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Original Article

Substituting Zinc Bacitracin Antibiotics with Symbiotics for Post-Peak Laying Hens

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■Keywords

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ABSTRACT

The objective of this study was to evaluate the effect of adding symbiotics to the diet of laying hens in the post-peak laying period on performance variables, egg quality, and nutrient digestibility. One hundred and ninety-eight 70-week-old Dekalb White laying hens were distributed in a completely randomized design with 6 treatments, each with 6 replications of 5 and 6 birds. The treatments were: corn and soybean meal (CSM); CSM + meat and bone meal (MBM); MBM + 0.05% zinc bacitracin additive (ZnBac); MBM + 0.1% Symbiotics in three phases: layer-type chick, pullet, and laying hen (Symb-S; Symb-G and Symb-L). Data were compared by Orthogonal Contrast. The CSM treatment showed better shell thickness when compared to MBM, and a better percentage of albumen. RF and BacZn showed better yolk coloration. ZnBac showed better yolk weight when compared to Symb-S. CSM and ZnBac increased red and yellow yolk colors and Symb-G had an effect for luminosity. The gross energy apparent metabolizability coefficient (GEAMC) was better for CSM and Simb-G. The crude protein apparent metabolizability coefficient (CPAMC) was better with MBM. The dry matter apparent metabolizability coefficient (DMAMC) was better for MBM, Symb-S, and Symb-L. Thus, it is possible to replace antibiotics with symbiotics for laying hens in the post-peak phase.

INTRODUCTION

For decades, antibiotics have been widely used as mechanisms to stimulate the immunocompetence of birds, control infectious diseases, act as a growth promoters, improve performance and feed efficiency, and make animals less susceptible to diseases (Gadde *et al.,* 2018; Al-khalaifa *et al.,* 2019).

Dietary supplementation of antibiotics at low levels is a common practice in the poultry industry. However, its inappropriate use can lead to the development of antibiotic-resistant bacteria and the accumulation of residues in poultry products, posing a threat to consumers (Tang *et al.,* 2017). This concern for consumers has led to a demand for new methods to protect intestinal health and improve bird performance (Najafabadi *et al.,* 2017).

Research has been carried out with the aim of replacing antibiotics with natural products that do not trigger bacterial resistance or leave residues in the final products (Al-Khalaifah, 2018; Barbalho *et al.,* 2023; Dong *et al.,* 2023; Ningsih *et al.,* 2023). One of the alternatives are symbiotics, a type of additive to poultry diets made of compounds derived from a combination of probiotics and prebiotics, which promote mutual effects on intestinal health, and lead to improvements in performance (Mohammed *et al.,* 2019; Ribeiro *et al.,* 2023).

According to (Ferket *et al.,* 2002), when prebiotics and probiotics are administered together the health of the gastrointestinal tract is

maintained, practically making it impossible for *E. coli, Clostridium,* or *Salmonella* to adhere. Prebiotics prevent the adherence of pathogenic microbiota to the intestinal epithelium, saturating the bacteria binding sites and eliminating them along with the stools. Probiotics, on the other hand, prevent inflammatory processes in the intestine, improving absorption rates, and minimizing energy expenditure to replace intestinal cells.

There are several studies with symbiotic components (pre and probiotics) in poultry feed (Deng *et al.,* 2020). However, there are still few studies on the use of symbiotics and their components to replace the use of antibiotics during the initial stages of laying hens, as well as on their impact on the post-peak laying period, which is characterized by a lower use of nutrients, and a decrease in egg production and quality.

Thus, the objective of the present research was to evaluate the effects of replacing bacitracin zinc antibiotics with a symbiotic supplement based on *Saccharomyces cerevisiae*, *Bifidobacterium bifidum*, *Bacillus subtilis, Enterococcus faecium, Lactobacillus acidophilus,* Glucans and Mannans in the diet of laying hens in different stages (chicks, pullets, and laying hens) on performance, egg quality, and nutrient digestibility during the post-peak laying phase.

MATERIALS AND METHODS

The birds used in this study were part of an ongoing study with similar experiments carried out in the breeding and rearing phase, , making it possible to redistribute the supplemented and non-supplemented animals and adjust the treatments for this experiment in the rearing and laying phases.

Experimental Site and Ethics Committee

The experiment was conducted at the Laboratory for Research with Birds of the Department of Animal Science at the Federal Rural University of Pernambuco, and it was approved by the local Animal Use Ethics Committee through process Number 060/2019.

