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PROTEIN VALUE OF PUMPKIN SEED CAKES IN RUMINANT NUTRITION

BJELANČEVINASTA VRIJEDNOST POGAČA SJEMENKI BUNDEVE ZA PREŽIVAČE

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SUMMARY

Chemical composition, in sacco protein degradability and in vitro protein digestibility (HCl-pepsin) of 14 samples of pumpkin seed cakes (PSC) was been examined. The concentrations of crude protein and crude fat varied from 546 to 658 and from 87 to 201 g/kg DM respectively. The concentrations of NDF, ADF and ADL varied from 88 to 416, from 49 to 137 and from 7 to 55 g/kg DM. The average effective protein degradability (EPD) in the rumen was 60.3% and varied from 46.8 to 84.1%. EPD was negatively correlated with NDF ($r=-0.63$, $P<0.05$). Strong correlation was also found between EPD and parameters of CIE (Commission Internationale de l'Eclairage) colour system. EPD was negatively related to CIE colour coordinate ($r=-0.70$, $P<0.005$) and positively to lightness L* variable ($r=0.60$, $P<0.05$) of PSC. PSC in dark brown colour had lower EPD than PSC in greenish-yellow colour. According to the shape of degradation curves, PSC can be divided into two groups. The first one, comprising PSC with lower EPD, was represented by a linear degradation curve and the second one, comprising PSC with higher degradability, by exponential one. The in vitro protein digestibility was high (from 94.8 to 97.4%) and correlated with the concentrations of ADF ($r=-0.80$, $P<0.001$) and ADL ($r=-0.74$, $P<0.01$). The concentrations of digestible undegraded dietary protein, which was calculated as a difference between effectively undegradable and in vitro indigestible protein, varied from 82 to 282 g/kg DM. It is concluded that differences which were probably due to time and temperature of roasting markedly affected the protein value of PSC for ruminants.

INTRODUCTION

The use of feedstuffs of animal origin in diets for ruminants has recently been banned in all European countries. Feedstuffs of animal origin, especially fish meal, which represented an important source of rumen undegradable protein, have to be replaced by alternative food of low protein degradability. Most protein rich supplements

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of plant origin like soybean meal, rapeseed meal and sunflower meal are characterised by relatively high protein degradability in the rumen. It has been found that protein degradability of soybean meal (Faldet et al., 1991; Mosimanyana and Mowat, 1992; Ljokjel et al., 2000) or rapeseed meal (Sommer et al., 1994; Dakowski et al., 1996) can be decreased by the heat treatment. Alternatively, solutions can possibly be found also among feed which is heat treated per se. Pumpkin seed cake is one of them.

Pumpkin seed cakes (PSC) are the by-product of pumpkin oil industry. In Slovenia, about 500 to 700 t of pumpkin seeds, about 70% of seeds without hulls (Cucurbita Pepo var. Styriaca) and about 30 % of those with hulls (Cucurbita Pepo) are processed yearly. More than one half of oil is produced by local oil-mills which do not have uniform technology of processing. Differences are mainly in grinding, temperature and duration of roasting and partly in squeezing.

Pumpkin seed cakes contain more than 50% of crude protein in dry matter (DLG, 1982; Wöhlbier and Jager, 1983). Crude protein concentration is affected mainly by the proportion of hulls. It has been established that seeds and oil seed cakes from Cucurbitae have relatively high protein digestibility (80 to 86%, Pal and Mahadevan, 1968; DLG, 1982; Wöhlbier and Jager, 1983). Pumpkin seed cakes have good flavour and therefore improve palatability of concentrates for ruminants. As far as we know, there is no information on protein degradability of PSC in the rumen. The aim of the present work has been to investigate rumen degradability and the enzymatic in vitro protein digestibility of PSC from different sources and factors which affect it.

MATERIAL AND METHODS

Samples of PSC (n=14) were provided by two big and ten smaller oil-mills from north-eastern Slovenia. Comparatively, one sample of soybean meal was included in experiment. Samples for chemical analyses and enzymatic digestibility were ground through a 1 mm screen and samples for determination of in sacco degradability through a 5 mm screen using laboratory hammer mill.

