

Effect of humic acid substances on proteolytic activity in intestine, digestibility of crude protein and protein content in the blood of broiler chickens

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Article Details: Received: 2020-10-18 | Accepted: 2020-11-27 | Available online: 2021-01-31

<https://doi.org/10.15414/afz.2021.24.mi-prap.159-163>



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The objective of the study was to investigate the effects of dietary intake of humic substances (HS) on the proteolytic activity and the digestibility of crude protein (CP) measured as the apparent assimilable mass coefficient of CP corrected for protein catabolism (AMCN) in the intestine as well as on the total protein and albumin content in the serum of broiler chickens (Cobb 500, $n = 120$). Chickens (groups A, B, C/negative control) were fed with mixtures with CP (g/kg DM) – Hyd1 230.20 (d 1–7), Hyd2 222.20 (d 8–28), Hyd3 209.40 (d 29–37) for 37 days. The humic substances were added into diets of experimental groups in the feed additive according to the content of humic/fulvic acids (HA/FA; g/kg) A 4.55/0.35, B 3.99/0.35, C 2.85/0.25. The body weights and feed consumption were measured once a week. The average daily weight gains and the feed conversion ratio were calculated. The dietary intake of HS had a positive effect on the increase of proteolytic activities in the intestinal apparatus and caused the significant enhancement of AMCN of birds from experimental groups on days 17, 24 and 31. However, the values of the total protein and the albumin in the serum were significantly decreased in the groups after intake of HA/FA 4.55/0.35 or 3.99/0.35 in the feed.

Keywords: gut of poultry, humates, enzymatic activity, crude protein, albumin

1 Introduction

Humates are raw materials utilisable in the plant and animal husbandry as sources of organic and mineral substances with the positive effects on their biological characteristics. These natural products are geological deposits located in the earth superficial layer which originated from the process of decomposition of plant and animal matter via the activities of microorganisms (McMurphy et al., 2011).

The feed additives with a positive effect on the production parameters of animals are could be the humic substances (HS) too. The enhancement of growth ability of animal depends on the sources of humic acids (HA) and fulvic acids (FA) present in HS. According to already published results, the improvements were observed in the case of production of broiler chickens in various parameters such as body weight, feed conversion, the retention of ashes, nitrogen, and energy as well as reduced crypt depth and increased length of the villi of the jejunal mucosa (Gomez-Rosales and Angeles, 2015). The scientific hypothesis for the performed experiment was based on the positive effects of HS on the enzymatic activities in the gastrointestinal apparatus which have the potential to improve the digestion of proteins in the gut of poultry. The study is aimed to investigate the effects of feed intake of HS preparation on the proteolytic activity and the digestibility of CP measured as the apparent assimilable mass coefficient of CP corrected for protein catabolism (AMCN) in the intestine as well as the protein content in the blood of broiler chickens.

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2 Material and methods

2.1 Chicken and diets

One-day-old broiler chickens of hybrid COBB 500 ($n = 120$, weight 50 g) (Mach Drubež, a.s., Litomyšl, Czech Republic) were divided into 3 experimental groups and a negative control. They were housed in four-floor pens (0.12 m² per broiler chicken) located in one experimental hall of the University of Veterinary Medicine and Pharmacy in Košice with constant access to feed and water. All groups were fed with mash diets (Agrocass plus, Ltd., Slovak Republic) for 37 days. The methionine was used as the first limiting amino acid. Chickens (groups A, B, C/negative control) were fed with mixtures Hyd1 230.20 (d 1–7), Hyd2 222.20 (d 8–28), Hyd3 209.40 (d 29–37) for 37 days. The diets were prepared and formulated without antibiotics and growth promoters. The anticoccidial agents were added into the starter and grower feed mixtures. The calculations of diets were performed according to the nutrient requirements and nutrient value of feeds for poultry (Kočí et al., 1994). HS (Humac Ltd., Slovak Republic) were added into feeds of three experimental groups – Humac natur (HN) for A and HN monogastric (HNM) for B and C group. The characteristics of the preparations HN/HNM: the size of particles up to 100 µm, max. moisture 15%, the content of humic acids (HA) min. 650/570, fulvic acids (FA) min. 50/50 g/kg, macroelements Ca 42.28/51.1, Mg 5.11/4.86, Fe 19.05/18.09 g/kg and microelements Cu 15/14.25, Zn 37/35.15, Mn 142/135, Co 1.24/1.18, Se 1.67/1.59 as well as Mo 2.7/2.57, V 42.1/40 mg/kg dry matter (DM). The inclusion levels of the HS in the diets according to the content of HA/FA in the feed additives were as follows: A 4.55/0.35, B 3.99/0.35, C 2.85/0.25 g/kg. The body weights (BW) of chickens and the feed consumption were evaluated once a week. The average daily weight gains (ADWG) and feed conversion ratio (FCR) were calculated. The samples of diets were analysed (Table 1) according to the official methods of the Association of Official Analytical Chemists (Cunniff, 1995). There were performed the analyses of DM, crude protein (CP), crude fat, starch and ash. The fibre was determined with the method by Van Soest et al. (1991). The mineral composition of the feed was analysed by atomic absorption spectrophotometry (AAS) (Van Loon, 1980). The quantitative determination of phosphorus was performed spectrophotometrically (Carvalho et al., 1998). The insoluble portion of ash in HCl was determined in the feed mixture as the residue of ash, after dissolving ash in diluted hydrochloric acid by weighing (Daněk et al., 2005). The metabolisable energy value of diets was calculated with the formula from the Commission Regulation (EC) No 152/2009 (European commission, 2009) according to the method of calculation and expression of energy value.

