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EVALUATION OF HEMATOLOGICAL AND BIOCHEMICAL BLOOD PARAMETERS IN GUINEA PIGS (CAVIA PORCELLUS) SUPPLEMENTED WITH NANOEMULSIONS OF ESSENTIAL OILS

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Abstract: The objective of this study was to evaluate the hematological and biochemical blood parameters of guinea pigs (*Cavia porcellus*) supplemented with nanoemulsions of essential oils (EO) of chincho and oregano. Fifty hybrid male guinea pigs of the Peruvian and Andean breeds (average weight 280 g) were randomly distributed in 5 experimental groups, with 10 replicates each: T1 (Control Group), T2 (base diet + APC: 200 ppm bacitracin zinc), T3 (base diet + 200 ppm chincho EO nanoemulsion), T4 (base diet + 200 ppm oregano EO nanoemulsion) and T5 (base diet + 200 ppm chincho and oregano EO nanoemulsion). Venous blood samples were taken during the benefit of the animals, 49 days after the beginning of the study. The hematological parameters evaluated were primary and secondary erythrocyte indices, absolute and relative leukocyte count. In blood biochemistry, glucose, triglycerides, cholesterol and total proteins were measured. Analysis of variance (ANOVA) and Tukey's test were applied. In the red series, T2 presented a significant difference ($p<0.05$) compared to T1 in MCV (Mean Corpuscular Volume). Within the leukocyte series, no significant difference was found; however, T1 exceeded the reference value in monocytes. In addition, a significant reduction ($p<0.05$) in glucose levels was observed in the T3 group in contrast to T1. Globulins of T3, T4 and T5 were significantly ($p<0.05$) higher compared to T1. However, the values remained within the accepted limits according to reference standards. It is concluded that adding oregano and chincho EO nanoemulsions to the guinea pig diet does not affect blood parameters and could be a natural alternative to the use of APC in animal feed.

Keywords: nanoemulsion, essential oils, hematological parameters, blood biochemistry, guinea pig.

INTRODUCTION

The guinea pig (*Cavia porcellus*) is a rodent mammal native to the Andean region of South America. Its meat is highly valued due to its high protein content of high biological value and low cholesterol (Flores *et al.,* 2017). The advantages of raising this species include a short reproductive cycle, precocity, prolificacy, high adaptability and versatile feeding (Verástegui, 2015). The guinea pig population has experienced a notorious increase in Peru, exceeding 18.7 million in 2018 according to data from the National Institute of Statistics and Informatics (INEI). Our country is considered the largest exporter of guinea pig meat with a 71.3% share in the foreign market. Between 1994 and 2018, guinea pig meat exports were high, being the highest in 2014 with 23 tons and with the USA as the main destination (99.9%) (MINAGRI, 2019). To boost this industry, new technologies are being developed in areas such as nutrition and feeding, reproduction, genetic improvement, management and production (Solari, 2010).

The increase in guinea pig meat production and its intensive raising has caused several problems, among them overcrowding and with it, an increase in infectious diseases (Herrera, 2022). To prevent the spread of these diseases, antibiotic growth promoters (APC) have been added to the concentrate formulation included in guinea pig diets for many years (Carro and Ranilla, 2002), and this measure is considered the main one to prevent high mortality rates due to salmonellosis (Ramírez *et al.,* 2021). However, the sustained use of antibiotics in small doses in animal production results in antibiotic residues in meat intended for human consumption (Flemming and Freitas, 2005).

Antimicrobial resistance is a global problem. The causes include the indiscriminate use of antibiotics, the use of these drugs in the feed of animals destined for human consumption without respecting the withdrawal period, among others (Serra, 2017). Since 2006, the use of APCs in animal husbandry was banned by the European Union (Vásquez *et al.,* 2019) and for years studies have been carried out and new alternatives have been developed to replace APCs in animal feed. Among these proposals are essential oils (EO) obtained from aromatic plants such as chincho *(Tagetes elliptica*) and oregano *(Origanum vulgare*).

EOs are secondary metabolites of plants, made up of a varied mixture of chemical compounds. There are studies on the use of EOs in the feed of different livestock species that have shown positive effects in terms of digestibility, feed conversion, productivity, antiparasitic and antioxidant effects (Martínez *et al.,* 2015).

