Energy Profile in Broiler Chickens Fed with Different Levels of Humates

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ABSTRACT— ***The objective of the study was the evaluation the effects of the peroral intake of humates (HS) added into feed mixtures on the energy metabolism of broiler chickens (Cobb 500). The selected parameters are concentrations of glucose, triglycerides and cholesterol analysed in the blood serum. There were used four groups of birds (A, B, C, negative control; n=120) in the experiment. Chickens were fed with ground diets (starter HYD1, grower HYD2, finisher HYD3) with supplements of HS from day 1 to 37 as follows A: Humac nature (HN) 7 g/kg, B: Humac nature monogastric (HNM) 7 g/kg, C: HNM 5 g/kg feed (Humac Ltd., Slovak Republic). The diets had the following content of crude protein: HYD1 230.20, HYD2 222.20, HYD3 209.40 g CP /kg dry matter). The statistical evaluation of results was performed by one-way analysis of variance and means were compared by the Student-Newman-Keuls test and correlation analysis. Higher concentrations (P<0.05) of triglycerides in blood serum were analyzed in groups A or B by 0.24 and 0.26 mmol/l, respectively, in comparison to the control group (y = 0.35830x + 0.58324). The values of cholesterol were at a lower level (P<0.05) in all 3 experimental groups by 1.07, 0.54 and 0.84 mmol/l (y = - 1.18073x + 2.75772). The positive effect of the intake of HS on glucose concentration was not confirmed when a higher concentration (P<0.05) was analyzed in the blood serum of the control group compared with groups A or C by 3.31 and 4.56 mmol/l (y = - 4.06420x + 13.37519), respectively.***

Keywords— Poultry digestion, Dry matter, Crude protein, Humic substances

1. INTRODUCTION

Interest in improving animal welfare and the problem of developing antimicrobial resistance affecting the health of the human population influenced changes in legislation and subsequently the ban on the use of antibiotic growth stimulants in the European Union by Regulation 1831/2003 (Castanon, 2007). The consequence of this ban was the deterioration of the productivity and health of animals, as well as the increase in intestinal diseases due to the lack of prophylactic effects of antibiotic substances in critical periods of animal husbandry (Pineda-Quiroga et al., 2017). Organic acids (Konieczka et al., 2021), plant extracts (Arpášová et al., 2018), probiotics and prebiotics (El-Ghany et al., 2022) and enzymes (Slominski, 2011) are currently used as alternative growth stimulators.

The scientific hypothesis was based on the confirmed positive effects of humates (HS) on the production parameters of food animals, which can potentially improve the energy metabolism of broiler chickens.

HS are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decomposition and transformation of plant and microbial residues in the process of humification.

Essential components of this process are plant lignin, its transformation products, polysaccharides, melanin, cutin, proteins, lipids, nucleic acids and fine coal particles (Bezuglova, 2019).

The experimental study aimed to analyze the effects of dietary intake of humates (HS) on selected parameters of energy metabolism: the concentration of glucose, triglycerides and cholesterol in the blood serum of broiler chickens.

2. MATERIALS AND METHODS

The feeding experiment was performed with 120 broiler chickens (Cobb 500, average weight 50 g) supplied from a commercial hatchery. They were randomly divided into 4 groups of 30 individuals each (A, B, C / negative control). Housing was carried out for 37 days in pens in one experimental poultry hall with constant access to feed and water. The pens were identical in terms of orientation and area (0.12 m^2 per chicken).

The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice.

There were used the following feed mixtures (Agrocass, Ltd., Slovak Republic) for the feeding of chickens: starter HYD1 230.20, grower HYD2 222.20, and final HYD3 209.40 g CP /kg dry matter. Antibiotics and growth stimulants were not added to the feeds. The starter and grower feed mixtures contained anticoccidials.

