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Herbal Mixtures based on Indian Medicinal Plants in Growing Lambs and Ruminal Environments

P.B. Razo-Ortíz[1], P.A. Hernández-García[2], G.D. Mendoza-Martínez[3], J.A. Martínez-García[3], G. Vázquez-Silva[4], C. Díaz-Galván[1], J.J. Ojeda-Carrasco[2], E. Espinosa-Ayala[2]

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ABSTRACT

Background: The prohibition of the use of antibiotics in lamb feeding requires the search for natural alternatives that can improve productive performance and ruminal fermentation parameters. Herbal mixtures can function as cost-effective substitutes for growth promoters since an extraction or purification process is not required.

Methods: An experiment was conducted to evaluate the effect of *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica* on the productive performance of finishing lambs. Thirty-six Hampshire x Suffolk male lambs (initial body weight (BW) 23.52±3.67 kg), in individual crates, were used for 45 days of evaluation after 15 days adaptation and were randomly assigned to treatments which consisted of dietary inclusion of the feed plant additive at 0.000, 0.250, 0.375 and 0.500% of dry matter (DM). Rations were sampled for DM, ash and crude protein (AOAC, 2005), neutral and acid detergent fibre (according to van Soest, 1991) analysis and metabolizable energy was estimated. The animals were weighed on days 1, 15 and 45 before feeding and the average daily gain (ADG) and feed conversion rate were calculated by daily recording of the dry matter intake. Contrasts no orthogonal were used to test linear or quadratic effects of the herbal mixture with initial body weight (BW) as a covariate for ADG and final BW.

Result: There was no effect (P>0.05) of herbal mixtures on ADG, final BW, feed efficiency, DM intake and feed conversion by phytobiotic dietary inclusion. Ruminal pH (P=0.07) increased quadratically in response to the herbal mixture dietary concentration. The acetate increased and propionate reduced, both quadratically (P<0.05). The evaluated doses of the polyherbal additive did not influence growth and feed efficiency of lambs.

Key words: Lambs, Performance, Phytobiotics, Polyherbal.

INTRODUCTION

In recent years, the use of plant additives rich in secondary metabolites in ruminant nutrition has been considered to improve animal performance (Jouany and Morgavi, 2007). Since the early 1990 s, the mixture of Indian plants *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica* in the feeding of domestic animals has shown positive effects on intake, digestibility and weight gain in calves (Wheller, 1994).

The banning of antibiotics and anabolics as feed strategies (Scarth *et al*., 2011) has led researchers to search for feed plant additives to replace the synthetic products (Budan *et al*., 2013). Some herbal mixtures from India have shown positive results in sheep production (Crosby *et al*., 2017; Martínez-Aispuro *et al*., 2019) and dairy cattle (Mendoza-Martínez *et al*., 2019). Therefore, the objective of this research was to evaluate the potential of an herbal mixture in the productive performance and ruminal environment of growing lambs with increasing levels of the feed plant additive.

MATERIALS AND METHODS

This experiment was performed according to the recommendations of the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 2012) and was conducted at the experimental facilities of the Autonomous University of the State of Mexico in UAEM Amecameca University Centre in January 2019.

[1]Doctorado en Ciencias Agropecuarias, Universidad Autónoma Metropolitana-Xochimilco, Coyoacán, Ciudad de México, México.
[2]Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca, Estado de México.
[3]Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco, Coyoacán, Ciudad de México, México.
[4]Departamento El Hombre y su Ambiente, Universidad Autónoma Metropolitana-Xochimilco, Coyoacán, Ciudad de México, México.

Corresponding Author: P.A. Hernández-García, Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca, Estado de México.
Email: pedro_abel@yahoo.com

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Animals and experimental rations

Thirty-six male Hampshire × Suffolk lambs (initial body weight of 23.52+3.67 kg) were assigned by a completely randomised design to treatments which consisted of dietary inclusion of the mixture at 0, 0.250, 0.375 and 0.500% of

dry matter (DM) of Mebogrow® (Nuproxa México, Querétaro, México) in a basal diet formulated for finishing lambs (Table 1; NRC, 2007). The herbal product was based on *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica.*

Experimental procedures

The lambs were individually housed in individual metabolic crates with a single feeder and nipple drinker. Feed was offered at 08:00 and 15:00 h; water and feed were provided *ad libitum*. Lambs had a 15 day adaptation period to the experimental diets and the experimental period lasted 45 days. Before starting each experiment, lambs were immunised against *Clostridium* (Bobact® 8, 2.0 mL $lamb^{-1}$ intramuscularly) and dewormed (Closantil® 5%, 20 mg kg^{-1} BW orally). Samples of feed were analysed for ash, DM and crude protein by macro Kjeldahl (AOAC, 2005); neutral and acid detergent fibre using the van Soest *et al.* (1991) procedures. Lambs were weighed on days 1, 15 and 45 before morning feeding. The response variables were daily feed intake, average daily gain (ADG), feed: gain ratio and final body weight (BW).

