https://doi.org/10.17221/114/2017-CJAS

Effect of Freeze-dried Pasture Herbage on Ileal Digestibility of Amino Acids and Fatty Acids in Chickens

Miloš Skřivan, Milan Marounek, Michaela Englmaierová, Eva Skřivanová, Kateřina Růnová*

Department of Nutrition Physiology and Animal Product Quality, Institute of Animal Science, Prague-Uhříněves, Czech Republic

**Corresponding author: skrivan.milos@vuzv.cz*

ABSTRACT

Skřivan M., Marounek M., Englmaierová M., Skřivanová E., Růnová K. (2018): **Effect of freeze-dried pasture herbage on ileal digestibility of amino acids and fatty acids in chickens**. Czech J. Anim. Sci., 63, 222–229.

The ileal digestibility of amino acids and fatty acids in young chickens fed control diet or experimental diets supplemented with freeze-dried pasture herbage at 20 or 40 g/kg was studied. Control diet contained wheat, maize, and soybean meal. Predominant species in the pasture herbage harvested in May were *Lolium perenne*, *Festuca pratensis*, and *Trifolium pratense*. Freeze-dried pasture herbage contained less protein (169 g/kg) and fat (24 g/kg) and more neutral detergent and acid detergent fibre (340 and 210 g/kg) and tannins (12.38 g/kg) than control diet. Concentrations of amino acids, except phenylalanine and threonine, were lower in pasture herbage than in control feed. In both the control feed and freeze-dried pasture herbage, unsaturated fatty acids occurred in higher proportions than saturated fatty acids. In freeze-dried pasture herbage linolenic acid was the main fatty acid. In chickens fed freeze-dried pasture herbage the ileal digestibility of amino acids and fatty acids decreased in a dose-dependent manner. Freeze-dried pasture herbage at 4% in diet had negative effect ($P < 0.05$) on the digestibility of amino acids and fatty acids in comparison with control diet. But there was no significant effect of 2% freeze-dried pasture herbage in diet on amino acids digestibility. This suggests that pasture herbage contains anti-nutritional factors that inhibit proteolysis and lipolysis. The effect of pasture herbage on digestibility was variable. In chickens fed diets containing 4% freeze-dried pasture herbage, apparent ileal digestibility of amino acids varied from 0.39 (cysteine) to 0.91 (methionine). Variability in the ileal digestibility of fatty acids was less pronounced (from 0.65 to 0.89).

Keywords: lyophilized pasture; broilers; digestibility; fat; protein; anti-nutritional factors

Most domesticated fowl were raised on pasture by the end of the 1950s. However, putting poultry out to pasture remains a popular management method for many small flock owners. Pastured poultry represent an economic option with minimal investment for housing equipment and maintenance. Good pasture herbage is a source of vitamins, antioxidants, and essential fatty acids (Elgersma et al. 2013), as well as protein and minerals (Skrivan and Englmaierova 2014; Wencelova et al. 2016). Unlike other pasture-grazing animals, poultry cannot digest plant cell walls because they lack the enzymes to break down the fibre. Nutritionally, the value of the pasture depends on the insects and seeds that are obtained from the pasture grasses. Sun et al. (2013), who reared broilers on grassland containing a large population of grasshoppers, found that insects represented

Supported by the Ministry of Agriculture of the Czech Republic (Project No. QJ1510192 and Project No. MZERO0718).

https://doi.org/10.17221/114/2017-CJAS

74.5% of the material present in the crop, whereas plant particles were only 10.1%. Other benefits of raising poultry on pasture include improved soil fertility by working the manure into the soil and weed control.

Growing consumer demand for high-quality food has increased interest in organic poultry production. Poultry reared in organic systems do not receive in-feed antibiotics or synthetic chemicals, live under high standards of animal welfare, and must have free access to pasture herbage.

