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Effect of Different Levels of Cellulose in the Diet on the Proteolytic Activity of the Pancreas in Broiler Chickens

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The experiment involved 36 Cobb broiler chickens and investigated the effect of different levels of cellulose preparation, Arbocel BWW-40, on the body weight, pancreas weight, content of protein in the pancreas gland and activity of trypsin and chymotrypsin in pancreas. The addition of cellulose to the bird diet showed a significant effect ($P<0.05$) on an increased body weight, content of protein in pancreas gland and the activity of proteolytic gland enzymes, which can stand for a more intensive protein metabolism in birds fed with a diet enriched with the Arbocel BWW-40 cellulose preparation.

Key words: Cellulose, trypsin, chymotrypsin, proteolytic activity, broiler chickens

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Carbohydrates constitute the main component of poultry feeds whose share in the diet ranges from 40 to 70% (MCNAB & BOORMAN 2002). The water-insoluble fraction (cellulose, hemicelluloses, lignin) has little effect on the nutritive value (0-3 kcal/gram) (BARTNIKOWSKA 1995), however, it affects mainly the alimentary tract, which is reflected in its anatomy and histology (FARNESS & SCHNEEMAN 1982; HARA *et al.* 1996), affects the digesta passage time (BURROWS *et al*. 1982) and the development of caecum bacteria (CAMPBELL *et al.* 1997). A proper bacterial fermentation in the caecum and maintenance of appropriate alimentary tract peristalsis require a 2-3% share of insoluble fibre in bird diet (SMULIKOWSKA 2002).

Research into chickens showed that a diet enriched with faba bean hulls, made mostly from cellulose, decreased the activity of trypsin in the ileal digesta and pancreas gland in birds (LONGSTAFF & MCNAB 1991). The experiment reported by SCHNEEMAN and GALLAHER (1980) which involved rats fed with 20%-cellulose diet demonstrated no positive effect of fibre added to the diet on the activity of enzymes in the pancreas. An inhibiting effect of fibre of different origin (alfalfa, cellulose, wheat and oat bran, xylans) on the activity of trypsin and chymotrypsin *in vitro* was shown by DUNAIF and SCHNEEMAN (1981), incubating human duodenal juice with different sources of non-starch polysaccharides.

When considering the results cited above, the main aim of the present research was to determine the effect of various levels of cellulose in broiler chicken diet on their final body weight and the activity of proteolytic enzymes of the pancreas.

Material and Methods

Experimental animals

The research was carried out at the laboratory of biological tests of the Department of Animal Physiology, the University of Technology and Agriculture in Bydgoszcz. The experiment covered 36 Cobb chickens, from 0 to 42 days of age, from a local commercial hatchery. The chickens were housed in an environmentally regulated room, raised compliant with recommendations provided in the Cobb broiler breeding guidebook and vaccinated following the dates defined by the chicken producer. Throughout the experiment broilers were fed *ad libitum* and had free access to water.

Experimental diets

Throughout the experiment the birds were fed with isocaloric and isonitrogenous corn-wheat-soybean meal diets. The feeding program applied

covered three periods during which adequate feed was administered: starter from 0 to 21 d; grower from 22 to 35 d and finisher from 36 to 42 d of age. The experimental factor was constituted by a varied share of Arbocel BWW-40 cellulose preparation by Rettenmaier Polska Sp. z o.o., which was added to the experimental diets. The preparation properties are given in Table 1. The content of crude fibre obtained from the diet components accounted for 3% in each feed mixture. A total amount of crude fibre obtained from feed components and Arbocel BWW-40 is given in Table 2.

Table 1

Arbocel BWW-40 properties (J. Rettenmaier & Söhne GMBH + Co)

Properties	Arbocel BWW-40
Color	White
Structure	Average-length fibres
Cellulose content (%)	About 99.5
Lignin content (%)	None
Ash content (%)	0.3
Bulk density (g/l)	About 130
Fibre length (ěm)	200
Taste	Neutral
Smell	Neutral
Whiteness (%)	About 86
Water absorption (%)	About 580

Table 2

Percentage of fibre in the control and experimental diets

Diet	Group			
	C	E1	E2	E3
Starter				
Fibre from diet components	3.00	3.00	3.00	3.00
Additive of cellulose	–	0.25	0.50	0.75
Grower				
Fibre from diet components	3.00	3.00	3.00	3.00
Additive of cellulose	–	0.35	0.60	0.85
Finisher				
Fibre from diet components	3.00	3.00	3.00	3.00
Additive of cellulose	–	0.45	0.70	0.95

