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Effect of the probiotic additive *Bacillus subtilis* and their endospores on milk production and immune response of lactating sows

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Forty eight Yorkshire-Landrace x L35 were used with a range between three and five farrowings for determining the effect of the probiotic additive of *Bacillus subtilis* and their endospores on milk production and immune response of lactating sows. Experimental treatments were: control without additive; three weeks of probiotic consumption (G-3), consumption for four weeks (G-4), before farrowing and during the 33 d of lactation. Indicators evaluated maintained in the normal parameters, according to the species and animal category. Total protein (g/L-1) (69.63, 77.56 and 74.86) differed between the control without additive and the G-3, while for immunoglobulin concentration (Igs G) (g/L-1) there was a significant increase between treated animals regarding the control (2.46, 2.84 and 2.89). In a similar way performed milk production at 7, 21 and 28 d. Results suggest the possibility of supplying the additive without worsening the productive performance, with positive influence on the sow's health.

Key words: *Bacillus subtilis, sows, health, milk production*

The management of lactating sows and their litters is an activity of great risk and high economic cost, due to the nutrient demands of suckling sows. This is of vital importance for not limiting milk production and with that litter growth and the sow's performance (Neil and Williams 2011).

During the first days and weeks the efficient consumption of colostrum and milk, respectively, is the determining factor for piglet survival in which also influences the type of epitheliochorionic placenta of the sow breeder that do not allow the transference of antibodies (Bérèterbide *et al.* 2006). Also, the amount and composition of the sow's milk supply the piglets the nutrients and protection they need for responding to the adversities during this period. A rapid growth is also guaranteed for them.

Considering these conditions, the objective of this study was to determine the performance of the milk production and health of lactating sows consuming a probiotic additive of *Bacillus subtilis* and their endospores.

Materials and Methods

The trial was developed in the pig experimental unit of the Institute of Animal Science (ICA), located in the province of Mayabeque, Cuba. Forty eight Yorkshire-Landrace x L35 sows were used. Animals had between three and five farrowings. For the analysis of data, a random block design was applied with three treatments consisting of a control, without additive, and two groups consuming the probiotic three (G-3) and four weeks before farrowing (G-4) and during the 33 d of lactation. Blocks were the weeks where animals and treatments were placed.

Animals received water ad libitum and the feed based on maize and soybean (table 1). Average feed consumption was 6 kg. Feed was supplied in two daily rations (8:00 a.m. and 3:00 p.m.). It was prepared weekly from the requirements of the category, according to NRC (1998). The feeding technology described in the Pig Rearing Handbook of the Institute of Swine Research (IIP) (2008) was followed.

Table 1. Composition in dry basis of the diet consumed by lactating sows

Ingredients	%
Maize meal	76.30
Soybean meal	20.00
Common salt	0.50
Calcium carbonate	1.00
Dicalcium phosphate	1.60
Vitamin-mineral premix	0.54
Choline	0.14

The probiotic additive was obtained from the Center of Biotechnological Studies of the University of Matanzas "Camilo Cienfuegos", according to the methodology described by Milián (2009). Its active principle was the strain C-34 of *Bacillus subtilis*. The additive was manually and homogenously mixed in the ration every week at a rate of 1 Lt^{-1} of feed, equivalent to 109 endospores/g^{-1} of concentrate.

Milk production in 10 sows per treatment was determined at 7, 14, 21 and 28 d, according to the technique of litter weighing, described by Salmon-Legagneur (1956). For evaluating health indicators, blood was extracted to 24 sows (8 sows/treatment) seven days after farrowing and the hematological indicators (hemoglobin and hematocrit) were measured. In addition, serum was obtained for establishing the concentration of total proteins and the quantification of the immunoglobulin (IgG) through automatic or programmable photometric equipment (COBAS INTEGRA 400 PLUS. Results from milk production and of immunological indicators measured were processed with the computing InfoStat system (Balzarini *et al.* 2012). For the differences between means, Duncan's (1955) multiple range test was used.

Results and Discussion

Table 2 shows the performance of the blood and immunological indicators determined. The parameters were maintained in the normal ranges for the species and animal category (Clark and Coofer 2008). However, there was an increase in the values of the indicators of total proteins and in the concentration of G immunoglobulins in the group of treated sows. This performance could be related to the strengthening of the immune system and with the good health of the sows by the probiotic action allowing greater immunity for passing on to the piglets.

The increase found of IgG was positive if taken into account the type of placenta shown by the sow (Kolb 1974), with the transplacental limitation of the antibodies. Moreover, according to Bérèterbide *et al.* (2006) after 24 h of the piglet birth, the production and concentration of immunoglobulins in the colostrums decreases rapidly and the intestinal wall became impermeable to the antibodies. Thus, the attainment of more IgG is important. Milián *et al.* (2013) reported on the capacity of sporulated Bacillus cultures in the production of IgG. Likewise, it is indicated that these additives are stimulants of the synthesis of T lymphocytes and cytoquines (Rajput *et al.* 2013). Lee *et al.* (2012) reported significant increases in the IgG concentration when assessing the effect of B. subtilis on poultry.

