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ScienceDirect
Livestock Science 113 (2008) 43 – 51
LIVESTOCK SCIENCE
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Effect of heat processing on ruminal degradability and intestinal disappearance of nitrogen and amino acids in Iranian whole soybean

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Received 4 January 2007; received in revised form 16 February 2007; accepted 20 February 2007

Abstract

The effects of heat processing (roasting and steeping) on nitrogen (N) fractionations, ruminal degradation and intestinal digestibility of N and amino acids (AA) in two Iranian whole soybean cultivars (Sahar and Williams) were determined using two ruminally fistulated and two intestinally cannulated Holstein steers by nylon bag techniques. The seeds were roasted at 140 to 145 °C using a drum roaster. A fraction of the seeds were cooled immediately and the rest were held in isolated barrels for 45 min (steeping). The non-protein N (NPN) and buffer soluble N (BSN) fractions in heat processed soybeans were found to be reduced significantly ($P<0.01$) compared to raw seeds. Soluble (a) and insoluble potentially degradable (b) fractions of N, showed a similar pattern in raw and heat processed soybeans (in both cultivars), but rate of degradation (c) was reduced dramatically in heat processed seeds (67 and 70% in Sahar and Williams cultivars, respectively). The ruminal degradability of N and AA of heat processed soybeans was reduced significantly ($P<0.001$) compared to raw seeds. However, among the individual AA some variation was found. Intestinal digestibility of N and AA (total and individual) was improved significantly ($P<0.001$) by roasting (16.9 and 12.3% for N and total AA, respectively) and steep-roasting (25.0 and 18.5% for N and total AA, respectively). Intestinal digestibility of N or AA, measured with or without ruminal pre-incubation, was the same. Total tract disappearance of N was not significantly different between raw and roasted seeds. Total tract disappearance of total AA was higher for raw than roasted seeds, due to higher protein degradability of raw seeds. Roasting and steep-roasting were effective methods of changing the site of digestion from rumen to small intestine and therefore the amount of digestible undegraded AA in the small intestine can be increased. There was no significant difference between the two soybean cultivars with respect to N and total AA ruminal degradability, intestinal digestibility and total tract disappearance. The interaction between cultivar and heat processing was also not significantly different.

Keywords: Amino acid; Digestibility; Heat processing; Mobile bag; Whole soybean

1. Introduction

Whole soybean is used as a high energy-protein supplement for dairy cows, but the protein is highly degradable by rumen microbes (Krishnamoorthy et al., 1982). Various chemical and physical processing has been suggested to decrease ruminal protein degradability. Heat processing is the most commonly used physical method (Plegge et al., 1985; Pena et al., 1986). Roasting whole soybean increased milk production by as much as 4.5 kg/d, 3.5% fat-corrected milk production by 4.0 kg/d, and milk protein by 0.09 kg/d, with no

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doi:10.1016/j.livsci.2007.02.017

reduction in milk fat production (Faldet and Satter, 1991). This improved production has been attributed primarily to an increase in escape of protein from ruminal degradation, thereby providing more amino acids (AA) for intestinal digestion and absorption. Modern systems for protein evaluation in ruminants are moving in the direction of predicting absorption of individual AA from the small intestine (Rulquin and Vèritè, 1993). Several studies (Erasmus et al., 1994; O'Mara et al., 1997; Van Straalen et al., 1997) indicated that ruminal disappearance of AA and protein degradability are not similar. Thus, knowledge of ruminal disappearance of individual AA is required when protein value is to be expressed on the basis of individual AA (Rulquin and Vèritè, 1993). On the other hand, excessive heat treatment may reduce the availability of certain AA particularly Lys. The determination of intestinal digestibility of individual AA is, therefore, of special importance in heat-treated feedstuffs.

There are studies in the literature on the effects of heat treatment on N and AA degradation and intestinal digestion of soybean meal (O'Mara et al., 1997; Harstad and Prestløkken, 2000; Danesh Mesgaran and Stern, 2005). However, there are limited published studies giving estimates of undegradable protein and especially AA ruminal and intestinal disappearance of heat processed whole soybean. Hence, the objective of this research was to elucidate the effects of roasting and steep-roasting on ruminal degradability and intestinal digestibility of N and especially AA of Iranian whole soybeans.

