ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 6 (1): 31-35, 2007
ISSN 1682-8356
© Asian Network for Scientific Information, 2007

Surface Area of the Tip of the Enterocytes in Small Intestine Mucosa of Broilers Submitted to Early Feed Restriction and Supplemented with Glutamine

A.V. Fischer da Silva[1], A. Maiorka[1], S.A. Borges[2], E. Santin[1], I.C. Boleli[3] and M. Macari[3]
[1]Universidade Federal do Paraná, Centro Politécnico, Setor de Ciências Biológicas,
Departamento de Fisiologia, Endereço: Rua Francisco H. dos Santos, sn,
Jardim das Américas, 81530-000 Curitiba, PR Brasil
[2]Faculdade de Ciências Biológicas e de Saúde - Universidade Tuiuti do Paraná - UTP
[3]Departamento de Morfologia, FCAV/UNESP, Jaboticabal, Brazil

Abstract: A total of 640 one-day-old male Cobb chicks were used to evaluate the effects of early feed restriction and glutamine on villi density and tip surface of enterocytes in the small intestine of broilers. A two-factor factorial experimental design with glutamine and feed restriction as main factors was used. Treatments consisted of quantitative feed restriction at 30% of *ad libitum* intake from 7 to 14 days of age, and glutamine addition at 1% in the diet from 1 to 28 days of age. Sections of the small intestine (duodenum, jejunum, ileum) were collected at 14 and 21 days of age for analyses by scanning and transmission electron microscopy. Villi density decreased with age and increased in cranial-caudal direction. Glutamine increased villi density in the small intestine. Microvilli density and height decreased with age. Glutamine increased microvilli width. The jejunum was the segment with the largest surface area of the tip of the enterocytes, followed by the duodenum and the ileum. Feed restriction decreased the surface area of the tip of the enterocytes in the small intestine at 14 and at 21 days of age. Glutamine supplemented in the feed increased the surface area of the tip of the enterocytes of the jejunum and ileum at 21 days of age.

Key words: Electron microscopy, feed restriction, glutamine, intestinal mucosa, microvilli amplification factor

Introduction

Although feed restriction and fasting managements are utilized in poultry production with the objective of reducing the broiler growth rate and fat deposition on carcass, the detrimental effects of these procedures on the intestinal mucosa of the animals are not frequently studied. Altmann (1972) described that fasting could reduce the mitotic activity of intestinal cells, while other researchers (Yamauchi *et al*., 1996; Shamoto *et al*., 1999 and Fischer da Silva, 2001) observed that feed restriction reduced villi height and crypt length in broilers, as well as the density, height and width of microvilli (Ferrer *et al*., 1991).

On the other hand, the density and size of villi and microvilli of small intestine are directly related with the absorption capacity of the birds (Macari, 1995). According to Ferrer *et al*. (1995), villi height and microvilli density are the best measurements to show the changes on surface area of absorptive cells.

Researchers recently showed that some amino acids in diets could reduce intestinal atrophy in patients submitted to parenteral feeding (Pierzynowski and Sjodin, 1998). Specially, glutamine has been utilized as an important energy source for the development of the intestinal mucosa (Windmueller and Spaeth, 1974). Glutamine was utilized to stimulate intestinal cell proliferation by improving ornithine carboxylase activity (Kandil *et al*., 1995; Fischer da Silva, 2001), thus increasing the absorptive surface of gastrointestinal mucosa and the utilization of dietary nutrients.

The objective of this study was to evaluate the ultrastructure of the intestinal mucosa of broilers submitted to early feed restriction (7 to 14 days of age) and supplemented with 1% of glutamine in diet.

Materials and Methods

This experiment was carried out with 640 one-day-old male Cobb chicks, submitted to usual broiler management practices. Water intake was offered *ad libitum* and the diets were isocaloric according with the National Research Concil (1994). Feed restriction was quantitative (30% of *ad libitum* feed intake) from 7 to 14 days of age, and 1% of glutamine was supplemented until 28 days of age.