Animals, trial designs, and experimental treatment

For the execution of the study, 198 birds of the Dekalb White® breed, aged 70 to 90 weeks, were distributed in a completely randomized design with 6 treatments and 6 replications, 3 of which containing 5 birds, and 3 with 6 birds (totaling 33 birds per treatment). Treatments consisted of two base diets, the first consisting of a corn and soybean meal without additives, called reference diet one – (RF), provided from the starter phase; the second, similar to the first, but with the inclusion of meat and bone meal, called reference diet two - (MBM), also provided from the starter phase; and two more diets, one with the same feed composition as reference diet II (containing MBM), but with the addition of 0.05% of the Zinc Bacitracin additive - (ZnBac), and the other with the addition of 0.1% of the Symbiotic additive - provided to three groups of animals, namely one group that already consumed the symbiotic since the first day of life, called Starter phase (Symb-S); other group of animals that consumed the symbiotic from the grower phase (Symb-G); and a final group of animals that started consuming the symbiotic at the beginning of the experiment, that is, in the laying phase (Symb-L). The Animals received water and feed *ad libitum* throughout the experimental period.

Symbiotic additive

The symbiotic supplement used had the following composition: prebiotics (mannans - 52.00 g/kg; glucans - 28.00 g/kg) and probiotics (*Saccharomyces cerevisiae* - 2.00×10^{11} cfu/kg, *Bifidobacterium bifidum* - 2.00×10^{11} cfu/kg, *Bacillus subtilis* - 2.88×10^{11} cfu/kg; *Enterococcus faecium* - 2.08×10^{11} cfu/kg; and *Lactobacillus acidophilus* - 1.04×10^{11} cfu/kg).

Experimental Diets

The diets were formulated according to the nutritional requirements of the birds, according to the DEKALB Line Guide (Dekalb, 2009) and the Brazilian Tables for Poultry and Swine (Rostagno *et al.* 2017) (Table 1).

Housing

The birds were housed in a masonry shed equipped with 64 metal cages (100 x 40 x 45cm) with four subdivisions, cup-type drinkers, and trough-type feeders. The temperature and relative humidity data were recorded by a thermo-hygrometer, obtaining averages equivalent to 31ºC and 72%, respectively (Figure 1). The lighting program adopted followed the recommendation of the breed manual, which was 12 hours of natural light + 4 hours of artificial light, totaling 16 hours of light.

Performance Variables

Egg weight (g), egg production (%), egg mass (g/bird/day), feed intake (g/bird/day), and feed conversion

Table 1 – Composition of experimental diets.

Ingredients (%)	RF	MBM	ZnBac	Symb-S/Symb-G/Symb-L
Corn 7.86%	60.164	60.118	60.118	60.118
Soybean meal 45%	24.391	22.973	22.973	22.973
Meat and bone meal 43%	------	1.491	1.491	1.491
Soy oil	1.059	1.058	1.058	1.058
Limestone	10.790	10.696	10.696	10.696
Dicalcium phosphate	0.500	-------	-------	------
Common salt	0.279	0.257	0.257	0.257
Sodium bicarbonate	0.150	0.150	0.150	0.150
Vitamin premix[1]	0.150	0.150	0.150	0.150
Mineral Premix[2]	0.050	0.050	0.050	0.050
DL-methionine	0.254	0.261	0.261	0.261
L-Lysine	0.039	0.052	0.052	0.052
Phytase[3]	0.006	0.006	0.006	0.006
Inert	2.170	2.738	2.688	2.638
Zinc Bacitracin	------	------	0.050	------
Symbiotic	------	------	------	0.100
Total	100	100	100	100
Calculated nutritional composition, %				
Metabolizable energy (kcal/kg)	2750	2750	2750	2750
Crude protein	15.989	15.989	15.989	15.989
Linoleic acid	1.869	1.863	1.863	1.863
Phosphorus available	0.370	0.370	0.370	0.370
Calcium	4.500	4.500	4.500	4.500
Sodium	0.207	0.207	0.207	0.207
Chlorine	0.232	0.228	0.228	0.228
Potassium	0.639	0.621	0.621	0.621
Digestible Amino Acids, %				
Methionine + cystine	0.749	0.749	0.749	0.749
Methionine	0.481	0.487	0.487	0.487
Lysine	0.764	0.764	0.764	0.764
Threonine	0.592	0.586	0.586	0.586
Tryptophan	0.200	0.194	0.194	0.194
Arginine	0.980	0.978	0.978	0.978
Leucine	1.311	1.295	1.295	1.295
Histidine	0.393	0.386	0.386	0.386
Phenylalanine	0.713	0.700	0.700	0.700
Phenylalanine + tyrosine	1.271	1.242	1.242	1.242
Glycine + serine	1.272	1.335	1.335	1.335
Valine	0.692	0.684	0.684	0.684

