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ABSTRACT

Short-chain fatty acids or volatile fatty acids (VFA; containing acetic, propionic, butyric, valeric, caproic, isobutyric and isovaleric acid) can be studied as indicators of digestion and carbohydrate fermentation in avian caeca. An experiment with a total of 320 growing turkey poults was carried out to determine VFA concentrations in the caeca at 4, 8 and 12 weeks of age. The diet of the turkeys consisted of pelleted maize, wheat, barley or oats as such or supplemented with beta-glucanase-xylanase enzyme. Concentration of acetic acid was the highest among VFAs, the concentration reaching from 0,104 to 0,458 mg/g digesta and the proportion from 58 to 76 per cent of total VFA. Concentrations of propionic acid and butyric acid reached from 0,0075 to 0,0705 mg/g digesta and from 0,0180 to 0,2454 mg/g digesta, respectively. Significant interacting effects of bird age, cereal species and enzyme supplementation occurred on concentration of most VFAs. Generally, concentrations were higher in older birds and in enzyme-supplemented diets.

Key words: volatile fatty acid, turkey, cereal, enzyme

TIIVISTELMÄ

Haihtuvia rasvahappoja (lyh. VFA; etikkahappo, propionihappo, voihappo, valeriaanahappo, kapronihappo, isovoihappo ja isovaleriaanahappo) voidaan tarkastella siipikarjan umpisuolissa tapahtuvan sulatuksen ja fermentaation indikaatttoreina. Tässä tutkimuksessa määritettiin VFA-pitoisuuksia kasvavien kalkkunoiden umpisuoliruokasulasta 4, 8 ja 12 viikon iässä. Eläinten ruokinta koostui rakeistetusta maissista, vehnästä, ohrasta tai kaurasta joko sellaisenaan tai beeta-glukanaasi-ksylanaasi-entsyymiseoksella täydennettynä. Haihtuvista rasvahapoista korkeimmat pitoisuudet olivat etikkahapolla (0,104 – 0,458 mg/g ruokasulaa; osuus VFA:sta tällöin 58 – 76 %). Propionihappopitoisuudet vaihtelivat välillä 0,0075 – 0,0705 mg/g, ja voihappopitoisuudet välillä 0,0180 – 0,2454 mg/mg. Linnun iällä, viljalajilla ja entsyymilisäyksellä oli merkitseviä yhdysvaikutuksia useimpiin VFA-pitoisuuksiin. Pitoisuudet olivat tyypillisesti korkeampia entsyymitäydennetyillä viljoilla.

Avainsanat: haihtuva rasvahappo, kalkkuna, vilja, entsyymi

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1 INTRODUCTION

A short-chain fatty acid or a volatile fatty acid (VFA) can be determined as a carboxyl acid with a carbon chain no longer than six carbon atoms. VFAs (especially acetic, propionic and butyric acid, or typically referred to as acetate, propionate and butyrate in physiological pH) are known to be produced in the intestine of poultry as a result of carbohydrate fermentation (see for instance Józefiak et al. 2004 for a review).

Fermentation may occur in the small intestine due to dietary inclusion of poorly digestible carbohydrates (Choct et al. 1996), and in this case VFA production can be considered as a sign of poor digestion. According to Yasar and Forbes (1999), soaking of feed had led to decreased digesta viscosity and improved performance of broiler chickens, as well as to decreased caecal VFA. However, fermentation in the hindgut may also, in the contrary, be associated to better utilisation of carbohydrates, thus indicating that even gallinaceous poultry may use fermentation and VFA production as a form of energy metabolism. Jamroz et al. (1996) have reported higher ceacal concentrations of VFA in birds when better feed utilisation had been observed. Our previous studies may be interpreted to confirm this assumption: higher VFA production has been observed in the ceaca of turkey poults at older age and when feed enzyme had been added to the diet, both of these factors leading to better feed utilisation and higher energy values as well (Palander, Näsi and Järvinen 2005).

In addition to the possible but somehow unclear roles of VFAs as substrates for energy metabolism in gallinaceous poultry, some individual VFAs may have interesting roles in regulating gut physiology or taking part to pathogen control. The positive function of some prebiotic bacteria like *Lactobacillus acidophilus* may also be associated to an effect on concentrations of different VFAs (Li et al. 2017).

Butyric acid (or actually butyrate) has also been introduced as feed additive in order to replace growth-promoting antibiotics (see for instance Aghazadeh & Taha Yazdi 2012; Matis et al. 2018). However, butyrate as well as acetate are also naturally produced in ceacal fermentation, and it would therefore be of interest to gather more information on effects of different diets on VFA profiles produced in the avian caeca. Most of the existing study has been carried out with broiler chickens or, in some rare cases, with chicks of laying hens (Martinez et al. 2006). Data for turkeys seems to be negligible. In the present study, concentrations of the main VFAs were investigated in growing turkeys at different ages fed different cereals with or without enzyme supplementation.

