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Oregano extract (*Origanum vulgare*) in female broiler chickens of free-range strain raised in the Western Amazon

Extrato de orégano (Origanum vulgare) em frangos de corte fêmeas de linhagem caipira criadas na Amazônia Ocidental

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ABSTRACT

This study aimed to evaluate the effect of oregano extract added to commercial diets on zootechnical performance parameters, carcass yield, immunological conditions, morphometry, and intestinal pH for free-range broiler chickens raised under sanitary challenge conditions. Three hundred chicks of the strain 'Heavy red' were used and distributed in a completely randomized experimental design with five treatments and six replications, totaling 30 experimental units, each consisting of 10 birds. The treatments were: T1: Basal diet without oregano extract (OE); T2: Basal diet with OE (150 mg/kg); T3: Basal diet with OE (250 mg/kg); T4: Basal diet with OE (350 mg/kg); T5: and Basal diet with OE (450 mg/kg). There was a significant effect on feed intake, weight gain, feed conversion, feed efficiency, heterophil/lymphocyte ratio (H/L), and intestinal morphometry. No significant differences were observed in viability, carcass yield, intestinal pH, lymphoid organs, gizzard, heart, and intestine. Oregano extract influenced the liver yield and abdominal fat of broilers. Overall, the inclusion of oregano extract at the level of 350 mg/kg provided better results.
Keywords: Phytogenic additives, Alternative poultry farming, Medicinal plants.

RESUMO

O objetivo desse trabalho foi avaliar o efeito do extrato de orégano adicionado à ração comercial, considerando os parâmetros de desempenho zootécnico, rendimento de carcaça, condições imunológicas, morfometria e pH intestinal de frangos de corte de linhagem caipira, criados sob condição de desafio sanitário. Foram utilizados 300 pintos da linhagem vermelho pesadão, que foram distribuídos em um delineamento experimental inteiramente casualizado - DIC, com cinco tratamentos e seis repetições, totalizando 30 unidades experimentais, constituídas por 10 aves cada. Os tratamentos foram: T1: Ração basal, sem Extrato de Orégano (EO); T2: Ração basal com EO (150 mg/Kg); T3: Ração basal com EO (250 mg/Kg); T4: Ração basal com EO(350 mg/Kg); T5: Ração basal com EO (450 mg/Kg). Houve efeito significativo no consumo de ração, ganho de peso, conversão alimentar, eficiência alimentar, relação heterofilo/linfócito (H/L) e morfometria intestinal. Não foram observadas diferenças significativas na viabilidade, no rendimento de carcaça, no pH intestinal, nos órgãos linfoides, moela, coração e intestino. Promoveu influência no rendimento de fígado e gordura abdominal dos frangos. A inclusão no nível de 350mg/kg proporciona melhores resultados.
Palavras-chave: Aditivos fitogênicos, Avicultura alternativa, Plantas medicinais

INTRODUCTION

Broiler production has become one of the most developed agricultural activities due to the quick return on invested capital. However, the profit depends on how the activities are carried out to achieve high production. According to Barbosa Filho et al. (2017), new techniques are increasingly being adopted to produce chickens that can grow rapidly in less time.

The use of antibiotic growth promoters (AGPs) in broiler diets has been widely implemented on farms as one of the main performance enhancers, compensating for problems such as high stocking density, stress, and poor sanitary conditions (FERKET, 2003). AGPs contribute to increased feed intake, weight gain, and feed conversion, thereby reducing the cost of bird production. However, despite the positive results for the sector, the use of these antibiotics has raised concerns regarding the possibility of transmission and proliferation of antibiotic-resistant microorganisms through food sources.

