Scientific Paper

Effect of zootechnical additives on productive and health indicators in broilers

Grethel Milián-Florido[1], Ana Julia Rondón-Castillo[1], Manuel Pérez-Quintana[2], Fátima Graciela Arteaga-Chávez[3], Ramón Boucourt-Salabarría[4], Yadileiny Portilla-Tundidor[5], Marlen Rodríguez-Oliva[1], Yoenier Pérez-Fernández[1] and Marta Elena Laurencio-Silva[1]

[1] *Universidad de Matanzas, Ministerio de Educación Superior Autopista a Varadero km 3½, Matanzas, Cuba*
[2] *Universidad Estatal Amazónica, Pastaza, Ecuador*
[3] *Escuela Superior Politécnica Agropecuaria de Manabí, Ecuador*
[4] *Instituto de Ciencia Animal, Mayabeque, Cuba*
[5] *Universidad Autónoma de España, Madrid, España*
E-mail: grethel.milian@umcc.cu

Abstract

In order to evaluate the effect of three zootechnical additives of *Bacillus subtilis* on productive and health indicators in broilers, a study was conducted in the poultry farm of the Institute of Animal Science –Mayabeque, Cuba–. For such purpose 200 broilers of the Cuban breeding hybrid EB_{34} were used, in a completely randomized design. Four treatments were studied: T1: control group (CG), T2: C-31, T3: C-34 and T4: E-44, at a dose of 10^9 endospores per gram of concentrate feed. The trial lasted 42 days, and hematological, immunological, productive and health indicators were evaluated. The use of these zootechnical additives did not cause any effect at 21 days on any of the indicators; however, at 35 and 42 days the weight of the bursa of Fabricius, the spleen and the hemagglutination titers to Newcastle vaccine increased ($p < 0,001$). There were improvements ($p < 0,001$) in the hematological indicators hemoglobin, hematocrit, globulins, albumins and total proteins at 35 and 42 days of age; as well as an increase of live weight at 35 ($p < 0,01$) and 42 days ($p < 0,001$), lower intake at 42 days ($p < 0,05$), better conversion at 35 ($p < 0,001$) and 42 days ($p < 0,05$), and higher feed efficiency at 35 and 42 days ($p < 0,001$). The indicators mortality and viability did not show differences among the treatments. It is concluded that the addition of these zootechnical additives improved the productive and health responses in broilers.

Keywords: *Bacillus subtilis*, intake, mortality, probiotics

Introduction

The addition of antibiotics to the concentrate feed during long periods is a habitual practice in the poultry industry in recent years. However, there is concern among poultry farmers, concentrate feed manufacturers, consumers and state regulating agencies about the current systems of egg and meat production, with the use of growth promoters of antibiotic origin in concentrate feeds (Linares, 2015; Sarangi *et al*., 2016).

Thus, alternatives are needed to growth promoters which are compatible with food security and with the consumer (Pérez *et al*., 2015; Zhang *et al*., 2016; Liu *et al*., 2016). Many natural products, including the zootechnical additives based on sporulated cultures of *Bacillus* spp., are proposed by different enterprises of the poultry sector to be used as viable alternatives (Nguyen *et al*., 2015; Hou *et al.,* 2015).

From such elements, the objective of this study was to evaluate the effect of three zootechnical additives on productive and health indicators in broilers.

Materials and Methods

The trial was conducted in the poultry farm of the Institute of Animal Science –Mayabeque, Cuba–. Two hundred broilers of the female line of the Cuban breeding bird EB_{34}, 1-42 days old, were used, distributed in a completely randomized design in which four treatments (additives) were included: T1: control group (CG), T2: C-31, T3: C-34 and T4: E-44.

The dose of zootechnical additives from cultures of the strains C-31, C-34 and E-44 of *Bacillus subtilis* subspecies subtilis (Milián *et al*., 2014), supplied to the animals, was 10^9 endospores per gram of concentrate feed; it was adjusted according to the proposal made by Milián (2009).

Feed and water were supplied *ad libitum*. The diet varied in its composition for the starter, growth and finishing stages, according to NRC (1994), as shown in table 1.

To determine *in vivo* the probiotic effect of the three zootechnical additives ten animals per treatment were selected and slaughtered (jugulation) in

Table 1. Composition of the starter, growth and finishing diets, supplied to the animals.

