Vol.56, n.2: pp. 249-258, March-April 2013
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Probiotics Association in the Suckling and Nursery in Piglets Challenged with *Salmonella typhimurium*

Esther Ramalho Afonso[1*], Larissa José Parazzi[1], Carolina Tobias Marino[1], Simone Maria Massami Kitamura Martins[2], Lucio Francelino Araújo[3], Cristiane Soares da Silva Araújo[1], **Flávio Garcia Vilela[1] and Aníbal de Sant'Anna Moretti[1]**
[1]Departamento de Nutrição e Produção Animal; Faculdade de Medicina Veterinária e Zootecnia; Universidade de São Paulo; Av. Duque de Caxias Norte, 225; 13635-900; Pirassununga - SP – Brasil. [2]Departamento de Reprodução Animal; Faculdade de Medicina Veterinária e Zootecnia; Universidade de São Paulo; Pirassununga - SP – Brasil. [3]Faculdade de Zootecnia e Engenharia de Alimentos; Universidade de São Paulo; Av. Duque de Caxias Norte, 225; 13635-900; Pirassununga - SP – Brasil.

ABSTRACT

This work aimed to study the probiotics association in 144 piglets from birth to 62 days old. In lactation, the design was completely randomized with two treatments, CTL, 1 mL of distilled water and ProbA, 5g in 15 ml of distilled water, both orally, and in the nursery in randomized block design, with 2x3 factorial arrangement of treatments, ProbA ProbB: 30g/ton of ProbB in the ration; CTL ProbB: 30g/ton of ProbB in the ration; ProbA ProbA, CTL CTL; ProbA CHA (challenged); CTL CHA. At 35 days of age the animal of the nursery were inoculated with Salmonella typhimurium orally. There was no effect of the parameters evaluated during the maternity. In nursery, the feed conversion was favorable to the ProbA. In the evaluation of fecal score, the challenged group had more diarrhea and increased elimination of S. typhimurium*. Results showed the positive action of probiotics when applied at birth by the direct influence on the formation of the intestinal microbiota.*

Key words: *Bacillus subtilis, Bifidobacterium pseudolongum, Lactobacillus reuteri*, Challenge. Piglets

INTRODUCTION

As the world population grows, greater is the demand of the food of animal origin, which is affecting the production system as a whole. In this context, the social, environmental and food security aspects should be considered. Particularly in swine production, the international trade is of 5.4 million tons of meat and generates an annual revenue of 11.9 billion dollars. The main importing countries are Japan, Russia, Mexico, South Korea and Hong Kong (Abipecs 2009). Brazil occupies a prominent position in the international market, placed among the countries that have a steady growth in pork production and exportation, with the constant search for new markets such as the European Union, Japan, china and South Korea (Abipecs 2011).

The indiscriminate use of growth promoters (antibiotics and chemotherapy) used in pig farming has been questioned by providing the possible selection of resistant strains, toxicity or allergies in humans (Palermo 2006). Despite the proven capacity to improve the performance, when used in sub-therapeutic doses as micro ingredients in the diets, the addition of antibiotics, as growth

[*]Author for correspondence: estherafonso@gmail.com

promoter in the rations, was banned by the European Union countries since 2006 (Brugalli 2003); it can only be used therapeutically, by veterinarian's prescription. These prohibitions led serious economic consequences in the production chain of the swine meat (Butolo 1999).

The animal production, in face of the increasing demands, has been adapting and creating substitution possibilities of the antimicrobial by others feed additives such as the prebiotics, probiotics, symbiotics, organic acids and vegetable extracts. This area of study has received increased attention by the researchers, with the need of further clarification on the effects of these alternative products on the cost and production performance.

Therefore, this study aimed to elucidate the effects and actions of the association of probiotics containing *Lactobacillus reuteri*, *Bifidobacterium pseudolongum* and *Bacillus subtilis*, added to the diet of piglets in the lactation and nursery. The performance, fecal score and sanitary aspects were evaluated in relation at the challenge programmed with *Salmonella typhimurium*.

MATERIALS AND METHODS

One hundred forty hybrid piglets of the lactation to nursery were used, from the Swine Research Laboratory (SRL), Faculty of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ-USP), Pirassununga Campus.

During the maternity, a completely randomized design composed by two treatments, Probiotic A (ProbA) and Control, was used where the experimental unit was the stall. The distribution of the treatments was intercalated within each maternity ward, where the probiotic (ProbA) was provided to the piglets before of the colostrum feeding. The same procedure was performed with the control (CTL) treatment, intercalating itself thus with the Probiotic A treatment. This adequacy aimed to standardize the treatments in the maternity ward.

The probiotic (ProbA) used was a commercial product in powder form composed by *Lactobacillus reuteri* (1.5x 10^9 CFU/g) and *Bifidobacterium pseudolongum* (1.5x 10^9 CFU /g) and the control (CTL) treatment received distilled water. At birth, 72 piglets were orally administrated the probiotic mixing the contents of the envelope of 5g in 15 mL of distilled water the other 72 piglets were orally administered 1 mL of distilled water. At 21 days of age, the same procedure was performed, in which the same dose of the two treatments was administered to the piglets.

The variables analyzed in the phase of maternity were: average weight (AW) in kilograms (kg) at birth, 7, 14 and 21 days of age and average daily weight gain (ADWG), in kg/animal /day, of the birth to 7days, 8 to 14 days and 15 to 21 days old.

