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Impact of a Polyherbal Mixture (*Withania somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinalis*) on Lamb Growth and Ruminal Fermentation

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Abstract

The aim of this study was to evaluate a polyherbal mixture with Withania somnifera, Ocimum sanctum, Tinospora cordifolia and Emblica officinalis on lambs growth and ruminal fermentation. Thirty crossbred lambs (initial body weight 21.461 ± 2.989) were randomly assigned in three treatments that consisted of rations with the polyherbal mixture at: 0.0, 0.5 or 1.0% of dry matter in a finishing ration with 85% concentrate during 60 days period with lambs fed individually. Digestibility, total VFA concentration, acetate proportion and ruminal pH were increased linearly (P<0.10) as the polyherbal mixture was increased in the diet. The proportion of butyrate was reduced linearly (P<0.01) and feed conversion was improved (quadratic effect P<0.07). The results indicate that the polyherbal mixture improved feed conversion and digestibility, increasing the fermentation activity in the rumen.

Keywords: *Digestibility, Feed plant additive, Lambs, Rumen*

INTRODUCTION

Polyherbal mixtures are an alternative to improve ruminant production (Martínez-Aispuro et al. 2019; Cañada et al. 2018; Mendoza et al. 2019 Gutiérrez et al. 2019) and can play an important role in replacing banned ionophores in several countries. However, the available products must be evaluated at different doses to determine the optimal conditions in order to obtain the benefits of these phytogens (Frankič et al. 2009).

A polyherbal based on medicinal plants from India (*Withania somnifera, Ocimum tenuiflorum, Tinospora cordifolia* and *Emblica officinalis*) combines secondary metabolites adding polyphenols, flavonoids and antioxidants that when were dosed to calves improved digestibility and growth (Velázquez et al. 2019). In another experiment, the polyherbal favored greater weight gains in goats during deworming (Roy et al. 2003). Tannins and saponins at certain concentrations can be positive by improving rumen fermentation but at high doses can cause negative effects (Jouany and Morgavi, 2007; Frutos et al. 2004; Patra, 2010). Phytogenic antioxidants have been recognized as an important additive to be included in rations in ruminants under stress conditions (Humer et al. 2018) and nutritional stress can occur in lambs with high grain diets where lambs do not always manifest the daily gain expected (Hernández et al. 2017). Therefore, the objective of this study was to evaluate the productive performance of lambs and some indicators of ruminal fermentation with different levels of a polyherbal mixture included in a finishing diet for lambs.

MATERIALS AND METHODS

A polyherbal mixture with *Withania somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinalis* was used (ImmuPlus® from Nuproxa México, Indian Herbs).

1. *In vitro* evaluation and chemical composition of polyherbal

The *in vitro* ruminal degradation kinetics of the polyherbal mixture was evaluated using the gas production technique (Theodorou et al. 1994) estimating the parameters: lag phase (h), maximum volume (Vmax; mL), gas production rate (S %/h) and mean time ($K_{0.5}$ h) as described by Rodríguez-Guerrero et al. (2018). The inoculum was obtained from two sheep fed alfalfa and concentrate (50:50), obtained with an esophageal probe, using 10 ml of ruminal fluid without particles and 80 ml of buffer solution (Goering and Van Soest, 1970) under anaerobic conditions incubated at 38°C. Gas pressure was recorded at 0, 2, 4, 6, 8, 10, 14, 18, 24, 30, 36, 42, 48, 60 and 72 hours of incubation (Blümmel and Lebzien, 2001). At the end of the fermentation, the residual dry matter (DM) was weighed to estimate the *in vitro* dry matter digestibility (IVDMD) (Osorio-Terán et al. 2015). Samples of the polyherbal were analyzed for DM, Crude Protein (CP), Ethereal Extract, Ash (AOAC, 1990) and Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) with the procedures of Van Soest et al. (1991).