Raw materials (%)	Starter (0-14 days)	Growth (15-28 days)	Finishing (29-42 days)
Corn meal	42,43	54,32	60,27
Soybean meal	43,88	33,68	28,58
Sunflower oil	8,80	7,28	6,52
Dicalcium phosphate	2,57	2,45	2,39
Calcium carbonate	0,74	0,72	0,71
Common salt	0,25	0,25	0,25
DL-methionine	0,33	0,30	0,29
Pre-mixture vitamins-minerals*	1,00	1,00	1,00
Indicator			
Metabolizable energy (MJ/kg)	13,38	13,38	13,38
Crude protein (%)	23,00	20,00	18,80
Calcium (%)	0,95	0,95	0,95
Assimilable phosphorus (%)	0,42	0,42	0,42
Methionine + cystine (%)	0,92	0,87	0,82

*One kilogram of feedstuff contains: vitamin supplement: vitamin A (10 000 UI), Vitamin D_3 (2 000 UI), vitamin E (10 mg), vitamin K_3 (2 mg), thiamin (1 mg)-B_1, riboflavin (5 mg)-B_2, pyridoxine (2 mg)-B_6, vitamin B_{12} (15,4 mg), nicotinic acid (125 mg), calcium pantothenate (10 mg), folic acid (0,25 mg) and biotin (0,02 mg), and mineral supplement: selenium (0,1 mg), iron (40 mg), copper (12 mg), zinc (120 mg), magnesium (100 mg), iodine (2,5 mg) and cobalt (0,75 mg).

each sampling, which was conducted at 21, 35 and 42 days. The selection was made taking into consideration the birds that had a live weight in the range of ± 10 % of the real average weight of each group of animals. The procedure to evaluate this biological activity in each sampling is described below:

Immunological indicators. The organs spleen and bursa of Fabricius were weighed on a Sartorius BL 1500 digital scale, to determine the relative weight (RW). The RW was calculated in each sample as: RW = (SW/LW) x 100 %; where SW is the sample (organ) weight and LW is the live weight of the animal.

In the case of the bursa of Fabricius the criterion expressed by Giambrone (1996) was applied, which classifies poultry according to the following ranges:

- At values of CG = [2, 4], the birds are normal.
- At values of CG ≤ 1, the birds are immunodepressed.

The titers for the Newcastle vaccine (HI) were determined by the beta method for microtitration with the commercial antigen La Sota (Sánchez, 1990).

Hematological indicators. To take the blood samples the collection method by draining off of the jugular vein, described by Sánchez (1990), was used. Ten samples per treatment were taken for the determinations of hemoglobin (Hg), hematocrit (Ht), globulins, albumins and total proteins, according to the methodology described by Lynch *et al.* (1969).

Besides these health indicators, the viability and mortality were evaluated according to the description in the Technical handbook for broilers (UCAN, 1998).

Productive indicators. The indicators live weight, relative carcass weight, feed intake, conversion and efficiency were evaluated according to the Technical handbook for broilers (UCAN, 1998).

Statistical processing of the *in vivo* evaluation. For the statistical analysis of the results a variance analysis was applied, using the statistical software INFOSTAT version 2012 (Di Rienzo *et al*., 2012). The difference among the means was determined through Duncan's (1955) multiple range comparison test. The mortality and viability values were transformed into.

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Results

Table 2 shows the performance of the immune response, measured through the immunological indicators at 21, 35 and 42 days, in broilers treated with three *B. subtilis* cultures.

Table 2. Immune response at 21, 35 and 42 days, in broilers treated with three *B. subtilis* cultures.

Indicator	Time (days)	Treatment				SE ±	Sign.
		T1: CG	T2: C-31	T3: C-34	T4: E-44		
Relative weight of the bursa of Fabricius	21	2,46	2,59	2,63	2,73	0,20	NS
	35	1,61^{a}	2,51^{b}	2,51^{b}	2,51^{b}	0,1	***
	42	1,56^{a}	2,71^{b}	2,72^{b}	2,53^{b}	0,20	***
Relative weight of the spleen	21	0,12	0,13	0,13	0,14	0,01	NS
	35	0,11^{a}	0,18^{b}	0,17^{b}	0,15^{b}	0,01	***
	42	0,09^{b}	0,24^{b}	0,23^{b}	0,23^{b}	0,01	***
Hemagglutination titers Newcastle vaccine	21	20,6	22,6	22,7	21,8	0,55	NS
	35	16,8^{a}	22,6^{b}	22,8^{b}	21,8^{b}	0,74	NS
	42	15,4^{a}	38,6^{b}	40,0^{b}	39,8^{b}	0,58	****

a, b: different letters in the same row significantly differ for p <0,05 (Duncan, 1955). *** p < 0,001.

