INFLUENCE OF SWINE PLASMA AND WHOLE EGG IN RATIONS OF WEANED PIGLETS ON STRUCTURE AND ULTRA-STRUCTURE OF INTESTINAL MUCOUS DEVELOPMENT

PLASMA SUÍNO E OVO INTEIRO EM RAÇÕES DE LEITÕES DESMAMADOS SOBRE A ESTRUTURA E ULTRA-ESTRUTURA DA MUCOSA INTESTINAL[1]

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SUMMARY

The objective of the study was to evaluate the inclusion of skim powdered milk, inclusion of increasing levels of swine plasma and whole egg, as well as soybean meal in diets of piglets that were weaned at 21 days old influence the development of the structure and ultra-structure of intestinal mucous. Plasma and whole egg replaced , respectively, 25, 50 and 75 and 15, 30 and 45% crude protein of skim milk. At 27 and 35 days old, the animals were slaughtered and intestine samples were collected for further measurements of villus height, crypt depth, villus:crypt ratio and villus density. The whole egg added to the ration to replace up to 45% the crude protein of skim milk, maintained the intestinal mucous of the piglets. The use of ration with high level of soybean meal damaged the structure and ultra-structure of the intestinal mucous, thus showing the necessity of limiting the inclusion of soybean meal into the weaning ration. The use of plasma stimulates the development of the intestinal mucous, independently of feed intake, showing the potential of this ingredient to promote an increase of the intestinal epithelium and the nutrient digestion and absorption areas as well.

KEY-WORDS: Intestinal villus. Electron micrographs. Nutrition. Swine.

RESUMO

Avaliou-se a inclusão de leite em pó, níveis crescentes de plasma suíno, níveis crescentes de ovo inteiro e alto nível de farelo de soja em dietas de leitões desmamados aos 21 dias de idade sobre o desenvolvimento da estrutura e ultra-estrutura da mucosa intestinal. O plasma e o ovo substituíram, respectivamente, 25, 50 e 75 e 15, 30 e 45% a proteína bruta do leite em pó. Aos 27 e 35 dias de idade os animais foram abatidos para coleta de amostras do intestino e posterior determinação da altura de vilosidade, profundidade de cripta, relação vilo:cripta e densidade de vilos. A utilização de ovo inteiro substituindo até 45% a proteína bruta do leite em pó possibilitou a manutenção do epitélio intestinal semelhante ($P<0,05$) ao leite em pó. A utilização de alto nível de farelo de soja proporcionou danos na estrutura e ultra-estrutura da mucosa intestinal, confirmando a necessidade de limitação da inclusão de farelo de soja na ração de desmama. O plasma estimula o desenvolvimento da mucosa intestinal, independentemente do consumo de ração dos leitões, indicando o potencial uso deste ingrediente em favorecer o aumento do epitélio intestinal e das áreas de digestão e absorção de nutrientes.

PALAVRAS-CHAVE: Elétron-micrografias. Nutrição. Suínos. Vilosidade intestinal.

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INTRODUCTION

Modifying the diet and pattern of feed intake at weaning, also changes the characteristics of the villi, indicating problems in the digestive and nutrient absorption capacity of the piglets. The decrease of the villi surface in the small intestine predisposes animals to poor absorption of nutrients, and possible dehydration and diarrhea as well (SCANDOLERA et al., 2005).

Research results have shown that diets containing lactose and proteins of animal origin such as plasma, red blood cells and egg (FIGUEIREDO, 2003) may decrease the severity of the effects resulting from weaning the piglets.

There is a positive correlation between post-weaning feed intake and intestine integrity morphology, which indicates the stimulating effect that ingested food has on the development of the intestinal epithelium (JIANG et al., 2000). Therefore, maintaining consumption pattern and continuing to supply specific ingredients and nutrients to the piglets, may help maintain the capacity of digestion and absorption of the intestinal epithelium after weaning.

The use of animal plasma in the diet has a positive effect on piglet performance (DIJK et al., 2001), intestinal mucosa and immunity system. This may be due to direct stimulation resulting from greater food intake or to the presence of factors and/or specific nutrients found in the plasma, such as immunoglobulins (GATNAU et al., 1995).

Similar to swine plasma, the whole egg is a food rich in immunoglobulins with high amino acid levels. The immunoglobulins present in the egg act on piglet microbial environment, thus becoming a potential source of protection (HARMON et al., 2000). However, the action mechanism of the plasma and egg added to the ration on the physiology of the digestive tract has not been completely elucidated yet. The understanding of this mechanism is important, since it will clarify how protein sources affect piglet performance .

