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Organic minerals, tributyrin, and blend of organic acids in the diet of commercial laying hens at the end of production

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Abstract

This study aimed to evaluate the responses in performance, egg quality, and health of internal organs of laying hens of Hisex White lineage. The birds were provided with diets supplemented with organic minerals (OM) + tributyrin, associated or not with a mixture of organic acids (benzoic formic, citric, and phosphoric), compared to a control treatment containing zinc bacitracin (28 ppm). In total, 160 laying hens of the commercial Hisex White lineage of 68 w and an average weight of 1.735 ± 0.025 kg were distributed in 40 experimental plots. The study employed a completely randomized design, with five treatments and eight replications/treatment. The experimental period was divided into seven production cycles of 21 days, totalling 147 experimental days. The variables analysed were egg production and loss, feed intake, mean egg weight, egg mass, feed conversion, and internal and external quality variables of eggs. At the end of the experimental period, the final weight of the birds was measured to evaluate the relative weight of liver and kidneys. No significant effect of the treatments on the productive performance and internal and external quality of the eggs was evident. The association of organic minerals with a mixture of organic acids and tributyrin did not influence the productive performance and internal and external quality of the shell of Hisex White laying hens in the period from 68 to 89 weeks of age.

Keywords: eggshell quality, mineral supplementation, organic mineral, poultry production
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Introduction

In poultry production, errors in the management of egg collection and processing, associated with a reduction in the quality of shell that happens naturally over time, can imply high losses during the process (Santos *et al*., 2021). Producers can face losses of up to 15% from production to the arrival of the product at the consumer. These losses usually happen due to inferior quality of the shell, which, among other factors, may be related to the nutrition of birds with regard to levels of calcium, vitamin D, available phosphorus, manganese and zinc, and amino acid levels in the feed (Delgado *et al*., 2017). Besides nutrition, environmental factors also interfere in the quality of the eggshell. This includes environmental temperature, since high temperatures interfere in the metabolism of eggshell formation by increasing respiratory rate, which induces respiratory alkalosis and can reduce feed

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intake (Valentim *et al.,* 2019). The age of birds is also a determining physiological factor in eggshell quality, since aging increases egg weight, but the amount of calcium carbonate deposition remains practically constant during the productive life of laying hens, resulting in a reduction in eggshell thickness. Moreover, laying hens at the end of productive life may show a reduction in carbon anhydrase activity, leading to a lower calcification of the eggshell (Oliveira *et al*., 2019). Therefore, it is essential that the nutrition of laying hens in the final productive cycle mitigate such effects that are reflected in large economic losses.

For proper nutrition, animals must ingest and absorb adequate amounts of nutrients, including minerals that for birds are considered of significant importance, since they participate in many biochemical processes, and are fundamental for the constitution of the eggshell, such as calcium, phosphorous, and manganese. Through the diet, the animals ingest 70% of calcium carbonate and other mineral requirements, and these compounds are important for eggshell formation (Burke, 1996; Waheed *et al*., 2019).

Nowadays, there is a growing interest in exploring factors that increase the absorption or metabolization of trace elements. Organic sources or chelated minerals have been used because they are more bioavailable. Chelated minerals are defined as being a mixture of mineral elements that are bound to some type of carrier, which can be an amino acid or polysaccharide, which has the ability to bind to the metal by covalent bonds via amino acids groups or oxygen, forming a cyclic structure (Pereira *et al*., 2009; Gayathri & Panda *et al*., 2018)

The supplementation of diets with organic minerals instead of inorganic minerals is present in several studies, mainly due to the better absorption of these when compared to inorganic minerals. Organic minerals, besides offering greater stability, benefit from biochemical protection against chemical reactions that may occur in the intestinal lumen, resulting in better bird performance and greater absorption and use of these minerals (Brito *et al*., 2006; Alagawany *et al*., 2021).

