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Microbiological Feces Count from Covies Fed Different Levels of Oregano in the Diet

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ABSTRACT

Background: Forage and feedstuff technologies with additives contributed to more efficient nutrition of herbivores. **Aim.** To evaluate the microbial content of feces from covies fed forages and feedstuffs containing various levels of oregano. **Materials and methods:** This paper consisted of two studies, one on El Romeral Farm, Guachapala Canton, the University of Cuenca, located on S 2º45’ 54” , W 78º42’ 58” , 2 254 m above sea level; and another at the Molihers food processing factory, in Ochoa Leon, on S 2º49’ 48” , W 78º59’ 12” , 2 600 m above sea level. The control (T1) consisted of feeds for covies (Molihers); the other treatment (T2) was based on Phytogenics, at a rate of 2 kg/ton. Treatment 3 (T3), used phytogenics (3 kg/ton); whereas treatment 4 (T4) contained phytogenics (5 kg/ton). All the treatments contained 13.5% protein. A completely randomized experimental design was used, along with one-way ANOVA. The microbiological composition of feces was measured. **Results:** The fecal bacterial burden was reduced (P <0.05) using oregano compared to the control. **Conclusions:** The nutrition of covies using alfalfa and feeds containing phytogenic additives such as oregano at 3 and 4 kg/t doses of

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the feed proved effective, the bacterial and parasitic counts in the feces were reduced due to the organic principles in the oregano supplemented.
Keywords: herbivores, diet, additives, coprology (*Source: AGROVOC*)

INTRODUCTION

Livestock is closely related to social and cultural development, so forage technologies must be under constant transformation to improve technology and contribute to good water and soil management (FAO, 2020). Covy raising is slowly gaining importance in livestock production with increased consumption in the Ecuadoran urban population, as well as in other Andean nations (Chela, 2015; Regalado and Usca, 2019; FAO, 2020), so many people are working on covy production as an economic alternative. This scenario calls for new research to improve production through nutrition and be able to raise farmer income.

Chela (2015) said that oregano contains essential oils rich in carvacrol, thymol, borneol, and linalool. Among the components of oregano, acids are very abundant. Calo et al. (2019) noted that it is a good source of **vitamins, such as** niacin (B3), which regulates digestive functions in the organisms, and beta-carotene. Oregano also contains **tannins** and **flavonoids**, which are responsible for many of the medicinal properties of the plant. Both substances are considered excellent antioxidants and essential oils, which may be accessed when the plant is ingested naturally (Regalado and Usca, 2019). Accordingly, this paper aims to evaluate the microbial content of feces from covies fed forages and feedstuffs containing various levels of oregano as a plant additive.

MATERIALS AND METHODS

Location of the experiments

Research was done in the province of Azuay, which consisted of two studies, one on El Romeral Farm, Guachapala Canton, the University of Cuenca, located on S 2º45’ 54” , W 78º42’ 58” , 2 254 m above sea level; and the other at the Molihers food processing factory, in Ochoa Leon, on S 2º49’ 48” , W 78º59’ 12” , 2 600 m above sea level.

Climate characteristics of the research areas on Farms El Romeral and Ochoa Leon (Molihers Facility)

Temperature and precipitations

Table 1 shows the climate values for EL Romeral in 2021, the maximum temperature was 22.21 ºC, while the minimum value was 12.1 ºC, and a mean temperature value of 16.16 ºC. Overall annual precipitation was 1 238 mm, with May as the month with the highest values and October as the driest. On Ochoa Leon, (Molihers facility) the maximum temperature was 14.67 ºC, while the minimum value was 8.75 ºC, and the mean temperature value was 11.42 ºC. Annual precipitation was 1 181 mm, with April and October as the rainiest months, and August with the lowest precipitation values.

Table 1. Maximum, minimum, and mean temperature (^{0}C) and precipitation (mm) values by quarter in 2021, in the two experimental areas.

