Impact of Wheat Non-Starch Polysaccharides on the Intestinal Wall and Microflora in Broilers

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Abstract
Some cereals like barley, oat, and wheat contain significant quantities of non-starch polysaccharides (NSP). The main problems generated by NSPs are related to their viscous nature, to the physiological and morphological effects on the digestive tract and to their interaction with the intestinal flora. The objective of this experiment was to assess the impact exerted by wheat non-starch polysaccharides (NSP) on the intestinal flora and wall in broilers. The experiment was carried out during 6 weeks. We created two experimental groups: the experimental group EG1 fed on forage without wheat in its structure, and the experimental group EG2 fed on combined forage including 40% wheat. At 6 weeks old, after broiler killing, we sampled the intestinal wall and determined lactic bacteria and coliforms. Wheat incorporation in a proportion of 40 % in the combined forage determines the increase of NSP content with 1.84 percentage points; the number of lactic bacteria decreases insignificantly in EG2, fed on forage including wheat. In the experimental variant EG2, the mean height of the jejunal mucosa vilosity was bigger (684.6μ) than in the variant EG1 (403.9 μ), and these were coated with a slightly-hypertrophic epithelium (32.4 μ); on some of the areas, the capillaries were ectasied and we could notice hemorrhagic regions.

Keywords: broilers intestinal microflora, intestinal wall, non starch polysaccharides,

1. Introduction

Non-starch polysaccharides (NSP) are composed of chemical compounds with different physical and chemical properties (cellulose, arabinoxylans, beta glucans and pectic polysaccharides with manans and gallactans). The antinutritional effects on monogastrics are different and in many cases extreme. Some cereals like barley, oat, wheat, contain significant quantities of NSP. The main NSP-generated problems are related to their viscous nature, to the physiological and morphological effects on the digestive tract and to their interaction with the intestinal microflora. Arabinoxylans are the main NSP in wheat and give rise to highly viscous conditions in the small intestine, interfering with digestion and absorption of nutrients [1]. Wheat is the preferentially grown cereal in many European countries. However, different wheat cultivars vary with respect to their apparent metabolisable energy (AME) values [2], which is the reason why the quality of wheat for broiler chickens in some cases may be questioned [2,3]. Rye is the cereal of light sandy soils, which prevail in Poland, where about 3.2 million tons of rye is used for feeding purposes. However, it is well known that despite the better amino acid composition of protein, the feeding value of rye is inferior as compared to other cereals, especially when fed to young chickens [4,5,6]. With respect to the composition of the carbohydrate fraction, wheat, rye and triticale all contain arabinoxylans (AX), a soluble fraction of non-starch polysaccharides (NSP). These substances may create a viscous environment within the intestinal

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lumen [7]. Increased viscosity is associated with lower apparent metabolisable energy [2] as well as nutrient absorption and incidence of wet and sticky droppings [7]. Supplementation of broiler diets with appropriate enzymes, capable of degrading the xylan backbone of the AX has been shown to improve the nutritive value of wheat- [8], triticale [9] and rye-based diets [5,6]; for broilers. The improvement of performance has been suggested to be due to the lowered viscosity. However, the mode of the NSPs degrading enzyme action is not completely understood [6,11]. The composition of the diet affects the gastrointestinal microflora in broiler chickens. In particular, the presence of viscous polysaccharides has been shown to increase the intestinal microbial activity associated with poor broiler growth performance [14,16, 17, 21]. An increased microbial bile acid deconjugation, which leads to impaired lipid digestion, has been suggested to be partly responsible for poor broiler performance. Feed enzymes can reduce the bacterial activity in the ileum by reducing the amount of nutrients available for microbial fermentation [5,18].

