Evaluation of Nutritive Value of Grass Pea Hay in Sheep Nutrition and Its Palatability as Compared with Alfalfa

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ABSTRACT

The nutritive value of grass pea (*Lathyrus sativus* L.) hay was evaluated based on its chemical composition, Gas production, fractioning of protein in CNCPS and AFRC systems, Metabolizable Energy (ME), rumen degradability through *in situ* technique and *in vitro* digestibility through Tilley and Terry method. The Crude Protein (CP), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) values of grass pea hay amounted to 232.4, 397 and 300.6 (g kg^{-1} DM) respectively. Condensed Tannins (CTs) and β-N-Oxalyl –L-α, β-diAmino Propionic acid (ODAP) amounted 0.2 and 11.8 (g kg^{-1} DM) respectively. Grass pea estimated ME ranged from 6.86 (MJ kg^{-1} DM) to 12.03 (MJ kg^{-1} DM) by different methods. Metabolizable Protein (MP) content was found 534.7 g kg^{-1} of CP. A high level of CP and MP content followed by a high content of ME along with a high palatability, cause grass pea to be introduced as an alternative to sheep forage in drought conditions.

Keywords: Condensed tannins, Grass pea, Nutritive value, ODAP, Palatability.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) has been a traditional crop used for both animal consumption as forage and grain, and for human consumption as food. The main qualities of this legume grain consist of its sturdiness, drought tolerance, and adaptability to a wide range of soil types, including the marginal ones (Yan *et al.,* 2006). In addition, high protein content makes this species be considered as a forage crop; indeed, economic yield under adverse environmental conditions has made grass pea a popular crop in subsistence farming in certain developing countries that suffer from extremely adverse weather conditions (Praveen *et al.*, 1994). Although rich in protein, the utilization of grass pea grain has been limited by the presence of a water-soluble non-protein amino acid, β-N-OxalylDiAminoPropionic acid (β-ODAP).

This Anti-Nutritional Factor (ANF) acts as a neurotoxin, crippling the lower limbs when consumed in large quantities during a prolonged period. It can cause the disease neurolathyrism, in monogastric animals and in humans (Hanbury *et al.*, 2000). This has led to the crop being excluded from agricultural improvement efforts. The other most frequently occurring ANFs in this legume are tannins, protease and amylase inhibitors, lectins, saponins, alkaloids, non-starch polysaccharides, vicine and convicine, as well as phytate (Hanbury *et al*., 2000). Environmental condition and the presence of such natural enemies as pests and insects can increase the ANFs in plant (Hanbury *et al.*, 1999). Most of these compounds are degraded in the rumen and so are not considered as serious nutritional problems for ruminants. However, condensed tannin (CT) can affect protein and cell wall digestibility (Scharenberg *et*

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al., 2007). Poland *et al*. (2003) reported that grass pea produces hay that is comparable with alfalfa hay in its nutrient make-up. No adverse effects were observed in gestating ewes fed grass pea hay instead of alfalfa hay. Certain *Lathyrus* species have recently been found suitable as multipurpose legume crops in Iran (Ahmadi *et al*., 2012). Nevertheless, data on nutrient content of grass pea hay with seedpods (GPHP) are still quite scarce as investigations are mostly concentrated on grass pea seeds and their nutritive values. There are only a small number of published studies that have determined nutritive value of GPHP. For these reasons, the present research aimed at the characterization and evaluation of GPHP nutritive value and its palatability as compared with alfalfa.

MATERIALS AND METHODS

Experimental Forage

Grass pea forage samples were taken from farms in Zanjan, Iran (latitude: 36° 12´ N, longitude: 49° 11´ E, altitude: 1570 m). Grass pea had been sown in mid August 2007, and harvested at its full-flowering stage. Representative samples were obtained from the core and edges of at least 25- 30 bales (1 kg from each) of dried forages. Forages were then cut into 3-5 cm pieces with subsamples taken for chemical analysis, through *in vitro* and *in situ* experiments. Same forage samples were used in *in vivo* digestibility trials and palatability determinations.