It has been shown in laying hens that dietary antioxidants are preferentially deposited in eggs, rather than in the body (Loetscher et al. 2014). This implies that the antioxidants of a pasture herbage are better used in laying hens than in broiler chickens. Nevertheless, broiler chickens also benefit from pasture. Grazing of broiler chickens improves animal welfare, reduces the need for supplementary feed, and improves qualitative parameters of meat (Grashorn 2010). Moderate levels of dehydrated pasture herbage in poultry diets improved breast skin yellowness and fatty acid composition without a major impact on broiler performance (Mourao et al. 2008). A consumer panel classified the meat from grazing broilers with greater scores for overall appreciation (Ponte et al. 2008). Skrivan et al. (2015) reported that the α-tocopherol concentration in breast meat doubled in chickens with free access to pasture herbage; moreover, the lightness and yellowness of the meat decreased, and the redness increased. The oxidative stability of the meat of pastured chickens increased. However, the pasture herbage is also the source of anti-nutritional factors such as tannins. The content of tannins varies with the growth stage and in the different organs of plants (Piluzza et al. 2014). Tannins can negatively affect the protein digestibility in monogastric animals (Garcia et al. 2004). Tannin applied at the dose of 1000 mg per kg caused negative changes in jejunal wall histology, disadvantageous microbial status of jejunum content, and a slight decrease in performance parameters in chickens (Jamroz et al. 2009).

The knowledge of the nutrition physiology of pastured poultry is incomplete. Thus, the objective of the present study was to determine the apparent ileal digestibility of amino acids and fatty acids in meat chickens fed a control feed containing wheat, maize, and soybean meal or an experimental feed supplemented with freeze-dried pasture herbage. In our previous study (Skrivanova et al. 2017), pasture herbage intake by chickens at the age of 36 days represented 2.7 g dry matter (DM)/chicken/day. Therefore, the experimental diets were supplemented with freeze-dried pasture herbage at 20 or 40 g/kg.

MATERIAL AND METHODS

Birds and diets. A total of 270 one-day-old cockerels (Ross 308) were used in this experiment. Chickens were kept in a pen on wood shavings (13.5 chickens per m^2). The room was provided with gas heating and ventilation with a temperature-controlled fan. The pen was equipped with nipple drinkers and pan feeders. The lighting programme from 1 to 7 days was 23 h light and 1 h darkness and then 16 h light and 8 h darkness. The chickens were fed the control diet (Table 1). At 28 days of age, 48 chickens with average live weight were individually relocated to balance cages and were divided into 3 groups (16 chickens per group). The first group received the control diet containing wheat, maize, soybean meal, and rapeseed oil (Table 1). The diets of two experimental groups, the second and the third group were supplemented with 20 and 40 g/kg of freeze-dried pasture herbage, respectively. The composition of the diets and freeze-dried pasture herbage is shown in Tables 1, 2, and 4. The pasture herbage came from the experimental grassland of the Institute of Animal Science (Prague-Uhříněves, Czech Republic). The pasture herbage was harvested in the half of May 2015. Predominant species were *Lolium perenne* and *Festuca pratensis* with 20% of *Trifolium pratense.* Feed and water were provided *ad libitum*. Titanium dioxide, as a dietary marker, was added to the diet at a rate of 5 g/kg at the expense of monocalcium phosphate for the determination of amino acid and fatty acid digestibility in the ileum. The chickens were fed these diets for eight days. The protocol of this experiment was approved by the Ethical Committee of the Institute of Animal Science (Prague-Uhříněves, Czech Republic).

Digesta collection. At the age of 36 days, chickens were starved for 1 h, given access to the feed for 2 h, and then immediately slaughtered by cervical dislocation. The body cavity was opened to reveal the lower gastrointestinal tract between Meckel's diverticulum and the ileocaecal junction. Ileal di-

https://doi.org/10.17221/114/2017-CJAS

gesta were collected from the distal two-thirds of ileum. Digesta were gently squeezed into a small plastic container, and the samples were immediately frozen and then freeze dried.