Oregano EO contains numerous substances such as flavonoids, tannins, triterpenes, thymol and carvacrol, the latter two components are the ones that contribute most to its antioxidant and bactericidal capacity (Saavedra, 2019). Due to its properties, it has been employed as a supplement in the feeding of production animals. Chincho (*Tagetes elliptica*) is a plant native to Peru and grows abundantly in the highlands of the department of Huánuco. The EOs of plants of the genus Tagetes have a high content of terpenes and sesquiterpenes, including β-ocimene, β-terpinene, myrcene, tagetones, dihydrotagetone and tagetenones (Cerrón *et al.,* 2023). There is very little literature on the use of chincho EO in animal feed.

The main disadvantage of ECs is that they are chemically unstable under environmental conditions, i.e., they can lose their antimicrobial and antioxidant properties when exposed to light, humidity, oxygen and elevated temperatures (Misharina *et al.,* 2003). In order to better preserve the components of EOs, several encapsulation methods have been proposed, among which nanoemulsions stand out (Ruiz, 2020). Unlike conventional emulsions, nanoemulsions are characterized by a very small droplet size (100-500 nm), which generates a notable decrease in the influence of gravity and, together with Brownian motion, prevents sedimentation during storage. In addition, it prevents flocculation between drops, allowing the system to remain dispersed without experiencing phase separation (Monroy and Pereira, 2020).

Although the application of EOs in food is a fact that is increasingly studied, one must keep in mind the possible toxicity if they are used in an inadequate way due to an overdose. For example, ingesting EO of eucalyptus, clove, cinnamon, and nutmeg can cause generalized depression in the Central Nervous System. In addition, narcotic and narcotic effects have been described for cumin, coriander, eucalyptus, nutmeg, thyme (Usano *et al.*, 2014).

In view of this problem, the assessment of hematological and biochemical parameters is a good tool to evaluate guinea pig health. However, their application in guinea pigs is limited (Vidalón, 2014)

There is scarce documented information about the addition of these EO nanoemulsions in guinea pig feed. Therefore, this work seeks to provide information about the inclusion of chincho (*Tagetes elliptica*) and oregano (*Origanum vulgare*) EOs in the form of nanoemulsions in guinea pig feed, evaluating hematological and blood biochemistry values.

MATERIALS AND METHODS

PLACE AND TIME

The study and fattening period was carried out for 7 weeks at the Research Unit of the Laboratory of Biochemistry, Nutrition and Animal Feeding (LBNAA) of the Faculty of Veterinary Medicine of the Universidad Nacional Mayor de San Marcos - Lima, San Borja. At the end of the fattening period, the guinea pigs were processed. The study was carried out between March and September 2022.

Obtaining the essential oil

The essential oils (EO) of oregano and chincho were extracted by steam distillation at the LBNAA facilities during February and March 2022. The samples, composed of leaves and flowers, were collected manually in the Junín region. A stainless steel distiller with a capacity of 3 kg of raw material was used for extraction. The distillation process lasted approximately 2 hours, after which the essential oil was separated with a decanting pear, dehydrated with anhydrous Na_2SO_4 and stored in amber bottles under refrigeration at 4°C until use

$$Rendimiento\ del\ AE\ (\%) = \frac{masa\ de\ AE\ obtenido\ (g)}{masa\ de\ materia\ (g)} \times 100$$

The yield of essential oils (%RAE) was determined by the gravimetric method, applying the formula proposed by Carhuapoma (2007).

Nanoemulsion processing

Three O/W nanoemulsions were formulated with essential oils of oregano, chincho and their combination (200 ppm, HLB 12) according to Marinkovic et al. (2022) with some adaptations. First (oily phase), the essential oil and Tween 80-20 (10% w/w) were mixed and agitated at 700 rpm for 15 min. Then, the oily phase was added dropwise to MiliQ water (% V/V) under continuous stirring (700 rpm, 30 min) in the dark. Finally, the mixture was homogenized with Ultraturrax (Ika 25) at 22000 rpm for 5 min.

ANIMALS UNDER STUDY

We used 50 male guinea pigs resulting from the crossbreeding of Peru and Andean breeds, weaned at 15 days of age, acquired from the IVITA Huaral Station, with an average of 280 g live weight. They were randomly distributed in 5 treatments (Experimental Unit) with 10 replications per treatment. The guinea pigs remained in quarantine for one week, apparently in good health. Each experimental unit will be maintained and fed independently.

INSTALLATIONS AND POWER SUPPLY

The animals were kept in 50 individual 0.20 m^2 ponds made of cement walls and floors. Prior to the arrival of the guinea pigs, the houses were cleaned and disinfected with quaternary ammonium and lime. After that, the bedding was placed with shavings and straw, according to MINAGRI (2019) indications. A container for water supply and another one for feed supply were placed in each pond.

