Effect of the High Fibre (8%) Layer Diets on Digestive Tract Integrity

Cristinel Panaite[1,2*], Dumitru Dragotoiu[2], Rodica Diana Criste[3], Tatiana Dumitra Panaite[3], Maria Gabriela Cornescu[3]

[1]BIOMIN Romania SRL, Zona Industrială, 550000-Sibiu, Europa Unită, 15, Romania,
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 011464-Bucharest, Mărăşti Blvd, 59, District 1, Romania,
[3]National Research-Development Institute for Animal Biology and Nutrition (IBNA), 077015-Balotesti, Calea Bucuresti, 1, Romania

Abstract

Although fibre is regarded by many nutritionists as a mere diluent of poultry diets, new experimental evidences suggest that the dietary fibre positively influences the digestive tract integrity. A 5-week feeding trial was conducted on 200 Tetra layers (28 weeks) assigned to 5 groups, housed in an experimental hall under controlled environmental conditions (temperature: 23.81±1.54°C; humidity: 64.76±11.93%), and 16h/24h light regimen. The control group (C) received a conventional diet (2780 kcal/kg metabolizable energy; 17.5% crude protein; 4.39% crude fibre). Compared to C formulation, E1 formulation included 23% sunflower meal, which increased the dietary fibre (8%). The other 3 experimental diet formulations differed from E1 formulation by the presence of phytoadditives or cellulolytic enzymes: E2 (0.015% enzyme); E3 (0.015% phytoadditive) and E4 (0.015% enzyme+0.015% phytoadditive). At the end of the experiment 30 hens were sacrificed (6 hens/group) to obtain jejunum samples for histological parameters. The results of the intestinal measurements revealed significantly (P≤0.05) lower values for experimental groups compared to C group. The lowest values registered for E1 group: villus height (583±99.74µm) and crypt depth (126±36.19µm). Mucosa thickness increased significantly (P≤0.05) in E3 group (275±70 µm) compared to C group (173±66.08 µm).

Keywords: crude fibre, enzyme, intestinal villi, laying hens

1. Introduction

By banning the use of antibiotics in animal feed as growth promoters, in many countries around the world has increased the incidence of enteric disorders in poultry. Nutritional strategies applied for poultry to reduce these problems were based on the use of natural additives, including whole grains and increasing the fibre level of poultry diets [1].

The prebiotic potential of fibre utilization is one way of stimulating the health of the intestine and, therefore, the lower use of antimicrobial growth promoters. In addition, the presence of raw materials rich in fibre increases the economic efficiency of the poultry production process. Typically, both in research and in the practice of poultry feeding, fibre has been considered a diluent of the diet [2] with negative effects related to the voluntary feed consumption and nutrient digestibility [3, 4]. However, some researchers believe that a minimum level of fibre must be included in animal diets to maintain normal

* Corresponding author: Panaite Cristinel Vasile
Email: cristinelpanaite@yahoo.com

physiological functions within the digestive tract [5-11]. Also, an increased level of fibre within the diet contributes to digestive organs development [12] and an increase in bile acid, hydrochloric acid and enzyme secretion [13, 14].
These amendments could lead to improved nutrient digestibility [12, 15, 16], growth performances [17, 18] and animal welfare [19, 20]. However, fibre is not included in the tables with nutrient requirements (NRC, 2012). Cellulose properties are determined by the properties of fibres [12, 21] and the cellulose usability is affected by species, hybrid, age.
In this context, an experiment was conducted on hens fed with diets rich in cellulose (8%) in order to assess the effects of these productive parameters on animal welfare and integrity of the digestive tract.