Crude protein (CP) was determined by the method of Kjeldahl and crude fat (fat) by extraction with tetrachloroethylene on Fosslet. Concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970) and enzymatic (HCl-pepsin) *in vitro* protein digestibility as described by Naumann et al. (1976).

Pumpkin seed cakes were visually ranged regarding their colour. The PSC in light yellow colour was given number 1 and the PSC in dark brown colour number 14. The colour was also measured by the use of Chroma Meter (Minolta 200 B). According to CIE (Commission Internationale de l'Eclairage), $L^*a^*b^*$ colour system was used in which L^* represents lightness variable, a^* the chromaticity coordinate between red and green colour and b^* chromaticity coordinate between blue and yellow colour.

Protein degradation was done by the in sacco method (Ørskov et al., 1980) using two fistulated non lactating Simmental cows. They were given meadow hay ad libitum and two kg of concentrate per day. Nylon bags (10x14 cm) of nylon cloth LT075 (Locker Wire Weavers, PO Box 161, Warrington WA1 25U, 45 µm pore size) were heat-sealed. About 6 g of sample was weighed in each bag and incubated in the rumen for 4, 8, 16, 24, and 48 hours. 4, 8 and 16 h incubations were done in two, 24 and 48 h incubations in four replicas. After the incubation, the bags were washed in domestic washing machine and dried at 60°C to constant weight. Samples from replicas were pooled before the CP determination.

In soybean meal and PSC with exponential degradation curve, data were fitted to the equation $p=a+b(1-e^{-ct})$ as described by Ørskov and McDonald (1979). In the equation mentioned above p represents CP disappearance from the bag at time t, a is soluble protein fraction, b is insoluble potentially degradable fraction and c is degradation rate of fraction b. Effective protein degradability (EPD) was calculated on the basis of degradation characteristics (a, b, c) and theoretical outflow rate $k=0.05\ h^{-1}$ as EPD = a+b+c/(c+k) (Ørskov and McDonald, 1979).

In PSC with linear protein degradation in the rumen, data were fitted to the equation p=a+Ct. The

coefficient C represents linear degradation rate of protein in the rumen and the meaning of p and a is the same as before. In this case the EPD was calculated as

$$EPD=\sum_{i=0}^{n}[p(t_{i+1})-p(t_i)]\,[f(t_i,t_{i+1})]$$

according to Kristensen et al. (1982). Value p is CP disappearance from the nylon bags at time ti and $f(t_i, t_{i+1})$ is the average proportion of protein which remains in the rumen until the period between t_i and t_{i+1} ie. $f(t_i, t_{i+1})=(e^{-kti}+e^{-kti+1})/2$.

In vitro organic matter digestibility (IVOMD) was estimated on the basis of gas production (GP ml/200 mg DM), CP (g/kg DM) and ash (g/kg DM) concentration according to Menke and Steingass (1987):

IVOMD=0.9991 GP+0.0595 CP+0.0181 Ash + 9.00

The net energy for lactation (MJ NEL/kg DM) was estimated on the basis of GP (ml/200 mg DM), CP, ether extract (EE) and nitrogen free extract (NFE, all in g/kg DM) concentration according to Menke and Steingass (1987):

NEL=0.0949 GP+0.0085 CP+0.0186 EE +
+0.0045 NFE-2.93

The in vitro gas production test was carried out according to Menke et al. (1979). Rumen liquid was taken from a fistulated cow which was fed standard diets throughout the trial (20% concentrates with minerals and vitamins and 80% hay ad libitum).

RESULTS AND DISCUSSION

Chemical composition of PSC is presented in Table 1. The concentration of CP was relatively high and varied from 546 to 658 g/kg DM. Variability was even greater in the concentration of fat (87 to 201 g/kg DM). Samples of PSC varied widely also in the concentrations of NDF (88 to 416 g/kg DM), ADF (49 to 137 g/kg DM) and ADL (7 to 55 g/kg DM). Concentrations of NDF, ADF and ADL were correlated with PSC colour. Chromaticity coordinate a^* of CIE colour system was positively related to concentrations of NDF ($r=0.87$, $P<0.001$), ADF ($r=0.71$, $P<0.01$) and ADL ($r=0.61$, $P<0.05$). That means that reddish-brown PSC contained more NDF, ADF and ADL than greenish yellow PSC.

