

Determination of the Nutritive Value of Unheated vs. Heat Processed Grass Pea Seed in Ruminants

A. Riasi^{1*}, M. Golizadeh², M. H. Fathi², N. Asadzadeh³, and A. Taghizadeh⁴

ABSTRACT

Samples of grass pea seed (*Lathyrus sativus*) were prepared and analyzed for some chemical composition and as well for their anti-nutritional factors. The rumen degradability coefficients and post ruminal digestibility of dry matter and crude protein of unheated vs. oven heated samples were determined, employing *in situ* and *in vitro* techniques. Results indicated that grass pea seed contained a considerable level of crude protein (36%). The process of heating reduced ($P < 0.05$) tannins and Oxalyl DiAminoPropanoic acid (ODAP) content in grass pea seeds. Heat processing, especially 3 hours of heating, increased ($P < 0.05$) the slowly degradable fractions of the seed. Ruminant disappearance of DM and CP occurred at a lower rate ($P < 0.05$) for the heated grass pea seed than for the unheated seed. Heat processing did not affect the production of some such nutritional parameters (estimated through gas production method) as *DOM*, *ME*, *NE_L*, *SCFA* and *MP* production ($P > 0.05$). It was concluded that grass pea seed was of a substantial potential as a protein source in ruminant nutrition with its heat processing resulting in positive effects on its DM and CP digestibility.

Keywords: Chemical Composition, Digestibility, Heat processing, *Lathyrus Sativus*.

INTRODUCTION

The rapidly growing demand for livestock products in Iran and a parallel increase in the need for raising domestic animals have drawn the attentions towards new feed resources. Grass pea (*Lathyrus sativus*) is a plant that typically grows in tropical and subtropical regions. This plant is very well adapted to adverse climatic conditions requiring very little management for its production. Grass pea seed is a major source of protein for large sections of the population in Asia and Africa (Campbell, 1997; Hanbury *et al.*, 2000). Recently, ICARDA has developed new grass pea lines

with the objective of improving its yield potential as well as adaptability. In a study, Ahmadi *et al.* (2012) who tested some 14 genotypes of grass pea, reported that grain yield ranged from 0.41 to 2.23 t ha⁻¹ in different regions in Iran.

The seed contains a relatively high level of protein (19–32.5% of DM), with adequate concentrations of most macro and micro elements as well as amino acids, particularly lysine. It is low in fat (1.6%) but its starch content (41.2%) supplies abundant energy (Low *et al.*, 1990; Hanbury *et al.*, 2000; Trombetta *et al.*, 2006). Like the other legume seeds, it contains such anti-nutritional elements as tannins, lectins,

¹ Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Islamic Republic of Iran.

* Corresponding author: e-mail: ariasi@cc.iut.ac.ir

² Department of Animal Science, College of Agriculture, University of Birjand, Birjand, Islamic Republic of Iran.

³ Research Section, Isfahan Education Department, Isfahan, Islamic Republic of Iran.

⁴ Department of Animal Science, College of Agriculture, University of Tabriz, Tabriz, Islamic Republic of Iran.



phytate and oligosaccharides. It also contains a non-protein amino acid, ODAP (β -n-Oxalyl- L- α , β -DiAminoPropanoic acid) (Hanbury *et al.*, 2000; Trombetta *et al.*, 2006). It is reported that grass pea seed contains 0.22-7.2 g kg⁻¹ of ODAP (Campble, 1997) and there are some different processing methods that can reduce its ODAP content (Tadelle *et al.*, 2003). Among different legume seeds, it appears that grass pea seed contains a relatively high soluble, and rapidly degradable CP (Gonzalez and Andres, 2003).

To the best of our knowledge there is only limited data regarding the chemical composition and nutritive value of Iranian grass pea seed. The objective of this experiment was to study the chemical composition, rumen degradability parameters and post-ruminal digestibility of unheated *vs.* heated grass pea seed through *in situ* and *in vitro* methods.

MATERIALS AND METHODS

Grass pea seeds were obtained from local market, Birjand, Iran. The climate in Birjand is classified as dry arid with average annual rainfall of 169.4 mm and maximum annual temperature of 40°C. After removing dust and foreign materials, the pea samples were divided to four parts. The first portion was used as an unheated sample and the rest heated on a baking oven (Memmert, UFE700, Germany). A thin layer of each sample was spread on the flat plates of an oven for heat processing at 120°C for 1, 2

and 3 hours, intervals. The different times selected were to study the time needed for heat processing or roasting of grass pea seed.

