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EFFECT OF SWEET CHESTNUT EXTRACT (FARMATAN®) ON THE DIGESTIBILITY AND BIOAVAILABILITY OF NITROGEN AND SOME MINERALS IN THE LABORATORY RATS

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Effect of sweet chestnut extract (Farmatan®) on the digestibility and bioavailability of nitrogen and some minerals in the laboratory rats

Study was conducted to investigate the dietary effect of hydrolysable tannin from sweet chestnut on the protein digestibility and bioavailability, dry matter and organic matter apparent digestibility and apparent digestibility and apparent bioavailability of some mineral elements. Ten male Wistar rats (98.9 g ± 25.6 g of body mass) were fed ad libitum with balanced diets. In the experimental diet 0.1 % of sweet chestnut extract (SCE) was added to the diet. Five days balance experiment takes place after fifteen days of adaptive period. SCE did not have any influence on the protein quality measurements or dry matter and organic matter apparent digestibility. Tannin significantly ($p < 0.05$) decreased the digestibility and bioavailability of Ca and Fe. The digestibility of Na was unchanged (97.76 % and 97.31 % in control and SCE group, respectively), but bioavailability significantly decreased in SCE group (53.16 %) as compared to the control group (74.17 %). On the contrary, the apparent digestibility of Se significantly increased, (64.25 %) in SCE group compared to the control group (52.31 %).

Key words: animal physiology; digestibility; bioavailability; protein; mineral elements; tannin; laboratory rats

Vpliv kostanjevega ekstrakta (Farmatan®) na prebavljivost in izkoristljivost dušika in nekaterih mineralov pri laboratorijskih podganah

V raziskavi smo proučevali učinek hidrolizirajočih taninov iz kostanja na prebavljivost in neto izkoristljivost beljakovin, navidezno prebavljivost suhe in organske snovi ter navidezno prebavljivost in izkoristljivost nekaterih mineralov. V poskus je bilo vključenih deset Wistar podgan moškega spola (98,9 g ± 25,6 g telesne mase), ki so dobivale uravnoteženo krmo po volji. V poskusno krmno mešanico smo dodali 0,1 % kostanjevega ekstrakta. Najprej je bilo 15 dnevno predposkusno obdobje, ki mu je sledil petdnevni bilančni poskus. Kostanjev ekstrakt ni imel vpliva na izmerjene parametre kakovosti beljakovin ali navidezno prebavljivost suhe in organske snovi. Tanin je statistično značilno ($p < 0,05$) zmanjšal navidezno prebavljivost in navidezno izkoristljivost Ca in Fe. Navidezna prebavljivost Na je bila podobna (97,76 % in 97,31 % v kontrolni oz. poskusni skupini). Navidezna izkoristljivost Na je bila statistično značilno manjša v poskusni skupini (53,16 %) v primerjavi s kontrolno skupino (74,17 %). Nasprotno je bila navidezna prebavljivost Se statistično značilno boljša v poskusni skupini (64,52 %) v primerjavi s kontrolno skupino (52,31 %).

Ključne besede: fiziologija živali; prebavljivost; izkoristljivost; beljakovine; minerali; tanin; laboratorijske podgane

1 INTRODUCTION

Tannins – the water-soluble polyphenolic compounds are present in numerous feeds such as fodder legumes, browse leaves, rape and their by-products, sunflower seeds, sorghum and fruits, used in animal nutrition, but also served as human foods. They are known to have two characteristics: they depress the protein digestibility (Mariscal-Landín et al., 2002, 2004) and exert a beneficial influence in the alimentary canal through a modulating activity on the gut microflora, due to their toxic effect to pathogenic parasites (Voravuthikunchai et al., 2004; Voravuthikunchai and Kitpipit, 2005).