1Vitamin Premix (supplies per kilogram of product): vit. D3, 2,500,000.00 IU; vit. A, 9,000.00 IU; vit.; vit. And, 20,000.00 IU; vit. K3 (Menadione) 2500.00 mg; vit. B1 (Thiamine) 2000.00 mg; B2 (Riboflavin) 6,000.00 mg; B6 (Pyridoxine) 3000.38 mg; B12 Cobalamin) 15,000.00 mg; Niacin (Ac. Nicotinico) 35,000.00 mg; Pantothenic Acid, 12,000,000 mg; Folic Acid, 1,500.00 mg; Selenium, 250.00 mg; Biotin, 100,000 mg. 2Premix Mineral (provides per kilogram of product): Copper, 20,000,000 mg; Iron, 100,000,000 mg; Manganese, 130,000,000 mg; Iodine, 2000.00 mg; Zinc, 130,000,000 mg. 3Phytase: 10,000 FTU/g. RF: Reference feed; MBM: Meat and Bone Meal; ZnBac: Zinc Bacitracin; Symb-S: Symbiotic since the first day of life; Symb-G: Symbiotic in the start phase; Symb-L: Symbiotic in the laying-hen phase.

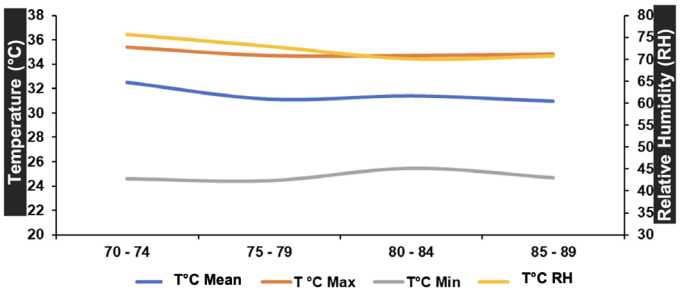

Figure 1 – Mean variations in temperature (T, °C) and relative humidity (RH, %) during the experimental period.

(kg of feed/dozen eggs and kg of feed/kg of eggs) were evaluated in the performance assessment. The eggs were collected twice a day (morning and afternoon), and then were counted and weighed.

Egg production was calculated as the ratio between the number of eggs produced and the number of birds housed. The egg mass was obtained by multiplying the average egg weight by the egg production; the result was then divided by 100 and expressed in grams of eggs per bird/day. The weekly feed intake was

calculated considering the amount of feed provided in the seven-day period, minus leftovers, divided by the number of birds housed per experimental unit. The feed corresponding to each experimental unit was weighed and packed in properly identified plastic buckets. In the case of birds that died during the period, the average intake of the plot was corrected.

To calculate feed conversion (g/bird/day), the average bird intake was divided by the egg mass obtained during the same evaluated period. Feed conversion per kg of feed/dozen eggs was obtained by dividing the average feed intake of the plot by the number of dozens of eggs produced.

Egg quality

On the last three days of each 28-day period, 3 eggs were selected per experimental unit, totaling 108 eggs. They were identified and then taken to the laboratory for evaluation of the egg quality parameters: candling eggs, egg weight (g), color of the yolk, albumen height (mm), albumen weight (g), yolk weight (g), shell weight (g), shell thickness (mm), yolk percentages, albumen, shell, and Haugh Unit score.

A candling scale from 1 to 4 was used for shell quality: 1 – excellent; 2 – good; 3 – thin shell, and 4 – cracked (BRASIL, 1990). To determine the height of the albumen, the eggs were broken, and their contents (white + yolk) placed on a flat and leveled surface. Then, the height of the albumen (mm) was measured by reading the value indicated by a caliper. To calculate the Haugh Unit, the values of egg weight (g) and albumen height (mm) were used, applying the formula HU = 100 x log (h - 1.7 x W0.37 +7.57), described by Card & Nesheim (1966), where W refers to egg weight and h to albumen height. Subsequently, the yolks were separated from the albumen and weighed on a precision scale.

