Assessment of a probiotic based on *Bacillus subtilis* and its endospores in the obtainment of healthy lungs of pigs

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In order to assess the effect of a probiotic based on *Bacillus subtilis* and its endospores on the obtainment of healthy lungs of pigs, 76 pre-fattening animals of 21 kg of average weight were slaughtered according to completely randomized design. Three groups were formed considering the descendent of the sows consuming the probiotic in the final gestation stage (21 and 30 d before the farrow) (1), during lactation (2) and post-weaning stage (3), at a rate of 109 endospores/g of concentrate. The hemoglobin, hematocrit and relative weight of the thymus and spleen were determined to 24 animals. The hematological indicators of hemoglobin and hematocrit were within the normal range for the category and animal species. The relative weight of the thymus (0.08 vs. 0.09 vs. 0.08) only differed ($P < 0.05$) in group 2. However, the weight of the spleen (0.18 vs1.50 vs. 1.62) was superior in the animals consuming the probiotic. The percentage of obtainment of healthy lungs was superior ($P < 0.01$) in the pigs receiving the product. The lungs, in the groups 2 and 3, increased in 35 and 33 percent units, respectively, in respect to the control. It is concluded that the consumption of the probiotic by the pregnant sow and the descendents favors the obtainment of healthy lungs. Extending these results to a higher number of pigs for the production of healthy lungs is recommended.

Key words: *pigs, healthy lungs, probiotic, Bacillus subtilis, endospores.*

It is known that, due to its compatibility with humankind, several parts of the swine organism can be used in human medicine (Roppa 2008). Surfacen is produced from healthy lungs of pigs. This medicine is necessary for treating the respiratory distress syndrome in newborns (RDSN) and other affections such as severe adult respiratory distress syndrome (SARDS), meconium aspirations, asthma, lung transplant, pneumonias, among others (Moreno *et al*. 1999)

In swine rearing, the respiratory affections are considered to be one of the main causes of death (Bulnes *et al*. 1986). They affect all countries and climates with intensive swine production, with prevalence values that may reach 100 % (Ciprian 2003 y Marco 2005). Therefore, optimizing the management and feeding of this species is necessary to reduce the losses due to this cause.

Prebiotics are living microorganisms that, in proper amounts in the diet, improve the intestinal microbial balance and, in general, the animal health (González and González-Martínez 2006). It is known that the genus *Bacillus spp.*. and its endospores have marked probiotic effects on stimulating the immunologic system, improving the digestive physiology, among other aspects (Milián *et al*. 2008).

The objective of this study was to assess the effect of a probiotic based on *Bacillus subtilis* and its endospores on the obtainment of healthy lungs.

Materials and Methods

The study was conducted in the swine integral unit of the Institute of Animal Science (ICA). A total of 76 pre-fattening animals of the commercial crossing Yorkshire-Landrace x L35 and both sexes were slaughtered according to a completely randomized design. They came from sows consuming the probiotic in the final stage of gestation and during the whole lactation.

The groups formed were: 1 control, 2 probiotic supply 21 d before farrow, 3 probiotic supply 30 d before farrow.

After weaning, at 33 d of age, the animals were allocated in collective pens, at a rate of 5 pigs/pen, and 8 replications each. The animals consumed the product for five weeks, when reaching the slaughtering weight at 68 d of age, with average of 21 kg. They were slaughtered two hours after consuming 600 g of feed.

The probiotic was ellaborated at the Univerisity of Matanzas "Camilo Cienfuegos", from strains of *Bacillus subtilis* and its endospores (Milián 2009). The product was manually mixed, homogeneously, at a rate of 109 endospores/g of concentrate (mL/kg of concentrate). A conventional diet (tables 1 and 2), covering the requirements of the category, was used according to NRC (1998) and to that indicated in the Manual of Technical Procedures for Swine Rearing (IIP 2008).

The indicators percentage (%) of healthy lungs and the main loss causes (%) were analyzed. The weight of the thymus and spleen, expressed in g kg^{-1} (weight relative to the live weight), was determined to 24 pigs (eight/treatment). Blood sampling was also obtained to determine the hemoglobin (Hb) through the cianometahemoglobin method and hematocrit (Hto), according to Wintrobe (1979).

The results of the relative weights of thymus and spleen were processed according to the statistical software Infostat 2001 (Balzarini *et al*. 2001). An analysis of proportion comparison was conducted for the data expressed in per cent (Font 2007) and Duncan's test (1955) was applied for $P < 0.05$ (Steel *et al*. 1997).

Table 1. Diet composition for pregnant and lactating sows fed with *Bacillus subtilis* and its endospores

Ingredients	Pregnant sows	Lactating sows
Maize, %	81.56	74.22
Soybean, %	15.00	22.0
Common salt, %	0.50	0.50
Calcium carbonate, %	1.20	1.00
Dicalcic phosphate, %	1.20	1.60
Premixture of vitamins and minerals, %	0.54	0.54
Colina		0.14
Total	100.00	100.00

Table 2. Bromatological analysis of the pre-fattenign pigs'diets fed with *Bacillus subtilis* and their endospores

	Bromatological analysis (%)
DM	89.55
CP	23.43
Ash	4.82
Calcium	1.55
Phosphate	1.00
Calcium/phosphate	1.55

Results and Discussion

Table 3 shows the results of the hematic frame. These values agree with those reported as normal for the species and animal category, according to Sotolongo *et al*. (2006) and Gélvez (2009).

