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Animal Health

Original

Evaluation of the Symbiotic Effect of PROBIOLEV® in Heavy Purebred Birds at Maturity

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ABSTRACT

Background: The zootechnical additives can substitute therapies with antibiotics and offer an effective alternative to producing healthy feeds sustainably. **Aim**. To evaluate the effect of the inclusion of a symbiotic additive (PROBIOLEV®) in the diet of birds, based on glucan oligosaccharides, mannans, and *Bacillus subtilis* E44. **Materials and Methods:** The experiment relied on a completely randomized design with two treatments: T-1 (basal diet and control) and T-2 (basal diet plus PROBIOLEV®). The production indicators, such as the percentage of birds included in hatching, the total number of eggs per bird, and per week, feed consumption and feed conversion, and health indicators like mortality and viability, were evaluated as well. **Results:** Compared to the control, the birds that received the symbiotic bio preparation in the diet produced more eggs (1516 and 176) throughout the experimental period. The inclusion of the additive was observed to improve all the indicators evaluated and caused benefits (P<0.05) in the percentage of birds in production, total eggs per bird (23.7 and 27.6), and egg conversion per feed consumed (6.3 and 5.4). Moreover, the application of the additive reduced mortality (29.7% and 12.5%) and increased bird viability (70 and 87%, respectively). **Conclusions**: The results of this study confirm the symbiotic potential of PROBIOLEV® in the improvement of production and health indicators of heavy purebred birds. This is a natural additive, which can be an interesting biotechnological choice for animal production in Cuba.

Keywords: feed additives, bird rearing, eggs (*Source*: *MeSH)*

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INTRODUCTION

The inclusion of probiotic, prebiotic, and symbiotic additives has spread out in the world's poultry production. These natural biotherapeutic agents are beneficial to health, with active biological properties and defined preventive and therapeutic capacities, which are a useful alternative to excessive antibiotic use to promote animal growth (El Jeni *et al.*, 2021; Melara *et al*., 2022).

The application of these additives maintains a beneficial microbiome for animal health. They are used to balance the intestinal environment and increase the population of beneficial bacteria that produce lactic acid and favor eubiosis. They also enhance metabolic and digestive processes, as well as the modulation of the immune system. These effects permit production increases, and therefore milk, meat, and egg availability and quality for the people (Milián *et al*., 2019; Iñiguez *et al*., 2021).

For several years, in Cuba, multidisciplinary groups have worked on the introduction of these biopreparations in animal production, not only due to the effects on animal yields and health but also due to the utilization of an economically viable technology for the Cuban conditions. In that sense, the Center for Biotechnological Studies (CEBIO), at the University of Matanzas (UM), obtained and patented a method for the manufacture of the symbiotic additive PROBIOLEV® (Pérez *et al*., 2006).

The evaluation of the symbiotic potential of this natural additive (PROBIOLEV®) was begun in broiler chicken and showed improvements in the response of some microbiological, fermentation, morphometric, hematological, immunological, and productive indicators (Pérez, 2000 and Pérez *et al.*, 2005). Although advantageous, it is not manufactured or applied at a large scale. However, stimulating Cuban agriculture is one of the most important ways to animal protein consumption.

Despite the fact that Cuban aviculture is not engaged in massive poultry meat production, there is a national poultry genetic project that stands out among several countries, including the industrial regions. This company looks to pure line selection and breeding processes, as well as breeding animals to ensure the gene bank and replacement of all basic birds in the nation. Hence, the aim of this paper was to evaluate the symbiotic effect of PROBIOLEV® in heavy purebred birds during Maturity.

MATERIALS AND METHODS

Preparation of symbiotic additive PROBIOLEV®: A volume of 25 L of PROBIOLEV® was obtained from distilled alcohol cream (*Saccharomyces cerevisiae*) at 20% dry matter (DM) and enzymatic crude of *Bacillus subtilis* E44, according to the method described by Pérez *et al.* (2006). The bio preparation was then stained to check for bacillus endospores and yeast cells and to measure the pH and the quality of the preparation. Then it was bottled in sterile plastic glasses with a screw cap (5 L) and was stored at room temperature until it was used.