2. Materials and methods

2.1. Soybean samples and heat processing

In this study two Iranian cultivars of soybeans (Sahar and Williams) were used. These are the most common cultivars used in dairy cow diets in Iran. The soybean seeds were fed into a turning cylindrical tunnel [turning speed of 2.5 (circle per minute) and diameter of tunnel of 50 cm] with a flame and blower at its end, so that they were exposed to burning air. The seeds traversed the cylinder tunnel (4-m length), so that the temperature of the beans exiting the roaster was 140 to 145 °C. Then, some seeds were gradually cooled (about 1 h) and the rest were immediately placed and held for 45 min in 100-L isolated barrels covered with canvas before being allowed to cool (Faldet et al., 1992b). So a total of 6 feed samples (2 soybean cultivars which were raw, roasted or steep-roasted) were used in the study.

2.2. Chemical analyses

The feed samples were dried using a forced-air oven at 96 °C for 48 h, ground to pass through a 2-mm screen, then analysed for total N (Kjeldahl method, Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden), acid detergent insoluble N ([ADIN], Licitra et al., 1996), acid detergent fibre ([ADF], Van Soest et al., 1991), neutral detergent insoluble N ([NDIN], Licitra et al., 1996), neutral detergent fibre ([NDF], Van Soest et al., 1991), ether extract ([AOAC, 2000], ID 920.39), and ash ([AOAC, 2000], ID 942.05) concentrations. Sodium sulphite and alpha amylase were not used in the NDF assay, and NDF was expressed as the ash free residue after extraction with boiling neutral solutions of sodium lauryl sulphate and EDTA. Non-protein N was estimated by incubating 2 g DM equivalent of feed samples in 3 M sodium tungstate solution and determining N in residual sediments (NPN was then calculated as the total N minus residual sediments N). Buffer soluble N (BSN) was determined similarly, but borate–phosphate solution was used.

Amino acid analysis of feeds and residues of ruminal degradation and intestinal disappearance was performed by cation-exchange chromatography using a Beckman System Gold amino acid analyzer with post-column ninhydrin detection (Clarke, 1993). Feed samples for analysis were hydrolyzed in 6 M HCl at 105 °C for 24 h in vacuo. The hydrolysates were dried in vacuo over NaOH and then taken up in sample diluent (Beckman) prior to their injection into the cation-exchange column.

2.3. In situ N and amino acids disappearance

Ruminal, small intestinal and total tract N and AA disappearance of feeds were evaluated using the in situ mobile bag technique (Subuh et al., 1996). Two ruminally fistulated (430 ± 10 kg) and two intestinally cannulated (400 ± 8 kg) Holstein steers were used in the study. Steers were fed a total mixed ration (TMR) twice daily (0900 and 1600 h). The TMR included (on DM basis) 2.70 kg alfalfa hay, 1.75 kg maize silage and 2.25 kg concentrate (barley 635.0, cottonseed meal 58.0, beet pulp 173.0, wheat bran 100.0, limestone 10.0, salt 4.0, vitamin–mineral supplement 5.0 and urea 15.0, g/kg DM) per steer per day. Predicted metabolisable energy and crude protein content of the TMR were 2.29 Mcal/kg and 136 g/kg (DM basis), respectively (NRC, 2001). In order to determine N degradability coefficients, 5 g DM equivalent of each feed sample (ground with a 2-mm screen mill) was placed in individual nylon bags (made of artificial silk cloth

Table 1
Chemical composition (g/kg of DM) of feeds for Sahar (S) and Williams (W) cultivars

	S			W		
	Raw	Roasted	Steep-roasted	Raw	Roasted	Steep-roasted
DM	920 (9.5)[a]	970 (9.0)	975 (9.9)	910 (10.1)	985 (9.0)	970.0 (12.2)
OM	945 (12.2)	946 (14.7)	948 (12.4)	942 (11.8)	950 (10.7)	944.0 (13.2)
N	59.0 (3.5)	60.9 (4.0)	60.4 (3.7)	57.0 (4.5)	60.1 (6.0)	60.0 (3.5)
EE[b]	175 (15.0)	185 (20.0)	190 (20.2)	185 (17.5)	210 (19.4)	205.0 (15.9)
NDF	220 (15.0)	168 (20.0)	170 (20.2)	240 (19.0)	195 (25.0)	191.0 (18.7)
ADF	155 (5.4)	130 (4.0)	123 (5.4)	65.0 (10.1)	141 (7.9)	142.0 (9.5)
NFC[c]	181 (8.0)	164 (12.0)	188 (15.0)	167 (9.0)	161 (20.0)	175.0 (7.7)

[a] Mean (S.E.M.).
[b] Ether extract.
[c] Non-fibre carbohydrate calculated as (NRC, 2001): 100−(N+NDF+EE+ash).