New alternatives have emerged to replace the use of traditional AGPs, such as biotechnology products including probiotics, prebiotics, synbiotics, organic acids, enzymes, and plant extracts. These products are natural, non-toxic, and do not induce microbial resistance. They can be used in feed to stabilize the microbiota without causing harm to health, nutrient absorption, animal performance, or consumer health. In recent years, some plant extracts have been studied as phytogenic additives in animal feed. One such extract is oregano oil (*Origanum vulgare*), which can increase nutrient digestibility, improve feed conversion, and promote the balance of microorganisms, thereby reducing the occurrence of pathogens in the intestine (JAMROZ & KAMEL, 2002; KAMEL, 2001). It is believed that adding oregano oil to feed can improve the zootechnical performance of broilers due to its beneficial properties.

Therefore, this study aimed to assess the effect of oregano extract added to commercial feed on zootechnical performance parameters, carcass yield, immunological conditions,

morphometry, and intestinal pH of free-range strain broiler chickens raised under sanitary challenge conditions.

MATERIAL AND METHODS

The study was conducted at the Poultry Farming sector of the Federal University of Acre - UFAC, in the Rio Branco campus, Acre, from November 2019 to January 2020, with a duration of 70 days. The project was approved by the Ethics Committee on Animal Use (CEUA-UFAC) of the Federal University of Acre, under protocol number 45/2018.

The experimental poultry house used had a fiber cement roof, with dimensions of 80 m², divided into 32 boxes of 1.0 m x 2.0 m each, separated by screens, with concrete walls measuring 0.30 meters and a height of 2.8 meters, and equipped with a lanternim.

The birds were distributed in a completely randomized experimental design (CRD), with five treatments and six repetitions, totaling 30 experimental units. Each unit was composed of 10 female birds from the free-range strain 'Heavy red' (1-day-old vaccinated female chicks), housed in a poultry litter composed of 50% reused and 50% new litter, to impose a sanitary challenge on the chickens. The treatments were:

- T1 – Basal feed without oregano extract (OE);
- T2 – Basal feed with oregano extract at 150 mg/Kg;
- T3 – Basal feed with oregano extract at 250 mg/Kg;
- T4 - Basal feed with oregano extract at 350 mg/Kg;
- T5 - Basal feed with oregano extract at 450 mg/Kg.

The product used was provided by ADVET Nutrição Animal, a company located in São Paulo, under the name Oregan OL. It was incorporated into the diet by mixing. Each box had a feeder, a water dispenser for ad libitum water and feed intake, and a 100W incandescent lamp to assist in heating. At 14 days, the birds were vaccinated against Newcastle disease via the ocular route.

To evaluate the zootechnical performance, the feed supply was recorded, and the weight of the remaining feed in each feeder was measured. The weight of the birds in each experimental unit was measured at 14, 28, 42, 56, and 70 days to determine the average feed intake (kg), weight gain (kg), and feed conversion ratio.

At the end of the experiment, on day 70, one representative bird from each box, selected based on weight, was chosen to evaluate blood collection parameters, yield, morphometry, and intestinal pH. The birds were properly identified for each treatment, separated, and subjected to a 12-hour fasting period before being slaughtered the following day.

Before slaughter, 2 ml blood samples were collected from one bird of each experimental unit by puncturing the ulnar vein and placed in tubes containing 10% EDTA (ethylenediaminetetraacetic acid) solution for leukogram analysis. The heterophil/lymphocyte (H/L) ratio was determined by counting 100 leukocytes from each blood smear, differentiating lymphocytes and heterophils, and dividing the number of heterophils found by the number of lymphocytes (GONZALEZ et al., 2003). Blood smears were stained using the Rapid Panoptic Method. The spleen and bursa of Fabricius were also weighed for immunological parameters at 70 days of age.

Regarding yield, at the end of the experiment, one bird from each experimental unit was separated for slaughter, identified, and subjected to a 12-hour fasting period with ad libitum

water. The next day, data on carcass yield (without feet, heads, and viscera) were collected, considering the weight of the clean carcass concerning the live weight after fasting. The yield of noble cuts (breast, thigh, and drumstick), viscera (liver, heart, gizzard, spleen, Bursa of Fabricius, intestine), and fat present in the celomatic region and around the gizzard of each chicken concerning the weight of the carcass was measured.