The wood of sweet chestnut contains hydrolysable tannins, which is particularly rich in esters of gallic acid with monosaccharides. Hydrolysable tannins can inter-

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act with proteins of saliva and feed, with sugars (Naurato et al., 1999), metals and others macromolecular substances (Barroga et al., 1985). The complexes – bonds of hydrolysable tannins with proteins, are less stable at low or high pH (< 3.0 and > 8.5), in comparison to condensed tannins. The binding of endogenous enzymes with tannins result in lowered digestibility of proteins (Charlton et al., 1996). Entering the digestive tract the tannins may form a thin film of insoluble, denaturized proteins covering the surface of mucous membrane of intestinal wall (Sell et al., 1985; Scalbert, 1991) and the thickness of created layer depends on dose of administrated tannin in the diet (Zhao et al., 2001; Yoshimura et al., 2007). This phenomenon can explain both protective and inhibitory functions of tannins.

The present study was undertaken with the purpose to determine the apparent digestibility of diet dry matter, organic matter and nitrogen and the amount of retained nitrogen, as well as the apparent digestibility of some mineral elements and apparent bioavailability of them.

2 MATERIAL AND METHODS

2.1 DIETS

Two diets were prepared, a control diet and a sweet chestnut extract (SCE) diet in which a fraction (1 g per kg) of wheat starch was replaced with SCE (Farmatan®). The protein source of diets was soybean meal, which is the main protein source of feedstuffs for monogastric animals. Diets contain 120 g of crude protein per kg. Both diets (Table 1) were designed to meet the nutrition requirements of growing rats (NRC, 1995). Weende analysis and the content of some minerals in the diets were performed.

2.2 ANIMALS AND EXPERIMENTAL PROCEDURE

All procedures were performed according to current legislation on animal experimentation in Slovenia. Permission for the experiment was granted by the Veterinary Administration of the Republic of Slovenia (VURS), under number 34401-57/2007/4. Ten male Wistar rats (98.9 g ± 25.6 g of body weight) were housed in individual balance cages placed in a room kept at about 21 °C and 55 % humidity (checked and recorded each day), with light automatically regulated on a 12-hours light/dark cycle starting at 7.00 a.m. Balance cages permit the collection of urine and faeces separately during the experiment. Rats have free access to drinking water and control or experimental diet. On the first day of the experiment were separated into two equal groups (n = 5). After the adaptation period (15 days) the 5 days balance experiment takes place.

Each day of the balance experiment, each animal received a new weighed daily meal and the residue from the day before was weighed. Body masses were recorded on the first day of the balance experiment, on the third day and on the last, fifth, day. Urine was collected in a bottle after filtration. This bottle contained 10 ml of 6 M HCl (decrease pH) for each cage to stop all reactions in the urine and to prevent losses of nitrogen. The faeces samples were collected in a different vessel. On the last day of the balance study, the urine was transferred to a prepared plastic bottle, weighed and stored at –20 °C until analyses were performed. Faeces samples were also stored in prepared plastic bags, weighed and frozen.

Before taking an aliquot of the sample of faeces for analysis, it was homogenized in a ceramic holder. Urine was homogenized by shaking to prevent stratification. In diets, faeces and urine nitrogen was determined by Kjeldahl method. Crude protein

***Table 1:** Composition of experimental diets (g/kg)*
***Preglednica 1:** Sestava krmnih mešanic (g/kg)*

	Control Kontrola	Sweet chestnut extract Kostanjev ekstrakt 0.1 %
Sweet chestnut extract / ekstrakt kostanja		1.0
Soybean meal / sojine tropine	220.0	220.0
Methionine / metionin	5.0	5.0
Wheat starch / pšenični škrob	649.0	648.0
Sunflower oil / sončnično olje	50.0	50.0
Cellulose / celuloza	50.0	50.0
Mono Ca phosphate / mono kalcijev fosfat	6.0	6.0
$CaCO_3$ / apnenec	9.0	9.0
NaCl / sol	1.0	1.0
Premix / premiks*	10.0	10.0
Sum / vsota	1000	1000

* Premix (mineral vitamin mixture) were prepared in Lek Veterina by our recipe / premiks (mineralno vitaminska mešanica) so nam pripravili v Lek Veterina po naši recepturi.