A two-factor factorial experimental design was utilized (glutamine x restriction), with 4 treatments with 4 replicates each, as follows: T1 - group with 1% of glutamine supplementation and diet offered *ad libitum*; T2 - 1% of glutamine supplementation and feed restriction; T3-without glutamine supplementation and *ad libitum* feeding; T4 - without glutamine supplementation and feed restriction. From 15 days of age, birds previously submitted to feed restriction were fed *ad libitum* until 49 days of age.

At14 and 21days of age, four birds per treatment were euthanized and samples of approximately 2-cm of

duodenum, jejunum and ileum were taken. Samples were fixed in 2.5% glutaraldehyde in buffer phosphate 0.1 M pH 7.4 overnight. After that, samples were washed in the same buffer and post-fixed in 1% diluted osmium tetraoxide for one hour. Then, samples were washed in phosphate buffer 0.1M, pH 7.4, and dehydrated in ethanol (50, 60, 70, 80, 90 and 100%), and then dried and recovered with gold to be electron microphotographed and analyzed by scanning electron microscopy. Villi number was measured for the average of 3 areas of 103269µm^2/sample.

For transmission electron microscopy, four segments of approximately 1 mm^2 were taken from duodenum, jejunum and ileum and fixed in 2.5% glutaraldehyde in phosphate buffer for 6 hours at 4°C, and postfixed in osmium tetraoxide 1% for 2 hours. Then, the samples were dehydrated in ethanol (50, 60, 70, 80 90 and 100%). Later, they were infiltrated with 1:1 ethanol-Epon 812 resin at room temperature for 2 hours and embedded in Epon 812 resin at 60°C for 72 hours. The samples were then sectioned, contrasted with lead citrate and uranyl acetate and electron-microphotographed by transmission electron microscopy.

Longitudinal sections of cell were evaluated by image analysis in order to measure: tip enterocytes diameter (µm), microvilli height and width (µm); and microvilli density (microvilli number/1µ2m). The average of each measurement corresponded to the measurements of 10 samples/bird.

The surface area of the tip of the enterocytes was calculated based on the tip diameter (Cd) of the cell and the extension factor of microvilli (EFM), according to Ferrer *et al.* (1995), using the equation: S=p x Cd2/4 x EFM, where EFM = π x H x d x D + 1 (L = microvilli height; d = microvilli width and D = microvilli number/um^2).

All data were submitted to ANOVA and multiple comparisons were done by the Tukey's test with 5% of significance.

Results and Discussion

Table 2 shows the results of villi density in the intestine of broilers at 14 and 21 days of age. We observed a reduction in villi number with age, and an increase in cranial-caudal direction (duodenum to ileum) in the birds submitted to *ad libitum* feeding without glutamine supplementation. Similar results were also presented by Yamauchi and Isshiki (1991), who observed that villi number was reduced in 10-day-old birds. Nevertheless, these results do not mean a reduced absorptive capacity/segment, but a higher development of villi. Reduced villi number/area related with age is followed by an increase in villi height (Macari, 1995; Fischer da Silva, 2001).

At 14 days of age, feed restriction reduced (P<0.05) villi numbers; however glutamine increased villi numbers in the jejunum and ileum (Fig. 2). At 21 days of age, feed restriction reduced villi numbers in the three segments of the small intestine. Glutamine increased villi numbers (P<0.05) in the duodenum and jejunum in 21-day-old birds submitted to *ad libitum* feeding. Rhoads *et al.* (1997) suggested that the glutamine effect could be a response to increased decarboxylase enzyme (ODC) activity. Fischer da Silva (2001) showed an increase in ODC expression in the small intestine of birds supplemented with glutamine in diet.

The results of microvilli height are shown in Table 2. Feed restriction reduced microvilli height in the duodenum and jejunum of birds at 14 and 21 days of age. On the other hand, an interaction between feed restriction and glutamine at 21 days was observed, showing that, although feed restriction reduced microvilli height, the presence of glutamine in this diet increased it.