2. Materials and methods

The experiment was performed on 60 chickens, distributed in two experimental variants. The chickens were fed as follows: during the first growth period, namely from eclosion to 3 weeks, the combined forage supplied 3157-3204 kcal metabolisable energy (EM) and a crude protein content of 22.91-22.94 %. In the second growth period, from 3 to 6 weeks, the combined forage supplied 3201.75-3244 kcal EM and 19.92-20.16 % CP. The experimental organization scheme and the nutritional characteristics of the forage mixtures used in this experiment are presented in table 1. To determine the nutritional value of the combined forage and the non-starch polysaccharide content, we used the standardized specific methods in concordance with the WEENDE scheme; the analyses were carried out in laboratories at the Department of Animal Nutrition and Alimentation, Faculty of Animal Sciences and Biotechnologies Timişoara and University College Dublin. To establish lactic bacteria and coliforms, the research material was inoculated in nutritional broth and then maintained in thermostat, at 37° C, for 24 hours. Then we determined the total germ number, with the help of Petri dish- cultures, making serial dilutions for each of the bacteria genera studied. Of each dilution, we performed inoculations on Petri dishes; the solid medium (agar), melted and chilled at 45°C, was then poured on these, by welding for a uniform medium distribution. The prepared Petri dishes, adequately marked for each dilution, were maintained at room temperature for medium solidification, and then introduced in the thermostat, at 37°C, for 24 hours. Germ number determination was performed by counting the developed colonies, considering that each germ generates one colony. The number of colonies determined on the Petri dish was then multiplied with the dilution titre, obtaining the number of germs per dilution. To determine the morphophysiological changes induced by the various NSP sources on the epithelial tissue, we sampled 1-2 cm^2 of duodenum, respectively jejunum from each chicken. The histological examination of the intestinal wall was carried out in concordance with the steps of sampling, washing, inclusion, sectioning and staining with haematoxylin-eosin, respectively trichromic Mallory staining. The testing of the difference significance between groups, regarding the production indices, was performed with the help of the Mann-Whitney-Wilcoxon test, and with the Minitab 14 software and the Duncan's test as well.

3. Results and discussion

According to tabular data, we obtained the content in soluble, insoluble and total NSP of the combined forage; these values are presented in table 2. According to table 2, we may conclude that the NSPs contents, during the growth period from eclosion to 3 weeks, had bigger values in the 40%-wheat combined forage, with 0.965 percentage points. As regards NSPi, these are 0.046 percentage points bigger in the same groups. During the growth period from 3 to 6 weeks, we may notice that the combined forage comprising 40% wheat had a NSPs content that was 1.18487 percentage points bigger than in the group fed forage without wheat. On the contrary, the NSPi contents decrease with 0.117 percentage points in the same groups. NSPt records a bigger increase, with 0.951 percentage points, in the first growth period, and with 0.972 in the second period, favouring the groups fed wheat-based forage as well. Of all NSP, wheat comprises the most arabinoxylans. We may observe that the 40%-wheat forage presented an arabinoxylan content of

1.17 percentage points bigger than in EG1, during the period from 0 to 3 weeks, and of 1.19 percentage points bigger, during the period from 3 to 6 weeks.

Table 1 Organization scheme of the experiment

Period 0-3 weeks	
EG1 CF (0 % wheat) EM (kcal/kg) 3204 CP 22.91%	EG2 CF (40 % wheat) EM (kcal/kg) 3157 CP 22.94%
Period 3-6 weeks	
EG1 CF (0 % wheat) EM (kcal/kg) 3244 CP 20.16%	EG2 NC (40 % wheat) EM (kcal/kg) 3201,75 CP 19.92 %

[1]EM=metabolizable energy,
[2]CP= crude proteine

Table 2 Combined forages content in non-starch polysaccharides (NSP)

Growth period	Specification	NSPs[1] (%)	Percentage differences	NSPi[2] (%)	Percentage differences	NSPt[3] (%)	Percentage differences	Arabino-xylans	Percentage differences
Period 0-3 weeks	0% wheat	0.788	-	9.46	-	10.248	-	4.16	-
	40 % wheat	1.693	0.905	9.506	0.046	11.199	0.951	5.33	1.17
Period 3-6 weeks	0 % wheat	0.865	-	9.55	-	10.415	-	4.37	-
	40 % wheat	1.8487	0.9837	9.5383	-0.117	11.387	0.972	5.56	1.19

