THE EFFECT OF HEMP BY-PRODUCTS FEEDING ON GUT MICROBIOTA AND GROWTH OF BROILER CHICKENS

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Abstract: The *Cannabis sativa* L. is an annual plant and well known as an important source of fiber, food, feed, dietary oil and medicine for thousands of years in many countries. The aim of the experiment was to study whether hemp by-products may affect the microbial colonization of the gut, because it would affect the absorption and utilization of nutrients also. This could have effect to performance of chickens. A total of 60 sexed Ross 308 hybrid cockerels were fattened on conventional deep litter system. The trial was conducted from day 10 to day 37 of chicken's age. Cockerels were divided into three equal groups with 2 replicates per treatment. There were 10 chickens per replicate pen. Cockerels were allocated randomly. The two experimental groups received feed mixtures containing 2.5% of hempseed expellers or 1% of pellets from technical hemp plant tops. The pellets from hemp plant tops are flowers and seeds with a bit of shives. It was crumbled before mixing into feed mixture. The third group was without hemp addition. On behalf of each bacterial species were not detected statistically significant differences ($P > 0.05$) between groups at 37th day of age. Based on our results we can conclude that the content of cannabidiol 0.03% and 0.15% (hempseed expellers and hemp plant tops, respectively) not affect monitored microbiological parameters of intestinal contents. It was achieved non-significant ($P > 0.05$) differences in average body weight in our study. The same trend was found in carcass yield.

Key Words: hempseed expellers, hemp plant tops, carcass yield, *Lactobacillus*, *E. coli*

INTRODUCTION

The *Cannabis sativa* L. is an annual plant and well known as an important source of fiber, food, feed, dietary oil and medicine for thousands of years in many western and eastern countries in Old World (Callaway 2004, de Padua et al. 1999). The two main proteins in hempseed are edestin and albumin. Both of these high-quality proteins are easily digested and contain nutritionally important amounts of all essential amino acids. In addition, hempseed has very high level of the amino acid arginine (Callaway 2004). Industrial hempseeds have a low content (~0.3%) of tetrahydrocannabinol (THC) which stimulates appetite (Konca et al. 2014, Hampson et al. 2000, Koch 2001). Cannabinol (CBN) is a metabolite of THC, with potential immunosuppressive and anti-inflammatory activities (Pubchem 2015). Another metabolite of THC is cannabidiol (CBD). It is reported that hempseed's cannabidiols have antimicrobial, immunomodulatory, antioxidative, antihypertensive and mineral binding activities (Korhonen and Pihlanto 2003). The nutrient composition of hemp by-products can be quite different. This statement confirms our previous study where the content of CBD in hempseed expellers was 0.017% and crude protein content was 27% (Stastnik et al. 2015).

With the increasing production of hemp (*Cannabis sativa* L.) it´s by-products are more often. These products can be used in animal nutrition. This study was aimed to investigate the possibility of use hemp by-products in animal nutrition. We decided to examined hempseed expellers and hemp plant tops, in our study. Thus, the aim of this experiment was to study whether hemp by-products may affect

the microbial colonization of the gut, because it would affect the absorption and utilization of nutrients also. This could have effect to performance of chickens. The addition of hempseed expellers at dose 15% negatively affected the growth of chickens in our previous experiment (Stastnik et al. 2015). So, we decide to include a lower dose of expellers to feed mixture now.

MATERIAL AND METHODS

The experiment was performed with 60 sexed Ross 308 hybrid cockerels which were fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 10 to day 37 of chicken's age. Room temperature and humidity were controlled. Lighting system was 18 hours light and 6 hours dark. Cockerels were divided into three equal groups with 2 replicates per treatment. There were 10 chickens per replicate pen. Cockerels were allocated randomly. The two experimental groups received feed mixtures containing 2.5% of hempseed expellers or 1% of pellets from technical hemp plant tops (groups HSE and HP, respectively). The pellets from hemp plant tops are flowers and seeds with a bit of shives. It was crumbled before mixing into feed mixture. The third group was without hemp addition (C).