At 21 days no significant differences were found in the immunological and hematological indicators among the birds treated with the *B. subtilis* cultures, or between them and the controls; for which it can be inferred that such cultures and their endospores did not influence the immune system at this age.

At 35 and 42 days it was observed that the bursa of Fabricius, the spleen and the hemagglutination titers for the vaccine response, measured through the Newcastle vaccine, were higher (p < 0,001) in the treatments with *B. subtilis*.

Table 3 shows the Hg and Ht levels at 21, 35 and 42 days; and the levels of globulins, albumins and total proteins at 35 and 42 days. At 21 days, for the indicators Hg and Ht no difference was observed among the groups under study, for which it can be stated that still at this level there were no significant values of erythrocytes to show a positive effect on the animals. Nevertheless, it was observed that at 35 and 42 days all the evaluated indicators were higher in the treatments where the additives were used (p < 0,001).

Table 4 shows the performance of the productive indicators. A response was observed in the birds from 35 and 42 days, with the exception of the carcass which was evaluated only at 42 days, without differences among the treated groups.

The results of mortality and viability during the rearing cycle of the broilers are shown in table 5; no differences were found among the treatments.

Table 3. Hematological indicators at 21, 35 and 42 days, in broilers treated with three *B. subtilis* cultures.

Indicator	Time (days)	Treatment				SE ±	Sign.
		T1: CG	T2: C-31	T3: C-34	T4: E-44		
Hemoglobin (g.dL^{-1})	21	7,38	8,10	7,81	8,21	0,2600	NS
	35	7,71^{a}	9,03^{b}	9,00^{b}	8,94^{b}	0,1329	***
	42	7,65^{a}	9,20^{b}	9,13^{b}	9,51^{b}	0,1688	***
Hematocrit (%)	21	0,25	0,27	0,26	0,28	0,0084	NS
	35	0,26^{a}	0,30^{b}	0,30^{b}	0,30^{b}	0,0045	***
	42	0,26^{a}	0,31^{b}	0,30^{b}	0,32^{b}	0,0055	***
Globulin (g.dL^{-1})	35	1,70^{a}	2,58^{b}	2,52^{b}	2,42^{b}	0.0935	***
	42	2,15^{a}	3,39^{c}	3,22^{cb}	3,13^{b}	0,0815	***
Albumin (g.dL^{-1})	35	1,56^{a}	2,89^{b}	2,24^{b}	2,40^{b}	0,0867	***
	42	2,32^{a}	2,39^{b}	2,80^{b}	2,71^{b}	0,0815	***
Total proteins (g.dL^{-1})	35	4,71^{a}	5,10^{b}	5,16^{b}	5,12^{b}	0,0893	***
	42	4,74^{a}	5,56^{b}	5,52^{b}	5,45^{b}	0,0871	***

a, b: different letters in the same row differ significantly for p <0,05 (Duncan, 1955.) *** p < 0,001.

Table 4. Productive indicators in broilers fed *ad libitum* with three zootechnical additives of *B. subtilis*.

Indicator	Period (days)	Treatment				SE±	Sign.
		T1: CG	T2:C-31	T3:C-34	T4:E-44		
Live weight (g)	21	521,9	540,5	548,9	544,1	21,4	NS
	35	1 122,6[a]	1 193,3[b]	1 247,4[b]	1 236,5[b]	22,3	**
	42	1 451,9[a]	1 685,9[b]	1 640,1[b]	1 678,5[b]	21,5	***
Intake (g)	21	921,7	954,68	955,9	956,2	12,3	NS
	35	2 370,1	2210,8	2 264,95	2 241,0	47,7	NS
	42	3 433,6[b]	3 340,0[a]	3 316,9[a]	3 372,4[ab]	22,2	*
Conversion	21	1,77	1,77	1,76	1,76	0,05	NS
	35	2,11 [b]	1,85 [a]	1,82 [a]	1,81 [a]	0,03	***
	42	2,37 [b]	1,98 [a]	2,03 [a]	2,01[a]	0,03	*
Weight increase (g)	21	483,9	502,5	510,9	506,1	21,4	NS
	35	1 084,6[a]	1 155,3[b]	1 209,4[b]	1 198,5[b]	22,3	**
	42	1 413,91[a]	1 647,85[b]	1 602,15[b]	1 640,51[b]	21,5	*
Efficiency (%)	21	56,7	56,6	57,3	56,9	1,7	NS
	35	47,4 [a]	54,0 [b]	55,07 [b]	55,2 [b]	0,7	***
	42	42,3 [a]	50,5 [b]	49,5 [b]	49,8 [b]	0,7	***
Carcass yield (%)	42	60,48	58,67	56,18	58,18	1,49	

[a,b] Different letters among treatments differ for $p < 0,05$ (Duncan, 1955). ** $p < 0,01$ *** $p < 0,001$ *$p < 0,05$.

