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Effect of a Polyherbal Additive on Performance and Parasite Infection of Hair Creole Ewes

M.A. Mejia-Delgadillo[1], H.A. Lee-Rangel[2], P.A. Hernandez-Garcia[3], A. Vazquez-Valladolid[2], H. Mendez-Cortes[2], J.E. Guerra-Liera[1], H.J. Lopez-Inzunza[1]

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ABSTRACT

Background: Gastrointestinal nematodes (GIN) infection represents several problems to livestock production. Control of GIN infection is usually achieved by the use of chemotherapeutic anthelmintic drugs. However, indiscriminate use of anthelmintics led to development of anthelmintic resistance. An alternative to chemical control is use of bioactive plants in animal feeds as natural anthelmintics. The objective of this experiment was to compare the effects of polyherbal additive containing high concentrations of saponins with a conventional chemical antiparasitic drug on performance and GIN counts of ewes.

Methods: Sixteen hair ewes (creole 19.05±0.895 kg initial BW) fed a basal diet, were randomly assigned to one of three treatments: 1) Doramectin (0.2 mg/kg BW, as positive control); 2) Polyherbal additive, Peptasan (4 grams/lamb/day) and 3) Control group that was not dewormed. The experiment was conducted for 28 days. At 0, 14, 21 and 28 parasite infections were assessed by fecal egg counts (FEC).

Result: The daily gain was positively correlated (P<0.01) with herbal product (261 g/d) and Doramectin (146 g/d), in contrast with the control group (33 g/d). The number of eggs per gram of feces (EPG) was significantly (P<0.01) reduced by Peptasan (90 EPG) compared to Doramectin (711 EPG) after 28 days of treatment. At the same time the EPG in control group was remained unchanged (4566 eggs/g). Infection with GIN can affect feed utilization and lamb performance and polyherbal products are alternative anthelmintics to reduce the parasitic infection in growing ewes.

Key words: Anthelmintic, Ewe growth, Herbal products, Nematodes, Phytobiotics.

INTRODUCTION

Gastrointestinal nematodes (GIN) in domestic ruminants are the main parasites that affect their productivity and health, causing economic losses in grazing systems worldwide (Maderos *et al*., 2010). Before the finishing period of lambs in intensive systems, it is common to use pharmaceuticals products to control nematodes and most of them are expensive and some of which experience resistance (Gradé *et al*., 2008). Therefore, the use of non-conventional alternatives such as medicinal plants as anthelmintics is important for control of parasites in sheep (Githiori *et al*., 2003), herbs could be a better alternative for such cases (Shrivastav *et al*., 2021). Extracts from medicinal plants are rich in secondary compounds that reduce GIN infection (Habibi *et al*., 2012). Some secondary components of the plants, such as Volatile Organic Compounds (VOCs) present in many herbal or tree species, can positively or negatively affect animal performance and rumen function, Karnani *et al*. (2021) mentioned that supplementation of herbal products enhance the productivity in goats by improving the ruminal environment. Therefore, the objective of this experiment was to compare the effects of a polyherbal product (Peptasan) with a conventional anti-parasitic drug (Doramectin) over 28 days on lamb growth and parasite infection.

MATERIALS AND METHODS

Flash gas chromatography electronic nose (FGC E-Nose)

The analysis was carried on Coordinación para la Innovacióny la Aplicación de la Ciencia y la Tecnología (CIACyT) of the Universidad Autonoma de San Luis Potosi. The FGC- E-Nose model Heracles II, equipped with an automatic injection unit HS100 (AlphaMOS®, Tolouse, France), was used to detect the VOCs of the Peptasan. The identifications were made using the Kovats index with a C6-C16 standard (Melucci *et al*. 2016).

Experimental animals and design

The experiment was conducted at the experimental Station

[1]Faculty of Agronomy, Autonomous University of Sinaloa, Km 17.5 Culiacán-El Dorado Highway, 8000, Mexico.
[2]Faculty of Agronomy and Veterinary Medicine, Biosciences Center, Autonomous University of San Luis Potosí, S.L.P., 78000, México.
[3]Autonomous Mexico State University, UAEM University Center, Amecameca, México.