In the nursery, the experimental design was randomized blocks according to the weight of the animals where the animals were considered: heavy with 8.0-9.0 kg; medium with 6.0-7.9 kg and light with 5.0 to 5.9 kg, with a 2x3 factorial arrangement of treatments, the experimental unit considered was the stall with three animals, constituting eight replicates per treatment.

The 144 piglets of the maternity were divided in two treatments ProbA and Control and distributed into three nursery ward, thus forming six treatments: Ward1: Probiotic A Probiotic B (ProbA ProbB) and Control Probiotic B (CTL ProbB); Ward 2: Probiotic A Probiotic A (ProbA ProbA) and Control Control (CTL CTL); Ward 3: Probiotic A Challenged (ProbA CHA) and control challenge (CTL CHA) (Table1).

In the nursery, pre-initial rations were provided (21 to 33 days old); initial 1 (34 to 54 days old) and initial 2 (55 to 62 days of age) (Table 2). Water and ration were provided ad libitum. The Probiotic B (ProbB) used was a commercial product composed by *Bacillus subtilis* (1.0 x 10^{10} CFU/g) and added in the ration at amount of 30g/ton of ration.

Twelve hours before the challenge programmed by the oral inoculation with *Salmonella typhimurium* in the concentration of $1.0x10^6$ CFU/mL (LSH90/05), the same piglets of the group ProbA and CTL in the maternity received the probiotic orally and distilled water at the same dose at the birth. The challenge was performed till 35 days of age. The challenged piglets remained isolated from the others. The fecal material was collected through the rectal swabs in the D0: day of inoculation (35 days); D7: 7 days after the inoculation (42 days) and D14: 14 days after the inoculation (56 days), for the isolation of *S.typhimurium.*

The performance variables analyzed were average weight (AW) to 34 (Period 1), 47 (Period 2), 54 (Period 3), 62 (Period 4) days of age. The ranges of average daily feed intake (ADFI) in

kg/animal/day, average daily weight gain (ADWG) in kg/animal/day and feed conversion (FC) considered were from 21 to 33 days (Period 1), 34 to 47 days (Period 2), 48 to 54 days (Period 3), 55 to 62 days (Period 4) and during the total period, respectively.
In the nursery phase, from 21 to 62 days of age, consistency of feces was observed daily and classified as: 1 = solid feces - normal; 2 = creamy feces - moderate diarrhea and 3 = liquid feces - severe diarrhea. The periods analyzed were: 34° (24 h before the challenge), 36^{o} (24 h after the challenge), 38^{o} day (48 h after the challenge), week 2 (from 39 to 48 days), week 3 (from 49 to 55 days) and week 4 (from 56 to 62 days), similar to the study performed by Wang et at. (2009).

Table 1 – Description of treatments in the maternity and nursery.

Maternity					
Prob A 72 piglets			CTL 72 piglets		
Nursery					
Room 1		Room 2		Room 3	
ProbA	CTL	ProbA	CTL	ProbA	CTL
ProbB	ProbB	ProbA	CTL	CHA	CHA
24 piglets that received ProbA in the maternity	24 piglets that not received ProbA in maternity	24 piglets that received ProbA in the maternity	24 piglets that not received ProbA in the maternity	24 piglets that received ProbA in maternity	24 piglets that not received ProbA in maternity

Table 2 - Composition in kilograms of the experimental diets administered during the nursery period.

Ingredients (kg)	**Experimental Diet**		
	Pre-initial	**Initial I**	**Initial II**
Soybean meal	333.32	333.80	328.00
Biscuit meal	120.00	60.00	-
Ground corn	-		598.00
Corn meal	428.64	533.52	-
Whey	40.00	10.00	-
Dicalcium phosphate	16.00	15.00	18.50
Calcitic limestone	-	5.40	7.00
Lactose	12.00	4.00	-
Sugar	30.00	30.00	40.00
Sodium chloride	3.20	2.60	6.00
Lysine	2.80	1.40	
Methionine	3.60	1.00	-
Phytase	0.12	0.06	-
Choline	0.32	0.22	-
Palatability	8.00	1.00	-
Vitamin premix	2.00	1.00	1.00
Mineral premix	-	1.00	1.50
Total (kg)	1000	1000	1000
	Nutritional Composition		
Dry matter (%)	89.66	89.62	90.21
Gross Energy (cal/g)	4341	4303	4282
Crude Protein (%)	21.62	22.46	21.94
Crude fiber (%)	3.55	3.38	5.07
Mineral matter (%)	5.26	6.19	7.41
Calcium (%)	0.58	0.93	0.94
Phosphorus (%)	0.52	0.67	0.62
Ether extract (%)	2.68	2.68	3.13

Data were analyzed using the computer program Statistical Analysis System (SAS Institute Inc. 2008), and previously verified the normality of the residuals by the Shapiro-Wilk test (PROC

UNIVARIATE). The data on the performance, culture and identification of *S. typhimurium* in the feces were subjected to the analysis of variance which separated as sources of variation the effect of block and treatment effect, and also added the factor repeated measures on time, relative to different periods of development of the animals. This analysis was performed using the MIXED procedure of SAS. The analysis for the time was only performed when the interactions between the effect of time and treatment effect were significant. Independent of the data studied within each time or not, the treatments were the decomposed by the use of orthogonal contrasts.