Table 5. Health indicators during the rearing cycle (%).

Indicator	Treatment				SE ±
	T1: CG	T2: C-31	T3: C-34	T4: E-44	
Viability	71,1 (88)	78,5 (95)	73,9 (91)	77,9 (95)	0,10
Mortality	18,33 (12,0)	10,31 (4,0)	16,04 (8,75)	10,87 (4,75)	0,08

The values between parentheses correspond to the original means.

Discussion

The three zootechnical additives showed a marked effect on the studied birds. The response on the immunological system and the hematological response coincide with the report by Ayala *et al*. (2015), when evaluating different microbial strains with probiotic effect; they can act as antigens and trigger certain immunity reaction, which is translated into a higher response of the immunological indicators in different animal categories.

Other studies conducted by Milián *et al*. (2013) and García *et al*. (2014) proved that probiotics can act as oral adjuvants, produce higher resistance to enteric infections, provide increased and sustained immune response against infectious organisms, accelerate the development and maturation of the immune system, increase the diversification of lymphocytes, and decrease the catabolic consequences of the infections that cause immunosuppression (Ayala *et al*., 2014). Such results coincide with the ones obtained in this study, because when evaluating the effect of the three zootechnical additives based on *B. subtilis* and their endospores on the activation of some of the above-mentioned mechanisms, there was an improvement of the immunological indicator in the studied birds.

For the indicator bursa of Fabricius, the results are within the normal indexes established by Giambrone (1996). According to this criterion, the studied broilers were not depressed; on the contrary, the increase of the immune system function of the birds was favored and there was a better immunostimulant response. However, in similar studies conducted by García *et al*. (2014) in fattening broilers, with a strain of *Wickerhamomyces anomalus* LV-6, no difference was found between the treatment and the control group for the indicator bursa of Fabricius, unlike for the spleen.

The HI titers reached showed the immunostimulant effect of these zootechnical additives. Hemagglutinin is part of the viral glycoprotein involved in the absorption of the Newcastle virus in target cells, for which the hemagglutination-inhibiting antibodies limit infection by this virus. It was observed that in the broilers there was a high relation between the titer of these antibodies and the protection of the immune system, for which it is considered a viable indicator that relates the efficacy of the vaccine with general immunity. Analogous results were found by Molnár *et al*. (2011), when evaluating this indicator in 255 broilers fed with a *B. subtilis* culture.

On the other hand, García *et al*. (2014) evaluated the probiotic effect of a *W anomalus* strain on some physiological and productive indicators in broilers. With the inclusion of this culture in the diet, they observed a good health status of the animals and improvement in the vaccine response ($p < 0,05$).

The Hg and Ht levels are given by the action of the *B. subtilis* cultures, as well as by the levels of folic acid, iron and other nutrients contributed by these additives. Ocampo *et al*. (2012) stated that the high hematocrit levels are directly related to an improvement in the nutritional quality of the diet.

The hemoglobin values coincided with the ones reported for birds, which are between 8 and 12 g.dL^{-1}, according to Edbahuer *et al*. (1990) and Gómez (1992). These authors found lower quantities of hemoglobin and hematocrit in the animals of the group treated with yeast as probiotic; yet, both were within the normal ranges (7,0-18,6 g.dL^{-1} of Hg and 23-55 % of Ht) for broilers, according to Jiménez *et al*. (1998). The HI titers for the Newcastle vaccine increased when the strain LV-6 was added, with regards to the control group. Such results indicate an improvement of the vaccine response of the animals and, thus, a stimulation of the immune response; which coincides with the report by Pedroso *et al*. (2012) with regards to the immunoregulatory action probiotics can exert.

Globulins are synthesized in the reticulum-endothelial system, which allows to infer that the results are closely linked to the immunospecific response from the action of albumins and globulins as part of the total proteins. For birds (males) the albumin values are reported to vary between 1,6 and 2,0 g dL^{-1} and the globulin values, from 1,8 to 3,0 g dL^{-1} (Salgado-Tránsito *et al.,* 2011). In this work both indicators were found in the above mentioned levels. The values of total proteins are related to the indicators of normal proteinemia (3,0-6,0 g dL^{-1}) of the birds (Díaz *et al.,* 2014).

