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Small Intestine Development of Laying Hens Fed Different Fiber Sources Diets and Crude Protein Levels

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Crude protein, fibers, intestinal development.

ABSTRACT

The objective of the presente study was to evaluate the effects on different dietary fiber sources and crude protein levels on the intestinal morphometry of commercial layers. Isa Brown® layers with 48 weeks of age were distributed in a completely randomized experimental design with a 3 x 2 + 1 factorial arrangement, resulting in seven treatments with seven replicates of eight birds each. At the end of the fourth experimental period (28 days each), birds were 64 weeks of age and were randomly chosen (two birds per replicate, totaling 14 birds per treatment), weighed and sacrificed by neck dislocation. Their intestine was dissected and the duodenum, jejunum and ileum were collected for subsequent analysis of intestinal morphometry. Treatments consisted of diets containing three different fiber sources (cottonseed hulls, soybean hulls or rice husks) and two crude protein levels (12% or 16%). Soybean hulls and 16% crude protein level promoted, in general, an increase in villus height and crypt depth in the three intestinal segments. In the duodenum, the control diet resulted in higher villus height and crypt depth relative to the diets containing fiber. In the jejunum, higher crypt depth values. In the ileum, dietary fiber increased villus height as compared to the control diet.

INTRODUCTION

The intestinal development of poultry has been subject of many studies in the last few years. The incidence of enteric disorders has increased due to restrictions on the inclusion of antibiotics and animal proteins in poultry diets, and therefore, management and nutritional changes have been recommended to overcome productivity losses caused by these diseases.

The recommendations include stimulating the development of the gastrointestinal tract (GIT) during the first stages of life, improving the digestibility of dietary nutrients, and to change the physical-chemical conditions of the intestinal content to obtain proper balance of the intestinal flora. This requires detailed knowledge on the influence of diet and feedstuff processing, as well the inclusion of natural additives on digestive physiology and on the development of the microflora.

Fiber-rich feedstuffs have currently been used as alternative ingredients in the diet of monogastric animals. Their influence on nutrient retention in the GIT has been much debated. Research has indicated that some fiber sources used in adequate quantities may enhance intestinal development, and hence, some productivity indicators (Montagne *et al.*, 2003).

The present study evaluated the addition of different fiber sources

MATERIALS AND METHODS

The experiment was carried out with 392 48-week-old Isa Brown® layers. Birds were selected as a function of body weight and of egg production during two weeks. Egg production was individually controlled for later distribution of layers into the experimental units and to equalize production in the beginning of the experimental period.

Birds were submitted to the experimental treatments from 48 to 64 weeks of age, which were divided in 28-day periods. Water and feed were supplied *ad libitum*, and feed intake was measured at the end of each period. A lighting program of 17 hours of light per day was adopted.

A completely randomized experimental design in a 3x2+1 factorial arrangement was applied, with three fiber sources (soybean hulls, rice husks, and cottonseed hulls) and two crude protein levels (12% and 16%). Each treatment included seven replicates, totaling 49 experimental units. The control treatment consisted of a corn and soybean meal-based diet, with no addition of any fibrous feedstuff.

Feeds were formulated to supply the nutritional requirements of layers according to the recommendations of Rostagno *et al*. (2005), except for crude protein (CP) and crude fiber (CF). The ingredient and nutritional compositions of the experimental diets are shown in Tables 1 and 2, respectively.

According to Rostagno *et al*. (2005), 0.71% digestible lysine and 0.47% digestible threonine supply the nutritional requirements of layers during egg production period. However, the analyses showed that feeds with 16% crude protein contained 0.81% digestible lysine and 0.53% digestible threonine, with no addition of synthetic amino acids.

Table 1 – Ingredient composition of the experimental diets.