[1] soluble non-starch polysaccharides
[2] insoluble non-starch polysaccharides
[3]total non-starch polysaccharides

Table 3 Evolution of the number of Lactobacillus and coliform bacteria in the Broiler chickens from the experimental variants

EG1		EG2	
Lactic bacteria	Coliforms	Lactic bacteria	Coliforms
$4.81x10^8$	$9.65x10^5$	$1.75x10^8$	$1.24x10^5$

The caecum is the most important fermentative segment in chicken gastric-intestinal tract, where the undigested carbohydrates may ferment with the help of the flora present here, being decomposed into organic acids and gas [11]. Fibre utilization in chicken diet influences chicken growth performances, and also the fermentation within caecum, causing even caecal hypertrophy [12]. [13] showed that a fibre-rich diet generated a 25%-increase of caecum size in turkeys, for example. In the experiment performed by Jozefiak, the poultry fed rye-based diet comprising high levels of soluble arabinoxylas had the biggest caecum weight and the biggest faeces amount. In this case, the supplementation with digestive enzymes caused the reduction of caecum weight nearly to the same level like in the other chicken groups. The enzyme supplementation of the wheat-based combined forage influences insignificantly the number of bacilli and coliforms within caecum [1]. We sampled the caecal content in order to determine microflora and the number of lactic bacteria and coliforms. These values are presented in table 3. According to this table, we may observe that the number of lactic bacteria decreases insignificantly in EG2, fed combined

forage including wheat, from 4.81 * 10^8 to 1.75 * 10^8 . Regarding coliform number, they decrease from 9.65 * 10^5 in EG1 to 1.24 * 10^5 in EG2. Intestinal viscosity enhancement may slow down the digestive passage, causing dramatic changes in the intestinal microbial balance by reducing oxygen pressure within the small intestine and by providing a stabile environment where the fermentative microflora may be constant (Wagner and Thomas, 1978). This determines intestinal microflora multiplication and consequently the increase of volatile fatty acids (AGV) and lactic acid production and the decrease of pH value (Hume et al., 1992; Ricke et al., 1982). Under normal conditions, the caecum represents the headquarters of the most processes of fermentation of the carbohydrate residual fragments from broilers' gastric-intestinal tract (Jozefiak et al., 2004). The mechanism glucanase enhances caecal fermentation with is unknown (Choct et al., 1999). Possibly low-molecular weight glucids have entered the caecum because of the reduced digestive viscosity, and rapidly fermented. Also, caecum filling and emptying may be enhanced by enzyme ability of stopping the digestive viscosity at caecum enter (Choct et al., 1999). In a study, F. Gao, 2007 did not find out any significant difference between the control group and the group fed supplemented enzymes in terms of coliform bacteria and lactobacilli within the caecal content, in the case of 21-day old chickens. This result is concordant with the results achieved by Engberg et al. (2004), who drew the conclusion that there are minor changes in the caecal content of lactobacilli and coliform bacteria. In the case of the individuals from the experimental group 1 **(**EG1**)**, the microscopic sections in duodenum and jejunum reveal the organization of this segment's wall in four superposed coatings: mucous, submucous, muscle and serous. The mucous presents villosities with the mean height of about 1905.3 μ, slightly widen at the base (fig. 1) and coated with a high mono-layered epithelium, with the mean height of 26.6μ, endowed with ribbed plateau. The inter-glandular areas are reduced, occupied with lax conjunctival tissue consisted of collagen fibres, fibroblasts, mono- and polymorph nuclear leukocytes and sanguine capillaries. Several infiltrative cells may be observed within the glandular (fig. 2) and villositar inter-epithelial regions, and also on the epithelium surface. The conjunctival tissue within the submucous coating comprises numerous vascular packages, and the muscle coating, consisted of two superposed layers of smooth muscle fibres, internally circular and externally longitudinal, has a mean width of about 174.3 μ. At jejunum mucous level, villosities range in size, but their mean height is 403.9 μ. The intestinal glands, with wide lumen, come from the base of villosities (fig. 2.); their epithelium is passed through by infiltrative cells, which can be observed in the glandular lumen, too. The lax conjunctival tissue within the villositar and interglandular corion (lamina propria) structure is reduced, it integrates the slightly ectasied capillary and lymphatic network. The mean width of the muscle coating, at this level, is about 150.0 μ.

