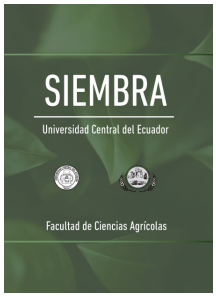
Evaluation of productive variables and intestinal integrity in Ross 308 AP male breeders, through the application of oregano oil (Origanum vulgare L.) in the drinking water during the breeding stage



Evaluación de variables productivas e integridad intestinal en machos reproductores Ross 308 AP, mediante la aplicación de aceite de orégano (Origanum vulgare L.) en el agua de bebida durante la etapa de cría

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Abstract

Oregano oil has established itself as a favorable alternative in poultry farms, due to its antibacterial and immunostimulant benefits, among others. Therefore, the objective of the research was to evaluate the productive variables and intestinal integrity in in male Ross 308 AP breeders, by applying oregano oil in the drinking water. We worked under a completely randomized design, with four treatments (T0 = 0 ml L^{-1}, T1 = 0,05 ml L^{-1}; T2=0,125 ml L^{-1} y T3 = 0,25 ml L^{-1}) and four replicates, the study variables were; in productive variables; a) mortality, b) weight gain, c) feed conversion, and d) tarsus length, and intestinal integrity; a) villus length, b) villus width, c) crypt width, d) crypt depth, e) width between villus, and f) width between crypts. The data obtained were analyzed by analysis of variance, coefficient of variation, Tukey test and Pearson's correlation. In the results, a significant difference was confirmed among all treatments (*P-value* = < 0.05), in turn, the 0.25 ml L^{-1} dose provided the best results in weight gain (141,88 g), feed conversion (1,91) and tarsus length (3,45 cm day^{-1}); however, in intestinal integrity, only the 0.125 and 0.25 ml L^{-1} doses promoted an increase in the length of the villi, (0,97 cm day^{-1} in both cases). The conclusion is that is beneficial in the productive variables, while, in intestinal integrity, only the highest doses generate positive effects on the length of the villi.

Keywords: oregano oil, drinking water, intestinal integrity, productive parameters, Ross 308 AP chickens.

Resumen

El aceite de orégano se ha establecido como una alternativa favorable dentro de las explotaciones avícolas, debido a sus bondades antibacterianas, inmunoestimulantes, entre otros. Por ello, el objetivo de la investigación fue evaluar las variables productivas e integridad intestinal en machos reproductores Ross 308, mediante la aplicación de aceite de orégano en el agua de bebida. Se trabajó bajo un diseño completamente al azar, con cuatro tratamientos (T0 = 0 ml L^{-1}, T1 = 0,05 ml L^{-1}; T2=0,125 ml L^{-1} y T3 = 0,25 ml L^{-1}) y cuatro repeticiones; las variables de estudio fueron, en variables productivas: a) mortalidad, b) ganancia de peso, c) conversión alimenticia, y d) longitud del tarso; en integridad del

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intestino: a) largo de vellosidad, b) ancho de vellosidad, c) ancho de la cripta, d) profundidad de la cripta, e) ancho entre vellosidades, y f) ancho entre criptas. Los datos obtenidos se analizaron mediante un análisis de varianza, coeficiente de variación, prueba de Tukey y correlación de Pearson. En los resultados, se confirmó diferencia significativa entre todos los tratamientos (*P-valor* < 0,05), a su vez, la dosis de 0,25 ml L^{-1} brindó los mejores resultados en ganancia de peso (141,88 g), conversión alimenticia (1,91) y longitud de tarso (3,45 cm $día^{-1}$); sin embargo, en la integridad intestinal, solo las dosis de 0,125 y 0,25 ml L^{-1} promovieron un incremento en el largo de la vellosidad (0,97 cm $día^{-1}$ en ambos casos). Concluyendo que cualquier tratamiento que utilice aceite de orégano es beneficioso en las variables productivas, mientras que, en la integridad intestinal, solo las dosis más elevadas generan efectos positivos en el largo de la vellosidad.

Palabras clave: aceite de orégano, agua de bebida, integridad intestinal, parámetros productivos, reproductores Ross 308 AP.