The feed of experimental chickens was supplemented with products containing humates (Humac Ltd., Slovak Republic). Humac natur (HN) was added to the feed of group A: 7 g/kg and Humac natur monogastric (HNM) to the feed of groups B: 7 g/kg and C: 5 g/kg. HN/HNM products had the following parameters: particle size up to 100 μm, maximum moisture 15% and humic acid content (HA) min. 650/570, fulvic acids (FA) min. 50/50 g/kg, Ca 42.28/51.1, Mg 5.11/4.86, Fe 19.05/18.09 g/kg, Cu 15/14.25, Zn 37 /35.15, Mn 142/165, Co 1.817/1 1.59 as well as Mo 2.7/2.57, V 42.1/40 mg/kg dry matter. Feed additives were added to feed mixtures according to content of HA/FA: A 4.55/0.35, B 3.99/0.35, C 2.85/0.25 mg/kg.

Table 1: Nutrients and components in experimental diets

Analyzed nutrients (g/kg)	**Feed mixture**		
	Starter HYD1	**Grower HYD2**	**Finisher HYD3**
Dry matter	1000.00	1000.00	1000.00
Crude protein	230.20	222.20	209.40
Fat	31.30	83.80	67.70
NDF	112.60	122.10	128.80
ADF	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
Ca	4.93	6.00	7.41
P	5.73	7.93	5.13
Na	2.98	1.93	1.60
Mg	2.86	3.06	3.11
K	9.03	8.83	8.49
Cu	0.0275	0.0578	0.0594
Zn	0.0229	0.0294	0.1336
Mn	0.0789	0.1472	0.1437
Metabolizable energy (MJ/kg)*	13.27	14.30	13.58
Components			
HYD1 / HYD2 / HYD3	maize, wheat, extracted soybean meal, vegetable oil, limestone, amino acids and their salts, $CaHPO_4$, lysine, methionine, mineral-vitamin premix		

*European Commission (2009); NDF – neutral detergent fibre, ADF – acid detergent fibre

Experimental diets were analyzed using official methods (Table 1) of the AOAC (Cunniff, 1995). Determinations of dry matter, crude protein (CP), fat, starch and ash were carried out. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed by the methods of Van Soest et al. (1991). Atomic absorption spectrophotometry (AAS) was used to determine the mineral composition of feeds (Van Loon, 1980). Phosphorus was quantified spectrophotometrically (Carvalho et al. 1998). Determination of the insoluble proportion of ash in HCl in the feed mixture was carried out (Daněk et al. 2005). The value of metabolizable energy (ME) was calculated by the equation according to the regulation of the European Commission (EC) no. 152/2009 (European Commission, 2009) according to the method for calculating and expressing the energy value.

The samples of blood serum were used for the analysis of the energy profile. The concentrations of glucose, triglyceride and cholesterol were measured with an ELIPSE device (AMS, Italy).

The results of the experiments were evaluated by one-way analysis of variance, means were compared by the Student-Newman-Keuls test and the correlation analysis (IBM SPSS Statistics, Version 24).

3. RESULTS AND DISCUSSION

The values of the parameters of the energy profile, namely the concentration of glucose, triglycerides and cholesterol in the blood serum of broiler chickens on the 37th day of the experiment are shown in Table 2. There was observed a positive effect of HS intake on the concentration of triglycerides and cholesterol.

Table 2. Evaluation of the parameters of energy profile in blood serum of broiler chickens on day 37

Parameter (mean ± SD, n = 32)	**A**	**B**	**C**	**K**
Glucose (mmol/l)	10.63 ± 1.022 ^{a}	12.03 ± 1.961 ^{ab}	9.38^{ab} ± 4.516 ^{a}	13.94 ± 0.774 ^{b}
Triglycerides (mmol/l)	0.83 ± 0.244 ^{a}	0.85 ± 0.129 ^{a}	0.74 ± 0.240 ^{ab}	0.59 ± 0.105 ^{b}
Cholesterol (mmol/l)	1.74 ± 0.238 ^{a}	2.27 ± 0.322 ^{a}	1.97 ± 1.088 ^{a}	2.81 ± 0.200 ^{b}

Means with different superscripts are significantly different ($^{a, b}$ P < 0.05)

In the case of triglycerides, higher concentrations (P < 0.05) were analyzed in groups A and B after an intake of 7 g/kg HS by 0.24 or 0.26 mmol/l compared to the control group. On the other hand, the determined cholesterol level was at a lower level (P < 0.05) in all 3 experimental groups by 1.07, 0.54 and 0.84 mmol/l. A positive effect of oral HS intake on glucose concentration could not be demonstrated, when its higher concentration (P < 0.05) was measured in the blood serum of the control group compared to groups A and C by 3.31 or 4.56 mmol/l.