with a 50-μm pore size and averaged 12 cm×19 cm). Incubation times were 1, 2, 3, 4, 8, 16, 24, 36, 48 and 72 h. Bags were placed in the dorsal sac of the reticulorumen of each steer (2 bags per each feed sample in each steer) after the 0900 h feeding. Immediately after incubation, the bags were hand washed thoroughly in cold running water. Two bags of each feed sample were washed without incubation in the rumen (0 h samples). The bags were dried in a forced-air oven (58 °C, 48 h) and weighed to determine DM disappearance. The residues were analysed for N content. For determination of N and AA ruminal and intestinal disappearance, 1.2 g DM equivalent of each feed sample was placed in small nylon bags (made of artificial silk cloth with a 48-μm pore size and averaged 3 cm×6 cm) with the lids stuck tightly and then the bags were inserted into plastic mesh cylinders (26 cm×8 cm, 0.57-mm pore size) and incubated in the rumen for 12 h (26 bags per each feed sample in each steer). After removal from the rumen, bags were washed using cold water and some bags (14 bags per each feed sample in each steer) were inserted into the small intestine via the intestinal cannula at the rate of one bag every 30 min, then removed from the voided faeces and rinsed in cold running water. The bags were dried in a forced-air oven (58 °C, 48 h) and weighed to determine DM disappearance. The residues in the bags after ruminal incubation and faecal collection were analysed for N and AA content.

2.4. Calculations and statistical analyses

The exponential equation of Ørskov and McDonald (1979) was fitted to values for ruminal degradation of N versus time. Curves were fitted using the NLIN procedure of SAS (SAS, 1999) to estimate the soluble (*a*) and insoluble potentially degradable (*b*) fractions, and rate of degradation (*c*). Effective degradation (ED) was calculated as a function of degradation and passage, assuming a constant fractional passage rate (k_p) of 0.08 h^{-1}: $ED=a+[(b\times c)/(c+k_p)]$. Calculations as described by Subuh et al. (1996) were used for intestinal digestibility and total tract disappearance of N and AA.

Experiments were designed as completely randomized with a factorial arrangement of treatments and the data were analysed using the General Linear Models procedure of SAS (SAS, 1999), with the factors in the model

Table 2
Effect of heat processing and cultivar (Sahar, S; Williams, W) on N fractions (g/kg of N) of feeds

	P				C			P×C
	NOP	ROS	STR	S.E.M.	S	W	S.E.M.	S.E.M.
Non-protein N	83.0 x	27.0 y	16.0 y	10.7**	44.0	41.0	8.8	15.2
Buffer soluble N	415.0 x	91.0 y	54.0 y	19.4 ***	193.0	181.0	15.8	27.4
Neutral detergent soluble N	854.0	896.0	894.0	58.9	884.0	879.0	48.0	83.2
Neutral detergent insoluble N	146.0	104.0	106.0	12.9	116.0	121.0	10.5	18.3
Acid detergent insoluble N	108.0	86.0	84.0	18.5	89.0	96.0	15.1	26.2

P: soybean processing; C: cultivar; P×C: interaction of processing and cultivar.
NOP: non-processing; ROS: roasting; STR: steep-roasting.
Means in the same row with no common letters differ ($P<0.05$).
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 3
Amino acid (AA) N (g AA-N of 100 g^{-1} N) and AA profile (g AA of 100 g^{-1} AA) of whole soybean samples (O) and the undegraded residues after 12 h incubation in the rumen (R) for Sahar (S) and Williams (W) cultivars