Chemical Composition

The samples were milled, then passed through a 1 mm sieve before chemical analysis. Dry Matter (DM) was determined by drying the samples at 105°C overnight (12 hours). Nitrogen content was determined through Kjeldahl method (Kjeldahl method, Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hogans, Sweden), while the other contents assessed according to the following instructions: Neutral Detergent and Acid Detergent Fibers ([aNDF and ADF], Editorial 2005), ether extract ([AOAC 2000], ID 920.39), ash ([AOAC 2000], ID 942.05), NFC (calculated, NRC 2001), Ca, Fe, and Mg ([AOAC 2000], ID 968.08), P ([AOAC 2000], ID 965.17), Na and K ([AOAC 2000], ID 956.01) and Cl ([AOAC 2000], ID 943.01). Total and condensed tannin (Khazaal *et al.*, 1996) ODAP (Hussain *et al.* 1994) contents of the unheated *vs.* heated grass pea seeds were also determined.

In situ Ruminal Degradability of DM and CP

Three Holstein heifers with ruminal fistula made of soft plastic attached to, were made use of as experimental specimens. They

Table 1. Ingredients of Total Mixed Ration (TMR) (DM basis).

Ingredients	% of ration	Ingredients	% of ration
Alfalfa hay	37.52	Cottonseed meal	4.423
Corn silage	11.21	Wheat bran	9.532
Wheat straw	18.76	Beet molasses	0.931
Barley grain	8.76	Calcium carbonate	0.326
Corn grain	4.40	Mineral and vitamin premix ^a	0.311
Soybean meal	2.19	Salt	1.637

^a Composition of mineral and vitamin premix: Ca= 140 g kg⁻¹; P= 70 g kg⁻¹; Mg= 20 g kg⁻¹; Fe= 2.4 g kg⁻¹; Na= 70 g kg⁻¹; Cu= 0.3 g kg⁻¹; Mn= 2.6 g kg⁻¹; Zn= 2.4 g kg⁻¹; Co= 0.1 g kg⁻¹; I= 0.1 g kg⁻¹; Se= 0.01 g kg⁻¹; Vitamin A= 400,000 IU kg⁻¹; Vitamin D= 100,000 IU kg⁻¹; Vitamin E= 100 IU kg⁻¹.

were individually housed in a standard concrete floored pen. Table 1 shows the ingredient composition of the Mixed Ration (TMR) offered to the heifers in two equal feedings at 08:00 and 16:00 hours. The forage to concentrate ratio was 67.39:32.51. The unheated vs. processed samples (1.5 g DM) were weighed into 6×10 cm polyester bags (45 μ pore size), 8 bags being prepared for each sample and each incubation time. Ruminal incubation times were 2, 4, 8, 16, 24 and 48 hours. All bags were inserted at the same time, just before the morning feeding (*i.e.*, 08:00 and 16:00 hours). Bags representing 2 and 4 hours were soaked in water (39°C for 15 minutes), before incubation. At the end of each incubation period, bags were rinsed with cold tap water until the rinsing water became clear. Zero time disappearance was obtained by washing the un-incubated bags in a similar way. All washed bags were dried in a forced-air oven at 65°C for 48 hours. Disappearance of DM and CP at each incubation time was estimated from the proportion remaining after incubation in the rumen. The N concentration of sample residues was determined through Kjeldahl method ([AOAC 2000], ID 976.06H).

Ruminal, Post-ruminal and Total Tract DM and CP Digestibility

Ruminal DM and CP disappearance was determined following the incubation of the samples in the rumen. For this purpose and as mentioned before the samples (eight bags for each sample) were suspended in the rumen of the three Holstein heifers. The bags were removed from the rumen 12 hours after incubation. Nitrogen (N) concentration of pre-incubated and incubated samples was determined through Kjeldahl method. Following ruminal incubation, the Daisy II incubator procedure including pepsin and pancreatin digestions were employed to evaluate the post-ruminal disappearance of ruminally undigested DM and CP (Gargallo *et al.*, 2006). Total tract of DM and CP

disappearance was estimated based upon the ruminal and post-ruminal DM and CP disappearance (Gargallo *et al.*, 2006).

In vitro Gas Production

Two ruminally fistulated rams, used as rumen fluid donor, were fed at 8:00 and 17:00 hours daily a diet of alfalfa hay and whole barley (60:40, DM basis) at calculated maintenance energy requirements. Rumen fluid was collected before the morning feeding and strained through four layers of cheesecloth into a pre-warmed Thermos flask. The samples were incubated *in vitro* with the rumen fluid in calibrated glass syringes following the procedures of Menke *et al.* (1979). Approximately 200 mg samples of each substrate were weighed into 100 ml calibrated glass syringes and incubated in a water bath at 39°C with 10 ml of strained rumen fluid along with 20 ml of McDougall medium to determine the volume of Gas Production (GP) at 24 hours post-incubation. Each sample was incubated triplicate in two series and in different weeks of time. Total gas values were corrected for blank incubation.