1. Introduction

At present, poultry farming has become the main source of protein production worldwide allowing low acquisition costs. It should be emphasized that the growing global demand for protein raised by 3.5 % between 1980 and 2030, so that strategies aimed at obtaining protein-rich products, such as poultry, meat and eggs, should be maximized (Sitio Avícola, 2015). It is known that per capita consumption of chicken meat reached 33.2 kg, while beef and pork maintain an average consumption of 10 kg (Petermann et al., 2018), as a direct consequence of considering poultry products as the most economical animal protein alternative (Campozano-Marcillo et al., 2021).

However, according to the Continuous Agricultural Surface and Production Survey 2020 (Instituto Nacional de Estadísticas y Censos [INEC], 2021), a decrease in poultry production was recorded in Ecuador, shifting from a broiler production of 34,884 birds in 2019 to 23,527 in 2020, resulting in a diminished availability of affordable animal protein (Verjel-Carrascal & Pacheco-Sánchez, 2018). Therefore, it seems highly necessary to implement strategies aimed at optimizing the economic and productive resources of poultry farms, particularly considering that the "food and nutrition" item accounts for 72 % of their total productive cost (El Heraldo, 2020).

As a consequence, in order to achieve better results within the areas of animal health and poultry nutrition, the use of antibiotics and growth promoters represents at present the most widely used alternative (Fonseca-García et al., 2017); however, the indiscriminate use of these chemical compounds, in many cases, caused resistance in the poultry, pushing widespreadly the need to reduce the use of antibiotics and growth promoters on poultry farms, in order to safeguard the integrity of consumers (Vázquez et al., 2019).

Plant additives are known as a good replacement for antibiotics and growth promoters, when considering both technical, economic and biological points of view because of their repealed residual nature (El Heraldo, 2020). As a consequence, through the use of essential oils, plant additives emerging as an effective alternative against pathogens, showing an effective and favorable impact on the production and management of poultry. For this reason, increasing interest has currently been recorded in biologically active plant compounds within poultry farms, due to their positive effects on growth, antibacterial activity, improvements in intestinal health, and meat quality (Méndez Zamora et al., 2015). It is worth considering that the benefits of these oils are provided by their antibacterial, antifungal, antiviral, antioxidant and immunostimulant activities (McKee & McKee, 2016).

For instance, oregano oil (*Origanum vulgare* L.), by containing within its main components carvacrol and thymol, has the ability to minimize the viscosity of digested food (Betancourt López, 2012), while, the presence of flavonoids, tannins and triterpenes enables this oil to fight bacterial, fungal, parasitic, microbial, viral, allergic, vasodilators, estrogenic, and inflammatory problems, among others (Loeza-Concha et al., 2019).

For all the above mentioned reasons, the object of this research was to evaluate more closely the productive variables and intestinal integrity in Ross 308 AP male breeders, through the application of oregano oil (*Origanum vulgare* L.) in the breeders drinking water.

2. Materials and Methods

The research was carried out on a farm belonging to AVESCA (a company dedicated to poultry farming), located in the parish of Amaguaña, canton Mejía, province of Pichincha, Ecuador; on Ross 308 AP heavy breeders

during the rearing stage.

The authors worked under a completely randomized design, consisting of four treatments, where oregano oil (commercial name "Regano") was added to the drinking water of the breeders. Four doses were used, forming the following treatments; T1 = 0 ml oregano oil/liter of water (ml L^{-1}), T2 = 0.05 ml L^{-1}; T3 = 0.125 ml L^{-1}, and T4 = 0.25 ml L^{-1}; with four replicates each, generating a total of 16 experimental units. For each experimental unit, rearing pens were used, which allow management of the breeder from day 1 to day 28, which were made from metal circles, placed on a bed of shavings inside the test house. Each experimental unit had 125 Ross 308 AP male breeders, one day old, with an average initial weight of 45 grams and a uniformity of 92 %, having two origins of grandmothers. Thus, equaling a total trial sample of 2,000 male breeders.