Table 1 Composition of the experimental diets

Analysed nutrients (g/kg)	Diets		
	Hyd1	Hyd2	Hyd3
Dry mater	100.00	100.00	100.00
Crude protein	230.20	222.20	209.40
Crude fat	31.30	83.80	67.70
ND fibre	112.60	122.10	128.80
AD fibre	54.60	62.50	68.10
Ash	57.30	60.60	52.10
Ash insoluble in HCl	2.10	1.80	2.40
Starch	485.60	446.80	448.60
Calcium	4.93	6.00	7.41
Phosphorus	5.73	7.93	5.13
Sodium	2.98	1.93	1.60
Magnesium	2.86	3.06	3.11
Potassium	9.03	8.83	8.49
Copper	0.0275	0.0578	0.0594
Zinc	0.0229	0.0294	0.1336
Manganese	0.0789	0.1472	0.1437
Metabolizable energy (MJ/kg)*	13.27	14.30	13.58

*Calculation based on Commission Regulation (EC) 152/2009; Hyd1/Hyd2/Hyd3 – maize, wheat, soybean meal GMO, vegetable oil, limestone, amino acids and their salts, monocalcium phosphate, mineral-vitamin premix; Hyd1 – the dried derivative of pig blood; Hyd2/Hyd3 – sunflower extracted meal, salt

2.2 Analysis of proteolytic activity and the digestibility of CP

The samples of faeces were placed into sterile tubes for digestive enzyme analyses. The preparation of samples for the quantification was performed as follows. One gram of fresh sample was diluted with 49 ml sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 mmol/l, HCl 0.5 mol/l, pH 7.0) and homogenised. The samples were subsequently taken for the measurement of nonspecific proteolytic activity (Broderick, 1987) with the substrate azocasein (Merck Ltd., Germany). The excreta from 48 chickens were sampled from the cloaca into sterile glass containers on 17, 24 and 31 days of age. The samples from two birds was pooled. Therefore the resulting number of samples was 24 in four groups. The quantification of DM, CP, ash and portion of insoluble ash in HCl was performed according to above-mentioned methods. The digestibility of CP in the intestine was measured as AMCN which was calculated according to the methods described by Gugliemo and Karasov (1993).

2.3 Analysis of protein content in the blood

The total protein and the albumin in blood were checked after the slaughter of 48 birds on day 37 of fattening. The samples of blood were taken from randomly selected chickens in groups from the wing vein into tubes without anticoagulants. Serum was separated after coagulation of blood samples at room temperature and centrifugation at 4,000 g for 15 min. Then the samples of serum from two birds were pooled and the resulting number of samples was 24. Biochemical parameters in blood serum were determined using automated biochemical analyser Ellipse (AMS, Rome, Italy) with standard kits (Dialab, Prague, Czech Republic) for the concentrations of total protein (g/l) and albumin (g/l).

2.4 Statistical analysis

Means of the results from the treatments were compared by one-way analysis of variance and by Tukey-Kramer multiple comparison test (IBM SPSS Statistics, Version 24).

3 Results and discussion

The results indicate that the dietary intake of HS can be effective in the enhancement of the performance of broilers. The final BW (g) was higher in the groups A $2,506.67 \pm 160.47$ ($P < 0.05$), B $2,490.25 \pm 166.39$ ($P < 0.05$), C $2,377.75 \pm 166.39$ ($P > 0.05$) in comparison to negative control $2,319.42 \pm 92.55$ on day 37. ADWG (g/day) was increased ($P > 0.05$) in the same experimental groups A 66.39 ± 16.85 , B 65.95 ± 17.77 , C 62.91 ± 18.06 compared to control 61.34 ± 17.31 in the time interval day 1–37. The similar improvement was observed by Arif et al. (2016). They determined the increase of the weight gains of broilers fed HA at 2.25 g/kg diet as a result of the improved feed conversion. Their inclusion level of HA was approximately one half compared to the value in our experiment.