The feed supplied daily consisted of a base diet of commercial balanced ration in pellets suitable for guinea pigs in the growth-fattening stage free of APC for T1, T3, T4 and T5, and a base diet with APC for T2. The diet was supplemented with fresh alfalfa at 10% of live weight. The balanced ration was offered in the morning hours equivalent to 10% of the live weight in dry matter along with fresh water *ad libitum.*

EXPERIMENTAL DESIGN

Treatments

Animals will be randomly distributed into 5 experimental groups with 10 replicates per treatment:

- T1: Base diet
- T2: Base diet + APC (200 ppm zinc bacitracin).
- T3: Base diet + 200 ppm* of chincho (*Tagetes elliptica*) EO nanoemulsion.
- T4: Base diet + 200 ppm* of oregano (*Origanum vulgare*) EO nanoemulsion.
- T5: Base diet + 200 ppm* of nanoemulsion of oregano and chincho EO combination.

(*) Moharreri et al., 2022.

Sample Collection and Analysis

Blood sampling was performed at the end of the fattening period (49 days). During the processing of the animals, approximately 2 to 5 mL of blood were collected and placed in tubes with EDTA for the hemogram and without EDTA for the blood biochemistry analysis, respectively. The tubes were labeled and transported at a temperature of 4-8° and processed the same day at the LBNAA of the FMV-UNMSM. The following hematological parameters were evaluated in the laboratory: hematocrit, hemoglobin, erythrocyte count, VCM, HCM, CHCM, total leukocyte count, differential leukocyte count. The biochemical parameters evaluated were as follows: Glucose, cholesterol, triglycerides, total proteins, albumin, globulin. All parameters were processed manually.

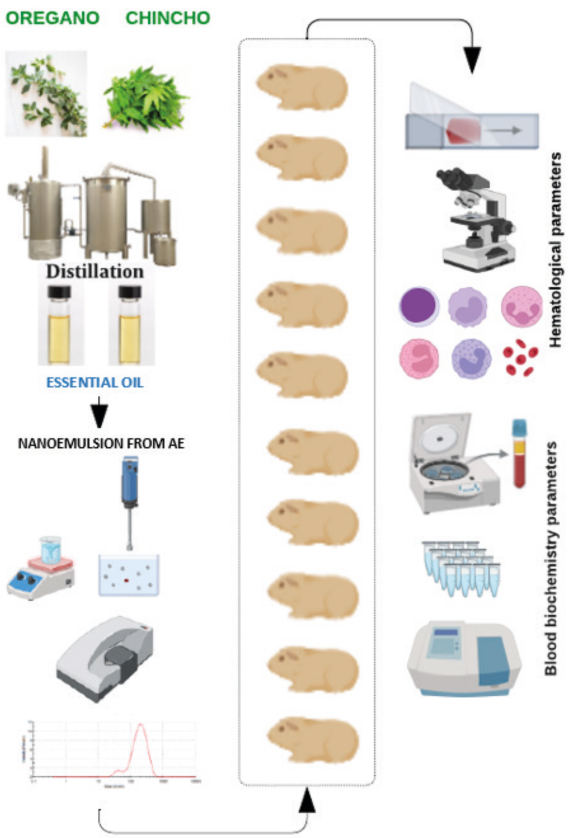

Figure 1. Graphical abstract.

Statistical Analysis

The data obtained as a result of the experiment were subjected to analysis of variance and Tukey analysis using the Statgraphics C program. XVI R© 3.5.3 (2019).

RESULTS

²SAMPLE	WEIGHT AE (g)	LEAF WEIGHT (g)	*R	%R
ORGAN	5.1049	3039	0.0016	0.1688 ± 0.010
CHINCHO	7.7927	3771.7	0.0020	0.2057 ± 0.014

Table 1. Percentage yields[1] of the essential oils

*R=performance

[1]values are given as mean ± standard deviation (n=3). [2]Species under study.

Parameters		Size (d.nm.)		Intensity	St Dev (d.n)
Z-Average (d nm)	145.2	Peak 1	208.6	93.2	92.58
PdI	0.273	Peak 2	42.76	6.8	10.07
Intercept	0.946	Peak 3	0.00	0.0	0.000
Result quality	Good				

Table 2. Nanoemulsion particle size and polydispersity analysis (PDI).