Eggshells were washed to remove all albumen and air-dried for a period of 48 hours for weighing and thickness measurement through a digital micrometer (iGaging, 0.1-0.00005). The albumen weight was obtained as the difference between the weight of the egg and the weight of the shell and yolk. The calculation of the percentage of yolk and shell was performed according to the weight of the yolk and shell in relation to the weight of the egg. The percentage of albumen was determined in relation to the weight of the egg through the difference by the formula 100 - (% yolk + % shell). The color of the yolk by the fan was measured on a scale of values from 1 to 15 (with 1 being the palest yellow and 15 being the most intense orange). The color of the yolk was determined with the aid of a colorimeter (Konica Minolta, model CR-400), which was previously calibrated on a white surface according to pre-established standards, operating under the CIELAB system (L*, a*, b*). L* stands for luminosity, ranging from white (L=100) to black (L=0); a* is the intensity of the red color, ranging from red (+a*) to green (-a*); and b* is the intensity of the yellow color, ranging from yellow (+b*) to blue (-b*).

Nutrient digestibility

In this experiment, the method of partial collection of excreta was used when the birds were 80 weeks old. Three days were used for adaptation to the experimental diets, and then three more days were used for the collection of excreta. An insoluble acid ash source (trade name Celite®), an indigestible indicator, was added (1%) to the experimental feeds in order to measure the digestibility of the nutrients according to the methodology described by Van Keulen & Young (1977).

The dry matter apparent metabolizability coefficient (DMAMC), the crude protein apparent metabolizability coefficient (CPAMC), the gross energy apparent metabolizability coefficient (GEAMC), apparent metabolizable energy (AME), and the apparent nitrogen-corrected balance coefficient (AMEn) were determined for the diets. Dry matter metabolizability (DMAMC) and crude protein (CPAMC) coefficients were calculated by using the formulas:

DMAMC = (DM intake – DM excreted) / DM intake X 100

CPAMC = [(%CP intake - %CP excreted) /%CP intake] x 100.

To determine the AME and AMEn values, the formulas proposed by Matterson *et al.* (1965) were used:

AME Reference feed (RF) = (GE intake – GE excreted) /DM intake

AME Feed = AME RF + (AME test – AME RF) / (g diet/g feed)

Nitrogen Balance (NB) = N intake – N excreted

AMEn Reference feed (RF) = (GE intake – GE excreted ± 8,22 x BN) /DM intake

CMAEB = (AMEn / Gross Energy) x 100

Statistical Analyses

The bird performance and egg quality data were analyzed by using the PROC GLM of the Statistical Analysis System version 9.4 program, and the averages

were compared by the orthogonal contrast method, using the following contrasts of interest: C1: RF vs MBM; C2: MBM vs ZnBac; C3: ZnBac vs Symb-S, C4: ZnBac vs Symb-G.; and C5: ZnBac vs Symb-L.

The statistical model used was the following:

$Yij= \mu+Ti+\varepsilon ij$

In which: Yij = observation, μ = average constant of the common population for all observations, Ti = effect of the diet and εij = random error term.

RESULTS

Performance

There was no significant effect of the treatments (p>0.05) for any of the performance variables studied (Egg weight - g; Egg production - %; egg mass - g/bird/day; Feed Intake - g/bird/day; Feed Conversion - Kg:Kg; Feed Conversion per Dozen Eggs - Kg/dz), as presented in Table 2.

Table 2 – Performance characteristics of birds in the post-peak laying phase (from 70 to 90 weeks of age) fed different experimental diets.

Treatments	Egg weight (g)	Egg production (%)	egg mass (g/bird/day)	FI (g/bird/day)	FC (kg:kg)	FC/Dozen (kg/dz)
RF	59.6	85.7	51.0	95.1	1.863	1.3
MBM	60.1	85.3	51.3	95.1	1.855	1.3
ZnBac	60.3	85.2	51.4	95.3	1.856	1.3
Simb-S	59.3	85.6	50.7	95.0	1.874	1.3
Simb-G	60.5	86.2	52.2	95.1	1.824	1.3
Simb-L	60.3	86.4	52.0	95.1	1.829	1.3
Overall Average	60.0	85.7	51.4	95.1	1.850	1.3
SEM	0.2	0.4	0.3	0.1	0.009	0.1
Effect of Contrasts (*p-value*)						
C1	0.5	0.7	0.8	0.9	0.8	0.8
C2	0.8	0.9	0.9	0.6	0.9	0.8
C3	0.1	0.8	0.5	0.6	0.6	0.6
C4	0.8	0.5	0.4	0.7	0.3	0.4
C5	0.9	0.4	0.5	0.7	0.4	0.3