Ingredients (%)	Cont.*	12% +SBH	16% +SBH	12% +RH	16% +RH	12% +CSH	16% +CSH
Ground corn	68.88	59.38	49.45	62.52	52.29	64.94	54.44
Soybean meal	19.45	15.58	25.78	14.49	24.80	13.99	24.36
Soybean hulls	-	8.56	7.54	-	-	-	-
Rice husks	-	-	-	7.56	6.66	-	-
Cottonseed hulls	-	-	-	-	-	6.20	5.46
Calcitic limestone	8.56	8.55	8.51	8.38	8.36	8.56	8.52
Soybean oil	1.13	5.53	6.78	4.63	5.96	3.87	5.28
Dicalcium phosphate	1.23	1.32	1.24	1.32	1.24	1.31	1.23
Salt	0.48	0.50	0.48	0.48	0.46	0.50	0.48
DL- methionine	0.15	0.23	0.13	0.24	0.14	0.23	0.14
L-threonine	-	0.06	-	0.07	-	0.07	-
L- lysine HCl	-	0.19	-	0.22	-	0.23	-
Vit + min supplement**	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

* - control feed, SBH- soybean hulls, RH- rice husks and CSH- cottonseed hulls. ** - Enrichment per kg of feed: 8,000 IU vitamin A, 2,500 IU vitamin D_3, 12.5 mg vitamin E, 1.00 mg vitamin K_3, 1.50 mg vitamin B_1, 3.4 mg vitamin B_2, 1 mg vitamin B_6, 20 mcg vitamin B_{12}, 0.25 mg folic acid, 0.1 mg biotin, 30 mg niacin, 12 mg calcium pantothenate, 12 mg Cu, 1.10 mg I, 48 mg Mn, 60 mg Zn, 0.23 mg Se, 60 mg Fe.

Table 2 – Nutritional levels of the experimental diets.

Nutrients	Cont.*	12%+SBH	16%+SBH	12%+RH	16%+RH	12% +CSH	16% +Cal
Metabolizable energy (kcal/kg)	2.900	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein (%)	14.78	12.00	16.00	12.00	16.00	12.00	16.00
Nitrogen (%)	2.36	1.95	2.56	1.95	2.56	1.95	2.56
ADF (%)	4.03	7.16	7.19	8.69	8.54	7.78	7.74
NDF (%)	10.80	14.54	14.15	15.42	14.92	15.03	14.59
Crude fiber (%)	2.40	5.00	5.00	5.00	5.00	5.00	5.00
Available phosphorus (%)	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Total phosphorus (%)	0.53	0.50	0.52	0.50	0.53	0.50	0.53
Calcium (%)	3.65	3.65	3.65	3.65	3.65	3.65	3.65
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Digestible lysine (%)	0.71	0.71	0.81	0.71	0.81	0.71	0.81
Total lysine (%)	0.75	0.74	0.86	0.74	0.84	0.74	0.83
Dig. methionine + cystine (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Total methionine + cystine (%)	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Digestible methionine (%)	0.38	0.42	0.37	0.42	0.37	0.42	0.37
Total methionine (%)	0.39	0.43	0.38	0.43	0.39	0.43	0.38

Table 3 presented the chemical analyses of the fibrous feedstuffs of the experimental diets.

Table 3 – Chemical analyses of the fibrous feedstuffs.

Ingredients	Soybean hulls	Rice husks	Cottonseed hulls
DM (%)	90.15	92.72	89.63
Ash (%)	4.95	19.82	2.78
EE (%)	1.23	0.21	1.31
CP (%)	11.28	2.40	4.25
N (%)	1.80	0.38	0.68
CF (%)	51.34	58.44	40.88
NNE (%)	21.35	11.85	40.41
NDF (%)	67.31	73.07	82.30
ADF (%)	54.94	60.25	56.59
Lignin (%)	1.60	18.15	15.55

At the end of the fourth experimental period, at 64 weeks of age, two birds per replicate, totaling 14 birds per treatment, were randomly selected, weighed, and sacrificed by neck dislocation. The intestine was dissected, and segments (duodenum, jejunum and ileum) were collected for intestinal morphometric analyses.

Two-cm long samples of the medial portion of the duodenum, jejunum and ileum were collected for morphometric evaluation of villi and crypts. Samples were fixed in Bouin solution (70% saturated picric acid solution, 25% formaldehyde and 5% acetic acid) for 24 hours and then processed according to routine methods for light microscopy. Samples were dehydrated in graded series of ethanol (70, 80, 90 and 100%), cleared in xylol, and embedded in paraffin. Six-µm thick tissue sections were stained with hematoxylin and eosin. Tissue section images at 10x magnification were obtained under a light microscope coupled to a Zeiss MC80 DX® camera. Thirty images per intestinal segment were analyzed per bird, using the software program Image J®. Villus height (VH) and crypt depth (CD) were measured in the images, and the results were expressed in µm. Villus height to crypt depth (VH/CD) ratios was calculated.