The poultry were handled and reared in sheds owned by Avesca, a commercial line belonging to Pronaca. The reception shed was prepared and accurately washed and disinfected; additionally, the sheds were evaluated by microbiological analysis, using drag swabs, in order to guarantee that the surfaces of each shed presented high asepsis indexes. The breeders came from grandparent flocks free of mycoplasma, belonging to the poultry genetics company Aviagen. At the time of reception they had a vaccination schedule, which consisted of Marek HVT - Rispens, Reovirus, Coccidia and Gumboro vaccines (in hatchery), and Salmonella Enteritidis, Infectious Bronchitis and Newcastle (in farm). A growth diet based on 2,900 kcal with 19 % crude protein was used throughout the research process. The administration of oregano oil (Regano) was carried out in a single daily intake, and subsequent administration of water *ad libitum*. During the whole research, 12 hours of artificial light and 12 hours of darkness were maintained, and an initial temperature of 30 °C was maintained at the reception through the use of combustion brooders, then it was gradually lowered by 1 degree per week, until reaching a final temperature of 26 °C.

The productive or zootechnical variables evaluated were as follows:

a) *mortality*, this data was recorded daily and evaluated on a weekly and cumulative basis, calculated using equation [1], and expressed as a percentage.

$$number\ of\ dead\ birds \Big/ total\ number\ of\ birds \times 100 \quad [1]$$

b) *weight gain*, this was evaluated cumulatively, by recording the difference between the final weight, and the initial weight of the birds expressed in grams, using an electronic floor scale (Bat1), which weighed 100 % of the treatments.

c) *Feed conversion*, this variable was determined by the equation [2].

$$feed\ consumption \Big/ (final\ weight\ -\ initial\ weight)) \quad [2]$$

d) *Tarsus length*, the length in centimeters of the male tarsus, from the intertarsal joint to toe number 1, was analyzed using the tarsus ruler administered by the commercial company Aviagen, the same supplier of the genetics under study. Two measurements were taken, one at the beginning of the trial and the other at the end of it, this variable was evaluated in 50 % of the population of each treatment.

At the laboratory level, the intestinal integrity or morphology of the intestine was evaluated by hiring private specialists belonging to the ANIMALAB Laboratory, the samples analyzed were two birds per treatment chosen at random. This was evaluated on days 1, 14 and 28; specifically, the jejunum was dissected and sent for analysis to the laboratory in a 10 % formalin solution. The variables evaluated were: a) villus length; b) villus width; c) crypt width; d) crypt depth; e) width between villi; and f) width between crypts; all measured in micrometers (µm). In the laboratory, the tissues were fixed in 10 % buffered water for 48 hours at 4 °C with kerosene upon arrival, then cut at 4 µm thickness and stained with hematoxylin-eosin; subsequently, washed and stored in ethanol: water (75:25, v:v). The sections were microdissected to determine the height and width of intestinal villi, as well as the depth and width of crypts. A Motic 29AX E250223, Model BA310 microscope was used, and by means of a Motic® Images plus 2.0 computerized image processor, lines were drawn from the base to the apex of the villi to measure the length between bases in order to determine the width and depth in µm.

We worked under a completely randomized design [DCA], an analysis of variance was performed under a confidence level of 95 %, where the coefficient of variation of the variables evaluated was taken into

consideration to determine the existence or not of homogeneity of the data collected, at the same time, in the variables that had a significant difference, the Tukey test of means was performed at 5 % and, finally, a Pearson correlation was elaborated with the variables evaluated and the results obtained.

3. Results and Discussion

3.1. Production or zootechnical variables

Regarding the zootechnical variables (Table 1) of the Ross 308 AP breeding males, a variation coefficient range of 2.67 to 1.58 % was identified in the variables weight gain, feed conversion and tarsus length, which allowed to expose high homogeneity in that data set, however, in the mortality variable, the variation coefficient covered 46.89 %.

Table 1. Coefficient of variation and P-value of zootechnical variables in Ross 308 AP breeding males.

Variable evaluated	Coefficient of variation (%)	Dose (*P-value*)
Mortality (%)	46,89	0,23
Weight gain (g)	1,58	0,00
Feed conversion	2,67	0,01
Tarsus length (cm day-1)	1,99	0,01

On the other hand, when generating a comparison on the results of P-value in the evaluated variables, significant differences emerged within the variables weight gain, feed conversion and tarsus length, respectively, in the applied dose, as shown in Table 1.