Table 2: *Chemical composition of experimental diets (g/kg DM)*
Preglednica 2: *Kemijska sestava krmnih mešanic (g/kg SS)*

	Control Kontrola	Sweet chestnut extract Kostanjev ekstrakt
Dry matter / suha snov (g/kg)	901.23	900.71
Crude protein / surove beljakovine	120.37	120.13
Crude fat / surova maščoba	59.68	59.45
Crude fiber / surova vlaknina	48.28	48.51
Crude ash / surov pepel	35.70	35.74
Non nitrogen extractives / brezdušični izvleček	735.97	736.18
Mineral elements / minerali		
P	3.40	3.61
Ca	10.79	7.77
Mg	0.97	0.94
K	5.44	5.44
Na	0.57	0.60
Zn (mg/kg DM) / (mg/kg SS)	21.42	22.42
Fe (mg/kg DM) / (mg/kg SS)	91.36	80.23
Cu (mg/kg DM) / (mg/kg SS)	11.88	12.00
Se (μg/kg DM) / (μg/kg SS)	133.95	131.82

(CP) was evaluated as N * 6.25. Dry matter and crude ash were determined in both diets and samples of faeces. Dry matter by placing a certain weight of diet or faeces in an oven at 105 °C to dry until a constant weight was reached. Crude ash content was determined by placing a certain amount of sample in a muffle furnace at 550 °C for ashing, followed by determination of the final weight. Mineral elements (Ca, P, Mg, K, Na, Zn, Cu and Fe) were determined in the diets and faeces, after ashing and preparation of an acid extract, by atomic absorption spectrometry. In urine, mineral elements were determined after microwave digestion (Milestone microwave laboratory systems). 2.5 g of homogenized sample of urine was placed in a vessel and 8 ml of 65 % HNO_3 added. The plate with vessels was placed in the microwave cavity, the temperature sensor connected and the program run. After completion of the program, the solution was cooled to room temperature and transferred to a marked flask. The different mineral elements (Ca, P, Mg, K, Na, Zn, Cu and Fe) concentrations in the clear solution were determined by atomic absorption spectrometry.

2.3 DATA ANALYSIS

Data were analyzed by the General Linear Models (GLM) procedure (SAS/STAT, 2000), taking into consideration the diet as the main effect. Data are expressed as

Table 3: *Effect of sweet chestnut extract on body mass, diet intake and growth rate (average ± standard deviation) (5 days balance experiment)*
Preglednica 3: *Vpliv kostanjevega ekstrakta na telesno maso, zauživanje krme in prirast (povpečje ± standardna deviacija) (bilančni poskus – 5 dni)*

	Control Kontrola (5)	Sweet chestnut extract Kostanjev ekstrakt (5)	*p*-value *p*-rednost
Initial body mass / telesna masa na začetku poskusa (g)	189.3 ± 31.6	188.6 ± 25.4	0.9693
Final body mass / telesna masa na koncu poskusa (g)	220.0 ± 30.6	221.3 ± 26.5	0.9419
Diet intake (g /day) / zaužita krma (g/dan)	19.8 ± 1.9	20.4 ± 1.4	0.5716
Growth rate (g/day) / prirast (g/dan)	6.1 ± 0.5	6.6 ± 0.5	0.2409
g diet per g growth rate / g krme za g prirasta (g/g)	3.26 ± 0.57	3.12 ± 0.12	0.6027

Table 4: *Excreted faeces and urine, consumed and excreted nitrogen and nitrogen balance (average ± standard deviation)*
Preglednica 4: *Izločeno blato in seč, zaužit in izločen dušik ter bilanca dušika (povprečje ± standardna deviacija)*

	Control Kontrola (5)	Sweet chestnut extract Kostanjev ekstrakt (5)	*p*-value *p*-vrednost
Consumed N (mg/5 day) / zaužit N (mg/5 dni)	1720 ± 160	1768 ± 119	0.6045
Excreted faeces (g/5 day) / izločeno blato (g/5 dni)	17.75± 2.13	18.68 ± 2.20	0.5176
N in faeces (mg/5 day) / N v blatu (mg/5 dni)	272 ± 20	263 ± 30	0.6207
Excreted urine (g/5 day) / izločen seč (g/5 dni)	67.2 ± 25.6	89.4 ± 22.4	0.1826
N in urine (mg/5 day) / N v seču (mg/5 dni)	435 ± 99	388 ± 40	0.3553
Nitrogen balance (mg/5 day) / bilanca dušika (mg/5 dni)	1318 ± 115	1422 ± 121	0.2016

least square means (LSM) ± standard deviation (SD). Significance was considered established at $p < 0.05$.