2. Materials and methods

A five-week experiment was performed on 200, Tetra SL hens, phase I (28 weeks) in the experimental halls from IBNA, Balotesti. The protocol for this study was approved by the ethic commission of IBNA Balotesti (decision nr. 52/30.07.2014).The experiment was performed in a hall with controlled environmental conditions (temperature: 23.81±1.54^{o}C; humidity 64.76±11.93% and ventilation: 23.85±3.036%). The light regimen was adequate to the age category (16h light/8h dark).
The layers, divided into 5 groups (C, E1, E2, E3, E4, respectively), were housed in metabolic cages, stacked on 3 tiers (4 hens/cage; 10 cages/group). Experimental cages configuration allowed daily monitoring and sampling of fodder and droppings remains. Feed and water were administered ad libitum. The formulations of the compound feeds used in this experiment (Table 1), considered the following: the objective of the experiment, species, hybrid, age and nutritional requirements of Tetra SL hybrid [22]. All the birds were fed compound feeds that had the same basic structure (maize, rice bran, sunflower soybean and sunflower meal). The control group (C) received a conventional diet (2780 kcal/kg; 17.5% ME, 17.5% CP, 4.39% CF).
Compared to C, experimental diets (E1, E2, E3 and E4) were differentiated by an input of 22.79% sunflower meal which increased the dietary fibre level (8%). Sunflower meal has a high content of crude fibre (22.5% CF): cellulase 28.7%; hemicellulase 11.00%; 8.9% lignin; 28.9% N.D.F. and 39.00% A.D.F. (optimizing diet database - Brill program).
Except E1 diet, fibre digestibility from the experimental diets was improved with and enzymatic product (BIOZYM M6000) and a plant mixture (DIGESTAROM) produced by BIOMIN Company.
Experimental diets E2, E3 and E4 differed from E1 by the cellulosic enzyme or/and the plant mixture: E2 (0.015% enzyme); E3 (0.015% phytoadditive) and E4 (0.015% enzyme+0.015% phytoaditive).
Biozym M6000 is a multi-enzyme feed additive having beta-xylanase (produced by immersed fermentation of a selected strain of Trichoderma longibrachiatum CNMC MA 6-10W) and beta-glucanase as active products. This product is used stabilised source of enzymes. Digestarom is a phytogenic feed additive designed to improve digestion and to maintain gut health. It is a standard mixture of essential oils, herbs, spices and plant extracts [23].
After compound feeds manufacturing (one batch/group/growing phase), a sample was collected from each batch for physical and chemical analysis.
Standardized methods complying with *Regulation (CE) 152/2009* (Sampling and analytical methods for the official inspection of feeds) and ISO standards were used to determine the nutrient concentration: gravimetric method for the dry matter (DM); the Kjeldahl method for the crude protein (CP); extraction in organic solvents for the ether extractives (EE); acid hydrolysis followed by alkaline hydrolysis for the crude fibre (CF); gravimetric method for the ash (Ash). During the experiment were monitored: average daily intake (g CF/hen/day); feed conversion ratio (kg CF/kg egg); laying percentage (%); average egg weight (g) and layer welfare. At the end of the experiment 30 hens were slaughtered (6 hens/group) and samples were taken from the jejunum for measurements concerning the integrity of the intestinal mucosa.

Table 1. Experimental diet structure

Specification	C	E1	E2	E3	E4
			%		
Corn	55.38	28.7	28.685	28.685	28.67
Rice bran	-	22.21	22.21	22.21	22.21
Soybean meal	22.97	10.11	10.11	10.11	10.11
Sunflower meal	8	22.79	22.79	22.79	22.79
Vegetal oil	1.76	4.5	4.5	4.5	4.5
Lysine	-	0.24	0.24	0.24	0.24
Dl - methionin	0.1	0.09	0.09	0.09	0.09
Calcium carbonate	8.96	8.68	8.68	8.68	8.68
Monocalcium phosphate	1.38	1.31	1.31	1.31	1.31
Salt	0.4	0.32	0.32	0.32	0.32
Vitamin- mineral* premix	1	1	1	1	1
Enzyme (BIOZIM M6000)	-	-	0.015	-	0.015
Phytoadditive (Digestarom)	-	-	-	0.015	0.015
Micofix	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100
Chemical analysis, theoretic calculation					
DM, %	87.11	88.35	88.35	88.35	88.35
ME, kcal/kg	2.780	2.750	2.750	2.750	2.750
CP, %	17.50	17.97	17.97	17.97	17.97
EE, %	3.55	5.97	5.97	5.97	5.97
Cellulose, %	4.39	8.00	8.00	8.00	8.00
Calcium, %	3.90	3.90	3.90	3.90	3.90
Phosphorus, %	0.69	0.73	0.73	0.73	0.73
- available, %	0.38	0.38	0.38	0.38	0.38
Sodium, %	0.17	0.01	0.01	0.01	0.01
Chlorine, %	0.30	0.10	0.10	0.10	0.10
Lysine, %	0.87	0.87	0.87	0.87	0.87
Methionine,%	0.40	0.39	0.39	0.39	0.39
Meth.+cys., %	0.70	0.67	0.67	0.67	0.67
Threonine,%	0.67	0.59	0.59	0.59	0.59
Tryptophan, %	0.20	0.20	0.20	0.20	0.20
Linoleic acid(c18:2)	2.22	3.26	3.26	3.26	3.26