The objective of the present work is to evaluate the development of the structure and ultra-structure of the intestinal mucosa of 28 and 35 day-old piglets that were weaned at 21 days old and fed diets containing swine plasma and whole egg as replacement for powder milk, and high levels of soybean meal as a protein source.

MATERIAL AND METHODS

In the experiment, 64 male and female hybrid piglets, weaned at 21 days old were used. The animals were housed in two brick warehouses divided into 36 individual stalls of 1.50 x 1.70 m, equipped with communicating bowl type drinker and semi-automatic feeder.

The experimental diets were formulated to meet or exceed the recommendations of NRC (1998). The isonitrogenous, isolisinics diets maintained amino acids levels and were supplemented with minerals and vitamins. Eight diets were used (Table 1), one contained powdered milk (PM) and the other soybean meal (SbM) as main protein source, another three with increasing levels of swine plasma (SP) and three diets with increasing levels of powdered whole egg (WE). Swine plasma (SP) levels of 25, 50 and 75%; while whole egg (WE) levels of 15, 30 and 45% were added to the diets to replace the powdered milk protein in the diet.

For the histological evaluation of the intestinal mucosa and ultra-structure of the duodenum, jejunum and ileum, the piglets were slaughtered at 28 and 35 days old (7 and 14 days post-weaning), with four replicates per treatment. After slaughter, two 1-cm segments were collected from each one of three portions of the intestine. Four piglets were slaughtered at the time of weaning and the gut samples collected corresponded to the control group.

Subsequently, these samples were opened at the mesenteric border, extended by the serosa and fixed in Bouin solution for 24 hours. After, they were transferred to 70% alcohol and processed by standard paraffin method. Sections of 4 to 6 μm were cut and stained according to hematoxylin and Harris-eosin technique. The measurements of villus height and crypt depth were performed in 30 villi, using the image analyzer Krontron Eletronik (Video Plan) magnified 230 times.

To evaluate the ultrastructure of the duodenum, jejunum and ileum and to determine villi density, the samples were fixed in 3% glutaraldehyde and 0.1 M sodium cacodylate buffer, pH 7.4 and post-fixed in 2% osmium tetroxide. Subsequently, the samples were dehydrated through a graded ethanol series, critical point dried using CO_2, then mounted on stubs coated with 32 nm metallic gold-palladium and examined under electron microscope to obtain the electron micrographs. In this analysis the data obtained from the ileum portion were discarded since the characteristics of the mucosa prevented an accurate count to determine the number of villi.

For statistical analysis, the control treatment PM was considered as level 0% of SP and level 0% of WE, totaling eight treatments and the following decomposition of orthogonal contrasts: C1- soybean meal vs other treatments; C2- plasma levels vs whole egg levels; C3- plasma linear regression; C4- plasma quadratic regression; C5- plasma cubic regression; C6- egg linear regression; C7- egg quadratic regression; and C8- egg cubic regression.

To evaluate the histological characteristics of duodenum, jejunum, and ileum, the randomized block design was used to control initial weight differences, in a factorial design with two slaughtering times, eight treatments and four repetitions.

The evaluation of the interaction between slaughtering age and treatments was performed using variance analysis, the mean slaughtering ages were compared by Tukey and the treatment means decomposed into orthogonal contrasts. Statistical

analysis was performed using the software PROCGLM, SAS (SAS Institute Inc., 1993).

RESULTS AND DISCUSSION

Table 2 shows villus mean height (VH), crypt depth (CD) and villus:crypt ratio (VC) in the portions of the duodenum, jejunum and ileum of piglets fed diets containing different protein sources, from 21 to 34 days old, slaughtered at 21, 28 and 35 days old.

There was significant correlation ($P<0.03$) between age and treatment for VH in the duodenum. As for age effect on the treatments, it was found that piglets consuming food with high SP levels, intermediate WE and SbM levels, VH increased with age.

The interactions between treatment and age, showed that 28 day-old piglets fed the SbM diet had lower duodenum VH compared to other treatment means (292.5 x 425.9 μm). At 35 days old, duodenum VH of piglets fed SbM was not significantly different than other treatments. These data can be explained by reduced feed intake of animals fed SbM diet during the first post-weaning week and the presence of allergenic factors that damage the intestinal mucosa. Soares et al. (2000) also reported lower villi heights in the proximal small intestine of piglets fed diets containing soybean meal compared to animals that were fed diets with powdered milk, one week after weaning.