Corresponding Author: H.A. Lee-Rangel, Faculty of Agronomy and Veterinary Medicine, Center for Biosciences, Autonomous University of San Luis Potosí, S.L.P., 78000, Mexico.
Email: hector.lee@uaslp.mx

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of the Cuajinicuilapa Campus of the Autonomous University of Guerrero, Mexico (from june 29 to 14 august 2015). Three experimental treatments: 1) Doramectin (0.2 mg/kg BW, subacutely); 2) Polyherbal additive (Nuproxa, Peptasan 32.4% total saponins), 4.0 g/d; 3) Control group that was not dewormed, were assigned at random to 16 ewes (Creole initial body weight 19.05±0.895 kg) in individual cages (0.80 × 1.2 m) with feeder, drinker and shed.

The ewes were weighed two consecutive days at the beginning of the experiment (days 0 and 1) and end of the experiment (days 27 and 28). The feed (Table 1) was provided at 08:00 and 15:00 h. All ewes had free access to feed, ensuring 100 g feed refusal per kg of the amount fed daily. Fecal samples were collected on days 0, 7, 14, 21 and 28 directly from the rectum of each ewe with a gloved finger and identified; the number of fecal eggs counts per gram of feces was counted using the modified McMaster technique (Arece *et al*., 2002). Daily feed intake, average daily gain (ADG) and feed conversion (ratio of kg gain/kg feed intake) were calculated for all lambs.

In vitro gas production

Samples of Peptasan (500 mg) were incubated in 100 mL amber flasks to estimate the kinetics of gas production with rumen fluid. The inoculum consisted of rumen liquor obtained as described by Miranda *et al*. (2017) using an esophageal probe from two sheep (34±1.6 kg BW) fed a 50:50 ratio of forage to concentrate. The inoculum was collected prior to feeding, mixed and strained through eight layers of cheesecloth into a flask flushed with CO_2. Then, 10 mL of particle-free rumen fluid was added to each bottle and 80 mL of buffer solution of Goering and Van Soest (1991) was added under anaerobic conditions. The flasks were incubated in a water bath at 38°C and the gas pressure was measured with a pressure gauge (Metron, Mode: 63100, Mexico) during incubation (Blümmel and Lebzien, 2001). Estimating the following parameters for gas production: lag phase (h), maximum volume (Vmax; mL/g MS of substrate) and rate (S; h^{-1}). The pressure values were transformed to gas volume with the linear regression equation V = (P + 0.0186)/(0.0237). Estimating the following parameters for gas production: lag phase (h), maximum volume (Vmax; mL/g MS of substrate) and rate (S; h^{-1}) of gas production with the model proposed by Menke and Steingass (1988):

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

Where,
Vo= Total gas produced.
v= Volume.
s= Gas production rate.
t= Time.
L= Lag phase.

After testing the variables for normality, the results were analyzed according to a completely randomized design where treatments were fixed effects (Steel *et al*., 1997). Means were compared with Tukey's test and differences among treatments were declared at P<0.05. Data were analyzed with the JMP7 software (Sall *et al*., 2012).

Table 1: Experimental diet (% dry matter basis).

Ingredient	% Inclusion
Cane molasses	10.00
Pangola grass	35.00
Urea	1.00
Soybean meal	5.00
Coconut meal	16.00
Corn grain	10.00
Mineral premix[1]	0.50
Mineral premix[2]	2.50
Total	100.00

[1]Biotecap: Ca 1.88%, P 2.20%, Mg 1.40%, Na 0.035%, Cl 0.15%, Se 18.65 ppm, Cr 5 ppm, Cu 500 ppm, Zn 2050 ppm, Mn 215 ppm, Co 13.50 ppm, I 23.80 ppm, Fe 5000 ppm. Live yeast 5% (*Saccharomyces cerevisiae* 2 × 10^{10} CFU/g). [2]Ovy ways: Se 590 ppm, Cr 990 ppm, Cu 1500 ppm, Zn 3000 ppm, Mn 3000 ppm, Co 30 ppm, I 30 ppm, live yeast cells 1.0 × 10^9 UFC/g, Vit E 40 UI.