Effective protein degradability which varied from 46.8 to 84.1% (Table 2, Fig. 1) was negatively correlated with the CIE a^* colour coordinate ($r=-0.70$, $P<0.005$) and the concentration of NDF ($r=-0.63$, $P<0.05$) or ADF (-0.60, $P<0.05$) and positively correlated with lightness L^* variable ($r=0.60$, $P<0.05$). We suppose that the colour of PSC, as well as EPD, depends mainly on the temperature and duration of roasting before pressing. Light greenish-yellow PSC had high protein solubility, high EPD and exponential degradation curves in the rumen. On the other side, EPD of PSC with of darker colour is lower while its protein degradation curves in the rumen are linear (Fig. 2). Cumulative protein degradation curves in the rumen are usually exponential. Linear degradation curves of protein from oil seed meals can be initiated by treatment with formaldehyde (Freer and Dowe, 1984) or combination of heat, blood and xylose (Mosimanyana and Mowat, 1992). The results of the present experiment are in disagreement with the results of Ganesh and Grieve (1990) who found out that soybean roasted at different temperatures differed neither in rapidly degradable protein fraction nor in extent or degradation rate of slowly degradable one. In comparison to soybean meal, relatively low protein degradability in PSC can also be related to amino acid composition. PSC protein contains relatively high proportion of sulphur containing amino acids (Zdunczyk et al., 1999), which are characterised by relatively low degradability in the rumen (Hancock et al., 1994).

Protein availability in the small intestine was estimated by in vitro HCl-pepsin method. Protein digestibility was high and varied from 94.8 to 97.4% (Table 3). It has been established that protein digestibility is negatively related to chromaticity coordinate a^* of CIE $L^*a^*b^*$ colour system ($r=-0.69$, $P<0.01$). It can be speculated that high roasting temperature affects adversely total tract protein digestibility. However, the adverse effect of roasting temperature on protein digestibility was considerably less pronounced than its favourable effect on lower protein degradability in the rumen. In vitro protein digestibility was also negatively correlated with the ADF ($r=-0.80$, $P<0.001$) and ADL ($r=-0.74$, $P<0.01$) concentration.

Table 1. **Concentration of crude protein (CP), crude fibre (CF), crude fat (Fat), ash (Ash), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) in pumpkin seed cakes of different origin**

Tablica 1. **Koncentracija sirovih bjelančevina (CP), sirove vlaknine (CF), sirovih masti (Fat), pepela (Ash), u neutralnom detergentu netopivih vlaknina (NDF) u kiselom detergentu netopivih vlaknina (ADF) i lignina (ADL) u pogača sjemenki bundeve različitog podrijetla**

Sample – Uzorak	CP	CF	Fat	Ash	NDF	ADF	ADL
	g/kg DM - g/kg ST						
11E	613	61	125	94	351	68	11
7E	605	66	133	93	213	87	26
3E	571	48	112	89	261	59	8
5E	560	36	187	92	88	49	7
4E	568	53	151	103	205	59	9
2E	578	50	201	101	364	66	16
1E	623	39	149	107	150	50	8
8L	546	47	189	86	403	105	43
9L	567	108	160	81	337	117	49
6L	577	42	193	109	236	60	12
12L	600	45	129	104	416	66	8
13L	609	60	94	132	396	77	19
10L	571	126	113	104	311	137	55
14L	658	54	87	124	268	79	13
Maximum – Najviše	658	126	201	132	416	137	55
Minimum – Najmanje	546	36	87	81	88	49	7
CV%	5	44	26	14	35	34	82
Average – Prosjek	589	60	145	101	286	77	20

Figure 1. **Relationship between effective protein degradability (EPD) and lightness (L^*) or chromaticity coordinate a^* of CIE $L^*a^*b^*$ colour system in pumpkin seed cakes**

Slika 1. **Regresijska veza između efektivne razgradljivosti bjelančevina (EPD) te svjetlosti (L^*) ili vrijednosti koordinate a^* CIE $L^*a^*b^*$ koloristićkog sustava u pogača sjemenki bundeve**