Standard Error of the Mean (SEM); Feed Intake (FI); Feed Conversion (FC).; Feed Conversion per Dozen Eggs (FC/Dozen); ZnBac: Zinc Bacitracin; C1: RF vs MBM; C2: MBM vs ZnBac; C3: ZnBac vs Symb-S; C4: ZnBac vs Symb-G; C5: ZnBac vs Symb-L; MBM: Meat and Bone Meal; RF: Reference feed; Symb-S: Symbiotic since the first day of life; Symb-G: Symbiotic in the start phase; Symb-L: Symbiotic in the laying phase.

Egg quality

The results found for egg quality are shown in Tables 3 and 4. Regarding the results of the color of the yolks, the birds that consumed the RF diet produced eggs with more intense red (a*) and yellow yolks, as compared with the yolks of the birds that consumed the diet with MBM. The egg yolks of birds that consumed bacitracin and the symbiotic supplement, regardless of the beginning of the use of the latter, showed greater color intensity for the same yolks already mentioned in the results found when using Minolta. Regarding lighting, higher values were obtained for the yolks of the birds that consumed symbiotic supplement since the pullet phase (Symb-G).

For the candling variables, there was a significant effect (p<0.05) for candling in C1, yolk color in C2, yolk weight in C3, and percentage of albumen in C1 (Table 4). For the other parameters there was no significant effect (p>0.05). For candling, the RF treatment was significantly better when compared to the MBM treatment. Yolk color was more intense for

Table 3 – L*, a* and b* values for measuring egg yolk colors obtained from laying hens in the post-peak laying phase.

	Minolta			Colorimetric fan
Treatments	L*	a*	b*	score
RF	54.112	-1.622	31.473	6.418
MBM	54.648	-2.115	29.757	5.495
ZnBac	54.452	-1.708	32.203	6.300
Symb-S	54.772	-1.475	32.310	6.290
Symb-G	55.378	-1.663	32.440	6.233
Symb-L	54.988	-1.685	32.918	6.382
Overall Average	54.725	-1.711	31.850	6.183
SEM	0.138	0.052	0.241	0.062
Effect of Contrasts (*p-value*)				
C1	0.243	0.003*	0.009*	<.0001*
C2	0.666	0.011*	0.001*	<.0001*
C3	0.483	0.132	0.865	0.928
C4	0.049*	0.767	0.706	0.549
C5	0.243	0.878	0.259	0.464

There was statistical difference (p<0.05) for the Orthogonal Contrast test; SEM: Standard Error of the Mean; L the luminosity; a* the intensity of the red color; b* the intensity of the yellow color. ZnBac: Zinc Bacitracin; C1: RF vs MBM; C2: MBM vs ZnBac; C3: ZnBac vs Symb-S; C4: ZnBac vs Symb-G; C5: ZnBac vs Symb-L; MBM: Meat and Bone Meal; RF: Reference feed; Symb-S: Symbiotic since the first day of life; Symb-G: Symbiotic in the start phase; Symb-L: Symbiotic in the laying phase.

Table 4 – Egg quality of laying hens in the post-peak phase of laying fed different experimental diets.

Treatments	Egg weight (g)	Yolk weight (g)	Eggshell weight (g)	Albumen weight (g)	Albumen height (mm)	Candling Eggs (score)	Shell Thickness (mm)	HU	Egg yolk (%)	Albumen (%)	Eggshell (%)
RF	59.781	16.322	5.570	37.398	7.102	2.605	0.394	83.985	27.672	62.553	9.358
MBM	60.063	16.428	5.538	38.098	7.203	2.862	0.388	84.582	27.358	63.425	9.217
ZnBac	60.285	16.493	5.725	38.068	7.073	2.720	0.398	83.748	27.373	63.470	9.498
Symb-S	58.808	16.048	5.577	36.663	6.865	2.628	0.397	82.655	27.352	63.194	9.485
Symb-G	60.357	16.333	5.663	38.358	6.772	2.688	0.392	81.637	27.073	63.060	9.393
Symb-L	59.723	16.250	5.643	37.830	6.455	2.627	0.394	79.910	27.223	63.330	9.447
Overall Average	59.836	16.320	5.621	37.736	6.912	2.688	0.394	82.753	27.342	63.166	9.401
SEM	0.265	0.061	0.036	0.238	0.112	0.032	0.173	0.744	0.124	0.111	0.051
Effect of Contrasts (*p-value*)											
C1	0.766	0.625	0.814	0.398	0.795	0.024*	0.278	0.820	0.487	0.018*	0.460
C2	0.814	0.754	0.154	0.971	0.740	0.198	0.071	0.750	0.973	0.902	0.129
C3	0.125	0.048*	0.254	0.095	0.594	0.401	0.903	0.676	0.964	0.473	0.941
C4	0.939	0.442	0.632	0.725	0.442	0.771	0.331	0.422	0.506	0.289	0.564
C5	0.553	0.245	0.527	0.772	0.121	0.393	0.564	0.149	0.739	0.703	0.776