Contrast 1 (C1) - effect of the administration of ProbA versus no administration of ProbA.

Contrast 2 (C2) - effect of the addition of ProbB versus non ProbB administration in ration.

Contrast 3 (C3) - effect of Challenge (CHA) versus Control (CTL).

Contrast 4 (C4) - interaction of the administration of probiotic ProbA with the addition of ProbB in ration.

Contrast 5 (C5) - interaction of the administration of ProbA with the Challenge.

The feces score data were subjected to the analysis of variance by the GLM procedure and the effect of treatment was separated by the Tukey test. The effects were considered significant at p <0.05.

RESULTS

In the maternity phase, there was no interaction between the time and the Probiotic A and Control treatments (p>0.05) as the treatment effect (p>0.05). Over the period, the average values were 3.44 kg (Control) and 3.56 kg (ProbA) (not shown data). For the average daily weight gain, there was interaction between the time and treatment (p = 0.0318) during 0 to 7 days of age. The piglets that received Probiotic A had higher average daily weight gain (0.175 kg/animal/day) when compared to the control (0.159 kg/animal/day). During the other periods, significant effects were not observed (unpublished data). On average, during the maternity, the daily weight gain was 0.204 kg/animal/day (Control) and 0.212 kg/animal/ day (ProbA).

In the nursery phase, there was a significant interaction between the time and treatment (p=0.0203) for the variable average weight (Table 3). At 33 days, significant effect was observed in C4 contrast (p = 0.0203).

In the group that received ProbA associated with ProbB, the average weight was higher when compared to non-association of the two probiotics (Fig. 1a). At 54 days, there was a significant effect in C2 contrast (p = 0.0484) where the group that received the ProbB had lower average weight (17.52 kg) compared to the treatments that not received (19.03 kg) (Table 3). During the same period, there was a significant effect in C4 (p = 0.0210). There was interaction between the administrations or not of ProbA and addition or not of ProbB, where the non-association of both the probiotics resulted in higher weight (Fig. 1b). At 62 days, there was significant change in C2 contrast (p = 0.0049), showing that the group ProbB (21.68 kg) presented a lower value compared to other treatments which did not receive Prob B (23.78 kg) (Table 3).

In relation to average daily feed intake (ADFI) (Table 4) significant effect of the interaction was observed between the time and treatment (p = 0.0098). In the period from 21 to 33 days, there was significant effect in C4 contrast (p = 0.0281), and the treatment ProbB associated with ProbA had the higher intake in relation to other treatments (Fig. 1c). By analyzing the period from 48 to 54 days, significant effect was observed for the contrasts, C1 (p = 0.0152) and C3 (p<0.001). The group that received ProbA (0.960 kg/animal/day) had the higher intake in relation to the treatments that did not receive ProbA (0.848 kg/animal/day). The Challenged Treatments (1.270 kg/animal/day) had higher intake compared to the control group (0.670 kg/ animal/day). There was significant change in the C1 and C2 contrast, during the period from 55 to 62 days, showing in the treatments that received ProbA (1.240 kg/animal/day) higher consumption in relation to the treatments without ProbA (1.161 kg/animal/day) (p = 0.0345). In the C2 contrast, it was observed that the treatments receiving ProbB (1.089 kg/animal/ day) had lower consumption compared to the Control (1.240 kg/animal/day) (p = 0.0113) (Table 4).

There was significant effect of the interaction between the time and treatment (p = 0.0014) for average daily weight gain (ADWG) (Table 5). During the period from 34 to 47 days, significant effect in C2 contrast was observed (p = 0.0206) where the ProbB group (0.338 kg/animal/day) had lower weight gain compared to other treatments that did not receive ProbB (0.422 kg/animal/day). There was significant change in C2 contrast, during the period from 55 to 62 days, showing lower average daily weight gain for the ProbB group (0.596 kg/ animal/day) compared to other treatments that did not receive the probiotic (0.730 kg/animal/day) (p = 0.0072).

There was significant effect of the interaction between the time and treatment (p<0.001) (Table 6) for feed conversion. There was significant change in the C2 contrast (p = 0.0356) during period from 34 to 47 days, where the ProbB group (1.91) had higher feed conversion than the other treatments (1.63). During period of 48 to 54 days, in C3 contrast, the Challenged group (1.80) showed higher feed conversion compared the control (0.97) (p<0.001). During the period from 55 to 62 days, there was a significant effect in C4 contrast (p = 0.0012), and the ProbA treatment not associated with ProbB showed better feed conversion than the other treatments (Fig. 1d).

