

A note on changes in the concentration of short-chain fatty acids in the caecal content of laying hens due to addition of fresh grass to the diet

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ABSTRACT

Twenty laying hens (Hy Line) kept in individual cages were randomly divided into two groups, B and BG. Both groups were fed a basal diet *ad libitum*, hens from group BG additionally received 25 g of fresh chopped grass per day in separate feeders. The experiment lasted twelve days, at the end all birds were slaughtered and the content of the caecum was sampled. Total and individual short-chain fatty acid (SCFA) concentrations in caecal contents were measured by high pressure liquid chromatography. The total amount of SCFA in caecal digesta of hens fed diets with added fresh grass was lower by 27% in comparison with group B ($P \leq 0.05$), however, the proportions between acetic, propionic and butyric acids were similar in both groups. In both groups the level of acetic acid was highest followed by propionic and butyric acids ($P \leq 0.05$).

KEYWORDS fresh grass, laying hens, caecal contents, short-chain fatty acids

INTRODUCTION

Compared with wild birds, the overall length of the gastrointestinal tract in domestic birds is much shorter. Therefore, good performance on commercial poultry farms is dependent on feeding highly digestible diets. However, in recent

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years increased interest has developed in free-range poultry production (Laszczyk-Legendre, 1999), where roughage might make up even up to 48% of a basal diet (Steenfeldt et al., 2001).

There is usually no problem in digestion of most monosaccharides as well as starch in the poultry gastrointestinal tract (GIT). Digestion of NSP fractions, which are the main component of dietary fibre, tends to be more variable due to their heterogeneous nature and lack of susceptibility to poultry digestive enzymes (Carré, 1993; Jamroz et al., 2001).

Dietary fibre is the sum of plant polysaccharides and lignin that are not hydrolysed by endogenous enzymes of the avian and mammalian digestive systems. However, these carbohydrates are hydrolysed by enzymes produced by the avian's GIT microflora. In most poultry species the ceacum is the main site of microbial fermentation (Goldstein, 1989; Pinchasov and Elmaliah, 1994; Vispo and Karasov, 1997; Jamroz et al., 1998). The final products of that process include short-chain fatty acids (SCFA), ammonia, carbon dioxide and methane (Marounek et al., 1999). Organic acids produced during dietary fibre breakdown are similar to those present in the rumen; these include butyric, iso-butyric, acetic, lactic, propionic, valeric and iso-valeric acids (Jamroz et al., 1994).

Diet and age are the main factors influencing fermentation processes (Carré, 1993). Changes of SCFA concentrations in the ceacum due to the amount and type of carbohydrate fractions were observed by many authors in different poultry species (Langhout and Schutte, 1996; Jamroz et al., 1996, 1998; Tsukahara and Ushida, 2000).

However, no data is available about fresh grass as a factor affecting caecal fermentation in laying hens. The objective of this experiment was to determine changes in SCFA concentrations in the caecal content of laying hens fed diets varying in fermentable carbohydrate levels due to addition of the fresh grass.

MATERIAL AND METHODS

The experiment was done on twenty brown Hy-Line laying hens, 48 weeks old, kept in individual balance cages. Birds were randomly divided into two feeding groups: B and BG. Both groups were fed a basal diet (Table 1) *ad libitum*, hens from group BG additionally obtained 25 g per day of fresh chopped grass in separate feeders, which amounted to about 5.5% of the consumption of the basal diet in group B. Grass was in the mid-maturity stage and consisted of the following species (%): perennial ryegrass (*Lolium perenne* L.) 50, red fescue (*Festuca rubra* L.) 40 and smooth-stalked meadow grass (*Poa pratensis* L.) 5. The experiment lasted from the 5th to the 17th of May, grass was cut five times a day from randomly chosen parts of a meadow, immediately chopped and offered in separate feeding boxes.