As to microvilli width, no significant differences between the treatments (Table 3) were observed at 14 days of age, but at 21 days, microvilli of the ileum of birds supplemented with glutamine were wider than in birds fed *ad libitum* without glutamine.

The results of microvilli density showed that feed restriction reduced microvilli number per area in the duodenum and ileum of 14-day-old birds. In the jejunum, at 14 days of age, microvilli number per area was lower in birds fed *ad libitum* and supplemented with glutamine as compared to birds from the other treatments. In 21-day-old birds, although microvilli number in the jejunum increased when glutamine was supplemented, this number was reduced in birds submitted to feed restriction.

The results of surface of the tip of the enterocytes indicates that the jejunum have the largest absorption area in the small intestine, followed by the duodenum and the ileum (Table 5). These results are consistent with the findings of Ferrer *et al.* (1995), in male White Leghorn chickens during the first and second week of life. Buddington and Diamond (1989) described an increase in monosaccharide absorption during the second week of age, and Rovira *et al.* (1994) showed an increase in absorption only in the jejunum during the two first weeks of life. However, these authors observed a higher intestine growth in all segments of small intestine during this period, suggesting a high metabolic demand in these young birds.

In the study presented herein, feed restriction reduced the surface area of the tip of the enterocytes of the small intestine of 14- and 21-day-old birds. In contrast, birds supplemented with glutamine and fed *ad libitum* showed larger tip surface area in the enterocytes of the jejunum and ileum in 21-day-old birds.

The magnitude of the surface area of the tip of the enterocytes is larger in duodenum and ileum in 14-day-old - birds than in 21-day-old birds. In contrast, in the

Table 1: Villus density (villus number/103269µm^2) per segment of small intestine of broilers at 14 and 21 days of age

Groups		Duodenum		Jejunum		Ileum	
Days of age		14	21	14	21	14	21
Feed *ad libitum* with glutamine		28	19	67	33	92	56
Feed restriction with glutamine		22	17	48	31	62	36
Feed *ad libitum* without glutamine		25	15	39	32	71	51
Feed restriction without glutamine		17	12	37	27	53	45
Glutamine	0.0%	25	18[a]	57[a]	32[a]	77[a]	48
	0.1%	21	13[b]	38[b]	29[b]	62[b]	46
Feed	Restriction	26	17[a]	53	32[a]	81[a]	54[a]
	Ad libitum	19	14b	43	29b	58[b]	41[b]
Coefficient of variation (%)		23.6	18.0	10.5	5.3	6.3	16.0

Table 2: Height microvilli (µm) of small intestine villi of broilers at 14 and 21 days of age

Groups		Duodenum		Jejunum		Ileum	
Days of age		14	21	14	21	14	21
Feed *ad libitum* with glutamine		3.41	1.86	3.48	2.31	1.83	1.43
Feed restriction with glutamine		2.78	1.74	2.22	2.16	1.68	1.23
Feed *ad libitum* without glutamine		3.20	1.92	3.04	2.24	1.84	1.46
Feed restriction without glutamine		2.30	1.63	2.47	2.14	1.43	1.06
Glutamine	0.0%	3.10[a]	1.80	2.85	2.24	1.76	1.33
	0.1%	2.75[b]	1.78	2.76	2.19	1.63	1.26
Feed	Restriction	3.30[a]	1.89[a]	3.26[a]	2.27	1.83	1.45
	Ad libitum	2.54[b]	1.69[b]	2.35[b]	2.15	1.56	1.15
Coefficient of variation (%)		7.3	2.3	7.1	5.2	13.2	20.1

Table 3: Microvilli width (µm) of small intestine villi of broilers at 14 and 21 days of age