3 RESULTS

In the chemical composition of both diets there are no significant differences (Table 2), which was expected since only 1 g of SCE was substituted with wheat starch in the experimental diet. There was a small variation in calcium and iron content, but the content of all minerals was within the frontier of the recommendations (NRC, 1995).

There was no effect of tannin on the rat's growth performance (body mass and diet intake). It was shown a slightly better, but not significant ($p = 0.2409$) average growth rate in the SCE group as compared to control (Table 3).

In the parameters of nitrogen balance no significant differences among the SCE group and control group were detected (Table 4). The favourable result is less nitrogen excreted in urine of SCE group as compared to control, which means that, when nitrogen (amino acids) is absorbed through the intestinal wall it is more applicable, than from the control group. The nitrogen balance was better in SCE group, since the nitrogen excreted through faeces was also slightly decreased as compared to control group.

The results of protein digestibility, protein biological value, net protein utilization and protein efficiency ratio (Table 5), and dry matter and organic matter apparent digestibility (Table 6) were slightly better in SCE group as compared to the control group, but the differences were not significant.

But there was more crude ash excreted in faeces of SCE group (67.1 mg/g faeces ± 6.4 g) as compared to the control (60.2 mg/g faeces ± 4.4 g), the difference was in the line of significance ($p = 0.0793$). The result of more ash in the faeces of SCE is significantly lower apparent digestibility of calcium (Ca) ($p < 0.0001$) and iron (Fe) ($p = 0.0028$), on the contrary selenium (Se) apparent digestibility was significantly increased in SCE group ($p = 0.0028$). On the digestibility of the other minerals, the addition of SCE in a diet had no influence (Fig. 1). Apparent bioavailability (Fig. 2) of Ca and Fe was also significantly decreased in SCE group as compared to

Table 5: *Apparent and true protein digestibility, protein biological value, net protein utilization and PER (average ± standard deviation)*
Preglednica 5: *Navidezna in prava prebavljivost beljakovin, biološka vrednost beljakovin, neto izkoristljivost beljakovin in PER vrednost (povprečje ± standardna deviacija)*

	Control Kontrola (5)	Sweet chestnut extract Kostanjev ekstrakt (5)	*p*-value *p*-rednost
Apparent protein digestibility (%) Navidezna prebavljivost beljakovin (%)	84.17 ± 0.59	85.09 ± 1.44	0.2208
True protein digestibility (%) Prava prebavljivost beljakovin (%)	89.85 ± 0.58	90.64 ± 1.62	0.3394
Protein biological value (%) Biološka vrednost beljakovin (%)	85.4 ± 4.1	88.7 ± 2.3	0.1569
Net protein utilization (%) Neto izkoristljivost beljakovin (%)	76.7 ± 3.6	80.4 ± 3.4	0.1329
PER (g growth rate/1g consumed CP) PER (g prirasta/1g zaužitih SB)	2.88 ± 0.42	2.96 ± 0.11	0.6870

***Table 6:** Apparent digestibility (AP) of dry matter and organic matter (average ± standard deviation)*
***Preglednica 6:** Navidezna prebavljivost suhe snovi in organske snovi (povprečje ± standardna deviacija)*

	Control Kontrola (5)	Sweet chestnut extract Kostanjev ekstrakt (5)	*p*-value *p*-vrednost
Consumed dry matter (mg/5 day) Zaužita suha snov (mg/5 dni)	89.29 ± 8.33	91.98 ± 6.20	0.5789
Excreted dry matter in faeces (mg/5 day) Izločena suha snov z blatom (mg/5 dni)	9.57 ± 1.00	9.68 ± 0.54	0.8370
AD of dry matter (%) NP suhe snovi (%)	89.3 ± 0.2	89.5 ± 0.6	0.5596
Consumed organic matter (mg/5 day) Zaužita organska snov (mg/5 dni)	86.11 ± 8.04	88.69 ± 5.98	0.5793
Excreted organic matter in faeces (mg/5 day) Izločena organska snov z blatom (mg/5 dni)	8.50 ± 0.83	8.43 ± 0.50	0.8861
AD of organic matter (%) NP organske snovi (%)	90.1 ± 0.1	90.5 ± 0.6	0.2340