Intestinal morphometry was obtained from 4 cm samples of the duodenum from the first intestinal loop of the selected birds for evaluation, one from each repetition. These samples were washed in a 10% formalin solution and kept therein for 24 hours. Then, they were placed in a new 10% formalin solution to prepare the slides.

The samples were cleaved to 1cm in length and opened completely for inclusion in the samples. They were then placed in a solution of 70% alcohol, four solutions of absolute alcohol, two of xylene, and two of paraffin with intervals of 25 minutes each, in an oven at a constant temperature of 60°C.

The samples were then blocked in paraffin, and cuts were made with a microtome to a thickness of 4µm. Afterward, the cuts were placed in a water bath at 40°C to distinguish the cuts and collected with the slides. They were then returned to the oven to remove excess paraffin and improve their adherence to the slide.

The Hematoxylin/Eosin method was used for histological staining, including deparaffinization, hydration, staining, dehydration, and clarification. The slides were then mounted. All processes were performed based on the protocols established by the Laboratory of Pathology and Wildlife of the Federal University of Acre.

Intestinal villi and crypts were visualized with a 40x objective, using an optical microscope (Leica 5002) and Leica Application Suite 3.0.0 software. Readings of 10 villi and 10 crypts per repetition were taken regarding their height and depth, respectively. The villus/crypt (V/C) ratio was obtained by dividing the height by the depth.

Samples of the duodenum and cecum from the slaughtered chickens at 71 days were also collected for pH measurement, according to the methodology of Fukayama et al. (2005).

Statistical analysis was performed using the SISVAR computational package described by Ferreira (2015), using the F-test for analysis of variance and Tukey's test for comparison of means, at a 5% probability level.

RESULTS AND DISCUSSION

Table 1 shows the data on feed consumption of chickens fed diets with different levels of oregano extract for each rearing period.

Table 1 – Feed consumption (in kg) of broilers fed diets added with different levels of oregano extract for each treatment and experimental period

Treatment	Period				
	1-14*	1-28*	1-42*	1-56*	1-70*
Control	0.24 ab	0.76 ab	1.74 a	3.25 b	4.74 b
150mg/Kg	0.24 ab	0.77 ab	1.85 c	3.29 b	4.90 c
250mg/Kg	0.22 a	0.77 ab	1.74 a	3.03 a	4.81 bc
350mg/Kg	0.23 ab	0.75 a	1.70 a	3.04 a	4.59 a

450mg/Kg	0.24 b	0.78 b	1.80 b	3.09 a	4.75 b
CV (%) **	5.70	2.42	1.60	1.59	1.22

*Means followed by the same letter in a column do not statistically differ from each other by Tukey's test (p>0.05). **CV (%): coefficient of variation.

According to the manual of the strain, at 70 days birds consume about 6.408 kg of feed. Here, feed consumption by birds during the entire experimental period was lower than the manual's recommendation, which is considered positive as feed expenses are the costliest. Added to that, the final weight of the birds was not affected, as shown in Table 2.

In this study, broilers fed diets supplemented with oregano extract (OE) consumed less than those fed only basal feed. This finding is consistent with those of Pulici et al. (2014), who also observed lower feed intake in birds treated with 0.03% isolated oregano essential oil compared to those receiving a diet containing salinomycin+0.05% essential oil, during the period from 1 to 35 days.

The results suggest that the experimental conditions were suitable for observing the effects of adding oregano extract to the bird feed, compared to the control treatment. According to Menten (2001), the addition of this extract must be tested under field conditions, under which health status is low and growth promoters may have effects on bird performance. To achieve this, the current experiment used reused bedding to impose a sanitary challenge on the animals. Thus, it is believed that this experimental condition helped to obtain the data related to feed consumption.