Data were submitted to analysis of variance using the General Linear Model (GLM) procedure of SAS® (2002) statistical package. When statistical significance was determined at 5% probability level, means were compared by the test of Tukey.

RESULTS AND DISCUSSION

In duodenum, when evaluating the development of the intestinal mucosa, it was observed that the interaction between fiber sources and crude protein levels (Table 4). The details of that interaction for the parameter VH shows that, within the fiber sources, crude protein levels had a significant effect only for rice husks, when the low crude protein level (12%) resulted in low VH development relative to the high crude protein level (16%). Rice husks contain high NDF, which, together with the low crude protein level, did not supply the protein required for cell renewal. This was not observed with soybean hulls, which contain high protein and low lignin levels. When crude protein levels were evaluated, only the low crude protein level (12%) influenced VH, with layers fed soybean hulls presenting higher VH as compared to the other fiber sources, showing that soybean hulls supplied nutrients and energy, promoting higher cell renewal and higher villi growth (Table 5).

As to CD, the details of the interaction indicated that there was a numerical effect of the evaluated crude protein levels (12 and 16%). Therefore, within crude protein level (12%), the diet with cottonseed hulls inclusion promoted higher CD development, but with statistical differences only relative to soybean hulls. Within the high crude protein level (16%), the diet with rice husks resulted in higher CD development, but statistically similar to soybean hulls. When fiber sources were evaluated, only cottonseed hulls presented significant effect, indicating the low crude protein level (12%) promoted higher CD development (Table 5).

The nutritional and physiological effects of dietary fiber depend on the amount of cell wall added to the diet, as well as on its chemical and structural composition. Fibrous diets with high NDF content have an abrasive action on the intestinal epithelium, as mentioned by Dierick *et al*. (1989), and may, in addition to increase endogenous nitrogen content in fecal content, increase cell renewal rate, which happens in the crypts, consequently, increasing crypt depth.

The parameter VH/CD was significantly affected ($p<0.01$) by fiber source (Table 4). Soybean hulls promoted higher VH/CD ratio relative to the diets containing cottonseed hulls and rice husks (Table 5). That parameter was also significantly influenced ($p<0.01$) by crude protein levels (Table 4). The comparison of mean show that the diets formulated with 16% crude protein promoted higher VH/CD ratio relative to the low crude protein level (12%) (Table 5).

When the results of the factorial arrangement were compared with the control treatment, significant

VH and CD (Table 4). Data analyses showed that layers fed the control diet presented higher VH and CD development relative to those fed different fiber sources and crude protein levels (Table 5). Several factors may have contributed for these results, particularly fiber quantity and quality and NDF and lignin dietary content. Less fermentable fiber sources may have stronger abrasive effects on the intestinal mucosa, reducing particularly villus height.

Table 4 – F values of VH (μm), CD (μm) and VH/CD of the duodenal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Variation source	Duodenum		
	VH	CD	VH/CD
Fiber source	17.78**	5.32*	16.86**
CP levels	3.39ns	23.34**	28.68**
Fiber source *vs.* CP level	17.34**	7.52*	2.55ns
Factorial x Control	21.58**	8.30*	0.000ns

**p<0.01; *p<0.05; ns = not significant (p>0.05).

Opposite to these findings, Moore *et al*. (1988) did not observe any significant changes in the intestinal cells of pigs fed high NDF levels. However, indications of intestinal epithelium damage were found in some animals, suggesting that some individuals may be more susceptible to the abrasive action of dietary fiber.

According to Cummings (1981), dietary fiber is fermented by bacteria present in the cecum, resulting in the production of short-chain fatty acids (SCFA), particularly acetate, propionate and butyrate, as well as of lactate, succinate, water, and different gases, depending on the fiber. Following intestinal absorption, SCFA play specific roles in the body. Acetate is transported to the liver and is used as energy source by muscles; propionate is converted in glucose in the liver and may also inhibit pathogens, such as Salmonella; and butyrate is the main energy source of metabolic activities, stimulating epithelial cell growth both in the small and large intestines.

In the jejunum, there was no interaction (p>0.05) between fiber sources and crude protein levels for any of the analyzed parameters (Table 6).