* Premix (mineral vitamin mixture) were prepared in Lek Veterina by our recipe / premiks (mineralno vitaminska mešanica) so nam pripravili v Lek Veterina po naši recepturi.

the control group. Sodium (Na) apparent bioavailability significantly decreased ($p = 0.0006$), even that apparent digestibility (Fig. 1) was similar in both groups, almost all Na was absorbed through the intestinal wall (97.7 and 97.3 % in control and SCE, respectively), but there was pretty high level of Na in urine, although some less in SCE group, where apparent bioavailability was significantly better (Fig. 2). There was also high level of Mg and K excreted in urine, however similar in both groups (Fig. 2).

4 DISCUSSION

Tannins can have a positive role in ruminant and also non-ruminant nutrition. The objective of adding tannins is to increase the supply of amino acids without prevent-

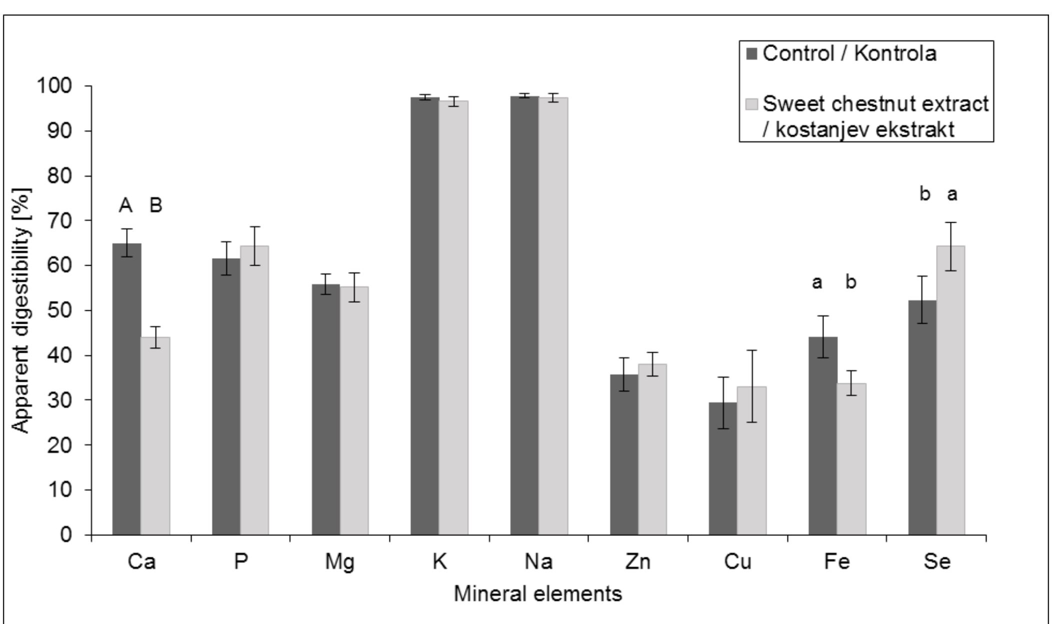

***Figure 1:** Apparent digestibility (%) of some mineral elements; bars with different letter differ significantly: A, B (p < 0.001); a, b (p < 0.05)*
***Slika 1:** Navidezna prebavljivost (%) nekaterih mineralov; stolpci označeni z različnima črkama se med seboj statistično razlikujejo: A, B (p <0,001); a, b (p < 0,05)*

ing hydrolysis of dietary proteins in the abomasums and intestine. But in the monogastric animals orally administration of hydrolysable tannins can cause many disorders in digestive tract such as hypersecretion of mucin out of the mucosa of the stomach and duodenum, thickening of the wall of the crop, necrosis, etc (Mueller-Harvey, 2006). But the question is what the level of tannins in the diet is and what the nature of the tannins is, condensed or hydrolysable, which have different effect.