*There was statistical difference ($p<0.05$) for the Orthogonal Contrast test; SEM: Standard Error of the Mean; HU: Haugh unit score; ZnBac: Zinc Bacitracin; C1: RF vs MBM; C2: MBM vs ZnBac; C3: ZnBac vs Symb-S; C4: ZnBac vs Symb-G; C5: ZnBac vs Symb-L; MBM: Meat and Bone Meal; RF: Reference feed; Symb-S: Symbiotic since the first day of life; Symb-G: Symbiotic in the start phase; Symb-L: Symbiotic in the laying phase.

RF, and lower for MBM in C1. In C2, the yolk color had a higher mean value for the MBM treatment as compared to ZnBac.

The treatment containing zinc bacitracin yielded higher yolk weight as compared to that of the birds that consumed a symbiotic supplemented diet since the start phase, but it did not differ from the eggs produced by birds with additive supplemented since the pullet and laying hen phases. For the percentage of albumen, birds fed with diets containing MBM had a higher value in comparison to those fed with RF.

Nutrient digestibility

The values of apparent metabolizable energy (AME), corrected for nitrogen balance (AMEn), and the apparent dry matter, crude protein, and crude energy metabolizability coefficients of the diets, are shown in Table 5.

The AME and AMEn values found for the RF and MBM diets did not show significant differences. On the other hand, when zinc bacitracin was added, the values were higher when compared to the same diet without zinc bacitracin (AME, p=0.102 and AMEn, p=0.085); in relation to the use of the symbiotic supplement in the diets, regardless of the inclusion phase, the apparent metabolizable energy metabolizability coefficient (AMEMC) was higher for the diets that had MBM in the diet than the diet based on corn and soybean meal (RF). The GEAMC was higher for birds that consumed symbiotics, and higher when it was included from the pullet and laying hen phases.

The diet with MBM provided better CPAMC values than the diet with only corn and soybean meal. The addition of Bacitracin provided a lower CPAMC value when compared to the diet without additives (P=0.028) and with the diet with bacitracin since the start phase (p=0.099). The DMAMC results were better for diets with MBM (p<0.0001), and with the addition of bacitracin the value was significantly lower (p=0.006). However, with the addition of symbiotics beginning from the chick, pullet and laying-hen phases, higher results were obtained (p=0.010; p=0.019 and p=0.095, respectively).

DISCUSSION

Studies have shown that the use of probiotic strains in poultry diets has improved productive performance (Wang *et al.,* 2020). Mikulski *et al. (*2020) reported that the use of probiotics increased the laying rate and feed efficiency by approximately 2.8%. In this study,

Table 5 – Apparent Metabolizable Energy Values (AME), Apparent Corrected for Nitrogen balance (AMEn), Gross Energy Apparent Metabolizability Coefficients (GEAMC), Crude Protein Apparent Metabolizability Coefficient (CPAMC), and Dry Matter Apparent Metabolizability Coefficient (DMAMC) of diets for laying hens in the post-peak phase, based on dry matter.