Besides the adoption of organic minerals, researchers and companies are interested in the use of organic acids. The use of organic acids is associated with the inhibitory effect on the development of pathogenic microorganisms and their influence on the availability of nutrients (Bastos-Leite *et al*., 2016). Such acids can improve the performance of the birds, since they function as antimicrobials. They reduce the pH of the digestive tract and stimulate the increase in pepsin secretion, which provides bactericidal and bacteriostatic action in the gastrointestinal tract (GIT). This promotes a lower binding capacity of bacteria with the intestinal wall of the bird and improves buffering capacity with cations, such as calcium, magnesium, iron, copper, and zinc, increasing their availability and retention (Michel *et al*., 2019).

Organic acids interfere in the intestinal microbiota and in the metabolisability of feed nutrients, besides presenting a trophic effect on the structure and development of the intestinal mucosa (Vasconcelos *et al*., 2016). Tributyrin is a triglyceride containing three molecules of butyric acid esterified to glycerol. This conformation blocks the butyrate from being absorbed before reaching the small intestine, because the lipases present in the small intestine are necessary to cleave the bonds of covalent esters. This additive (tributyrin) has been used to improve the intestinal health of birds, ensuring greater absorption of nutrients, improving productive performance, and guaranteeing feed intake (Hansen *et al*., 2021); tributyrin is more palatable than butyrate.

Several studies indicate an increase in the size of villi and crypt depth and, consequently, the area of nutrient absorption. Van Immerseel *et al*. (2004) and van der Aar *et al*. (2017) reported that organic acids are readily available energy sources to enterocytes (gut cells that account for the final digestion of food and transepithelial transport of nutrients) and thus, stimulate an increase in the rate of cell proliferation and increase the absorption of nutrients by the GIT.

This study aimed to evaluate the effectiveness of diets supplemented with the organic minerals zinc and manganese (OM) + tributyrin with or without a blend of organic acids (benzoic, formic, citric, and phosphoric acids), compared to a control treatment containing zinc bacitracin (28 ppm) on the productive performance, losses, and internal and external quality of eggs of commercial laying hens at the end of the production cycle.

Materials and Methods

The study was executed at the Aviculture Laboratory of the Federal Institute of Minas Gerais – Bambuí, from July to December, 2018. The project was subjected, analysed, and approved by the Ethics Committee on the Use of Animals – CEUA, under project no. 13/2018.

To perform the experiment, 160 light laying hens of the Hisex White lineage, at 68 weeks of age and an average weight of 1.735 ± 0.025 kg, were distributed in a completely randomized design, with five treatments of eight replicates with four birds per repetition. Birds were housed in 40 cages, with dimensions of 50 cm × 45 cm × 45 cm, at a stocking density of 450 cm^2/bird. The treatments used during the experiment were:

Treatment 1: control treatment (diet formulated based on the nutritional requirements of the Hisex White lineage) + growth-promoting antibiotic (GPA – zinc bacitracin, 28 ppm);
Treatment 2: control treatment + organic microminerals (OM) + tributyrin + GPA;
Treatment 3: control treatment + OM + tributyrin + organic acid complex + GPA;
Treatment 4: control treatment + OM+ tributyrin;
Treatment 5: control treatment + OM+ tributyrin + complex organic acids.

The organic micromineral product (OM) is a compound of organic minerals, zinc, and manganese, associated with tributyrin. This product was included in the feed formula at a dosage of 3 kg/ton of feed, which provided 125 mg of butyric acid/kg of feed, 24 mg organic manganese/kg feed and 18 mg organic zinc/kg feed. The organic acid was a blend of organic acids composed of benzoic, formic, citrus, and phosphoric acids. The growth-promoting antibiotic used was zinc bacitracin at the dosage of 28 g/t of feed. An analysis of the possible pathogenic microorganisms present in the bedding and in the breeding environment of the birds was not performed.

The feeding was executed twice a day with water supply via a nipple drinker system. Temperature and relative humidity were measured every 15 minutes using a datalogger (Instrutherm HT-70). Table 1 shows the temperature means during the experimental periods.

