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Morphological Changes of the Intestinal Mucosa of Broilers and Layers as Affected by Fasting Before Sample Collection

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ABSTRACT

The present study aimed at evaluating the histo-morphological changes resulting from different fasting periods before the collection of tissue samples in different segments of the small intestine (duodenum, jejunum and ileum) of 7-d-old male chicks of a broiler and a layer strain. A completely randomized experimental design in in a 2x7 factorial arrangement, being two strains with different growth rates (Ross 308 and *HyLine*® W36) and seven fasting periods (0, 2, 4, 6, 8, 10 and 12 hours), with six replicates, totaling 84 birds. The comparison of the morphometrics of the duodenum, jejunum and ileum of broiler and layer chicks demonstrated faster digestive tract development in broilers relative to layers. The fasting period caused morphological changes in the liver and small and large intestines in both strains. Therefore, it must be highlighted that in studies involving organ weights and intestinal morphometrics, birds must not be submitted to fasting before tissue collection.

INTRODUCTION

The growth rate of broilers selected for fast growth is considerably influenced by intestinal development (Smith *et al*., 1990). After hatching, the small intestine of poultry grows faster, weight-wise, than total body mass. In broiler, small intestine relative growth reaches its peak between six and 10 days of age, independently of the presence of absence of food (Mateos *et al*., 2004; Sklan, 2004). However, feed intake stimulates the development of the gastrointestinal tract (GIT) (Gracia *et al*., 2003), and duodenum develops earlier than the jejunum and the ileum (Uni *et al*., 1999).

In broilers, the biochemical and morphological development, and consequent maturation of the small intestine, occur during the first 10 days of life. Villi area and size rapidly increase between one and two days of age, and then their growth rate gradually decreases, reaching a plateau between five and 10 days post-hatch (Uni *et al*., 1996). However, the proliferation of intestinal epithelial cells in broilers is not limited to the crypts; it also occurs along the villi during the first week of life (Uni *et al*., 1998a). The changes that occur in the intestine prepare the chicks to use the nutrients supplied by exogenous feeding (Uni *et al*., 1998b).

Fasting hatchlings for 24 hours negatively affects broiler performance at market age, and this is related mainly to its inadequate gastrointestinal tract development (Sklan, 2001; Gonzales *et al*., 2003). Therefore, due to the importance they have for the future performance of broilers, the first few hours and the first week of life of broilers chicks have been subject of many studies on the morphometrics of the gastrointestinal tract, particularly of the duodenum, jejunum and ileum in the small intestine (Macari *et al*., 2002).

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The current methods of morphometric evaluation of small intestine segments require fasting the birds for at least 12 hours before slaughter in order to reduce contamination risks and to promote complete emptying of the gastrointestinal tract. Fasting aims at preventing imprecisions, as the physical presence of feed particles in the intestinal lumen may cause structural destruction of the villi, resulting in incorrect morphometric measurements, and hence, imprecise evaluation. However, the intestinal epithelium is also affected by the absence of feed, as observed by Yamauchi *et al*. (1996), who found significant duodenal villi height reduction in broilers exposed to 24-h fasting.

During the first week of life – a critical development phase in the development of the newborn – the absence or presence of exogenous food changes the structure of villi in the intestinal mucosa both of mammals, such as rats (Ross & Mayhew, 1984), and of poultry (Tarachai & Yamauchi, 2000). However, the rate in which these changes occur is considerably different among animal species and classes: it is much faster in poultry, particularly in broilers selected for high growth rate and feed efficiency (Smith *et al*., 1990).

According to Sakamoto & Yamauchi (2000), three hours after re-feeding of chicks submitted to fasting is sufficient to observe clear changes in the small intestine villi structure, indicating the speed in which the gastrointestinal tract responds to the absence or presence of stimuli, reducing or increasing cell turnover. Tarachai & Yamauchi (2000) also determine that the stimuli are mostly chemical, and not physical, as the structural changes result from the enteral absorption of nutrients.

Different studies evaluating the morphometric characteristics of the small intestine mucosa detected that fasting previous to tissue collection may impair the collection of data relative to observations not related with feed withdrawal. Therefore, fasting may influence the correct interpretation of experiments evaluating conditions that may affect the integrity and the morphology of the digestive tract.

The objective of the present study was to establish de maximum fasting period to which broiler and layer chicks may be submitted that does not cause morphological changes in different segments of the small intestine (duodenum, jejunum, and ileum) as well as in other organs.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Nutrition Laboratory of the School of Veterinary Medicine and Animal Science of UNESP, Botucatu campus.

A total of 120 one-day-old male chicks, being 60 Ross® 308 broilers and 60 *HyLine*® W36 layers, was used. Chicks were vaccinated in the hatchery against Marek's disease.

Chicks were housed in 12 battery divisions, each being 0.30 m high, 0.95 m wide, and 0.50 m deep, located in the two central levels of a four-level battery. Ten randomly-distributed chicks from the same strain were housed in each division.