	S						W					
	Raw		Roasted		Steep-roasted		Raw		Roasted		Steep-roasted	
	O	R	O	R	O	R	O	R	O	R	O	R
Total AA-N	80.43	76.04	81.11	78.41	80.64	78.00	80.51	76.34	80.72	79.07	80.84	77.57
Essential AA	49.56	50.73	48.49	48.6	48.56	48.57	48.79	49.85	48.25	48.41	48.44	48.61
Arg	9.14	8.54	8.14	7.86	8.12	7.82	8.99	8.30	8.44	8.20	8.64	8.43
His	3.05	3.07	3.14	3.15	3.23	3.25	2.77	2.81	3.01	3.02	3.17	3.18
Ile	4.82	5.13	4.82	5.03	5.18	5.41	5.03	5.39	5.14	5.38	5.23	5.48
Leu	9.94	10.69	9.51	9.45	9.57	9.44	9.34	9.96	9.11	9.07	9.13	9.01
Lys	6.62	6.51	5.95	5.79	5.74	5.62	6.71	6.42	6.17	5.96	6.04	5.80
Met	1.23	1.30	1.64	1.69	1.60	1.64	1.30	1.40	1.53	1.59	1.41	1.45
Phe	5.44	5.74	5.30	5.43	5.2	5.31	5.83	6.20	5.74	5.90	5.45	5.65
Thr	3.60	3.78	3.98	4.06	3.86	3.94	3.82	4.07	3.87	3.94	3.98	4.03
Val	5.72	5.97	6.01	6.13	6.06	6.14	5.00	5.30	5.24	5.34	5.39	5.57
Non-essential AA	50.44	49.27	51.51	51.4	51.44	51.43	51.21	50.15	51.75	51.59	51.56	51.38
Ala	5.00	5.16	4.63	4.73	4.37	4.49	4.84	5.08	4.52	4.58	4.32	4.43
Asp	12.84	12.28	13.40	12.92	13.82	13.43	13.02	12.54	13.03	12.64	13.31	12.72
Glu	19.94	18.80	20.13	20.15	19.99	20.03	19.86	18.66	20.11	20.04	19.91	20.03
Gly	3.90	4.03	4.03	4.15	4.11	4.19	4.19	4.38	4.32	4.46	4.28	4.36
Ser	5.33	5.35	5.38	5.39	5.54	5.55	5.46	5.46	5.73	5.76	5.60	5.61
Tyr	3.43	3.64	3.94	4.06	3.61	3.74	3.84	4.03	4.04	4.11	4.14	4.23

consisting of heat processing (3), soybean cultivars (2) and their interaction. The main effects of factors and their interaction were compared using a Duncan test.

3. Results

3.1. Feed chemical composition

Organic matter (OM) content was similar for both raw and heat processed soybeans (both cultivars), indicating that it was not affected by heat processing. Similarly, little difference was observed in N and ether extract content among raw and heat processed soybeans (Table 1). Neutral detergent fibre and ADF content of roasted soybeans were lower than raw soybeans (both cultivars). Heat processing decreased significantly ($P<0.001$) the BSN content of seeds (Table 2). The level of NPN was also lower in heat processed ($P<0.01$) compared to raw soybean seeds. Steeping intensified the effect of heat on reducing NPN and BSN but differences were not significant. The NDIN content of raw soybeans was more

Table 4
Degradation constants and effective degradability (%) of N of feeds for Sahar (S) and Williams (W) cultivars

	S			W		
	Raw	Roasted	Steep-roasted	Raw	Roasted	Steep-roasted
a [a]	0.40	0.39	0.39	0.47	0.40	0.38
	(0.022) [b]	(0.011)	(0.010)	(0.016)	(0.013)	(0.019)
b	0.61	0.62	0.62	0.52	0.60	0.61
	(0.024)	(0.027)	(0.026)	(0.018)	(0.035)	(0.042)
c	0.09	0.03	0.03	0.10	0.03	0.03
	(0.011)	(0.003)	(0.003)	(0.011)	(0.004)	(0.006)
ED [c]	72.29	55.91	54.91	75.89	56.36	54.63

[a] *a*, *b* and *c* = fitted parameters for N degradability (Ørskov and McDonald, 1979).
[b] Mean (S.E.M.).
[c] Effective degradability was calculated as a function of degradation and passage, assuming a rate of passage constant (k_p) of 0.08 h^{-1}: $ED=a+[(b\times c)/(c+k_p)]$.

Table 5
Mean values of ruminal N disappearance of feeds for Sahar (S) and Williams (W) cultivars at different incubation times

Time	S			W		
	Raw	Roasted	Steep-roasted	Raw	Roasted	Steep-roasted
0	0.427	0.404	0.405	0.508	0.410	0.441
1	0.447	0.422	0.421	0.519	0.418	0.446
2	0.532	0.426	0.421	0.555	0.436	0.454
3	0.582	0.445	0.452	0.590	0.475	0.453
4	0.573	0.450	0.468	0.608	0.490	0.473
8	0.643	0.469	0.472	0.757	0.492	0.496
16	0.899	0.591	0.552	0.941	0.596	0.584
24	0.977	0.699	0.705	0.957	0.662	0.731
36	0.983	0.783	0.768	0.972	0.766	0.770
48	0.993	0.920	0.916	0.988	0.933	0.906
72	0.994	0.936	0.923	0.986	0.935	0.936

Table 6
Effect of heat processing and cultivar (Sahar, S; Williams, W) on rumen degradability (%) of feed N and disappearance of total and individual AA after 12 h incubation in the rumen