There were not observed significant differences in FCR (kg/kg) during the experiment. The measured values were in A 1.65 ± 0.29 , B 1.64 ± 0.19 , C 1.53 ± 0.26 and in control 1.51 ± 0.17 . The positive results of feed utilization are in coincidence with the partial improvement of nutrient digestibility and the protection of commensal gut microflora (Windisch et al., 2008).

The addition of HS had a positive effect on the increase of proteolytic activities in the intestinal apparatus (azocasein $\mu\text{g/ml/min/g}$) in B by 0.53 on day 17, in A, B and C groups by 11.81, 8.50 and 7.30 on day 24 as well as in B and C groups by 5.44 and 5.47 on day 31 compared to control (Table 2). According to Jamdar and Harikumar (2005), the chicken intestine possesses proteolytic activities (cathepsin B, D, H, L, aminopeptidases and alkaline proteases). Kinetic studies employing specific inhibitors indicated that the degradation (90–94%) of proteins at acidic pH is governed by pepstatin sensitive proteases.

Similarly, the significant increase of AMCN ($P < 0.05$) was observed in C by 21.74% on day 17, in B by 21.88% on day 24 and in control by 26.03% on day 31 in accordance with the data in the Table 2. Comparable results were got by Ozturk et al. (2014) observed the effect of HS (7.5, 15 and 22.5 g/kg) on performance and the utilization of nutrients in Ross chicks. They concluded that 15 and 22.5 g/kg of HS significantly increased the digestibility of nutrients.

As for the concentration of total protein in serum (g/l), the values were decreased in A and B groups by 9.10 and 8.07 ($P < 0.001$) compared to control (Table 2). The comparable results were obtained in the case of the albumin concentration in serum when this parameter was reduced by 4.65 g/l ($P < 0.001$) in A group in comparison to control. Total proteins include blood plasma proteins synthesized mainly in the liver such as albumin, clotting proteins,

and globulins with the significant extrahepatic role (Grieninger and Granick 1975). The results revealed a lower hepatoprotective effect of the added HS.

Table 2 Impact of the intake of HS on the proteolytic activity in the digestive apparatus and AMCN of broiler chickens ($n = 6$, mean \pm SD) Group

	Proteolytic activity (azocasein) (mg/ml/min/g)			AMCN			Total protein in blood (g/l) d37	Albumin in blood (g/l) d37
	d 17	d 24	d 31	d 17	d 24	d 31		
A	24.37 ^a \pm 2.19	29.34 ^a \pm 1.40	12.55 ^a \pm 2.44	0.60 ^{ab} \pm 0.08	0.60 ^{ab} \pm 0.09	0.58 ^{ab} \pm 0.13	22.28 ^c \pm 2.88	7.23 ^c \pm 1.35
B	26.66 ^a \pm 3.88	26.03 ^a \pm 2.13	19.29 ^a \pm 3.09	0.58 ^{ab} \pm 0.08	0.62 ^{ab} \pm 0.07	0.73 ^b \pm 0.04	23.31 ^c \pm 2.22	8.88 ^{ac} \pm 1.51
C	25.10 ^a \pm 3.19	24.83 ^a \pm 3.74	19.32 ^a \pm 5.01	0.69 ^b \pm 0.06	0.64 ^b \pm 0.02	0.64 ^{ab} \pm 0.10	26.34 ^{ac} \pm 5.51	9.20 ^{ac} \pm 2.82
Control	26.13 ^a \pm 1.97	17.53 ^a \pm 9.74	13.85 ^a \pm 0.81	0.54 ^a \pm 0.04	0.50 ^a \pm 0.03	0.54 ^a \pm 0.05	31.38 ^a \pm 2.46	11.88 ^a \pm 1.56

Means with different superscript letters differ significantly a, b $P < 0.05$, a, c $P < 0.001$ (mean \pm SD), AMCN – apparent assimilable mass coefficient of CP corrected for protein catabolism, d – day

4 Conclusions

The dietary intake of humic substances (HS) had a positive effect on the increase of proteolytic activities in the intestinal apparatus and caused the significant enhancement of AMCN of birds from experimental groups on days 17, 24 and 31. However, the values of the total protein and the albumin in the serum were significantly decreased in the groups after intake of HA/FA 4.55/0.35 or 3.99/0.35 in feed.

Acknowledgement

This study was supported by the grant from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic for the project VEGA-1/0402/20.

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