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DIGESTIBILITY AND PERFORMANCE OF BROILER CHICKENS FED DIETS WITH HUMIC SUBSTANCES

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Abstract

The objective of the study was to investigate the effects of dietary intake of humic substances (HS) on the ileal digestibility of dry matter (DM) and crude protein (CP). One-day-old broiler chickens (Cobb 500, n=120, average weight 50 g) were divided at random into 4 equal groups (A, B, C / negative control). Chickens were fed with mixtures with the content of CP - BR1 230.20, BR2 222.20, BR3 209.40 $g.kg^{-1}$ DM for 37 days. The applied additives HN/HNM (Humac Ltd., Slovak Republic) contained humic acids (HA) min. 650/570 and fulvic acids (FA) min. 50/50 $g.kg^{-1}$. The humic substances were added into diets of experimental groups according to the content of HA/FA in the feed additives A 4.55/0.35, B 3.99/0.35, C 2.85/0.25 $g.kg^{-1}$. The body weights (BW) and feed consumption were assessed once a week. The average daily weight gains (ADWG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) were calculated. The ileal chymus, sampled from 48 birds after slaughter, was used for the quantification of DM, CP, ash and ash insoluble in HCl on day 37. The production parameters of chickens were from day 1 – 37 as follows. The average finishing body weights were A 2506.67 ± 160.47, B 2490.25 ± 166.39, C 2377.75 ± 133.19 and control 2319.42 ± 92.55 g. The average weight gains achieved the values A 66.39 ± 16.85, B 65.95 ± 17.77, C 62.91 ± 18.06 and control 61.34 ± 17.31 $g.day^{-1}$. The average daily feed intakes were A 108.97 ± 50.11, B 116.91 ± 55.35, C 108.85 ± 51.44 and control 106.83 ± 46.97 $g.day^{-1}$ from day 1 – 37. The addition of humic substances caused the increase of ileal digestibility in B group compared to control: DM 38.32 ± 0.18 / 30.94 ± 2.41% ($p < 0.05$) and CP 40.39 ± 0.05 / 34.29 ± 0.26% ($p < 0.05$). The dietary intake of feed additives containing HS seems to have a positive effect on finishing BW and the ileal digestibility of DM and CP in the case of the minimal inclusion level of HA/FA 3.99/0.35 $g.kg^{-1}$.

Keywords: gut of poultry, humates, dry matter, crude protein, ileum

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Introduction

The feed additives with the antimicrobial characteristics were used for the improvement of production parameters of livestock and poultry (*Dibner and Buttin*, 2002). The antibiotic growth promoters were banned in the EU with the Regulation 1831/2003 (*Castanon*, 2007) because of the negative residual effects of animal products used in human nutrition on health and the increased bacterial resistance.

The alternative growth promoters to antimicrobials which are used nowadays are organic acids (*Ur Rehman et al*., 2016), plant extracts (*Abd El-Hack and Alagawany*, 2015; *Dhama et al*., 2015), probiotics and prebiotics (*Alagawany et al*., 2018) and enzymes (*Griggs and Jacob*, 2005). As far as the essential oils are concerned, these are important as growth promoters of poultry (*Levkut et al*., 2010, 2011).

The additional 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; *Arif, M. et al*., 2019).

According to the International Humic Substances Society (2007), HS are major components of the natural organic matter (NOM) in soil, water and in geological organic deposits such as lake sediments, peats, brown coals and shales. These are responsible for the characteristic brown colour of decaying plant debris and contribute to the brown or black colour in surface soils. As for formation, HS are complex and heterogeneous mixtures of polydispersed materials created by biochemical and chemical reactions during the decay and transformation of plant and microbial remains in the process called humification. The important components of this process are plant lignin, its transformation products, polysaccharides, melanin, cutin, proteins, lipids, nucleic acids and fine char particles. HS in soils and sediments are divided into following fractions: HA, FA and humin. The strong base (NaOH or KOH) are used for the extraction of HA and FA from the soil and other solid phase sources. Because of the insolubility of HA at low pH, they are precipitated by adding strong acid.

According to the results of *Marcin et al*. (2020), the addition of HS combined with urea had a positive effect on the amylolytic and cellulolytic activities in the rumen fluid of sheep. Higher activities of digestive enzymes (amylase, lipase, protease) were observed after feeding of broiler chickens with FA (*Mao, Y*., 2019). It is in agreement with the length of the mucosal villi of the jejunum and the increased gut length due to dietary inclusions of HS into broiler feed (*Vašková et al*., 2018; *Taklimi et al*., 2012).

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 potential to improve digestion of proteins, starch and cellulose in the gut of poultry.

Therefore, the study aimed to investigate the effects of dietary intake of a preparation containing HS on the basic production parameters and the ileal digestibility of the dry matter (DM) and the crude protein (CP) of broiler chickens.