	P				C			P×C
	NOP	ROS	STR	S.E.M.	S	W	S.E.M.	S.E.M.
N	54.5 x	23.0 y	24.0 y	2.76***	32.3	35.3	2.25	3.90
Essential AA								
Arg	60.0 x	27.4 y	27.9 y	1.27***	37.7	39.2	1.04	1.80
His	56.5 x	24.8 y	25.1 y	1.02***	34.6	36.3	0.83	1.44
Ile	54.0 x	21.7 y	22.6 y	0.89***	31.7	33.8	0.73	1.26
Leu	53.8 x	25.5 y	25.0 y	1.02***	34.3	35.3	0.83	1.44
Lys	58.2 x	27.4 y	27.6 y	0.92***	36.3 y	39.1 x	0.75*	1.30
Met	54.0 x	22.4 y	23.0 y	1.26***	32.5	33.7	1.03	1.78
Phe	54.3 x	23.1 y	24.0 y	0.96***	32.9	34.7	0.78	1.36
Thr	54.4 x	23.7 y	24.5 y	0.82***	33.1 y	35.2 x	0.67*	1.16
Val	54.7 x	23.6 y	23.0 y	1.27***	33.4	34.1	1.04	1.80
Non-essential AA								
Ala	55.2 x	23.8 y	23.2 y	0.96***	33.2	34.9	0.79	1.36
Asp	58.6 x	27.6 y	26.4 y	1.02***	37.2	37.9	0.83	1.44
Glu	59.4 x	25.2 y	25.4 y	0.79***	35.7 y	37.7 x	0.64*	1.12
Gly	55.2 x	22.7 y	23.5 y	0.92***	33.1	34.5	0.75	1.30
Ser	56.8 x	24.8 y	24.2 y	0.96***	34.8	35.8	0.78	1.36
Tyr	54.6 x	23.2 y	23.4 y	0.74***	32.3 y	35.1 x	0.61*	1.05
Total AA	56.9 x	25.1 y	25.1 y	0.97***	34.9	36.5	0.79	1.37

P: soybean processing; C: cultivar; P×C: interaction of processing and cultivar.
NOP: non-processing; ROS: roasting; STR: steep-roasting.
Means in the same row with different letters differ.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

than for heat processed seeds, however the difference was not significant.

Differences in total AA and AA profile between raw and roasted soybeans were relatively small in both cultivars (Table 3) and heat processing of soybean had no systematic effect on the proportion of total AA. In both cultivars the relative contribution of Lys, Arg and Ala in processed soybeans was lower and Met and Tyr was higher than raw soybeans.

3.2. Ruminal degradation of N and disappearance of AA

The curve for N disappearance for all treatments showed an exponential trend as described by the Ørskov and McDonald (1979) equation. The soluble (*a*) and insoluble potentially degradable (*b*) fractions were similar for raw and heat processed soybeans, so heat processing did not alter these parameters (Table 4), but rate of degradation (*c*) and hence ED of N were affected by heat processing for both cultivars. For the Sahar cultivar, roasting and steep-roasting decreased degradation rate of N by 68% and 70%, and in the Williams cultivar by 73% and 67%, respectively (Tables 4 and 5).

Ruminal degradation of N and disappearance of total and individual AA in raw and heat processed soybeans are shown in Table 6.

3.3. Intestinal disappearance of N and AA

Intestinal disappearance of N and AA measured both with and without rumen pre-incubation are shown in Tables 7 and 8.

Roasting increased the intestinal disappearance of N and total and individual AA significantly ($P<0.001$) and steeping intensified it, showing beneficial effects of steeping beyond roasting. Also, the extent of disappearance for total AA was greater than N for all feed samples both with or without rumen pre-incubation.

3.4. Total tract disappearance of N and AA

The results for total tract disappearance of N, individual and total AA are shown in Table 9. There was

Table 7
Effect of heat processing and cultivar on intestinal disappearance (%) of N, individual and total AA after ruminal pre-incubation for Sahar (S) and Williams (W) cultivars