Analyses. The feed crude protein content was measured using a Kjeltec Auto 1030 instrument (Tecator, Sweden). The fat content in the diet was determined by extraction with petroleum ether using a Tecator Extraction System 1045 Soxtec (Tecator). Dry homogenized diets were ashed at 550°C, and the ash was dissolved in 3 M hydrochloric acid. Total P in the solution was determined using a vanadate-molybdate reagent (AOAC International 2005; method No. 965.17). The Ca concentration in the hydrochloric acid extract was measured by atomic absorption spectrometry using a Solaar M6 instrument (TJA Solutions, UK). Phytate P contents of the diets were determined by a capillary isotachophoretic method (Duskova et al. 2001). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods of Van Soest et al. (1991) using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, USA). Samples of the feeds and the ileal contents of the lower ileum (n = 16) were hydrolysed with 6 M HCl at 110°C for 23 h and analysed on an AAA ion exchange analyser (Ingos Ltd., Czech Republic). Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, after oxidation with performic acid at 0°C for 1 h. A post-column derivatization using ninhydrin was used.

The fatty acid (FA) composition of the diet and the digesta (n = 16) was determined after the

Table 1. Ingredient and analyzed composition of control diet, freeze-dried pasture herbage, and diets supplemented with freeze-dried pasture at 2% and 4% (g/kg)

Item	Control	Freeze-dried pasture herbage	Pasture herbage	
			2%	4%
Wheat	390.0		403.0	398.0
Maize	232.0		209.8	188.7
Soybean meal	305.5		305.0	310.0
Freeze-dried pasture herbage	0		20.0	40.0
Cellulose	9.0		4.0	0
Rapeseed oil	30.0		30.0	35.0
Monocalcium phosphate[1]	13.5		13.0	13.0
Limestone	10.5		10.5	10.5
Sodium chloride	2.0		2.0	2.0
DL-Methionine	1.5		1.6	1.7
L-Lysine HCl	1.0		1.1	1.1
Vitamin-trace mineral premix[2]	5		5	5
Analysed nutrient content				
Crude protein	206	169	210	200
Ca	9.6	5.1	9.6	9.5
Fat	53.6	24.0	52.9	53.1
NDF	93.2	340	104.5	118.1
ADF	22.1	210	27.4	31.6
Tannins	0.38	12.38	0.63	0.88
Non-phytate P	3.5	4.0	3.5	3.5
Calculated ME_N (M/kg)	12.6	5.4	12.6	12.6

NDF = neutral detergent fibre, ADF = acid detergent fibre, ME_N = metabolizable energy

[1]titanium dioxide, as a dietary marker, was added to the diet at a rate of 5 g/kg at the expense of monocalcium phosphate

[2]supplied per kg of diet: retinyl acetate 3.6 mg, cholecalciferol 13 µg, niacin 40 mg, α-tocopheryl acetate 50 mg, menadione 3 mg, thiamine 3 mg, riboflavin 5 mg, pyridoxine 4 mg, cyanocobalamin 40 µg, calcium pantothenate 12 mg, biotin 0.15 mg, folic acid 1.5 mg, choline chloride 250 mg, ethoxyquin 100 mg, iron 50 mg, copper 12 mg, iodine 1 mg, manganese 80 mg, zinc 60 mg, selenium 0.2 mg

https://doi.org/10.17221/114/2017-CJAS

chloroform-methanol extraction of total lipids (Folch et al. 1957). The alkaline trans-methylation of the FA was performed as described by Raes et al. (2003). The gas chromatography of the methyl esters (FAME) was performed using an HP 6890 chromatograph (Agilent Technologies, Inc., USA) with a programmed 60 m DB-23 capillary column (150–230°C) and a flame-ionisation detector; the split injections were performed using an Agilent autosampler. One-microlitre samples of FAME in hexane were injected at a 1 : 40 split ratio. The separation was achieved using the following column temperature program: initially, the column was operated at 60°C for 7 min, and then the temperature was programmed at 20°C/min to 110°C and held for 4 min, programmed at 10°C/min to 120°C and held for 4 min, programmed at 15°C/min to 170°C, programmed at 2°C/min to 210°C and held for 13.5 min, and finally programmed at 40°C/min to 230°C and held for 7 min. The fatty acids were identified by the retention times compared with the standards. The PUFA 1, PUFA 2, PUFA 3, and 37 Component FAME Mixes (Supelco, USA) were used as standards.

