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Enzymatic Activities in the Intestinal Apparatus of Chickens after Peroral Intake of Humates

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Abstract:*The effects of dietary intake of humates on the proteolytic, amylolytic and cellulolytic activities in the intestine of broiler chickens Ross 308 (starting weight 37.79 ± 0.58 g) were analyzed. Three experimental (A, B, C) and one control groups of birds (n=120) were fed with mixtures Hyd1 236.0 (day 1 - 10), Hyd2 222.40 (day 11 - 30), Hyd3 193.30 (day 31 - 42) g.kg^{-1} crude protein. Humates (HM or HN) were added into feed mixtures: A group – 0.7% HM containing an absorbent of mycotoxins, B group – 0.7% HN without the absorbent, C group – 0.3% HM and the control without humates. There were measured body weights and feed consumption, as well as the average daily weight gains and the feed conversion ratio, were calculated once a week. On one hand, the positive effects of 0.7% humates were observed on the increase (P <0.05) of proteolytic on day 21 (B) by 7.92 (azocasein μg.ml^{-1}.min^{-1}.g^{-1}), cellulolytic on day 28 (A) by 0.89 (glucose μmol.l^{-1}.min^{-1}g^{-1}) in the intestine of broiler chickens measured in the excrement. On the other hand, the decrease (P <0.001) of amylolytic activities was observed on day 33 (A) by 1.58 and (B) by 1.43 (glucose μmol.l^{-1}.min^{-1}g^{-1}). In the case of intake of 0.3% humates, the proteolytic or cellulolytic were decreased in C on day 21 or 28 by 16.39 and 2.41, respectively. The recommended level of added humates into a feed of chickens is 0.7%.*

I. INTRODUCTION

Humates are complex mixtures of heterogeneous organic compounds of biotic origin that have undergone extensive transformations since they were first produced by plants. Lignin is probably an important precursor and based on solubility, humates can be classified into humic acids (insoluble below pH 2), fulvic acids (soluble at any pH), and humin (insoluble in water). Because of the complexity and irregularity of humates, and the pathways of their formation, they should not be considered as strictly defined molecules, but can rather be characterized by average properties [1].The positive effect of humates on the growth and feed conversion of broiler chickens was demonstrated in the study performed by [2]. Humate supplementation increased the feed conversion efficiency and egg production in hens but did not effect on feed intake [3]. Sopoliga et al. [4] reported the positive influence of humates on egg production and the quality of quails and pheasants as well. According to Yeo [5], the improvement in weight gain and better feed conversion may be related to promotional effects on metabolic processes of digestion and utilization of nutrients.

The scientific hypothesis was based on the positive effects of humates on the detoxication of exogenous and endogenous poisons, stabilization of intestinal microflora, prevention of digestive problems, activation of

metabolism and feed supplementation with mineral compounds in the nutrition of broiler chickens. The study aimed to investigate the effects of the peroral intake of preparations of humates on the proteolytic, amylolytic and cellulolytic activities in the intestine of broiler chickens.

II. Materials and Methods

2.1 Chicken and diets

One hundred and twenty, one-day-old broiler chickens of hybrid Ross 308 were delivered from a commercial hatchery. They were divided at random into 4 groups of 30 animals (control / A, B, C). The values of the starting body weight (BW, g) were the following A 37.67 ± 0.88, B 37.29 ± 0.51, C 38.75 ± 0.35 and the negative control 37.45 ± 0.53. The chickens were housed in four floor pens located in one experimental hall of the University of Veterinary Medicine and Pharmacy in Košice with constant access to feed and water. The pens were identical concerning the same direction and the same area (0.12 m^2 per broiler chicken). All groups were fed with mash diets (Barbara Sp. Z o.o., Turza, Poľsko: Hyd1 – 236.0, Hyd2 – 222.4, Hyd3 - 193.3 $g.kg^{-1}$ crude protein (CP) in the feeding periods: 1 – 10, 11 – 30, 31 - 42 days. The methionine was used as the first limiting amino acid. The diets were prepared and formulated without antibiotics and growth promoters. The anticoccidial agents were added to the starter and grower feed mixtures. The calculations of diets were performed according to the nutrient requirements and nutrient value of feeds for poultry [6].

