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The Level of Major Proteins and Minerals in the Blood Serum of Chickens Fed Diets with Pure Cellulose

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The aim of the research was to determine the concentration of total protein and its fractions as well as the concentration of selected mineral components in the blood serum of male broiler chickens Cobb 500 fed diets with different cellulose content. Blood samples were collected for examination from the birds' pterygoid canal veins on their 42 day of age. There was no influence of cellulose preparation on the content of total protein and its fractions: albumins, $alfa_1$-, $alfa_2$-, beta-, gamma-globulins, albumin to globulin ratio, inorganic phosphorus, sodium, potassium, chloride and iron concentrations in blood serum. The highest calcium concentration ($P<0.05$) was detected in the blood serum of males fed a diet with the highest cellulose content (0.75-0.95%). Simultaneously, a tendency of increased calcium content was observed along with an increased amount of pure cellulose in diets. The lowest magnesium level ($P<0.05$) was observed in the blood of birds fed diets with the lowest amount of cellulose (0.25-0.45%). The magnesium level in the blood of birds fed a diet with higher amounts of cellulose did not considerably differ from the control group. Results from the current study suggest that introduction of a limited amount of pure cellulose into the diet of broiler chickens does not affect total protein concentration and protein fractions but can influence the mineral content in the blood serum.

Key words: Chicken, pure cellulose, blood serum, biochemical indices.

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Due to a number of physiological functions, both blood plasma proteins and minerals are significant indicators of animal health and performance. One of the major factors affecting the protein profile and concentration of minerals in blood is nutrition. Cereal-based feeds with high protein and energy concentration commonly administered to chickens usually contain substantial amounts of non-starch polysaccharides (NSP), mainly derived from cell walls in the endosperm and the outer seed hulls. Numerous studies showed that the soluble fraction of NSP characterized by high viscosity had an antinutritive effect and decreased both digestibility and nutrient absorption (SMITS *et al*. 1997; LANGHOUT *et al*. 1999; SMULIKOWSKA 2002). Unlike the soluble NSP fraction, the fraction of insoluble fibre (mainly cellulose and lignin), considered to be "energy diluent", reduces the concentration of nutrients in poultry feeds rendering no negative effect on digestibility. During the last decade, researchers revealed that a small amount of insoluble fibre was indispensable in broiler diet in order to ensure the proper growth of birds and correct functioning of their digestive tracts (SMULIKOWSKA 2002). There are several factors that may bring about better utilization of feed ingredients (HETLAND & SVIHUS 2001; SVIHUS & HETLAND 2001; HETLAND *et al.* 2003; BOGUSŁAWSKA-TRYK 2005; JIMÉNEZ-MORENO *et al.* 2009; SARIKHAN *et al.* 2010). These include an increase in the weight of individual segments of the digestive tract, gizzard activity, pancreatic enzyme activity and a higher concentration of bile acids in jejunum, positive changes in the morphology of the small intestine mucosa resulting in a larger absorption surface. It is well-known that dietary fibre has the ability to precipitate minerals in the digestive content which decreases their absorption (VAN DER AAR 1983; AGUNBIADE & LONGE 1998; GREGER 1999; SMULIKOWSKA 2002). In view of the studies mentioned above, it seems justified to ask whether cellulose, as the major component of insoluble fibre fraction (considering its physico-chemical properties), can affect the protein profile and mineral concentration in the blood of broiler chickens. We were also interesred in the extent of this interaction.

The aim of the present study was to determine the level of total protein, its fractions and selected minerals in the blood serum of chickens fed diets with different levels of pure cellulose.

Material and Methods

Experimental animals

The study was conducted under a research protocol approved by the Local Ethical Committee in Bydgoszcz (Poland).

The study was conducted on 36 male Cobb meat-type chickens from a local hatchery. After delivering broilers to the biological tests laboratory, they were weighed and randomly assigned to one of four experimental groups. Each group consisted of nine birds (9 birds per cage). The broilers were housed in litter-floored cages, in a room with automatically controlled temperature, humidity and air exchange. The birds were provided with continuous light. Rearing was conducted according to the technological recommendations for the breed. Throughout the experiment, the broilers were fed *ad libitum* and had free access to water.