Tannins were determined as described in FAO/IAEA Working Document (2000). Finely ground samples were suspended in aqueous acetone (70%), subjected to ultrasonic treatment, centrifuged, and total phenols determined by Folin-Ciocalteu reagent. Standard tannic acid solution was used for calibration and total phenols were expressed as tannic acid equivalents. Tannins in the extract were removed by precipitation with polyvinyl pyrrolidone. Remaining phenol compounds were determined once more and the difference between both determinations corresponded to tannins.

Apparent ileal digestibility was calculated by comparing the ratio of nutrient and Ti in the diet and in the ileal digesta. Titanium content was measured on a UV spectrophotometer following the method of Short et al. (1996). The amino acid and fatty acid recovery (mg/g feed) represents part of dietary amino acid and fatty acid recovered in the terminal ileum and it is calculated on the basis of amino acid and fatty acid concentration in mg/g feed and ileal digestibility.

Statistical analyses. The data from the experiments were analysed using the analysis of variance (ANOVA) with the General Linear Models (GLM) procedure in the SAS software (Statistical Analysis System, Version 9.2, 2003). One-way ANOVA was used. Differences were considered significant at $P < 0.05$. The results summarized in the tables are presented as the mean and the standard error of the mean (SEM).

RESULTS

Freeze-dried pasture herbage contained less crude protein and fat but more fibre than the control diet containing wheat, maize, soybean meal, and rapeseed oil (Table 1). The metabolizable energy content in the freeze-dried pasture herbage was less than one half of that in the control feed. The concentration of tannins in freeze-dried pasture herbage, control feed, and feeds supplemented with freeze-dried pasture herbage at 2% and 4% was 12.38, 0.38, 0.63, and 0.88 g/kg, respectively. The concentrations of all the amino acids except phenylalanine and threonine were higher in the control feed than in the pasture herbage (Table 2). Glutamic acid was the main amino acid both in

Table 2. Concentration of amino acids (AA) in control diet, freeze-dried pasture herbage, and diets supplemented with freeze-dried pasture herbage at 2% and 4% (g/kg, as fed)

Amino acid	Control	Freeze-dried pasture herbage	Pasture herbage	
			2%	4%
Essential AA				
Arginine	13.8	7.5	12.4	12.1
Histidine	5.7	3.1	5.2	5.1
Isoleucine	9.6	6.4	8.6	8.4
Leucine	16.5	11.4	14.7	14.5
Lysine	14.4	8.9	13.3	12.8
Methionine	3.8	2.7	3.8	3.8
Phenylalanine	6.3	11.0	10.0	9.8
Threonine	6.8	7.8	7.0	6.9
Valine	10.8	8.1	9.6	9.6
Non-essential AA				
Alanine	9.1	8.8	8.3	8.2
Asparagic acid	22.1	12.5	19.1	18.7
Cysteine	3.8	1.7	3.1	3.0
Glycine	8.9	7.3	8.0	7.8
Glutamic acid	45.0	16.2	41.3	40.5
Proline	13.6	6.8	12.2	12.0
Serine	9.3	5.4	8.5	8.4
Tyrosine	6.0	4.2	6.1	6.0
ΣAA	205.5	129.8	191.2	187.6

https://doi.org/10.17221/114/2017-CJAS

Table 3. Coefficient of apparent ileal digestibility and recovery of amino acids (AA) at the terminal ileum (mg/g feed) of broilers fed control diet and diets supplemented with freeze-dried pasture herbage (PH) at 2% and 4%