It was proved that, generally, this probiotic did not alter or produced any pathology in the animals under study.

The relative weight of the thymus and spleen is shown in table 4. These two organs are the ones of highest interest in the immune processes during the first stage of the animals' live (Onifades 1997). Significant differences were found in the thymus, with superior weight only in the pigs from the sows consuming the product 21 d before the farrow.

Apparently, this increase was due to the better functioning of the immune system of those animals consuming the probiotic and its endospores. As referred in the literature, these cultures are immunomodulators. Thus, a superior immunestimulating response is obtained (Milián 2009).

The relative weight of the spleen increased in groups 2 and 3, in respect to the control. Similar results were obtained by Martínez (2011), who stated that the presence of dead and alive yeasts in the composition of soluble-dehydrated grains favors the increase noted, probably due to the probiotic effect. This result is closely related with the higher protection developed in the animals consuming the probiotic. According to Perdigón *et al*. (2006), the spleen is a peripheral organ where the cells involved in the defense of the organism are stored and execute their function.

Table 5 evidences that the production of healthy lungs was higher in the experimental periods consuming the probiotic (table 3). The amount of healthy lungs increased in 35 and 33 percent units in groups 2 and 3, in respect to the control. It can be inferred that reducing the staying of the animals in the pens to only 35 d, achieved by the effect of *Bacillus subtilis* and its endospores as a growth promoter additive, diminished the risks of respiratory lesions affecting the lungs.

Up to date, only the relation between the production of healthy lungs and the different seasons, sex, age and final liveweight has been reported. However, the possibility of using any non-antibiotic additive or any product influencing on the obtainment of healthy lungs has not been reported.

In the groups with the prebiotic culture, there was lower incidence of respiratory disorders. Ross and Katan

Table 3.Performance of the blood indicators of pigs consuming a probiotic based on *Bacillus subtilis* and their endospores

Indicators	Experimental groups			EE ±	Physiológical ranges	SE ±	Physiological ranges
	Control	21 d before parturition	30 d before parturition				
Hg, g.dL^{-1}	11.78	12.18	12.35	0.23	10-16		
Hto, %	35.50	36.63	37.13	0.62	30-45		

Table 4. Relative weight of the thymus and spleen of pigs consuming a probiotc based on *Bacillus subtilis* and their endospores

Indicators	Experimental groups			SE (±) Signif
	Control	21 d before parturition	30 d before parturition	
RW/thymus, g kg^{-1}	0.08^{a}	0.09^{b}	0.08^{a}	0.004*
RW/spleen, g kg^{-1}	0.18^{a}	1.50^{b}	1.62^{b}	0.09***

^{ab} Means with different letters differ among them at *P < 0.05 ***P < 0.001

Table 5. Performance of the healthy lungs in pigs with a probiotic based on Bacillus and their endospores

Indicators	Experimental groups			Signif.
	Control	21 d before parturition	30 d before parturition	
Total of slaughtered animals	22	30	24	
Amount of healthy lungs obtained	12	27	21	
Percentage of healthy lungs	54.55a ±8.69	90.00b ±7.44	87.50b ±3.32	***

[ab] Means with different letters differ among them at ***P < 0.001

(2000) stated that the beneficial effect of probiotics is produced when they are consumed in proper amounts and the ecosystem of the microorganisms inhabiting in the intestine is modified. This generates a beneficial microbial balance translated into a better health and stimulation of the immune response of the animal, achieved in this study due to the benefic action of the endospores (Milián 2006).

Pneumonia was the main cause of loss in the control group (table 6). The 90 % reached coincides with the reports of Bulnes *et al*. (2004) in a study conducted in Cuban slaughter houses. This demonstrates that pneumonia is one of the pathological processes attacking pigs the most (Cabrera *et al*. 2006).

The loss percentages due to hemolytic lungs were related with the slaughtering, which is not an indicator of affection or of the physiological state of the animal.

It is concluded that the presence of a probiotic culture, based on *Bacillus subtilis* and its endospores, in the feeding of sows and their descendents favors the production of healthy lungs and reduces the losses due to pneumonia.

Table 6. Main causes of the diminishing of healthy lungs

Causes	Experimental groups			Sign.
	Control	21 d before parturition	30 d before parturition	
Neumonia (%)	90.00	66.00	33.00	
Hemolytics (%)	10.00	33.00	66.00	
SE±	15.81[a]	28.86[b]	28.86[b]	***

[ab] Means with different letters differ among them at ***P < 0.001

The use of *Bacillus subtilis* and its endospores increased the activity of the thymus and the spleen. There was a better immune response in the pigs and higher protection for the animal. No alterations were found in the hematologic indicators assessed.

Acknowledgement

Thanks are given to the workers of the swine unit, especially those of the Experimental Unit of Meat Processing (slaughter house) and the Laboratory of Analytic Chemistry of the Institute of Animal Science for their help in this study. Special thanks are given to Alejandro Albelo, a technician of the Department of Management and Feeding of Monogastrics.

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Received: January 10, 2013