When the fiber factor was independently analyzed, the results show a significant effect (p<0.05) of fiber sources on VH (Table 6). Soybean hulls promoted higher VH values relative to the other evaluated fiber sources (Table 7). This may have been caused by the protein content and low lignin levels of soybean hulls, which thereby may have supplied more nutrients and energy for cell renewal. This effect was observed in *in-vitro* assays with soybean hulls that showed 96% dry matter digestibility, suggesting that its fibrous fraction is highly digestible, despite consisting of 70% cell wall (Zambom *et al*., 2001).

Crude protein levels significantly (p<0.01) influenced the parameters VH, CD and VH/CD in the jejunum (Table 6). The high crude protein level (16%) promoted higher VH and CD development and reduced VH/CD ratio values (Table 7).

There was a significant interaction (p<0.05) between the factorial arrangement results *versus* the control treatment for the parameter CD (Table 6). The analysis of data demonstrated that the birds fed the control diet presented lower CD development as compared to those fed different fiber sources and crude protein levels (Table 7). Jin *et al*. (1994) reported that, due to the abrasive action of fiber on the intestinal epithelium, the use of fibrous feedstuffs in the diet reduces villus height and increases crypt depth in an attempt to compensate villi cell loss, as the crypts are responsible for cell renewal.

Table 5 – Means and coefficients of variation of VH (μm), CD (μm) and VH/CD ratio of the duodenal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Parameter	CP level	Soybean hulls	Rice husks	Cottonseed hulls	Mean	Control
VH						
	12%	1588Aa	1325Bc	1459Ab	1458	
CV=10.82	16%	1573Aa	1567Aa	1367Ab	1502	1629**
	Mean	1581	1446	1414	1480	
CD						
	12%	256Ab	279Aab	285Aa	273.65	
CV=14.77	16%	245Aab	266Aa	225Bb	245.64	282*
	Mean	251	273	255	260	
	12%	6.3	4.8	5.3	5.4 B	
VH/CD CV=17.38	16%	6.6	6.0	6.2	6.3 A	5.8ns
	Mean	6.4 a	5.4 c	5.74 b	5.8	

Means followed by capital letters in the same column and small letters in the same row are different by the test of Tukey. Comparison of means between the factorial

Table 6 - F values of VH (μm), CD (μm) and VH/CD of the jejunal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Variation source	Jejunum		
	VH	CD	VH/CD
Fiber source	5.51*	1.11ns	2.49ns
CP levels	38.56**	75.43**	10.93*
Fiber source *vs.* CP level	0.76ns	2.57ns	1.81ns
Factorial x Control	1.14ns	5.17*	1.56ns

**p<0.01; *p<0.05; ns= not significant (p>0.05).

The parameters VH and VH/CD were

Table 7 – Means and coefficients of variation of VH (µm), CD (µm) and VH/CD ratio of the jejunal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Parameter	CP level	Soybean hulls	Rice husks	Cottonseed hulls	Mean	Control
VH						
	12%	819	752	771	780 B	
CV=10.87	16%	887	858	845	863 A	803ns
	Mean	853 a	804 b	807 b	822	
CD						
	12%	148	144	136	142 B	
CV=14.56	16%	167	177	173	172 A	147*
	Mean	158	161	154	157	
	12%	5.6	5.3	5.8	5.6 A	
VH/CD CV=16.49	16%	5.4	5.00	5.00	5.1 B	5.6ns
	Mean	5.5	5.14	5.4	5.3	

Means followed by capital letters in the same column and small letters in the same row are different by the test of Tukey. Comparison of means between the factorial arrangement and the control treatment are also presented. **p<0.01; *p<0.05; ns = not significant (p>0.05).

fiber sources and crude protein levels in the ileum (Table 8). The details of the interaction for the parameter VH show that, within fiber sources, crude protein levels presented significant effect only for soybean hulls and rice husks, with the high crude protein level (16%) promoting higher numerical VH value. Within CP levels, only the level of 16% was significant, with cottonseed hulls resulting in the lowest VH (Table 9). In addition of containing high NDF and lignin levels, cottonseed hulls also presents anti-nutritional factors, which may interfere in nutrient digestibility, particularly of proteins, and may indeed destroy intestinal villi, reduce VH, and not supply the amount of energy required for cell renewal.