Calculations and Statistical Analysis

Degradation of DM and CP was calculated using the equation of Ørskov and McDonald (1979) as: $P = a + b(1 - e^{-ct})$, where P is the disappearance rate at time t , a the rapidly degradable DM or CP fraction, b the slowly degradable DM or CP fraction in the rumen, c the rate constant of degradation of b , and t representing the time of incubation. Effective Degradabilities of DM (EDDM) and CP (EDCP) were estimated, using the equation of Ørskov and McDonald (1979) as: $EDDM \text{ or } EDCP = a + [b \times c / (c + k)]$, where k is the fractional outflow rate from the rumen (per hour) while a , b , and c as described above. The k values used to calculate $EDDM$ and $EDCP$ were 0.04, 0.05, and 0.06 h⁻¹ (Ørskov, 1979).



The values of Digestible Organic Matter (DOM), Metabolisable Energy (ME) and Net Energy of Lactation (NE_L) were calculated through the equation of Menke and Steingass (1988) as: $DOM (\%) = 9 + 0.9991 GP + 0.0595 CP + 0.0181 A$, $ME (MJ kg^{-1} DM) = 1.06 + 0.157 GP + 0.084 CP + 0.22 CL - 0.081 A$, and $NE_L (MJ kg^{-1} DM) = -0.36 + 0.1149 GP + 0.0054 CP + 0.0139 CL - 0.0054 A$. Short Chain Fatty Acids (SCFA) were determined by the equation reported by Makkar (2005) as: $SCFA (mmol 200 mg^{-1} DM) = 0.0222 GP - 0.00425$. The production of microbial protein (MP) was calculated as 19.3 microbial nitrogen per Kg DOM (Czerkawski, 1986). GP was the net gas production (ml 200 mg⁻¹ DM) after 24 hours incubation while CP, A and CL represented Crude Protein, Ash and Crude Lipid in g 100 g⁻¹ DM, respectively.

The data were analyzed through SAS (1993) using the mixed model procedure as a completely randomized design.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of unheated and heated grass pea seed are presented in Table 2. Results indicated that grass pea seed contained a relatively high CP (about 360 g kg⁻¹). The CP content of grass pea seed evaluated in this study was higher than that reported in the previous reports (Hanbury *et al.*, 2000; Trombetta *et al.*, 2006). Aletor *et al.* (1994) on selected lines of three *Lathyrus* species, reported that average CP content of *L. sativus* was 32.5%. The differences arising among studies may be related to genetic variations (Campbell, 1997). Grass pea seed contained higher levels of NFC, Na, Mg and Fe, but lower contents of EE, Ca, P, Cl and K as compared with the reported data for soybean meal (NRC, 2001). The other nutrients (ash, NDF, ADF, and hemicellulose) of grass pea seed were relatively the same as those reported for soybean meal (NRC, 2001). The sample

Table 2. Chemical composition (DM basis) of unheated vs. heated grass pea seed (n= 3, per sample).^a

Composition (g kg ⁻¹)	Grass pea seed				SEM
	Unheated	1 hour heated	2 hours heated	3 hours heated	
DM	930 ^b	950 ^a	953 ^a	952 ^a	4.1
CP	361	358	360	355	7.2
EE	13.2 ^a	9.7 ^b	9.6 ^b	9.5 ^b	1.1
Ash	42.4	43.0	43.1	44.4	1.5
aNDF	185.4	182.7	184.4	183.7	11.3
ADF	74.5	76.0	76.1	75.8	2.5
Hemicellulose	110.9	106.7	108.6	107.9	8.4
NFC	397.1	406.6	402.9	407.4	19.1
Ca	0.61	0.62	0.60	0.61	0.02
P	3.1	3.2	3.0	3.1	0.09
Na	2.6	2.8	2.8	2.5	0.04
Cl	0.5	0.5	0.48	0.47	0.10
K	14.2	14.8	14.5	14.5	0.9
Mg	8.4	8.6	8.6	8.2	0.8
Fe	0.413	0.420	0.409	0.431	0.03
Total tannin	0.323 ^a	0.173 ^b	0.194 ^b	0.231 ^{ab}	0.017
Condensed tannin	0.177 ^a	0.098 ^b	0.079 ^b	0.130 ^{ab}	0.019
ODAP ^b	3.84 ^a	2.89 ^b	2.93 ^b	2.88 ^b	0.33

^a Mean in the same row with different letters are different (P< 0.05), ^b β-Oxalyl-DiAminoPropionic acid.

studied here was generally low in Ca and P which confirms the previous data (Hanbury *et al.*, 2000; Low *et al.*, 1990).