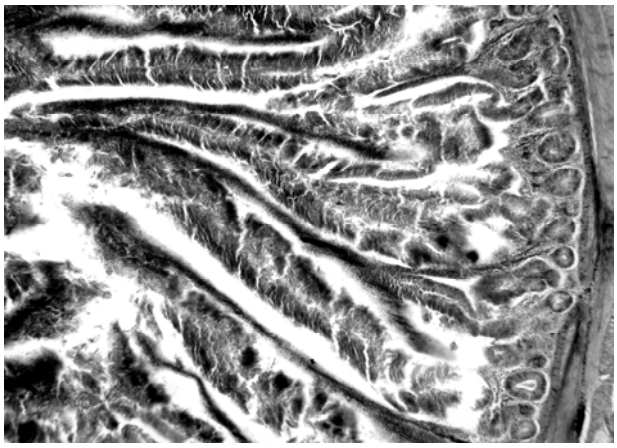

Figure 1 Experimental group (EG1) - section through duodenum, overview
(100x; Mallory trichromic staining)

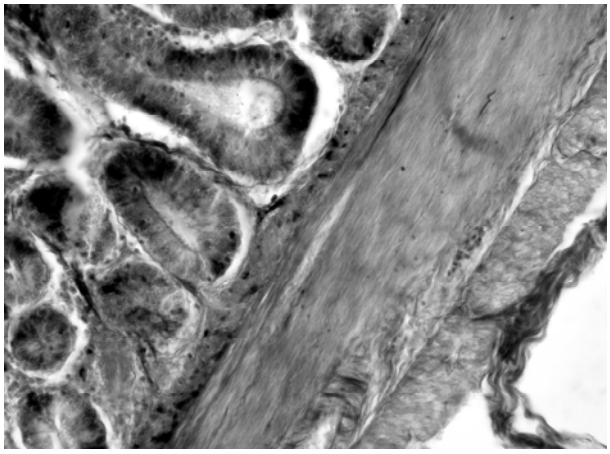

Figure. 2. Experimental group (EG1)– section through duodenum - intestinal glands and interglandular corion with infiltrated and sanguine capillary
(400x; Mallory trichromic staining*)*

In the case of the individuals within the experimental group EG2, the duodenal mucosa presents villosities with a mean height of about 1090.8 μ, slightly branched (fig. 3), coated with a slightly hypertrophic epithelium, predominantly consisted of absorbent prismatic cells, with a mean height of about 32.6μ. Among the absorbent cells, there are a reduced number of caliciform cells, which are more numerous at glandular epithelium level, and migrating leukocytes as well. The periglandular regions are reduced, occupied by lax conjunctival tissue, the support for a hardly-visible capillary network and of an abundant leukocytary infiltrate. There is, in the villositar corion, a strong leukocytary infiltrate, and the capillary network becomes slightly hypertrophic; however the very well developed structural element of the villositar corion is represented by the smooth muscle fascicle or the Brüke's muscle (fig. 3). The muscularis has a mean width of about 132.1 μ.

