Assessment of parasitic and productive parameters on sheep with a ration supplemented with leaves of Guazuma Ulmifolia in the Mexican tropics

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ÉVALUATION DE PARAMETRES PARASITAIRES ET PRODUCTIFS SUR DES MOUTONS NOURRIS AVEC UNE RATION ENRICHIE AUX FEUILLES DE *GUAZUMA ULMIFOLIA* AU MEXIQUE

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Travail de fin d'études
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OBJECTIF DU TRAVAIL

Ce travail de fin d'étude a pour objectif d'évaluer l'impact d'une supplémentation de ration avec une plante locale (*Guazuma Ulmifolia*) ayant un potentiel fourrager sur les parasites gastro-intestinaux, le poids et l'état corporel de moutons dans la région sud du Mexique.

RESUME

Les infections parasitaires gastro-intestinales (nématodes, protozoaires) représentent un problème sérieux pour la production de petits ruminants. Ce problème est actuellement aggravé par la résistance aux produits anthelminthiques. Ainsi le besoin d'alternatives de contrôle plus durables a augmenté. L'usage de de fourrages bioactifs avec des tanins condensés a présenté des résultats encourageants. La plante mexicaine locale *Guazuma ulmifolia* a été citée dans des études ethnovétérinaires et sélectionnée naturellement par les moutons (races Blackbelly et Pelibuey). Ainsi, l'objectif était d'évaluer l'impact de cette plante inclus dans l'aliment sur les parasites gastro-intestinaux et les performances de ces animaux. Vingt-deux moutons ont été répartis au hasard en deux groupes: un groupe témoin sans *G. ulmifolia* et un groupe test dont la ration contenait des feuilles fraiches de *G. ulmifolia* (30% de la matière sèche de l'alimentation totale). Pendant trente jours, le poids, l'état corporel et la charge parasitaire gastro-intestinale ont été évalués. Les résultats n'ont montré aucune activité anthelminthique et anticoccidienne significative, de même qu'un impact non significatif sur le poids. Cependant, suivant l'analyse bromatologique de *G. ulmifolia*, son utilisation comme complément alimentaire dans le but de corriger les déficits en protéines et calcium de régimes de moindre qualité est justifié.

ASSESSMENT OF PARASITIC AND PRODUCTIVE PARAMETERS ON SHEEP FED ON A RATION SUPPLEMENTED WITH LEAVES OF GUAZUMA ULMIFOLIA IN MEXICO

AIM OF THE WORK

The aim of this work is to assess the impact of ration supplementated with a local plant (*Guazuma Ulmifolia*) having a forage potential on gastrointestinal parasites, weight and body condition of sheep in the southern region of Mexico.

SUMMARY

Gastrointestinal (GI) parasite infections (nematodes, protozoa) represent a serious problem in small ruminant production. This issue is currently potentiated by anthelmintic resistance. Thus, the need for more sustainable control alternatives increased. The use of bioactive forages with condensed tannins (CT) has shown encouraging results. The local Mexican plant *Guazuma ulmifolia* is cited in ethnoveterinary studies and naturally selected by sheep (pelibuey breed). *Guazuma ulmifolia* contains a certain amount of CTs. Therefore the objective of this study was to evaluate the impact of *G. ulmifolia* in sheep diet on animal performance and GI parasites. Twenty-two sheep were randomly distributed in two groups: a control group without *G. ulmifolia* and a group receiving a diet which contained *G. ulmifolia* fresh foliage at 30% of the dry matter of the total diet. For thirty days, weight, body condition and GI parasite load were assessed. The results showed no significant anthelmintic and anticoccidials effects ($p > 0.05$) as well as no significant impact on weight during the thirty days experiment. Nevertheless, considering the bromatological analysis of *G. ulmifolia,* its use as a supplement on diet in order to adjust protein and calcium deficits of poor quality diet is justified.

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Table of contents

1. INTRODUCTION 8

2. MATERIALS AND METHODS 9

2.1. Ethical mention 9

2.2. Study area. 10

2.3. Animals. 10

2.4. Experimental design. 10

2.5. Laboratory Analysis and calculations 11

2.6. Statistical analysis 12

3. RESULTS 12

3.1. Nutrient values of *C. nlemfuensis* and *G. ulmifolia* and diet-associated analysis. 12

3.2. Overall variable parameters responses to the treatment 14

3.3. Kinetic parameter treatment response to treatment 15

3.4. Parasitological load response to treatment 16

4. DISCUSSION 17

4.1. Bromatological composition and nutritional value (energy, protein) 17

4.2. Variable parameters 18

5. CONCLUSIONS 20

6. BIBLIOGRAPHY 20

1. INTRODUCTION

In Mexico, ovine production has increased within recent years, probably due to the growing demand for meat mainly in the central region of the country. In addition to the high profitability of the species, sheep are small prolific ruminant animals which adapt easily to various environments and make adequate use of the resources available in each region of the country (Partida *et al.*, 2013).

In southeastern Mexico, livestock is managed extensively and greatly promoted by government institutions. Thereby the vegetation has been strongly impacted and the original landscape modified, in which corn crop fields have been replaced by pastures (Nahed *et al.*, 2010).

The use of tree and shrub foliage with forage potential in the feeding of ruminants has been used as an indirect alternative feed mainly in the time of forage shortage. This alternative has aroused the interest of some researchers (Sosa *et al*., 2004 ; Pinto-Ruíz *et al*., 2014 ; Martínez-Alfaro *et al*., 2014 ; Martínez-Alfaro *et al*., 2015). Moreover, it has been documented that trees dispersed in pastures or as a live fence perform multiple functions in production units (Nahed *et al*., 2010).

In previous studies, surveys were made with producers in the region allowing the identification of the most locally used tree and shrub species with forage potential (Martínez-Alfaro *et al*., 2014 ; Martínez-Alfaro *et al*., 2015), highlighting the social importance of *Guazuma ulmifolia* tree.