Results indicated that the unheated grass pea seed contained lower levels of total and condensed tannins (0.323 and 0.177 g kg⁻¹, respectively). Deshpande and Campbell (1992) reported that condensed tannin of *L. sativus* lines ranged from 0 to 4.38 g kg⁻¹ and some varieties of grass pea seed may contain 7.7 g kg⁻¹ of condensed tannin (Hanbury *et al.*, 2000). It has been shown tannin content of grass pea seed is reduced through some different processing methods (Ramachandran and Ray, 2008). In the present study, 1 and 2 hours heat processing resulted in better effects in the reduction of total and condensed tannin of the seed. However, there was no difference observed among the 1, 2 and 3 hours of heat treatment as regards tannin contents.

Grass pea seed made use of in the current study may be considered as a medium ODAP containing variety. Campble (1997) reported that ODAP levels of grass pea seed varied from 0.22 to 7.2 g kg⁻¹. Thus a large range of variability in ODAP can exist in germplasm collection of grass pea. Hanbury *et al.* (2000) in a review reported that ODAP concentration can vary widely both within and between the species of grass pea seed. Heating the seeds at their different life times decreased ($P < 0.05$) the ODAP level. However, there was no difference observed between or among the duration times of heating as regards ODAP. Tadelle *et al.* (2003) who studied the effects of different processing methods on ODAP content of grass pea seeds reported that roasting had only marginal reduction effect on ODAP content, but soaking for 24 hours was slightly more effective and while cooking for 20 minutes resulted in clearly more reduction in ODAP. These findings confirm the results of the present study. Although it is reported the rumen may be a source of bacteria capable of metabolizing ODAP, however Peng and Brooker (2000) noted that the common ruminal *Prevotella* species and *P. ruminicola* B14, had no effect on

degradation of ODAP during *in vitro* incubation.

DM and CP Degradability

The degradability parameters and effective degradability of DM and CP of grass pea seeds are presented in Table 3. The rapidly degradable DM and CP fractions in the unheated grass pea seed was recorded as the highest (0.55 and 0.70) ($P < 0.05$) while increase in the time of heating process linearly decreased these fractions. High level of rapidly degradable DM fraction of the non-heated grass pea seed could be the result of its Non-Starch Carbohydrate (NSC) quantity and quality and its low NDF and ADF concentrations (Gonzalez and Andres, 2003). Gonzalez and Andres (2003) compared CP degradability fractions of different legume seeds. They reported that grass pea seed contained a relatively high soluble CP (about 50 g kg⁻¹ DM) as well as a rapidly degradable CP fraction (52.5%). The low values observed for slowly degradable CP fraction of the unheated samples may be attributed to the tannin content and/or the unknown anti-nutritional factors in the present study's samples (McSweeney *et al.*, 2001; Gurbuz *et al.*, 2008). Processing the seeds, especially 2 and 3 hours of heating, considerably increased ($P < 0.05$) slowly DM and CP degradable fractions. Literature information on the *Lathyrus* genus degradability and the effects of processing upon it are scarce. Overall, the rapidly degradable CP fraction of the unheated and the different time durations of heating grass pea seed were higher than those reported for soybean meal (Ørskov, 1992) which should be considered in ration balancing.

The *EDDM* and *EDCP* estimated at 0.04 h⁻¹ were not significantly different between the unheated and heated grass pea seeds. But, the values obtained at 0.05 and 0.06 h⁻¹ outflow rates were the highest ($P < 0.05$) for unheated grass pea seed. Madsen and Hvelplund (1987) observed a linear and quadratic response of *EDCP* to increase of

**Table 3.** *In situ* DM and CP degradation parameters and effective degradability of unheated and heated grass pea seed.

	Grass pea seed				S.E.M.
	unheated	1h heated	2h heated	3h heated	
Parameters ^a					
<i>a</i> (g/g)	0.55 ^a	0.46 ^b	0.40 ^{bc}	0.38 ^c	0.02
<i>b</i> (g/g)	0.45 ^b	0.49 ^{ab}	0.70 ^a	0.68 ^a	0.07
<i>c</i> (h ⁻¹)	0.03	0.02	0.02	0.02	0.01
Effective degradability (g/g) ^b					
EDDM4	0.73	0.61	0.61	0.59	0.06
EDDM5	0.71 ^a	0.59 ^b	0.58 ^b	0.56 ^b	0.04
EDDM6	0.69 ^a	0.57 ^{ab}	0.56 ^{ab}	0.54 ^b	0.05
Parameters					
<i>a</i> (g/g)	0.70 ^a	0.65 ^a	0.61 ^{ab}	0.55 ^b	0.03
<i>b</i> (g/g)	0.35 ^b	0.30 ^b	0.56 ^a	0.64 ^a	0.02
<i>c</i> (h ⁻¹)	0.02 ^b	0.05 ^a	0.02 ^b	0.02 ^b	0.01
Effective degradability (g/g) ^c					
EDCP4	0.81	0.81	0.80	0.75	0.05
EDCP5	0.81 ^a	0.80 ^a	0.76 ^{ab}	0.69 ^b	0.03
EDCP6	0.80 ^a	0.78 ^{ab}	0.74 ^{ab}	0.67 ^b	0.04

Mean in the same row with different letters are different (P<0.05).