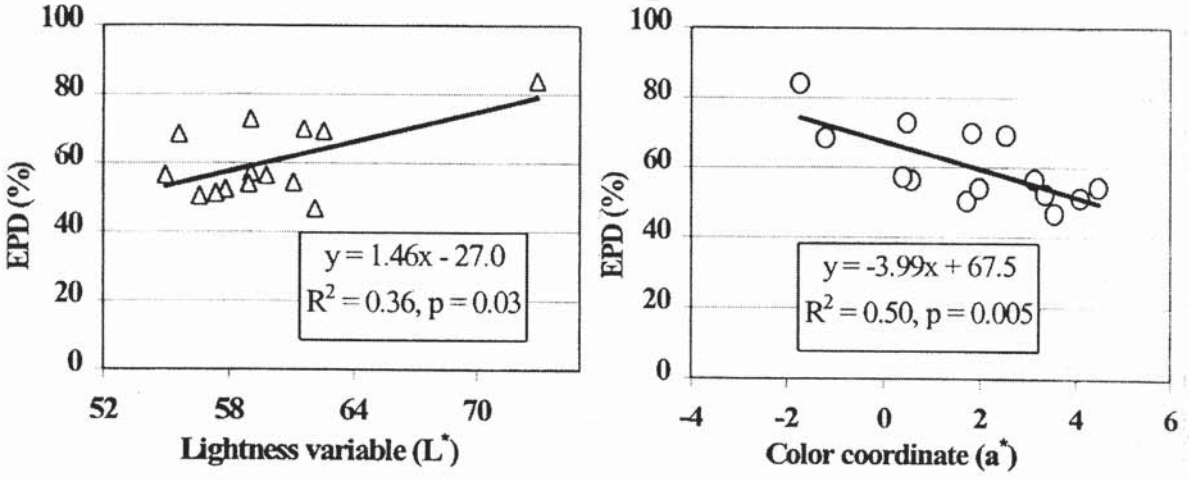

Figure 2. **Protein degradation curves of PSC with exponential (, n=7) or linear (, n=7) degradation characteristics in comparison with soybean meal (, n=1)**

Slika 2. **Značajke razgradnje bjelančevina u pogača sjemenki bundeve s eksponentnim (, n=7) ili linearnim (, n=7) značajkama razgradnje u usporedbi sa sojinom sačmom (, n=1)**

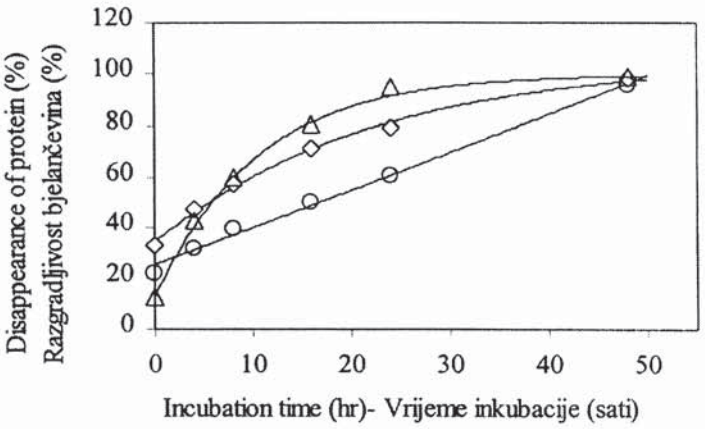

Table 2. **Coefficients of exponential (a, b, c) or linear (a, C) degradation curves, effective protein degradability (EPD) and coordinates of CIE L*a*b* colour system in pumpkin seed cakes of different origin**

Tablica 2. **Koeficienti eksponentne (a, b, c) ili linearne (a, C) jednadžbe razgradnje, efektivna razgradljivost bjelančevina (EPD) i koordinate CIE L*a*b* koloristićkog sustava u pogača sjemenki bundeve različitog podrijetla**