The analyzed sample has an average size of 145.2 nm and a homogeneous distribution (PdI = 0.273), suggesting a well-stable nanoemulsion. A PdI of less than 0.3 indicates a relatively homogeneous distribution, which is desirable in nanoemulsions and colloidal systems (Danaei et al. 2018; Solans et al. 2005)

Table 3 shows the averages obtained for the erythrocyte and leukocyte parameters of the guinea pig corresponding to the 5 treatments evaluated in this investigation. Within the erythrocyte series, the T2 group (83.6b ± 2.9) was the only one that presented a significant difference ($p<0.05$) with respect to the control group (78.1a ± 4.4) on the VCM parameter. Regarding HCM, groups T2 (27.9bc ± 0.8) and T4 (28.1c ± 2) presented significant difference ($p<0.05$) compared to the control group (26.4a ± 1.4). No significant difference ($p>0.05$) was evidenced in the leukocyte series between the experimental and control groups in any of the parameters measured.

Table 4 describes the blood biochemical parameters of the guinea pigs belonging to the 5 treatments evaluated in this study. The results indicate that there is no significant difference ($p>0.05$) for triglycerides, cholesterol, total protein and albumin parameters between the different treatments and the control group. However, the T3 group (119.6a ± 23.6) showed significant difference ($p<0.05$) compared to the control group (144.1b ± 17.5) for glucose level. While groups T3 (2.8b ± 0.8), T4 (2.5b ± 0.8) and T5 (2.7b ± 0.9) evidenced significant difference with respect to the control group (1.8a ± 0.6) for globulin level.

DISCUSSION

In this study, the yield of *Tagetes elliptica* essential oil was 0.2057% (Table 1), a result similar to that reported by Cerrón et al. (2023) in the Junín region, with 0.23%. In contrast, Segovia and Suárez (2010) obtained higher values (0.49%) in the Ancash region, while studies by Huaraca et al. (2021) in Andahuaylas and Ruiz and Salazar (2021) in Lima reported lower yields of 0.048% and 0.041%, respectively.

On the other hand, *Origanum vulgare* essential oil presented a yield of 0.1688% (Table 1), lower than the 0.25% obtained by Garcia and Pinto (2018) in Arequipa and significantly lower than the 1.31% recorded by Carhuallanqui et al. (2020) in the Junín region. Differences in essential oil yield may be influenced by environmental factors such as altitude, temperature, humidity and light, as well as soil quality, water availability and extraction conditions and methods employed (Ruiz and Salazar, 2021).

	TREATMENTS[2]					
	T1	T2	T3	T4	T5	*Reference values
Erythrocytes (x106/µl)	5.6 ± 0.2	5.5 ± 0.3	5.6 ± 0.2	5.3 ± 0.3	5.6 ± 0.3	4.06 - 6.02
Hemoglobin (g/dl)	14.7 ± 0.5	15.3 ± 1.1	15.5 ± 0.8	14.9 ± 0.9	14.9 ± 1.1	10.1 - 15.1
Hematocrit (%)	43.4 ± 1.8	45.7 ± 3.1	45.3 ± 2.4	43.7 ± 3.3	43.8 ± 3.0	33.8 - 48.8
VCM (fl)	78.1a ± 4.4	83.6b ± 2.9	80.9ab ± 5.3	82.0ab ± 6	78.4a ± 3.0	77.5 - 88.7
HCM (pg)	26.4a ± 1.4	27.9bc ± 0.8	27.7abc ± 1.8	28.1c ± 2	26.6ab ± 1.5	24.2 - 27.2
CHCM (%)	32.9 ± 0.5	32.3 ± 0.8	32.2 ± 1.4	32.2 ± 1.2	31.9 ± 1.5	28.3 - 32.4
Leukocytes (x103/µl)	4.6 ± 1.3	4.76 ± 1.9	4.66 ± 1.42	4.88 ± 1.8	4.49 ± 1.47	2.7 - 10.1
Neutrophils (x103/µl)	1584.72±727.9	1925.47 ± 869.7	1628.21±941.4	1553.43±708.7	1537.08±907.8	400 - 4302
Lymphocytes (x103/µl)	2555.07±653.8	2429.96 ± 1159.7	2255.15±628.9	2744.36 ± 1251	1537.08±585.1	1420 - 8403
Monocytes (x103/µl)	464.05 ± 378.4	254.9 ± 147.8	335.7 ± 198.4	371.34 ± 259.4	199 ± 148.3	0 - 373,7
Eosinophils (x103/µL)	161.88 ± 184	144.88 ± 99.5	201.38 ± 209.9	197.18 ± 165.4	114.541±114.5	2,7 - 363,6
Basophils (x103/µl)	0	0	0	0	0	0 - 60,6

Table 3. Hematological parameters of guinea pigs[1] (*Cavia porcellus*) supplemented with different concentrations of *Tagetes elliptica* and *Origanum vulgare* EO, in Lima, Peru.