TABLE 1

Composition and nutritional value of basal diet and grass, g · kg⁻¹ dry matter

Components	Content	
Maize	581.5	
Soyabean meal (46% CP)	237.0	
Rapeseed meal	50.0	
Soya oil	20.0	
Limestone	80.0	
DL-methionine (20%)	4.0	
NaCl	3.0	
Dicalcium phosphate	19.5	
Mineral - vitamin premix	5.0	
Chemical composition	Basal diet	Grass
crude protein (N x 6.25)	170.9	187.1
crude fat	56.1	29.1
crude ash	149.7	109.1
crude fibre	48.6	243.3
monosaccharides	3.35	32.0
starch	492.5	24.4
hemicellulose	103.1	255.2
NDF	171.6	533.0
ADF	68.4	277.8
TDF	127.4	618.3

After 12 days of feeding all of the birds were slaughtered by cervical dislocation, the abdominal cavity was opened, and caecal digesta samples were collected for SCFA analysis.

Chemical analysis and calculations

The chemical composition of the diet and average sample of fresh grass was determined according to AOAC (1990). The content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined according to Van Soest (1963), monosaccharides according to Åman and Graham (1990). The hemicellulose, content was calculated as the difference between NDF and ADF contents. The level of total dietary fibre (TDF) was estimated as dry matter minus crude protein, fat, ash, starch, and monosaccharide contents.

SCFA analysis was conducted by the modified method of Wielen et al. (2000). Approximately 0.4 g of caecal material was diluted in 1.6 ml of sterile deionized water, mixed, centrifuged for 10 min at 13,400 x g and 12.5 µl of xylitol solution (internal standard: 0.586 M xylitol in 1.5 M HCl) were added to 700 µl of the sample. Subsequently, samples were again mixed and centrifuged for 10 min at 13.400 x g

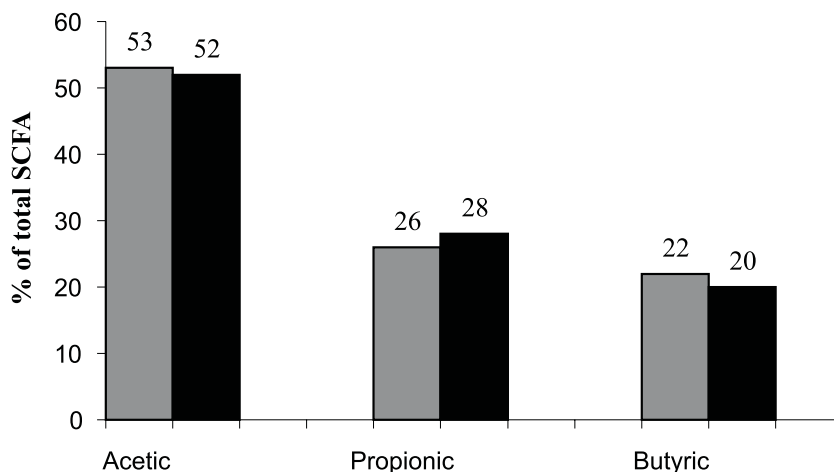


Figure 1. Proportions of acetic, propionic and butyric acids in the caecal contents of laying hens, in % of total SCFA

before HPLC analysis. Ten microliters of the sample were injected to a HPLC (Waters 2690), equipped with an absorbance detector (Waters 2487) and organic acid column (300x7.8 mm) from Bio-Rad (Animex HPX-87-H Ion exclusion column). The column and auto-sampler were operated at constant temperatures, 35°C and 5°C, respectively. The column was operated with 0.005 M H₂SO₄ at 0.6 ml/min as the eluent, in 95 x 10⁵ Pa. Concentrations of SCFA in caecal samples were compared and calculated with usage of the Volatile Free Acid Standard Mix (46975-U by Supelco).