Where:* 1 kg premix vitamin mineral contains: (1350000 IU/kg vit. A; 300000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit.B1; 480 mg/kg Vit.B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine 18 mg/kg selenium; 6000 mg/kg.

From each hen was collected a fragment of 5-6 cm from the jejunum level (intestinal segment situated between the terminal portion of the duodenal loop and Meckel's diverticulum). Fixation of samples was done by immersing for 24 hours in a 10% formaldehyde solution. Subsequently, the samples were histoprocessed, included in paraffin, sectioned at microtome, at the thick of 5 mm and the histopathological sections were coloured by haematoxylin and eosin method. The measurements were made using a micrometre, with 4x objective. Eleven measurements have been done for each sample: height of five intestinal villi, depth of five intestinal crypts and thickness of intestinal mucosa, in a single point.

The intestinal villi height was considered the distance between the apical region and villous basis. The depth of an intestinal crypt was considered the distance between the intestinal villous base and the crypt base. The values were expressed in micrometers (μm).

Statistical analysis: The analytical data were compared by variance analysis (ANOVA), using StatView for WINDOWS (SAS, version 6.0). The differences between the average values within the groups were considered significant for $P<0.05$. The results were expressed as mean±SD for all measurements.

3. Results and discussion

Table 2 data show that the hens of the control group (C) had a significantly (P<0.05) higher average daily intake than those of the experimental groups (E1, E2, E3, E4). Thus, it results that the addition of sunflower meal high in cellulose, reduced the average daily intake, similar results to those presented by [24].
According to these researchers, the average daily intake decreased due to increase of dietary fibre content. Alternatively, the hens of group E3 involving dietary phytoadditive had a better feed intake than those who had dietary addition of enzyme and Mycofix (E2) and enzyme and phytoadditive (lE4) (Table 2). There were no differences between groups in terms of laying percentage (Table 2), but the average egg weight was significantly (P<0.05) higher in the hens from C group compared to E2, E3, E4 (Table 2).

Table 2. Effect of the high fibre layer diets on productive parameters (average values/group)

Specification	C	E1	E2	E3	E4	SEM	p* value
Average daily feed intake, gCF/head/day	124.34 ^{bcde}	117.25 ^{a}	119.49 ^{ad}	116.03^{ace}	119.91^{ad}	0.538	<0.0001
Feed conversion ratio, Kg CF/kg egg	2.13^{ce}	2.10^{ce}	2.32^{abd}	2.09^{ce}	2.41^{abd}	0.022	<0.0001
Laying percentage, %	94.98	91.92	95.13	94.34	95.13	0.701	0.5539
Average egg weight, g	62.81b^{cde}	61.78^{ade}	61.31^{ade}	60.26^{abc}	60.49^{abc}	0.143	<0.0001

*Values with the different superscript in the same raw are statistically different (P<0.05)

Haematological parameters determined to evaluate the health status of poultry, are presented in Table 3. Note that the number of erythrocytes and the percentage of haematocrit was significantly (P<0.05) higher in C group compared to E1, E2, E3 groups, but lower than the haematological values of reference [25]. Monocytes were present in a significant higher percentage (P<0.05) in the blood of E1 hens compared to that from E4.