Chicks were brooded using 250-W infrared lamps, one per division. Water and feed were supplied *ad libitum* in two nipple drinkers and a trough feeder per division.

Birds were distributed according to a completely randomized experimental design in a 2x7 factorial arrangement, being two strains with different growth rates (Ross® 308 and *HyLine*® W36) and seven fasting periods (0, 2, 4, 6, 8, 10 and 12 hours), with six replicates each. Feed was based on corn and soybean meal, and was formulated according to the recommendations of Rostagno *et al*. (2005).

When birds were seven days of age, feed supply was interrupted, and at every two hours, until 12 hours of fasting were completed, one bird per replicate was sacrificed by neck dislocation, totaling 84 birds, representing seven collection periods x 12 birds (six broilers and six layers).

In order to evaluate organ weights and intestinal morphometrics, the heart, gizzard, proventriculus, liver, pancreas, small intestine and the large intestine, including the ceca, were collected and weighed immediately after collection. The gizzard was opened and weighed after its content was removed. After removal, the small and large intestines were cut in segments, weighed and measured The small intestine was measured between from the site where the duodenum emerges from the gizzard and the beginning of the ceca, and the large intestine length included the length of the colon and the rectum, adding the length of the ceca. Organ relative weight was calculated relative to live body weight. Chicks were weighed before sacrifice.

Two segments measuring approximately 3.0 cm of the duodenum, jejunum and ileum were cross sectioned and longitudinally opened by the mesenteric edge. Samples were washed in phosphate buffer solution at 0.1 M (pH 7.4) and fixed in Bouin solution for three days. Samples were then trimmed to eliminate the torn

edges, and remained for further 24 hours in the fixing solution. Samples were then washed in ethanol at 70% to remove the fixing solutions, dehydrated in graded series of alcohol, cleared in xylol, and embedded in "paraplast". Four semi-serial sections with 7-m thickness were placed in each slide. Slides were died using the method of the periodic acid of Schiff (PAS).

Using an image capture and analysis system (Image-Pro Plus version 4.5, 0.27), villi height and perimeter and crypt depth were measured and goblet cells were counted in duodenum, jejunum and ileum sections. Villus height was measured from the basal region, which starts at the higher portion of the crypts, until villus tip, whereas crypt depth was measure from the base up to the crypt-villi transition region (Carrijo *et al.*, 2005). Perimeter was measured around the border where microvilli were located (Uni *et al.*, 1995). Goblet cell number and goblet cell:epithelial cell ratio were determined by counting 500 epithelial cells and all goblet cells per slide.

The obtained results were submitted to analysis of variance (ANOVA), using the GLM procedure of SAS statistical software (1996).

RESULTS AND DISCUSSION

There was no significant interaction between fasting period and bird strain regarding organ weight and intestinal length (Table 1) or any influence of fasting period on gizzard, proventriculus or pancreas relative weights. Fasting period reduced the relative weights of the liver, small intestine and large intestine ($p < 0.01$). The relative weights of the liver and large intestine linearly decreased ($p < 0.01$) with fasting period, and are expressed by the following: Liver (%) = 4.63 – 0.08 x Fasting ($R^2 = 0.31$) and LI (%) = 1.81 – 0.05 x Fasting ($R^2 = 0.28$), respectively. The change in relative small intestine weight as a function of fasting period was quadratic and is expressed by the equation SI (%) = 9.34 – 0.69 x Fasting + 0.04 x Fasting2 ($R^2 = 0.60$).

Large intestine length linearly decreased ($p < 0.01$) as a function of fasting period and it is expressed by the equation: LI (cm) = 17.48 – 0.24 x Fasting ($R^2 = 0.15$). These results are consistent with those obtained by Gonzales *et al*. (2003), who observed that fasting periods of 18 and 36 hours reduced the relative weight of the small intestine in broiler chicks. However, those authors also observed a reduction in small intestine length, which was not the case in the present experiment.

Strain affected the relative weights of the gizzard, pancreas and large intestine, with higher values ($p < 0.01$) obtained in layer chicks compared with broiler chicks, which, in turn, presented longer small and large intestines ($p < 0.01$).

There was no interaction between fasting period and strain for villus perimeter, crypt depth and goblet cells/epithelial cells ratio in the duodenum, jejunum and ileum (Table 2). Fasting linearly increased ($p < 0.01$) goblet cells/epithelial cells ratio in the jejunum, which is expressed by the equation: G/E = 30.12 + 0.36 x Fasting ($R^2 = 0.09$), indicating the defense adaptation

Table 1 - Relative organ weight* (%) and intestinal length (cm) of seven-day-old chicks as a function of fasting period and strain.