Table 1 Mean temperature (°C) and relative humidity (RH, %) during experimental periods

Period*	Mean temperature (°C)	Mean RH (%)
1	20.31	62.05
2	20.33	62.04
3	20.28	61.83
4	20.31	62.05
5	20.32	62.05
6	20.32	62.06
7	20.3	62.08
Overall mean	20.31	62.02

Period* Mean of the 21 days of each trial period

The light program used was 16 h daily of natural lighting + artificial lighting. During the experimental period, the feeds were formulated based on corn and soybean meal and were iso-nutritive except for the addition of the evaluated additives. The formulation followed the nutritional recommendations of the Hisex White lineage (Tables 2 and 3).

Table 2 Experimental ingredients used for laying hens of the Hisex White lineage in the period from 68 to 89 weeks of age.

Ingredients: (kg)	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Corn	628.21	625.2	625.85	625.39	626.05
Soybean meal (45%)	256.67	256.67	256.67	256.67	256.67
Limestone	98.33	98.33	96.67	98.33	96.67
Dicalcium phosphate	10	10	10.01	10	10
Sodium Chloride	3.6	3.6	3.6	3.6	3.6
DL- methionine	0.6	0.6	0.6	0.6	0.6
Cl Choline 60%	0.4	0.4	0.4	0.4	0.4
Px mineral[1]	0.5	0.5	0.5	0.5	0.5
Px vitamin [2]	1	1	1	1	1
Phytase 5000 FTU	0.5	0.5	0.5	0.5	0.5
Zinc Bacitracin 15%	0.19	0.19	0.19	0	0
Blend of organic acids[3]	0	0	1	0	1
Bentonite	0	2.5	2.5	2.5	2.5
Butyric acid 50%	0	0.25	0.25	0.25	0.25
Organic manganese 16%	0	0.15	0.15	0.15	0.15
Organic zinc 16%	0	0.1125	0.1125	0.1125	0.1125
Total (kg)	1	1	1	1	1

[1] Guaranteed levels per kg mineral premix: copper 20 g, iron 96 g, iodine 1.4 mg, manganese 156 g, selenium 500 mg, zinc 110 g

[2] Guaranteed levels per kg vitamin premix: folic acid 100 mg, pantothenic acid 6 mg, antioxidant 1 mg, biotin 5 mg, niacin 20 g, vitamin A 4.9 IU, vitamin B1 250 mg, vitamin B12 6 mg, vitamin B2 3 mg, vitamin B6 150 mg, vitamin D3 1.5 IU, vitamin E 8, IU vitamin K 1.2 IU.

[3] Guaranteed levels of organic acid mixture: benzoic acid: 159.2 g/kg; formic acid: 229.54 g/kg; citric acid: 81.675 g/kg; phosphoric acid: 198 g/kg; calcium (minimum): 185 g/kg; phosphorus (minimum): 65 g/kg.

Treatment 1: control treatment (diet formulated based on the nutritional requirements of the Hisex White lineage) + growth-promoting antibiotic (GPA – bacitracin zinc, 28 ppm). Treatment 2: control treatment + organic microminerals (OM) + tributyrin + GPA. Treatment 3: control treatment + OM + tributyrin + organic acid complex + GPA. Treatment 4: control treatment + OM + tributyrin. Treatment 5: control treatment + OM + tributyrin + organic acid complex

Table 3 Calculated nutritional levels of experimental diets for the respective treatments

Nutrient	Units	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Crude Protein	%	17.1075	17.0835	17.0888	17.085	17.0903
Total Methionine	%	0.3196	0.3191	0.3192	0.3192	0.3193
Total Met+Cis	%	0.6084	0.6074	0.6076	0.6074	0.6077
Total Lysine	%	0.9207	0.9199	0.92	0.9199	0.9201
Lys. Dig. birds	%	0.8023	0.8016	0.8017	0.8016	0.8018
Met. Dig. birds	%	0.3007	0.3003	0.3004	0.3003	0.3004
Met+Cis Dig. birds	%	0.5416	0.5407	0.5409	0.5408	0.5409
Tre Dig. Birds	%	0.1919	0.1917	0.1918	0.1917	0.1918
Tre Dig. Birds	%	0.5829	0.5821	0.5823	0.5822	0.5824
Sodium	mg/kg	1.666	1.666	1.664	1.666	1.664
Chlorine	mg/kg	2.731	2.73	2.73	2.73	2.73
Calcium	%	4.175	4.175	4.111	4.175	4.112
Total phosphorus	%	0.596	0.596	0.596	0.596	0.596
Phosphorus available:	%	0.362	0.361	0.362	0.362	0.362
Butyric acid 50%	mg/kg	0	125	125	125	125
Organic manganese 16%	mg/kg	0	24	24	24	24
Organic zinc 16%	mg/kg	0	18	18	18	18
Inorganic manganese	mg/kg	78	78	78	78	78
Inorganic zinc	mg/kg	55	55	55	55	55
Choline	mg/kg	1.062	1.061	1.061	1.061	1.061
AME birds	kcal/kg	2.706.6	2.696.6	2.698.8	2.697.2	2.699.4