Oviedo-Rondón (2006) observed a lower feed consumption in chickens fed diets enriched with phytogenic additives compared to those receiving commercial antibiotics between 14 and 19 days. Meanwhile, Jang et al. (2007) found no significant differences in feed consumption, weight gain, and feed conversion of birds at 35 days of age when fed with basal feed, antibiotics, or a mixture of essential oils containing the active ingredient thymol - Crina Poultry (one of the components of oregano essential oil). However, the authors suggested that the absence of differences between treatments may have been due to the lack of a sanitary challenge in the chicken-rearing environment.

Table 2 presents the data on the weight gain of broilers fed different levels of oregano extract during each rearing period.

Table 2. Weight gain (in kg) of broilers fed diets added with different levels of oregano extract for each treatment and experimental period

Treatment	Period				
	1-14*	1-28*	1-42*	1-56*	1-70*
Control	0,13 b	0,40 b	0,84	1,31 b	1,93
150mg/Kg	0,15 a	0,41 b	0,87	1,48 a	1,98
250mg/Kg	0,14 ab	0,42 b	0,83	1,40 ab	1,96
350mg/Kg	0,15 a	0,42 b	0,83	1,39 ab	1,92
450mg/Kg	0,14 ab	0,44 a	0,84	1,39 ab	1,93

CV (%) **	6,60	3,10	3,79	4,43	2,13

DNS No significant difference between means within the column. *Means followed by the same letter in the column are not statistically different from each other by Tukey's test (p>0.05). **CV (%): coefficient of variation.

According to the manual of the strain, the average weight of birds on the 14th day is 0.188 kg, and it can reach 2.464 kg on the 70th day. However, in all experimental periods, the weight of broilers was lower than the recommended in the manual.

Thus, the active potential of oregano extract in birds' diets was evident, as its addition provided the highest average live weight in the final breeding periods. This corroborates with the results obtained by Badiri and Saber (2016), who observed that adding different doses of oregano essential oil (50, 100, 200, and 400 mg) to the Japanese quail's feed resulted in the lowest dose of oil providing the highest weight gain in the final breeding phase (42 days), with all levels added being higher than the control treatment.

Based on our findings, an addition of 350 mg/kg is suggested since it provided the lowest feed consumption without causing any losses in the final weight of broilers. Fotea et al. (2010) confirmed that all groups receiving oregano oil (0.3%, 0.5%, and 1%) had superior results than the control group (no oil addition) when evaluating diets of commercially bred broiler chickens. The best weight gain was achieved at 42 days by adding 1% oregano oil to the diet. On the other hand, Fukayama et al. (2005) did not observe significant differences (p>0.05) in feed consumption, weight gain, and feed conversion parameters of Cobb 500 chickens fed with doses of 0.025%, 0.050%, 0.075%, and 0.100% oregano essential oil in their feed.

Table 3 displays the data on feed conversion of broilers fed diets with different levels of oregano extract in each rearing period.

Table 3. Feed conversion (kg/kg) in broilers fed diets added with different levels of oregano extract for each treatment and experimental period

Treatment	Period				
	1-14*	1-28*	1-42 DNS	1-56*	1-70 DNS
Control	1.76 b	1.89 b	2.09	2.48 b	2.46
150mg/Kg	1.59 a	1.88 ab	2.14	2.23 a	2.48
250mg/Kg	1.58 a	1.84 ab	2.10	2.15 a	2.45
350mg/Kg	1.54 a	1.80 ab	2.05	2.19 a	2.40
450mg/Kg	1.77 b	1.76 a	2.15	2.23 a	2.46
CV (%) **	5.48	3.78	3.71	4.69	2.83

DNS No significant difference between means within the column. *Means followed by the same letter in the column are not statistically different from each other by Tukey's test (p>0.05). **CV (%): coefficient of variation.

Jamroz and Kamel (2002) showed that oregano increases nutrient digestibility, positively affecting intestinal microbiota and reducing pathogen adherence, resulting in improved feed conversion.

The addition of 350mg/kg of oregano extract provided the best conversion up to the last breeding phase, although it was not significant in the final period, it was the best in the previous periods. The non-significant performance (p>0.05)

may be explained by the fact that in this period, the birds were already heavier with an increase in abdominal fat, and therefore, their metabolism might have been diverted to fat production. A high amount of fat was observed in the birds' carcasses with a high coefficient of variation (CV), indicating a significant variation between the results, as shown in Table 5.