Table 1 shows chemical composition of used hemp by-products. The used hempseed expellers contained 0.03% of cannabidiol (CBD) and pellets from hemp plant tops contained 0.15% of CBD. The content of tetrahydrocannabinol (THC) and cannabinol (CBN) are non-detectable in feed either in feces. The compositions of experimental rations are presented in Table 2. The rations were calculated according to the Recommended nutrient content in poultry diets and nutritive value of feeds for poultry (Zelenka et al. 2007). The chickens were fed *ad-libitum.* The live weight was measured every week during the trial.

Table 1 Chemical composition of used hemp by-products

	HSE	***HP***
Dry matter (g/kg)	100	100
Gross energy (MJ/kg)	20.54	17.52
Crude protein (g/kg)	306.4	157.7
Ether extract (g/kg)	96.2	68.9
Crude fibre (g/kg)	367.1	288.7
Crude ash (g/kg)	74.2	163.8
CBD* (%)	0.03	0.15

**CBD - cannabidiol*

At the end of experiment 6 birds were selected randomly from each group, weighed and slaughtered. Microbial analyses were carried out at the 37th days of broilers age. Fresh digesta were obtained from the lower ileum. The digesta were diluted into a fluid state with saline solution. Weighed digesta (3 g) was homogenised in centrifuged tube. Into Petri dishes 1 ml of sample was inoculated. Three groups of microorganisms were determined. The *Escherichia coli* (*E. coli*) on Rapid Ecoli 2 agar (Biorad, USA) for 24 hrs at 37 ˚C. *Enterococci* spp. on Slanetz-Bartley agar (Merck, Germany) for 72 hrs at 37 ˚C. *Lactobacilli* spp. cultivated anaerobically on MRS agar (Biokar Diagnostics, France) for 48 hrs at 37 ˚C. After cultivation, counts of microorganisms were expressed as log CFU/g (Colony Forming Unit). Feathers were removed and chickens were eviscerated. Carcass yield was calculated. Breast muscle and leg muscle were deboned and weighted in these selected chickens. These values were calculated by the percentage of live weight.

Table 2 Composition of feed mixtures (g/kg)

Component	C	HSE	HP
Wheat	400	400	400
Soybean meal	260	260	260.5
Maize	205	182.3	192
Rapeseed oil	40.5	45	44
Premix*	30	30	30
Hempseed expellers	0	25	0
Hemp plant tops	0	0	10
Wheat gluten	31	24.4	29.5
Maize starch	20	20	20
Monocalciumphosphate	7.5	7	7.5
$CaCO_3$	3	2.8	3
L-Lysine	1.5	2	2
DL-Methionine	1.5	1.5	1.5
Chemical composition - as fed (per kg of diet)			
Dry matter (g)	880	880	880
AME (MJ)	12.61	12.60	12.58
Crude Protein (g)	200.7	198.4	195.7
Ether extract (g)	60.4	61	62
Crude fibre (g)	45.8	55.2	48.6
Crude ash (g)	58.6	57.1	55.5

** Premix contains (per kg): lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2500 mg; zinc 3400 mg; manganese 4000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450000 mg; calciferol 166700 IU; tocoferol 1500 mg; vit K 350 mg; thiamine 140 mg; B2 230 mg; B6 200 mg; B12 1000 mg; biotin 7 mg; niaciamid 1200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6000 mg; salinomycin sodium 2333 mg.*

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). One-way analysis (ANOVA) was used. To ensure evidential differences Scheffe´s test was applied and $P < 0.05$ was regarded as statistically significant difference.

RESULTS AND DISCUSSION

The mean bodyweight of chickens during the experiment is presented in Table 3. In accordance with the performance targets for ROSS 308, the average body weight of cockerels would be 2,493 g at 37 days of age (Aviagen Group 2014). Whereas non-significant ($P > 0.05$) the highest average body weight 2,216 g was achieved in control group in our study at the day of slaughter. In accordance with this, Afzali et al. (2015) found inclusion of extruded hempseed into feed mixture for broilers had no significant effect on performance. In another study Barani et al. (2015) observed that addition of hempseed at dose 10% significantly decreased feed intake and body weight of broilers. On the other hand, Khan et al. (2009) found that mean body weight gain and dressing percentage of chickens was significantly higher ($P < 0.05$) in group with 20% cannabis addition against to the control group at the end of the experiment. Nevertheless, our results of earlier trial (Stastnik et al. 2015) suggested that addition of hempseed expellers at dose 15% negatively affected the growth of chickens, because the final body weight (at 37 days of age) with part of hempseed cakes in feed mixture was significantly lower ($P < 0.05$). A higher proportion (15%) also worsened feed conversion ratio. Data of carcass yield were not affected ($P > 0.05$) by higher inclusion of hempseed cakes.