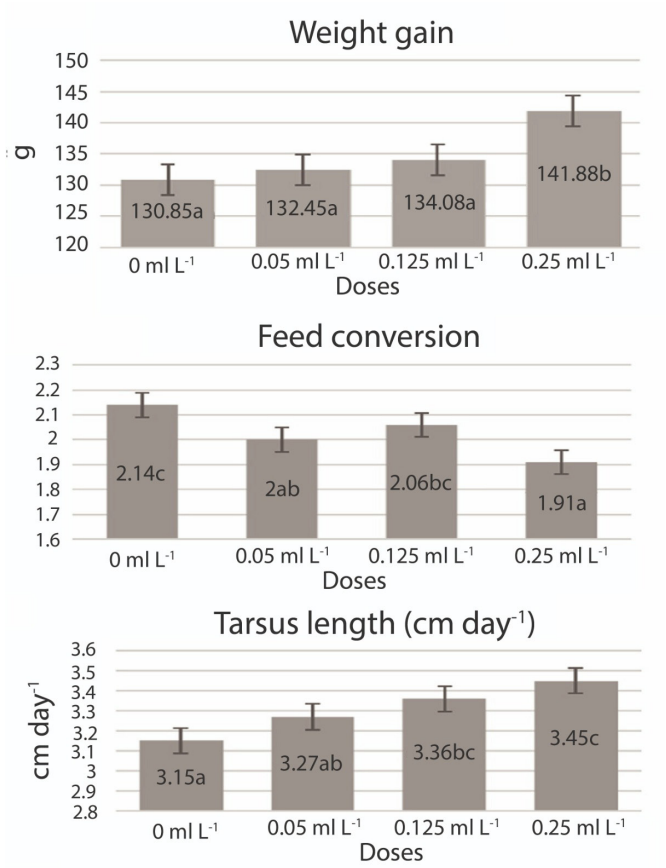

Figure 1. Tukey test on the variables weight gain, feed conversion and tarsus length, when comparing different doses of oregano oil.*
* Means with a common letter are not significantly different.

As it can be appreciated in Figure 1, when comparing the weight gain and the oregano oil dose used results confirmed that the dose 0.25 ml L^{-1} was the only one that expressed significant difference (141.88 g) compared to the others, thus confirming that the highest dose of the trial provided the best results. This result is consistent with those found in Sánchez-Zamora et al. (2019) and Silva-Vázquez et al. (2018). These studies, when evaluating the use of oregano oil in the diet of broilers (0.2 g kg^{-1} = 0.2 ml L^{-1} of oregano oil), concluded that the use of the aforementioned oil promotes broiler weight gain by improving feed intake, as its application in the diet improves its palatability. At the same time, it tends to stimulate digestive secretions, increases antioxidant properties, generates antimicrobial capacities and strengthens the immune system of the birds, which is why oregano oil is considered a growth promoting agent of natural origin (Cázares-Gallegos et al., 2019).

Conversely, when taking into account feed conversion, a clear difference was identified between the doses 0 ml L^{-1} (2.14c) and 0.25 ml L^{-1} (1.91a), while the use or not of oregano oil in the feed diet was not relevant in the remaining doses (Figure 1). This confirms that the use of oregano oil with the highest dose of the trial generates positive effects, allowing to obtain greater efficiency within the feed conversion, which is corroborated by Hernández-Coronado et al. (2019), who assure that the use of this oil has the capacity to improve the feed conversion and digestibility of the consumed feed, specifically in the ileal treatment, which directly influences the productive scope of fattening poultry. According to Reyer et al. (2017), a dose of 0.1 % of oregano oil added in the poultry feed diet, has the capacity to generate efficiency in feed conversion and morphometry at hepatic level, representative characteristics of an antibiotic growth promoter product. It should be emphasized that the dose has a high significance in the results obtained, since in the research proposed, the dose of 0.125 ml L^{-1} presented a feed conversion of 2.06, which was apparent to the result of Ibarra-Espain et al. (2020), who, when applying the dose of 0.1 ml L^{-1} obtained a feed conversion of 1.98, for which reason, said author recommends increasing the dose of oregano oil in the diet of chickens.