Roofchaee et al. (2011) found that adding 600 and 1200mg/kg of oregano essential oil to the diet significantly improved the feed conversion of broiler chickens at 42 days. This corroborates with the results obtained by Lee et al. (2003), who discovered that carvacrol (a bioactive component of essential oil) improved feed conversion in broiler chickens by increasing food efficiency, resulting in greater growth. Conversely, Eler et al. (2019) observed no significant results in feed conversion in male Cobb 500 broilers when adding oregano essential oil (300, 600, and 900mg/kg) to their diet. These authors observed that between 1 and 39 days the best conversion was seen in chickens that did not receive the oil.

According to the strain manual, the ideal feed conversion is 1.66 at 14 days and 2.60 at 70 days. The results obtained in this experiment were better than recommended, with an average of 1.60 at 14 days in the first breeding phase and 2.50 in the last breeding phase. This brings positive results to the poultry sector, as a better feed conversion than recommended in the manual is desirable, as it can reduce production costs. It is worth noting that the zootechnical viability of the chickens was 100%, as no mortalities were observed in any treatment during the entire experimental period.

Our results of carcass, breast, thigh, and drumstick yields after the slaughter at 71 days showed no significant difference between the analyzed treatments. These results are in line with Fukayama et al. (2005), who also did not observe significant differences in carcass, breast, and abdominal fat yields when adding oregano essential oil to birds' diets at 42 days of age. However, Isabel and Santos (2009) found that adding 100 ppm of a mixture of cloves and cinnamon significantly ($p>0.05$) improved the birds' breast yield. Dias (2011) also found that birds receiving 300 mg/kg of oregano essential oil in their diet had a higher carcass yield than the treatment without the addition.

Table 4 shows the data on edible offal (liver, heart, and gizzard), intestines, and abdominal fat of the birds at 71 days.

Table 4. Yields of the liver (%), heart (%), gizzard (%), intestine (%), and abdominal fat (%) for birds of all treatments at 71 days of age

Treatment	Offal yield (%)				
	Liver*	Heart ^{DNS}	Gizzard ^{DNS}	Intestine ^{DNS}	Abdominal fat*
Control	1.64 b	0.49	2.19	3.71	4.07 a
150mg/Kg	1.64 b	0.46	2.20	3.63	4.81 ab
250mg/Kg	1.64 b	0.47	1.87	3.54	3.73 a
350mg/Kg	1.21 a	0.49	2.03	3.16	4.13 a
450mg/Kg	1.48 ab	0.47	2.21	3.49	5.81 b
CV (%) **	15.08	16.04	14.26	11.95	15.68

^{DNS} No significant difference between means within the column. *Means followed by the same letter in the column are not statistically different from each other by Tukey's test ($p>0.05$). **CV (%): coefficient of variation.

Significant differences (p<0.05) were observed among treatments in liver yield and abdominal fat content of the birds. The addition treatments of 350 and 450mg/kg resulted in the lowest liver weight averages, while the treatment with the highest extract addition dose (450mg/kg) showed the highest fat content in the carcass. Conversely, Demir and Kilinc (2006) observed a decrease in abdominal fat in birds fed diets with plant extracts of thyme and garlic at 21 days.

Kirkpinar et al. (2010) did not observe any differences in carcass, breast, and organ yield when evaluating treatments with diets containing oregano oil, garlic, and a mixture of both. In this study, significant differences (p<0.05) were observed in liver yield, with reduced liver weight in birds in treatments with higher extract additions (350 and 450mg/kg). These results are consistent with Koiyama (2012), who also observed reduced liver size in birds fed with added copaiba oil in the diet, compared to the treatment without addition, which may be associated with some form of hepatic intoxication, according to the author. Thus, the extract in some way reduced the challenge imposed by the reuse of litter, resulting in a reduction in hepatic overload, as reflected by lower liver weights compared to other treatments.