Rumen fluid samples were collected on day 60 by oesophageal probe before feeding (50 ml) and pH measured immediately and then acidified with metaphosphoric acid and frozen until analysis of volatile fatty acids (VFAs) by gas chromatography (Erwin *et al*., 1961).

Statistical analysis

The results are presented as the means and standard error of the means (SEM). The Shapiro-Wilk's test was used to determine normal distribution and the results were analysed as a completely randomised design. The initial BW was tested as a covariate and the coefficients were estimated by an interactive matrix program to test linear or quadratic effects of herbal mixture concentration with the R Software (Mirman, 2016). Statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSION

Initial BW was not significant as a covariate. Final BW, ADG, daily intake and feed conversion were not affected ($P>0.10$, Table 2) by dietary inclusion of the herbal mixture. The pH showed a quadratic response ($P<0.10$), increasing with the intermediate concentrations. However, statistical differences were observed in the ruminal volatile fatty acids (VFA s). The acetate increased (linear and quadratic effect $P<0.05$) and propionate reduced (quadratic effect; $P<0.05$). Butyric acid and total VFA s were not affected by the inclusion of the herbal mixture ($P>0.05$).

The lack of response in this experiment contrasts with previous reports where the animals responded under stress conditions. Misra and Agrawala (2005), who evaluated the same phytobiotic as support for the treatment of metabolic problems in ruminants, found positive responses and found that the variation in feed consumption between days decreased. Polyherbal products, due to the content of their metabolites, are sometimes used for their antibiotics, immunomodulators and immunostimulants, growth promoters, methane reducers and anthelmintic properties

Table 1: Experimental diets and chemical composition of the experimental ration.

	MeboGrow [a], %			
	0	0.250	0.375	0.500
Ingredients, %				
Corn, grain	60.000	59.750	59.625	59.500
Corn, straw	22.0	22.0	22.0	22.0
Soybean paste	7.0	7.0	7.0	7.0
Molasses	5.0	5.0	5.0	5.0
Corn, gluten	3.0	3.0	3.0	3.0
Urea	1.0	1.0	1.0	1.0
Mineral premix[b]	1.0	1.0	1.0	1.0
Sodium bicarbonate	1.0	1.0	1.0	1.0
Polyherbal additive	0.0	0.250	0.375	0.500
Chemical composition, %				
Dry matter	87.70	87.47	87.36	87.25
Ash	4.21	4.20	4.21	4.23
Crude protein	14.71	14.47	14.46	14.45
Neutral detergent fibre	23.78	23.56	23.81	23.72
Acid detergent fibre	12.69	12.70	12.76	12.77
Metabolizable energy[c], $Mcal^{-1}$	2.836	2.828	2.825	2.819

[a] MeboGrow formulated with *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica*. [b] Mineral premix: 27% calcium, 3% phosphorus, 0.75% magnesium, 4.2% sulphur, 6.5% sodium, 0.05% potassium, 978 mg/kg iron, 15 ppm cobalt, 5 ppm copper, 25 ppm zinc, 2.25 ppm molybdenum, 1 ppm selenium, 160 ppm iodine. [c] estimated with NRC (2007) values.

Table 2: Effect of the concentration of herbal mixture on the productive performance of lambs and ruminal fermentation.

	MeboGrow[a], %				SEM	*P value*	
	0	0.25	0.375	0.5		Linear	Quadratic
Initial weight, kg	23.60	23.38	23.26	23.91	1.208	0.92	0.73
Final weight, kg	37.82	37.49	38.28	37.40	0.987	0.91	0.85
Daily intake, kg/d	1.458	1.430	1.578	1.474	0.0443	0.37	0.79
ADG, g	0.337	0.329	0.375	0.325	0.0231	0.86	0.82
Feed conversion	4.45	4.49	4.58	4.05	0.229	0.68	0.96
Ruminal pH	6.69	7.04	6.96	6.89	0.108	0.16	0.07
Total VFA s, mmol/L	74.68	68.89	73.68	73.52	7.318	0.94	0.61
Acetic, %	47.09	52.11	51.79	50.31	1.152	0.02	0.01
Propionic, %	44.29	39.47	39.77	42.02	1.291	0.11	0.01
Butyric, %	8.61	8.40	8.43	7.66	1.016	0.53	0.71

SEM, Standard error of the mean; ADG, average daily gain; VFA s, volatile fatty acids. [a]Formulated with *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica*.