Treatments	AME (kcal/kg)	AMEn (kcal/kg)	GEAMC (%)	CPAMC (%)	DMAMC (%)
RF	3.206	3.177	85.883	67.079	78.937
MBM	3.185	3.153	87.629	70.391	81.934
ZnBac	3.220	3.190	86.619	68.674	80.699
Symb-S	3.203	3.172	87.498	69.958	81.922
Symb-G	3.225	3.192	88.083	69.502	81.754
Symb-L	3.204	3.171	87.519	69.004	81.444
Overall Average	3.207	3.176	87.221	69.101	81.188
SEM	12.822	12.599	0.348	0.532	0.321
			Effect of Contrasts (*p*-value)		
C1	0.307	0.239	0.003*	<.0001*	<.0001*
C2	0.102	0.085	0.085	0.028*	0.006*
C3	0.419	0.389	0.132	0.099	0.010*
C4	0.837	0.929	0.015*	0.284	0.019*
C5	0.441	0.371	0.123	0.668	0.095

*There was statistical difference ($p<0,05$) for the Orthogonal Contrast test; SEM: Standard Error of the Mean; ZnBac: Zinc Bacitracin; C1: RF vs MBM; C2: MBM vs ZnBac; C3: ZnBac vs Symb-S; C4: ZnBac vs Symb-G; C5: ZnBac vs Symb-L; MBM: Meat and Bone Meal; RF: Reference feed; Symb-S: Symbiotic since the first day of life; Symb-G: Symbiotic in the start phase; Symb-L: Symbiotic in the laying phase.

symbiotic diets provided better nutrient metabolization results, which resulted in more pigmented yolks and thicker eggshells. This corroborates the studies by Ray *et al.* (2022), who reported that using feed with the addition of probiotics resulted in higher productivity.

The current study suggests that using the symbiotic supplement since the start phase can promote a better metabolization of nutrients, especially crude proteins, in the end of the laying-hen phase, given the higher CPAMC results for the diets of birds that consumed symbiotics since chicks versus those that received zinc bacitracin.

Although no significant differences were noted regarding low feed conversions for birds that consumed symbiotic supplement, it is usually observed that, at this phase, a drop in egg production is often accompanied by a drop in feed intake, which is difficult to control even under experimental conditions. In this study, due to the higher metabolizable energy values, it was observed that it would be possible to reduce the feed supply for the birds that consumed the symbiotic supplement, which could further reduce the feed conversion rates of these birds.

To some extent, the use of prebiotics may stimulate the immune response and reduce the effect of stress in laying hens (Tang *et al.,* 2017). This would improve the productive performance of the birds and their health status, since prebiotics attract cells and other immune components to the intestinal tract, increasing the barrier against antigens in the mucosa (Sheoran *et al.*, 2018). However, in this study, no performance improvements were observed. A positive effect was only found in some egg quality variables, which are presented in Table 4.

The present study corroborates the one carried out by Najafabadi *et al.* (2017) with 70-week laying hens using prebiotics, where no significant effect (P>0.05) was found for the variables of egg weight, egg production, egg mass, and feed intake. This result may be related to the age of the hens, as with advanced age the physiological conditions of the digestive tract are developed, and the morphological and gastrointestinal microbial conditions become stable, with no alteration.

It is possible to say that prebiotics can be effective under certain conditions, such as enteric diseases (Murate *et al.,* 2015), and heat stress (Cheng *et al.*, 2019), which can occur in the poultry industry. Different responses to these additives may occur because of age, diet, intestinal microflora, types of prebiotic diets, or other environmental conditions (Hajati & Rezaie, 2010; Patterson & Burkholder, 2003).

According to Bozkurt *et al.* (2012), the production performance of laying hens was not affected by the addition of Mannan Oligosaccharides (MOS), or by the addition of essential oils to the diet. However, Chen *et al.* (2005) found that commercial prebiotics improved the performance of laying hens.

According to Güçlü (2011), probiotics and prebiotics additives to quail diets improved egg production and eggshell thickness, and positively affected hatchability in quail farming. Mostafa *et al.* (2015) found a significant effect on the performance of the chicks

supplemented with Mannan Oligosaccharides (MOS), depending on the ways that it was included in their diets in the initial phase. Body weight, body weight gain, feed intake, feed conversion, mortality, and percentage of carcass yield were unaffected by dietary inclusion of prebiotics, probiotics and symbiotics when compared to un-supplemented control diets in broilers (Sarangi *et al.,* 2016).

There was an effect of the reference diet and the diet containing zinc bacitracin on the yolk color variable. Studies demonstrate that higher concentrations of pigmenting agents (mainly carotenoids) in the ingredients of diets cause increases in yolk color intensity (Sjofjan *et al.,* 2020). Thus, we could say that the diets that caused these effects did so for being richer in carotenoids, which is the case of the RF diet (that contained a greater amount of corn) in comparison with the MBM diet. On the other hand, other additives that balance the gastrointestinal microbiota can enhance the absorption of these pigmenting agents.