Results and Discussion

The compounds identified in the FGC- E-Nose are shown in Fig 1. In total, 104 volatile compounds were identified, but acetaldehyde, 2-propanol, 1-propanol, octane, cyclohexane, (Z)-2-octenal, citronellal, 4-vinylguaiacol, trans-2-undecenal, butane, 2,3-pentadenione, isopropyl

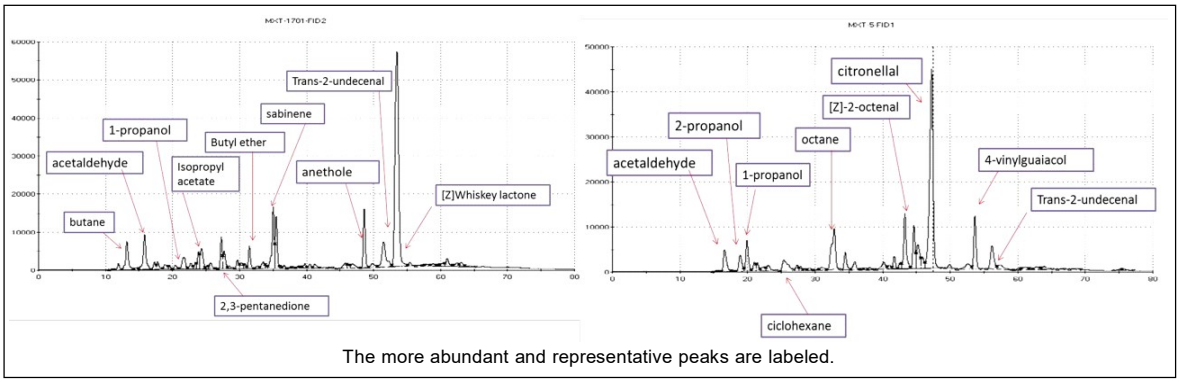

Fig 1: Representative chromatograms to detect VOCs present in the herbal extract.

Table 2: Lamb performance and egg count.

Item	Treatment			SEM	*P*-value
	Control	Herbal extract	Doramectin		
Initial BW kg	18.10	19.11	19.97	0.895	0.71
Final BW kg	19.04^{b}	26.43^{a}	24.06^{ab}	1.016	0.005
ADG g	33^{c}	261^{a}	146^{b}	20.55	0.0001
DM intake kg/day	0.420 ^{c}	1.325^{a}	0.871^{b}	0.0809	0.0001
Feed conversion	21.38^{b}	5.35^{a}	6.15^{a}	2.949	0.037
FEC/gram feces					
Day 0	4389	3978	4267	421.3	0.92
Day 7	4477^{a}	2100^{b}	2155^{b}	373.7	0.008
Day 14	4444^{a}	1000^{b}	1233^{b}	413.4	0.0001
Day 21	4566^{a}	333^{b}	755^{b}	452.2	0.0001
Day 28	4611^{a}	90^{c}	711^{b}	496.9	0.0001

SEM: Standard error of the mean; BW: Body weight; ADG: Average daily gain; DM: Dry matter; FEC: Fecal egg counts.

Table 3: *In vitro* gas production parameters and the chemical composition of the herbal extract (Peptasan).

Item	Peptasan	Standard deviation
Volume maximum gas, mL g^{-1} DM	265	4.6
Rate of gas production, h^{-1}	0.0416	0.001
Lag phase, h	-2.72	0.12
$K_{0.5,}$ h	16.60	0.73

DM: Dry matter; $K_{0.5}$: Time required to obtain half of the volume maximum.

acetate, butyl ether, anethole, sabinene and (Z) whiskey lactone were in higher concentration.

Significant (P<0.05) reduction in fecal egg counts were noticed in ewes treated with Peptasan, the herbal extract decreases a nematode count than in ewes treated with Doramectin, on days 21 and 28. No change in fecal egg counts was noticed in control groups. The predominant nematode species were strongyles (*Trichostrongylus, Haemonchus, Oesophagostomum*). Higher fecal egg counts resulted in reduced daily gain, decreased intake and impaired feed efficiency (Table 2). Gonzalez-Garduño *et al*. (2014) found that in grazing conditions the *H. contortus* and *C. curticei* are the most common gastrointestinal nematodes in tropical areas of Mexico, peptasan showed an anthelmintic effect against these parasites. In previous study, extracts with saponins (12.8-13.2%) and phenolic compounds also reduced nematode FEC but a lesser degree (up to 54%) (Mejia *et al*., 2014). Similar to the present observation the maximum reduction (97.41%) in FEC in sheep naturally infected with paramphistomes was observed on day 21 after treatment with 20 mg/ ml *Acacia concinna* extract (Priya *et al*., 2013).