Table 3. The effect of the high fibre layer diets on haematological parameters (average values/group)

Specification	MU	C	E1	E2	E3	E4	SEM	p*value
Red blood cells (RBC)	M/µL	2.01^{bcd}	1.83^{a}	1.84^{a}	1.85^{a}	1.88	0.026	0.1452
Hematocrit (HCT)	%	26.25^{bcd}	23.25^{a}	22.75^{a}	23.25^{a}	23.75	0.437	0.0663
Hemoglobin (HGB)	g/dL	8.73	9.53	8.25	8.10	7.60	0.395	0.6519
Mean corpuscular volume (MCV)	fL	130.45	127.53	123.73	126.00	126.63	1.282	0.6166
Mean corpuscular hemoglobin (MCH)	pg	43.48	52.10	44.53	43.80	40.73	2.045	0.5213
Mean corpuscular hemoglobin concentration (MCHC)	g/dL	33.35	41.11	36.19	34.78	32.08	1.692	0.5318
Heterophile (%)	%	61.00	60.25	63.00	65.50	57.00	1.986	0.7704
Lymphocytes (%)	%	35.00	35.75	32.75	29.75	34.50	1.855	0.8844
Monocyte (%)	%	2.67	2.00^{e}	3.33	4.00	6.25^{b}	0.634	0.1961
Eosinophils (%)	%	2.00	2.50	2.33	2.33	2.25	0.311	0.9927

*Values with the different superscript in the same raw are statistically different (P<0.05)

As it can be noticed in table 4, the intestinal villus height of C hens was significantly (P≤0.05) higher than in the experimental groups. Also, the crypts depth measured on intestinal samples from C group were significantly (P≤0.05) higher compared to those samples derived from experimental groups. Mucosa thickness of C group was significantly (P≤0.05) lower compared to E3 group (addition of dietary Digestarom).

Researcher like [26] observed deeper crypts in jejunum and ileum of 28 days broilers, when the diet was supplemented with dietary fiber very viscous (4% xanthan gum). These effects were seen in villus, but only at ileal level. Another researcher [27] reported a linearly reduced height of villus depending on DF level, derived from sunflower meal. The similarly results was obtain by [21] who reported an improvement of villus

height in broilers (aged 15 days) fed with 2.5% peas shells diets. However, an increasing level of pea's shells (7.5%) had an opposite effect to those presented in Table 4. Researcher like [26] hase reported deep crypts, but there are no differences in cell proliferation when dietary sources of fibre with high viscosity were included.

Table 4. Effect of the high fibre layer diets on morphological integrity of intestinal mucosa (average values/group)

Specification	C	E1	E2	E3	E4	SEM	p*value
Villus height (µm)	860.00^{bcde}	583.00^{a}	627.00^{a}	678.00^{a}	633.00^{a}	19.448	<0.0001
Crypt depth(µm)	201.00^{bcde}	126.00^{a}	138.00^{a}	156.50^{a}	133.00^{a}	5.623	<0.0001
Muscular layer thickness(µm)	173.33^{d}	195.00	200.00	275.00^{a}	215.00	14.433	0.2363

*Values with the different superscript in the same raw are statistically different (P<0.05)

The microscopic appearance of jejunum of control groups did not present pathological alterations. Thus, the intestinal wall appears composed of mucosa, submucosa, muscular layer and serosa (Figure 1). The intestinal mucosa shows long villus, with numerous goblet cells and intestinal crypts are short and open in the space between the villus (Figure 2). At the level of lamina propia, the presence of lymphocytes, also heterophils, eosinophils can be noticed. In certain areas (Figure 3), it can be observed the presence of numerous lymphocytes, arranged in the form of aggregates, which represents the lymphoid tissue associated to the gut (GALT). Muscular layer consists of an external layer with longitudinal fibres and an internal layer with circular fibres. The submucosa is very thin.