On the other hand, when considering tarsus length (Figure 1), the 0.25 ml L^{-1} dose was established as the best option (3.45 cm day^{-1}) compared to the other doses. According to Yupanqui Quispe (2017), the length of the tarsus has a direct relationship with the weight and age of the bird, that is, as one of the mentioned variables increases, the remaining ones follow the sequence, which is corroborated with the results present in the trial. Similarly, length of the tarsus, the skeletal integrity of the birds is indirectly stimulated (Han et al., 2015), which tends to expose the bone quality of the animal, and the capacity to absorb and digest the nutrients provided in the diet (Savoldi et al., 2015), thus confirming the efficacy of oregano oil, being the best dose 0.25 ml L^{-1}, since it managed to provide the best results in all the zootechnical parameters evaluated.

When generating a Pearson correlation (Table 2) on the zootechnical variables evaluated, a high positive correlation was identified between weight gain and tarsus length (0.69); that is to say, it is highly probable that any of these variables allows the prediction of the results of the other (Pita-Fernández & Pértega-Díaz, 2002). It should be emphasized that the correlation between tarsus length and weight gain obtained was higher than that identified by Chincoya et al. (2018), whom calculated a correlation of 0.45 when describing the morphology of birds, thus assuming that the addition of oregano oil to the drinking water positively influences the zootechnical variables. Complementarily, through the results presented, it was determined that weight gain and feed conversion maintain a closely negative correlation (-0.79), which describes an inverse proportional relationship, in which, when the values of one variable increase, automatically, the other tends to reduce proportionally (Retes Cálix & Salazar Guamán, 2014).

Table 2. Pearson correlation of zootechnical variables in breeding males Ross 308 AP.

Variables evaluated	Mortality (%)	Weight gain (g)	Feed conversion	Tarsus length (cm day^{-1})
Mortality (%)	1	0,05	0,12	0,59
Weight gain (g)	-0,49	1	2,60E-04	2,90E-03
Feed conversion	0,41	-0,79	1	4,00E-04
Tarsus length (cm/ 1 day)	-0,14	0,69	-0,78	1

3.2. Intestinal integrity or bowel morphology

When considering the morphology of the intestine, as shown in Table 3, the variables evaluated conserved a range of 0.43 to 2.89 % in terms of the coefficient of variation, thus affirming high homogeneity within the

data obtained. When considering the P-value to identify the existence or not of significant differences between the variables studied, it was found that only the length of the villus tends to show variation when comparing the doses applied.

Table 3. Coefficient of variation and P-value of intestine morphology in Ross 308 AP breeding males.

Variables evaluated (µm)	Coefficient of variation (%)	Dose (*P-value*)
Hair length	2,89	0,00
Width of villi	0,78	0,38
Crypt width	1,44	0,22
Crypt depth	0,43	0,46
Width between villi	0,86	0,24
Width between crypts	0,86	0,24

Regarding the morphology of the intestine (Figure 2), when taking into account the dose applied, it was confirmed that there is a smaller increase (0.7 cm day-1) when working with the doses 0 and 0.05 ml L^{-1}, while there is greater development of the villi (0.97 cm day^{-1}) when using the doses of 0.125 and 0.25 ml L^{-1}. This affirms the efficacy of oregano oil within the intestinal area of the bird, ratified by Shiva et al. (2012), who mention that oregano oil improves the absorption system by increasing the length of the intestinal villi. In turn, Méndez Zamora et al. (2015) argue that the length of the villi is related to the decrease in feed conversion, which was corroborated with the results obtained in the present research. Finally, Bauer et al. (2019) argue that oregano oil at 1 and 2 % on the feed diet has the capacity to increase the length of the villi, a statement that supports the accuracy of the results presented in Table 3.