2. Finishing-Growth period test and ruminal fermentation

The experiment was carried out in the metabolic unit of INIFAP CENID (Ajuchitlán, Querétaro, Mexico) with 30 cross lambs (creole x pelibuey) with an initial body weight of 21.461 ± 2.989 which were assigned in three treatments consuming the polyherbal mixture: 0.0, 0.5 or 1.0% of DM mixed in a basal ration (dry basis) with corn grain (60.0%), corn stover (22.0%), soybean meal (7.0%), cane molasses (5.0%), corn gluten (3.0%), urea (1.0%) and mineral premix (2.0%) formulated for finishing lambs (NRC, 2007) with 2.8 Mcal of metabolizable energy (ME) and 14.5% of CP. The lambs were individually fed for 60 days. Before starting the experiment, the lambs were dewormed, vaccinated and had a period of 15 days of adaptation to the experimental diet.

Starting on day 50 and for 5 consecutive days, fecal grab samples were collected to estimate the apparent digestibility of DM using insoluble acid ash as an internal marker (Van Keulen and Young, 1977). Before feeding on day 60, a sample of ruminal fluid was obtained using an esophageal probe; the pH was immediately measured and then was acidified to perform the analysis of volatile fatty acids (VFA) by gas chromatography (Erwin et al. 1961).

3. Statistical analysis

The parameters of the *in vitro* ruminal degradation kinetics test were estimated by regression with the Menke and Steingass (1988) model where:

$$Vo = Vmax / (1+ e (2-4 * s * (t-L)))$$

The results of the lamb test were analyzed as a completely randomized design testing the linear and quadratic effects of the concentration of the polyherbal mixture with orthogonal contrasts, using the R software (Mirman, 2014).

RESULTS AND DISCUSSION

1. *In vitro* evaluation and chemical composition of polyherbal

The composition of the polyherbal and the fermentation kinetics are presented in Table 1. *In vitro* gas production indicated that half of the polyherbal mixture was fermented at 15.4 hours despite its low cell wall content. This indicated that some secondary metabolites act on the rumen and others may have post-ruminal effects. Based on *in vitro* gas equations (Menke and Steingass, 1988), the polyherbal ME content estimated was 3.042 Mcal/kg. The same polyherbal was analyzed by Priyadarshini et al. (2012) who reported similar composition values (CP 6.96%, fat 1.57%, organic matter 92.20%) but estimated a lower ME value (2.96 Mcal/kg). *In vitro* gas production indicates that the polyherbal has a degradation time similar to that of another polyherbal made with plants from India (Rodríguez-Guerrero et al. 2018) and similar to three herbs

that contain tannins and saponins (Singh et al. 2018) confirming its resistance to ruminal degradation like other polyherbal mixtures elaborated in India with positive results in ruminants (Godinez-Cruz et al. 2015; Martínez-Aispuro et al. 2019).

Table 1. Chemical composition and in vitro gas production parameters of the polyherbal mixture (*Withania somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinalis*)

Variable	Mean
Dry matter, %	91.47
Ash, %	10.41
Protein crude, %	7.37
Neutral detergent fiber, %	18.25
Acid detergent fiber, %	5.39
Ethereal extract, %	2.79
Ca, %	1.36
P, %	0.23
Fermentation kinetics parameters	
Maximum volume, mL	264
Fermentation rate, %/h	3.76
Lag phase, h	-0.031
$K_{0.5}$, h	15.40

Ca: calcium; P: phosphorus; $K_{0.5}$: Time required reaching half of the maximum fermentation volume.

2. Finishing-Growth test and ruminal fermentation

Only feed conversion was improved from the variables recorded in the lamb's growth-finishing test (quadratic effect, P<0.07; Table 2). Other polyherbal in India has improved the growth of finishing lambs (Orzuna-Orzuna *et al.* 2019; Godínez-Cruz et al. 2015; Martínez-Aispuro et al. 2019) but some of those herbals provide vitamins or their precursors. Other phytogenic formulations have not shown responses in productive variables recorded in lambs (Chaves et al. 2008).