Sample – Uzorak	a	B	c	C	EPD	L*	a*	b*
	%		h^{-1}		%			
11E[1)]	18.7	78.3	0.0340	-	50.4	56.6	1.73	16.0
7E	20.5	78.8	0.0416	-	56.3	59.8	0.58	18.6
3E	30.7	67.7	0.0688	-	69.9	61.6	1.83	18.3
5E	34.9	64.8	0.0538	-	68.4	55.6	-1.20	17.2
4E	33.9	65.0	0.0748	-	72.8	59.0	0.48	18.4
2E	36.7	61.4	0.0566	-	69.3	62.6	2.53	19.2
1E	56.0	42.5	0.0970	-	84.1	73.0	-1.73	19.1
8L[2)]	30.1	-	-	1.39	56.5	55.0	3.13	18.5
9L	19.7	-	-	1.45	46.8	62.1	3.55	20.9
6L	29.3	-	-	1.48	57.2	59.1	0.40	17.9
12L	26.0	-	-	1.42	52.3	57.9	3.35	17.6
13L	22.3	-	-	1.52	51.2	57.4	4.10	17.3
10L	26.4	-	-	1.48	54.3	61.1	4.48	19.2
14L	23.5	-	-	1.61	53.9	59.0	1.98	16.8
Maximum - Najviše	56.0	78.8	0.0970	1.61	84.1	73.0	4.48	20.9
Minimum - Najmanje	18.7	42.5	0.0340	1.39	46.8	55.0	-1.73	16.0
CV%	33.0	18.6	34.9	4.9	17.9	7.4	105.5	6.7
Average – Prosjek	29.2	65.5	0.0609	1.48	60.3	60.0	1.80	18.2
SBM	12.3	87.6	0.0970	-	70.1	-	-	-

[1)] E - protein degradation characteristics described using exponential equation
- značajke razgradnje bjelančevina opisane eksponentnom funkcijom
[2)] L- protein degradation characteristics described using linear equation
- značajke razgradnje bjelančevina opisane linearnom funkcijom
SBM - Soybean meal - sojina sačma

Table 3. **Enzymatic in vitro protein digestibility (EIVPD), concentrations of undegraded dietary protein (UDP) and digestible undegraded protein (DUDP), in vitro organic matter digestibility (IVOMD) and concentration of net energy for lactation (NEL) in pumpkin seed cakes of different origin**

Tablica 3. **Encimatska in vitro probavljivost bjelančevina (EIVPD), koncentracija nerazgradljivih bjelančevina (UDP) i probavljivih nerazgradljivih bjelančevina (DUDP), in vitro probavljivost organske tvari (IVOMD) i koncentracija neto energije za laktaciju (NEL) u pogača sjemenki bundeve različitog podrijetla**

Sample - Uzorak	EIVPD	UDP[1)]	DUDP[2)]	IVOMD	NEL
	%	g/kg DM- g/kg ST		%	MJ/kg DM-ST
11E[1)]	96.3	304	282	70.1	7.35
7E	96.4	264	243	72.5	7.62
3E	95.7	172	147	68.2	7.22
5E	97.4	177	162	71.4	8.31
4E	96.8	154	136	74.3	7.95
2E	96.9	177	159	65.6	7.77
1E	97.2	99	82	75.1	7.99
8L[2)]	96.4	238	218	63.9	7.71
9L	94.9	302	273	64.8	7.18
6L	96.6	247	227	70.5	8.06
12L	96.4	286	265	70.7	7.43
13L	96.5	297	276	71.9	6.76
10L	94.8	261	231	67.4	6.54
14L	96.8	303	282	76.5	7.16
Maximum – Najviše	97.4	304	282	76.5	8.31
Minimum – Najmanje	94.8	99	82	63.9	6.54
CV%	0.8	29	30	5.5	6.80
Average – Prosjek	96.4	234	213	70.2	7.50
SBM	-	160	-	-	-

UDP=CP(100-EPD)/100;
DUDP=UDP-CP (100-EIVPD)/100
SBM - Soybean meal - sojina sačma

Concentration of undegradable dietary protein (UDP) in PSC varied from 99 to 304 g/kg DM and was on average considerably higher (234 g/kg DM) than in sample of soybean meal (160 g/kg DM, Table 3). The concentration of digestible UDP (DUDP) in PSC, which was calculated by subtracting in vitro indigestible protein from effectively undegradable protein, varied from 82 to 282 g/kg DM (Table 3) and was negatively correlated with the lightness L^* ($r=-0.58$, $P<0.05$) and positively correlated with the colour coordinate a^* ($r=0.64$, $P<0.05$) and the concentration of NDF ($r=0.59$, $P<0.05$). Concentration of DUDP depends mainly on its colour and related EPD whereas the CP concentration and in vitro protein digestibility do not markedly affect it. Estimated digestibility of UDP was relatively high, and varied from 82.5 to 93.0%.