On this purpose studies on sheep ingestive behavior of tropical trees with forage potential have been conducted. It has been concluded that both foliage of *G. ulmifolia* (Sosa *et al*., 2004; Martínez-Alfaro *et al*., 2014 ; Martínez-Alfaro *et al*., 2015) and fruits (Pinto-Ruíz *et al*., 2014), have a higher preference rate in sheep and are valuable from a nutritional point of view.

However one of the major issues of sheep production, as a meat source, is the sensibility of the species to endoparasitism. GI parasitic infections have a strong impact on the health and production of small ruminants and therefore on the profitability of farms via the losses generated. Without proper control of internal parasites, fecundity, milk yield and carcass value suffer and, in severe cases, death may occur (Pugh *et al*., 2021).

In order to restrain this problem, anthelmintics use is an effective control solution. However the prevalence of anthelmintic resistance has risen sharply within recent years all around the world including Mexico (Herrera-manzanilla *et al*., 2017 ; Mondrag *et al*., 2019 ; Santiago-figueroa *et al*., 2019). Besides, even if new molecules could emerge, resistance builds up quickly (De

Graef *et al*., 2013) and this issue would remain. Thus alternatives control strategies, more sustainable, are necessary to restrain this important problem for the future (Abbott *et al*., 2012). One part of the sustainable integrated management strategy studied is the use of bioactive forages that contain tannins. Indeed it has been shown that condensed tannins have beneficial antiparasitic benefits that include anthelmintic (Terrill *et al*., 2012 ; Hoste *et al*., 2015 ; Hoste *et al*., 2016), and anticoccidial effects (Kommuru *et al*., 2014 ; Saratsis *et al*., 2016). In addition studies have shown that CTs have also nutritional benefits due to the protection of dietary protein from excessive fermentation in the rumen. This benefits include improved growth, milk yields, fertility, and tolerance to some intestinal parasites (Mueller-Harvey *et al*., 2019).

Some studies over *G. ulmifolia* revealed that this local Mexican plant contains a certain range of CTs concentration. Values of their analyses made with foliage vary from 0.23% to 6.5% DM (León-Castro *et al*., 2015 ; Pinto-Ruiz *et al.*, 2014; Feedbase, 2020).

Investigations on the plant showed various medical qualities such as antidiabetic properties (Caballero-George *et al*., 2002), hypotensive and vasorelaxant activity (Magos *et al*., 2008), antiulcer (Berenguer *et al*., 2007), antibacterial activities (Camporese *et al*., 2003), and antiviral activity (Felipe *et al*., 2006). However, up to now, only a few information on the effects of *G. ulmifolia* on GI parasites are available in the literature. Only one study investigated the impact of *G. ulmifolia* included at 10% of the total diet dry matter over *Haemonchus contortus* on kids (León-Castro *et al*., 2015). This study, made in the state of Guerrera, Mexico, compared *G. ulmifolia* with two other most common tropical tree foliage *Pithecellobium dulce* and *Acacia cochliacantha*. It was concluded that *P. dulce* and *A. cochliacantha*, which present higher CTs concentrations, have potential control over *H. contortus*, by contrast to *G. ulmifolia*.

Thereby the objective of this study was to evaluate the effect of higher diet complementation of *G. ulmifolia* on sheep GI parasites and productive aspects through its CT content and its nutritional value.

2. MATERIALS AND METHODS

2.1. Ethical mention

In the absence of proper regulation on the use of animals for research and animal welfare during experiments in Mexico, the protocols were carried out according to the best practices usually accepted by the Ethical Committee of University of Liège (Belgium) when conducting similar experiments.

2.2. Study area.

The study was carried out in the Sacrosanto Ranch, Municipality of Jiquipilas, Chiapas, located at 16 ° 34´20´´ LN and 93 ° 30´50´´ LW and at an altitude of 610 meters above sea level. According to the Kôppen Classification, modified by García (2004) the climate is Aw (w) (i): it is temperate subhumid C (W2) (W) with rains in summer, which corresponds to the study period. The type of vegetation is tropical forest with tree species with potential forage of secondary succession such as *G. ulmifolia* the presence of which is natural in the Central Region of Chiapas, Mexico.

2.3. Animals.

Twenty-two pelibuey sheep were used. They were randomly allocated to two homogeneous groups in terms of physiological status (parasitology), age (between 1 and 2 years old) and nutritional status (weight, corporal score) (Table I).

Table I. Group repartition of the studied animals according to initial parameters.

Parameters	Group		SEM	P>t
	G0	G1		
Age (year)	1.6	1.6	0.2	1
Weight (kg)	28.4	28.4	2.1	1
BCS (/5)	2.8	2.9	0.2	0.8
Trichostrongyle-type eggs (EPG)	202	277	115	0.7
Eimeria spp. oocysts (OPG)	430	493	141	0.7
Strongyloides spp. larvated eggs (EPG)	93	132	62	0.7

G0: sheep fed with only hay of Cynodon nlemfuensis. G1: sheep fed with foliage of G. ulmifolia and hay of C. nlemfuensis. BCS: Body Condition Score. EPG: egg per gram. OPG : oocysts per gram. SEM : standard error of the mean.

2.4. Experimental design.

The animals were provided with mineral salts and water *ad libitum*. The control group (G0) was only fed with hay of Estrella of Africa (*Cynodon nlemfuensis*). The tested group (G1) received a blend of 30% on DM basis of fresh foliage of *G. ulmifolia* in addition to 70% hay of *Cynodon nlemfuensis*. As the feeding level of the forages was not known, a value of 2.1% body weight DM was chosen (INRA, 2018). In order to limit the possible deficiencies in DM requirements the diet offered was calculated based on the value of 3% body weight DM per day. *C. nlemfuensis*, as hay, was assumed containing around 95% of DM and *G. ulmifolia*, as fresh foliage, 50% of DM. Each group was offered two meals per day. The experiment lasted for 30 days. Body Condition Score (using the Waldn-Brown scoring) (Thompson *et al*., 1994) and weight were measured 3 times (on d0, 19 and 29) and individual coprological analyses were done twice (d-1 and d-2) before the trail starting and every 5 days thereafter. Feed refusal was measured daily: *G. ulmifolia* individually at each meal, and *C. nlemfuensis* per group once a day.