Table 2. Effect of the level of the polyherbal mixture on the productive behavior of lambs and ruminal fermentation parameters

	Polyherbal mix % DM				*P value*	
	0.0	0.5	1.0	SEM	Linear	Quadratic
Initial weight, kg	20.60	21.78	21.95	0.960	0.34	0.68
Final weight, kg	32.11	33.84	34.13	0.964	0.15	0.54
DM intake, g/d	1116.5	1141.5	1060.2	31.53	0.21	0.18
ADG, g	190.9	201.3	203.2	8.16	0.29	0.67
Feed conversion	5.87	5.78	5.35	0.193	0.07	0.47
Digestibility, %	67.32	68.53	73.55	2.602	0.10	0.55
Ruminal pH	6.17	6.27	6.53	0.073	0.001	0.36
Total VFA, mM	101.40	104.61	110.96	3.183	0.04	0.69
Acetate, %	44.40	45.37	47.82	0.887	0.01	0.50
Propionate, %	42.26	41.64	40.92	0.653	0.15	0.95
Butyrate, %	13.32	12.98	11.25	0.471	0.004	0.24

SEM: standard error of the mean; DM: dry matter; AGD: daily weight gain; VFA: volatile fatty acids.
Polyherbal mix made with *Withania somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinalis*.

The digestibility of the DM, total concentration of VFA, the proportion of acetate and ruminal pH (Table 2) increased as the concentration of the polyherbal in the ration was increased (linear, P<0.10) while the proportion of butyrate was linearly reduced (P<0.01). The mechanisms of action by which the polyherbal

mixture improved digestibility are unknown, but *in vitro* studies with other polyherbal confirm that tannins, saponins and antioxidants increase digestibility (Singh et al. 2018) but at certain concentrations, these metabolites can reduce digestion depending on the chemical characteristics of each molecule (Frutos et al. 2004).

The metabolites of the mixture affected ruminal fermentation as reported with other herbal products (Makkar, 2005). Changes in acetate and butyrate indicate that polyherbal affected some microbial populations (bacteria or protozoa). Some polyherbal plants have shown antimicrobial activity against pathogens in dairy cattle (Shafi et al. 2016; Mushtaq et al. 2018) and probably could affect bacteria of the genus *Butirivibrio* (producers of butyrate) in the rumen (Kopecný et al. 2003). Amylolytic bacteria could also be reduced as observed by Neubauer et al. (2018) with other phytogens resulting in increased production of acetate. The higher acetate concentration can also be explained by the increase in ruminal pH in response to the polyherbal that could improve the conditions for cellulolytic activity (Russell and Dombrowski, 1980; Miwa et al. 1997).

Although only the chemical composition of nutritional fractions was determined, the herbs in the mixture provide various secondary compounds such as polyphenols and flavonoids with antioxidant capacity (Cecchini et al. 2014), *Withania somnifera* contains flavonoid catechin with antioxidant properties (Alam et al. 2011) and a glycoprotein (glycolitanolide) with antimicrobial activity (Tiwari et al. 2014), *Emblica officinalis* has hydrolysable tannins with low molecular weight compounds (Ghosal et al. 1996) with effects on ruminal fermentation and metabolism of the composition of nutritional fractions was nitrogen (Śliwiński et al. 2002; Frutos et al. 2004; Hess et al. 2006) and *Tinospora cordifolia* provides alkaloids, glycosides and phenolics with antioxidant properties (Premanath and Lakshmidevi, 2010; Bhuwan and Sushmita, 2016). These compounds explain the longer mean degradation time compared to conventional forages.

Natural antioxidants contribute to improving ruminal fermentation (Naziroğlu et al. 2002; Wei et al. 2015). *In vitro* experiments indicate that the addition of natural vitamin E increased the concentration of VFA, propionate, gas production, protozoal activity, synthesis of microbial protein and digestibility by 8 to 9% (Belanche et al. 2016).

In relation to ciliates, some secondary compounds reported in herbs have anthelmintic activity (Kamaraj et al. 2011) and these could reduce rumen protozoa allowing greater growth of bacteria (Ortega and Mendoza, 2003). Saponins inhibit protozoans and their population affects the fermentation pattern (Patra, 2010).

Unlike essential extracts and oils, polyherbal compounds are mixtures of whole plants or specific parts; whole plants contain most of the molecules or nutrients that are actively responsible for their biological effects (Frankič et al. 2009) and due to this diversity of metabolites, the mechanisms of action in the animals that consume them and their microbiota are multiple and complex.

CONCLUSION

The polyherbal mixture with *Withania somnifera, Ocimum sanctum, Tinospora cordifolia* and *Emblica officinalis* improved the feed efficiency utilization, increased digestibility of nutrients and ruminal fermentation.

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