The above mentioned has led to greater concentration of proteins and gammaglobulins in the milk (Salmon *et al.* 2009). Although in this study the composition of this fluid was not determined it can be inferred that is due to a lower cellular rechanging, propitiated by a good intestinal health and higher production of enzymes which brings about a more efficient absorption process. This favors, thus, the increase of available nutrients that improve some biological functions by the additive action.

The weight of the sows at farrowing and at weaning as well as the weaning-estrus interval did not varied between the experimental groups as set out in table 3. Weight loss during lactation differed for the control group and the G-4 corresponding to sows consuming the probiotic four weeks before farrowing. However, all values performed according to the Pig Rearing Handbook (IIP 2008) and to NRC (1998) for the category of multiparous lactating breeders.

Previous results coincide with the study realized by Georgoulakis *et al.* (2004 who observed lower weight loss on adding *B. toyoi* in the diet of lactating sows. Thus, productive and reproductive advantages are evidenced on including probiotic additives in the feeding of sows, which could be related to the gestational anabolism. This process allows the sow to maintain energy, protein, vitamins and minerals for using them during lactation (Salmon-Legagneur and Rerat 1962).

In table 4 is shown the milk production of sows at 7, 14, 21 and 28 d of lactation. There was increase in the groups consuming the additive in the last gestation period and during lactation, except at 14 d, time in which production did not vary regarding the control. This increase could be associated to the adequate use of the nutrients of the diet, due to better digestibility bringing about benefits for intestinal health. All this is related to the probiotic activity and its effect on the most important physiological process executed by the breeder during the lactation period.

In literature consulted there was no information on the use of probiotic strains on the amount of milk produced. However, Barros *et al.* (2011) reported an increment in the protein concentration in the sow's milk at 21 d of lactation, when Bacillus spp. was mixed with a probiotic based on mannanoligosaccharides. Thus,

Table 2. Protein profile of the biochemical indicators of lactating sows treated with the probiotic additive

Indicators	Control without Additive	*Bacillus subtilis* additive		SE (±) Signif.
		G-3	G-4	
Hemoglobin, g L^{-1}	11.00	11.47	10.95	0.22 P = 0.0671
Hematocrit, %	36.27	36.50	36.37	0.01 P = 0.0861
Total proteins, g L^{-1}	69.63[a]	77.56[b]	74.86[ab]	1.79 P = 0.0166
Albumen, g L^{-1}	34.78	37.43	35.56	2.55 P = 0.7557
Albumen/globulin rel.	1.10	1.14	1.23	0.20 P = 0.9064
IgG, g L^{-1}	2.46[a]	2.84[b]	2.89[b]	0.11 P = 0.0378

Normal parameters (Clark and Coofer 2008): Hb 10-16 gL-1, Hto 30-45 %
abMeans with different letters in each row differ at P < 0.05 (Duncan 1955)

Table 3. Productive performance of lactating sows consuming Bacillus subtillis during the last third of gestation (G-3 and G-4) and lactation

Indicators	Control without Additive	*Bacillus subtilis* additive		SE (±) Signif.
		G-3	G-4	
LW at farrowing, kg	176.87	175.39	176.82	1.27 P=0.9650
LW at weaning, kg	163.05	162.82	165.69	2.53 P=0.5040
LW loss in lactation, kg	13.82[b]	12.57[ab]	11.13[a]	0.81 P=0.0477
Weaning-estrus interval, d	8.58	8.83	9.52	0.25 P=0.1220

ab Means with different letters in each row differ at P < 0.05 (Duncan 1955)

Table 4. Performance of milk production of sows consuming the additive during the last third of gestation (G-3 and G-4) and lactation

Milk production, kg.d^{-1}	Control without Additive	*Bacillus subtilis* additive		SE (±) Signif.
		G-3	G-4	
At 7 d	5.22[a]	5.68[b]	5.64[b]	0.08 P=0.0007
At 14 d	6.28[a]	6.51[b]	6.57[b]	0.10 P=0.1219*
At 21 d	8.03[a]	8.44[b]	8.62[b]	0.09 P=0.0003
At 28 d	7.26[a]	7.86[b]	7.73[b]	0.08 P=0.0001

ab Means with different letters in each row differ at P < 0.05 (Duncan 1955)

the way of action of these additives on this effect is not known with exactitude. Studies considering this subject matter are required.

Mean daily milk productions increased as lactation stage advanced, with values of approximately 8 kg at 21 d, time coinciding with the peak of maximum production, according to the literature. Later, a reduction of milk secretion was observed at 28 d. Lodge (1972) indicated the need of attaining higher milk production during the first three weeks of life of the litters, which coincides with the phase of little solid feed consumption. In addition, during the course of the lactation days, the amount and quality of the sow's milk become insufficient. For that reason the importance of the concentrate to cover the nutritional requirements of the pigs (Martínez 2011).

It is concluded that the inclusion of the *Bacillus subtilis* additive in the diet of lactating sows is beneficial for milk production during the first three weeks of lactation and increases the concentration of immunoglobulins G. This is reflected in a better immunological response that determines good health for the sows.

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