2.5. Laboratory Analysis and calculations

GI parasites counts were measured according to MacMaster's cell count technique (Thienpont *et al*., 1986). Feces was collected in the morning and conserved individually in a cool place with ice. Coprological analyses on pooled samples were then made during the day.

The Chemical composition analyses of the feed samples were carried out according to the procedures of AOAC (2000). Crude Protein (CP) was determined by the Kjeldahl method (N x 6,25); crude fiber (CF) by the method of Weende (method no. 978.10). The dry matter content was determined from a test sample of 5g maintained at a temperature of 105 °C for 24h (AFNOR, 1982). Crude ash was determined from a test sample of 1g incinerated in an oven at 550°C during 4 h. Fractions of Neutral Detergent Fiber (NDF) were determined according to the technique proposed by Van Soest and collaborators (1991). Finally, major macrominerals such as calcium, potassium, sodium, magnesium were determined by atomic absorption spectrophotometry. Phosphorus was measured by colorimetry. The determination of trace elements such as (Cu, Fe, Zn, Mn) was carried out by atomic absorption spectrophotometry.

The determination of tannins was made using methylcellulose precipitable tannin assay (MCP) for the total tannins (Mercurio *et al*., 2007), and the ethanolysis method for the CTs.

The calculation of forages net energy, intestinal digestible protein (IDP) and rumen degradable nitrogen balance (RDNB) were performed according to INRA (2018).

The substitution rate of *G. ulmifolia* was calculated according to the followed formula: [(G0 *C. nlemfuensis* intake – G1 *C. nlemfuensis* intake) / *G. ulmifolia* intake].

2.6. Statistical analysis

Adequate allocation of animals in groups was verified according to Student's t test. For serial data a mixed model was used (SAS, 2004) allowing to take into account compound symmetry covariance structures. Multiples comparisons were performed according to the Tukey test.

3. RESULTS

3.1. Nutrient values of *C. nlemfuensis* and *G. ulmifolia* and diet-associated analysis.

The substitution rate of *G. ulmifolia* was 53%, allowing to consider that the supplement showed a "concentrated-type effect".

The chemical composition of *G. ulmifolia* foliage is reported in (Table II). *G. ulmifolia,* with a concentration of 19g/kg DM, could be considered as a very good source of calcium. Nevertheless calcium excess could interact with zinc absorption and, to a lesser extent, copper (Meschy, 2010 ; NRC, 2007).

In terms of energy, *G. ulmifolia* with value higher than 500 KJ net energy / kg DM was considered as a high-quality forage whereas hay of *C. nlemfuensis* as a medium/poor- quality forage.

In this experiment in both sheep group energy DM offered were more than adequate. However energy intake was slightly lower than the requirement for G0 (Figure 1). This deficit of ingested energy increased during the 10 last days of the experiment (average 2254 KJ/d).

In terms of protein composition, *G. ulmifolia,* with 15% DM crude protein, was a valuable forage protein source. The calculated IDP is 9%DM, thus RDNB shows a moderate excess (66.5g) (Table II). In our experiment in both sheep groups protein supplies and intakes were adequate (Figure 1).

In this assessment in both sheep group, mineral supplies were sufficient and the possible deficiency of sodium and zinc were compensated by mineral salt rock. Nevertheless with 30% of *G. ulmifolia* the ratio Ca/P at 3.6 was higher than the norms (1.1-2.1) (NRC, 2007).

Condensed tannins concentration was 1.05% DM for *G. ulmifolia*. Medium intake of *G. ulmifolia* was 213gr DM per day. Sheep from G1 thus were provided on average of 0.075gr DM CT per kg per day.

Table II. Chemical composition, energy and protein values of G. ulmifolia (fresh foliage) and C. nlemfuensis (hay) used in the study.

Parameters	*G. ulmifola (foliage)*		*C. nlemfuensis (hay)*	
	Average	SD	Average	SD
Dry matter (% Fresh matter)	38.7	0.8	83.0	0.9
Crude ash (% DM)	10.7	1.3	10.5	2.7
Crude protein (% DM)	15.4	2.3	6.9	2.3
Crude fiber (% DM)	22.9	2.4	34.5	4.6
NDF (% DM)	62.7	3.8	68.5	8.2
Total tannins (% DM)	0.79	0.05	/	
Condensed tannins (% DM)	1.05	0.04	/	
Calcium (g/kg DM)	19,0	5,0	4,2	0,5
Phosphorus (g/kg DM)	3,1	0,4	1,7	0,9
Potassium (g/kg DM)	18,6	2,3	14,9	0,5
Sodium (g/kg DM)	0,1	0,1	1,1	1,1
Magnesium (g/kg DM)	4,4	0,7	1,7	0,3
Copper (mg/kg DM)	30,2	18,5	7,3	5,0
Iron (mg/kg DM)	86,5	23,8	368,5	261,9
Manganese (mg/kg DM)	49,9	5,5	79,4	8,4
Zinc (mg/kg DM)	31,0	14,4	32,7	6,4
Net Energy (KJ/kg DM)	5693	72	4726	106
IDP (% DM)	9.0	0.5	6.2	0.8
RDNB (g)	66.5	21.9	-9.8	7.9

SD: standard deviation, NDF: Neutral Detergent Fiber, IDP : Intestinal Digestible Protein (according to INRA system), RDNB : Rumen Degradable Nitrogen Balance, SD : standard deviation.