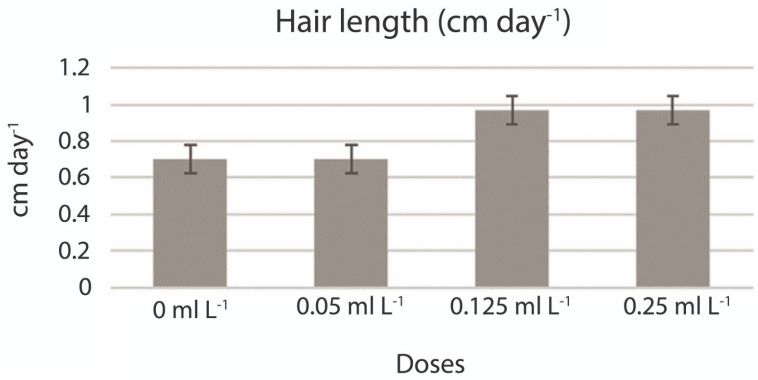

Figure 2. Tukey test on the variables referring to the morphology of the intestine, when comparing different doses of oregano oil.

* Means with a common letter are not significantly different.

Through Table 4, with Pearson's correlation, a high (0.94) positive correlation was identified between the width of the villi and the variables: width between villi and crypt width, i.e. The three above mentioned variables are dependent on each other, therefore allowing to conclude that by using oregano oil in the drinking water, influence on the increase of the width of the villi and crypt is recorded, in contrast with the results identified by Chávez et al. (2016), whom -when evaluating the consumption of prebiotics-, confirmed that the width of the villi is inversely proportional to the width of the crypt, that is, as one increases, the other decreases. Based on this information, it may be confirmed that the width of the villi and the width of the crypts are dependent on the feed diets and their components, this is corroborated by Madrid-Garcés et al. (2018), whom -when evaluating various feed diets adding different doses of antibiotics, additives and oregano oil-, concluded that each component has an independent effect within the intestinal conditions of the birds. It is important to emphasize that the longer and wider the villi, the greater the bird's capacity to absorb nutrients (Skoufos et al., 2016), this confirms that the inclusion of oregano oil in the drinking water is beneficial to improve the nutrient absorption capacity of poultry.

Table 4. Pearson correlation in intestine morphology in Ross 308 AP breeding males.

	Hair length (µm)	Villi width (µm)	Crypt width (µm)	Crypt depth (µm)	Width etween villi (µm)	Width between crypts (µm)/
Hair length (µm)	1	0,46	0,33	0,02	0,35	0,35
Villi width (µm)	0,31	1	0,08	0,36	6,30E-04	6,30E-04
Crypt width (µm)	0,4	0,64	1	0,45	4,70E-03	4,70E-03
Crypt depth (µm)	0,8	0,37	0,31	1	0,35	0,35
Width between villi (µm)	0,38	0,94	0,87	0,38	1	0
Width betweencrypts (µm)/	0,38	0,94	0,87	0,38	1	1

4. Conclusions

As to what regards the production or zootechnical variables, it was possible to identify that there is a direct influence on the poultry when oregano oil is applied in the drinking water, in both the weight gain, the feed conversion as well as the tarsus length variables, being in all cases the 0.25 ml L^{-1} dose the one that provided the best results. It is worth considering that even the minimum dose (0.05 ml L^{-1}) generated better results compared to the treatment where this oil was not applied.

In turn, when considering the morphology of the intestine, the only variable where a positive effect was achieved by the inclusion of oregano oil in the drinking water was in the length of the villi, showing that the dose of 0.05 ml L^{-1} generated the same results as when nothing was applied, that is, this dose did not generate any effect on this variable. On the other hand, the doses of 0.125 and 0.25 ml L^{-1} provided the same increase in the length of the villi (0.97 cm day^{-1}), so that it can be concluded that, in economic terms, it is more profitable to use the dose of 0.125 ml L^{-1} if the producer's objective is to influence the growth of the intestinal villi of the breeders.

Contributor roles

- Alexis Lenin Lalaleo Borja: conceptualization, data curation, formal analysis, fund acquisition, research, methodology, project management, resources, software, visualization, validation, writing - original draft, writing - review and editing.
- Lucia Monserrath Silva Deley: supervision, validation, writing - review and editing, writing - original draft.

Ethical implications

The authors state that the AVESCA company authorized the test in its facilities and the use of the results for scientific dissemination, and that the dissected broodstock were destined for sale for consumption because they did not reach the raising weight.

Conflict of interest

The authors declare that they have an employment relationship with AVESCA.

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