Humates (Humac Ltd., Slovak Republic) were added into feeds as follows: A – 0.7% Humac containing an absorbent of mycotoxins (HM), B – 0.7% Humac natur AFM (HN), C – 0.3% HM and the negative control without addition of humates. Therefore, 0.3 or 0.7% of feed used for experimental groups has created the preparation with humates. The characteristics of the applied additives were the following: the size of particles up to 100 μm, max. moisture 15%, the content of humic acids (HA) min. 650, fulvic acids (FA) min. 50 $g.kg^{-1}$, macroelements Ca 42.28, Mg 5.11, Fe 19.05 $g.kg^{-1}$ and microelements Cu 15, Zn 37, Mn 142, Co 1.24, Se 1.67 as well as Mo 2.7, V 42.1 $mg.kg^{-1}$ dry matter (DM). The body weights (BW) of chickens and the feed consumption were assessed once a week. All procedures were performed with the animals approved by the Animal ethics committee of the University of Veterinary Medicine and Pharmacy in Košice according to Directive 2010/63/EU [7].

2.2 Feed analysis

The samples of diets were analysed (Table 1) according to the methods of the Association of Official Analytical Chemists [8]. There were performed analyses of DM, CP, crude fat, starch and ash. The fibre was determined with the method by Van Soest et al. [9]. The mineral composition of the feed was analysed by atomic absorption spectrophotometry (AAS) [10]. The quantitative determination of phosphorus was performed spectrophotometrically [11]. The metabolisable energy value of diets was calculated with the formula from the Commission Regulation (EC) No 152/2009 [12] according to the method of calculation and expression of energy value.

Table 1. Composition of the experimental diets.

	Diets		
Analysed nutrients (g kg^{-1})	**Hyd1**	**Hyd2**	**Hyd3**
Dry mater	880.50	882.2	900.00
Crude protein	236.0	222.40	193.30
Crude fat	25.20	30.30	52.70
ND fibre	31.12	38.20	38.70
Ash	62.0	62.23	52.10
Starch	459.17	487.87	509.10
Calcium	8.84	9.42	8.01
Phosphorus	5.86	3.58	4.39
Sodium	1.36	1.41	1.22
Potassium	9.54	7.8	8.0

Copper	0.05963	0.06064	0.03478
Zinc	0.10789	0.09748	0.09667
Manganese	0.2385	0.23577	0.13667
Lysine	12.0	11.50	11.50
Methionine	5.1	5.0	5.0
Metabolizable energy (MJ kg^{-1})*	12.86	13.08	13.02

*Calculation based on Commission Regulation (EC) 152/2009

	Ingredients in diets
Hyd1 / Hyd2 / Hyd3	maize, wheat, soybean meal GMO, rapeseed meal, vegetable oil, limestone, sodium phosphate, sodium chloride, hydrogen sodium carbonate, mineral-vitamin premix, enzymes: 6-phytase E.C. 3.3.26 FTU 500.kg^{-1}, endo-1,4 beta xylanase (E161) EPU 2200.kg^{-1}
Hyd1 / Hyd2	Salinomycine 70 mg.kg^{-1}

2.3 Analysis of enzymatic activities

The enzymatic activities in the intestinal apparatus were checked on days 7, 14, 21, 28 and 33 of age. The samples of excreta were placed into sterile tubes for digestive enzyme analyses. Subsequently, the fresh sample of 0.3 g was diluted with 5 ml sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 mmol.l^{-1}, HCl 0.5 mol.l^{-1}, pH 7.0) and homogenised. The prepared samples were subsequently taken for the measurement of nonspecific proteolytic activity [13] with the substrate azocasein (Merck LTD., Germany). The analyses of amylolytic or cellulolytic activities were performed in a 1.0 ml sample according to the method of Lever [14] for the determination of reducing carbohydrates. The methyl hydroxyethylcellulose (Merck Slovakia Ltd.) or the soluble starch (Fisher Slovakia Ltd.) were used as substrates.

2.4 Statistical analysis

The data are expressed as means ± the standard deviation (SD) of single values. The results from the treatments were compared by the one-way analysis of variance. Significance was declared at $P < 0.05$.

III. Results and Discussion

The results indicate that the dietary intake of humates can be effective in the enhancement of the enzymatic activities in the intestinal apparatus of broiler chickens. The addition of 0.7% humates into a feed of birds had a positive effect on proteolytic activity. The increase of azocasein degradation was observed by 7.92 μg.ml^{-1}.min^{-1}.g^{-1} in the samples of excreta in the B group compared to the negative control on day 21 (HN) (table 2). According to Jamdar and Harikumar [15], 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 addition of 0.7% humates, in the preparation HM, enhanced the cellulolytic activity by 0.89 glucose μmol.l^{-1}.min^{-1}.g^{-1}in A group on day 28. The positive results of the enzymatic activities are in coincidence with the feed utilization and the partial improvement of nutrient digestibility [16].