Treatment 1: control treatment (diet formulated based on the nutritional requirements of the Hisex White lineage) + growth-promoting antibiotic (GPA – bacitracin zinc, 28 ppm). Treatment 2: control treatment + organic microminerals (OM) + tributyrin + GPA. Treatment 3: control treatment + OM + tributyrin + organic acid complex + GPA. Treatment 4: control treatment + OM + tributyrin. Treatment 5: control treatment + OM + tributyrin + organic acid complex

For the formulation of the diets, all the ingredients used in the preparation were sent to the laboratory where nutritional analyses of the raw materials were performed. The ingredients evaluated were corn, soybean meal, degummed soybean oil, limestone, and dicalcium phosphate. Besides the ingredients used in the formulations, samples of experimental diets were collected for laboratory analysis (Rostagno *et al*., 2017).

During the production phase (seven production cycles of 21 days each) the following performance characteristics were evaluated: feed intake (g/bird/day); egg production percentage (%); mean egg weight (g); percentage of egg loss (%); feed conversion by egg mass (kg/kg); feed conversion per dozen eggs (kg/dozen).

The total number of eggs produced was evaluated by recording daily, in the morning and afternoon. The amount of broken, cracked, dirty eggs (faeces and dirt), and eggs with blood stains on the eggshell were also daily evaluated, since they are an important variable to check the effect of treatments on egg losses in the experimental period. The percentage of losses was measured by dividing the total number of eggs with problems (dirty faeces and blood, cracked, or broken) produced in the week divided by the total eggs produced in the week, expressed as a percentage. At the end of the seven experimental periods, the mean of all weekly losses was measured. The measurement of total losses was made considering the sum of cracked, dirty, and broken eggs.

To evaluate egg quality characteristics, the experiment was divided into seven cycles of 21 days, so that at the end of each cycle two healthy eggs per repetition (n = 8) were collected on two consecutive days. The characteristics evaluated were: specific gravity (g/cm^3); eggshell resistance to breakage (unit); yolk percentage; percentage of albumen; eggshell percentage; eggshell thickness (mm); yolk colour determined using DSM YolkFan^{TM} equipment; and Haugh Unit calculation. For specific gravity, the eggs were immersed in saline solutions ranging from 1,065 to 1 g/cm^3 of density with gradient of 0.005 between them, with solutions prepared according to the recommendations of Freitas *et al*. (2004).

Break resistance analyses were performed using the Digital Egg Tester DET6000 equipment. Eggshell thickness (mm) was measured at three points of the equatorial region of the eggshells with the aid of a digital calliper and expressed in millimetres. Haugh units were determined from the data on egg weight and albumen height, using a formula proposed by Alleoni & Antunes (2001):

$HU = 100 \log (H+7.57-1.7W0,37)$,

where: H = height of the albumen (mm) and W = egg weight (g).

At the end of the experiment, all birds in the plot were weighed and one bird per plot was chosen (depending on the mean weight of the plot) to obtain the relative weight of the liver and kidneys, which were determined in relation to the live weight of the bird [(organ weight/live weight) × 100]. The weighing of the birds of the plot occurred at 17:30 so that most birds had laid to eliminate the interference of this weight in the determination. These birds were submitted to a previous fast of 4 hours before weighing. The selected birds were identified by the tibia and slaughtered by cervical displacement. Birds were manually eviscerated and their liver and kidneys were separated. For liver weights, the gallbladder was discarded. A semi-analytical scale with accuracy of 0.01 g was used for organ weighing.