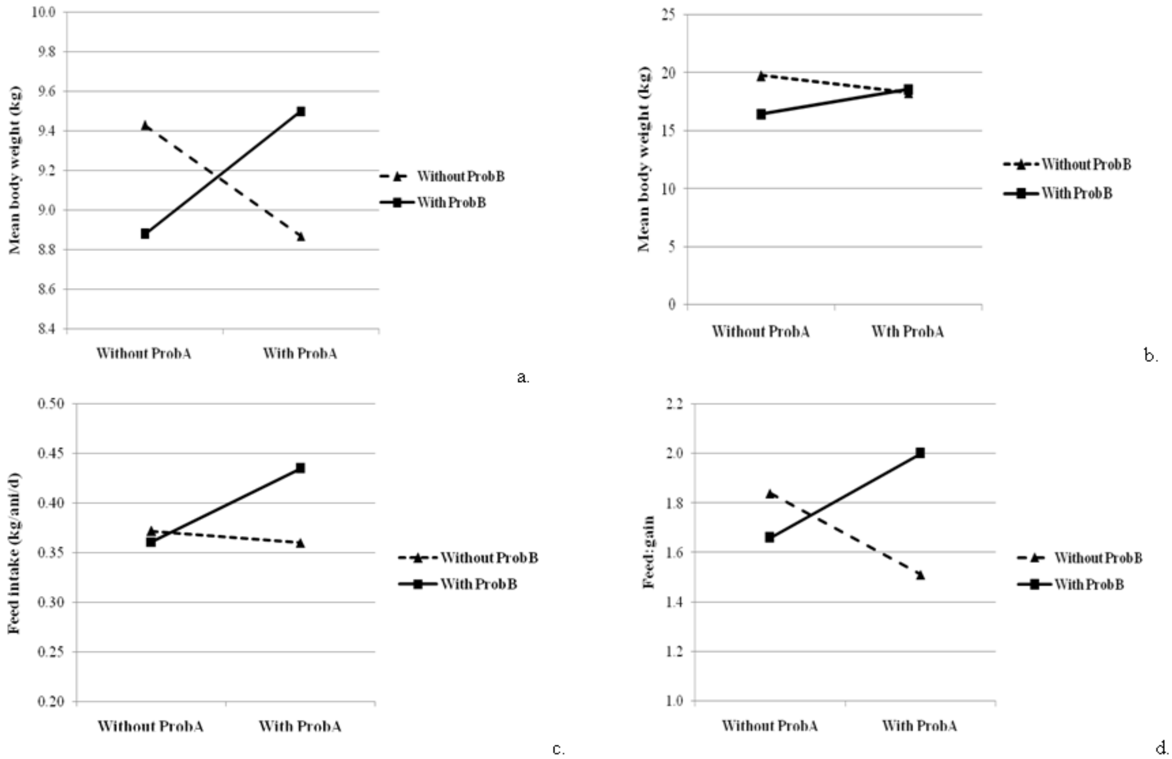

Figure 1 - Interaction of the average weigh between probiotics A and B to 33 (a.) e 54 (b.) days of age, interaction of the average daily intake between probiotic A and B in the period of 21 to 33 days of age (c.) and interaction of the feed conversion between probiotics A and B in the period of 55 to 62 days of age (d.).

Table 3 – Mean values and coefficients of variation of the variable average weight (AW), in kilograms, of piglets between 33 and 62 days of age, and the probabilities of contrasts.

Period (days)	Treatments*						Mean	CV***	Contrasts****				
	ProbA ProbB	CTL ProbB	ProbA ProbA	CTL CTL	ProbA CHA	CTL CHA			1	2	3	4	5
33	9.50	8.88	8.87	9.43	8.99	9.76	9.24	18.17	NS**	NS**	NS**	0.0203	NS**
47	14.38	12.78	14.95	14.30	13.60	14.80	14.14	18.22	NS**	NS**	NS**	NS**	NS**
54	18.57	16.47	18.29	19.78	18.48	18.80	18.40	17.43	NS**	0.0484	NS**	0.0210	NS**
62	22.59	20.78	23.92	23.64	23.47	23.63	23.01	14.54	NS**	0.0049	NS**	NS**	NS**

Probability of interaction time and treatment (p=0.0203).* Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA;CTL CHA. **NS: Not significant. *** Coefficient of variation *** *Contrasts: C1: effect of the administration of ProbA versus non administration of ProbA; C2: effect of the addition of ProbB in the diet versus non administration of ProbB in the diet; C3: effect of Challenge (CHA) versus Control (CTL); C4: interaction of the administration of probiotic ProbA with addition of ProbB in the diet; C5: interaction of the administration of ProbA with Challenge (CHA).

Table 4 - Mean values and coefficients of variation of the variable average daily feed intake (ADFI), in kilograms/animal/day, of piglets between 21 and 62 days of age, and the probabilities of contrasts.

Period (days)	Treatments*						Mean	CV***	Contrasts****				
	ProbA ProbB	CTL ProbB	ProbA ProbA	CTL CTL	ProbA CHA	CTL CHA			1	2	3	4	5
1 (21-33)	0.435	0.361	0.360	0.372	0.369	0.401	0380	17.87	NS**	NS**	NS**	0.0281	NS**
2 (34-47)	0.655	0.601	0.674	0.642	0.635	0.688	0.650	13.02	NS**	NS**	NS**	NS**	NS**
3 (48-54)	0.882	0.658	0.701	0.640	1.296	1.245	0.900	35.68	NS**	NS**	<0001	NS**	NS**
4 (55-62)	1.119	1.058	1.305	1.175	1.295	1.251	1.200	14.84	0.0345	0.0113	NS**	NS**	NS**

Probability of interaction time and treatment (p=0.0098). * Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA;CTL CHA. **NS: Not significant. ***Coefficient of variation ****Contrasts: C1: effect of the administration of ProbA versus non administration of ProbA; C2: effect of the addition of ProbB in the diet versus non administration of ProbB in the diet; C3: effect of Challenge (CHA) versus Control (CTL); C4: interaction of the administration of probiotic ProbA with addition of ProbB in the diet; C5: interaction of the administration of ProbA with Challenge (CHA).