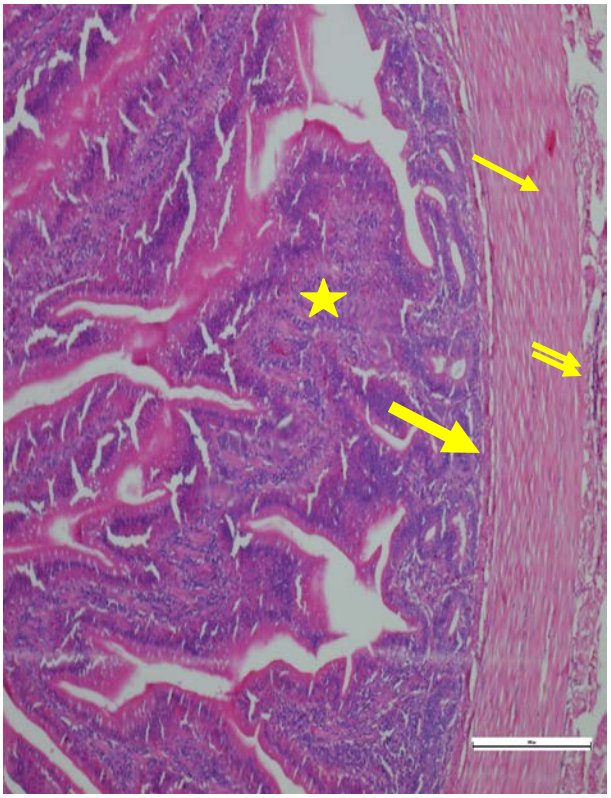

Figure .1 The layers of the intestinal wall: mucosa (star), submucosa (thick arrow), musculoasa (sageata subtire) si serosa (doublé arrow). HE, 10x