C – control group
E1, E2, E3 – experimental group

Measurments

On the 42 d of the experiment all the birds were weighed and then slaughtered in order to remove pancreas glands. Immediately after being removed from the body cavity, the prepared pancreas was cleaned carefully from connective tissue, weighed, frozen in liquid nitrogen and kept at –28°C. The frozen pancreas was homogenized for 10 min at 10000 rpm, in a 0.15M solution of NaCl containing 0.1% Triton X-100, at the weight to volume ratio of 1:10. The content of protein in the pancreas expressed in mg/g of the tissue was determined following LOWRY's method (1951), using UV-VIS 3100 apparatus by SHIMADZU, with a ready-kit by Sigma, taking measurements of the absorbance at a wavelength of 750 nm. The activity of proteolytic enzymes was assayed with the method developed by HUMMEL (1959) using an UV-VIS 3100 apparatus by SHIMADZU, with reagents by Sigma. To assay the activity of trypsin, Tosyl-L-arginins-methyl ester (TAME) was used as a substrate, and the measurement was made at a wavelength of 247 nm. When assaying the activity of chymotrypsin, N-benzoyl-L-Tyrosine ethyl ester (BTEE) was used as a substrate and the measurement was made at a wavelength of 256 nm. Prior to determining the enzymatic activity, homogenates were activated with enterokinase for 30 min at 25°C.

Statistical analysis

The results obtained were analysed statistically, applying Duncan's test, using Statistica software (1998). The significance of differences was verified at the significance level of $P<0.05$.

Results and Discussion

The body weight measurements made on the 42 d of the experiment (Table 3) showed higher values of this parameter in chickens of experimental groups, as compared with the control group; the body weight of chickens of E1 and E3 groups was significantly higher ($P<0.05$). A similar final body weight of Cobb chickens on the 42 d of experiment was recorded by SZYMECZKO (2000), using the same additives as applied here (Arbocel BWW-40) to broiler chicken diets. The current work also recorded a higher body weight in all the experimental groups, as compared with the control group, however the differences were not confirmed by statistical calculations. SZYMECZKO (2000) suggests that better effectiveness of rearing in experimental groups must have been due to the effect of cellulose added to the diet on an enhanced effectiveness of digestion processes and absorption of different nutrients. This concerns mostly exogenous amino acids: arginine, lysine and methionine, which is confirmed by research into other animal species (LI *et al.* 1994; MUIR *et al*. 1996). Another study carried out in rats confirmed a significant effect of cellulose added to the diet neither

Table 3

Body weight, pancreas weight and the content of protein in the pancreas of broilers fed dietary treatments

Parameter	Group			
	C	E1	E2	E3
Body weight (g)	2120±158[1)]	2317±111*	2216±179	2293±191*
Pancreas weight (g)	2.7±0.7	2.8± 0.2	2.6± 0.5	2.8±0.6
Pancreas protein (mg/g of pancreas)	89.0±16.2	122.6±17.5*	129.1±15.0*	122.3±12.2*

C – control group
E1, E2, E3 – experimental group
[1)] results are expressed as mean value ± SD
* significant differences, as compared to the control group (C) at $P<0.05$.

on the final body weight (OKU 1995) nor on the animal body weight gains (FARNESS & SCHNEEMAN 1992; SATCHITHANANDAM *et al.* 1996). Different results were reported by BRAGADO *et al*. (2001) who demonstrated a significantly lower body weight in rats fed with 15% cellulose feed, as compared with the animals fed with a standard laboratory diet.

The weight of the pancreas in all the bird groups investigated was similar (Table 3). Also the reports by LONGSTAFF and MCNAB (1991) noted no effect of the additive of fibre from faba bean hulls on the chicken pancreas weight. The present results and those reported by the authors cited above correspond to the results of experiments involving other animal species. No differences in the pancreas gland weight in rats were reported by SCHNEEMAN and GALLAHER (1980), applying 20% additive of cellulose to the diet, FORMAN and SCHNEEMAN (1980), making the animal diet richer with 5% pectin and SCHNEEMAN *et al*. (1982), adding 20% of wheat bran to the experimental feed.