Table 5 - Mean values and coefficients of variation of the variable average daily weight gain (ADWG), in kilograms/animal/day, of piglets between 21 and 62 days of age, and the probabilities of contrasts.

Period (days)	Treatments*						Mean	CV***	Contrasts****				
	ProbA ProbB	CTL ProbB	ProbA ProbA	CTL CTL	ProbA CHA	CTL CHA			1	2	3	4	5
1 (21-33)	0.296	0.294	0.274	0.285	0.282	0.298	0.288	18.49	NS**	NS**	NS**	NS**	NS**
2 (34-47)	0.376	0.299	0.469	0.375	0.354	0.389	0.377	29.10	NS**	0.0206	NS**	NS**	NS**
3 (48-54)	0.699	0.618	0.661	0.729	0.812	0.667	0.698	22.84	NS**	NS**	NS**	NS**	NS**
4 (55-62)	0.575	0.616	0.804	0.656	0.715	0.690	0.676	23.02	NS**	0.0072	NS**	NS**	NS**

Probability of interaction time and treatment (p=0.0014). * Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA;CTL CHA. **NS: Not significant. ***Coefficient of variation *** *Contrasts: C1: effect of the administration of ProbA versus non administration of ProbA; C2: effect of the addition of ProbB in the diet versus non administration of ProbB in the diet; C3: effect of Challenge (CHA) versus Control (CTL); C4: interaction of the administration of probiotic ProbA with addition of ProbB in the diet; C5: interaction of the administration of ProbA with Challenge (CHA).

Table 6 - Mean values and coefficients of variation of the variable feed conversion (FC) of piglets between 21 and 62 days of age, and the probabilities of contrasts.

Period (days)	Treatments*						Mean	CV***	Contrasts****				
	ProbA ProbB	CTL ProbB	ProbA ProbA	CTL CTL	ProbA CHA	CTL CHA			1	2	3	4	5
1 (21-33)	1.37	1.24	1.32	1.32	1.30	1.36	1.32	8.40	NS**	NS**	NS**	NS**	NS**
2 (34-47)	1.77	2.06	1.51	1.76	1.85	1.68	1.77	20.69	NS**	0.0356	NS**	NS**	NS**
3 (48-54)	1.33	1.07	1.11	0.83	1.74	1.87	1.33	37.83	NS**	NS**	<0001	NS**	NS**
4 (55-62)	2.00	1.66	1.51	1.84	1.82	1.82	1.78	15.86	NS**	NS**	NS**	0.0012	NS**

Probability of interaction time and treatment (p<0.0001). * Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA;CTL CHA. **NS: Not significant. ***Coefficient of variation *** *Contrasts: C1: effect of the administration of ProbA versus non administration of ProbA; C2: effect of the addition of ProbB in the diet versus non administration of ProbB in the diet; C3: effect of Challenge (CHA) versus Control (CTL); C4: interaction of the administration of probiotic ProbA with addition of ProbB in the diet; C5: interaction of the administration of ProbA with Challenge (CHA).

The animals of the programmed challenge showed normal feces in days 36 and 34 before the challenge. At 38 day, and 48 h after the inoculation with *S. typhimurium*, there was no interaction between the treatments additive and room (p>0.05), however it has effect of challenge treatment (p = 0.0172), with high scores. In the evaluation during three weeks there was not interaction between additive and room (p> 0.05). In relation to week 2, the animals of the room 1, creamy feces, and room 3, liquid feces, had scores higher than the room 2 (p <0.001). In week 3 and 4, the score of feces for room 3, liquid feces, was higher compared to rooms 1 and 2, creamy feces in both, which did not differ (p <0.05) (Table 7).

For the variable frequency of the presence of *S.typhimurium*, at 42 and 56 days, significant difference in C3 contrast was observed (p=0.0003 and p=0.0069, respectively), in the treatments that received the programmed challenge a higher frequency of presence of the bacteria in relation to control group (Table 8).

Table 7 – Means values and standard deviation of fecal score of piglets in different periods.

Period (days)	Room 1		Room 2		Room 3		****P		
	ProbA** ProbB	CTL** ProbB	ProbA** ProbA	CTL** CTL	ProbA** CHA	CTL** CHA	R*	A*	A*R*
34°	2.50 ± 0.71	1.00 ± 0	1.00 ± 0	1,00 ± 0	1.13 ± 0.35	1.13 ± 0.35	NS***	NS***	NS***
Mean [1]	NS**		NS**		NS**				
36°	2.00 ± 0.76	2.13 ± 099	2.13 ± 0.83	2.25 ± 0.89	1.88 ± 0.83	2.38 ± 0.74	NS***	NS***	NS***
Mean [1]	NS**		NS**		NS**				
38°	1.75 ± 1.04	2.50± 0.93	1.75 ± 0.46	1.50 ± 0.53	2.38 ± 0.74	2.38 ± 0.74	0.0172	NS***	NS***
Mean[1]	2.125[ab]		1.625[b]		2.375[a]				
Week 2(39-48)	2.43 ± 0.34	2.53 ± 0.34	2.00 ±0.31	1.98 ± 0.57	2.78 ± 0.21	2.77 ± 0.24	<0001	NS***	NS***
Mean[1]	2.484 [a]		1.992 [b]		2.773 [a]				
Week 3(49-55)	2.34 ± 0.35	2.48 ±0.27	2.22 ± 0.41	2.23 ± 0.60	2.81 ± 0.29	2.92 ± 0.13	<0001	NS***	NS***
Mean [1]	2.414[b]		2.226 [b]		2.867 [a]				
Week 4(56-62)	1.66 ± 0.41	1.70 ± 0.28	1.55 ± 0.30	1.38 ± 0.42	2.07 ± 0.56	2.02 ± 0.39	0.0011	NS***	NS***
Mean [1]	1.678 [b]		1.464 [b]		2.044[a]				