^a *a*, rapidly degradable fraction; *b*, slowly degradable fraction; *c*, rate constant of degradation of *b* fraction.

^b EDDM, effective degradability of DM. EDDM4, EDDM5 and EDDM6 were calculated as $k = 0.04, 0.05, 0.06$ h⁻¹ ruminal, respectively (k is the ruminal outflow rate).

^c EDCP, effective degradability of CP. EDCP4, EDCP5 and EDCP6 were calculated as $k = 0.04, 0.05,$

0.06 h⁻¹ ruminal, respectively (k is the ruminal outflow rate).

buffer solubility of CP. The high values of *EDCP* of grass pea seed may be indicative of a high rumen microbial activity, which is not affected by its relatively low tannin content and the proportion of ADIN in total N. It is reported that the effects of tannins are associated with their ability to combine with dietary proteins, cell wall polymers such as cellulose, hemicellulose and pectin, as well as minerals thus either retarding or preventing their microbial digestion (Gonzalez and Andres, 2003; Gurbuz *et al.*, 2008; McSweeney *et al.*, 2001).

Ruminal, Post-ruminal and Total Tract DM and CP Digestibility

Mean ruminal, post-ruminal and total tract of DM and CP disappearance of unheated vs. heated grass pea seeds are presented in

Table 4. Results indicate that the unheated grass pea seed possesses the highest ruminal DM and CP disappearance (P< 0.05). The unheated seed bore a lower (P< 0.05) post-ruminal CP disappearance than the heated seeds. However, there was no difference observed between the times of heating from this stand point. These results showed that heat treatment may increase the intestinal availability of essential amino acids originated from the grass pea seed (Trombetta *et al.*, 2006).

Total tract DM and CP disappearance of grass pea seed was affected by the heat processing. However, 3 hours heated sample made no difference with the unheated one, making one believe that the lower ruminal DM and CP disappearances of 3 hours heated sample were compensated for in the intestine.

Table 4. Ruminal, post-ruminal and total tract disappearance of dry matter and crude protein (g kg⁻¹ of DM and CP, respectively) of unheated vs. heated grass pea seed.^a

	Grass pea seed					SEM
	Unheated	1 hour heated	2 hours heated	3 hours heated		
Ruminal ^b						
DM	758 ^a	573 ^b	570 ^b	566 ^b		51
CP	843 ^a	723 ^b	677 ^b	669 ^b		21.8
Post-ruminal ^c						
DM	404 ^b	428 ^b	412 ^b	562 ^a		57.8
CP	643 ^b	776 ^a	783 ^a	844 ^a		25.2
Total tract						
DM	848 ^a	754 ^b	748 ^b	812 ^{ab}		34.1
CP	944 ^{ab}	938 ^{ab}	931 ^b	948 ^a		3.5

^a Mean in the same row with different letters are different ($P < 0.05$); ^b DM and CP disappearance from polyester bags after 12 hours incubation in the rumen of steers, ^c Intestinal disappearance of the ruminal undegraded DM and CP.

In vitro Gas Production

The *in vitro* gas production parameters for unheated vs. heated grass pea seeds are presented in Table 5. Krishnamoorthy *et al.* (1995) suggested that *in vitro* gas production technique should be considered for estimated metabolizable energy in tropical feedstuffs. The results obtained here show that the heat processing did not affect the gas production parameters (GP, DOM, ME, NEL, SCFA, and MP) of grass pea seed. Because, gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate, the carbohydrate quality of the sample used here may have non-affected by heat processing. On the other hand, Gurbuz *et al.* (2008) who tested the effect of condensed tannin on *in vitro* digestibility of some forage legumes reported negative correlations between content of condensed tannin and gas production, *DOM* and *ME*. The disagreement may be attributed to the low condensed tannin content in the samples used herein this research.

The 24 hours *GP* volume from the present samples was higher than those in the data reported by Maheri-Sis *et al.* (2008) for two

other tropical legume seeds (Kabuli chickpea= 78.66 and Des chickpea= 73.96 Ml 200 mg⁻¹ DM). Getachew *et al.* (2004) reported that feed CP level was negatively correlated with gas production. However, other studies with different types of feeds (i.e., CP ranging from 32 to 487 g kg⁻¹ DM) have revealed no effect of CP level on gas production (Blümmel *et al.*, 1999).