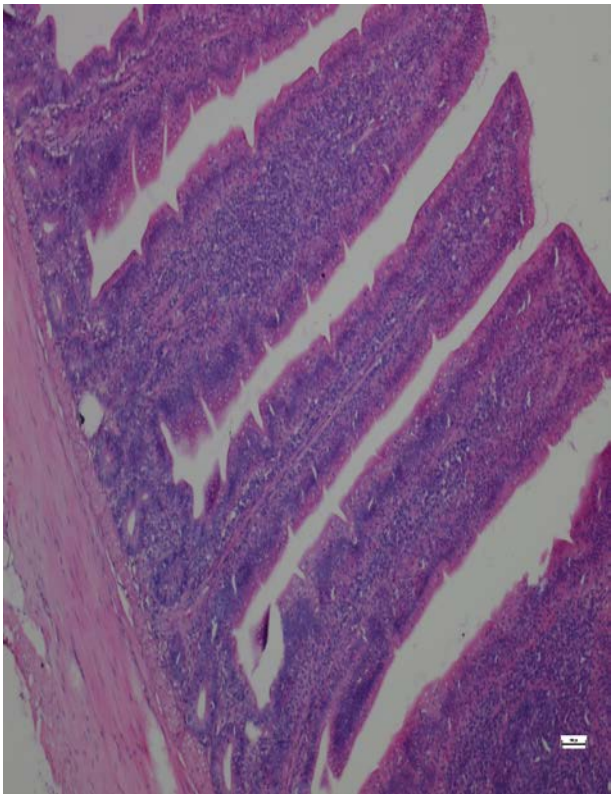

Figure 2. Normal aspect of villus and crypts

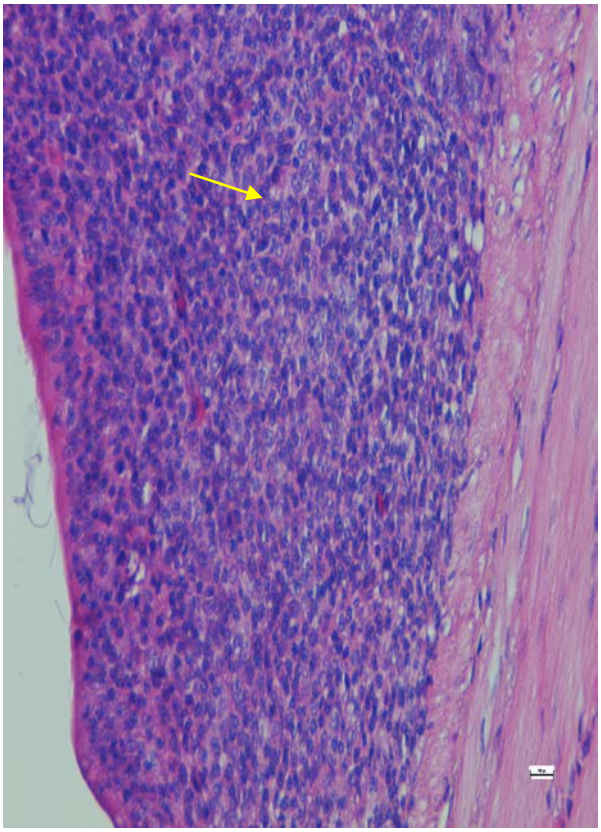

Figure 3. The lymphoid tissue associated to the gut (GALT), represented by dense lymphocytes located at lamnia propria level (arrow). HE, 10x

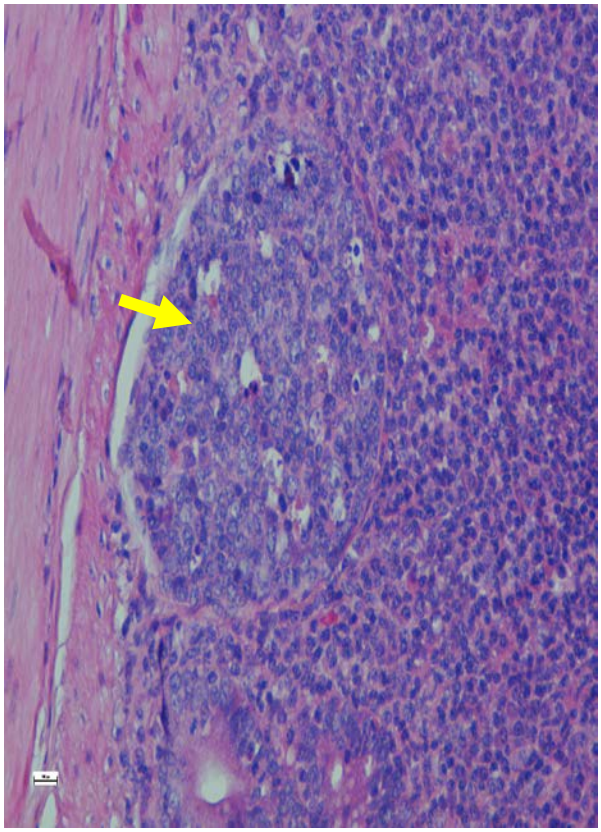

Figure 4. GALT hyperplasia associated with accumulation of histiocyte nests (large cells with round, euchromatic nucleus, abundant cytoplasm, weak eosinophile HE, 10x