Plants natural products and essential oil components such as citronellal, linalool, thymol, carvone, anethole, cinnamaldehyde, sabinene, terpenes and phenylpropenes have been shown to have a significant potential for helminth (Barros *et al*., 2009; El-Bahy and Bazh, 2015; Kaitiki *et al*., 2017) and insect (Dambolena *et al*., 2016) control. Nonetheless, owing to difference in the techniques used by different researchers and diverse chemical structure of natural compounds, collation of the effects for the evaluated compounds is difficult, which in turn causes difficult to ascribe their activities to a specific chemical compound. One of the principal VOCs in the herbal product is the [Z] whiskey lactone, which can attack the larval stages of important parasites of sheep and cattle *in vitro*. Doramectin is a broad-spectrum macrocyclic lactone belonging to the ivermectin class; though, crucial in combating nematode parasites in sheep, persistence of drug in tissues up to 28 days post-treatment and withdrawal period limit its usage (Perez *et al*., 2008). Further, the resistance of nematodes to macrocyclic lactones has become a serious challenge to the effective control of nematodes in small ruminants (Borges *et al*., 2015). Doramectin enter the environment through feces of the treated animals and evaluations of the long-term impacts are required (Beynon, 2012).

The effect of anthelmintic treatment on body weight of sheep has been studied by various workers. Treatment with benzimidazoles and macrocyclic lactones (Perez *et al*., 2008; Miller *et al*., 2012) and herbal products (Grade *et al*., 2008; Junkuszew *et al*., 2015) significantly reduced the parasites and significantly increased lamb body weight compared to the parasitized group in accordance to the present study. Drug residues and resistance are the major constraints for the use of chemical therapeutic agents such as benzimidazoles and macrocyclic lactones. The addition of thyme essential oils in Japanese quail feed ration increased live body weight and improved gut microflora (Khaksar *et al*., 2012). The beneficial effects are attributed to active constituents, anethole.

The *in vitro* gas production indicated that herbal plant additive has a mean ruminal degradation of 16.6 hours (Table 3). *In vitro* gas production parameters indicated that herbal Peptasan saponins could be acting in the rumen and the lower tract against different parasites. None of the parameters of *in vitro* gas production (Table 3) appeared to indicate that saponins in the herbal product negatively

affected ruminal fermentation and they showed kinetic parameters similar to or greater than three browse species containing tannins and other compounds that consumed by small ruminants (Camacho *et al*. 2010). Stewart *et al*. (2015) evaluated gas *in vitro* production characteristics of *Juniperus pinchotii*, J. *monosperma, J. ashei* and *J. virginiana* and found that the concentrations of secondary plant compounds should not cause adverse effects on digestibility or *in vitro* parameters. VOCs from peptasan could improve animal performance, health, metabolism, end products and rumen microbial function (Min *et al*., 2012).

Secondary compounds have different effects on rumen fermentation; they reduce rumen protozoa and increase microbial protein synthesis (Patra and Saxena, 2011), Wang *et al*. (2011) reported that saponins could increase VFA production and proportion of propionic acid and reduced ammonia concentration in agreement with Jafari *et al*. (2020) who found that polyphenols decrease the ruminal ratio of acetic/propionic. Saponins in the herbal product could have had this type of stimulatory effect and this may partly explain the superior daily gains and feed conversions observed in lambs receiving the herbal product. Studies conducted with *Acacia concinna* extracts confirm that this plant reduces ruminal methane production without adversely affect *in vitro* dry matter digestibly (Bunglavan *et al*., 2010).

Conclusion

It was concluded that herbal plant additive, Peptasan with high concentrations of saponins can be used at a dose of 4 g/d in intensive lamb diets during the adaptation period to control parasites. The results of *in vitro* degradation appear to indicate that Peptasan do not have negative effects on ruminal fermentation. The use of Peptasan as an anthelmintic improved weight gain and feed efficiency in lambs over the 28-day feeding period.

Conflict of interest

All authors declare that they have no conflict of interest.

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