High correlation has been observed between Short Chain Fatty Acid (SCFA) and gas production (Beuvink and Spoelstra, 1992). However, the grass pea seeds of higher *GP* values used in the present study were of a lower *SCFA* production potential in comparison with Kabuli chickpea and Des chickpea as reported by Maheri-Sis *et al.* (2008). These discrepancies could be due to differences between chemical compositions of the tropical seeds (especially their soluble carbohydrates, CP, NFC and NDF) (Menke and Steingass, 1988; Getachew *et al.*, 2004). Paya *et al.* (2007) using *in vitro* gas production reported that *ME* and *SCFA* production potential of corn grain were 2.4 Mcal Kg⁻¹ DM and 0.98 mmol 200 mg⁻¹ DM, respectively. These data are close to *ME* and *SCFA* of grass pea seed that should be considered in the future studies. The *ME* of grass pea seed was near to the data reported for sunflower meal (2.3 and 2.24 Mcal kg⁻¹, respectively). However the *NE_L*

**Table 5.** *In vitro* gas production parameters from unheated vs. heated grass pea seed.^a

Parameters ^b	Grass pea seed				SEM
	Unheated	1 hour heated	2 hours heated	3 hours heated	
Gas volume (ml 200 mg ⁻¹ DM)	177.6	172.7	177.0	178.0	5.3
DOM (g Kg ⁻¹ DM)	467.1	457.2	465.9	467.6	12.3
ME (Mcal Kg ⁻¹ DM)	2.3	2.24	2.27	2.26	0.19
NE _L (Mcal Kg ⁻¹ DM)	0.93	0.91	0.93	0.93	0.1
SCFA (mmol 200 mg ⁻¹ DM)	0.784	0.763	0.782	0.786	0.1
MP (g kg ⁻¹ DOM)	56.38	55.15	56.2	56.4	5.5

^a Mean in the same row with different letters are different ($P < 0.05$); ^b GP= Net Gas Production after 24 hours incubation; DOM= Digestible Organic Matter; ME= Metabolisable Energy; NE_L= Net Energy of lactation; SCFA= Short Chain Fatty Acid, and MP= Microbial Protein production.

of grass pea seed was noted lower than those reported for most of such protein sources as sunflower (0.93 vs. 1.38 Mcal kg⁻¹), or soybean meal (0.93 vs. 2.13 Mcal kg⁻¹) (NRC, 2001).

CONCLUSIONS

The CP content of grass pea seed evaluated in this study (36.1%) was higher than that reported in the previous works (32.5%), so it can be considered as a protein source in animal nutrition. Grass pea seed carried low levels of total and condensed tannins. However, heat processing reduced its tannins' contents. Non heat-treated grass pea seed contained higher rapidly degradable portion of CP. Heat treatment decreased the rapidly degradable DM and CP fractions of grass pea seed. Ruminal, post ruminal and total tract disappearance of DM and CP of grass pea seed was affected by heat processing. Heat processing for different time intervals decreased the CP disappearance in rumen, but it increased the total tract digestibility of CP after 3 hours of heating. The results indicated that the processing did not affect the estimated energy availability and microbial protein production in the seed. Other processing methods to enhance the nutritive value of grass pea seed should be put to test in the future studies.

REFERENCES

1. Ahmadi, J., Vaezi, B., Shaabani, A. and Khademi, K. 2012. Multi-environment Yield Trials of Grass Pea (*Lathyrus sativus* L.) in Iran Using AMMI and SREG GGE. *J. Agr. Sci. Tech.*, **14**: 1075-1085.
2. Aletor, V. A., Abd El Moneim, A. M. and Goodchild, A. V. 1994. Evaluation of the Seeds of Selected Lines of Three *Lathyrus* Species for b-N-Oxalylyamino-L-Alanine (BOAA), Tannins, Trypsin Inhibitor Activity and Certain *In-vitro* Characteristics. *J. Sci. Food Agric.*, **65**: 143-151.
3. AOAC. 2000. *Official Methods of Analysis*. 17th Edition, Official Methods of Analysis of AOAC International, Gaithersburg, MD, USA.
4. Beuvink, J. M. W. and Spoelstra, S. F. 1992. Interactions between Substrate, Fermentation and Products, Buffering Systems and Gas Production upon Fermentation of Different Carbohydrates by Mixed Rumen Microorganisms *In vitro*. *Appl. Microbiol. Biotechnol.*, **37**: 505-509.
5. Blummel, M., Aiple, K. P., Steingass, H. and Becker, K. 1999. A Note on the Stoichiometrical Relationship of Short Chain Fatty Acid Production and Gas Evolution *In vitro* in Feedstuffs of Widely Differing Quality. *J. Anim. Physiol. Anim. Nutr.*, **81**: 157-167.
6. Campbell, C. G. 1997. Grass Pea. International Plant Genetic Resources Institute. IPK, Corrensstrasse 3. 06466 Gatersleben, Germany.