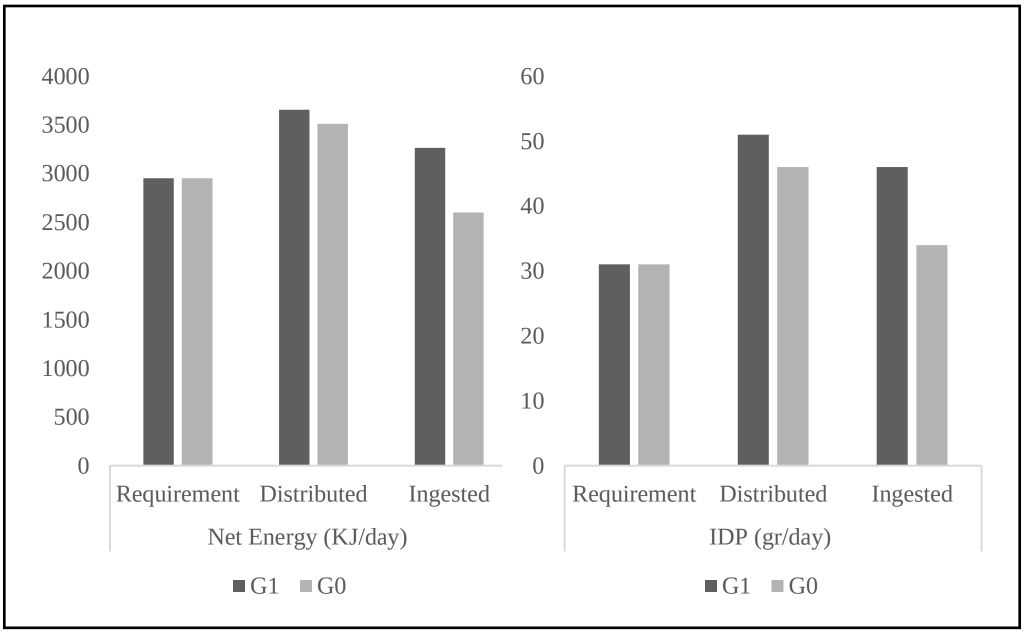

Figure 1. Energy and protein requirement, distributed and ingested according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis). IDP : Intestinal Digestible Protein (according to INRA system).

3.2. Overall variable parameters responses to the treatment

The effect of *G. ulmifolia* on animal performance and parasite counts (averages on the entire experiment time) are reported in Table VI. No significant differences were observed between G0 and G1 for all the parameters recorded.

Table VI. Overall mean weight, BCS, parasitic loads of sheep offered hay of C. nlmenfuensis alone or supplemented with fresh foliage of G. ulmifolia.

Parameters	Group		SEM	Pr>F
	Control	Test		
Weight (kg)	27,8	28,6	1,9	0,77
BCS (/5)	2,6	2,8	0,2	0,32
Trichostrongyle-type eggs (EPG)	562	791	282	0,57
Eimeria spp. oocysts (OPG)	338	353	94	0,91
Strongyloides spp. larvated eggs (EPG)	141	113	60	0,75

SEM : standard error of the mean. EPG : eggs per gram. OPG : oocysts per gram. G0: sheep feed without G. ulmifolia. G1: sheep feed with foliage of G. ulmifolia (and C. nlemfuensis).

3.3. Kinetic parameter treatment response to treatment

The BCS evolution over time is reported in Figure 2. There were significant effects of time ($p < 0.01$) and interaction treatment x time ($p < 0.05$). On day 19 the BCS was significantly higher for G1 when compared to G0. However, on day 29 this difference became no significant.

No significant effect of the time over weight in general nor difference between group weights over time were underlined although a trend was noticed for the time impact ($p > 0.1$). Moreover a gap between the groups seemed to emerge. Indeed whereas G0 follows a downward slope, the test group appeared to be more stable (Figure 3).

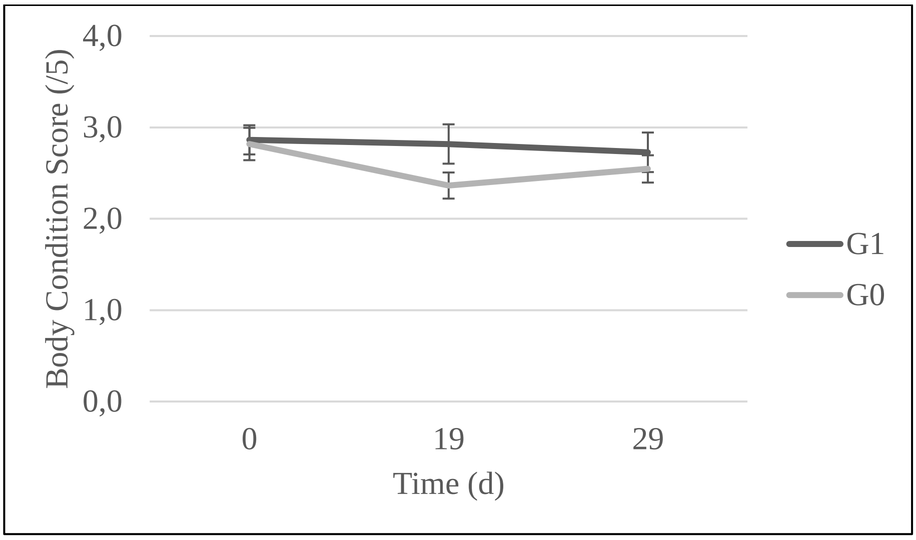

Figure 2. Body Condition Score of sheep according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis) in the time.

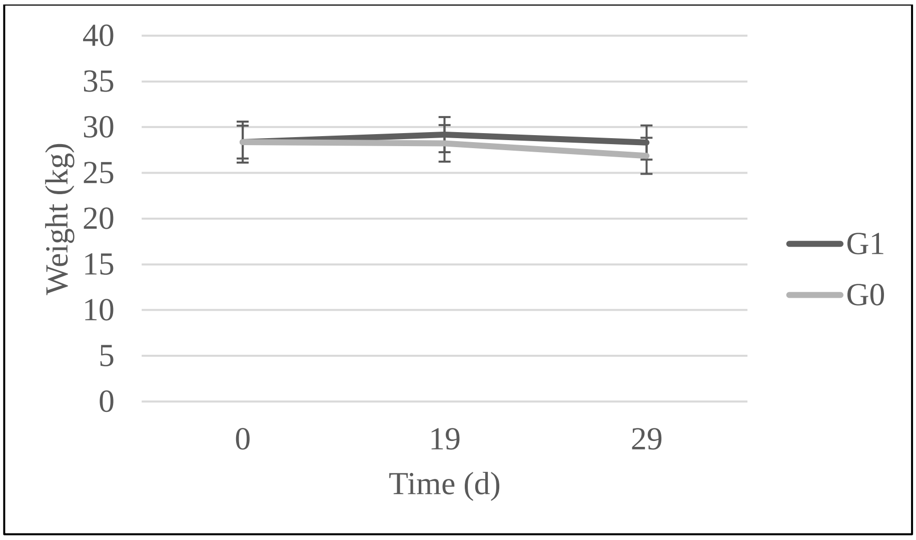

Figure 3. Weight of sheep according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis) in the time.