Materials and methods

Chickens and diets

One hundred and twenty one-day-old broiler chickens of hybrid COBB 500 (Mach Drubež, a.s., Litomyšl, Czech Republic) (average weight 50 g), were delivered from a commercial hatchery. Birds were divided into 4 groups of 30 animals (A, B, C / negative control). They were housed in four-floor pens located in one experimental hall of the University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic) 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 (Agrocass plus, Ltd., Slovak Republic) with CP content: BR1 – 230.20, BR2 – 222.20, BR3 – 209.40 $g.kg^{-1}$ DM) for 37 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 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 of chickens - Humac natur (HN) for A and Humac natur monogastric (HNM) for B and C group. These additives were absent in the feeds for the control group. The characteristics of the applied preparations HN/HNM were the following: 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 $g.kg^{-1}$, macroelements Ca 42.28/51.1, Mg 5.11/4.86, Fe 19.05/18.09 $g.kg^{-1}$ 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 $mg.kg^{-1}$ DM. The inclusion level of the additives in the diets of experimental groups was measured according to the content of HA/FA in the feed additives: A 4.55/0.35, B 3.99/0.35, C 2.85/0.25 $g.kg^{-1}$. The body weights (BW) of chickens and the feed consumption were evaluated once a week. The average daily weight gains (ADWG), the average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated.

Feed analysis

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, 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.

Check of digestibility

The ileal digestibility was checked after the slaughter of 48 birds on day 37. The samples of ileal chymus from each slaughtered chicken were applied into sterile glass containers. Subsequently, each 30 g sample was pooled from the ileal chymus of two chickens. Therefore the resulting total number of analysed samples was 24. The quantifications of DM, CP, ash and ash insoluble in HCl were performed by the above-mentioned methods.

The ileal digestibility was calculated according to the method of *Kadim and Moughan* (1997). The content of the insoluble portion of ash was used as the indicator. The digestibility was determined by calculating the analysed content of nutrients in feed and ileal chymus according to the formula:

Nutrient digestibility (%) =

$$= \frac{\text{nutrient content in diet/indicator in diet - nutrient output in ileum/indicator in ileum}}{\text{nutrient content in diet/indicator in diet}} * 100$$

***Table 1*: Composition of the experimental diets**

	Diets		
Analysed nutrients (g.kg^{-1})	BR1	BR2	BR3
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^{-1})*	13.27	14.30	13.58

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

	Ingredients in diets
BR1 / BR2 / BR3	maize, wheat, soybean meal GMO, vegetable oil, limestone, amino acids and their salts, monocalcium phosphate, mineral-vitamin premix
BR1	the dried derivative of pig blood
BR2 / BR3	sunflower extracted meal, salt

Statistical analysis

The data are expressed as means ± standard deviation (SD) of single values (IBM SPSS Statistics, Version 24). Means of the results from the treatments were compared by one-way analysis of variance. Treatment means were statistically compared by Tukey-Kramer multiple comparison test. Significance was declared at $P < 0.05$.

Results and discussion

The considerable interest has been in the use of HS as a feed additive to enhance performance and nutrient digestibility of poultry (*Domíngues-Negrete et al.,* 2019).

The present results indicate that the dietary intake of HS is effective in the enhancement of the performance of broilers. The values of BW and ADWG are shown in *Table 2*. ADWG was improved by the higher amount of the added HS 0.7% into feed, or the inclusion level of HA/FA in the feed additives A 4.55/0.35 and B 3.99/0.35 by 7.61% in A and by 6.99% in B groups compared to control at day 1 – 37. In the case of individual BW, there were observed the only one significant decrease on day 7 when this parameter was lower in A group by 9.7 g ($p < 0.05$) in comparison to the negative control. The finishing BW was higher in A or B groups compared to control by 187.25 and 170.83 g, respectively.

***Table 2*: Impact of feeding of humic substances on the growth parameters of broiler chickens**

Group	BW (g)							ADWG ($g.day^{-1}$)
	d 1	d 7	d 14	d 21	d 28	d 35	d 37	d 1 – d 37
A	50.0^{a} ± 3.75	182.07^{b} ± 4.66	488.97^{a} ± 65.44	963.07^{a} ± 120.89	1571.48^{a} ± 280.39	2311.92^{a} ± 367.72	2506.67^{b} ± 160.47	66.39^{a} ± 16.85
B	50.0^{a} ± 2.65	188.82^{ab} ± 5.85	512.75^{a} ± 67.64	986.37^{a} ± 116.20	1636.63^{a} ± 210.66	2326.56^{a} ± 292.20	2490.25^{b} ± 166.39	65.95^{a} ± 17.77
C	50.0^{a} ± 3.35	192.27^{ab} ± 7.18	521.5^{a} ± 45.87	989.33^{a} ± 102.5	1585.17^{a} ± 286.5	2281.93^{a} ± 326.51	2377.75^{ab} ± 133.19	62.91^{a} ± 18.06
Control	50.0^{a} ± 4.15	191.73^{a} ± 8.41	514.86^{a} ± 54.30	985.57^{a} ± 100.03	1633.0^{a} ± 193.26	2291.79^{a} ± 320.88	2319.42^{a} ± 92.55	61.34^{a} ± 17.31