	P				C			P×C
	NOP	ROS	STR	S.E.M.	S	W	S.E.M.	S.E.M.
N	61.5 z	74.0 y	82.0 x	1.13***	72.0	73.0	0.92	1.60
Essential AA								
Arg	71.6 z	82.2 y	87.7 x	1.20***	79.4	81.6	0.98	1.70
His	72.4 z	81.8 y	87.5 x	1.23***	80.4	80.8	1.00	1.74
Ile	64.5 z	79.0 y	83.8 x	1.07***	75.6	76.0	0.88	1.52
Leu	68.4 z	80.0 y	84.8 x	1.10***	76.9	78.6	0.90	1.56
Lys	72.4 z	77.0 y	81.5 x	1.00***	75.5 y	78.4 x	0.82*	1.42
Met	66.4 z	74.2 y	79.8 x	1.36***	71.5 y	75.5 x	1.11*	1.92
Phe	65.8 z	72.2 y	79.2 x	1.16***	70.5 y	74.3 x	0.95*	1.64
Thr	63.5 z	74.8 y	83.2 x	1.22***	72.7	75.1	0.99	1.72
Val	62.1 z	71.5 y	78.0 x	1.12***	68.7	72.4	0.91*	1.58
Non-essential AA								
Ala	61.4 z	67.8 y	76.6 x	1.19***	66.6 y	70.6 x	0.97*	1.68
Asp	68.5 z	76.2 y	83.2 x	1.20***	73.0 y	78.9 x	0.98**	1.70
Glu	68.4 z	78.3 y	84.0 x	1.10***	75.2 y	78.7 x	0.90*	1.56
Gly	62.4 z	72.0 y	77.0 x	0.93***	69.0 y	71.7 x	0.76*	1.32
Ser	68.6 z	74.5 y	80.2 x	1.12***	73.0	75.9	0.91	1.58
Tyr	63.0 z	73.2 y	80.0 x	0.96***	70.7 y	73.5 x	0.79*	1.36
Total AA	67.2 z	76.6 y	82.5 x	1.12***	74.0	76.8	0.92	1.59

P: soybean processing; C: cultivar; P×C: interaction of processing and cultivar.
NOP: non-processing; ROS: roasting; STR: steep-roasting.
Means in the same row with different letters differ.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 8
Effect of heat processing and cultivar on intestinal disappearance (%) of N, individual and total AA without ruminal pre-incubation for Sahar (S) and Williams (W) cultivars

	P				C			P×C
	NOP	ROS	STR	S.E.M.	S	W	S.E.M.	S.E.M.
N	64.6 z	71.9 y	81.8 x	1.00***	71.9	73.5	0.82	1.42
Essential AA								
Arg	71.2 z	81.9 y	89.0 x	0.96***	80.1	81.3	0.78	1.36
His	72.2 z	81.6 y	85.2 x	1.02***	78.7	80.7	0.83	1.44
Ile	64.7 z	78.0 y	83.4 x	0.92***	75.3	75.4	0.75	1.30
Leu	67.6 z	79.8 y	83.2 x	0.83***	76.5	77.3	0.68	1.18
Lys	72.1 z	76.6 y	82.0 x	0.78***	75.9	77.9	0.63	1.10
Met	67.1 z	74.6 y	80.6 x	1.10***	72.7	75.4	0.90	1.56
Phe	66.5 z	71.6 y	78.9 x	0.91***	70.6 y	74.1 x	0.74*	1.28
Thr	62.2 z	74.8 y	83.0 x	1.02***	72.3	74.3	0.83	1.44
Val	63.6 z	70.9 y	79.0 x	0.99***	69.6 y	72.8 x	0.81*	1.40
Non-essential AA								
Ala	60.7 z	68.6 y	76.0 x	0.82***	66.2 y	70.7 x	0.67**	1.16
Asp	68.5 z	75.5 y	82.2 x	0.85***	73.1 y	77.7 x	0.69**	1.20
Glu	69.0 z	78.8 y	84.9 x	0.89***	76.8	78.3	0.73	1.26
Gly	63.2 z	71.8 y	76.5 x	0.69***	62.9 y	71.9 x	0.56*	0.98
Ser	66.2 z	74.5 y	80.1 x	0.98***	72.8	74.4	0.80	1.38
Tyr	62.2 z	75.0 y	80.8 x	0.68***	71.9	73.4	0.55	0.96
Total AA	67.4 z	76.4 y	82.6 x	0.88***	74.4	76.5	0.72	1.25

P: soybean processing; C: cultivar; P×C: interaction of processing and cultivar.
NOP: non-processing; ROS: roasting; STR: steep-roasting.
Means in the same row with different letters differ.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

no significant difference between the two soybean cultivars with respect to N and AA total tract disappearance. The interaction between cultivar and heat processing was not significantly different either.

4. Discussion

4.1. Feed chemical composition

Pichard and Van Soest (1977) stated that NDIN roughly represents the slowly degradable and undegradable fractions in plant cell walls. Whether roasting increases the solubility of cell-bound protein in neutral detergent solution is not known. Another reason for the lower NDIN values for roasted soybean could simply be due to lower concentration of hulls. The ADIN content of heat processed and raw soybeans followed a similar trend.

