is also important to mention that feeding kids (10 % of dietary DM basis) with the fresh foliage of *P. dulce* and *A. cochliacantha* with tannin content between 8.8 to 13.1 % and phenols between 13.4 to 24.8 % (Table 1) decreased the excretion of *H. contortus* eggs in their feces due to increased consumption of secondary compounds (Figure 2) without affecting the concentration of white blood cells, similar to that reported by Paolini *et al.* (2003), Lange *et al.* (2006) and Rivero *et al.* (2012). What has been discussed is important because it has been determined that the tannins and saponins have significant negative effects on hematocrit and hemolytic activity that causes loss of hemoglobin and may result in death of the animal (Wang *et al.* 2007, Mahgoub *et al.* 2008). The higher concentration of eosinophils in the blood of male kids of T1 and T2 was attributed to the immune response of animals against the strong parasitic infestation developed by gastritis (Table 3 and Figure 1), similar to that reported by Schwarz *et al.* (2013) and Blackie (2014).

Productive response of male kids

The higher dry matter intake and weight gain in animals of T3 and T4 (Figures 3 and 4) was attributed to the decrease in parasite load (*H. contortus*) (Figure 1) that prevented the development of gastritis caused by damage of the parasite to the mucosa of the digestive tract, which favored food digestion and nutrient absorption and increased dry matter intake caused by supplying 10 % (dry matter basis of the diet) *Pithecellobium dulce* and *A. cochliacantha* fresh foliage (Table 2), similar to that reported by Hoste *et al.* (2005). *In vivo* studies in small ruminants suggest at least 30 - 40 g of condensed tannins per kilogram of dry matter (DM) to observe anti-parasitical activity (Hoste *et al.* 2006). Waghorn (2008) and Patra and Saxena (2010) reported that tannins in low concentrations (3 - 6 %) show a positive effect on diet digestion and animal growth. Olivares *et al.* (2013a) observed that the use of *P. dulce* and *G. sepium* foliage increased dry matter intake and weight gain in male kids.

CONCLUSIONS

The secondary compound content of foliage of *P. dulce* and *A. cochliacantha* had higher activity for *H. contortus* control in kids, without affecting the health of the animals, and indirectly increased their dry matter intake and total weight gain. Animals under treatments T1 and T2 were the most parasitized, reducing the blood cell concentration and productive response in kids.

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