Experimental conditions and treatments: The experiment was done at the Sierra Maestra Poultry Farm, in Pedro Betancourt municipality, in Matanzas province. The *in vivo* evaluation of the symbiotic bio preparation was performed for 90 days between May and July 2021. In that period, the mean temperature was 32 ºC ±; the max temperature was 35 ºC ± 1, whereas the minimum temperature value was 30 ºC ± 3. The average relative humidity was 72% ± 3.

The experiment was based on a completely randomized design with two treatments: T-1 (basal diet or control) and T-2 (basal diet plus PROBIOLEV®), using a 75 mL dose per kg of the concentrate. The population consisted of 128 mature heavy purebred female birds B4 (162--252 day-old), distributed in 64 birds per treatment.

Bird feeding: The feed consumed was supplied as a meal, once a day, early in the morning. The diet composition (breeding feed) is shown in table 1. The animals were given water (*ad libitum*), treated with 0.1% calcium hypochlorite, and administered through automatic nipples; the feed was served in linear troughs. The feedstuff was supplied at 163 g/bird a day, according to the standard set by UCAN -IIA (1998).

Table 1. Nutrient composition and proportion in the diet of heavy breeding animals

Nutrients	Measure unit	Proportion	
		Minimum	Maximum
Crude protein	%	15.5	18.8
Metabolizable energy	MJ/kg	11.30	11.70
		2700	2800
Fiber	%	2	8
Fat	%	2	6
Linoleic acid	%	1.0	1.0
Calcium	%	1.2	1.5
Available phosphorus	%	0.45	0.90
Di-calcium phosphate	%		1.90
Thiamine	%	0.74	0.75
DL-methionine	%	0.37	0.5
Methionine + cysteine	%	0.65	0.63

PROBIOLEV® (symbiotic additive) was supplied daily (a single frequency) in the T2 diet. It was applied directly in the troughs above the feed supplied. The dose of the additive (75 mL.Kg of the feed) was set according to the criteria suggested by Pérez (2000).

Bird handling: Animal handling was the same for the two animal groups in terms of feeding, lighting, technician, and environmental conditions. It was based on the Technical Manual Nº 7 UCAN -IIA (1998) for this species and category under evaluation. The birds were placed in 48 X 24 cm cages with, and 24 cm of front troughs, one bird per cage. Each cage was labeled according to the bird's numbering. It consists of a code beneath the wing, which is given to every bird to control production and breeding at that stage.

The adult birds were exposed to 16 hours of light a day, alternating natural and artificial light. The birds were artificially inseminated. The semen from the male was collected quickly, without trauma to the animal, maintaining quality and effectiveness. The insemination is performed using a plastic rod to introduce approximately 0.25 ml of semen per female. The time between the collection and insemination was not longer than 20 minutes. A male was used every 10-15 females, and the inoculation was repeated between four and five days later.

Determination of the production and health indicators: To determine the *in vivo* effect of the bio preparation on the production indicators, the following was checked weekly: eggs per bird, average egg production per treatment, number of breeding birds, feed consumption, and egg conversion per feed kg consumed. Meanwhile, mortality and viability were observed throughout the experimental period. These indicators were calculated as described in the Technical Manual for broiler chicken breeding (UCAN- IIA, 1998).

Statistical analysis The evaluation of laying bird percentage and mortality/viability comparison relied on the binomial comparison proportion. The comparison of weekly egg production per bird was made with the mean comparison test (Student-t) Both tests were run on STATGRAPHICS Plus, 5.0 (Statgraphics, 2002).

RESULTS

Table 2 shows the percentage of breeding birds by treatment. There were differences during the first four weeks. In that case, the highest number (P<0.05) of females that started laying corresponded with the group that received the symbiotic bio preparation in the diet. No significant differences were found between the groups in the other weeks (T-1 and T-2). Besides, from the fifth week on, 100% of the birds were laying eggs. It was achieved at the same time in the two groups (six weeks), with all the animals under production. However, PROBIOLEV® increased egg production in the birds treated, compared to the control group.