In the case of the amylolytic activity, there was observed the negative effect of 0.7% humates with a decrease in groups A and B by 1.58 or 1.43 glucose μmol.l^{-1}.$min^{-1}g^{-1}$on day 33 (HM or HN). The reason for decreased amylolytic activities in the intestine is not the toxicity of humates from the point of view of HA and FA. According to Dai et al. [17] and Murbach et al. [18], the oral administration of HA and FA may have higher safety.

The values of the final BW (g) were the following A 2510.54 ± 304.71, B 2349.0 ± 271.89, C 2528.69 ± 249.94 and the control 2606.38 ± 289.02 on day 42.

Table 2. Proteolytic, amylolytic and cellulolytic activities in the excreta of intestinal apparatus of broiler chickens (n = 6, mean ± SD) on days 7, 14, 21, 28, 33.

Group	**Proteolytic activity (azocasein μg.ml^{-1}.min^{-1}.g^{-1})**

	d7	**d14**	**d21**	**d28**	**d33**
A	$54.54^{a} \pm 13.86$	$71.91^{a} \pm 6.33$	$54.39^{ab} \pm 3.24$	$32.08^{a} \pm 1.58$	$62.57^{a} \pm 5.33$
B	$59.89^{a} \pm 12.38$	$69.79^{a} \pm 1.57$	$64.01^{b} \pm 7.97$	$42.62^{a} \pm 5.69$	$54.83^{a} \pm 5.08$
C	$55.23^{a} \pm 3.54$	$66.42^{a} \pm 0.32$	$39.70^{a} \pm 3.34$	$37.93^{a} \pm 5.69$	$48.62^{a} \pm 3.36$
Control	$67.99^{a} \pm 10.53$	$69.04^{a} \pm 11.47$	$56.09^{ab} \pm 11.53$	$42.07^{a} \pm 6.64$	$62.99^{a} \pm 11.13$
	Amylolytic activity (glucose μmol.l^{-1}.min^{-1}g^{-1})				
	d7	d14	d21	d28	d33
A	$2.13^{a} \pm 0.33$	$2.79^{a} \pm 1.45$	$3.68^{a} \pm 0.29$	$2.13^{a} \pm 0.50$	$1.27^{a} \pm 0.23$
B	$2.67^{a} \pm 0.78$	$1.78^{a} \pm 0.27$	$3.99^{a} \pm 1.24$	$1.77^{a} \pm 0.38$	$1.42^{a} \pm 0.18$
C	$2.32^{a} \pm 0.37$	$2.32^{a} \pm 0.35$	$2.88^{a} \pm 1.03$	$4.01^{a} \pm 2.03$	$2.39^{c} \pm 0.42$
Control	$2.70^{a} \pm 0.30$	$2.26^{a} \pm 0.32$	$4.68^{a} \pm 0.76$	$3.09^{a} \pm 0.44$	$2.85^{c} \pm 0.36$
	Cellulolytic activity (glucose μmol.l^{-1}.min^{-1}g^{-1})				
	d7	d14	d21	d28	d33
A	$6.43^{a} \pm 0.70$	$2.04^{a} \pm 0.78$	$4.04^{a} \pm 0.41$	$8.84^{b} \pm 1.59$	$3.24^{a} \pm 0.88$
B	$6.13^{a} \pm 1.68$	$1.94^{a} \pm 0.77$	$3.60^{a} \pm 0.44$	$7.21^{ab} \pm 1.32$	$3.51^{a} \pm 0.29$
C	$7.11^{a} \pm 1.64$	$2.67^{a} \pm 0.16$	$3.80^{a} \pm 0.88$	$5.54^{a} \pm 0.42$	$4.89^{a} \pm 1.54$
Control	$5.45^{a} \pm 0.70$	$2.97^{a} \pm 0.90$	$4.52^{a} \pm 1.67$	$7.95^{ab} \pm 1.51$	$4.76^{a} \pm 1.15$

Note: Means with different superscript letters differ significantly $^{a, b}P < 0.05$, $^{a, c}P < 0.001$ (mean ± SD).

IV. Conclusion

The dietary intake of 0.7% humates had a positive effect on the increase of the proteolytic (day 21), cellulolytic (day 28) but not on the amylolytic activities (day 33) in the intestinal apparatus of broiler chickens. The content of 0.3% humates in the feed was not sufficient and the decrease of proteolytic and cellulolytic activities was observed. The recommended level of added humates into a feed of chickens is 0.7%.

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