Experimental area	Indexes	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	VC (%)
El Romeral	Maximum temperature (^{0}C)	18-20	18-22	17-20	20-22	11.2
	Minimum temperature (^{0}C)	10-11	11-12	10-11	11-13	8.6
	Precipitation (mm)	100-121	110-140	38-65	118-170	14.3
Ochoa Leon	Maximum temperature (^{0}C)	18-20	17-21	17-20	20-22	9.3
	Minimum temperature (^{0}C)	10-12	11-12	11-12	12-13	12.5
	Precipitation (mm)	118-125	116-136	78-38	70-82	15.7

Table 2. Bromatological analysis of the feeds used in Covies

Parameters	T1	T2	T3	T4
Overall humidity (%)	11.36	12.22	12.17	12.1
Dry matter (%)	88.64	87.78	87.83	87.9
Protein (%)	10.03	9.77	10.27	10.95
Fiber (%)	14.35	14.21	14.26	14.14
Fat (%)	5.03	5.12	5.09	5.18
Ash (%)	12.13	11.54	11.6	11.25
Organic matter (%)	87.87	88.46	88.4	88.75

Source: SETLAB laboratory results, October 2021.

The conventional feedstuffs manufactured at Molihers contained phytogenic additives for covies and were formulated by linear programming (Table 2), using the following raw materials: yellow corn (national), imported soy paste, palmiste, alfalfa meal, molasses, and phytogenic additives at 2, 3, and 5 kg/t, as a substitute of growth promoters.

Experimental design to evaluate the feedstuffs in covies

To evaluate covy response when consuming the feedstuff with growth additives, 96 covies were purchased at 30 days of age approximately, all from the same breed and farm. The experimental material (covies) selection, the animals' weight, and the similarities were within a given range. At 8 days upon receiving the animals (adaptation period), they were tagged on their ears individually. The *in vivo* trials lasted 115 days (July 8th-October 31st, 2021); 48 animals were in El Romeral, and another 48 covies were at Molihers. Each facility was properly cleaned before the experiments. At 10 days, the animals were vaccinated with cuy-vac 0.5ml subcutaneously. Their initial weights were recorded using a precision balance fortnightly until the end of the experiment. A completely randomized design with four replicas was used, which included 16 experimental units (cage), 100 cm long x 50 cm wide, and 45 cm high, with a capacity for three individuals. A total of 32 cages were installed in the two experimental areas. The animals were fed daily with 333.3 g of alfalfa and 33.3 g of feedstuff per individual. Water was administered *ad libitum*. The animals were cleaned and disinfected every two weeks.

Experimental units:

Treatments: The experiment consisted of three treatments with the feedstuff, using increasing doses (2 kg, 3 kg, and 5 kg of phytogenic additives), and one control treatment (Molihers feedstuff containing chemicals), plus alfalfa in all cases.

T (control) Alfalfa + Conventional Molihers feedstuff.

T2 Alfalfa + feedstuff containing 2 kg of the phytogenic material (oregano)/TM.

T3 Alfalfa + feedstuff containing 3 kg of the phytogenic material (oregano)/TM.

T4 Alfalfa + feedstuff containing 5 kg of the phytogenic material (oregano)/TM.

Microbiological laboratory analysis of feces

The parameters evaluated in the feces analysis of covies were Total bacterial, total coliforms, fecal coliforms, aerobic mesophiles, mold and yeasts, and Eimeria OPG, determined 115 days after the end of the experiment, and were analyzed at SETLAB in October 2021. A simple analysis of variance (ANOVA) was performed to analyze the results in all the variables. The normality assumptions (Shapiro-Wilks test $P<0.05$) were evaluated in each model. The statistical significance among the treatments was measured using the Bonferroni test to control the type I, through SPSS ® VERSION 25, (2018).

RESULTS AND DISCUSSION

The new commercial additives from plants, such as aromatic plant extracts and their refined elements, were studied for possible application in future alternative feedstuff applications (Chandran and Athulya 2021; Saleena *et al.*,2021). Because of the lack of residues and acceptance as nutritional insurance, they offer several benefits over conventional antibiotics. These chemical products and their mixtures demand further research about their chemical and biological properties since reliable information is scarce in terms of how this improves the capacity of animals to digest nutrients and remove bacteria, fungi, and other parasitic forms from their intestinal tract. So far, dried preparations have succeeded in a variety of formulations (Usca *et al*.,2019; Uddin *et al.,*2021). The main factors to be considered are variations in the inclusion levels and their effects on the nutrition and health of small herbivores, ruminants, and birds (Chandran 2021 Chandran, and Athulya 2021; Saleena *et al.,*2021; Sharun *et al,* 2021; Shen *et al.,*2021; Uddin *et al.* 2021; Alajil *et al.,* 2022).