The decrease in the content of some individual AA due to heating found in this experiment is in agreement with data concerning heating of soybeans. An increase in temperature from 117 to 154 °C caused total Lys to decrease from 2.3 to 1.7% of DM (Faldet et al., 1992b). Lys is usually the most sensitive AA to heat processing and is often lost at levels 5–15 times greater than other AA (Dakowski et al., 1996), but our results show less negative effects on Lys. There are only very limited results published on the effect of heat processing of whole soybean on its Lys content. However, greater negative effects are reported from experiments with formaldehyde-treated soybean meal (Crooker et al., 1986). The reason for the increased proportion of some AA such as Met, Tyr and Thr in heated soybeans is not totally clear.

4.2. Ruminal degradation of N and disappearance of AA

The significant reduction in the rate of degradation (c) and hence ED of N by heat processing showed it appeared to increase by-pass protein from soybean and thereby increase the amount of available AA in the small intestine, provided that the digestibility of by-pass protein does not decrease with heat treatment. Previous studies also reported that heat processing reduced the rate of disappearance of DM and N of whole soybean

Table 9
Effect of heat processing and cultivar on total tract disappearance (%) of N, individual and total AA for Sahar (S) and Williams (W) cultivars

	P				C			P×C
	NOP	ROS	STR	S.E.M.	S	W	S.E.M.	S.E.M.
N	82.4	79.9	86.2	1.51	81.8	83.9	1.23	2.14
Essential AA								
Arg	88.6	87.1	91.1	0.99	88.3	89.6	0.81	1.40
His	87.9	86.3	90.6	1.00	88.3	88.3	0.82	1.41
Ile	83.6 y	83.5 y	87.5 x	0.94*	84.2	85.6	0.77	1.33
Leu	85.4	85.1	88.6	0.95	85.8	86.9	0.78	1.34
Lys	88.4 x	83.2 y	86.6 x	0.84*	84.9	87.2	0.69	1.20
Met	84.5	80.0	84.4	1.25	81.4	84.4	1.02	1.77
Phe	84.4 x	78.6 y	84.2 x	1.04*	80.8 y	83.9 x	0.85*	1.47
Thr	83.3 y	80.8 y	87.3 x	1.02*	82.8	84.8	0.83	1.44
Val	82.8 x	78.2 y	83.0 x	1.12*	79.9	82.7	0.91	1.58
Non-essential AA								
Ala	82.6 x	75.4 y	82.0 x	1.12*	78.4 y	81.6 x	0.89*	1.55
Asp	86.9 x	82.8 y	87.6 x	1.00*	83.7 y	87.8 x	0.82*	1.42
Glu	87.2 x	83.7 y	88.1 x	0.90*	85.0 y	87.6 x	0.73*	1.27
Gly	83.1 x	78.3 y	82.4 x	0.89*	80.2	82.4	0.73	1.26
Ser	86.4 x	80.8 y	85.0 x	0.98*	83.0	85.1	0.79	1.38
Tyr	83.2 x	79.4 y	84.6 x	0.85*	81.1 y	83.6 x	0.69*	1.20
Total AA	85.8 x	82.4 y	87.0 x	0.97*	84.0	86.1	0.79	1.37

P: soybean processing; C: cultivar; P×C: interaction of processing and cultivar.
NOP: non-processing; ROS: roasting; STR: steep-roasting.
Means in the same row with different letters differ.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

(Faldet and Satter, 1991; Hsu and Satter, 1995; Aldrich et al., 1997).

In this study, heat processing reduced ruminal disappearance of total AA to the same extent and trend as N, indicating that ruminal degradation of N can be used for the determination of ruminal disappearance of total AA. However, among the individual AA there was variation in ruminal disappearance, so that, in both raw and heat processed seeds Arg, Lys, Glu and Asp disappeared to a relatively high degree, whereas Leu, Ile, Met, Phe, Tyr, Thr, and to some extent, Val and Ala disappeared to a relatively low degree. The effect of rumen exposure on the extent of rumen disappearance of individual AA is reflected in the change in AA profile from the original feed. A fall in proportion results in higher disappearance than average, whereas an increase in proportion will result in lower than average disappearance. Based on literature values, Arg is known to be very sensitive to fermentation, whereas peptide bonds with branch-chained AA are highly resistant to hydrolysis (Rulquin and Vèritè, 1993). The effects of rumen exposure on individual AA disappearance obtained in sacco are not consistent and there are discrepancies between experiments, but the majority of the experiments indicate that, in highly degradable protein sources like soybean meal, Arg, Glu and Lys have higher extents of rumen disappearance than average, whereas Met and the branch-chained AA (Leu, Ile and Val) disappear less extensively than average (Harstad and Prestløkken, 2000). Numerous publications show the effect of ruminal exposure on disappearance of AA in soybean meal (Erasmus et al., 1994; Weisbjerg et al., 1996; O'Mara et al., 1997; Van Straalen et al., 1997) but we are not aware of studies that evaluated AA disappearance of heat processed whole soybean. Our results were in accordance with that observed in a study by Romagnolo et al. (1994), who suggested that hydrophobicity of proteins might be associated with reduced degradability in the rumen. Although, that study was on protein fractions, it is interesting to note that hydrophobic amino acids such as Leu, Ile, Phe, Met, Val, Tyr and Ala in the present study in general disappeared to a lower extent than more hydrophilic amino acids such as Arg, Lys, Glu and Asp. Thus, their suggestion may be of some relevance for individual amino acids as well. This trend was the same for both cultivars.