The results of the duodenal and cecal pH of birds slaughtered at 71 days did not show a significant difference for the different levels of oregano extract and control used. This is consistent with Fukayama et al. (2005), who also did not observe any differences in the intestinal pH of industrial broiler chickens fed a diet containing oregano essential oil in any of the analyzed periods, as according to the authors, there was no increase in bacteria to disrupt intestinal health, due to good conditions in the rearing environment.

The pH measurements of the ceca were close to those described by Sturkie (1965) and Santos (2003), which were 5.7 and 6.9, respectively. Thus, it can be concluded that the addition of the extract at all doses used did not cause any alteration in the pH of both the duodenum and ceca, where all averages are within the parameters considered normal.

On the other hand, Madrid et al. (2017) observed that higher doses of oregano oil in the diet of industrial broiler chickens resulted in lower intestinal pH values, with the control treatment obtaining the highest values. They also observed higher pH values in the duodenum compared to the ceca.

Table 5 and Figure showed the results on intestinal morphometry, such as villus height, crypt depth, and villus-to-crypt ratio (V/C) of the duodenum samples obtained from birds slaughtered at 71 days.

Table 5. Villus height, crypt depth, and villus-to-crypt ratio (V/C) of the duodenum in free-range broiler chickens at 71 days of age fed with oregano extract

Treatment	Height and depth (µm)		V/C*
	VILLI*	CRYPTS*	
Control	76.23 b	14.16 c	5.39 c
150mg/Kg	81.71 b	12.13 b	6.84 bc
250mg/Kg	83.20 b	12.02 b	6.99 bc
350mg/Kg	101.85 a	10.16 a	10.11 a
450mg/Kg	87.36 b	10.16 a	8.64 b

CV** (%)	9.18	8.58	14.43

[NS] Non-significant differences among means in the column. *Means followed by the same letter in the column do not differ from each other by Tukey's test (p>0.05). **CV (%): coefficient of variation.