The data of the experimental variables evaluated were subjected to variance analysis using the tools of the SISVAR®, program, with the means compared by the Tukey test at 5% probability. All variables were evaluated to verify the normality of errors using Shapiro Wilk test, and for those that showed a normal distribution of errors. A data transformation of (x + 1)0,5 was used.

Results and Discussion

The results showed no effect (P >0.05) of the experimental treatments on the variables of productive performance, weight of birds, and egg losses (Table 4).

Table 4 Productive performance of Hisex White birds receiving different experimental diets in the period from 68 to 89 weeks of age

Variables analysed	Treatments					CV (%)[6]	p-value
	T1[1]	T2[2]	T3[3]	T4[4]	T5[5]		
Egg production (%/bird/day)	85.67	84.21	88.38	85.16	85.42	2.83	0.5535
Feed intake (g/bird/day)	118.1	114.8	125.4	115.9	117.1	7.84	0.1838
Egg weight (g)	65.28	67.97	65.07	65.10	64.15	2.61	0.5643
Egg mass (g)	55.91	54.72	57.5	55.33	54.78	2.66	0.5879
Mean weight birds 89 week (g)	1783	1678	1755	1694	1771	3.38	0.5318
CA (kg/kg)	2.127	2.112	2.182	2.153	2.151	2.25	0.9012
CA (kg/dozen)	1.664	1.592	1.652	1.692	1.653	2.13	0.8991
Total losses (%)	1.28	1.71	1.24	1.92	1.36	15.55	0.5188
Dirty eggs with faeces (%)	0.36	0.68	0.34	0.6	0.56	14.85	0.5095
Dirty eggs with blood (%)	0.55	0.54	0.39	0.73	0.24	15.09	0.4325
Broken eggs (%)	0.37	0.46	0.48	0.55	0.54	10.93	0.8347
Cracked eggs (%)	0	0.026	0.026	0.047	0.023	3.19	0.4353

*Means followed by different letters on the same line differ statistically using the Tukey test (*P* <0.05)
Treatment 1: control treatment (diet formulated based on the nutritional requirements of the Hisex White lineage) + growth-promoting antibiotic (GPA – bacitracin zinc, 28 ppm); Treatment 2: control treatment + organic microminerals (OM) + tributyrin + GPA; Treatment 3: control treatment + OM + tributyrin + organic acid complex + GPA; Treatment 4: control treatment + OM + tributyrin; Treatment 5: control treatment + OM + tributyrin + organic acid complex; [6]CV: coefficient of variation

The performance of birds was unaltered using experimental diets supplemented with OM and GPA (growth-promoting antibiotic) and/or organic acid complex, or not. Few studies have done research with laying birds in association with such food additives. The literature indicates a variation in the results on organic acids or organic minerals in poultry diets, as they are dependent on the concentration and combinations of organic acids and buffer capacity of the diets used, bioavailability of the source of organic minerals used, as well as the environmental challenge of pathogenic microorganisms. This justifies the similarity between the treatments used in the current study. Currently, to attend global concerns on microbial resistance, healthy products, and environmental sustainability, poultry chain production without antibiotics is essential.

Possibly one of the main causes of the non-significant differences between treatments is due to the absence and/or low challenge of pathogenic microorganisms. Besides the items mentioned above, the nutritional levels of organic minerals used in the diet may have met the requirement of the birds, since studies such as those conducted by Chantiratikul *et al*. (2008) prove that the substitution of 70–100% of the inorganic minerals by organic minerals was able to maintain the productive performance of birds without harm. Ghasemi *et al*. (2022) reported that 66% and 100% chelated compound-based trace mineral supplementation improved lipid profiles and oxidative status in laying hens.