Statistical analysis was performed using the SAS (1996) System ver. 6.12. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The following SCFAs were present in caecal digesta samples of laying hens in the present study: acetic, propionic, butyric, iso-butyric, iso-valeric and valeric acids. Jamroz et al. (1994, 1998) also found similar SCFAs in caecal digesta in chickens, geese and ducks. In group B acetic, propionic and butyric acids made up 50, 24 and 21%, in group BG, 47, 26 and 17%, respectively, of the total SCFA amount. Only acetic and butyric acid concentrations differed significantly ($P \leq 0.05$) between groups B and BG.

In both groups the acetic acid concentration was markedly higher ($P \leq 0.05$) than of the other SCFAs (Figure 1), similarly as reported in chickens, ducks and geese (Jamroz et al. 1994, 1998), in some wild birds (Foley et al., 1989; Grajal et al., 1989; Dawson et al., 2000) and in other monogastric animals (Sunvold et al., 1995).

TABLE 2

Concentration of short chain fatty acids in caecal content of laying hens, mMol/kg

Group		Acetic	Pro-pionic	n-butyric	Iso-butyric	Iso-valeric	Valeric	Total
B	Mean	119.8 ^a	57.7	49.5	3.5	4.4	5.0	239.0
	SD	5.57	7.57	5.03	2.04	1.65	0.38	
BG	Mean	82.0 ^b	45.5	29.2	9.1	2.9	6.3	175.1
	SD	9.82	5.22	5.78	5.54	3.65	1.05	

^{a,b}- means in the column with different superscripts are significantly different at $P \leq 0.05$

SD- standard deviation

The total concentration of SCFAs (Table 2) decreased by 27% ($P \leq 0.05$) after addition of grass, which might be a result of reduced microbial activity, as SCFAs are the main products of microbial fermentation in poultry caeca (Marounek et al., 1999). No significant changes in the mutual proportions of acetic, propionic and butyric acids were observed (Table 2), which may indicate that caecal microflora had altered activity, while interactions between populations were stable. Probably caecal microorganisms of laying hens, similarly as in ruminants, need a longer time to adapt to a new type of diet.

Microbial activity in the caeca of geese, galliforms and ratites (other than the emu) is primarily fermentative (Vispo and Karasov, 1997) and SCFA concentrations are relatively high. However, the total SCFA concentration found in caecal digesta of laying hens in the present study was much higher compared with levels found in the caecal digesta of geese or ducks (Jamroz et al., 1998) fed a triticale-based diet. The literature about changes of caecal microflora and SCFA concentrations in laying hens is limited and more experiments with longer periods of feeding grass are needed.

CONCLUSIONS

Individual short-chain fatty acids found in the caecal content of laying hens were similar to those observed in other domestic and wild avian species, however, their total concentrations were higher. Addition of fresh grass to the diet affected caecal fermentation processes, resulting in lower total SCFA concentration, without changing the mutual proportions of acetic, propionic and butyric acids.

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STRESZCZENIE

Wpływ dodatku trawy do diety na koncentrację krótkołańcuchowych kwasów tłuszczowych w treści jelit ślepych kur niosek

Dwadzieścia kur niosek (Hy Line) podzielono losowo na dwie grupy: B i BG. Przez dwanaście dni ptaki obydwóch grup otrzymywały dietę podstawową do woli, w grupie BG dodatkowo w oddzielnych karmidlach świeżą, pociętą trawę w ilości 25g/ptak/dzień. W 12 dniu doświadczenia wszystkie ptaki ubito i pobrano treść jelit ślepych. W treści jelit ślepych oznaczono stężenie krótkołańcuchowych kwasów tłuszczowych. W grupie otrzymującej dodatek trawy stężenie krótkołańcuchowych kwasów tłuszczowych w treści jelit ślepych było niższe o 27% w porównaniu z grupą B ($P \leq 0,05$), jednak proporcje między kwasami: octowym, propionowym i masłowym nie zmieniły się istotnie. W treści jelit ślepych w obydwóch grupach największe było stężenie kwasu octowego, następnie propionowego i masłowego ($P \leq 0,05$).