When villi slough, cells are replaced by the migration of crypt cells, which multiply in a hyperplasic process (Argenzio, 1993). Therefore, an increase in crypt depth was expected in the present study, but this was not observed.

The details of the interaction showed that only soybean hulls, within crude protein levels, influenced the parameter VH/CD in the ileum, which values were higher when the high protein level (16%) was used. Within the evaluated crude protein levels, the level of 16% had a significant effect on the VH/CD ratio in the ileum, as well as soybean hulls, which also promoted higher levels of this ratio (Table 9).

There was a significant effect (p<0.05) of crude protein levels on the analyzed parameters (Table 8), with higher VH and CD development when 16% crude protein was fed (Table 9), which is consistent with the findings of the duodenum and jejunum in the present

derive not only from the amount supplied, but also of protein quality, that is, amino acid concentration and balance (Gonzales, 2002). In addition, the presence of nutrients in the intestinal lumen stimulates villus and crypt growth (Moran, 1985).

The obtained data are consistent with the findings of Otutumi *et al*. (2008), who analyzed the effects of different crude protein levels (15, 20, 25 and 30 %) on the intestinal morphometry of meat-type quails and observed a linear increase only in ileal villus height.

Fiber sources significantly influenced (p<0.05) (Table 8) the parameter CD, with rice husks and cottonseed hulls promoting higher CD values (Table 9). Rice husks and cottonseed hulls have high lignin and NDF contents, which may have abrasive action on the villi, as mentioned above. Diets with high NDF content may reduce villus height and increase crypt depth.

The analysis of the factorial arrangement results *versus* the control treatment showed interactions (p<0.05) for the parameter VH and VH/CD ratio in the ileum (Table 8). It was observed that birds fed the control diet presented lower VH development and VH/CD values relative to those fed different fiber sources and crude protein levels (Table 9).

This contrasting behavior is related to the main functions of the intestinal segments. In the duodenum, amino acid hydrolysis is incomplete, and therefore, there is minimal absorption. In addition, in poultry, most membrane carriers are located in the ileum, which is the main site of amino acid absorption (Rutz, 2002), explaining the different villus height in the ileum relative to the duodenum and the jejunum.

Table 8 - F values of VH (µm), CD (µm) and VH/CD of the ileal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Variation source	Ileum		
	VH	CD	VH/CD
Fiber source	4.48*	6.84*	9.91**
CP levels	34.64**	5.28*	3.46ns
Fiber source *vs*. CP level	3.62*	0.99ns	3.75*
Factorial x Control	37.30**	0.28ns	15.42*

**p<0.01; *p<0.05; ns= not significant (p>0.05).

Means followed by capital letters in the same column and small letters in the same row are different by the test of Tukey. Comparison of means between the factorial arrangement and the control treatment

Table 9 – Means and coefficients of variation of VH (µm), CD (µm) and VH/CD ratio of the ileal portion of the small intestine of commercial layers fed different fiber sources and two crude protein levels.

Parameters	CP levels	Soybean hulls	Rice husks	Cottonseed hulls	Mean	Control
VH						
	12%	586Ba	591Ba	585Aa	587	
CV=9.70	16%	664Aa	647Aa	605Ab	639	543**
	Mean	625	619	595	613	
CD						
	12%	131.39	142	136	136 B	
CV=12.70	16%	133.97	146	148	143 A	141ns
	Mean	133b	144a	142a	140	
	12%	4.5Ba	4.2Aa	4.4Aa	4.4	
VH/CD CV=15.54	16%	5.0Aa	4.5Ab	4.2Ab	4.6	3.9*
	Mean	4.8	4.3	4.3	4.5	

Means followed by capital letters in the same column and small letters in the same row are different by the test of Tukey. Comparison of means between the factorial arrangement and the control treatment are also presented. **p<0.01; *p<0.05; ns = not significant (p>0.05).

CONCLUSIONS

Considering the different fiber sources associated to the different crude protein levels used in the present study, it is concluded that soybean hulls associated to 16% crude protein level promoted the best results for the analyzed intestinal development parameters.

When the diets containing fiber were compared to a control diet not containing fiber, it was observed that the diet with no fiber addition promoted the best intestinal development parameter results in the duodenum; however, this was not found in the jejunum and ileum, where the diets with the addition of fiber resulted in better intestinal development.

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