2 MATERIAL AND METHODS

VFA profiles of caecal digesta were analysed using caecal samples from growing turkeys at different ages. Generally, the experiment had been designed for investigating nutrient digestibility and energy values of diets based on different cereals, and effects of enzyme supplementation and bird age on these parameters, which data (including also total VFA figures shortly presented) had been reported as early as in the previous decade (Palander et al. 2005).

2.1 Animals

A total of 320 growing turkey poults (B.U.T. 8) were used. For the trial, the 1-d-old birds were randomly allocated to 32 wire cages in a temperature-controlled room. Thus, four replicates for each feeding treatment were formed. Poults of both sexes were evenly distributed into each cage. At the beginning of the trial there were 10 birds (5 males and 5 females), after the first intestinal sampling 5 birds (2 males and 3 females) and from the second sampling to the end 2 birds (1 male and 1 female) in each cage. The care and use of the experimental animals followed accepted guidelines and had been evaluated and approved by the Ethical Committee of University of Helsinki.

2.2 Diets and feeding

The experimental feeding had originally been designed in order to determine digestibility and energy values for different cereal species, and did not represent a balanced diet for growing turkeys. The ground, pelleted cereals were fed as such during the experimental periods. See Palander et al. (2005) for the chemical composition of the pelleted cereals.

The birds were fed the experimental feeds during the trial periods at 4, 8 and 12 weeks of age according to the following schedule at each trial period: an accustoming period (I) of two days, a fasting period (I) of 12 h and an accustoming period (II) of one day, which were followed by an excreta collecting period of 24 h, an intervening time of one day, a fasting period (II) of 12 h, and finally a feeding period of 5 h before slaughtering and intestinal sample collection. During the accustoming period (I) the birds were gradually adopted to the experimental diets, which were used thereafter during the trial periods, while during the intervening time between the experimental periods commercial complete diets suited to the age of the birds were used.

2.3 Samples

On the slaughtering days at 28, 56 and 84 d of age a half of the remaining birds of each cage (approximately 1:1 of males and females) were stunned mechanically and killed by cervical dislocation. The body cavity was opened, the caeca dissected and gently emptied. The caecal contents of the birds of each cage were pooled to form one sample per cage, and the samples were immediately frozen.

2.4 Analysis

For the analysis of VFA, the samples were melted, 2,5 to 5 g of caecal digesta was diluted to the volume of 25,0 ml using 1.09 M formic acid solution, and acetic (ethanoic), propionic (propanoic), butyric (butanoic), valeric (pentanoic), and caproic (hexanoic) as well as branch-chained isobutyric (2-methyl-propanoic) and isovaleric (3-methyl-butanoic) acid content was analysed by gas chromatography with a HP 5890 Series II Gas Chromatograph fitted with a 10 m x 0.53 mm capillary column. The analyses were carried out in duplicate.

2.5 Calculation and statistics

A sample of one cage was considered as an experimental unit. Total VFA concentration was calculated as the sum of acetic, propionic, butyric, isobutyric, valeric, isovaleric and caproic (if detected) acids. Concentration of total VFA, concentrations of individual acids, and proportions of acetic, propionic and butyric acid and the sum of the branch-chained acids of the total VFA were subjected to an analysis of variance (performed with General Linear Model procedure for repeated measures of SPSS software) according to a model, which included cereal species and enzyme supplementation as within-subject factors and the effect of bird age as a repeat factor as well as interactions of these terms and terms representing the unexplainable error. For a curvilinear age effect, a quadratic polynomial contrast for age was also applied.

3 RESULTS AND DISCUSSION

Total VFA concentrations (the sum of the detected VFAs) have been presented and discussed in the earlier paper by Palander et al. (2005). The concentrations expressed in mg/g digesta varied from 1.61 in wheat at 8 weeks of age to 7.29 in oats supplemented with beta-glucanase-xylanase enzyme at 12 weeks of age and expressing a significant effect of age and enzyme supplementation. The increasing effect of enzyme supplementation on total VFA production has been mentioned also by Martinez et al. 2006, Masey-O'neill, Singh and Cowieson 2014, Kim et al. 2016 and Bedford 2018.