Table 2. Percentage of laying birds by week

Weeks	Number of birds laying eggs		P-Value
	T-1	T-2	
1	14.06	32.81	0.0015
2	28.12	51.56	0.0055
3	68.75	92.19	0.0010
4	84.37	95.31	0.0423
5	96.87	98.43	0.7171
6	100	100	1.0
7	100	100	1.0
8	100	100	1.0
9	100	100	1.0
10	100	100	1.0
11	100	100	1.0

Table 3 shows the laying performance depending on bird incorporation.

Table 3. Effect of PROBIOLEV® on weekly egg production by bird

Weeks	Average of eggs laid		P-Value
	T-1	T-2	
5	2.74	2.75	0.472
6	2.80	2.84	0.054
7	3.03	3.48	0.011
8	2.56	3.14	0. 002.
9	3.04	3.81	0.001
10	3.08	3.89	0.002
11	3.25	3.85	0.002

Egg laying by group underwent differences ($P<0.05$), with the highest number found in the treated group (Table 3). Throughout the experimental period (eleven weeks), the overall production was 1516 and 1767 eggs (control T-1, and treatment T-2, respectively). Besides, upon egg count by bird, the best results were observed in the group treated with the symbiotic additive (27.6), and the conventionally-treated (23.7), or control group.

Another production indicator in the experimental period was feed consumption, with no differences between the groups. This behavior was caused by the fact that the adult birds have a proportional diet (163 g a day), and eat all the feed supplied. However, an analysis of egg conversion by feed consumed showed that the favorable results corresponded to the group under PROBIOLEV®. In this case, the feed conversion was better (5.4) by comparing to the control group (6.3), which only ate the conventional feed.

The feed conversion efficiency, whether in muscular mass or egg production has a significant impact on the intensive animal breeding industry, as feedstuffs account for most of the production costs (Stanley and Hughes, 2012). In that sense, the scientific community claims that when zootechnical additives are included in the feed, there are improvements in digestion, absorption, and conversion of the nutrients in the diet. It was also corroborated that when the animals have a high percentage of beneficial microorganisms, conversion decreases, with favorable results (Dowarah *et al*., 2018; Rondón *et al.*, 2020).

The composition of PROBIOLEV® consists of glycan and mannan oligosaccharides: which are animal growth promoter prebiotics (Jahanian and Ashnagar 2015; Lourenço *et al*., 2016), and viable cells of *B. subtilis* and its endospores, a probiotic microorganism that favors higher levels of beneficial intestinal biota, and activates immune response (Díaz *et al*., 2017 y Milián *et al.*, 2019). This symbiotic bio preparation was supplied stably since the first day of age. It permitted the presence of healthy components in the bird's gastrointestinal tract (GIT).

Jeong and Kim (2014) said that the biopreparations made from cultures of *Bacillus* spp. and its endospores are well accepted. This bacterium produces hydrolytic enzymes that enhance feed digestion and absorption, and increase the population of lactic acid bacteria in the GIT, with an ensuing production of AGCC, lactic acid, and stimulation of the immune response. Hence, these biopreparations lead to greater efficiency in feed conservation, uniformity of body weight, and production yields of the animals that consume them.

Several experiences have shown that the inclusion of *B. subtilis.* strains in the diet of birds plays a significant role in the reduction and prevention of pathogenic intestinal colonizing, resulting from the inhibitory activity of antibiotics and bacteriocins produced by this strain. Moreover, the supply of de *Bacillus* spp. and its endospores, offers a better microbial balance (eubiosis) in the GIT, with a greater absorption range, and it creates favorable conditions for the development of beneficial microbial species, such as *Lactobacillus* spp. (Pérez *et al*., 2012; Rodríguez *et al.*, 2016; Adhikari *et al*., 2019).

Research done by Corrigan *et al*. (2015) demonstrated that prebiotic substances (mannans and glucans) manage to fix the lectin receptors of bacteria, blocking pathogens from nesting in the digestive tract. This event takes place through a process of competitive exclusion which means there is a recognition and increase of the beneficial population, and therefore, a reduction of harmful microorganisms. Once bacterial fixation is stopped, mannans form a sort of armor, drag the bacteria to the back tract, and are expelled from the body through the feces. This effect is observed from the early stages of the animals that receive the treatment.