3.4. Parasitological load response to treatment

Coprological analyses allowed to identify three main GI parasite's types: helminths (trichostrongyle-type and *Strongyloides spp.*) and protozoa (*Eimeria* spp.).

Trichostrongyle-type eggs load (OPG) showed no significant difference between G0 and G1 at any time (Figure 3). However, the average trichostrongyle-type eggs showed a linear increase ($p < 0.001$).

Eimeria spp. oocysts (OPG) load also showed no significant difference between G0 and G1, at any time. However, a significant effect of the time (Pr>F, $p < 0.05$) was observed: the proportion of oocysts decreased in the second part of the experiment.

Finally, the load of *Strongyloides spp.* eggs (figure 5) showed no significant difference between G0 and G1 at any time but parasite load peaks on day 25.

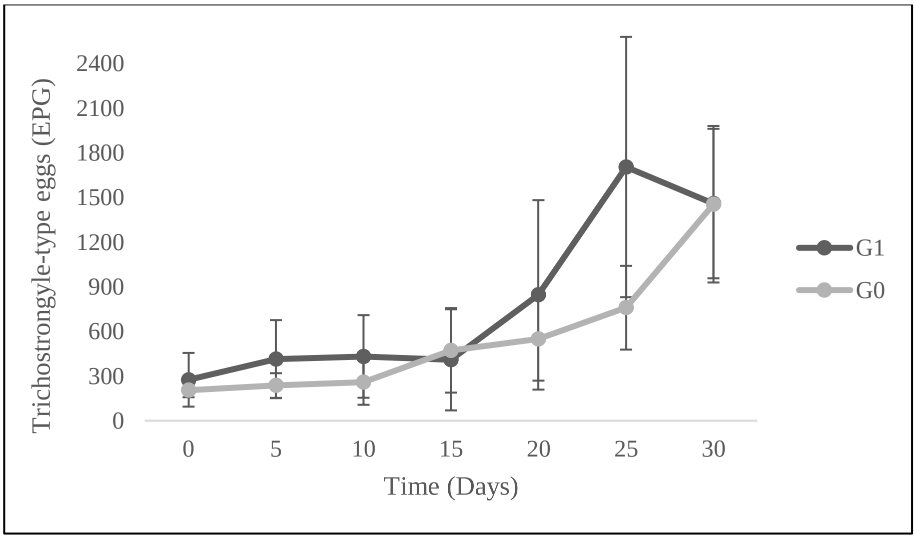

Figure 3. Parasitic load of Trichostrongyle-type eggs of sheep according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis) in the time.

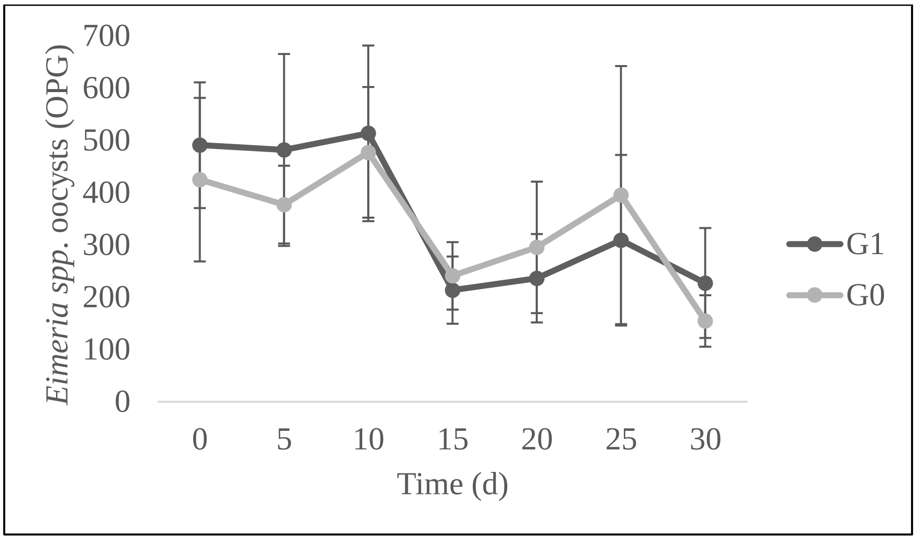

Figure 4. Parasitic load of Eimeria spp. oocysts (OPG) of sheep according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis) in the time.

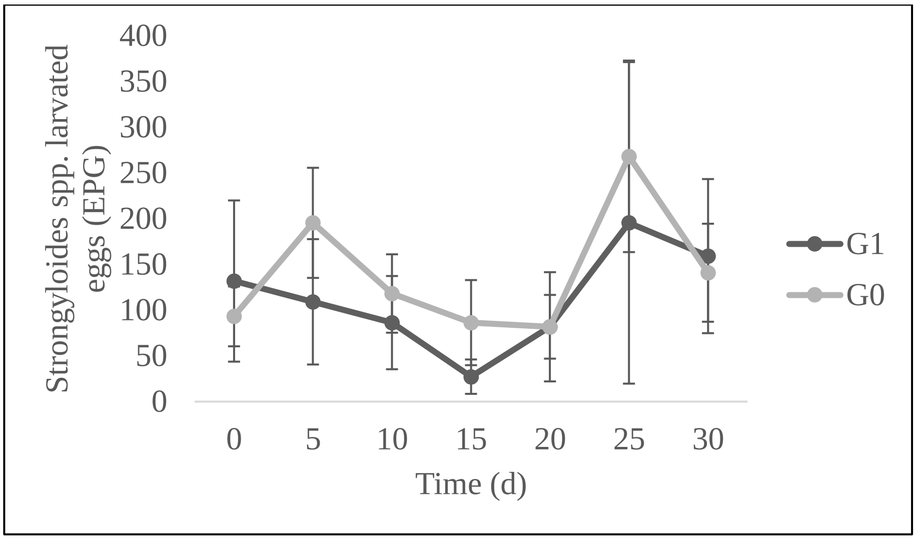

Figure 5. Parasitic load of Strongyloides spp. larvated eggs of sheep according to the studied group (G1 : with foliage of G. ulmifolia and hay of C. nlemfuensis, G0 : with only hay of C. nlemfuensis) in the time.