4.3. Intestinal disappearance of N and AA

The results indicate that pre-incubation in the rumen does not influence the digestibilities obtained to any notable extent. It is a question if intestinal digestibility is independent of rumen incubation (Volden and Harstad, 1995); however, there are many published data that show for protein rich feedstuffs such as soybean meal and fish meal, rumen pre-incubation has no or minor effect on protein digestibility measured using the mobile bag technique, and pre-incubation is needed just for starch containing feeds (Hvelplund et al., 1992; Volden and Harstad, 1995).

The higher extent of disappearance of total AA than N for all feed samples is in accordance with the results of Dakowski et al. (1996), who found higher total AA digestibility compared with N for rapeseed meal, and the results of Skórko-Sajko et al. (1994) for roughages, but contradicts the results of Zebrowska et al. (1997) and Weisbjerg et al. (1996) who found the same intestinal digestibility for N and total AA in soybean meal and SoyPass®. Improvement in small intestinal digestibility of N and (or) AA has been reported for ruminants fed roasted (Tice et al., 1993) and extruded soybean (Stern et al., 1985). Chemical assays and rat growth models have indicated an improvement in the quantities of postruminally available lysine in roasted versus raw whole soybean (Faldet et al., 1992a). Results of the study by Aldrich et al. (1997) suggested that destruction of the trypsin inhibitor is probably responsible for increased small intestinal digestibility of N and AA that has been observed in cattle fed roasted or extruded whole soybean and in our experiment. The trypsin inhibitor is bound within the soybean and not subject to solubilization in ruminal fluid, but it is subject to ruminal degradation reflective of DM disappearance (Aldrich et al., 1997). The presence of trypsin inhibitor in the duodenum at 5 to 6 h followed by a large spike in trypsin concentration at 10 to 12 h after administration (Baintner et al., 1993) suggests that ruminants are susceptible to the effects of trypsin inhibitor reported for non-ruminants and that trypsin inhibitor in dietary ingredients can reach the small intestine.

Amino acid disappearance of undegraded protein varies, especially if the protein sources are treated to protect against degradation (Mupeta et al., 1997). In our experiment variations in digestibility of the individual AA of intact samples and residues after rumen incubation were found. However, heat processing of soybean increased the intestinal digestibility of all AA and this shows an effective protection of protein within the rumen without harmful effects on intestinal N and AA digestibility. These results agree with those reported by Aldrich et al. (1997) who found higher true digestibility of individual and total AA by cecectomized roosters with soybean roasted at various temperatures and extruded.

4.4. Total tract disappearance of N and AA

Total tract disappearance of N was not significantly different between raw and roasted seeds. However, total tract disappearance of total AA was higher for raw than for roasted seeds, due to higher AA disappearance of raw seeds, and roasting and steep-roasting were effective methods of changing the site of digestion from rumen to small intestine and therefore the amount of digestible undegraded AA in the small intestine can be increased. These results are in accordance with the results of Faldet et al. (1992b) and Erasmus et al. (1994).

5. Conclusions

Heat processing (roasting at 140–145 °C and roasting plus steeping for 45 min) of whole soybean reduced ruminal degradation of N and disappearance of individual and total AA and increased small intestinal disappearance of N and AA, perhaps due to the destruction of trypsin inhibitor. Consequently, these processing methods appear to shift the site of N and AA digestion from the rumen to the small intestine and increase the amount of undegraded AA digested in the small intestine. Steeping improved the small intestinal and total tract digestibility of N, total and individual AA beyond the effects of roasting. The determination of AA ruminal and intestinal disappearance of raw and especially heat processed whole soybean in this study may be useful when formulating diets.

Acknowledgements

Financial support was provided by the Excellent Centre for Animal Science at the University of Mashad in Iran and by the Centre for Nutrition Modelling at the University of Guelph in Canada.

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