The inclusion of this bio preparation (PROBIOLEV®) in the diet changed the intestinal ecology of birds, which was related to improvements in the percentage of egg laying by week, eggs per bird, and egg conversion per feed kilogram consumed, compared to the animals that did not receive the microbial additive. In this case, the eggs were used for breeding. Accordingly, in this study, 1 064 and 1 189 fertile eggs were collected from the treatment and control groups, respectively. This result showed the positive effect of the additive used in this animal species and category.

For years, small-scale efforts have been made to evaluate the beneficial effects of zootechnical additives for breeding these particular birds. Suárez (2013) and Milián *et al*. (2021) conducted experiments to evaluate the probiotic effect of a *Lactobacillus salivarius* strain (PROBIOLACTIL®), and *Bacillus subtilis* strain E44 (SUBTILPROBIO®), in purebred heavy lines P8 and E1, respectively. The birds that ingested the probiotic bacteria showed better results than the control animals. These results coincided with the findings of the current research, though in purebred heavy lines B4.

Rodríguez *et al*. (2019) evaluated the antibacterial effect of PROBIOLEV® in male Ross 300 chickens exposed to *Salmonella enterica* FVE1284, and demonstrated that the application of this symbiotic additive lowered the infection caused by pathogenic bacteria ($p<0.05$), and increased the beneficial bacterium population ($2.47x10^{11}$) (*Lactobacillus* spp.). Furthermore, it generated a favorable physiological response by improving the fermentative patterns in the cecum (pH 6.6), and stimulated the immune state through bigger lymphoid organs (the bag of Fabricio and spleen), and the concentration of immunoglobulin M ($1.12\ g.L^{-1}$) in the birds' blood plasm.

These results can be explained by the fact that upon administration, probiotics and prebiotics induce several mechanisms in the gastrointestinal tract (GIT) that favor the balance of intestinal microorganisms, thus producing a better response to the digestive processes in the host.

Table 4 shows the proportions of mortality and viability. In both indicators, T-1 differed from T-2. The birds that consumed the bio preparation underwent a decline in mortality, and higher viability than the control group. The group of birds that did not consume the additive, showed lower viability and failed to reach the percentage required (85%) for the line's standard, used as a comparison criterion. One of the marked effects of zootechnical additives is to have a positive effect on the intestinal microbiota, toward a reduction in the presence of harmful germs, while the presence of beneficial bacteria rose (Li *et al*., 2016; Medina *et al*., 2017). Hence, viability was favorable, thus reducing the number of deaths.

Table 4. Health indicators at 252 days of age

Health indicators	Proportions		P-Value
	T 1	T 2	
Mortality	0.30[b]	0.13[a]	0.019
Viability	0.70[b]	0.88[a]	0.012

These results coincided a great deal with the findings of the current study and were reported by Rodríguez *et al.* (2016) upon evaluation of the effect of a probiotic mix containing *Lactobacillus salivarius* C65 and *Bacillus subtilis* E44 in the same species. The authors observed that its inclusion in the diet of birds showed improvements in mortality and viability. In that case, the studies led to greater income in terms of purebred heavy line (B4) breeding at starting.

The outcome at maturity in this bird line is proportional to the utilization of the zootechnical additive PROBIOLEV® since the beginning of breeding. The inclusion of this bioproduct in the diet had a positive effect on animal health and productivity. Rodríguez *et al*. (2016) said that the administration of probiotic microorganisms in the early stages of birds leads to a modification of the intestinal ecology, which contributes to greater nutrient absorption capacity and better yield responses, which matched the findings of the current study.

CONCLUSIONS

The results of this study confirm the symbiotic potential of PROBIOLEV® in the improvement of production and health indicators of heavy purebred birds. This natural additive is an interesting biotechnological choice for animal production in Cuba.

RECOMMENDATIONS

The authors suggest further study of the inclusion of zootechnical additives in bird nutrition, to contribute to animal production in Cuba.

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REFERENCES

Adhikari, B., Hernandez-Patlan, D., Solis-Cruz, B., Kwon, Y. M., Arreguin, M. A., Latorre, J. D., & Tellez-Isaias, G. (2019). Evaluation of the antimicrobial and anti-inflammatory properties of Bacillus-DFM (Norum™) in broiler chickens infected with Salmonella Enteritidis. *Frontiers in veterinary science, 6,* 282. https://www.frontiersin.org/articles/10.3389/fvets.2019.00282/full

CAN-IIA. (1998). Instructivo técnico de ponedoras y sus reemplazos. Ministerio de la Agricultura. Unión Combinado Avícola Nacional. Instituto de Investigaciones Avícolas. La Habana, Cuba.