4. DISCUSSION

4.1. Bromatological composition and nutritional value (energy, protein)

Medium refusal rate of *G. ulmifolia* was 2.2% of the total weight offered, it decreased over time (reaching 0.9% at the end of the experiment), indicating a great appetence for the foliage of this tropical tree.

G. ulmifolia proof to be a valuable forage source. Rich in protein, calcium and having low substitute rate, *G. ulmifolia* foliage supplementation in a poor diet allows to cover metabolizable energy (ME) and digestible protein (MP) supplies while sparing forage. Assessment calculation of the more adequate percentage of *G. ulmifolia* foliage in order to establish this equilibrium was 16% of the diet DM on the basis of energy, IDP and RDNB equilibrium. So, in comparison with the study approach, a sparing use of *G. ulmifolia* is possible while keeping the improvement in maintenance performance. Moreover *G. ulmifolia* contributed to elaborate an optimum diet, thus helping to avoid nutritional deficiencies. This is an important point considering the fact that protein, mineral or other diet deficits impact ruminal flora quality (Agabriel, 2010). Also a balanced feeding improves resistance to parasites. A sheep in an undernourishment state (protein in particular) is less resilient and resistant to GI parasites (Pugh *et al*., 2021). By contrast, subclinical parasitism can jeopardize nutrient requirements. Indeed, in general, due to the greater use of amino acids to mount the immune response and the increased GI tract tissue protein turnover, internal parasitism has an impact on the MP requirement, which seem to be relatively greater than the ME requirement (Lui *et al.*, 2003). Consequently the optimum ME: DP dietary ratio (characterized by RDNB) is influenced. In this way it has been shown that CTs, by binding to dietary protein and reducing rumen proteolysis, increased the proportion of dietary protein in small intestines and consequently protein absorption (Waghorn *et al*., 2008). Their benefits are more likely when dietary protein exceeds requirements, which was the case in this experiment (Table IV). However this CTs capacity seemed to be correlated with both structure of proteins and CTs themselves. Indeed in recent studies summarized in Mueller-Harvey's review (2019) the mean degree of polymerization length (mDP) of CT (which might be associated with the specific CT type in certain cases) seemed to be correlated with the bioactive effect on proteins.

Thus, the only proof of the presence of condensed tannins, even in important proportions, is not sufficient to warrant benefits due to a certain type of CTs.

4.2. Variable parameters

Unlike other studies (Rubio *et al*., 2004), supplementation with *G. ulmifolia* has shown no significant effect on weight gain. However, alongside the experiment time weight differences between G0 and G1 increased numerically. The evaluation time was short in this experiment (added to the absence of adaptation period for sheep) but the percentage of refusal of G. ulmifolia decreased over time. This could explain the fact that no significant differences were noticed. Moreover, as a consequence of nutritional issues mentioned, GI nematode infection could have a significant impact on the metabolic status of sheep, including weight and BCS. This clinical impact increased alongside with the parasitic load. In this experiment the parasitic load of nematodes increased during the experiment, reaching a significant parasitosis at > 1000 EPG (500 to 1000 EPG being considered as a medium infestation and > 1000 EPG as a severe infestation according to Chartier and collaborators (2000). This gradual increase in parasite load after 15 days of experience could be partly explained by the climate that impacts the development of helminth. Thus, an infestation could have occurred on pasture at the start of the rainy season (i.e from June) before the sheep batching. The prepatent period varying between 3 and 5 weeks according to type of helminth (Chartier *et al*., 2000), a significant consecutive excretion of eggs in feces could have gradually taken place from the second part of the experiment (i.e mid-July). This progression could also be explained by the important stress linked to the allotment in a limited space. Besides there were one-year-old animals in the groups on which parasitic impact is greater. In older animals repeated parasitic exposures allow the establishment of immunity and thus a parasitic resistance (Pugh *et al*., 2021; Zajac, 2006).

Lack of time to adaptation, stress (little space, human interaction) and environmental contamination due to space concentration could impact the experiment but evaluation of nutritional supplies and intakes such as minerals, protein and energy suggested no severe deficiency likely to impact parasitological evolution. The higher proportion of *G. ulmifolia* in the diet in comparison to the study of León-Castro and collaborators (2015) (30% versus 10% DM of the total diet respectively) let expect a possible effect would emerge. However, as well as the older study, the present supplementation with *G. ulmifolia* shown no significant antiparasitic effect. When comparing the chemical compositions of *G. ulmifolia* foliage in both experiments, the concentration of CT was higher in León-Castro and collaborators experiment (2015) (4.1% DM versus 1.1% DM presently). This difference highlighted the fact, described in the review of Mueller-Harvey (2019), that CT concentration varies greatly not only between plant species but as well between accessions. Yet the variation of CT according to the analysis technique is also to notice (Zeller, 2019). Nevertheless the parasitological impact of CT in diet fluctuates also according to the CT type (structural composition, ratio procyanidin-type / prodelphinidin-type, mDP) (Mueller-Harvey *et al*., 2019). Thus, the lack of significant antiparasitic impact could have been due to an insufficient concentration or to an inadequate structural type of the CTs contained in *G. ulmifolia* foliage.

5. CONCLUSIONS

G. ulmifolia has proven to be a valuable protein and calcium forage source. Its use as a diet complement to correct protein deficit in a basic-medium ration is valuable. Considering that adequate nutrient intake is very important for sheep productivity and resistance to parasitological infection.

In this short time period study, with the proposed *G. ulmifolia* supplementation, no significant impact of supplementation on weight and BCS was revealed.