From the studies in pig (Mariscal-Landín et al., 2004; Antongiovanni et al., 2007) it was expected the depression of nitrogen or dry matter digestibility. However, it depends on the level of tannins in the diet, 0.5 % show significant difference, but 0.25 % addition was too small for significance (Antongiovanni et al., 2007). Mariscal-Landín et al., (2004) also suggested that the nitrogen digestibility depends more on the protein profile of the diet then on the level of tannins using different sorghum samples with up to 1 % of tannin per kg diet. In chicken Jamroz et al. (2009) didn't found statistical difference in body mass or feed conversion by adding sweet chestnut tannins in the diet (0.25, 0.5 and 1 %), although the microbial status of small intestine and colon was changed with the application of tannin and the largest dose also cause the negative changes in jejunum wall histological picture. The fact is that in those studies the level of tannin in a diet was higher as in our study, 0.1 % of SCE (Farmatan®).

The use of natural extract of chestnut wood (0.15, 0.20 and 0.25 %) didn't have influence on the digestibility or nitrogen balance. The use of up to 0.20 % has even a positive effect on a growth performance, especially in young broiler chicks (Schiavone et al., 2008), which is in accordance with our results on laboratory rats, where the growth rate was slightly better (Table 3) in SCE group ($p = 0.2409$) as compared to the control. Sell et al. (1985) obtained the opposite result, where dry matter digestibility significantly decreased in the groups with high tannin (condensed) sorghum added to the diet for rats. They also observed a slight elevation of mucin secretion, but no histopathological lesions. Tamir and Alumot (1970) added carob extracts in a diet for rats. The hot water extract of green carobs contain hydrolysable tannins, which were added in the level of 4 % in a diet and there was no significant effect on the growth or nitrogen excretion, but tannins from ripe carobs (condensed tannins) caused a highly significant growth depression. It is very important what kind of tannins is added in a diet, not only the level. In a study of Shahkhalili et al. (1990) the tannins from cocoa have no net effect on nitrogen excretion through faeces, the tea and carob tannins have marked effect. This study suggests that diets rich in polyphenols (tannins) from varying sources influence faecal N excretion to varying extents. The direct comparison of the effect of different diet (or study) with tannins on the basis of the level of tannins should be interpreted with caution, since the

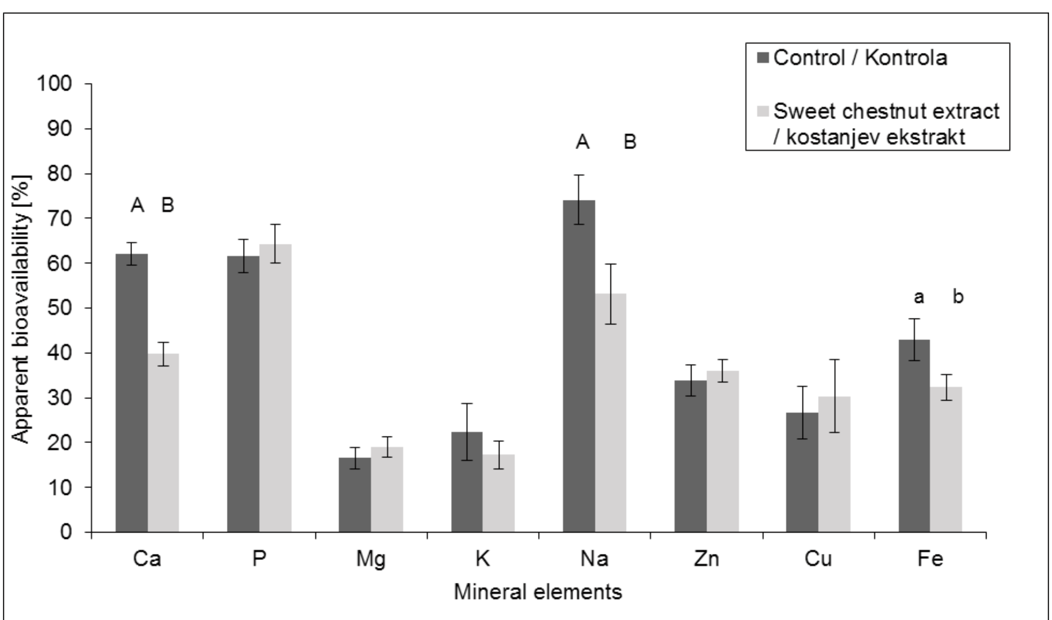

Figure 2: *Apparent bioavailability (%) of some mineral elements bars with different letter differ significantly: A, B ($p < 0.001$); a, b ($p < 0.01$)*
Slika 2: *Navidezna izkoristljivost (%) nekaterih mineralov; stolpci označeni z različnima črkama se med seboj statistično razlikujeta: A, B (p <0,001); a, b (p < 0,01)*