From the point of view of glucose concentration in the blood serum, similar results were obtained by Rath et al. (2006), who observed a statistically significant reduction after supplementing feed with HS.

Demeterova et al. (2009) did not observe significant differences in the concentration of glucose and cholesterol in the serum of broiler chickens. On the other hand, they observed a statistically significant decrease in the level of triglycerides (P < 0.001) after the intake of HS 7 g/kg of the feed mixture.

The opposite statistically significant values (P < 0.05) of the mentioned biochemical parameters were experimentally analyzed by ELnaggar and El-Kelawy (2018) too. The level of serum glucose increased in experimental groups of chickens after the addition of HS 1 g/kg feed and the concentrations of triglycerides decreased if HS were at the level of 1, 2 and 4 g/kg feed. In agreement with our results, they observed a decrease in cholesterol after the intake of HS at the level of 1 to 4 g/kg feed.

Table 3. Polynomial regression analysis of the parameters of energy profile in blood serum of broiler chickens on day 37

Parameter (mean ± SD, n = 32)	**Regression analysis**	**P**	**r**
Glucose	y = - 4.06420x + 13.37519	0.0270	- 0.39702
Triglycerides	y = 0.35830x + 0.58324	0.0038	0.49766
Cholesterol	y = - 1.18073x + 2.75772	0.0039	- 0.49536

P – level of significance, r – correlation coefficient

The reason for the lower concentration of glucose in the blood of chickens in our experiment may be the several times higher level of HS in the feed affecting the absorption in the digestive tract and subsequently the concentration of glucose in the blood. In the case of cholesterol, there were not observed differences between supplementations at the level of 5 or 7 g/kg.

Similar results were obtained by Arif et al. (2016), who observed a reduction in LDL cholesterol after supplementing feed with humic acids.

The presented evaluation of the results of the energy profile of broiler chickens was also confirmed by polynomial regression analysis. The calculated regression equations are demonstrated in Table 3. According to the interpretation of the correlation coefficient **r** by Hinkle et al. (2003), the values of all correlation coefficients are in the interval from 0.3 to 0.5 and thus a low correlation was confirmed in the case of all three parameters. A negative correlation was evaluated in the regression analysis of glucose ($P < 0.05$) and cholesterol ($P < 0.01$).

4. CONCLUSIONS

Dietary intake of humates at the level of 7 g/kg of feed positively increased the level of triglycerides and decreased the concentration of cholesterol in the blood serum of broiler chickens. A positive effect on the concentration of glucose in the serum was not manifested, its reduction was observed. The values of these three parameters were consistent with the results of the polynomial regression analysis of energy metabolism.

5. ACKNOWLEDGEMENT

The experimental study and its evaluation were financed by grants VEGA-1/0402/20 and KEGA-006UVLF-4/2022.

6. REFERENCES

[1] Arif, M., Rehman, A., Abd El-Hack, M.E., Arain, M.A., Haseebarshad, M., Zakriaand, H.M., Abbasi, I.M., "Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks", Indian Journal of Animal Sciences, vol. 86, no. 9, pp. 1073-1078, 2016.

[2] Arpášová, H., Pistová, V., Hrnčár, C., Fík, M., Haščík, P., "The impact of the humic acid and phytobiotics on performance and carcass parameters of broiler chickens", Acta fytotechnica et zootechnica, vol. 21, no. 4, pp. 173-178, doi: 10.15414/afz.2018.21.04.173-178, 2018.

[3] Bezuglova, O., "Molecular structure of humus acids in soils", Journal of Plant Nutrition and Soil Science, vol. 182, pp. 676-682, doi: 10.1002/jpln.201900043, 2019.