Experimental results using organic minerals in diets for laying hens are controversial. Similar studies have shown no differences in the productive performance of commercial laying hens receiving diets with organic minerals compared to birds receiving diets supplemented with inorganic minerals (; Figueiredo *et al*., 2013; Carvalho *et al*., 2015). The substitution of inorganic by organic minerals in diets for laying hens improved egg mass, feed conversion to egg mass, and feed conversion per dozen eggs when 66% and 100% of inorganic minerals was replaced by organic minerals in the diet (Gheisari *et al*., 2011).

The current study showed higher production and egg mass in the second cycle in laying hens fed with organic minerals (Zn, Cu, and Mn) in relation to diets with inorganic minerals, without differences in feed intake, feed conversion by mass and dozen eggs, and egg weight (Fernandes *et al*., 2008; Gheisari *et al*., 2011). Another study indicated no effect of supplementation with 250 ppm and 500 ppm of a mixture of organic minerals based on Zn, Mn, and Se on egg production, feed intake, feed conversion, and egg mass of light commercial laying hens in the first production cycle (Kazempour *et al*., 2017).

Other studies show that supplementation with organic acids in the diet of laying hens increased the egg production percentage, mainly by using citric and butyric acids and that the reduction of phosphorus supplementation available in diets supplemented with organic acids improved productive performance when compared with the negative control diet without the inclusion of acids (Jahanian & Golshadi, 2015). Wang *et al*. (2021) observed the effects of 250 ppm and 500 ppm of butyric acid glycerolipid supplementation in corn-based and wheat-based diets for commercial laying hens. Wang *et al*., (2021) observed that supplementation with 500 ppm improved the production and mass egg of laying hens receiving wheat-based diets in comparison to the inclusion of 250 ppm of the product. This effect occurred due to the decrease in the intestinal *Escherichia coli* count, with less effect of the organic acid supplementation levels on the performance of birds receiving the diet based on corn and soybean meal.

In the current study, tested product contained tributyrin in its formula, which is an additive widely used in poultry diets. Tributyrin reduces pathogenic bacteria in the digestive tract in the initial phase and throughout the breeding period (Alves *et al*., 2015). In the literature, tributyrin (besides its function as a substitute for growth promoters) acts by improving the development of the intestinal mucosa, increases intestinal villi length, and the retention of calcium and phosphorus from the diet. As such, it is an important ally in the nutrition of birds (Hansen *et al*., 2021). The current study did not consider the intestinal microbial population or the evaluation of intestinal villi due to the lack of structure and resources for performing such analyses.

The addition of organic or inorganic minerals and limestone granulometry in the diets of light commercial laying hens improved some external characteristics of eggs without affecting the productive performance and bone characteristics of birds (Fouladi *et al*., 2018). To evaluate the effect of the association of different organic acids (acetic, lactic, and butyric acids) in diets of Japanese quail, Wang *et al.* (2021) observed a beneficial effect of the association of such acids on the productive performance of birds receiving such diets compared to the basal diet without the inclusion of acids (Alleoni *et al*., 2011). The authors found an improvement from lactic acid compared to the other organic acids in terms of a greater decrease in the population of intestinal pathogenic bacteria, and improvements in biochemical parameters of the liver and blood, as well as quality parameters and the number of eggs produced.

This study did not observe an effect (P >0.05) of the treatments on the internal and external quality of the eggs, except for the percentage of albumen (P <0.05) (Table 5).

Table 5. Mean external and internal quality of eggs in the evaluation period, relative weight of the kidneys and liver and body weight at 89th week age of Hisex White birds receiving different experimental diets in the period from 68 to 89 weeks of age