Castillo, Y., & Miranda, I. (2014). COMPAPROP: Sistema para comparación de proporciones múltiples. *Rev. Protección Veg.*, *29*(3), 231-234. ISSN 1010-2752.

Corrigan, A., de Leeuw, M., Penaud-Frézet, S., Dimova, D., & Murphy, R. A. (2015). Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. *Applied and environmental microbiology, 81*(10), 3460-3470. https://europepmc.org/articles/pmc4407213/bin/aem.04194-14_zam999116247so1.pdf

Díaz-López, E. A., Ángel-Isaza, J., & Ángel, D. (2017). Probióticos en la avicultura: una revisión. *Revista de Medicina Veterinaria,* (35), 175-189. https://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-93542017000300175

Dowarah, R., Verma, A. K., Agarwal, N., Singh, P., & Singh, B. R. (2018). Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. *PloS one*, *13*(3), e0192978. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192978

El Jeni, R., Dittoe, D. K., Olson, E. G., Lourenco, J., Corcionivoschi, N., Ricke, S. C., & Callaway, T. R. (2021). Probiotics and potential applications for alternative poultry production systems. *Poultry Science, 100*(7), 101156. https://www.sciencedirect.com/science/article/pii/S0032579121001905

Iñiguez Heredia, F. A., Espinoza Bustamante, X. E., & Galarza Molina, E. L. (2021). Use of probiotics and organic acids as stimulants of the developpment of broilers. Alfa *Revista de Investigación en Ciencias Agronómicas y Veterinaria, 5*(14), 166-172.

www.scielo.org.bo/scielo.php?pid=S2664090220210002001668&script=sci_abstract&tlng=en

Jahanian, R., & Ashnagar, M. (2015). Effect of dietary supplementation of mannan-oligosaccharides on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in Escherichia coli-challenged laying hens. *Poultry science*, *94*(9), 2165-2172. https://www.sciencedirect.com/science/article/pii/S0032579119322540

Jeong, J. S., & Kim, I. H. (2014). "Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers." *Poultry science*. *93*, 3097-3103. DOI: 10.3382/ps.2014-04086

Li, Y., Xu, Q., Huang, Z., Lv, L., Liu, X., Yin, C., & Yuan, J. (2016). Effect of Bacillus subtilis CGMCC 1.1086 on the growth performance and intestinal microbiota of broilers. *Journal of applied microbiology*, *120*(1), 195-204. https://sfamjournals.onlinelibrary.wiley.com/doi/abs/10.1111/jam.12972

Lourenço, M. C., De Souza, A. M., Hayashi, R. M., Da Silva, A. B., & Santin, E. (2016). Immune response of broiler chickens supplemented with prebiotic from Sacharomyces cerevisiae challenged with Salmonella enteritidis or Minnesota. *Journal of Applied Poultry Research*, *25*(2), 165-172. https://www.sciencedirect.com/science/article/pii/S105661711930220X

Medina-Saavedra, T., Arroyo-Figueroa, G., Herrera-Méndez, C., & Mexicano-Santoyo, L. (2017). *Bacillus subtilis* as a probiotic in poultry farming: relevant aspects in recent research. *Abanico Veterinario*, *7*(3), 14-20. https://www.medigraphic.com/cgi-bin/new/resumenI.cgi?IDARTICULO=73330

Melara, E. G., Avellaneda, M. C., Valdivié, M., García-Hernández, Y., Aroche, R., & Martínez, Y. (2022). Probiotics: Symbiotic Relationship with the Animal Host. *Animals*, *12*, 719. https://www.mdpi.com/2076-2615/12/6/719/pdf?version=1647078156