At 35 days old, the similarity between VH of animals fed diet with SbM and other treatment means, can be explained by the piglet need to adapt to the diet containing SbM.

At 28 days old, increasing SP levels and duodenum VH fitted quadratically, according to the equation $Y_{AV} = 394.45 + 2.8298X - 0.048X^2$ ($R^2 = 0.80$). The highest VH was estimated at 419.01 μm, when SP replaced 29.48% protein of the powdered milk.

The observed increase of VH, in the duodenum, when 25 and 50% swine plasma protein was added to the diet, shows that plasma stimulates directly villus growth in the duodenum at 28 days of age, since feed consumption between piglets fed diets with 25 and 50% of SP and PM was similar. The decreasing VH with increasing SP level is possibly associated with lower feed intake, since consumption was 23.10% lower compared to PM diet.

At 35 days old, SP levels increased linearly with duodenum VH, according to the equation $Y_{AV} = 363.09 + 1.367X$ ($R^2 = 0.80$). These results suggest that specific factors or nutrients found in the SP, stimulate the development of intestinal villi, thus showing one of the mechanisms by which the plasma added to piglet diet at an early stage can improve performance. The observed increase of duodenum VH when SP increased, resulted in 35-day old piglets with mean VH absolute values similar to control group (488.4 x 475.9μm). These results showed that the use of plasma in the weaning diet resulted in a quick recovery of the intestinal epithelium. Klurfeld (1999) reported that the gastrointestinal tract is sensitive to quantitative and qualitative changes in the diet and that the presence of specific nutrients in the intestinal lumen can also influence its growth and development.

Age ($P<0.02$) and egg quadratic regression ($P<0.03$) affected significantly duodenum crypt depth. As slaughtering age increased, duodenum CD also increased. The comparison of duodenum CD of the control group with that of 28 and 35 day-old piglets, showed that in the first week CD remained unchanged, suggesting that food restriction interfered with crypt cell division, while the increase observed at 35 days indicated an increased rate of crypt cell production. According to Vente-Spreeuwenberg et al. (2003) the decrease in crypt cell production results in villi atrophy, a mechanism attributed to inadequate intake of energy and protein.

The levels of WE had a quadratic effect on CD, independent of slaughtering age, according to the equation $Y_{PC} = 234.8951 + 2.9535X - 0.076515X^2$ ($R^2 = 0.86$), with inflection point maximum estimated at 263.40 μm when WE replaced 19.3% of PM protein. These results suggest that replacing PM by WE protein at levels above 19.30% resulted in lower crypt cell proliferation, thus indicating better preservation of the intestinal epithelium.

Age and treatment ($P<0.05$) affected significantly the V:C ratio in the duodenum portion. As slaughtering age increased, the V:C ratio of piglets fed diet containing 75% SP also increased. The higher V:C ratio indicates a higher absorption capacity of the intestinal villi, which confirms the positive effect of SP on the intestinal mucosa. When evaluating the effect of treatments at each slaughtering age, at 28 days, there were significant differences for the plasma an egg quadratic regressions, and at 35 days, for plasma and egg levels and for plasma cubic regression.

At 28 days old, increasing levels of SP influenced quadratically the V:C ratio in the duodenum, according to the equation $Y_{RVC28} = 1.69280 + 0.0174966X - 0.000265060X^2$ ($R^2 = 0.63$). These results show the positive effect of SP on intestinal morphology and consequently, on intestinal absorption ability. The highest estimated V:C ratio was 1.98, when 33.01% PM protein was replaced by SP, at 28 days.

At 35 days old, SP levels influenced cubically the V:C ratio according to $Y_{RVC35} = 1.58533 + 0.375264X - 0.00124907X^2 + 0.0000111595X^3$ ($R^2 = 1.00$). Increasing WE levels influenced quadratically the V:C ratio in the duodenum of 28 day-old piglets according to $Y_{V:C} = 1.78643 - 0.0289536X + 0.000707950X^2$ ($R^2 = 0.84$). The lowest V:C ratio estimated was 1.490 when 20.45% of PM protein was replaced by WE.