Means followed of different letters in the same row differ by Tukey test (p<0.05).Classification: 1= solid tool – normal; 2= creamy stool – moderate diarrhea and 3= liquid stool – severe diarrhea. Means[1]: means obtained by different additives. * R: Room; A: Additive; A*R: Additive*Room. **Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA; CTL CHA. ***NS: not significant. ****P: probabilities.

Table 8 – Means values and coefficients of variation of the variable culture and identification of *Samonella typhimurium* in the feces at percentage (%) in various periods and probability of contrasts.

Age (days)	Treatments*						Mean	CV***	Contrasts****				
	ProbA ProbB	CTL ProbB	ProbA ProbA	CTL CTL	ProbA CHA	CTL CHA			1	2	3	4	5
35	0	2	2	0	0	1	0.09	0	NS**	NS**	NS**	NS**	NS**
42	18	28	19	9	46	76	0.33	150.3	NS**	NS**	0.0003	NS**	NS**
56	32	34	26	25	63	65	0.41	121.2	NS**	NS**	0.0069	NS**	NS**

Probability of interaction time and treatment (p=0.1012). * Treatments: ProbA ProbB; CTL ProbB; ProbA ProbA; CTL CTL; ProbA CHA;CTL CHA. **NS: Not significant. ***Coefficient of variation. *** *Contrasts: C1: effect of the administration of ProbA versus non administration of ProbA; C2: effect of the addition of ProbB in the diet versus non administration of ProbB in the diet; C3: effect of Challenge (CHA) versus Control (CTL); C4: interaction of the administration of probiotic ProbA with addition of ProbB in the diet; C5: interaction of the administration of ProbA with Challenge (CHA).

DISCUSSION

Results of this study showed that the response of the piglets during the nursery phase was of somehow positive. There was a significant effect of the administration of the ProbA in the average daily weight gain. Correa (2010) conducted a study on the commercial farm with the administration of *L. reuteri* ($1.5x10^9$ CFU/g) and *B. pseudolongum* ($1.5x10^9$ CFU/ g) orally before the ingestion of colostrum. There was no significant effect on the average weight and average daily weight gain. These results occurred probably, due to the good sanitary status of the farm where the study was conducted. Abe et al. (1995) used *B. pseudolongum* and *L. acidophilus* as probiotics in piglets during suckling and nursery and found weight gain during suckling.

These results suggested that the administration of probiotics, soon after birth could be effective, since the gut at birth may already be colonized and the probiotic bacteria acted avoiding that the pathogenic colonized the intestinal mucosa and the result of this action was reflected in a better absorption nutrients and immunoglobulins of the colostrum, enabling better viability of the piglet, and minor loss of piglets, particularly in its first days of life (Abrahão et al. 2004).

Similar the nursery period at 33 days, the interaction of the administration of ProbA and ProbB showed the positive variable average weight. This was probably due the presence of both the bacteria which complemented the intestinal microbiota positively in the piglets. However, at 54 days and in others, there was no interaction, suggesting that during the bacteria presented a positive impact in the microbiota, and afterwards a symbiotic that did not affect the weight.

In nursery phase, for intake during the first week of 21 to 34 days, there was an increase of this variable with the association of the two probiotic. In relationship to feed conversion, the association interaction showed unfavorable result. These findings were contrary to those reported by Budiño et al. (2006), in which the animals receiving the probiotic treatment containing *B. licheniformis* and *B. subtillis* principally, had lower average daily intake of feed compared to the control treatment with basal diet, and feed conversion was not influenced.

In the final period of nursery, from 55 to 62 days, better feed conversion was observed in the ProbA group. These results suggested that the animals receiving the ProbA in the maternity and 12 h before the programmed challenge was in a favorable state with the microbiota already established and stable. Silva et al. (2006) when using probiotic composed by *Pediococcus acidilactici* with or without *B. subtilis*, also observed better feed conversion compared to the control group, but found no differences in the final weight, daily weight gain and daily feed intake.

Despite the results with probiotics have been analyzed the highly variable, Steward and Chesson (1993) literature studies using different types of probiotics and concluded that on average, there was an increase of 4.8% in average daily weight gain of piglets in the initial phase.

Studies performed by Bruno (2008) and Parazzi (2010) showed that the performance of the animals treated with the vegetable extract and probiotics, respectively, in the nursery period were lower to the treatment containing antimicrobial, but the same did not occur for the period of growing and finishing, where the treatments became similar. Therefore, it is possible that the probiotics showed action in medium and long-term in the performance compared to antimicrobials. These studies showed that the probiotic with or without other parameters should extend to the growing and finishing period demonstrate the potential of these additives that could be used in the market.