(Lillehoj *et al*., 2018). The terpenes, phenols, polyphenols, carotenoids, oligosaccharides and vitamins from plant species can act as growth promoters (Cardinali *et al*., 2015; Dalle Zotte *et al*., 2016) and effects depend on the metabolite and its mechanism of action. Additionally, the addition of herbs, species and bioactive compounds have beneficial effects in feed formulated for ruminants on the antimicrobial and immune properties (Greathead, 2003; Cardozo *et al*., 2006; Patra *et al*., 2012; Oh and Hristov, 2016). Research evaluating the dietary inclusion of a polyherbal formula in lambs reported that feed intake, daily weight gain and final weight increased due to secondary plant metabolites (Mejia-Delgadillo *et al*., 2021). The inclusion of herbs and plants in the rations for lambs can increase the digestibility of DM and the daily gain due to the fermentative changes that their compounds can cause in their ruminal microbiota; thus, improving daily weight gain (Wanapat *et al*., 2012; Celia *et al*., 2016).

The response in ruminal pH will depend on the type of plant and dose, therefore, results from other herbs can not be compared directly. Rao *et al*. (2016) found a constant decrease in the pH in the rumen fluid as the inclusion of *Azadirachta indica* increased, which contrasts with what is observed here. Tannins in plants can affect ruminal biohydrogenation (Khiaosa-Ard *et al*., 2009) and tannins and phenols can affect the rumen protozoa (Bhatta *et al*., 2012), which could modify starch digestion and indirectly change rumen pH (Ortega and Mendoza, 2003).

Changes in VFA s will depend on the effects of herbs on the populations of bacteria and protozoa. Previous *in vitro* evaluations of the same polyherbal mixture showed increases in the production of acetic and propionic acids (Sardar *et al*., 1997); however, this showed increments in acetic and a reduction in the propionic proportion, which is energetically undesirable and explains the lack of response in daily gain. Phytobiotic compounds could improve ruminal microbiota by functioning as antimicrobials and/or growth promoters. Some tannins can alter the bacterial balance by affecting hydrogen cycles through the inhibition of oxidative phosphorylation in bacterial mitochondria, altering the hydrogen bonds or chelating mineral ions essential for bacterial metabolism (Scalbert, 1991).

Although benefits are expected from herbals, they are not always observed. Wanapat *et al*. (2008) evaluated the inclusion of *Allium sativum* in the rations for ruminates, finding increases in propionic acid, decreases in the acetic-propionic ratio and degradation of ruminal nitrogen, associated with reduced rumen protozoa. Changes in rumen protozoa have been related to improvement in ruminal parameters (Wanapat *et al*., 2012). The changes detected in acetate and propionate do not lead to a possible reduction of methane, although this mixture contains plants that have been evaluated *in vitro* and have decreased methanogenesis (Gupta *et al*., 2017) but *in vitro* protozoa do not survive. *Chebulic myrobalan* has been evaluated in an *in vitro* fermentation and has been reported to influence the decrease in methane production (Heidarian-Miri *et al*., 2013). *Terminalia bellirica* was controlled in *in vitro* fermentations to measure its methane inhibitory capacity, discovering that it has a large number of tannins and concluding that these could act directly on the arches without altering the rumen protozoa (Bhatta *et al*., 2012).

One of the main plants of this mixture is *Azadirachta indica*, which is present in other polyherbals (Martínez-Aispuro *et al*., 2019; Razo-Ortíz *et al*., 2020). *Azadirachta indica* is one of the most studied plants for its metabolites and the major components are terpenes and limonoids with a demonstrated antimicrobial activity and has been used in ethnoveterinary treatments in different species; in ruminants for udder infections and foot root and lice (Ogbuewu *et al*., 2011). The higher acetate concentration and the higher pH with herbs could be indicators of a higher cellulolytic digestion, but this was not manifested in lambs' performance as observed in other feed additives which contain *Azadirachta indica* (Martínez-Aispuro *et al*., 2019; Razo-Ortíz *et al*., 2020). This means that the formulas and their

proportions can result in combinations that may or may not be successful.

CONCLUSION

The evaluated doses of the polyherbal additive based on *Chebulic myrobalan*, *Terminalia bellirica* and *Azadirachta indica* did not improve growth and feed efficiency of lambs, even while having some effects on the rumen fermentation pattern.

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Declaration of conflict of interest

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript and in the decision to publish the results.

Authors' contributions

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

Bioethics and biosecurity committee approval

Research on animals was approved in accordance with the rules of the institutional committee on animal use of the Autonomous University of the State of Mexico campus UAEM Amecameca University Centre and in accordance with the regulations of the Law of Animal Protection of the State of México.

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