Considering the antiparasitological and nutritional benefits of CT present in *G. ulmifolia* foliage no evidence of effects could have been highlighted in a significante way. A longer experiment would have been interesting to confirm this. Moreover a structural composition analysis (ratio PC/PD, mDP) of CTs proper to *G. ulmifolia* would be necessary in order to investigate whether the lack of effect could be ascribed due to an insufficient concentration or to the CT type which enters in its composition.

6. BIBLIOGRAPHY

Abbott, K. A., Taylor, M., Stubbings, L. A. (2012) Sustainable worm control strategies for sheep: A technical manual for veterinary surgeons and advisers, 4th Edition. http://www.scops.org.uk/content/SCOPS-Technical-manual-4th-Edition-June-2012.pdf. Accessed June 29, 2019.

AFNOR. (Ed.), 1982. Norme NF U44-171 : Sludge, organic soil conditioners, growth media, determination of dry matter. AFNOR Editions.

AOAC. (Ed.), 2000. Official Methods of Analysis. 17th edition. Association of Official Analytical Chemist, Arlington, 74 pp.

J. Agabriel. (Ed.), 2010. Alimentation des bovins, ovins et caprins: Besoins des animaux, valeurs des aliments: Tables Inra 2007, mise à jour 2010. Editions Quae, Versailles, 315pp.

Berenguer, B., Trabadela, C., Sánchez-Fidalgo, S., Quílez, A., Miño, P., De la Puerta, R., Martín-Calero, M. J., 2007. The aerial parts of *Guazuma ulmifolia* Lam. protect against NSAID-induced gastric lesions. Journal of ethnopharmacology 114(2), 153–160.

Caballero-George, C., Vanderheyden, P.M., De Bruyne, T., Shahat, A.A., Van den Heuvel, H., Solis, P.N., Gupta, M.P., Claeys, M., Pieters, L., Vauquelin, G., Vlietinck, A.J., 2002. In vitro inhibition of [3H]-angiotensin II binding on the human AT1 receptor by proanthocyanidins from *Guazuma ulmifolia* bark. Planta medica 68(12), 1066–1071.

Camporese, A., Balick, M.J., Arvigo, R., Esposito, R.G., Morsellino, N., De Simone, F., Tubaro, A., 2003. Screening of anti-bacterial activity of medicinal plants from Belize (Central America). Journal of ethnopharmacology 87(1), 103–107.

Chartier, C., Itard J., Morel P., Troncy P. (Eds), 2000. Précis de parasitologie vétérinaire tropicale. Editions Tec et Doc, Paris, 774pp.

De Graef, J., Claerebout, E., Geldhof, P., 2013. Anthelmintic resistance of gastrointestinal cattle nematodes. Vlaams Diergeneeskd. Tijdschr 82, 113–123.

Feedbase, 2020. Bastard cedar (*Guazuma ulmifolia*), aerial part, fresh: Individual data on dry matter basis. http://www.feedbase.com/feedsample.php?Lang=E&name=5723&databasis=2 Consulté le 1 avril 2020

Felipe, A.M., Rincão, V.P., Benati, F.J., Linhares, R.E., Galina, K.J., de Toledo, C.E., Lopes, G.C., de Mello, J.C., Nozawa, C., 2006. Antiviral effect of *Guazuma ulmifolia* and Stryphnodendron adstringens on poliovirus and bovine herpesvirus. Biological & pharmaceutical bulletin 29(6), 1092–1095.

García, E. (Ed), 2004. Modificaciones al sistema de clasificación climática de Köppen. 5ª Edición. Universidad Nacional Autónoma de México, Instituto de Geografía, Mexico, 97 pp.

Herrera-Manzanilla, F.A., Ojeda-Robertos, N.F., González-Garduño, R., Cámara-Sarmiento, R., 2017. Veterinary parasitology: regional studies and reports gastrointestinal nematode populations with multiple anthelmintic resistance in sheep farms from the hot humid tropics of Mexico. Vet. Parasitol. Reg. Stud. Reports 9, 29–33.

Hoste, H., Torres-Acosta J.F.J., Sandoval-Castro C.A., Mueller-Harvey I., Sotiraki S., Louvandini H., Thamsborg S.M., Terrill T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. Vet. Parasitol. 212, 5–17.

Hoste, H., Torres-Acosta J.F.J., J. Quijada, I. Chan-Perez, M.M. Dakheel, D.S. Kommuru, Terrill T.H., Mueller-Harvey I. 2016. Interactions between nutrition and infections with *Haemonchus contortus* and related gastrointestinal nematodes in small ruminants. In: R.B. Gasser and G. Von Samson-Himmelstjerna. (Eds), Advances in parasitology. Vol. 93. *Haemonchus contortus* and Haemonchosis: Past, present and future trends. Academic Press, New York, pp. 239–351.

INRA, 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, 640pp.

Kommuru, D.S., Barker T., Desai S., Burke J.M., Ramsay A., Mueller-Harvey I., Miller J.E., Mosjidis J.A., Kamisetti N., Terrill T.H., 2014. Use of pelleted sericea lespedeza (*Lespedeza cuneata*) for natural control of coccidia and gastro-intestinal nematodes in weaned goats. Vet. Parasitol. 204, 191–198.

León-Castro, Y., Olivares-Pérez, J., Rojas-Hernández, S., Villa-Mancera, A., Valencia-Almazán, M.T., Hernández-Castro, E., Córdova-Izquierdo, A., Jiménez- Guillén, R., 2015. Effect of three fodder trees on *Haemonchus contortus* control and weight variations in kids. Ecosistemas y Recur. Agropecu. 2, 193–201.

Lui, S.M., Master D.G., 2003. Potential impact of nematode parasitism on nutrient partitioning for wool production, growth and reproduction in sheep. Aust. J. Exp. Agric. 43, 1409-1417

Magos, G. A., Mateos, J. C., Páez, E., Fernández, G., Lobato, C., Márquez, C., Enríquez, R. G., 2008. Hypotensive and vasorelaxant effects of the procyanidin fraction from *Guazuma ulmifolia* bark in normotensive and hypertensive rats. Journal of ethnopharmacology 117(1), 58–68.