Amino acid	AA digestibility			SEM	AA recovery			SEM
	control	PH 2%	PH 4%		control	PH 2%	PH 4%	
Essential AA								
Arginine	0.83[a]	0.80[a]	0.66[b]	0.025	11.5[a]	9.9[a]	8.0[b]	0.321
Histidine	0.78[a]	0.71[ab]	0.60[b]	0.027	4.4[a]	3.7[ab]	3.1[b]	0.203
Isoleucine	0.75[a]	0.70[a]	0.55[b]	0.030	7.2[a]	6.0[a]	4.6[b]	0.267
Leucine	0.79[a]	0.76[a]	0.62[b]	0.027	13.0[a]	11.2[a]	9.0[b]	0.407
Lysine	0.86[a]	0.83[a]	0.73[b]	0.020	12.4[a]	11.0[a]	9.3[b]	0.270
Methionine	0.95[a]	0.94[a]	0.91[b]	0.006	3.6[a]	3.6[a]	3.5[b]	0.023
Phenylalanine	0.80[a]	0.78[a]	0.67[b]	0.023	5.0[a]	7.8[a]	6.6[b]	0.198
Threonine	0.70[a]	0.62[a]	0.45[b]	0.037	4.8[a]	4.3[a]	3.1[b]	0.255
Valine	0.78[a]	0.73[a]	0.58[b]	0.030	8.4[a]	7.0[a]	5.6[b]	0.301
Non-essential AA								
Alanine	0.78[a]	0.72[a]	0.57[b]	0.030	7.1[a]	6.0[a]	4.7[b]	0.258
Asparagic acid	0.77[a]	0.72[a]	0.58[b]	0.029	17.0[a]	13.8[a]	10.8[b]	0.583
Cysteine	0.69[a]	0.54[a]	0.39[b]	0.043	2.6[a]	1.7[a]	1.2[b]	0.146
Glycine	0.74[a]	0.67[a]	0.50[b]	0.034	6.6[a]	5.4[a]	3.9[b]	0.283
Glutamic acid	0.85[a]	0.83[a]	0.72[b]	0.021	38.5[a]	34.3[a]	29.2[b]	0.893
Proline	0.81[a]	0.78[a]	0.65[b]	0.025	11.0[a]	9.5[a]	7.8[b]	0.316
Serine	0.76[a]	0.70[a]	0.54[b]	0.031	7.1[a]	6.0[a]	4.5[b]	0.364
Tyrosine	0.72[a]	0.69[a]	0.53[b]	0.030	4.3[a]	4.2[a]	3.2[b]	0.181

[a,b]values in the same row and section with different superscripts differ significantly at $P < 0.05$

the control diet and in the freeze-dried pasture herbage. The total amount of amino acids in the control feed and pasture herbage was 206 and 130 g/kg, respectively. In diets supplemented with freeze-dried pasture herbage, the apparent ileal digestibility of all amino acids was decreased in a dose-dependent manner. Freeze-dried pasture herbage (4% in diet) had negative effect ($P < 0.05$) on the digestibility of amino acids in comparison with control diet. Due to the lower digestibility of amino acids, the recovery of amino acids at the terminal ileum in broilers fed diets supplemented with pasture herbage was decreased. The effect of pasture herbage on the ileal digestibility of amino acids was variable. In broilers fed a diet supplemented with 4% freeze-dried pasture herbage the ileal digestibility of cysteine was decreased by 43.5%. The ileal digestibility of methionine in the same broilers was reduced by only 4.2%.

Both in the control diet and freeze-dried pasture herbage, unsaturated fatty acids occurred in higher proportions than saturated fatty acids (Table 4). In the control feed, oleic acid was the main fatty acid, followed by linoleic acid. In freeze-dried pasture herbage polyunsaturated fatty acids prevailed, and linolenic acid was the main fatty acid, followed by linoleic and palmitic acid. The apparent ileal digestibility of saturated fatty acids was lower than the digestibility of unsaturated fatty acids (0.67 to 0.81 vs 0.82 to 0.96). Again, in diets supplemented with freeze-dried pasture herbage, the apparent ileal digestibility of fatty acids and the recovery of fatty acids at the terminal ileum decreased in a dose-dependent manner (Table 5). A significantly ($P < 0.05$) negative effect on digestibility of the fatty acids had 4% of freeze-dried pasture herbage in diet.