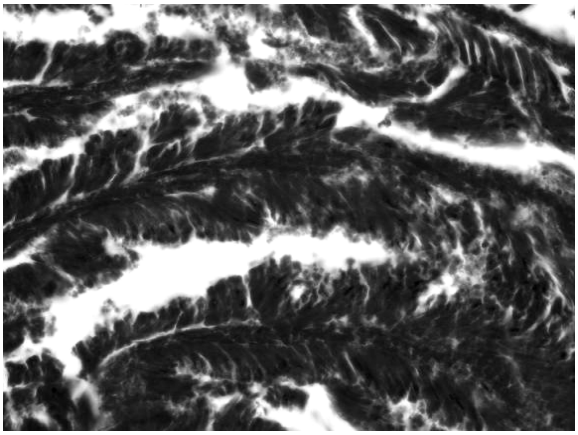

Figure. 3. Duodenum EG2 – intestinal villosity with slightly branched aspect
(400x; Mallory trichromic staining)

Jejunal **mucous' villosities**, in the case of the individuals within the experimental group **EG2**, present a widen aspect, with coated, short lateral branches, like the ones in duodenum, with a slightly hypertrophic epithelium. Mean cell height is about 32.4 μ. Mean vilosity height is about 684.6μ. The interglandular corion structure includes the conjunctival stroma rich in collagen fibres and dispersed fascicles of smooth muscle fibres. Also, the periglandular and submucous capillary network is slightly hypertrophic. In some regions, capillaries are ectasied and we could notice hemorrhagic areas (fig. 4). The villositar lamina propria is abundant in leukocytary infiltrate and Brüke's muscle fascicle.

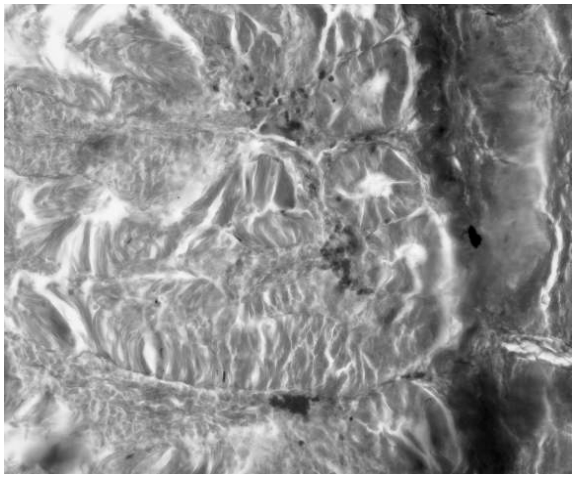

Figure. 4 Jejunum EG2 - ectasied capillary and hemorrhagic areas in the mucosa corion
(400x; trichromic Mallory staining)

The analysis of the histomorphometric data proves that, in the case of the experimental variant EG2, the mean height of the jejunum mucous villosities is bigger (684.6μ) than in the group EG1 (403.9 μ) and these are coated with a slightly hypertrophic epithelium (32.4 μ). Also, in the case of EG2, the periglandular and submucous capillary network is slightly hypertrophic; in some regions, capillaries are ectasied and we may notice hemorrhagic areas. The villositar corion is abundant in leukocytary infiltrate and Brüke's muscle fascicle, and we could notice a few caliciform cells and very many infiltrative cells in the structure of the glandular epithelium.

4. Conclusions

As a conclusion, we may say that wheat incorporation in the combined forage structure in a proportion of 40 % determines some changes. In these terms, the NSPs content increases; the **arabinoxylan** content increases, too, during the period 0-3 weeks and also during the period 3-6 weeks. The number of lactic bacteria insignificantly decreases in EG2, fed wheat-based forage. In the case of the experimental variant EG2, the mean height of the jejunum mucous villosities is bigger than in the variant EG1; these villosities are coated with a slightly hypertrophic epithelium. Also, in the case of EG2, the periglandular and submucous capillary network is

slightly hypertrophic; in some regions, capillaries are ectasied and we may notice hemorrhagic areas.

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