Table 3 Mean live weight of broilers per trial (g)

Day of age	n	10	17	24	31	37
		Mean ± standard error				
C	20	221 ± 5.57	535 ± 10.45	1,023 ± 20.97	1,623 ± 32.41	2,216 ± 47.30
HSE	20	230 ± 5.21	530 ± 11.75	915 ± 31.42	1,521 ± 46.44	2,141 ± 62.58
HP	20	239 ± 4.98	534 ± 12.86	928 ± 31.44	1,471 ± 42.10	1,998 ± 52.36

Differences between groups are not statistically significant (P > 0.05)

Table 4 shows the mean content of *E. coli, Lactobacillus* and *Enterococcus* in gut of broiler chickens. On behalf of each bacterial species were not detected statistically significant differences (P > 0.05) between groups at 37th day of age. Based on our results we can conclude that the higher content of cannabidiol not affect monitored microbiological parameters of intestinal contents. Jakubcová et al. (2014) in experiment with supplementation of chamomile extract found that lactic acid bacteria were higher in the group without chamomile extract. On the other hand, Lichovnikova et al. (2014) observed in their trial with added red grape pomace (*Vitis Vinifera* L.) that this had a positive effect on the content of *Lactobacillus*.

Table 4 The mean content of microorganism's log CFU per grams of digesta of the broilers

Group	n	*Escherichia coli*	*Lactobacillus* spp.	*Enterococcus* spp.
		Mean ± standard error		
C	6	6.28 ± 0.491	9.10 ± 0.187	6.47 ± 0.117
HSE	6	6.65 ± 0.402	9.36 ± 0.380	6.55 ± 0.072
HP	6	6.56 ± 0.218	8.79 ± 0.116	6.66 ± 0.064

Differences between groups are not statistically significant (P > 0.05)

The highest carcass yield was found in the control group (Table 5) but differences between groups were not significant (P > 0.05). The lowest value was observed in experimental groups. Carcass yield stated in the technological procedure for ROSS 308 (Aviagen Group 2014) is the 72.08% for 2,200 g of live weight. Percentages of breast muscle of body weight (Table 5) were non-significant (P > 0.05) highest for control group (21.30 ± 0.982%), while the lowest values were observed in experimental groups again (18.0 and 18.13%, respectively). In the manual of hybrid Ross 308 (Aviagen Group 2014) is stated similar percentage of breast muscle of body weight to our results. Percentages of thigh muscle of body weight was attempted the highest for HSE group (Table 5). The manual for the hybrid Ross 308 (Aviagen Group 2014) indicates a yield of leg meat 16.03% for 2,200 g live weight. The differences among groups in slaughtering yields were not statistically significant (P > 0.05). Similar results achieved Eriksson and Wall (2012) who conducted an experiment with organic broilers with supplementation of hempseed cakes at dose 100 and 200 g/kg in feed mixture (grower and finisher, respectively). They observed non-significant differences in carcass yield among experimental and control groups.

Table 5 Carcass yield (%)

Group	n	Carcass weight	Breast meat	Leg meat
		Mean ± standard error		
C	6	68.77 ± 0.712	21.30 ± 0.982	14.24 ± 0.310
HSE	6	66.84 ± 1.082	18.00 ± 0.987	14.40 ± 0.438
HP	6	66.03 ± 1.387	18.13 ± 1.364	15.14 ± 0.625

Differences between groups are not statistically significant (P > 0.05)

CONCLUSION

The addition of hempseed expellers and hemp plant tops had no positive or negative effect to growth of chickens. On behalf of each bacterial species were not detected differences between control and experimental groups at 37th day of chickens age. So, we can conclude that the content of cannabidiol in amount 0.03% and 0.15% (hempseed expellers and hemp plant tops, respectively) not affect monitored microbiological parameters of intestinal contents, based on our results. Consequently, it was achieved

non-significant differences in average body weight in our study. The same trend was found in carcass yield.

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