In relation to the effects of the programmed challenge, it was observed that the average daily intake was influenced, as the consumption of the challenged animals was higher in subsequent weeks to challenge. This might have occurred by reducing the consumption in the early days of post-challenge, and followed by a gradual increase to compensate for the days post-challenge. Loughmiller et al. (2007) report that there was a consumption concentrated in the first three days post-challenge, followed by a gradual increase that tried to compensate the consumption. This study conducted investigations of weekly intake, which was differently from those of Balaji et al. (2000) and Turner et al. (2002) who performed the measurements daily post-challenge. This could have revealed a decrease in consumption after the challenge. Opposite results found by Bruno (2008) and Parazzi (2010) demonstrated no influence of the challenge in the intake, which might be due to the dose of *S. typhimurium* (1.0×10^5 CFU) they used, a lower concentration of inoculation that was used in this study, and it could be a factor that may unfavorably the detection of possible differences among the studies.

Associating the performance results with the clinical (score of feces) and sanitary (elimination of *S.typhimurium*) characteristics, can be inferred that at the treatments ProbA CHA e CTL CHA, the weight was influenced by the higher frequency of pasty and liquid feces, compared to other treatments. The higher frequency of pasty and liquid feces score carry a degree of dehydration and negative consequences, such as loss of weight and a decrease at the piglets immunity. This fact provides the action of pathogens that cause different degrees of intestinal mucosa inflammation, which could lead to a lower nutrient absorption that would slow the piglets growth and development (Etheridge et al. 1984; Nabuurs et al. 1993).

The treatments challenged, although they have higher frequency of days with diarrhea showed equivalent performance to others. These results suggest that some factors might have contributed to the improvement of performance, such as lowest dose of the inoculated agent, high health status of the farm, for this to be an experimental farm, and a lesser degree of inflammation of the intestinal mucosa post-challenge.

Kyriakis et al. (1999) conducted a study with the bacteria *B. licheniformis* and *B. toyoi* and observed lower incidence of diarrhea in relation to the control treatment. Similarly, Huyanate et al. (2006) found that animals that received treatment with bacteria and probiotic yeasts in high doses also have lower incidence of diarrhea. On the other hand, other authors did not find the same answers. Budiño et al. (2006), did not observe differences in the incidence of diarrhea in piglets who received *B. licheniformis* and *B. subtilis*, in the same way that Santos et al. (1998) did not observe differences in the incidence of diarrhea when they administered *Lactobacillus sp*.

Therefore, the results in the literature are controversial with respect to that variable, suggesting that differences found may be related to different types of probiotic bacteria, the dosage of the same, the amount of viable esporulo, besides the sanitary condition.

Regarding the elimination of *S. typhimurium* was observed that the room challenged of 35 days of age showed a higher frequency of the presence of the bacteria to 42 days and this result remained with high frequency up to 56 days of age.

However, in research with different inclusion concentration of this study, Szabó et al. (2009) found that the weaned piglets that received probiotic containing *Enterococcus faecium* presented more fecal elimination of *Salmonella* in relation to basal control treatment. The animals were challenged with the same agent, but at a high concentration (1.0 x 109 CFU).

These results and associated comments reflect the multifactorial nature of situations and complex comparative interpretation, as having different environments that depict the health status of the farm gives origin to different answers by different infection pressure, both from the quantitative and qualitative aspect. In addition, must take into consideration other factors such as the strain used in the probiotic, the quantity of inclusion of microorganisms, duration of the administration, interaction with other drugs, manufacture and storage of products, as how it was considered by Menten and Pedroso (2005). The probiotic administered may not be part of the natural microbiota of the host or can be present in insufficient number to form a colony in the gastrointestinal tract and thus being able to establish a relation of symbiosis with the host animal as observed by Dale (1992) and Collins and Gibson (1999).

Therefore, aspects that require further investigation to better clarify the actions of probiotics, as they suggest the ability of these products to improve the animal performance through modifications and stabilization in the intestinal microbiota in a given period of time.

During In the suckling phase, treatment with ProbA presented a viable alternative for the use of probiotics for differential gain obtained mainly in the first week of life of the piglets.

The performance of the animals who received the ProbA during the nursery was significantly better compared to other treatments, which indicates that probiotics could be a satisfactory alternative.

The performance of animals in the nursery was influenced against challenge with *Salmonella typhimurium*, held to 35 days old. The challenge scheduled reproduced a distinct condition existing in the experimentations environment, bringing a positive aspect of the experimental model.

REFERENCES

Abe F, Ishibashi N, Shimamura S. Effect of administration of Bifidobacteria and Lactic Acid Bacteria to newborn calves and piglets. *J Dairy Sci.* 1995; 78(12): 2838-2846.

Abipes - Associação Brasileira Da Indústria Produtora E Exportadora De Carne Suína [Internet]. 2009 dec 10 [update 2009 dec 10]. Available from: http://www.abipecs.org.br/uploads/relatorios/relatorios-associados/ABIPECS_relatorio_2009_pt.pdf.

Abipes - Associação Brasileira Da Indústria Produtora E Exportadora De Carne Suína. Principais Destinos – Dados Anuais [Internet]. 2011 ago 05]. Available from: http://www.abipecs.org.br/uploads/relatorios/mercado-externo/destinos/dados-anuais/JULHO_11_PRINCIPAIS_DESTINOS.pdf

Abrahão AAF, Vianna WL, Carvalho LFOS, Moretti, AS. Causas de mortalidade de leitões neonatos em sistema intensivo de produção de suínos. *Braz J Vet Res An Sci.* 2004; 41: 86-91.