DISCUSSION

The amino acid concentration in grasses and other forage crops is related to phenophase and harvest time, as confirmed by Homolka et al. (2008). The analytical determination of the nutrient content is decisive in the experiment presented here.

https://doi.org/10.17221/114/2017-CJAS

Table 4. Concentration of fatty acids (FA) in control diet, freeze-dried pasture herbage, and diets supplemented with freeze-dried pasture herbage at 2% and 4% (g/kg, as fed)

Fatty acid		Control	Freeze-dried pasture herbage	Pasture herbage	
				2%	4%
C10:0	capric	0.008	0.008	0.008	0.008
C12:0	lauric	0.018	0.226	0.027	0.224
C14:0	myristic	0.555	0.172	0.520	0.493
C16:0	palmitic	7.032	3.702	6.885	6.764
C17:0	margaric	0.191	0.045	0.189	0.178
C18:0	stearic	5.073	0.707	4.925	4.742
C20:0	arachidic	0.106	0.033	0.104	0.103
C21:0	heneicosanoic	0.005	0.004	0.005	0.005
C22:0	behenic	0.001	0.001	0.001	0.001
C24:0	lignoceric	0.017	0.009	0.018	0.017
ΣSFA		13.006	4.907	12.682	12.535
C14:1	myristoleic	0.079	0.058	0.078	0.077
C16:1	palmitoleic	0.439	0.405	0.436	0.442
C18:1	vaccenic	0.771	0.296	0.769	0.771
C18:1	oleic	16.15	1.015	16.11	16.13
C18:2	linoleic	13.30	3.52	12.99	13.19
C18:3	linolenic	3.732	12.70	4.02	4.38
C20:1	eicosenoic	0.201	0.045	0.202	0.199
C20:2	eicosadienoic	0.016	0.026	0.015	0.015
C20:5	eicosapentaenoic (EPA)	0.056	0.294	0.050	0.049
C22:6	docosahexaenoic (DHA)	0.018	0.020	0.018	0.019
ΣUFA		34.762	18.379	34.688	35.272
UFA/SFA ratio		2.67	3.75	2.74	2.86

SFA = saturated FA, UFA = unsaturated FA

The lower amount of amino acids in the freeze-dried pasture herbage than in the control feed suggests a higher amount of non-protein nitrogen. Linolenic acid followed by palmitic acid and linoleic acid were the main fatty acids in red clover (Weenink 1961). Our results are consistent with the results of Elgersma et al. (2013), who reported that linolenic acid was the main fatty acid in legume species, lucerne, and perennial ryegrass-white clover mixture.

The fact that digestibilities of amino acids and fatty acids were significantly reduced in broilers fed freeze-dried pasture herbage suggests that the pasture herbage contains some anti-nutritional factors that are able to inhibit proteolysis, such as trypsin inhibitors or tannins. Clarke and Wiseman (2005) reported that the apparent ileal digestibility of amino acids did not correlate with trypsin inhibitor activity, and the presence of tannins is therefore a more plausible explanation. Tannins are widely distributed in plants (Mangan 1988). Tannins form complexes with proteins, reduce their degradation, and increase faecal N excretion (Orlandi et al. 2015). The concentration of tannins was high in freeze-dried pasture herbage (12.38 g/kg), but low in supplemented feeds (0.63 and 0.88 g/kg). Whether tannins at this concentration inhibit intestinal enzymes remains to be determined.

Moreover, differences in digestibility of individual amino acids were noted, especially at 4% of freeze-dried pasture herbage in diet. Lower ileal digestibility of cysteine (0.39) than methionine (0.91) may be associated with greater cysteine resistance to hydrolysis.