[4] Carvalho, L. H. M., De Koe, T., Tavares, P. B., "An improved molybdenum blue method for simultaneous determination of inorganic phosphate and arsenate", Ecotoxicology and Environmental Restoration, vol. 1, no. 1, pp. 13–19, 1998.

[5] Castanon, J.I.R., „History of the use of antibiotic as growth promoters in European poultry feeds", Poultry Science, vol. 86, no. 11, pp. 2466–2471, doi:10.3382/ps.2007-00249, 2007.

[6] Cunniff, P., Official Methods of Analysis of Association of Official Analytical Chemists, 16th edn., AOAC International, Airlington, Va, USA, 1995. ISBN: 9780935584547

[7] Daněk, P., Paseka, A., Smola, J., Ondráček, J., Bečková, R., Rozkot, M., "Influence of lecithin emulsifier on the utilisation of nutrients and growth of piglets after weaning", Czech Journal of Animal Science, vol. 50, no. 10, pp. 459–465, doi:10.17221/4245-CJAS, 2005.

[8] Demeterová M., Mariščáková R., Pistl J., Naď P., Šamudovská A., "The effect of the probiotic strain Enterococcus faecium DSM 7134 in combination with natural humic substances on performance and health of broiler chickens", Berliner und Münchener tierärztliche Wochenschrift, vol. 122, no. 9-10, pp. 370-377, doi:10.2376/0005-9366-122-370, 2009.

[9] El-Ghany, W.A., Abdel-Latif, M.A., Hosny, F., Alatfeehy, N.M., Noreldin, A.E., Quesnell. R.R., Chapman, R., Sakai, L., Elbestawy, A.R., "Comparative efficacy of postbiotic, probiotic, and antibiotic against necrotic enteritis in broiler chickens", Poultry Science, vol. 101, no. 8, 101988, doi: 10.1016/j.psj.2022.101988, 2022.

[10] Elnaggar, A.Sh., El-Kelawy, M.I. "Effect of humic acid supplementation on productive performance, blood constituents, immune response and carcass characteristics of Sasso chicken", Egyptian Journal of Animal Production, vol. 55, no. 1, pp. 75-84, doi:10.21608/EJAP.2018.93258, 2018.

[11] European Commission, „Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed", Official Journal of European Union, vol. 54, pp. 1-130.

[12] Hinkle, D.E., Wiersma, W., Jurs, S.G., Applied statistics for the behavioral sciences, Boston, USA: Houghton Mifflin, 756 pp., 2014. ISBN: 9780618124053

[13] Konieczka, P., Józefiak, D., Kinsner, M., Smulikowska, S., "Effects of high-moisture corn preserved with organic acids combined with rapeseed meal and peas on performance and gut microbiota activity of broiler chickens", Animal Feed Science and Technology, vol. 280, 115063. doi: 10.1016/j.anifeedsci.2021.115063, 2021.

[14] Pineda-Quiroga, C., Atxaerandio, R., Ruiz, R., Garcia- Rodríguez, A., "Effects of dry whey powder alone or combined with calcium butyrate on productive performance, duodenal morphometry, nutrient digestibility, and ceca bacteria counts of broiler chickens", Livestock Science, vol. 206, pp. 65–70, doi:10.1016/j.livci.2017.10.001, 2017.
[15] Rath, N.C., Huff, W.E., Huff, G.R., "Effects of humic acid on broiler chickens. Poultry Science", vol. 85, no. 3, pp. 410-414, doi:10.1093/ps/85.3.410, 2006.
[16] Slominski, P., "Recent advances in research on enzymes for poultry diets", Poultry Science, vol. 90, no. 9, pp. 2013-2023, . http://doi.org/10.3382/ps.2011-01372, 2011.
[17] Van Loon, J. C., "Analytical Atomic Absorption Spectroscopy, Selected methods", New York: Academic Press, New York, 348 pp, 1980. ISBN: 9780323154864
[18] Van Soest, P. J., Robertson, J. B., Lewis, B. A., "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition", Journal of Dairy Science, vol. 74, no. 10, pp. 3583–3597, doi:10.3168/jds.S0022-0302(91)78551-2, 1991.