Balaji R, Wright KJ, Hill CM, Dritz SS, Knoppel EL, Minton JE. Acute phase responses of pigs challenged orally with *Salmonella typhimurium. J Anim Sci.* 2000; 78:1885-1891.

Budiño FEL, Thomaz MC, Kronka RN, Tucci FM, Fraga AL, Scandolera AJ, Huaynate RAR, Nadai A, Correia RC. Efeito da adição de probiótico e/ou prebiótico em dietas de leitões desmamados sobre o desempenho, incidência de diarréia e contagem de coliformes totais. *Braz J Vet Res An Sci*. 2006; 43: 59-67.

Butolo JE. Uso de aditivos na alimentação de aves: frangos de corte. In: Simpósio Sobre as Implicações Sócio-Econômicas do Uso de Aditivos na Produção Animal. Piracicaba; 1999. Anais... Piracicaba: CBNA, 85-98.

Brugalli I. Alimentação alternativa: a utilização de fitoterápicos ou nutracêuticos como moduladores da imunidade e desempenho animal. In: Simpósio Sobre Manejo e Nutrição de Aves e Suínos. Campinas; 2003. Anais... Campinas: CBNA, 167-182.

Bruno DG. Efeito de um fito composto no desempenho de leitões submetidos ao desafio experimental com *Salmonella typhimurium* **[**PhD Thesis]. Pirassununga: University of São Paulo; 2008.

Collins MD, Gibson GR. Probiotics, prebiotics and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nut.* 1999; 69: 1052S. Suplemment, 1.

Corrêa VS, Caramori Júnior JG, Vieites FM, Abreu JG, Barros DS. Probiótico líquido para leitões lactentes em diferentes idades. *Rev Bras Saúde Prod Anim.* 2010; 11 (3): 827-837.

Dale N. Probióticos para aves. *Avi Prof.* 1992; 10(3): 88-89.

Etheridge RD, Seerley RW, Wyatt RD. The effect of diet on performance, digestibility, blood composition and intestinal micro flora of weaned pigs. *J Anim Sci.* 1984; 58:1396-1402.

Kyriakis SC, Tsiloyiannis VK, Vlemmas J, Sarris, K, Tsinas AC, Alexopoulos C, Jansegers L. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. *Res Vet Sci.* 1999; 67(3): 223-228.

Loughmiller JA, Dritz SS, Nelssen JL, Tokach MD, Goodband RD, Moser SA, De La Llata M. Effects of *Salmonella typhimurium* Challenge on swine Growth, Nitrogen Balance, Insulin-like growth factor-I and Acute phase proteins. *American J Anim Vet Sci.* 2007; 2(1): 11-22.

Menten JFM, Pedroso AA. Fatores que interferem na eficácia de probióticos. In: Conferência Apinco De Ciência E Tecnologia Avícolas, Santos; 2005. Anais... Santos: Fundação Apinco de Ciência e Tecnologia Avícolas, 41-53.

Nabuurs MJ, Hoogendoorn A, Van der Molen EJ, Van Osta AL. *Villus* height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. *Res Vet Sci*. 1993; 55:78-84.

Palermo JN. Uso de medicamentos veterinários: Impactos na moderna avicultura. In: Simpósio Brasil Sul De Avicultura, Chapecó; 2006. Anais... Chapecó, 70-78.

Parazzi LJ. Efeito da combinação de probióticos na dieta de leitões desafiados com *Salmonella* Typhimurium [PhD Thesis]. Pirassununga: University of São Paulo; 2010.

Santos MS, Ferreira CLLF, Gomes PC, Santos JL, Pozza PC. Avaliação da administração de Lactobacillus sp. No desempenho de leitões na fase de aleitamento e creche. In: Reunião Anual Da Sociedade Brasileira De Zootecnia. Botucatu; 1998. Anais... Botucatu: SBZ, CD-ROM.

Sas. STATISTICAL ANALISYS SYSTEM. SAS User's Guide. Version 9.2 Edition. Cary: SAS Institute, Inc., 2008.

Silva CA, Hoshi EH, Pacheco GD, Briganó MV. Avaliação de probióticos (*Pediococcus acidilactici* e *Bacillus subtilis*) após o desmame e efeitos no desempenho dos leitões, *Semina Ciênc Agrár*. 2006; 27(1):133-140.

Stewart CS, Chesson A. Making sense of probiotics. *Pig Vet J*. 1993; 31:11-33.

Szabó I, Wieler LH, Tedin K, Scharek-tedin L, Taras D, Hensel A, Appel B, Nockler K. Influence of a Probiotic Strain of *nterococcus faecium* on *Salmonella enteric* Serovar Typhimurium DT104 Infection in a Porcine Animal Infection Model. *Appl Environ Microbiol*. 2009; 75(9): 2621–2628.

Turner JL, Dritz SS, Higgins JJ, Herkelman KL, Minton JE. Effects of a *Quillaja saponaria* extract on growth performance and immune function of weanling pigs challenged with *Salmonella typhimurium*. *J Anim Sci*. 2002; 80: 939–1946.

Wang A, Yu H, Gao X, Li X, Qiao S. Influence of *Lactobacillus fermentum* I5007 on the intestinal and systemic immune responses of healthy and *E. coli* challenged piglets. *Antonie Van Leeuwenhoek*. 2009; 96:89-98.

Received: October 06, 2011;
Revised: August 14, 2012;
Accepted: December 17, 2012.