The results of digestibility measurements are influenced by the flow of endogenous amino acids into ileum. The endogenous protein reaching the distal ileum consists primarily of biliary secretions

https://doi.org/10.17221/114/2017-CJAS

Table 5. Coefficient of apparent ileal digestibility and recovery of fatty acids (FA) at the terminal ileum (mg/g feed) of broilers fed control diet and diets supplemented with freeze-dried pasture herbage (PH) at 2% and 4%

Fatty acid	FA digestibility			SEM	FA recovery			SEM
	control	PH 2%	PH 4%		control	PH 2%	PH 4%	
Saturated FA								
Myristic	0.78[a]	0.71[a]	0.55[b]	0.023	0.43[a]	0.37[a]	0.27[b]	0.012
Palmitic	0.81[a]	0.70[b]	0.49[c]	0.018	5.70[a]	4.82[b]	3.31[c]	0.124
Margaric	0.67[a]	0.65[a]	0.47[b]	0.026	0.13[a]	0.12[a]	0.08[b]	0.005
Stearic	0.75[a]	0.69[a]	0.51[b]	0.020	3.80[a]	3.40[a]	2.42[b]	0.099
Arachidic	0.80[a]	0.71[b]	0.48[c]	0.016	0.08[a]	0.07[b]	0.05[c]	0.002
Unsaturated FA								
Myristoleic	0.87[a]	0.82[a]	0.63[b]	0.028	0.07[a]	0.06[a]	0.05[b]	0.002
Palmitoleic	0.84[a]	0.76[a]	0.65[b]	0.032	0.37[a]	0.33[a]	0.29[b]	0.014
Vaccenic	0.91[a]	0.84[b]	0.70[c]	0.021	0.70[a]	0.65[b]	0.54[c]	0.016
Oleic	0.87[a]	0.79[b]	0.76[b]	0.027	14.05[a]	12.73[b]	12.26[b]	0.436
Linoleic	0.92[a]	0.85[b]	0.71[c]	0.019	12.24[a]	11.04[b]	9.36[b]	0.369
Linolenic	0.96[a]	0.89[b]	0.67[c]	0.022	3.58[a]	3.58[b]	2.93[c]	0.088
Eicosenoic	0.82[a]	0.76[a]	0.55[b]	0.034	0.16[a]	0.15[a]	0.11[b]	0.007
Eicosapentaenoic (EPA)	0.86[a]	0.77[b]	0.61[c]	0.017	0.05[a]	0.04[b]	0.03[c]	0.001

[a–c]values in the same row and section with different superscripts differ significantly at $P < 0.05$

and mucin, which is largely resistant to proteolysis (Moughan and Schuttert 1991). In 6-week-old broilers, the endogenous flow of amino acids represented 12 g per kg of DM intake (Ravindran and Hendriks 2004).

CONCLUSION

Freeze-dried pasture herbage at 40 g/kg in diet of chickens significantly decreased the ileal digestibility of both amino acids and fatty acids. This suggests that the pasture herbage contains anti-nutritional factors such as tannins and fibre that inhibit proteolysis and lipolysis.

REFERENCES

AOAC (2005): Official Methods of Analysis of AOAC International. 18th Ed. AOAC International, Gaithersburg, USA.

Clarke E., Wiseman J. (2005): Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. Animal Feed Science and Technology, 121, 125–138.

Duskova D., Marounek M., Brezina P. (2001): Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. Journal of the Science of Food and Agriculture, 81, 36–41.

Elgersma A., Soegaard K., Jensen S.K. (2013): Fatty acids, α-tocopherol, β-carotene, and lutein contents in forage legumes, forbs, and grass-clover mixture. Journal of Agricultural and Food Chemistry, 61, 11913–11920.

FAO/IAEA (2000): Quantification of Tannins in Tree Foliage. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

Folch J., Lees M., Stanley G.H.S. (1957): A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226, 497–509.

Garcia R.G., Mendez A.A., Sartori J.R., Paz I.C.L.A., Takahashi S.E., Placia K., Komiyama C.M., Quinteiro R.R. (2004): Digestibility of feeds containing sorghum, with and without tannin, for broiler chickens submitted to three room temperatures. Brazilian Journal of Poultry Science, 6, 55–60.

Grashorn M.A. (2010): Research into poultry meat quality. British Poultry Science, 51, 60–67.