Regalado and Usca (2019) in an experiment using different levels of oregano inclusion in the feedstuff, concluded that the best treatment was 2% oregano inclusion, with significant differences ($P <0.05$) in healthier animals, and greater cost-effectiveness, thus reducing the parasitic burden in the feces. Chela (2015), using different levels of oregano in growing and fattening animals, reported benefits in fattening and a reduction of the parasitic burden in the animals. The oregano extracts showed activity against bacteria and fungi, according to other

studies. Calo *et al.* (2019); Uddin *et al.* (2021), and Alajil *et al.,* (2022) noted that the genus Origanum and other leafy plants have activity against Salmonella, Escherichia, and Staphylococcus, as well as in fungi, such as Candida.

Results of microbiological tests to feces at EL Romeral

In EL Romeral, the total bacterial count CFU/g (Table 3) had significant differences (P<0.05) from treatment 1 (control) against T2, T3, and T4, the lowest values with no differences among themselves. This can be explained because oregano is an aromatic plant containing thymol and carvacrol, with bactericidal actions (Mayorga, 2016; Uddin *et al.,* 2021; Alajil *et al.,* 2022).

The fecal coliforms CFU/g showed significant differences (P<0.05) T, T2, and T3 against T4, which with greater oregano inclusion could have a better performance, and confirmed by other papers already mentioned (Chela, 2015; Mayorga, 2016). It is important to say that the control with the commercial feedstuff contains growth promoters and antibiotics, such as Flavomycin, Bacitracin, and Salinomycin to control Eimeria spp.

Table 3 Results of microbiological analysis in feces from covies (CFU/g Log) fed at different oregano levels in the feedstuff, in El Romeral.

Indexes	T1	T2	T3	T4	SE	Sig	VC (%)
Total Bacteria	8.05[b]	6.89[a]	6.97 [a]	6.83 [a]	0.5	*	12.31
Fecal Coliforms	4.60[b]	4.60 [b]	4.72 [b]	4.33 [a]	0.4	*	9.07
Total Coliforms	5.87 [a]	5.74 [a]	5.79 [a]	6.03 [a]	0.5	ns	6.56
Aerobic Mesophiles	5.98 [a]	5.60 [a]	5.99 [a]	6.03 [a]	0.4	ns	11.42
Mold and yeasts	3.58 [a]	4.06[c]	3.97 [c]	3.90 [b]	0.3	*	12.08
Eimeria	1.90 [a]	1.80 [a]	1.84 [a]	1.97 [a]	0.2	ns	9.35

a, b. unequal scripts in the rows indicate significant differences among the treatments (P<0.05), (ns) non-significant differences.

Also, it is important to say that no medication was used to control microorganisms in the experimental animals, possibly because oregano is an aromatic plant containing thymol and carvacrol, which have bactericide properties, and help maintain the proper digestive microflora (Mayorga, 2016), with improvements in feed digestion and an active role in the activation of the pancreas and intestines, optimizing antioxidant enzymes like superoxide dismutase and catalase and improving intestinal microvilli (Chela, 2015; Uddin et al.,2021).

In total coliforms, CFU/g (Table 3), in T1, no significant differences were observed (P< 0.05) from the other treatments T2, T3, and T4. The same behavior was found in the aerobic mesophile microorganisms, which confirms the effect of the additive with a germicide capacity coming from thymol and carvacrol, which increase the permeability of the bacterial membrane by ions and protons, the destruction of the lipid membrane thanks to the synergy of their components. (Grondona *et al*., 2014; Chela 2015; Mayorga, 2016).

Concerning molds and yeasts, EL Romeral (Table 3) showed statistical differences (P<0,05) from T1, with the lowest value among all the other treatments. Likewise, T2 and T3 had differences (P<0,05) against T4 and did not differ from each other, but for fungi and yeasts, the

compounds of oregano were the least effective against these organisms (Grondona *et al*., 2014; Chela 2015). Mayorga (2016) and Alajil et al. (2022) said the role of pancreatic and intestinal activators that optimize antioxidant enzymes like superoxide dismutase. Besides, other authors noted that intestinal microvilli are improved (Chela, 2015; Regalado, and Usca, 2019).

In Ochoa, the total bacterial count CFU/g (Table 4) T1 had no significant differences compared to T2 and T3, or between each other. Still, they differed ($P<0.05$) from T4, where the increase of oregano levels, as described by Carbajal (2012), underwent the fastest effects. Fecal coliforms (Table 4) showed no significant differences ($P> 0.05$) caused by the bactericidal effect from carvacrol, thymol, and tannins in more specific populations of bacteria (Chela, 2015; Chandran, 2021). In total coliforms, there were significant differences ($P<0.05$) between T2 and the rest of the treatments, which did not differ from each other, and that was the least effective level in the experiment. The aerobic mesophiles (Table 4) showed no significant differences ($P>0,05$) among the treatments.