At 35 days old, the average V:C ratio of piglets fed diets with increasing levels of SP was higher compared to the ones fed increasing levels of WE, confirming that the nutrients present in the plasma resulted in better integrity of the intestinal mucosa of post-weaning piglets. A higher Villus:Crypt ratio shows that a greater surface area is exposed to the lumen for absorption of nutrients.

The jejunum VH, CD, and V:C ratio increased significantly ($P<0.01$) as slaughtering age increased. The SP treatment influenced cubically VH ($P<0.03$),

WE quadratically the CD (P<0.02) and SbM vs other treatments on the V:C ratio. The comparison of villi characteristics of 28-day old piglets and control group showed 20.05% reduction of jejunum mean VH, while for 35 day-old piglets, VH mean absolute value was 1.60% higher than the control group. On the other hand, jejunum crypt depth (CD) increased from 28.39 to 48.19%, respectively, for 28 and 35-day old piglets compared to control group.

These results are consistent with reports by several authors, who observed changes in post-weaning VH and CD, as age increased. Among them, Cera et al. (1988) who reported VH reduction, between three and seven days post-weaning. Also Soares et al. (2000) compared the VH and CD means of piglets weaned and slaughtered at 21 days old (control group) with piglets submitted to different treatments that were slaughtered at 28 and 35 days, and observed that VH decreased 25.2% average and CD increased 58.2% average. The authors justified that the lower reduction of VH was due to the fact that piglets were fed ration in the maternity.

The SP levels in the diet influenced cubically the VH of the jejunum, according to $Y_{AV} = 388.6375 – 5.7179X + 0.26883X^2 - 0.002485X^3$ ($R^2 = 1.00$), while CD was similar. Although this cubic response does not have a biological explanation, it indicates a lack of fitting to the quadratic regression model, and confirms that diet consumption as well as the presence of specific nutrients influenced both regeneration and growth of piglet intestinal epithelium. The observed increase of VH, when 25 and 50% of PM protein was replaced by SP, confirms the stimulus of the proteins or peptides on the intestinal epithelium, while the decreasing VH when 75% SP was used, is related to the lower VH at 28 days old, resulting from lower feed intake. This confirms how dry matter intake stimulates the development of the gastrointestinal tract, as demonstrated by Cera et al. (1988) and McCracken et al. (1999).

The positive results that animal plasma supplemented feed had on jejunum VH and CD of post-weaning piglets were also reported by Gatnau et al. (1995). These authors observed an increase of intestinal villi surface area of animals fed diets supplemented with 8% swine plasma, which indicated that growth stimulating factors are present in the immunoglobulin fraction contained in the plasma.

However, the results reported in this study disagree with Carlson & Veum (2000) who found no effect of plasma or vegetal peptide on the jejunum VH of post-weaning piglets. While, in the same study, CD was smaller and there was a tendency for greater V:C ratio in the intestine of 28 days-old piglets fed diets supplemented with plasma and vegetal peptide compared to control group.

Some authors stated that plasma beneficial effects are associated with the presence of active immunoglobulins. However, the absorption of intact protein post-weaning is unlikely, these favorable effects observed are more likely to be related to the peptides formed after hydrolysis of the proteins present in the immunoglobulin fraction and/or by specific amino acid concentrations, which exerts a stimulating effect on intestinal enterocytes (EWTUSHIK et al. ,2000).

The increasing WE levels influenced quadratically CD, according to $Y = 179.7139 + 2.7658X – 0.05585X^2$ ($R^2 = 0.99$), and the maximum CD was estimated at 213.96 μm when 24.76% PM protein was replaced by WE. As it was observed for the duodenum portion, the replacement of PM protein by WE at levels higher than 24.76% decreased the proliferation of epithelial cells and favored mucosal integrity.

The mean V:C ratio in the jejunum of piglets fed SbM supplemented diet was lower than the other treatments. The results are due to SbM effect on diet consumption during the first week and soybean allergenic factors that promoted partial destruction of the villi, with consequent reduction of VH.

Villus height and the V:C ratio increased with increasing slaughtering age. Contrary to what was observed in the duodenum and jejunum, VH of piglet ileum at 28 days was higher than that of the control group, which shows that the distal portion of the intestine is less susceptible to weaning and nutrition.

Different levels of WE influenced cubically the mean CD of ileum according to $Y_{PC} = 193.4050 – 5.2235X + 0.3410X^2 - 0.005234X^3$ ($R^2 = 1.00$), also indicating a lack of fitting to another regression mathematical model.