Variables analysed	Treatments						
	T1^{1}	T2^{2}	T3^{3}	T4^{4}	T5^{5}	CV (%)^{6}	p-value
Eggshell thickness	0.392	0.395	0.395	0.386	0.404	0.67	0.3996
Specific gravity (g/cm^{3})	1.081	1.063	1.082	1.082	1.081	0.6	0.4812
Eggshell resistance (kg/cm^{2})	3.648	4.079	3.941	4.046	4.059	8.13	0.3581
Yolk colour	5.51	5.51	5.71	5.44	5.64	4.55	0.1843
Haugh Unit	93.31	90.08	91.65	90.30	91.89	2.61	0.0808
Shell (%)	11.93	11.78	11.80	12.11	12.04	2.63	0.2266
Albumen (%)*	56.09	56.23	56.29	55.10	55.50	1.55	0.0654
Yolk (%)	31.98	31.96	31.92	32.8	32.45	2.44	0.1742
Liver weight 89-weeks of age (g)	39.83	33.26	40.52	34.49	38.21	15.32	0.1614
Kidney weight 89-weeks of age (g)	5	5.50	5.89	5.24	5.24	19.25	0.1856
% liver at 89-weeks of age	2.23	1.98	2.36	2.02	2.18	15.45	0.5188
% kidney at 89-weeks of age	0.28	0.33	0.34	0.31	0.30	19.94	0.5318

*Means followed by different letters on the same line differ statistically using Tukey's test (P <0.05).
Treatment 1: control treatment (diet formulated based on the nutritional requirements of the Hisex White lineage) + growth-promoting antibiotic (GPA – bacitracin zinc, 28 ppm); Treatment 2: control treatment + organic microminerals (OM) + tributyrin + GPA; Treatment 3: control treatment + OM + tributyrin + organic acid complex + GPA; Treatment 4: control treatment + OM + tributyrin; Treatment 5: control treatment + OM + tributyrin + organic acid complex; CV: coefficient of variation

The association of the evaluated additives did not improve the external quality of the eggs possibly due to a low challenge by pathogenic microorganisms. The level of supplementation of organic minerals in diets of laying birds, according to Carvalho *et al*. (2015), can be supplemented to up to 70% of the NRC requirement without harming the internal and external quality of the eggs. Eberhart *et al*. (2021) also did not observe effects of dietary supplementation with organic or inorganic minerals on specific gravity; percentages of albumen, yolk and shell; shell thickness; and percentage of broken and cracked eggs in commercial laying hens in the second production cycle.

Alves *et al*. (2015) only observed effects of organic minerals in diets for laying hens on the variable shell thickness, where they obtained better results; an effect of mineral type on the specific gravity of the eggs was not observed. Figueiredo *et al*. (2013) observed that the substitution of inorganic by organic minerals in diets for laying hens provided better specific gravity and shell thickness when 66% and 100% of inorganic minerals was replaced by organic minerals.

A lower number of broken eggs, fine shells, and a higher percentage of yolk and solids in the yolk were observed in eggs of the laying hens receiving supplementation of 250 ppm and 500 ppm of organic minerals (Zn, Mn, and Se) in the diet; values of specific gravity, Haugh unit, and percentage and solid content in the albumen were not affected (Fernandes *et al*., 2008).

Kazempour *et al*. (2017) observed an improvement in the eggshell thickness of laying hens receiving diets with organic acids, with no influence on the resistance to breakage of these eggs. Janani an *et al*. (2015) did not find effects of supplementation of 2.5 g/kg and 5 g/kg of butyric acid glycerides in the diet on the thickness and strength of the shell, Haugh unit, index, and yolk colour of eggs from commercial laying hens. Dong *et al*. (2022) concluded in his work that inorganic trace minerals may be effectively replaced by low levels of complex organic trace minerals in laying hens during the late production stage. Corroborating these findings, Yang *et al*. (2021) concluded that diets supplemented with the organic trace minerals at 50% of the NRC profile in laying hens promoted optimum laying performance, mineral deposition, and reduced mineral excretion.

Further studies should be executed to evaluate the association of organic mineral complexes and their relationship with organic acid blends along with effective health challenges to promote greater efficiency of the active ingredients of these products.

Conclusion

The association of organic minerals with a mixture of organic acids and tributyrin did not influence the productive performance and internal and external quality of the eggshell of Hisex White laying hens in the period from 68 to 89 weeks of age.

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Authors' contributions

AG, DAM, LFSM: Planned the experimental project, data collection, writing, statistical analysis, and interpretation. LCM: Assisted in data collection and writing. JAVF: worked on statistical analysis and helped in interpretation of results. AAA, JKV, HFO: Writing, revising, and formatting the manuscript.

Conflicts of interest

No authors have a conflict of interest relative to this work.

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