7. Czerkawski, J. W. 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, UK, PP236.
8. Deshpande, S. S. and Campbell, C. G. 1992. Genotype Variation in BOAA, Condensed Tannins, Phenolics and Enzyme Inhibitors of Grass Pea (*Lathyrus sativus*). *Can. J. Plant Sci.*, **72**: 1037-1047.
9. Udén, P., Robinson, P. H. and Wiseman, J., 2005: Use of Detergent System Terminology and Criteria for Submission of Manuscripts on New or Revised, Analytical Methods as well as Descriptive Information on Feed Analysis and/or Variability. *Anim. Feed Sci. Technol.*, **118**: 181-186.
10. Gargallo, S., Calsamiglia, S. and Ferret, A. 2006. Technical Note: A Modified Three-step *In vitro* Procedure to Determine Intestinal Digestion of Proteins. *J. Anim. Sci.*, **84**: 2163-2167.
11. Getachew, G., Robinson, P. H., De Peters, E. J. and Taylor, S. J. 2004. Relationships between Chemical Composition, Dry Matter Degradation and *In vitro* Gas Production of Several Ruminants Feed. *Anim. Feed Sci. Technol.*, **111**: 57-71.
12. Gonzalez, J. and Andres, S. 2003. Rumen Degradability of Some Feed Legume Seeds. *J. Anim. Res.*, **52**: 17-25.
13. Gurbuz, Y., Kaplan, M. and Davies, D. R. 2008. Effect of Condensed Tannin Content on Digestibility and Determination of Nutritive Value of Selected Some Native Legumes Species. *J. Anim. Vet. Adv.*, **7(7)**: 854-862.
14. Hanbury, C. D., White, C. L., Mullan, B. P. and Siddique, K. H. M. 2000. A Review of the Potential of *Lathyrus sativus* L. and *Lathyrus Cicera* L. Grain for use as Animal Feed. *J. Anim. Feed Sci. Technol.*, **87**: 1-27.
15. Hussain, M., Chowdhury, B., Haque, A., Wouters, G. and Campbell, C. G. 1994. A Comparative Study of the Orthophthalaldehyde Method for Neurotoxin, 3-N-Oxalyl-2,3-DiAminoPropionic Acid (ODAP) as Modified by Various Laboratories. *Phytochem. Anal.*, **5**: 247-250.
16. Khazaal, K., Parissi, Z., Tsiouvaras, C., Nastis, A. and Orskov, E. R. 1996. Assessment of Phenolics-related Antinutritive Levels Using the *In vitro* Gas Production Technique: A Comparison between Different Molecular Weight of Polyvinylpyrrolidone or Polyethylene Glycol. *J. Sci. Food. Agric.*, **71**: 405-414.
17. Krishnamoorthy, U., Soller, H., Steingass, H. and Menke, K. H. 1995. Energy and Protein Evaluation of Tropical Feedstuffs for Whole Tract and Ruminant Digestion by Chemical Analysis and Rumen Inoculum Studies *In vitro*. *Anim. Feed Sci. Technol.*, **52**:177-188.
18. Low, R. K. C., Rotter, R. G., Marquardt, R. R. and Campbell, G. C. 1990. Use of Khesari (*Lathyrus sativus*) in Chicks Diets. *J. Brit. Poult. Sci.*, **31**: 615-625.
19. Madsen, J. and Hvelplund, T. 1987. Values of Feed Protein Degradability and Possible Methods for Its Estimation in Routine Laboratories. In: "*Feed Evaluation and Protein Requirements Systems for Ruminants*", (Eds.): Jarrige, R. and Alderman G., Commission of the European Communities, Brussels, PP. 97- 110.
20. Maheri-Sis, N., Chamani, M., Sadeghi, A.A., Mirza-Aghazadeh, A. and Aghajanzadeh-Golshani, A. 2008. Nutritional Evaluation of *Kabuli* and *Desi* Type Chickpeas (*cicer arietinum* L.) for Ruminants Using *In vitro* Gas Production Technique. *Afri. J. Biotechnol.*, **7(16)**: 2946-2951.
21. Makkar, H. P. S. 2005. *In vitro* Gas Methods for Evaluation of Feeds Containing Phytochemicals. *Anim. Feed Sci. Technol.*, **123-124**: 365-377.
22. McSweeney, C. S., Palmer, B. D., McNeill, M. and Krause, D. O. 2001. Microbial Interactions with Tannins: Nutritional Consequences for Ruminants. *Anim. Feed Sci. Technol.*, **91**: 83-93.
23. Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. The Estimation of the Digestibility and Metabolizable Energy Content of Ruminant Feeding Stuffs from the Gas Production When They Are Incubated with Rumen Liquor. *J. Agric. Sci.*, **93**: 217-222.
24. Menke, K. H. and Steingass, H. 1988. Estimation of Energetic Feed Value Obtained from Chemical Analysis and *In vitro* Gas Production Using Rumen Fluid. *Anim. Res. Dev.*, **28**: 7-55.
25. National Research Council (NRC). 2001. *Nutrient Requirements of Dairy Cattle*. Seventh Revised Edition, National Academic Science, Washington, DC, USA, PP381.
26. Ørskov, E. R. and McDonald, I. 1979. The Estimation of Protein Degradation in the