The mean villi density (VD) per area of piglets being fed different protein sources, from 21 to 34 days old and slaughtered at 21, 28 and 35 days old are represented in Table 3. There was a 29.96% reduction of villus density, from 28 to 35 days old. Considering the control group, at 28 days old there was 23.32% increase of villus density, while at 35 days old, the number of villi per area was 13.7% lower than the control group. No significant difference was observed (P>0.05) between VD of piglets consuming SbM supplemented diet compared to the means from other treatments. Higher VD was observed in piglets fed diet supplemented with increasing SP levels, when compared to animals consuming diets with increasing levels of WE (63 x 56), which shows that growth factors, peptides or specific nutrients present in the plasma also affected cellular proliferation of intestinal villi.

The use of increasing SP levels to substitute the protein from PM, influenced cubically the VD of the piglet duodenum, according to $Y_{DV} = 55.4958 + 1.2366X – 0.04854X^2 + 0.0004775X^3$ ($R^2 = 1.00$). The highest VD in absolute value was observed in the duodenum of 28 day-old piglets fed diet with 75% of SP, which confirms plasma ability to stimulate the intestinal mucosa and suggests an increase of the absorption area, since a greater area of the mucosa was exposed and in contact with the intestinal lumen.

The use of increasing levels of WE to partially replace the protein from PM did not influence (P>0.05) VD of piglet duodenum slaughtered at 28 and 35 days old. The VD of the jejunum was significantly affected by age (P<0.01) and treatments (P<0.01). There was a statistically significant difference for contrast 4 (P<0.01).

Table 1 – Proximate and calculated composition of experimental diets.

Ingredients	PM	SP 25%*	SP 50%*	SP 75%*	WE 15%*	WE 30%*	WE 45%*	SbM
Powdered milk (PM)	23,50	17,47	11,43	5,30	20,48	17,46	14,45	8,74
Swine plasma (SP)	-	2,95	5,90	8,94	-	-	-	-
Whole egg (WE)	-	-	-	-	2,54	5,08	7,62	-
Soybean meal (SbM)	18,00	18,00	18,00	18,00	18,00	18,00	18,00	30,00
Lactose	2,34	5,48	8,63	11,83	3,91	5,48	7,05	10,03
Corn	43,10	43,50	43,81	44,00	40,96	38,82	36,67	38,41
Limestone	0,09	0,50	0,90	1,30	0,12	0,15	0,17	0,12
DL-methionine	0,22	0,24	0,26	0,27	0,20	0,18	0,16	0,26
Bicalcium phosphate	1,77	1,41	1,05	0,72	1,85	1,93	2,01	2,21
Inert (kaolin)	1,10	0,97	0,84	0,82	2,06	3,01	3,97	-
L-lysine HCl	0,39	0,41	0,43	0,46	0,40	0,40	0,41	0,51
L-threonine	0,20	0,20	0,19	0,19	0,20	0,20	0,20	0,25
L-tryptophan	0,02	0,02	0,01	-	0,03	0,03	0,03	0,03
Common salt	0,33	0,20	0,07	-	0,35	0,38	0,40	0,57
Sugar	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Antioxidant	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Vitamin mix **	0,60	0,60	0,60	0,60	0,60	0,60	0,60	0,60
Mineral mix ***	0,24	0,24	0,24	0,24	0,24	0,24	0,24	0,24
Soybean oil	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
Zinc oxide	0,22	0,22	0,22	0,22	0,22	0,22	0,22	0,22
Sodium carbonate	0,81	0,58	0,36	0,07	0,78	0,75	0,72	0,74
Total	100,00	100,00	100,00	100,00	100,00	100,00	100,00	100,00
Calculated Composition								
EM (kcal/kg)	3.500	3.500	3.500	3.495	3.500	3.500	3.500	3.444
Calcium	0,85	0,85	0,85	0,85	0,85	0,85	0,85	0,85
Fiber, %	2,19	2,18	2,17	2,16	2,13	2,08	2,02	2,83
Total phosphorus, %	0,80	0,80	0,80	0,80	0,80	0,80	0,80	0,80
Fat, %	7,92	7,95	7,98	8,00	8,65	9,38	10,11	7,90
Lactose, %	14,0	14,00	14,00	14,00	14,00	14,00	14,00	14,00
Lysine, %	1,60	1,60	1,60	1,60	1,60	1,60	1,60	1,60
Methionine+cystine, %	0,90	0,90	0,90	0,90	0,90	0,90	0,90	0,90
Methionine, %	0,62	0,60	0,58	0,57	0,60	0,59	0,58	0,59
Crude protein, %	20,50	20,50	20,50	20,51	20,50	20,50	20,50	20,50
Threonine , %	1,02	1,02	1,02	1,02	1,02	1,02	1,02	1,02
Tryptophan, %	0,28	0,28	0,28	0,28	0,28	0,28	0,28	0,28
Sodium, %	0,63	0,63	0,63	0,63	0,63	0,63	0,63	0,63
Chlorine, %	0,46	0,46	0,46	0,49	0,46	0,46	0,46	0,46
Potassium, %	0,86	0,77	0,67	0,58	0,82	0,77	0,73	0,83