In all the experimental groups the determined content of protein in the pancreas was significantly higher ($P<0.05$), as compared with the control group (Table 3). The concentration of protein in duodenal juice depends on the type of secretion stimulus and is often used as an indicator of the enzymatic activity of the gland (KONTUREK 1976). SCHNEEMAN and RICHTER (1993) noted a significant increase in the concentration of protein in the pancreas gland in rats fed with a diet enriched with oat bran. Increased pancreas weights and increased concentration of protein in the gland were also reported by ISAKSSON *et al.* (1983), administering wheat bran-supplemented feed to rats for 10 days. Yet other reports show a significant daily increase in the total pancreas protein production in pigs fed meal with 40% of wheat bran added (LANGLOIS *et al*. 1987). The authors relate this phenomenon to a considerably increased (115%) volume of the duodenal juice secreted and probably an intensified pancreas protein biosynthesis. An increased volume of secreted duodenal juice and a significantly higher content of protein in duodenal juice were also reported in growing pigs fed with a diet which contained 2% potato fibre (JAKOB *et al*. 2000). However, in experiments with rats no significant effect of a 15 and 20% additive of cellulose and 20% of wheat bran added to animal diet on the content of protein in the pancreas gland was observed (BRAGADO *et al.* 2001; SCHNEEMAN & GALLAHER 1980; SCHNEEMAN *et al*. 1982).

Table 4

Activity of trypsin and chymotrypsin in the pancreas gland of broilers fed dietary treatments

Activity (IU / mg of protein)	Group			
	C	E1	E2	E3
Trypsin	8.2±6.2[1)]	19.0±8.5*	28.5±8.7*	18.0±4.4*
Chymotrypsin	16.2±6.2	28.4±5.3*	30.0±4.7*	19.4±6.6

C– control group
E1, E2, E3 – experimental group
[1)] results are expressed as mean value ± SD
* significant differences, as compared to the control group (C) at $P<0.05$

The activity of trypsin and chymotrypsin in the pancreas gland in broiler chickens is given in Table 4. The current study observed a significantly higher ($P<0.05$) activity of both proteolytic pancreas enzymes in all the experimental groups, as compared with the control group. The present results are confirmed by nutritional experiments involving other sources of fibre. Applying a 20% additive of wheat bran to the rat diet, SCHNEEMAN *et al*. (1982) also noted an almost two-fold significant increase in the activity of trypsin and non-

significant increase in the activity of chymotrypsin in the pancreas. Investigating the composition of duodenal juice in cannulated pigs, a significant 58% increase in the activity of trypsin and 59% of chymotrypsin was identified in the animal group fed with a 40% additive of wheat bran (LANGLOIS *et al*. 1987). In an experiment carried out on dogs fed with feed containing a wheat bran additive, as much as a 350% increase in the activity of chymotrypsin was noted (STOCK-DAMGE *et al*. 1984). An increased activity of trypsin in duodenal juice in pigs was also due to added potato fibre (JAKOB *et al.* 2000). Applying a 20% additive of pure cellulose to rat feed resulted in a slight decrease in the activity of trypsin and a slight increase in the activity of chymotrypsin in the pancreas, as well as a significant decrease in the activity of both enzymes in the ileal digesta (SCHNEEMAN & GALLAHER 1980). In an experiment reported by SCHNEEMAN and GALLAHER (1980), the content of cellulose in the diet exceeded considerably its level in feed mixtures used in the present study. The experiments carried out so far do not provide a clear-cut explanation of the effect of fibre on exocrine pancreatic function. Based on the present work it can only be stated that it depends on the kind of fibre consumed, its amount in the diet and the length of the administering period. Changes which occur in the pancreas and in its exocrine activity are probably due to the effect of fibre contained in the diet on releasing the hormones of alimentary tract (HASIK & BARTNIKOWSKA 1987), such as: CCK, secretin, gastrin, VIP and glucagon. These hormones together with acetylcholine and noradrenaline act as intermediaries in the mechanisms activating pancreatic secretion (KONTUREK 1976; SOMMER & KASPER 1981). The relationship between the presence of fibre in the diet and releasing CCK is confirmed by research carried out in humans (MÖSSNER *et al*. 1992; BOURDON *et al*. 2001). It is worth noting that the significantly higher activity of both trypsin and chymotrypsin in E1, E2 and E3 groups recorded in the present investigation closely corresponds to a higher content of protein in the pancreas in those groups which can show a more intensive enzymatic proteins synthesis in the pancreas gland due to the experimental factor studied. Furthermore, as mentioned earlier, chickens of all the experimental groups demonstrated a higher body weight, as compared with control group, which shows the benefits of the diet nutrients. BOTERMANS and PIERZYNOWSKI (1999) report that an increase in the body weight in growing pigs is positively correlated with an increase in the exocrine pancreas secretion. At the same time SZYMECZKO (2000) observed that chickens which obtained in feed mixtures the same level of Arbocel BWW-40 as in the current work, showed lower feed consumption, a higher slaughter yield, a higher meat yield and a lower fat content in the body.

Based on the present results, a favorable effect of pure cellulose on broiler chicken organism is noted, showing an increased body weight and a higher content of protein in the pancreas and a higher activity of proteolytic enzymes of the pancreas gland.

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