According to Garcia *et al.* (2002), pigmentation results from the deposition of xanthophylls in the egg yolk. Sources of carotenoid pigments can be natural, such as those from the corn group and others, ranging from yellow to red, or they can be artificial. Since there was increased nutrient absorption with the use of additives, it is possible to relate them to the effect of pigmentation in the yolk.

A study carried out by Ribeiro *et al.* (2010) using antibiotics, mannan oligosaccharides, and organic acids - associated with MOS in diets for commercial laying hens at the stage of 32 to 52 weeks of age - concluded that there was no significant effect on yolk color. Likewise, Maia *et al.* (2002) did not find a significant effect on yolk color with the inclusion of *Saccharomyces cerevisiae* in diets of commercial laying hens at 30 weeks of age, thus supporting the result found in the present study.

However, Pamplona (2020), when studying the effect of probiotic additives in the diet of commercial laying hens between 67 and 70 weeks of age, obtained a significant effect on yolk color. Yet, from 55 to 58 weeks of age, no significant difference was found for yolk color.

In the present study, an effect was found in the RF and MBM treatments for the percentage of albumen, with no effect in the other treatments containing the antibiotic and the symbiotic.

Thus, we corroborate the work of Lemos *et al.* (2014), who reported that the percentage of albumen and yolk indices in quail eggs were not influenced by the incorporation of different feed additives.

According to Bertechini (2006), performance-enhancing additives provide better results in challenging sanitary conditions. In this study, there was a low microbial challenge. Thus, the reduction of these challenges may have been responsible for the results obtained, making the improvement caused by the inclusion of additives imperceptible.

In the present study, there was no significant effect for shell thickness and albumen weight in the MBM treatment. A study by Shahir *et al.* (2014) demonstrated that there were no significant effects on the quality of the eggs of birds that consumed diets supplemented with commercial prebiotics, corroborating the present research.

However, Mohan *et al.* (1995), and Nahashon *et al.* (1994) report a small improvement in shell thickness. Shell thickness increased significantly, probably due to high nutrient absorption, Ca deposition, and reduction of the gastrointestinal tract caused by prebiotics, which could have an effect on the eggshell (Swiatkiewicz *et al.,* 2010; Sharifi *et al.,* 2011; Najafabadi *et al.,* 2017).

Furthermore, some of the microbial species, such as *Lactobacillus sporogenes*, have been shown to increase the absorption and concentration of Ca in the blood, thus improving eggshell thickness (Panda *et al.,* 2008). Zarei *et al.* (2011) report that feed additives had beneficial effects on egg quality characteristics, namely eggshell weight and shell thickness. Yet, Bozkurt *et al.* (2012) indicate that egg quality, except for shell thickness, was significantly affected by diet additives.

Meng *et al.* (2010) showed that oligosaccharide supplementation in diets for laying hens improved DM and CP digestibility. Furthermore, Sonmez & Eren (1999) stated that weight gain and feed efficiency from prebiotic supplement products are, in part, due to nutrient utilization in the gastrointestinal tract. Good digestibility by MOS supplementation can be attributed to improvements in morphological indices of the intestinal epithelium, as indicated by Baurhoo *et al.* (2007), who reported that dietary supplementation of MOS increased villus height and the number of goblet cells in the jejunal epithelium.

For the variables apparent metabolizable energy (AME) and apparent nitrogen-corrected metabolizable energy (AMEn), there was no significant effect. This corroborated the work of Lima *et al.* (2011) who conducted a study with laying hens submitted to food restriction and observed that energy metabolism had a

linear effect on AME, demonstrating that there was no significance in AMEn.

The present study obtained results regarding crude protein and dry matter similar to those found by Li *et al.* (2016) when they studied the supplementation of Xylo oligosaccharides (XOS) in laying hen diets. They observed that there were no significant differences in the apparent digestibility of crude proteins, dry matter, phosphorus, and energy. However, XOS supplementation can significantly increase apparent calcium digestibility, making it very important, especially for laying poultry. According to the same authors, to explain the differences in these results one should explore the influence of XOS on the digestibility of laying hens, mainly in cases of low nutrition.

CONCLUSION

The use of the symbiotic additive for laying hens in the post-peak laying phase achieved the purpose of replacing the zinc bacitracin antibiotic. When included from the start phase, it is possible to obtain better results for the DMAMC. In the pullet phase, it is possible to obtain even better results for GEAMC, and for yolk luminosity.

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