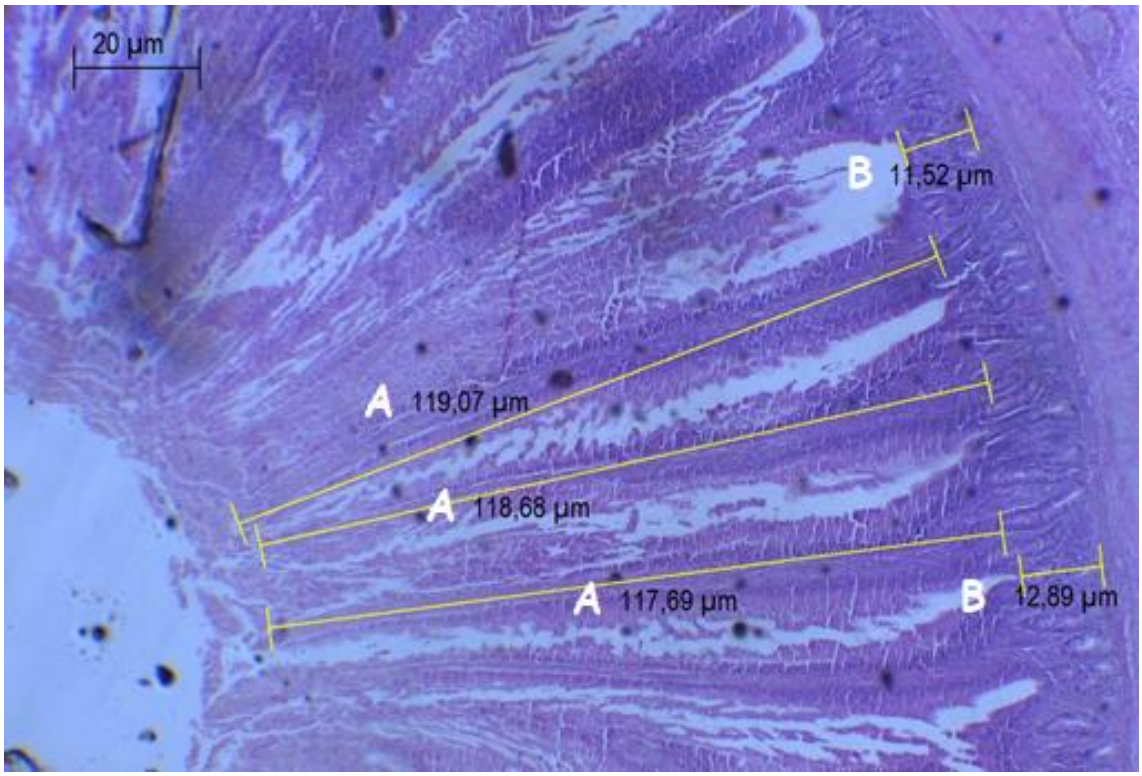

Figure 1. Villus height (A) and crypt depths (B) of female free-range broiler chickens slaughtered at 71 days of age.

The dose of 350mg/kg of extract continued to stand out among treatments, as it provided the highest average of villus height, the lowest average of crypt depth (i.e., shallower crypts), and a high V/C ratio. According to Qaisrani et al. (2014), these factors indicate good intestinal health, as higher villus height and shallower crypts, and consequently, a higher V/C ratio, provide ideal conditions for better nutrient absorption with lower energy loss.

Table 6 shows the data regarding the immune parameters, as well as the heterophil/lymphocyte ratio (H/L), and relative weights of the spleen and bursa of Fabricius.

Table 6. Blood parameters (H/L ratio) and relative weights of the spleen (g) and bursa of Fabricius (g) of broilers fed diets added with different levels of oregano extract as a function of the treatment at 71 days

Treatment	H/L *	Spleen [NS]	Bursa [NS]
Control	1.38 b	2.67±0.21	4.67±0.24
150mg/kg	1.42 b	2.50±0.18	5.17±0.43
250mg/kg	2.19 c	2.33±0.21	4.50±0.30
350mg/kg	0.53 a	2.83±0.13	4.33±0.17
450mg/kg	1.23 ab	2.67±0.19	4.33±0.41
CV (%) **	31.38	22.21	26.92

[DNS] No significant difference among treatments for any of the parameters evaluated ($p>0.05$), indicating that the addition of oregano extract doses had no impact on the immune health of birds.

Considering a normal heterophil/lymphocyte (H/L) ratio of 1/2 (LAGANÁ et al., 2007) and potential microbiological load due to litter reuse, significant differences ($p<0.05$) were observed in the H/L ratio in all treatments. This indicates that birds were strongly challenged due to a high H/L ratio in some treatments. We noted that the birds that responded best to the challenge posed by reused litter were those receiving 350mg/kg of the extract, as they showed a ratio closest to the normal indicated by Laganá et al. (2007). These results are consistent with those of Toghyani et al. (2011), who reported that higher doses of herbal additives can stimulate immune responses.

No significant effect ($p>0.05$) on the weight of lymphoid organs (bursa and spleen) was observed in birds of all treatments. This is in line with Barreto et al. (2008) and Toghyani et al. (2010), who also did not observe differences in the weight of these organs with diets containing different phytogenic additives. However, in these studies, the authors did not subject the chickens to a challenging condition. Ri et al. (2017) also did not observe significant effects on the size of lymphoid organs in industrial broilers fed powdered oregano in the diet. In contrast, Fukayama et al. (2005) observed a significant effect on the size of the spleen in birds with the addition of 0.025% of oregano essential oil, where they showed a higher spleen weight than birds in the control treatment.

CONCLUSIONS

Adding oregano extract to the diet of free-range broiler chickens provides improvements in zootechnical performance, and H/L and V/C ratios, without influencing viability, carcass yield, visceral organs, or intestinal pH. However, it does have an impact on liver yield and abdominal fat. The most effective dosage of the extract is 350mg/kg.

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