In order to obtain complete information on protein value of PSC, the effect of roasting temperature on the concentration of individual

amino acids in DUDP fraction should be investigated. Regarding the results taken from the literature, PSC contain considerable amounts of sulphur containing amino acids (Hancock et al., 1994; Zdunczyk et al., 1999). However, in comparison to soybean meal, they contain less lysine (Zdunczyk et al., 1999). Due to thermal lability of some amino acids it would be necessary to investigate the amino acid composition of the protein fraction which escapes degradation in the rumen.

Concentration of NEL varied from 6.5 to 8.3 MJ/kg dry matter. Average energy concentration (7,5 MJ NEL kg^{-1} DM) was considerably higher than those reported in DLG (1982) tables. The latest is probably a result of relatively high fat and low crude fibre concentration (Table 1). Relatively low crude fibre concentration indicates that PSC were made mainly from seeds without hulls. Concentration of NEL was negatively related to crude fibre concentration ($r=-0.70$, $P<0.01$).

CONCLUSIONS

Protein value of PSC for ruminants is high but variable. Due to close relation between the colour of the PSC on one and the concentrations of UDP and DUDP on the other hand, it is concluded that variability is probably due to time and temperature of roasting before pressing out the oil. Regarding literature data on high concentrations of sulphur containing amino acids, it can be assumed that PSC can provide an important source of mentioned amino acid for ruminants.

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SAŽETAK

Kod 14 uzoraka pogača sjemenki bundeve (PSC) istraživani su kemijski sastav te in sacco razgradljivost i in vitro probavljivost (HCl-pepsin) bjelančevina. Sadržaj sirovih bjelančevina kretao se od 546 do 658 g/kg, a sirovih masti od 87 do 201 g/kg suhe tvari (ST). Sadržaj NDF varirao je od 88 do 416 g/kg, ADF od 49 do 137 g/kg i ADL od 7 do 55 g/kg ST. Efektivna razgradljivost bjelančevina (EPD) u buragu kretala se od 46.8 do 84.1%, a u prosjeku iznosila je 60.3%. EPD je u negativnoj korelaciji s NDF ($r=-0.63$, $P<0.05$), a utvrđene su i korelacije između EPD i CIE (Commission Internationale de l'Eclairage) parametara koloristićkog sustava. EPD je dakle u negativnoj korelaciji s vrijednošću koordinate a^* CIE $L^*a^*b^*$ sustava ($r=-0.70$, $P<0.005$) i u pozitivnoj korelaciji sa svjetlošću L^* ($r=0.60$, PO.05) u PSC. PSC tamno smeđe boje imaju nižu EPD nego PSC zeleno-žute skupine. Prema formi funkcije razrađivanja bjelančevina PSC može se podijeliti u dvije skupine. Kod prve skupine, koju zastupaju PSC s niskom SPD, značajke razgradnje bjelančevina mogu se opisati linearnom funkcijom, a kod druge skupine koju zastupaju PSC visoke razgradljivosti s eksponentnom funkcijom. In vitro probavljivost bjelančevina je bila visoka (od 94.8 do 97.4%) i u negativnoj korelaciji s ADF ($r=-0.80$, $P<0.001$) i ADL ($r=-0.74$, $P<0.01$). Sadržaj probavljivih nerazgradljivih bjelančevina, koje su izračunate kao razlika između efektivno nerazgradljivih i in vitro neprobavljivih bjelančevina, varirao je od 82 do 282 g/kg ST. Ustanovljeno je da su razlike u bjelančevinastoj vrijednosti PSC za preživače vjerojatno pouzrokovane različitom dužinom i temperaturom prženja.