[1]values are given as mean ± standard deviation (n=50), lowercase letters and different superscripts in the same row indicate significant difference ($p<0.05$). [2]T1 (control: base diet), T2 (base diet + APC: 200 ppm zinc bacitracin), T3 (base diet + 200 ppm* of *Tagetes elliptica* EO nanoemulsion), T4 (base diet + 200 ppm* of *Origanum vulgare* EO nanoemulsion), T5 (base diet + 200 ppm* of *Tagetes elliptica* and *Origanum vulgare* EO combination nanoemulsion). *Zimmerman *et al.* (2015).

Parameter	**TREATMENTS[2]**					
	T1	**T2**	**T3**	**T4**	**T5**	***Reference Values**
Triglycerides (mg/dl)	58.3 ± 29.8	46.5 ± 4.7	48.9 ± 4.8	50.3 ± 3.6	52.5 ± 11.0	10 - 70
Cholesterol COLT(mg/dl)	52.9 ± 7.2	52.1 ± 8.1	56.3 ± 13.4	60.9 ± 9.8	55.1 ± 16.7	48 - 284
Glucose (mg/dl)	144.1^{b} ± 17.5	138.8^{b} ± 15.6	119.6^{a} ± 23.6	140.3^{b} ± 16.2	139.4^{b} ± 17.0	138 - 167
Total Proteins (g/dl)	5.1 ± 0.5	5.1 ± 0.3	5.5 ± 0.7	5.2 ± 1.1	5.5 ± 0.9	5.1 - 5.4
Albumin (g/dl)	3.3 ± 0.6	2.8 ± 0.6	2.7 ± 0.3	2.8 ± 0.4	2.8 ± 0.4	2.7 - 3
Globulin (g/dl)	1.8^{a} ± 0.6	2.2^{ab} ± 0.7	2.8^{b} ± 0.8	2.5^{b} ± 0.8	2.7^{b} ± 0.9	1.7 - 2.6

Table 4. Blood biochemistry parameters in guinea pigs[1] (*Cavia porcellus*) supplemented with *Tagetes elliptica* and *Origanum vulgare* EO *in* Lima, Peru.

[1]values are given as mean ± standard deviation (n=50), lowercase letters and different superscripts in the same row indicate significant difference ($p<0.05$). 2T1 (control: base diet), T2 (base diet + APC: 200 ppm zinc bacitracin), T3 (base diet + 200 ppm* of *Tagetes elliptica* EO nanoemulsion), T4 (base diet + 200 ppm* of *Origanum vulgare* EO nanoemulsion), T5 (base diet + 200 ppm* of *Tagetes elliptica* and *Origanum vulgare* EO combination nanoemulsion). *Genzer *et al.* (2019).

The use of essential oil nanoemulsions in animal nutrition represents an innovative approach to boost health and performance in animal husbandry. Due to their tiny particle size and high surface-to-volume ratio, these nanoemulsions promote efficient delivery and absorption of bioactive substances (Qingqing *et al.,* 2019). They have been widely proven to possess antimicrobial and anti-inflammatory effects, making them useful in various areas, including the food and pharmaceutical industries (Donsi and Ferrari, 2016). However, they face significant challenges such as improving manufacturing processes, confirming their safety and effectiveness, and selecting stabilizers and bioactive agents that do not compromise health. It is also crucial to further investigate the positive environmental impacts that nanoemulsions can have on food preservation. Future research should focus on refining production and use procedures to optimize the preservative capabilities of nanoemulsions, while ensuring their safety and efficacy. The therapeutic value of natural ingredients in animal health, especially in the livestock industry where health directly influences productivity, is of great importance and should not be underestimated.

Although there are several reports on the use of essential oils in animal feed, there are insufficient bibliographic resources regarding their application in the form of nanoemulsions in guinea pig feed.