Experimental diets

During the 6-week experiment, all birds were fed with isocaloric and isonitrogenous commercial mixtures based on maize, wheat and soybean meal. All chickens were fed starter up to the 21st day, grower from the 22nd to 35th day and then finisher diets from the 36th to 42nd day of age. The calculated content of crude protein (%) and metabolisable energy (MJ kg^{-1}) in the diets was as follows: starter 23.0, 12.6, grower 21.0, 13.3, finisher 19.0, 13.5. All mixtures were formulated to meet the minimum nutrient requirements for broiler chickens (NRC 1994). Experimental diets varied in the amount of pure cellulose (Arbocell BWW-40, J. Rettenmaier & Söhne GMBH + Co) incorporated into the mixtures. The composition and properties of the cellulose preparation are presented in an earlier publication (BOGUSŁAWSKA-TRYK 2005). The percentage of crude fibre (CF) derived from the mixture ingredients amounted to 3% in each feed. The total amount of crude fibre obtained from feed components and Arbocell BWW-40 is presented in Table 1.

Chemical analysis

At the end of the feeding experiment (42nd day of age), blood samples from the pterygoid canal veins of all chickens were collected for analysis. After coagulation, the blood samples were centrifuged at 3,000 rpm for 10 min, and the obtained serum was stored in a freezer at -20^{o}C for further analysis. In the blood serum, the following parameters were determined: total protein and its fractions (albumins, alfa$_1$-, alfa$_2$-, beta-, gamma-globulins), calcium (Ca), inorganic phosphorus (Pi), sodium (Na), potassium (K), chloride (Cl), magnesium (Mg) and iron (Fe). The albumin/globulin ratio was calculated. The total protein content was determined by the colorimetric method with the use of the Clinilab analyzer. The level of protein fractions was estimated with the use of the agar gel electrophoresis method, Beckman Company equipment and the application notes from the Beckman Coulter Company. The Ca, Pi, Mg and Fe contents were determined with the Clinilab analyzer and the application notes from the Merieux Company. The Na, K and Cl concentrations in the blood serum were measured with the use of the AVL-988-3 analyzer and ionoselective electrodes.

Statistical analysis

The results were analysed statistically with the use of the Statistica software program (STANISZ 1998). The *post hoc* Duncan test was applied. The significance of differences was set at $P<0.05$.

Results and Discussion

The proteins of blood serum constitute a vital indicator of the general health condition of any living organism. All the digestion and metabolism disorders that may result from malnutrition can be diagnosed by examining changes in the blood serum protein concentration (MONTAGNE *et al*. 2003).

Table 1

Calculated level of crude fibre (%) in broiler diets

Diet	Group			
	C	E1	E2	E3
Starter	3.00	3.25	3.50	3.75
Diet fibre	3.00	3.00	3.00	3.00
Additive of cellulose	–	0.25	0.50	0.75
Grower	3.00	3.35	3.60	3.85
Diet fibre	3.00	3.00	3.00	3.00
Additive of cellulose	-	0.35	0.60	0.85
Finisher	3.00	3.45	3.70	3.95
Diet fibre	3.00	3.00	3.00	3.00
Additive of cellulose	–	0.45	0.70	0.95

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

Table 2

Blood serum proteins (g l^{-1}) of broiler chickens ($\bar{x}$ ± SD)

Parameters	Group			
	C (n=9)	E1 (n=9)	E2 (n=9)	E3 (n=9)
Total protein	30.40± 3.57	30.33± 1.67	31.40± 1.47	30.36± 1.94
Albumin	15.81± 2.09	15.38± 0.60	16.28± 1.57	15.89± 1.03
Globulin	14.65± 0.89	14.97± 0.51	15.15± 0.61	14.46± 0.75
– $alpha_1$-globulin	3.40± 0.72	3.56± 0.82	3.38± 0.92	3.19± 1.00
– $alpha_2$-globulin	3.76± 0.69	3.67± 0.52	3.88± 0.62	3.50± 0.48
– beta-globulin	2.48± 1.01	2.51± 0.44	2.59± 0.30	2.29± 0.42
– gamma-globulin	5.01± 1.21	5.23± 0.28	5.30± 0.66	5.48± 1.22
Albumin: Globulin	1.08± 0.02	1.03± 0.01	1.07± 0.03	1.10± 0.02

