Development of the digestive organs in piglets born from sows consuming probiotic before farrowing and during lactation

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Morphometry characteristics of the digestive organs were determined in 24 piglets of 68 d of age and 21 kg of live weight, from a commercial cross and born from sows treated with *Bacillus subtilis*, before farrowing (0, 21 and 30 d) and during lactation (33 d). There was no effect of treatment on the relative fresh weight of the tract and digestive organs, although the small intestine showed low correlation ($r = 0.237$; $P < 0.10$), weighing less as the treatment days elapsed from 44.8 to 40.9 g/kg LW. The relative fresh weight of the small intestine was correlated with the gastrointestinal tract ($r = 0.936$, $P < 0.001$) and represented, as average, 59.1 % of the weight of the entire tract in this life stage of the animals. The linear density of the small intestine showed significant decrease ($P < 0.05$) when the treatment was extended in time from 0.60 to 0.53 g/cm. It is suggested that morphometry measurements of the alimentary canal could constitute an indicator of the response of piglets born from sows treated with *Bacillus subtilis* before farrowing and during lactation.

Key words: *piglets, Bacillus subtilis, probiotic, gastrointestinal tract, morphometry*

Since the first studies were developed with pigs to which additives of antibiotic type were supplied, it was demonstrated changes associated with this type of treatment in the small intestine of young animals (Braude *et al*. 1955 and Visek 1978) even though this fact was not always admitted (Rosen 1995). Usually, it has been evidenced that, together with the best animal performance traits, the small intestine decreases its weight and also shortens. Generally, this decrease tallies with that taking place in the entire tract.

The improvement in the performance traits could be ultimately due to lower energy request for maintenance of the digestive system according to Yen *et al*. (1985) and Nyachoti *et al*. (2000). This is particularly important in the first life stages of the piglet (Lallés *et al*. 2007), in which adequate correspondence between the structure and function of the gastrointestinal tract (Lykke *et al*. 2012) must exist. However, the utilization of antibiotics as growth promoters (AGP) has been a controversial topic in the animal production field.

The undesirable collateral effects derived from their use have turned them into a forbiden object by the European Community. Therefore, work is currently realized for searching new alternatives for the substitution of AGP. The use of probiotic preparations seems to constitute a viable option as additive for promoting growth.

The objective of this study was determining, in piglets, the effect of supplying a probiotic based on *Bacillus subtilis* and their endospores to define the sows before farrowing and during lactation.

Materials and Methods

The morphometry characteristics of the gastrointestinal tract divided in stomach, small and large intestine were determined in 24 piglets from a commercial Yorkshire-Landrace-L 35 cross. Animals were of 68 d of age and 21 kg of live weight, and came from sows treated with *Bacilus subtilis* before farrowing (0, 21 and 30 d) and during lactation (33 d). After weaning piglets continued probiotic consumption. The product was homogeneously hand mixed at a rate of 109 endospores/g of concentrate.

Sows were individually housed. Diets were prepared according to the requirements (NRC 1998) of the in-pig sow and according to the characteristics recommended by the pig rearing handbook (IIP 2008), with an average consumption of 3 kg/d (table 1).

After slaughtering the piglets, a laparotomy was practiced for isolating and extracting the gastrointestinal tract divided into three sections (stomach, small intestine and large intestine). The different digestive segments were carefully separated from the mesentery and were

Table 1. Composition of the diets of the sows (% DM)

Indicator	Diets	
	Gestation	Lactation
Ingredients		
Maize meal	81.56	74.22
Soybean meal	15.00	22.00
NaCl	0.50	0.50
$CaCO_3$	1.20	1.00
$CaPO_4 2H_2O$	1.20	1.60
Choline chloride	-	0.14
Vitamins and minerals[1]	0.54	0.54
Analysis		
N x 6.25	13.12	15.96
ME, kJ/kg DM	12.95	13.25

[1]According to the requirements of NRC (2012)

empty weighed, after eliminating the digesta and once washed (Ly 1979). For the weighing a scale with one gram appreciation was used. Additionally, the length of the small and large intestines were determined by means of a metallic tape measure, with 0.1 cm accuracy. Linear density of the intestines was calculated through the division of the fresh weight by its length. All weights and lengths were adjusted to the live weight of the animals for avoiding the source of variation (Ly 1979, Ly *et al*. 2012).

The technique of variance analysis was applied for mean comparison (Steel *et al*. 1997) according to a simple classification with three treatments and eight replications. Treatments agreed with the days of supplying the additives to the sows before farrowing, continuing in lactation and after weaning. Duncan's (1955) multiple range test was applied for mean comparison in the cases where the analysis of variance was significant ($P < 0.05$). Also, analysis of correlation and regression was made. The statistical package InfoStat (Balzarini *et al*. 2001) was used for data processing.

The theoretical suppositions of the analysis of variance and the homogeneity were analyzed by Levene's (1960) tests for the normality of the errors and by that of Shapiro and Wilk (1965) for the variables percentage of stomach, small and large intestines and fulfilling with the suppositions. Thus, the parametric analysis of variance was applied.

Results and Discussion

The relative weights of the digestive segments examined are shown in table 2. There was no treatment effect on the relative fresh weight neither in the digestive organs nor in the gastrointestinal tract, although the small intestine showed low correlation ($r = 0.237$; $P < 0.10$), since it was less heavy with the increase of the days of treatment from 44.8 to 40.9 g/kg LW. Frequently, in the literature is reported that when beneficial microorganisms or antibiotic type additives are included in the diet, these influenced on the digestive and absorptive processes taking place at the intestine and thus, in the decrease of this organ (Izat *et al*. 1990 and Rondón 2009).