Table 1. Acetic acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	0,356	0,345	0,164	0,310	0,180	0,190	0,300	0,358	0,013
8 weeks	0,330	0,319	0,132	0,104	0,113	0,350	0,424	0,408	0,011
12 weeks	0,262	0,329	0,184	0,374	0,231	0,398	0,394	0,458	0,013
Statistics: n=4 for each diet*age combination									
P(age)	0,009								
P(age quadratic)	ns								
P(age*cereal*enzyme)	0,047								
P(age*cereal)	0,001								
P(age*enzyme)	ns								
P(overall ceral*enzyme)	0,014								
P(overall cereal effect)	<0,001								
P(overall enzyme effect)	<0,001								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Table 2. Proportion (percentage) of acetic acid of total volatile fatty acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	72,33	73,52	84,15	76,41	66,13	68,90	62,51	68,50	1,76
8 weeks	69,51	69,92	85,41	79,46	76,19	61,90	64,44	68,63	1,28
12 weeks	68,87	68,64	67,69	66,91	68,74	58,21	65,76	63,48	1,57
Statistics: n=4 for each diet*age combination									
P(age)	0,012								
P(age quadratic)	0,065								
P(age*cereal*enzyme)	ns								
P(age*cereal)	ns								
P(age*enzyme)	ns								
P(overall ceral*enzyme)	ns								
P(overall cereal effect)	0,001								
P(overall enzyme effect)	ns								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Concentration of acetic acid is presented in Table 1 and proportion of acetic acid of total VFA in Table 2. For all diets and ages, concentration of acetic acid was highest among VFAs, the proportion reaching from 58 to 76 per cent of the total VFAs. In the study of Kim et al. (2016), a positive effect of a xylanase enzyme supplementation on acetic acid production had been observed, but in the current study, the interacting effects complicated the interpretation of the main effects. Age of the birds, cereal species and enzyme supplementation had significantly interacting effects ($P<0,05$) on acetic acid concentration indicating divergent trends in different diets. Acetic acid concentration was highest in the oats diet supplemented with enzyme at 12 weeks of age, and in this diet, age as well as enzyme supplementation had increasing effects on acetic acid concentration. For instance for wheat, however, the age and enzyme effects were unclear or inconsistent.

For proportion of acetic acid of all VFA, significant interactions were not found. The proportion was higher in the younger birds ($P<0,05$) and generally different between cereals ($P<0,01$), being higher in wheat and lower in barley and oats. Thus, it could be interpreted, that acetic acid proportion seemed to be higher when total VFA concentration was lower.

Table 3. Propionic acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	0,0520	0,055	0,0157	0,0219	0,0245	0,0141	0,0496	0,0355	0,004
8 weeks	0,0705	0,0564	0,0075	0,0157	0,0173	0,0442	0,0650	0,0398	0,003
12 weeks	0,0394	0,0448	0,0146	0,0194	0,0245	0,0354	0,0302	0,0445	0,003
Statistics: n=4 for each diet*age combination									
P(age)	ns								
P(age quadratic)	0,064								
P(age*cereal*enzyme)	ns								
P(age*cereal)	ns								
P(age*enzyme)	ns								
P(overall ceral*enzyme)	ns								
P(overall cereal effect)	<0,001								
P(overall enzyme effect)	ns								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Table 4. Proportion (percentage) of propionic acid of total volatile fatty acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	10,95	11,37	4,77	4,70	7,36	5,16	9,52	6,83	0,78
8 weeks	14,96	12,01	3,33	6,52	9,78	7,88	10,11	6,60	0,91
12 weeks	10,31	9,27	4,66	3,30	5,04	4,86	4,87	6,14	0,53
Statistics: n=4 for each diet*age combination									
P(age)	0,064								
P(age quadratic)	0,047								
P(age*cereal*enzyme)	ns								
P(age*cereal)	ns								
P(age*enzyme)	ns								
P(overall ceral*enzyme)	ns								
P(overall cereal effect)	<0,001								
P(overall enzyme effect)	ns								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Table 5. Butyric acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	0,0745	0,0706	0,0263	0,0449	0,0719	0,0697	0,1163	0,1223	0,006
8 weeks	0,0633	0,0780	0,0180	0,0305	0,0314	0,1726	0,1532	0,1405	0,006
12 weeks	0,0683	0,0997	0,0738	0,1488	0,1008	0,2454	0,1508	0,2118	0,011
Statistics: n=4 for each diet*age combination									
P(age)	<0,001								
P(age quadratic)	0,035								
P(age*cereal*enzyme)	ns								
P(age*cereal)	0,081								
P(age*enzyme)	0,009								
P(overall ceral*enzyme)	0,007								
P(overall cereal effect)	<0,001								
P(overall enzyme effect)	<0,001								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Table 6. Proportion (percentage) of butyric acid of total volatile fatty acid concentration in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	15,17	13,27	9,63	9,41	24,52	24,61	26,65	23,37	0,87
8 weeks	13,44	16,05	9,17	11,65	18,47	30,46	23,67	23,51	1,14
12 weeks	18,10	19,54	25,49	28,51	24,62	35,56	28,12	28,30	1,36
Statistics: n=4 for each diet*age combination									
P(age)	<0,001								
P(age quadratic)	0,009								
P(age*cereal*enzyme)	0,036								
P(age*cereal)	0,027								
P(age*enzyme)	ns								
P(overall ceral*enzyme)	ns								
P(overall cereal effect)	<0,001								
P(overall enzyme effect)	ns								
M = maize, W = wheat, B = barley, O = oats, +E = supplemented with beta-glucanase-xylanase enzyme									