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

The concentration of the total protein and its fractions in the blood serum of chickens fed diets with different pure cellulose content is presented in Table 2. No significant effect of the cellulose preparation Arbocel BWW-40 was detected on the total protein concentration and its fractions in the blood of 42-day-old broiler chickens. Both the protein (30.33-31.40 g l^{-1}) and the albumin content (15.38-16.28 g l^{-1}) fell into the range of physiological values defined for 6-week-old broiler chickens which may suggest that there is no negative influence of the pure cellulose additive on bird health (ROSS *et al*. 1978; BOWES *et al*. 1989; KRASNODĘBSKA-DEPTA & KONCICKI 2000). Numerous experiments carried out on broiler chickens took into consideration the nutritional value and physico-chemical characteristics of a dietary fibre which is, as a feed ingredient, a non-homogeneous substance (MONTAGNE *et al*. 2003; SARIKHAN *et al.* 2009). Until now, studies have focused on examining the properties of soluble dietary fibre fractions and their effect on the performance of chickens. The insoluble fraction, the major component of which is cellulose, has been basically regarded as digestive tract filler. There is no information in the literature on the effect of pure cellulose on the blood serum protein and its fractions in chickens. The research carried out by SARIKHAN *et al*. (2009) on broiler chickens fed diets with the non-soluble fibre preparation in which the ADF fraction constituted 86.5%, did not demonstrate any interaction between the additive and the protein and albumin content in blood. Laboratory tests conducted on mice showed that even long-term administration of 3% pure cellulose in diets did not affect the relative weight of the digestive tract organs (KANAUCHI *et al*. 2000). This did not result in any pathological changes in liver or kidneys and did not substantially influence protein metabolism parameters such as protein, albumin, BUN and creatinine. Also high, a 15% pure cellulose additive in diet did not influence the total protein level and its fractions in the blood serum of rats (AGUNBIADE & LONGE 1998). An experiment by CAO *et al*. (2003) revealed that the pure cellulose content in the diet of Single Comb White Leghorn chickens should not exceed 3.5%. A higher concentration (10%) has a negative effect on both the production results and nitrogen retention in birds. According to these authors, this is due to a considerable increase in the ingesta volume and intestinal feed passage time which reduces the time of nutrient exposition to digestive enzymes. In the present study in which the fibre levels and the pure cellulose additive in the diet fed to Cobb chickens (Table 1) are comparable with those quoted by CAO *et al*. (2003) and SARIKHAN *et al*. (2009), the levels did not affect the blood serum proteins. However, as it was shown in our earlier studies, they had a beneficial effect on pancreas proteolytic enzyme activity, protein content in the pancreatic gland as well as growth performance in birds (BOGUSŁAWSKA-TRYK 2005). This may suggest that the cellulose additive in the diet results in an increased protein metabolic rate.

The mineral content in the blood serum of birds is considerably dependent on its mineral concentration in feeds as well as factors influencing the degree of their absorption in the digestive tract. Mineral homeostasis is regulated by both neural

and humoral mechanisms. Depending on its type and level in the diet, the dietary fibre can have an inhibitory or stimulating effect on intestinal mineral absorption (VAN DER AAR *et al*. 1983; KANAUCHI *et al.* 2000; MONTAGNE *et al.* 2003; JIMÉNEZ-MORENO *et al.* 2009). In the present study, considerable differences ($P<0.05$) in calcium and magnesium content were detected in the blood serum of chickens fed diets with the pure cellulose additive in comparison to the control group (Table 3). The highest calcium level (2.38 mmol l^{-1}) was recorded in the serum of the E3 group fed a diet with a 0.75-0.95% cellulose level. It is emphasized that the calcium concentration in the blood showed a gradual increase along with an increasing level of cellulose in the diet. The lowest magnesium concentration (0.83 mmol l^{-1}) ($P<0.05$) was observed in the blood serum of broilers from group E1 fed a diet with the lowest cellulose additive (0.25-0.45%). The magnesium content in the blood of chickens fed diets with a higher cellulose level did not differ significantly from the control group. There were no significant differences in Pi, Na, K, Cl and Fe concentration in the blood of the experimental birds and the level of all analyzed minerals remained in a wide range of the physiological values defined for 6-week-old meat type chickens (ROSS *et al*. 1978; BOWES *et al*. 1989; KRASNODĘBSKA-DEPTA & KONCICKI 2000; SILVA *et al*. 2007). It is well-known that soluble dietary fibre fractions, undergoing bacterial fermentation in caecum and colon, favourably affect the digestive tract ecosystem leading to both an increased population of *Bifidobacterium* and *Lactobacillus* and a reduced number of pathogenic bacteria (*E. coli, Salmonella, Clostridium*) (SMULIKOWSKA 2002; XU *et al*. 2003). Consequently, there is an increase in short-chain fatty acid production. Medium acidification increased the soluble fraction of minerals in the digesta which facilitated their availability and absorption (BRONNER & PANSU 1999; COUDRAY *et al*. 2003). The effect of insoluble fractions of fibre, including cellulose, on mineral absorption in chickens has scarcely been investigated. Experiments conducted on laboratory animals revealed that cellulose in *in vitro* conditions diminished Ca and Mg ion absorption properties and even a prolonged consumption of diets with 3% cellulose content did not influence the electrolyte concentration in the blood serum of rats (KANAUCHI *et al.* 2000). The experiments on chickens showed that enriching the diets of broiler chickens with 3% oat hulls had a positive effect on the total retention of all the dietary nutrients including crude ash (JIMÉNEZ-MORENO *et al.* 2009). This phenomenon can be attributed to the fact that insoluble fibre molecules remain longer in the upper segments of the digestive tract thus stimulating the gizzard (HETLAND & SVIHUS 2001; HETLAND *et al*. 2005), intensifying the gastro-duodenal reflux (HETLAND *et al.* 2003), and finally, contributing to HCl secretion (GUINOTTE *et al.* 1995). The researchers mentioned that the low pH of the gizzard not only improves pepsin activity but also leads to elevated solubility of minerals in feeds which enhances their absorption. A study determining the mineral status in chickens fed different types of fibre did not show that a high (8%) cellulose level in diet had any effect on the Ca and Mg content in blood serum of 25-day-old birds (VAN DER AAR *et al.* 1983). Similarly, SARIKHAN *et al*. (2009) did not confirm the effect of fibre-based diet with 86.5% ADF fraction on the Ca concentration in blood of 3-week-old birds. However, they did observe a