Homolka P., Koukolova V., Nemec Z., Mudrik Z., Hucko B., Sales J. (2008): Amino acid contents and intestinal digestibility of lucerne in ruminants as influenced by growth stage. Czech Journal of Animal Science, 53, 499–505.

https://doi.org/10.17221/114/2017-CJAS

Jamroz D., Wiliczkiewicz A., Skorupinska J., Orda J., Kuryszko J., Tschirch H. (2009): Effect of sweet chestnut tannin (SCT) on the performance, microbial status of intestine and histological characteristics of intestine wall in chickens. British Poultry Science, 50, 687–699.

Loetscher Y., Kreuzer M., Messikommer R.E. (2014): Late laying hens deposit dietary antioxidants preferentially in the egg and not in the body. The Journal of Applied Poultry Research, 23, 647–660.

Mangan J.L. (1988): Nutritional effects of tannins in animal feeds. Nutritional Research Reviews, 1, 209–231.

Moughan P.J., Schuttert G. (1991): Composition of nitrogen-containing fractions in digesta from the distal ileum of pigs fed a protein-free diet. The Journal of Nutrition, 121, 1570–1574.

Mourao J.L., Pinheiro V.M., Prates J.A.M., Bessa R.J.B., Ferreira L.M.A., Fontes C.M.G.A., Ponte P.I.P. (2008): Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poultry Science, 87, 733–743.

Orlandi T., Kozlovski G.V., Alves T.P., Mesquita F.R., Avila S.C. (2015): Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of Acacia mearnsii tannin extract. Animal Feed Science and Technology, 210, 37–45.

Piluzza G., Sulas L., Bullitta S. (2014): Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. Grass and Forage Science, 69, 32–48.

Ponte P.I.P., Rosado C.M.C., Cresco J.P., Crespo D.G., Mourao J.L., Chaveiro-Soares M.A., Bras J.L.A., Mendes I., Gama L.T., Prates J.A.M., Ferreira L.M.A., Fontes C.M.G.A. (2008): Pasture intake improves the performance and meat sensory attributes of free-range broilers. Poultry Science, 87, 71–79.

Raes K., De Smet S., Balcaen A., Claeys E., Demeyer D. (2003): Effect of diets rich in N-3 polyunsaturated fatty acids in Belgian Blue double-muscled young bulls. Reproduction Nutrition Development, 43, 331–345.

Ravindran V., Hendriks W.H. (2004): Endogenous amino acid flows at the terminal ileum of broilers, layers and adult roosters. Animal Science, 79, 265–271.

Short F.J., Gorton P., Wiseman J., Boorman K.N. (1996): Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59, 215–221.

Skrivan M., Englmaierova M. (2014): The deposition of carotenoids and α-tocopherol in hen eggs produced under a combination of sequential feeding and grazing. Animal Feed Science and Technology, 190, 79–86.

Skrivan M., Pickinpaugh S.H., Pavlu V., Skrivanova E., Englmaierova M. (2015): A mobile system for rearing meat chickens on pasture. Czech Journal of Animal Science, 60, 52–59.

Skrivanova V., Tumova E., Englmaierova M., Chodova D., Skrivan M. (2017): Do rearing system and free-range stocking density affect meat quality of chickens fed feed mixture with rapeseed oil? Czech Journal of Animal Science, 62, 141–149.

Sun T., Long R.J., Liu Z.Y. (2013): The effect of a diet containing grasshoppers and access to free-range on carcase and meat physiochemical and sensory characteristics in broilers. British Poultry Science, 54, 130–137.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Weenink R.O. (1961): Acetone-soluble lipids of grasses and other forage plants. I.-Galactolipids of red clover (Trifolium pratense) leaves. Journal of the Science of Food and Agriculture, 12, 34–38.

Wencelova M., Varadyova Z., Pristas P., Cobanova K., Placha I., Kisidayova S. (2016): Effects of diet supplementation with herbal blend and sunflower seeds on fermentation parameters, microbial population, and fatty acid profile in rumen of sheep. Czech Journal of Animal Science, 61, 551–559.

Received: 2017–11–21
Accepted after corrections: 2018–04–19