Groups		Duodenum		Jejunum		Ileum	
Days of age		14	21	14	21	14	21
Feed *ad libitum* with glutamine		0.07	0.07	0.08	0.10	010	0.09
Feed restriction with glutamine		0.07	0.07	0.08	0.09	0.10	0.09
Feed *ad libitum* without glutamine		0.07	0.07	0.09	0.09	0.09	0.07
Feed restriction without glutamine		0.07	0.07	0.09	0.08	0.09	0.07
Glutamine	0.0%	0.07	0.07	0.08	0.09	0.10	0.09[a]
	0.1%	0.07	0.07	0.09	0.08	0.09	0.07[b]
Feed	Restriction	0.07	0.07	0.09	0.10	0.09	0.08
	Ad libitum	0.07	0.07	0.09	0.08	0.09	0.08
Coefficient of variation (%)		6.8	4.0	8.4	9.2	6.6	10.1

Table 4: Microvilli density (microvilli number/um^2) of small intestine villi of broilers at 14 and 21 days of age

Groups		Duodenum		Jejunum		Ileum	
Days of age		14	21	14	21	14	21
Feed *ad libitum* with glutamine		77	67	29	67	71	38
Feed restriction with glutamine		67	76	48	23	48	21
Feed *ad libitum* without glutamine		87	75	39	80	80	43
Feed restriction without glutamine		71	72	75	25	45	42
Glutamine	With	79	72	38[b]	45	62	29[b]
	Without	72	74	57[a]	52	59	43[a]
Feed	Restriction	82[a]	71	34[b]	73[a]	75[a]	41
	Ad libitum	69[b]	74	61[a]	22[b]	46[b]	31
Coefficient of variation (%)		12.0	12.1	8.1	27.0	15.6	20.0

Table 5: Tip surface area (µm^2) of small intestine enterocytes of broilers at 14 and 21 days of age

Groups		Duodenum		Jejunum		Ileum	
Days of age		14	21	14	21	14	21
Feed *ad libitum* with glutamine		992	545	1276	2321	775	406
Feed restriction with glutamine		731	392	440	327	537	170
Feed *ad libitum* without glutamine		987	566	1258	1568	852	240
Feed restriction without glutamine		551	314	644	295	361	176
Glutamine	With	862	468	858	1324[a]	656	288[a]
	Without	769	440	951	931[b]	606	208[b]
Feed	Restriction	990[a]	555[a]	1267[a]	1944[a]	813[a]	323[a]
	Ad libitum	641[b]	353[b]	542[b]	311[b]	449[b]	173[b]
Coefficient of variation (%)		14.0	9.3	13.3	14.3	17.0	22.3

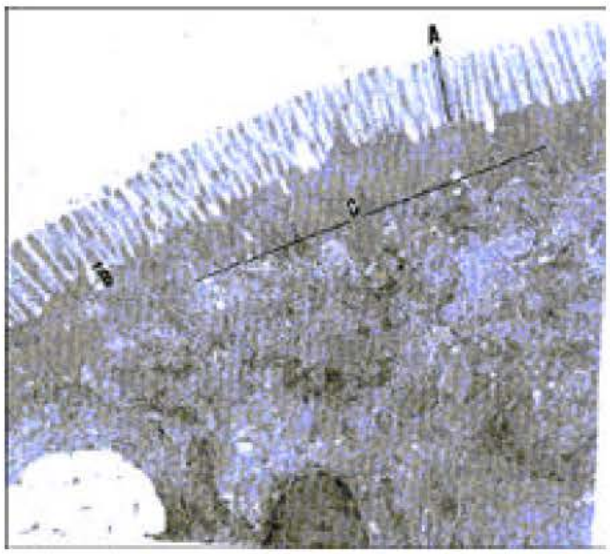

Fig. 1: Transmission electron-microphotography of the duodenum in a chick at 21 days of the age, (A) microvilli height, (B) microvilli width, (C) enterocytes diameter (X20000)