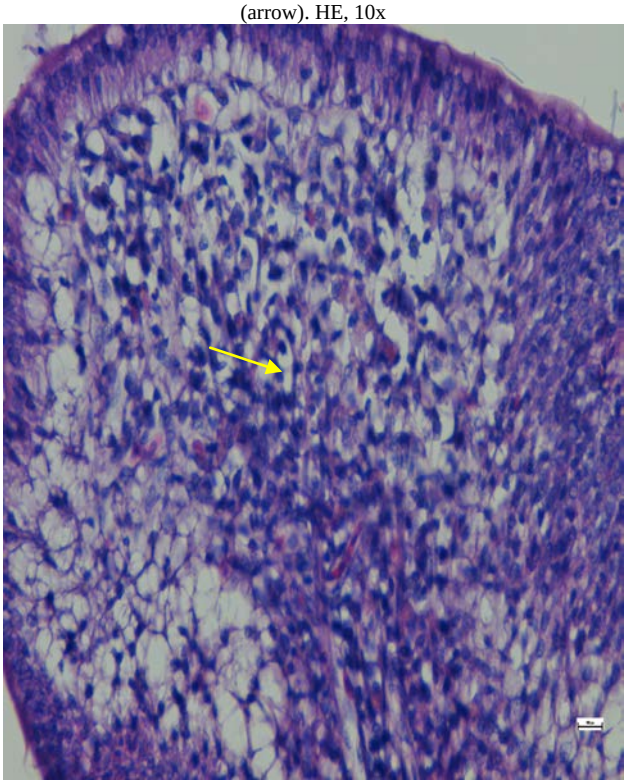

Figure 5. Fluid of edema accumulation at the level of lamnia propia, that it is gaining a tenuous aspect (arrow). HE, 40x

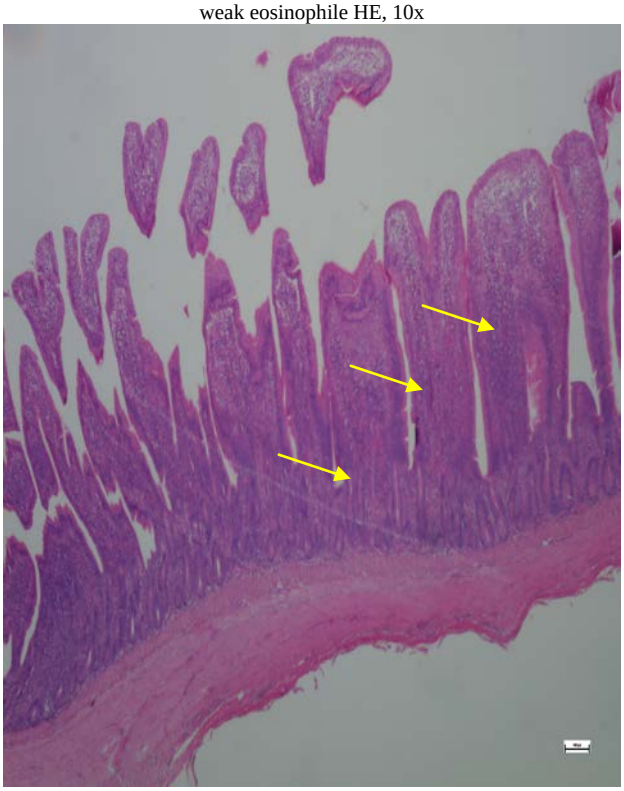

Figure 6. Villus shortening and thickening (arrows). HE, 10x

The histopathological changes diagnosed in individuals from the experimental groups exemplify the data from Table 4, documenting villus shortening and thickening (Figure. 6). Among the effects of the high fibre diet we noticed the infiltration of lamina propia with mononuclear inflammatory cells (lymphocytes, plasma cells) and polymorphonuclear cells (heterophils), fluid of oedema accumulation at the level of lamnia propia, that it is gaining a tenuous aspect (Figure 5), blood vessels hyperemia, GALT hyperplasia associated with accumulation of histiocytic nests (large cells with round, euchromatic nucleus, abundant cytoplasm, weak eosinophil) (Figure 4). In certain histopathological sections, intestinal bacterial flora could be observed in the form of adherent bacteria on the surface of enterocytes.

4. Conclusions

The addition of dietary sunflower meal in experimental groups (8% cellulose) reduced significantly ($P \leq 0.05$) the average daily intake and egg weight compared with the group that received a conventional diet (4.39% cellulose). In contrast, there were no significant differences between the five groups in terms of laying percentage. The height of villus from large intestine in the control group (4.39% cellulose) was significantly ($P \leq 0.05$) higher than that from the experimental groups (8% cellulose). The depth of crypts measured in intestinal samples collected from C group was significantly ($P \leq 0.05$) higher than the depth of crypts measured in intestinal samples collected from experimental groups. The presence of cellulolytic enzyme and/or herbal mixture did not influence villus height and crypts depth measured in the intestinal samples taken from hens from the experimental groups E2, E3 and E4.

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