Concentration of propionic acid, proportion of propionic acid of total VFA, concentration of butyric acid and proportion of butyric acid of total VFA are shown in Tables 3, 4, 5 and 6, respectively. Concentrations of propionic acid were lower than those of butyric acid and were highest for propionic acid in maize and oats diets (P<0,001) and for butyric acid in oats diets (P<0,001). For butyric acid, however, significant interactions with age and enzyme (P<0,01) as well as with cereal and enzyme (P<0,01) occurred, thus complicating the interpretation of the data. Enzyme seemed to increase butyric acid production especially in older birds, and more clearly in barley and wheat than in the other cereals. The latter fact is in accordance with the findings of Jamroz et al (1996) and Palander et al. (2005), which indicated a positive correlation of VFA production and feed utilisation: Generally, enzyme supplementation affects partly by reducing viscosity of small intestinal digesta, and viscosity is more typical for wheat and barley, which contain more soluble non-starch polysaccharides (see for instance Hetland, Choct & Svihus 2004; Bedford 2018). Overall, concentrations of propionic and butyric acids seemed to follow curvilinear increasing age trends (P<0,05). The proportions of individual acids of total VFA are naturally negatively correlating with each other, and as maize produced higher proportions of propionic acid (P<0,001), the proportion of butyric acid was clearly higher in oats and barley (P<0,001). A probable explanation might be the generally different chemical composition of these cereals (barley and oats containing remarkably more fibre). The particle size after grinding was not determined in the study, but one might in addition speculate, that the particle size may differ between the ground cereals and on its behalf have an effect on the amount and type of substance, which enters to the ceaca and thus regulate fermentation.

The data regarding the sum of branch-chained VFAs is shown in Table 7. The figures were low and probably inconsistent, and typically, not all of the determined compounds were detected to remarkable extent. In addition, abnormality of residual distribution of the data seemed evident (P<0,05). Therefore, statistical comparison of the branch-chained VFA data is not presented.

Table 7. Concentration of branch-chained volatile fatty acids in caecal digesta of turkeys at different ages (mg/g digesta) (n = 4 for each diet*age combination).

Age	M	M+E	W	W+E	B	B+E	O	O+E	SEM
4 weeks	0,00309	0,00338	0,00220	0,00329	0,00301	0,00267	0,00301	0,00269	0,001
8 weeks	0,00306	0,00289	0,00211	0,00384	0,00116	0,00185	0,00240	0,00112	0,001
12 weeks	0,00405	0,00395	0,00256	0,00249	0,00139	0,00167	0,00241	0,00177	0,001

M = maize, W = wheat, B = barley, O = oats,
+E = supplemented with beta-glucanase-xylanase enzyme

On the basis of the present results in turkeys and the published literature regarding chickens, the effects of cereal species and enzyme supplementation on VFA production seem evident (Martinez et al. 2006; Kim et al. 2016). However, other diet-related or even not diet-related factors have been reported to affect caecal fermentation, such as pelleting (Engberg, Hedemann & Jensen 2002), coccide (*Eimeria*) contamination (Wu et al. 2016) or rearing system and stocking density (Li et al. 2017).

Applications and significance of caecal fermentation and VFA production may be related to intestinal health by controlling pathogen growth and necrotic enteritis. For instance, the results of Poole et al. (2003) suggested that VFA reduction could be associated with persistence of a pathogen *E. coli* strain. Li et al. (2017) have also reported changes in VFA profiles due to *Clostridium perfringens* (a typical pathogen causing necrotic enteritis) infection. In addition, *Lactobacillus acidophilus* (a probiotic) had increased concentration of butyrate in the ileum and increased lactate and valerate in the ceacum. Also Mookiah et al. (2014) reported that prebiotics and probiotics reduced pathogen growth and besides increased caecal VFA production. Han et al. (2018) have also mentioned a connection with serum immunoglobulin level and caecal VFA as a result of probiotics inclusion.

VFA figures for growing turkeys are not generally found in literature for comparison. Therefore, literature regarding broiler chickens had to be taken as reference. In future, it would be of interest to compare VFA production and VFA profiles of these species to investigate the possible differences and the effects of the longer growing period of turkeys on the results.

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