The results found from the inclusion of the three zootechnical additives in the indicators live weight, intake, conversion, weight increase and efficiency showed a multifactorial probiotic response; and an expression of the favorable physiological status was observed in the birds which propitiated higher digestibility, nutrient absorption and bioavailability of nutritional compounds in blood. It triggered a favorable process related to an improvement of productive and health indicators.

The response in the indicator live weight was directly related to the inclusion of the three zootechnical additives, which allowed the treated birds to make a more efficient use of the nutrients contributed in the consumed feedstuff, obtaining higher live weight with a similar feed intake. Studies conducted by Milián *et al*. (2013), Ayala *et al*. (2012) and Iser del Toro (2016) proved that when zootechnical (probiotics, prebiotics, nutraceuticals, organic acids, enzymes and others) are used, there is a positive relation between the incorporation of small doses in the diets and the increase of live weight.

Hou *et al*. (2015) used a probiotic based on *Lactobacillus reuteri* in pigs and found positive responses in the live weight, as well as in the health indicators, due to the decrease in the incidence of diarrhea. Nevertheless, not in all cases the results of the study showed the authenticity of the use of probiotics, hence they were not seen sometimes as viable alternatives in animal husbandry.

The increase of live weight at 21 days responded to the instability of the intestinal microbiota in the initial stage, which is subject to large alterations during the first weeks of life in birds. This result coincides with the one reported by Salvador (2012), when evaluating the effect of a probiotic based on *Lactobacillus acidophilus*, *Pediococcus acidilacticii* and inactivated *Saccharomyces cerevisiae* in broilers, which had higher body weight gain (1 982,5 *vs*. 1 806 g) when compared with the control groups after 35 days of experiment.

Similar result was obtained by Aguavil (2012) when evaluating the effect of a native probiotic based on *L. acidophilus* and *B. subtilis*, which influenced positively the weight gain (2 710 g *vs*. 2 586,7 g) and feed conversion (1,78 *vs*. 1,92), and decreased the mortality rate (2,69 *vs*. 5,5 %) with regards to the control. As collateral result the absence of gastrointestinal parasites was reported, which contributed to improve the health status, because the broilers were free from *Escherichia coli, Eimeria* spp. and *Salmonella* spp.

Ortiz (2013), when adding the probiotic ECOBIOL *(Bacillus amyloliquefaciens* CECT 5940) in 480 broilers (Arbor Acres Plus), obtained better yield, because the final weight (2 575 g) was achieved in 2,5 days less than in the control group; this author stated that probiotics can improve conversion (1,98 *vs*. 2,06) and efficiency index (259 *vs*. 242), as well as decrease mortality rate (6,28 *vs*. 6,77 %).

In this work there were no differences in the mortality rate between the treatments and the control group, result that is associated with the conditions under which the experiment was conducted. The work was done with healthy broilers, which contributed to the fact that no infectious outbreak occurred in the experimentation sheds, throughout the stage. In addition, the established biosafety and comfort measures, according to the "Technical handbook" (UCAN, 1998), were followed; likewise, the vaccination system, correct management of the birds and concentrate feed quality were correctly applied.

Vélez-Zea (2013), in a study with replacement pullets in the first 42 days of age, observed that in the group to which probiotic based on *Lactobacillus* spp. was supplied the reduction of mortality in 2,1 % occurred, compared with the control.

On the other hand, Lei (2015) evaluated the effect of diet supplementation of *Bacillus amyloliquefaciens* based on direct microbial feeding, in 281 male broilers (Arbor Acres), during 42 days. Higher results were found in the productive indicators: weight gain (2 079 *vs.* 1 985 g), feed intake (3 771 *vs*. 3 692) and feed conversion (1,81 *vs*. 1,86) with regards to the control, which was translated into a decrease of mortality. Similar results were obtained by Boaro (2015), when evaluating probiotics in 720 male broilers of the Cobb breed.

Similarly, when evaluating the addition of a homemade microbial culture in the feeding diet of broilers, Gamboa (2014) and Gonzalez (2016) found a better weight gain (2 902,1 *vs*. 2 564 g), higher feed conversion (1,86 *vs*. 2,13) and decrease of mortality (3,3 *vs*. 8,3 %).

It is concluded that the zootechnical additives C-31, C-34 and E-44 of *B. subtilis* and their endospores show real possibilities for their use in animal husbandry in Cuba, because they constitute new zootechnical additives with probiotic activity, which improve the immunological, physiological, productive and health responses of broilers.

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