The present study evaluated the impact of supplementation with essential oil nanoemulsions of chincho and oregano on blood hematological and biochemical parameters in guinea pigs. The encapsulation of bioactive compounds such as essential oils in nanoemulsions can significantly improve several characteristics, such as oxidative stability, thermostability, shelf life, biological activity, as well as control the volatility and progressive release of these essential oils (Pino and Aragüez, 2021). The use of high-pressure homogenization has been shown to be highly efficient in the creation of biocompatible essential oil nanoemulsions, and the process results in smaller oil droplets that increase the biocompatibility of the final formulations (Moreno, 2013). The versatility of nanoemulsions as a delivery system is further demonstrated by their ability to encapsulate essential oils with different viscosities and concentrations, ensuring the adaptability of this technology to a broad spectrum of therapeutic applications. Nanoemulsion supplementation of guinea pigs involves a meticulous and precise approach to ensure bioavailability and efficacy of treatment. These findings have important implications for the application of nanoemulsions in the food industry, where stability and bioactivity are paramount. However, the main reports were limited by the need for standardized protocols to ensure that the data collected reflect true physiological changes rather than artifacts of the collection process. Future research should focus on optimizing both the homogenization process and the emulsification system to achieve a desirable balance between nanoemulsion stability and enhancing the potential of essential oils, paving the way for their use as fortified ingredients in the food industry. Overall, these new findings on essential oil nanoemulsions contribute to the continued advancement of knowledge in the field and have the potential to revolutionize transdermal delivery and natural substitutes for synthetic preservatives.

The availability of data on hematological and biochemical values in guinea pigs is limited. However, among the various studies reviewed, the work of Zimmerman *et al.* (2015) stands out as the most complete and exhaustive in this field, considering its values as referential for using juvenile guinea pigs, similar to the present work.

The erythrocyte count, hemoglobin, hematocrit, MCV and MCHC were within the reference ranges. However, T2 (base diet + APC: 200 ppm zinc bacitracin) presented a significantly higher value ($83.6^b \pm 2.9$) with respect to the control group ($78.1^a \pm 4.4$) in MCV. This is probably due to the fact that polychromasia (presence of immature erythrocytes) is a normal finding in this species. The HCM of groups T2 ($27.9^{bc} \pm 0.8$), T3 ($27.7^{abc} \pm 1.8$) and T4 ($28.1^c \pm 2$) is above the reference range of Zimmerman *et al.* (2015). However, when contrasting these values with the range referred by Vidalón (2014) for the HCM parameter (23.2 - 33.5), they are considered within acceptable limits.

Regarding the leukocyte series, most of the parameters were within the referential range established by Zimmerman *et al.* (2015). The leukocyte count was very similar in all groups, no significant difference was found. The T1 or Control group presented the highest monocyte count (464.05 ± 378.4), which exceeds the referential range (0 - 373.7). Monocytosis is related to chronic inflammatory processes, neoplasms or degenerative diseases (Nelson and Couto, 2020). One of the weaknesses of the study lies in the lack of evaluation of hematological and biochemical parameters at the beginning of the investigation, which prevents having initial values to compare with those obtained at the end of the study. This lack of initial data makes it difficult to interpret the changes observed and limits the ability to determine the precise impact of the variables analyzed in the guinea pig population studied.

The 5 experimental groups presented lymphocyte and eosinophil values within the reference range and none of them showed the presence of basophils.

The assessment of biochemical parameters helps us to identify metabolic alterations, feed deficiencies and the general health status of the animal (Matute, 2019). It is of great importance to know the biochemical parameters of guinea pigs to determine the clinical condition of the animal, to ensure an adequate nutritional balance and to be able to monitor the efficacy of treatments and the patient's prognosis (Arrúa *et al.*, 2023). The parameters evaluated in the present study are within the range established by Genzer *et al.* (2019), except for T3 glucose ($119.6^a \pm 23.6$), which turned out to be a lower value with respect to the control group with significant difference ($p<0.05$) and below the referential range. This hypoglycemia can be attributed to the fact that the samples from this group were the last to be collected and processed. To avoid these pre-analytical errors in the future, it is recommended to use gray cap tubes (with sodium fluoride and potassium oxalate).

Triglyceride, cholesterol, total protein, albumin and globulin values are within the ranges mentioned by the reference author.

This work evaluated the hematological and biochemical parameters of guinea pigs supplemented with nanoemulsions of chincho and oregano essential oil. The purpose was to detect any significant alteration that could indicate signs of toxicity due to the use of these additives in guinea pig feed. The results showed values within the reference ranges, suggesting that essential oil nanoemulsions could be an effective alternative to improve production and prevent diseases in these animals. In addition, they represent a natural alternative to the use of APC in animal feed

CONCLUSION

The inclusion of essential oils of chincho and oregano in the form of nanoemulsions in guinea pig feed does not cause differences in hematological and biochemical values in the evaluated treatments.

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