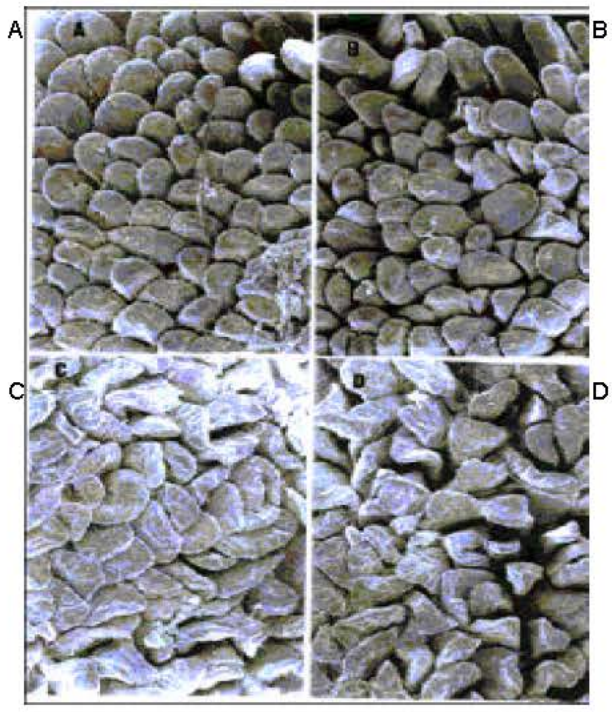

Fig. 2: Scanning electron-microphotography of the ileum of chicks at 14 days of the age, showing higher villi density in the treatments with glutamine (A; B). (A) *Ad libitum* feeding with glutamine; (B) Feed restriction with glutamine (higher density); (C) *Ad libitum* feeding without glutamine; (D) Feed restriction without glutamine (X100).

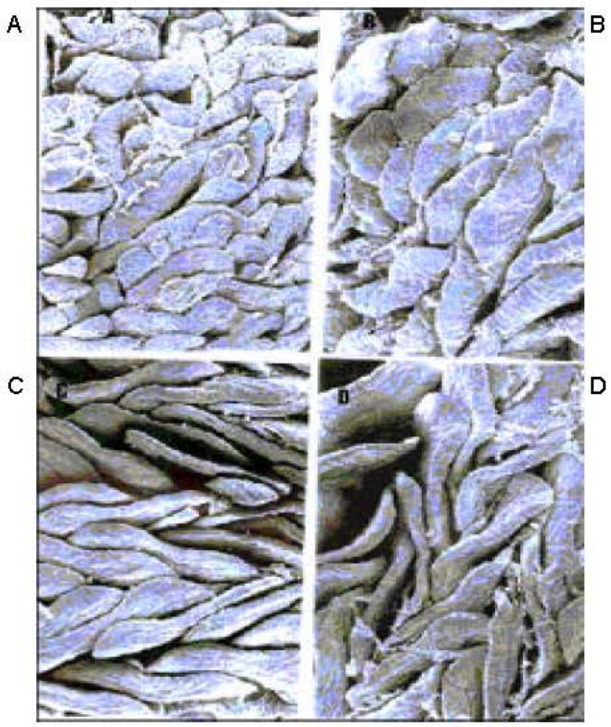

Fig. 3: Scanning electron-microphotography of the jejunum of chicks at 21 days of the age, showing higher villi density in the treatments with glutamine (A; B). (A) *Ad libitum* feeding with glutamine; (B) Feed restriction with glutamine (higher density); (C) *Ad libitum* feeding without glutamine; (D) Feed restriction without glutamine (X100).

jejunum, the magnitude of the tip surface area is larger in 21-day-old birds as compared to 14-day-old birds. The same tendency was found for microvilli density in these segments in both ages. Ferrer *et al.* (1995) suggested a progressive growth of microvilli following the maturation of enterocytes.

Glutamine increases the height and width of the microvilli of the small intestine, suggesting an increase of the surface area of absorption of the tip of the enterocytes. As the capacity of digestive tract is a restrictive factor to feed intake, digestion and absorption of the nutrients (Sell *et al.*, 1991), the trophic effect of glutamine could be a good alternative to improve the capacity of the intestinal tract, and consequently the performance of the birds.