Means with different superscript letters differ significantly $^{a, b}$ $P < 0.05$ (mean ± SD),
BW – body weight, ADWG – average daily weight gain, d – day

The similar improvement of weight gains was observed by *Arif et al.* (2016). They determined the increase of this parameter of broilers fed HA at 2.25 $g.kg^{-1}$ diet as a result of the improved feed conversion. Their dosage of HA was approximately one half compared to our inclusion levels. On the other hand, the statistical insignificance of ADWG could be partially supported by the findings of

Karaoğlu et al. (2004). They observed that supplementation of HS and HA in the diet of broiler chickens did not influence the body weight gains.

ADFI and FCR are presented in *Table 3*. There were not observed significant differences in FCR during the experiment. The improvement of ADFI was evaluated in the experimental groups: A and C on day 7 (by 0.6% and 4.02%), B on day 14 and 21 (by 4.39% and 5.39%), A and B on day 28 (by 4.26% and 7.59%), A, B and C on day 35 (by 6.64%, 17.13% and 12.22%) as well as in B and C on day 37 (by 5.38% and 2.10%).

The positive results of feed utilization are in coincidence with the partial improvement of nutrient digestibility and the protection of commensal gut microflora caused by HS (*Windisch et al*., 2008). However, gut health is a very important factor in the utilization and conversion of nutrients of growing chickens (*Islam et al*., 2008).

***Table 3*: Impact of feeding of humic substances on the feed consumption and conversion of broiler chickens**

Group	ADFI ($g.day^{-1}.chicken^{-1}$)						FCR ($kg.kg^{-1}$)
	d 7	d 14	d 21	d 28	d 35	d 37	d 1 – d 37
A	28.11	60.39	105.82	147.09	167.20	145.18	$1.65^{a} \pm 0.29$
B	27.29	66.54	112.17	152.38	188.36	154.71	$1.64^{a} \pm 0.19$
C	29.11	60.42	100.95	135.24	177.83	149.52	$1.53^{a} \pm 0.26$
Control	27.94	63.62	106.12	140.82	156.10	146.38	$1.51^{a} \pm 0.17$

Means with different superscript letters differ significantly $^{a, b}$ $P < 0.05$ (mean ± SD),
ADFI – average daily feed intake, FCR – feed conversion ratio, d – day

The addition of HS had a positive effect on the increase of ileal digestibility of DM or CP at the inclusion level of HA/FA into diets at minimum 3.99/0.35 $g.kg^{-1}$ feed (*Table 4*) on day 37. As for DM digestibility, there was observed the higher value in the B group compared to control by 7.38%. The significant difference was observed between groups B and C by 14.66% ($P < 0.05$).

Similarly, in the case of the CP digestibility, the higher value was determined in the B group compared to control by 6.1% and the significant difference was observed between groups B and C ($P < 0.05$) by 7.60% too. The digestibility of nutrients present in the chymus depends on the enzymatic activities in the small intestine which could be positively influenced by HS.

This statement can be confirmed by the observation of *Terry et al.* (2018) when HS had a positive effect on the apparent total tract digestibility. HS are can improve the digestibility of energy in the ileum which is dose-dependent but on the contrary, the ileal digestibility of N was not affected (*Gomez-Rosales and Angeles*, 2015).

***Table 4*: Ileal digestibility of DM and CP analysed in ileal chymus after intake of HS sampled from 48 broiler chickens on 37 day (samples were pooled to n = 6, mean ± SD)**

Group	DM (%)	CP (%)
A	$33.27^{ab} \pm 5.26$	$37.99^{ab} \pm 1.42$
B	$38.32^{a} \pm 0.18$	$40.39^{a} \pm 0.05$
C	$23.66^{b} \pm 0.56$	$32.79^{b} \pm 0.66$
Control	$30.94^{ab} \pm 2.41$	$34.29^{b} \pm 0.26$

Means with different superscript letters differed significantly: ^{a,b} $P < 0.05$,
DM – dry matter, CP – crude protein, HS – humic substances

Conclusion

The dietary intake of HS seems to have a positive effect on the ileal digestibility at the inclusion level HA/FA at minimum 3.99/0.35 $g.kg^{-1}$. There were observed the increased digestibility of DM by 7.38% ($p < 0.05$) and CP by 6.1% ($p < 0.05$) compared to negative control. The finishing individual BW of experimental groups were positively influenced in A by 187.25 ($p < 0.05$) and in B groups by 170.83 g ($p < 0.05$).

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