The bactericidal properties attributed to oregano are for both Gram-negative and Gram-positive bacteria (Grondona *et al.,* 2014; Winska et al., 2019; Chandran, 2021; Uddin et al., 2021), which explains the behavior of anaerobes, as described by Marino et al. (2011) that oregano has bactericidal activity (Al-Quitani et al., 2022), justifying the behavior of the control and T4, in greater values for these parasites, with differences for T2 and T3, with more favorable effects.

Anuranj *et al.* (2022) and Shehata *et al.* (2022) noted that oregano has bactericidal activity, which also indicates the possible germicidal effects, and justifies the behavior against the control in values without differences for Eimeria, where no significant differences were observed among the treatments resulting from the presence of the parasite. Thus, for molds and yeasts ($P<0.05$) in T3 with the lowest value and the other treatments which did not differ among themselves, due to their anti-fungal and antimicrobial effects. The antiparasitic effect of oregano is its oil, which produces hydrophobicity that permits entry to the cell membrane, killing microorganisms. (Chalan, 2016; Anuranj *et al.,* 2022; Shehata *et al.,* 2022; Ajide *et al.,* 2023).

Feedstuff additives are ideal to increase animal production. Natural medicines from herbs, spices, and various plant extracts have garnered special attention as possible natural growth promoters (Al-Mufarrej *et al.,* 2019; Alqhtani et al., 2022; Anuranj *et al.,* 2022; Shehata *et al.,* 2022; Ajide *et al.,* 2023). Since they are natural, non-toxic, residue-free, and easily found, the beneficial effects, such as appetite stimulant, increased digestive enzyme digestion, immunostimulant, anti-microorganism, and antioxidant, make them suitable as natural additives for birds and small herbivores (Anuranj *et al.,* 2022).

One of the main issues affecting feed manufacturers through the years is conservation. Today, there is greater interest in improving feeds for small animals, using antimicrobial compounds (Delaquis *et al.,* 2002; Anuranj *et al.,* 2022; Shehata *et al.,* 2022).

In recent years, the anti-microbial and preserving capacities of plant extracts, like essential oils, have been studied (Calo *et al*., 2015; Valdiviezo-Ugarte *et al.,* 2019; Winska *et al.,* 2019;

Chandra,2021). Essential oils are liquid and volatile aromatic extracts from plant parts, like leaves, roots, flowers, seeds, fruit, wood, or the whole plant (Hyldegaard *et al.* 2012; Salena, 2022). Initially, these compounds were used in medicine and cosmetics, but eventually, more nutritional studies were done focusing on their antimicrobial activity.

Table 4. Results of microbiological analysis in feces from covies (CFU/g Log) fed at different oregano levels in the feedstuff, in Ochoa Leon

Indexes	T1	T2	T3	T4	SE	Sig	VC (%)
Total Bacteria	7.21 [a]	7.59 [a]	7.62[a]	8.48 [b]	0.3	*	10.22
Fecal Coliforms	3.92 [a]	3.88 [a]	3.86 [a]	3.73 [a]	0.3	ns	9.15
Total Coliforms	5.97 [a]	7.60[b]	6.38 [a]	6.15 [a]	0.4	*	12.04
Aerobic mesophiles	4.57 [a]	4.92 [a]	4.88 [a]	4.85 [a]	0.3	ns	8.67
Mold and yeasts	3.65[b]	3.57 [b]	2.81 [a]	3.56 [b]	0.2	*	8.55
Eimeria	1.85 [b]	1.57 [a]	1.54 [a]	1.89 [b]	0.2	*	9.71

a, b. unequal scripts in the rows indicate significant differences among the treatments (P<0.05) and non-significant differences.

CONCLUSIONS

The nutrition of covies using alfalfa and feeds containing organic additives such as oregano at 3 and 4 kg/t doses of the feed is viable since the bacterial and parasitic counts in the feces were reduced due to the organic principles in the oregano supplemented.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: FGB, FRAJ, MPG, CFSD, JSFR, GPMC; data analysis and interpretation: FGB, FRAJ, MPG, CFSD, JSFR, GPMC; redaction of the manuscript: FGB, FRAJ, MPG, CFSD, JSFR, GPMC.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.