Conclusions: Feed restriction causes atrophy of intestinal mucosa and reduces the surface area of the tip of the enterocytes in the small intestine.

Glutamine increases villus density, microvilli width and surface area of the tip of the enterocytes in the small intestine of broilers.

Villus density decreases with age and increases according to the location of the segments in cranial-caudal direction of the small intestine.

Microvilli height of the small intestine decreases with age.

The jejunum has the largest tip surface area in the small intestine, followed by the duodenum and the ileum.

References

Altmann, G.G., 1972. Influence of starvation and refeeding on mucosal size and epithelial renewal in rat small intestine. J. Anat., 133: 391-400.

Buddington, R.K. and J.M. Diamond, 1989. Ontogenetic development of intestinal nutrient transporter. Ann. Rev. Phisiol., 51: 601-619.

Ferrer, R., J.M. Planas, M. Durfort and M. Moreto, 1991. Morphological study of the caecal epithelium of the chiken (Gallus Gallus Domesticus L.). Br. Poult. Sci., 32: 679-691.

Ferrer, R ., J.L. Planas and M. Moreto, 1995. Cell apiccal surface area in enterocitytes from chicken small and large intestine during development. Poult. Sci., 74: 1995-2002.

Fischer da Silva, 2001. Efeitos da restrição alimentar precoce e da glutamina no desenvolvimento e na mucosa intestinal em frangos. Tese de Doutorado, Universidade Estadual Paulista/Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, 77p.

Kandil, H.L., R.A. Argenzio, W. Chen, H.M. Berschneider, A.D. Stiles, J.K. Westwich, R.A. Rippe, D.A. Brenner and J.M. Rhoads, 1995. L-glutamine and l-asparagine stimulate ODC activity and proliferatio in a porcine jejunal enterocyte line. Am. J. Physiol., 269: G591-G599.

Macari, M., 1995. Mecanismos de proliferação e reparação da mucosa gastrointestinal em aves. Anais -1° Simpósio de coccidiose e enterite. Campinas -SP.

National Research Council, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC., 155p.

Pierzynowski, S.G. and A. Sjodin, 1998. Perspectives of glutamine and its derivatives as feed additives for farm animals. J. Anim. Feed Sci., 7: 79 -91.

Rhoads, J.M., R.A. Argenzio, W. Chen, R.A. Rippe, J.K. westwick, A.D. Cox, H.M. Berschneider and D.A. Brenner, 1997. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. Am. J. Physiol., 272: G943-G953.

Rovira, N., M.E. Soriano and J.M. Planas, 1994. Ontogenic and regional changes in kinetic constants of α-methyl-D-glucoside transport in chicken small intestine. Biochem. Soc. Transpor, 22: 262S.

Sell, J.L., C.R. Angel, F.J. Piquer, E.G. Malarino and H.A. AL-Batshab, 1991. Developmental patterns of selcted characteristics of teh gastrintestinal tract of yong turkey. Poult. Sci., 70: 1200-1205.

Shamoto, K., K. Yamauchi and H. Kamisoyama, 1999. Morphological alterations of the duodenal villi inchicks refed rice bran or grower mash fasting. Jap. Poult. Sci., 36: 38-46.

Windmueller, H.G. and A.E. Spaeth, 1974. Uptake and metabolism of plasma glutamine by the small intestine. J. Biol. Chem., 249: 5070-5079.

Yamauchi, K., H. Kamisoyama and Y. Isshiki, 1996. Effects of fasting and refeeding on structures of the intestinalvilli and epithelial cells in White Leghorn hens. Br. Poult. Sci., 37: 909-921.

Yamauchi, K.E. and Y. Isshiki, 1991. Scanning electron microscopic observations on the intestinal vili in growing white leghorn and broiler chickens from 1 to 30 days of age. Br. Poult. Sci., 32: 67-78.