The relative fresh weight of the small intestine was strongly correlated with that of the gastrointestinal tract ($r= 0.936$; $P < 0.001$) and represented, as average, 59.1 % of the weight of the entire tract in this life stage of the animals (Reis de Souza *et al*. 2007). This interdependence is shown in figure 1. Results obtained coincide with those reported by Braude *et al*. (1955) and Visek (1978).

In table 3 are shown data corresponding to the matrix of correlation between the different digestive organs and the GIT. All interdependencies were positive, although those obtained with the large intestine did not attain significance.

The relative fresh weight of the small intestine (y, g/kg live weight) was strongly correlated ($r= 0.929$; $P < 0.001$) with that of the gastrointestinal tract and represented, as average, 59.1 % of the weight of the entire tract in this life stage of the animals. This interdependency is presented in figure 1.

The morphometry response found could be associated with the first pig life stages when the digestive tract growth, in general, and of the small intestine, in particular, undergoes a marked hypertrophic activity (Ly 1979, Cranwell 1995, Adeola and King 2005 and Reis de Souza *et al*. 2005), due to the development of hydrolytic enzymes and intestinal mucosa.

The measurements of length and linear density of the intestines are presented in table 4. The length of both intestines was numerically lower in the groups of animals consuming the probiotic regarding the control,

Table 2. Effect of the probiotic supply to sows on the gastrointestinal[1] tract weight of piglets

Indicator	Days of the additive supply before farrowing			SE ±
	-	21	30	
n	8.0	8.0	8.0	-
Live weight, kg	21.1	21.4	21.5	-
Relative fresh weight, g/kg live weight				
Stomach	9.86	10.01	9.21	0.58
Small intestine	44.87	40.68	40.92	3.77+
Large intestine	18.85	20.63	19.69	1.84
GIT	73.58	71.31	69.82	5.14
Percentile contribution, % GIT				
Stomach	13.4	14.0	13.2	0.84
Small intestine	61.0	57.1	58.5	1.86
Large intestine	25.6	28.9	28.3	1.87
GIT	100.0	100.0	100.0	100.0

[1]Fresh weight of the empty organs
+P< 0.10

Table 3. Pearson's matrix of correlation for the digestive organs in piglets treated with a probiotic (n = 24)

Indicator	STO	SI	LG
SI	0.550*		
LI	0.139	0.326	
GIT	0.582*	0.936*	0.831

STO, SI, LI and GIT are stomach, small intestine, large intestine and gastrointestinal tract in that order. All measurements are expressed in fresh g/kg live weight $P < 0.05$ for $r > 0.500$ in absolute values

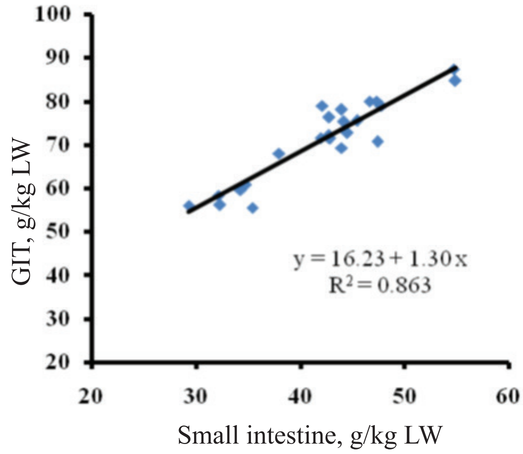

Figure 1. Interdependency between empty weight of the small intestine and that of the entire tract in piglets (± 3.14***)

but without significant effect of the treatment. On the other hand, the linear density of the small intestine did not show significant decrease ($P < 0.05$) when the treatment was prolonged in time, from 0.60 to 0.53 g/cm. This slimming of the small intestine wall is in accordance with the statement of Visek (1978) of the absence of inflammatory processes by beneficial modifications in the jejunum and ileum environment of the animals. In contrast, the linear density of the caecum and colon increased ($P < 0.05$) with the days of treatments, with a different and opposite reaction to that occurring in the small intestine, perhaps due to a greater N rechange in the large intestine tissue (Nyachoti *et al*. 2000). It must be highlighted that piglets treated longer with the probiotic were precisely those showing better health and zoogenic response (Ayala *et al*. 2012).

Table 4. Effect of a probiotic supply to sows in longitudinal measurements of the gastrointestinal tract of piglets

Indicator	Days of additive supply before farrowing			SE ±
	-	21	30	
n	8	8	8	-
Live weight, kg	21.1	21.4	21.5	-
Length, cm/kg of live weight				
Small intestine	79.17	73.03	76.24	4.19
Large intestine	15.68	12.97	13.85	1.47
Both intestines	94.85	86.00	90.09	5.16
Linear density, g/cm/kg live weight				
Small intestine	0.60[a]	0.58[ab]	0.53[b]	0.03*
Large intestine	1.20[a]	1.64[b]	1.45[ab]	0.15*
Both intestines	0.89[a]	1.10[b]	0.99[b]	0.07*

[1]Fresh weight of the empty organs. *$P < 0.05$

[ab]Means in the same row with different letters differ significantly at $P < 0.05$ (Duncan 1955)

The absence of significant influence in some morphometry indicators could be a consequence of the great variability found in the population examined, in view of the fact that in this experiment eight individuals were studied per treatment. Evidently, the longitudinal measurements and the linear density seem to be more accurate than the adjusted one, concerning the intestine of pigs.

It is suggested that the morphometry measurements of the alimentary canal could be an indicator of the trophic response of piglets to the treatment of the in-pig sow with probiotics before farrowing and after this.

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