Milián, G., Rodríguez, M., Días, D., Rondón, A. J., Pérez, M. L., Boucourt, R., & Beruvides, A. (2019). Evaluación del aditivo zootécnico SUBTILPROBIO® C-31 en la alimentación de gallinas ponedoras en una unidad de producción comercial. *Cuban Journal of Agricultural Science*, *53*(2), 161-168. http://scielo.sld.cu/scielo.php?pid=S2079-34802019000200161&script=sci_arttex-t&tlng=pt

Milián, G., Rodríguez, M., González, O., Rondón, A. J., Pérez, M. L., Beruvides, A., & Placeres, I. (2021). Evaluación del aditivo zootécnico SUBTILPROBIO® E-44 en indicadores productivos y de salud en aves líneas puras pesadas en condiciones de producción. *Cuban Journal of Agricultural Science*, *55*(1), 67-75. http://scielo.sld.cu/scielo.php?pid=S2079-34802021000100007&script=sci_arttext&tlng=pt

Pérez, M. (2000). Obtención de un hidrolizado de crema de levadura de destilería y evaluación de su actividad probiótica. TPhD Thesis. Instituto de Ciencia Animal, San José de las lajas, Mayabeque, Cuba.

Pérez, M., Laurencio, M., Milián, G., Rondón, A. J., Arteaga, F., Rodríguez, M., & Borges, Y. (2012). Evaluación de una mezcla probiótica en la alimentación de gallinas ponedoras en una unidad de producción comercial. *Pastos y Forrajes*, *35*(3), 311-320. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942012000300007

Pérez, M., Milián, G., Piad, R., González, R., Bocourt, R., & Savón, L. (2006). Hidrolizado de fondaje de cubetas de destilerías de alcohol con un crudo enzimático de la cepa de Bacillus licheniformis E-44 y su procedimiento de obtención. Patente No. 23179, (Int. cl. 8) A-23-J1/00, 3/30. Int. cl, 8.

Pérez, M., Piad, R., Bocourt, R., Milián, G., Medina-Medina, E., Savon, L., & Laurencio, M. (2005). Actividad prebiótica y probiótica de un hidrolizado enzimático de crema de destilería en pollos de ceba prebiotic and probiotic activity of an enzymatic hydrolysed of alcohol distillery cream in broilers actividade prebiótica e probiótica dun hidrolizado enzimático de crema de destilería en polos de ceba. *CYTA-Journal of Food*, *5*(1), 42-47. https://www.tandfonline.com/doi/abs/10.1080/11358120509487670

Rodríguez, M., Florido, G. M., Castillo, A. J. R., Rodríguez, A. B., & Chávez, F. A. (2019). Artículo original Actividad antibacteriana del aditivo simbiótico PROBIOLEV® en pollos de ceba infectados con Salmonella enterica. Revista de la Sociedad Venezolana de Microbiología, 39, 34-40.

Rodríguez, M., Milián, G., Boucourt, R., Beruvidez, A., & Crespo, E. (2016). Evaluation of a probiotic mixture in the started birds feeding of heavy pure breeds B4 in a production unit. *Cuban Journal of Agricultural Science*, *49*(4). http://cjascience.com/index.php/CJAS/article/view/493

Rondón, A., González, J., Rodríguez, M., Milián, G., Martínez, M., Beruvides, A., & Vera, R. (2020). In vitro metabolic activity of Lactobacillus salivarius and its effect on productive and health indicators of lactating calves. *Cuban Journal of Agricultural Science*, *54*(2). http://www.cjascience.com/index.php/CJAS/article/view/957

Stanley, D., & Hughes, G. (2012). El anuncio. *Microbiología y Biotecnología Aplicada*, *96*, 1361-1369.

Statgraphics. (2002). Statgraphics Plus version 5.1. Statgraphic Technical Support Center. Manugistics, Inc., Rockville, Maryland, USA.

Suárez, M. I. (2013). Evaluación a escala de producción del efecto probiótico del bio-preparado PROBIOLACTIL en pollos de inicio de reproductor pesado. Eng. Thesis. Universidad de Matanzas. Cuba.

AUTHOR CONTRIBUTION STATEMENT

Research conception and design: MRO, DGC, NRR, AJRC, GMF, ABR; data analysis and interpretation: MRO, DGC, NRR, AJRC, GMF, ABR; redaction of the manuscript: MRO, DGC, NRR, AJRC, GMF, ABR.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.