- Rumen from Incubation Measurements Weighted According to Rate of Passage. *J. Agric. Sci.*, **92**: 499-503.
27. Ørskov, E. R. 1992. *Protein Nutrition in Ruminants*. Academic Press, London, UK, PP.175.
28. Paya, H., Taghizadeh, A., Janmohammadi, H. and Moghadam, G.A. 2007. Nutrient Digestibility and Gas Production of Some Tropical Feeds Used in Ruminant Diets Estimated by the *In vivo* and *In vitro* Gas Production Techniques. *Am. J. Anim. Vet. Sci.*, **2(4)**: 108-113.
29. Peng, H. H. and Brooker, J. D. 2000. Isolation of ODAP-degrading Bacteria from the Sheep Rumen. *Lathyrus Lathyrism Newsletter*, **1**: 33.
30. Ramachandran, S. and Ray, A. K. 2008. Effect of Different Processing Techniques on the Nutritive Value of Grass Pea, *Lathyrus sativus* L., Seed Meal in Compound Diets for Indian Major Carp Rohu, Labeo Rohita (Hamilton), Fingerling. *Arch. Pol. Fish.*, **16**: 189-202.
31. SAS Institute Inc. 1989. *SAS/STAT User's Guide: Version 6*. 4th Edition, SAS Institute Inc., Cary, North Carolina.
32. Trombetta, M. F., Mattii, S., Pasquini, M. and Falaschini, A. 2006. Evaluation of the Digestibility of *Lathyrus sativus* in Growing Pigs. *Ital. J. Anim. Sci.*, **5**: 147-153.
33. Tadelle, D., Alemu, Y., Nigusie, D. and Peters, K. J. 2003. Evaluation of Processing Methods on the Feeding Value of Grass Pea to Broilers. *Inter. J. Poult. Sci.*, **2(2)**: 120-127.

تعیین ارزش تغذیه ای دانه خلر خام و حرارت داده شده برای نشخوارکنندگان

۱. ریاسی، م. گلی زاده، م. ح. فتحی، ن. اسد زاده، و ا. تقی زاده

چکیده

نمونه هایی از دانه خلر (*Lathyrus sativus*) جمع آوری شد و برخی ترکیبات شیمیایی و فاکتورهای ضد تغذیه ای آن اندازه گیری گردید. ضرایب تجزیه پذیری و قابلیت هضم پس از شکمبه ای ماده خشک و پروتئین خام دانه های حرارت داده شده و حرارت داده نشده با روش های درون کیسه ای و آزمایشگاهی تعیین شد. نتایج به دست آمده نشان داد که دانه خلر دارای مقدار قابل توجهی پروتئین خام (حدود ۳۶ درصد) است. محتوی تانن و اگزالیل دی آمینوپروپانوئیک اسید (ODAP) دانه خلر حرارت داده شده بطور معنی داری ($P < 0.05$) کاهش یافت. حرارت دادن، بویژه به مدت ۳ ساعت، بخش کند تجزیه دانه خلر را افزایش ($P < 0.05$) داد. نرخ ناپدید شدن شکمبه ای ماده خشک و پروتئین خام دانه های خلر حرارت داده شده کمتر از نمونه خام بود ($P < 0.05$). حرارت دادن بر برخی پارمترهای تغذیه ای برآورد شده با روش تولید گاز شامل قابلیت هضم ماده آلی، انرژی قابل متابولیسم، انرژی خالص شیردهی، قابلیت تولید اسیدهای چرب فرار و تولید پروتئین میکروبی تاثیر معنی داری نداشت ($P > 0.05$). نتایج به دست آمده بیانگر پتانسیل خوب دانه خلر به عنوان یک منبع پروتئینی در تغذیه نشخوارکنندگان بود و حرارت دادن اثرات مثبتی بر قابلیت هضم ماده خشک و پروتئین خام آن داشت.