	Relative weight. %		Length. cm				Small intestine	Large intestine
	Gizzard	Proventr.	Liver	Pancreas	Small intestine	Large intestine		
Fasting (F)	ns	ns	p<0.01	ns	p<0.01	p<0.01	ns	p<0.01
0	4.98	1.12	4.79a	0.61	9.51a	1.71ab	76.6	17.3a
2	5.47	1.14	4.49ab	0.60	7.90b	1.81a	74.2	17.4a
4	5.36	1.21	4.16bc	0.58	7.04c	1.57abc	70.5	16.4a
6	5.27	1.13	3.98c	0.59	6.56c	1.49cb	70.6	15.8ba
8	5.17	1.16	4.09bc	0.65	6.35c	1.29cd	74.6	16.5a
10	5.28	1.11	3.95c	0.60	6.33c	1.34cd	71.2	14.6b
12	5.39	1.23	3.76c	0.55	6.32c	1.18d	70.5	14.5b
Strain (S)	p<0.01	ns	ns	p<0.01	ns	p<0.01	p<0.01	p<0.01
Broiler	5.10B	1.13	4.18	0.53B	7.14	1.29B	82.8A	17.8A
Layer	5.44A	1.18	4.16	0.66A	7.15	1.69A	62.4B	14.4B
Interaction (FxS)	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.8	11.5	11.1	20.2	13.3	18.8	7.4	9.1

* Relative organ weight (%) = (organ weight live weight) x 100. a,b,c Means in the same column followed by different small letters are significantly different by the SNK test. A,B Means in the same column followed by different capital letters are significantly different by the F test. ns = not significant.

Table 2 - Villus perimeter (VP), crypt depth (CD), and goblet cell:epitelial cell ratio (G/E) in the duodenum, jejunum and ileum, as a function of fasting period and strain.

	Duodenum			Jejunum			Ileum		
	VP. m	CD. m	G/E[1]	VP. m	CD. m	G/E[1]	VP. m	CD. m	G/E[1]
Fasting (F)	ns	ns	ns	ns	ns	p<0.01	p<0.05	ns	ns
0	1989	108	25	1003	87	29b	681b	71	38
2	2015	101	25	1010	83	31ab	703ab	73	38
4	2035	107	27	1072	94	32ab	761ab	73	40
6	1976	98	26	1061	86	34a	797ab	78	40
8	1983	104	25	1187	95	32ab	808a	78	38
10	1813	99	27	1034	91	32ab	714ab	72	39
12	1920	102	29	1214	89	35a	732ab	77	40
Strain (S)	p<0.01	ns	ns	p<0.01	p<0.05	p<0.01	p<0.01	ns	p<0.01
Broiler	2065A	103	27	1168A	93A	35A	801A	76	42A
Layer	1853B	102	26	1001B	85B	30B	686B	73	36B
Interaction (FxS)	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	14.7	13.6	15.1	16.4	14.9	12.3	13.6	16.1	11.0

1 - Count of 500 epithelial cells and all goblet cells per bird. a,b,c - Means in the same column followed by different small letters are significantly different by the SNK test. A,B - Means in the same column followed by different capital letters are significantly different by the F test. ns = not significant.

of the intestinal mucosa to fasting. Fasting period also promoted a quadratic increase ($p < 0.01$) in villus perimeter in the ileum, expressed by the equation: $VP = 669.86 + 33.60 \times Fasting - 2.46 \times Fasting^2$ ($R^2 = 0.10$). However, the low R^2 value indicates that this increase in villus perimeter is not closely related to fasting period. There was no effect of fasting on villus perimeter in the duodenum or in the jejunum. These results are different from the findings of Yamauchi *et al*. (1996) and Gonzales *et al*. (2003), who observed that up to 18 hours of fasting immediately after hatch reduced villi height and crypt depth in the duodenum, jejunum and ileum of broiler chicks. Those authors suggest that these changes may affect the performance of broilers at 42 days of age. However, it should be considered the those authors worked with broiler chicks up to 48 hours after hatch and with longer fasting periods than those applied in the present experiment.

Shakamoto & Yamauchi (2000) reported that changes in villi height and enterocyte area can be observed submitted to fasting during post-hatching phase, and that these changes can be used to estimate intestinal function and their negative effect on body weight gain.

The remaining evaluated characteristics of the different small intestine segments were not affected by fasting period ($p > 0.05$). Broiler chicks presented higher ($p < 0.01$) villus perimeter in the duodenum, jejunum and ileum compared with the layer chicks. In addition higher ($p < 0.05$) crypt depth values in the jejunum and goblet cells to epithelial cells ratio values were determined in the jejunum and ileum ($p < 0.01$) of broiler chicks relative to layer chicks (Table 2). Consistent with the data presented by Macari *et al*. (2002), these results demonstrates the broiler and layer strains present different intestinal mucosa development rates: broilers have larger villi and deeper crypts, which may favor their higher body weight gain.

CONCLUSIONS

When the morphometrics of the duodenum, jejunum and ileum of broilers and layers the pre-starter growth phase, i.e., from hatch to seven days of age, was compared, the faster development of the intestinal tract of broilers relative to layers was evidenced. Fasting period promoted liver weight and small and large intestine morphological changes both in broilers and layers. Fasting birds before tissue collection in studies involving organ weight and intestinal morphometrics is not recommended.

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