* percent of powdered milk protein replaced in the diet.

** Vitamin mix – guaranteed levels per kg of product: vit A 4,000,000 UI, vit D_3 1,000,000 UI, vit E 10,000 mg, vit K_3 3,000 mg, vit B_{12} 9,000 mcg, vit B_2 3,800 mg, biotin 40 mg, calcium pantothenate 6,000mg, niacin 14,000 mg, choline 100g, antibiotic 150 g, antioxidant 60g, vehicle q.s. 1,000 g.

*** Mineral mix- guaranteed levels per kg of product: Fe 40,000 mg, Cu 35,000 mg, Mn 20,000 mg, Zn 40,000 mg, Co 360 mg, I 840 mg, Se 120 mg, vehicle q.s.1,000g.:

Table 2 – Villus height (VH), crypt depth (CD) in µm, villus:crypt ratio (V:C) in the duodenum, jejunum and ileum of piglets at 21, 28 and 35 days old, fed diets supplemented with powdered milk (PM), swine plasma (SP), whole egg (WE) and soybean meal (SbM).

	CT** (21 d)	PM	SP 25%*	SP 50%*	SP 75%*	WE 15%*	WE 30%*	WE 45%*	SbM	Mean [AGE]	VC (%)
Duodenum											
VH [IDxTRA]	475,9										17,71
28 d [C1,C4]		401,3a	414,6a	436,4a	329,6b	366,3a	312,5b	390,6a	292,5b	368,0	
35 d [C3]		380,9a	383,0a	409,5a	488,4a	407,6a	435,2a	385,1a	420,1a	413,7	
Mean		391,1	398,8	422,9	409,0	386,9	373,8	387,8	356,3		
CD [TRA]	225,8										18,86
28 d		229,8	234,6	211,3	237,1	228,8	235,1	203,6	181,6	220,2B	
35 d		247,0	217,8	238,2	235,4	274,4	295,0	215,2	240,1	245,3A	
Mean [C7]		238,4	226,2	224,7	236,3	251,6	265,0	209,4	210,8		
V:C [IDxTRA]	2,108										20,52
28 d [C4,C7]		1,754a	1,780a	2,089a	1,453b	1,608a	1,458a	1,949a	1,675a	1,720	
35 d [C2,C5]		1,585a	1,917a	1,734a	2,082a	1,529a	1,488a	1,801a	1,748a	1,735	
Mean		1,670	1,848	1,911	1,767	1,568	1,473	1,875	1,711		
Jejunum											
AV [TRA]	442,9										16,17
28 d		358,0	357,1	430,0	318,2	349,5	337,6	365,9	316,6	354,1 B	
35 d		419,3	392,7	498,4	529,2	440,5	492,7	421,8	405,1	450,0 A	
Mean [C5]		388,7	374,9	464,2	423,7	395,0	415,2	393,9	360,9		
PC [TRA]	140,9										15,78
28 d		172,8	190,3	193,5	179,8	195,9	173,5	181,4	160,5	180,9 B	
35 d		187,2	183,0	195,0	201,8	219,6	253,1	200,1	230,9	208,8 A	
Mean [C7]		180,0	186,7	194,3	190,8	207,8	213,3	190,8	195,8		
V:C [TRA]	3,14										24,17
28 d		2,131	1,925	2,207	1,748	1,822	1,955	2,048	2,043	2,001 B	
35 d		2,250	2,337	2,627	2,639	2,050	2,003	2,120	1,780	2,288 A	
Mean [C1]		2,190	2,131	2,417	2,193	1,936	1,979	2,084	1,911		
Ileum											
AV	243,5										16,31
28 d		292,6	314,2	289,6	255,7	299,3	264,9	306,2	269,0	286,4 B	
35 d		333,6	311,0	308,2	283,8	343,0	349,9	343,1	344,6	327,8 A	
Mean		313,1	312,6	298,9	269,8	321,1	307,4	324,7	306,8		
CD [TRA]	150,3										16,62
28 d		183,0	234,2	174,9	165,6	178,1	184,9	159,2	160,1	180,0	
35 d		203,8	181,5	181,4	197,0	170,1	219,6	184,5	204,4	193,2	
Mean [C8]		193,4	207,9	178,2	181,3	174,1	202,3	171,9	182,3		
V:C	1,620										23,14
28 d		1,643	1,360	1,658	1,548	1,713	1,445	1,956	1,707	1,630 B	
35 d		1,657	1,729	1,743	1,501	2,039	1,603	1,894	1,751	1,730 A	
Mean		1,650	1,544	1,694	1,524	1,876	1,524	1,925	1,729		