tannins are determined by different methods and there is a possibility that not all the tannins were extractable by one or another method and the condensed and hydrolysable tannin act different. The complexity and variability among experimental condition make it difficult to compare (Piluzza et al., 2013).

Bioavailability of trace minerals can be influenced by various dietary compounds, which include both inhibitors and enhancers of absorption. Tannins are known to form insoluble complex with divalent metal ions such as iron, regarding them less available for absorption, as is also our result (Fig. 1). Tannins supposed to be responsible for low availability of iron in legume seeds in rats (Rao and Prabhavathi, 1982, Garcia-Lopez et al., 1990) and high iron-binding capacity of extracts from seed coats of coloured-flowering varieties, but not white flowering varieties of faba bean (Griffiths, 1982). Presence of 0.4 % of polyphenolic extracts (green tea, chokeberry or honeysuckle) in the perfusion liquids significantly reduced the absorption of minerals (Ca, P, Mg, Na and K) from small intestine of rats, mainly due to depression of the water absorption. In the nutritional experiment with the same amount and source of polyphenolic extract the apparent digestibility of Zn and Cu decreased, but not of the other minerals and in spite of all that the mineral concentration of bone was not affected (Frejnagel and Wroblewska, 2010). In rabbits low (1.4 % catechin equivalent) or high (3.5 % catechin equivalent) sorghum tannin diets decreased calcium apparent absorption, but not magnesium (Al-Mamary et al., 2001). Those were condensed tannins, which in a great deal inhibit the digestive enzyme activities and high dose depressed growth rate and increase diet consumption. Tannic acid in the level 5 g, 10 g, 15 g or 20 g per kg of diet for rats reduce the Fe absorption, but not the Zn, Cu and Mn absorption (Afsana et al., 2004), which is in accordance with our results. Numerous dietary and host factors interact to affect the bioavailability of mineral nutrients. There are significant differences between monogastric animals in their sensitivity to antinutritional factors, such as proteinase inhibitors or lectins (Huisman et al., 1990). It may be assumed that such differences also exist with regarding to polyphenol compounds. The attention should be paid to the adaptive response of animals to dietary tannins. Rats and mice also show a specific adaptive response by increasing secretion of prolin rich protein by the parotid gland when tannins are present in the diet (Jansman, 1993). It is very likely that relatively small differences in protein or apparent mineral digestibility and bioavailability between control and SCE group were in the adaptive response of rats to the tannin.

To our knowledge, there was no publication of increased digestibility of Se after the addition of tannin in the diet. It is most likely that Se could be changed from one to another species during digestion or it form insoluble complex with tannin during passage through digestive tract and it was not detected in the lab. Cuderman and Stibilj (2010) found decreased Se(IV) response during storage, after 4 days only 7 % of Se(IV) was present in the water extract and enzymatic hydrolysis caused almost 50 % depression. It is most likely that Se reacted with matrix components (tannin) and this could result in a misidentifications and inaccurate determined values, which could be the real reason for low determined value for Se in faeces.

5 CONCLUSIONS

Addition of 0.1 % of sweet chestnut extract did not depressed diet intake or growth rate, on the contrary, the values were slightly increased. SCE had no influence on the protein quality measurements, dry matter or organic matter digestibility. The apparent digestibility of macro minerals was in average higher as compared to micro minerals, with significant depression of Ca and Fe apparent digestibility in SCE group. More than 95 % of K and Na was absorbed in both groups, but around 80 % of K was excreted through urine in both groups. Apparent bioavailability of Ca, Na and Fe was significantly decreased in SCE group. Surprising the apparent digestibility of Se increased after the addition of SCE, but likely untrue. Further studies are necessary to investigate what really occurs in nitrogen and mineral metabolism after the tannin addition.

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