Martínez-Alfaro, J.C., Valdés-Oyervides, F.J., Ruíz-Zarate, F., 2014. Preferencia y conducta ingestiva de la oveja Pelibuey sobre el follaje de árboles tropicales. In : Universidad Autónoma de Chiapas (Ed.), Proceedings of the 1st Congreso Mesoamericano de Investigación., Tuxtla Gutiérrez, Chiapas, October 1-3 2014.

Martínez-Alfaro, J.C., Valdés-Oyervides, F.J., Ruíz-Zarate, F., Nahed-Toral, J. 2015. La utilización de follaje arbóreo en la alimentación y conducta ingestiva de la oveja Pelibuey. In : Centro de convenciones (Ed.), Proceeding the 2nd Tropical Livestock World Congress.

Tampico, Tamaulipas. Febuary 15-17 2015.

Mercurio, M.D., Dambergs, R.G., Herderich, M.J., Smith, P.A., 2007. High throughput analysis of red wine and grape phenolics - Adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. J. Agric. Food Chem. 55, 4651–4657.

INRA, 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, 640pp.

Meschy, F., 2010. Nutrition minérale des ruminants. Editions Quae, Versailles, 208 pp.

Mondragón-Ancelmo, J., Olmedo-Juárez, A., Reyes-Guerrero, D.E., Ramírez-Vargas, G., Ariza-Román, A.E., López-Arellano, M.E., Mendoza de Gives P., Napolitano, F., 2019. Detection of gastrointestinal nematode populations resistant to albendazole and ivermectin in sheep. *Animals* 9 :775, doi:10.3390/ani9100775

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W.F., Salminen, J.P., Skøt, L., Smith, L.M.J., Thamsborg, S.M., Totterdell, P., Wilkinson, I., Williams, A.R., Azuhnwi, B.N., Baert, N., Brinkhaus, A.G., Copani, G., Desrues, O., Drake, C., Engström, M., Fryganas, C., Girard, M., Huyen, N.T., Kempf, K., Malisch, C., Mora-Ortiz, M., Quijada, J., Ramsay, A., Ropiak, H.M., Waghorn, G.C., 2019. Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. Crop Sci. 59, 861–885.

Nahed, T.J., Gomez, H., Pinto, R., Guevara, F., Medina, F., Ibrahim, M., Grande, D., 2010. Research and development of silvopastoral systems in a village in the buffer zone of the Ocote Biosphere Reserve, Chiapas, Mexico. Research Journal of Biological Sciences 5, 499-507.

National Research Council (Ed.), 2007. Nutrient requirements of small ruminants sheep, goats, cervids, and new world camelids. National Academies Press, Washington, 362pp.

Partida de la Peña (Ed.), J.A., Braña, V.D., Jiménez S.H., Ríos, R.F.G., Buendía, R.G., 2013. Producción de carne ovina - Libro Técnico No. 5. Centro nacional de investigación disciplinaria en fisiología y mejoramiento animal – Instituto nacional de investigaciones agrícolas forestales y pecuarias, Ajuchitán, 116 pp.

Pugh, D.G., Baird, A.N., Edmondson M.A, Passler, T. (Eds), 2021. Sheep, goat, and cervid medicine. 3rd edition. Elsevier, Edinburgh, 1575 pp.

Pinto-Ruiz R., Gómez-Castro H., Guevara-Hernández F., Hernández-Sánchez, D., Ruíz-Sesma, B., 2014. Preferencia y Conducta ingestiva de ovinos alimentados con frutos arbóreos tropicales. Revista Científica RCV-LUZ. 27(2), 158-163.

Ribéreau-Gayon, P., Stonestreet, E., 1966. Le dosage des tannins du vin rouge et la détermination de leur structure. Chimie Analytique 48, 188-196.

Rubio, E.E.S., Pérez Rodríguez, D., Reyes, L.O., Buenfil, G.Z., 2004. Evaluación del potencial forrajero de árboles y arbustos tropicales para la alimentación de ovinos. Téc.Pecu.Méx. 42, 129–144.

Santiago-figueroa, I., Lara-bueno, A., González-garduño, R., López-arellano, M.E., Luis, J., Rosa-arana, D., Maldonado-simán, E.D.J., 2019. Veterinary parasitology : regional studies and reports anthelmintic resistance in hair sheep farms in a sub-humid tropical climate , in the Huasteca Potosina, Mexico. Vet. Parasitol. Reg. Stud. Reports 17, 100-292.

Saratsis, A., Voutzourakis N., Theodosiou T., Stefanakis A., Sotiraki S., 2016. The effect of sainfoin (*Onobrychis viciifolia*) and carob pods (*Ceratonia siliqua*) feeding regimes on the control of lamb coccidiosis. Parasitol. Res. 115, 2233–2242.

SAS Institute Inc. (Ed), 2004. SAS/STAT® 9.1 User's guide. SAS Institute Inc. Cary, 5136 pp.

Sosa, R.E.E., Rodríguez, P.D., Ortega, R.L., Zapata, B.G., 2004. Evaluación del potencial forrajero de árboles y arbustos tropicales para la alimentación de ovinos. Téc. Pec. Méx. 42(2), 129-144.

Terrill, T.H., J.E. Miller J.E., Burke J.M., Mosjidis J.A., Kaplan, R.M., 2012. Experiences with integrated concepts for the control of *Haemonchus contortus* in sheep and goats in the United States. Vet. Parasitol. 186, 28–37.

Thienpont, D., Rochette, F., Vanparijs, O.F.J. (Eds), 1986. Diagnosing Helminthiasis by Coprological Examination. 2nd Edition. Janssen Research Foundation, Beerse, 205 pp.

Thompson J., Meyer H. (Eds), 1994. Body condition scoring of sheep. OSU Extension Catalog, Oregon State University, Corvallis, 4 pp.

Van Soest, P. J., Robertson J. B., Lewis B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583−3597

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production: Progress and challenges. Anim. Feed Sci. Technol. 147, 116–139.

Zeller, W.E., 2019. Activity, purification, and analysis of condensed tannins: Current state of affairs and future endeavors. Crop Sci. 59, 886–904.

Zajac A.M., 2006. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. Vet Clin Food Anim. 22, 529-541.