Table 3

Mineral content in the blood serum of broiler chickens ($\bar{x}$ ± SD)

Parameters	Group			
	C (n=9)	E1 (n=9)	E2 (n=9)	E3 (n=9)
Calcium, mmol l^{-1}	2.00± 0.25	2.08± 0.23	2.19± 0.22	2.38*± 0.10
Phosphorus, mmol l^{-1}	2.07± 0.20	2.02± 0.14	2.17± 0.16	2.18± 0.20
Sodium, mmol l^{-1}	155.19± 6.98	151.06± 3.60	153.82± 3.84	151.97± 2.38
Potassium, mmol^{-1}	3.27± 0.77	3.28± 1.00	3.51± 1.07	3.82± 0.96
Chloride, mmol l^{-1}	114.29± 5.73	110.78± 2.43	112.61± 2.13	112.49± 1.97
Magnesium, mmol l^{-1}	0.91± 0.06	0.83*± 0.07	0.86± 0.09	0.95± 0.07
Iron, µmol l^{-1}	22.91± 2.09	22.29± 3.56	22.58± 3.57	23.36± 2.80

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

* Significant differences, as compared to the control group (C) at $P<0.05$.

considerable rise in the calcium content in 42-day-old chickens, which may have been caused by a higher concentration of carboxylic acids from the fermentation of indigestible carbohydrates in colon. The latter enhance the absorption of minerals, mainly of calcium and magnesium. Most insoluble fibre components, including cellulose and its derivatives, are highly resistant to bacterial fermentation processes (KLURFELD 1999). According to JAMROZ *et al.* (2001) and SMULIKOWSKA (2002), 20-35% non-soluble dietary fibre (NDF) is digested in the digestive tracts of broiler chickens, whereas the digestibility of the ADF fraction barely reaches 2%. Our results show that there is a higher degree of calcium absorption in chickens fed diets with the pure cellulose additive which may be the result of slight cellulose fermentation in the caecum of experimental birds. On the basis of the available literature, it is difficult to explain the reason for the lowered magnesium content in the blood of the E1 group, the more so that the mechanisms of Ca and Mg absorption are alike (BRONNER & PANSU 1999; COUDRAY *et al*. 2003). One of the reasons could involve the magnesium present in the metabolism of organic proteins. Our previous research (SZYMECZKO 2000; BOGUSŁAWSKA-TRYK 2005) revealed that 0.25-0.45% cellulose additive in the diet had a positive effect on muscle protein synthesis and growth of birds which can be ascribed to faster Mg accumulation in muscle cells.

The results show that introducing limited amounts of pure cellulose into the diet of broiler chickens does not affect either the total protein concentration or the protein fractions in blood serum. An increase in the calcium content in blood can signify a higher degree of Ca absorption from the digestive tract in birds fed diets supplemented with pure cellulose.

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