* % replacement protein from powdered milk, **CT – control slaughtered at the weaning day (21 days old).
Tested contrasts: C1: SbM vs other treatments; C2: SP levels vs WE levels; C3: SP Linear Regression (Reg); C4: quadratic regression SP; C5: Cubic Reg SP; C6: Linear Reg WE; C7: Quadratic Reg WE; C8: Cubic Reg WE.
ID x TRA – Interaction age x treatment: age effect within treatment, a,b – means in the same row followed by different letters differ by F test at 5%. Treatment effect within age: C1, C2, C3, C4 ,C7 - contrasts significant at 5%;
AGE – Age effect: A, B – means in the same column followed by different letters differ by F test at 5% . TRA – Treatment effect: C1,C5,C7, C8- contrast significant at 5%.

Table 3 – Villus density (nº/µm^2) in the duodenum, jejunum, ileum of piglets at 21, 28 and 35 days old, fed diets supplemented with powdered milk (PM), swine plasma (SP), whole egg (WE) and soybean meal (SbM).

	CT** (21 d)	PM	SP 25%*	SP 50%*	SP 75%*	WE 15%*	WE 30%*	WE 45%*	SbM	Mean AGE	VC (%)
Duodenum TRA	56										19,84
28 d		61	77	60	92	56	71	69	76	69,01 A	
35 d		50	48	51	55	53	37	49	44	48,33 B	
Mean C2, C5		55	63	56	77	55	54	59	60		
Jejunum TRA	34										23,89
28 d		80	60	64	106	72	91	70	96	78,92 A	
35 d		51	44	46	61	58	51	39	46	50,10 B	
Mean C4		67	52	56	80	65	74	54	84		
Ileum	nd										
28 d		46	58	71	59	72	61	64	64		
35 d		nd	nd	nd	nd	nd	nd	nd	nd		

* % replacement protein from powdered milk, **CT – control slaughtered at the weaning day (21 days old), nd – non-determined.
Tested contrasts: C1: SbM vs other treatments; C2: SP levels vs WE levels; C3: Linear Regression (Reg) SP; C4: quadratic regression SP; C5: Cubic Reg SP; C6: Linear Reg WE; C7: Quadratic Reg WE; C8: Cubic Reg WE.
AGE - Age effect: A, B – means in the same column followed by different letters differ by F test at 5% .
TRA – Treatment effect: C2, C4, C5 – contrasts statistically significant at 5%.

There was no statistically significant difference in the jejunum VD, among piglets fed diet supplemented with SbM and the average for other treatments. The mean jejunum VD between the piglet group fed diets supplemented with SP or WE were similar.

The increasing levels of SP influenced quadratically VD, in the jejunum portion of the intestine, according to $Y_{DV} = 62.7343 – 1.0450X + 0.017254X^2$ ($R^2 = 0.99$). The piglets fed diets with 25 and 50% SP had VD of 22.39 and 16.42%, respectively, lower than the piglets fed diet with PM. The diet supplemented with 75% SP had VD 0f 19.40% higher than the piglets that consumed PM diet.

The use of increasing levels of WE did not influence the VD of the jejunum, confirming previous results showing that, like PM, WE is an effective source of protein for piglets, and does not compromise the villi of the intestinal epithelium.

CONCLUSIONS

The supplementation of piglet diet with powdered egg maintains the intestinal epithelium. The use of plasma stimulates the intestinal mucosa, regardless of piglet feed intake. However, the use of soybean meal should be limited in the post-weaning diets.

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