Laboratory Analysis

Dry matter was determined by the samples being dried at 105°C overnight and their ash by having the samples ignited in muffle furnace (AOAC, 2000). Ether Extract (EE) content was determined through Sokstech automated apparatus (AOAC, 2000), Total nitrogen (N) content through Kjeldahl method while crude protein determined as $N \times 6.25$. Neutral Detergent Fiber (NDF), NDFom and Acid Detergent Fiber (ADF) contents were also determined using automated Fibertech Foss Tecator 1010 apparatus (Van Soest *et al*., 1991), Neutral Detergent Insoluble Nitrogen (NDIN), Acid Detergent Insoluble Nitrogen (ADIN), were determined by measuring nitrogen contents of NDF and ADF, as described above. Sodium sulfite was not included in Neutral Detergent Solution (NDS). Mineral composition was determined using atomic absorbency according to Miles *et al*. (2001). The β-ODAP content was determined through HPTLC method according to Tarade *et al*. (2007) procedure. Standard β-ODAP was supplied by Lathyrus Technologies, Hyderabad, India. β-ODAP was extracted, using water and HCL. The acetonitrile was then densitometrically determined at 500 nm on Camag II densitometer. Total extractable Phenolics (TP), Total Tannin (TT) and Condensed Tannins (CT) were determined using folin-ciocalteu reagent, insoluable PolyVinyl PolyPyrrolidone (PVPP) and butanol-HCl method as described by Makkar (2000). All chemical analyses were carried out in triplicate except for TP, TT and CT, which were replicated 10 times. Relative Feed Value (RFV) was obtained from the estimates of Dry Matter Digestibility (DMD) and Dry Matter Intake (DMI) (Moore and Undersander, 2002):

DMI (% BW)= 120/(NDF, % DM)

DMD (% DM) = 88.9-0.779 (ADF, % DM)

RFV= (% DDM×%DMI)/1.29

or

RFV= [(88.9-0.78×ADF%))×(120/NDF%)]/1.29

Gas Production and Estimated Parameters

Rumen fluid was obtained from four rumen fistulated animals fed twice daily with a diet containing, alfalfa hay (60%) and

concentrate (40%). The forage samples (200 mg dry weight) were incubated in triplicate in rumen fluid in calibrated glass syringes following the procedures of Menke *et al.,* (1979) using 100 ml calibrated glass syringes. Rate and extent of gas production was determined by reading gas volumes before incubation (0) and 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. Cumulative gas production data were fitted to the exponential equation of Ørskov and McDonald (1979):

$$y= a+b \ (1-e^{-ct})$$

Where, *y* is the gas produced at time "*t*"; *a* the gas production from the immediately soluble fraction (ml); *b* gas production from the insoluble fraction (ml); *c* the gas production rate constant for the insoluble fraction (*b*); *a+b* the potential gas production (ml), *t* the incubation time (h). The metabolizable energy (ME MJ kg^{-1} DM) content of forage was calculated using equations of Menke and Steingass (1988) as follows:

ME (MJ kg^{-1} DM)= 15.33-0.0152 MADF(g kg^{-1} DM)

ME (MJ kg^{-1} DM)= 2.2+0.136 GP_{24}+0.0057CP+0.00029 CF^2

ME (MJ kg^{-1} DM) =2.2+0.136 GP24+0.057CP+0.0029 CP^2

OMD (%)=14.88+0.889 GP_{24}+0.45 CP+0.0651 ash

DOMD (%)= OMD (%)×OM (%)

ME (MJ kg^{-1} DM) = 0.0157 DOMD (%)

Where, *GP_{24}* represents 24 hours net Gas Production (ml 200 mg^{-1}); *CF*: Crude Fat; *CP*: Crude Protein, *MADF*: Modified ADF.

The potential Dry Matter Intake (DMI kg day^{-1}) of grass pea was determined while using the equation of Blümmel and Ørskov (1993) as follows:

DMI (kg day^{-1})= 1.66+0.49a+0.0297b-4c

CNCPS Protein Fractioning and Metabolizable Protein System

Protein fractioning of grass pea was determined according to procedures by Licitra *et al.,* (1996). *A* and *B1* fractions

were determined through TriChloroacetic Acid (TCA) and borate-phosphate buffer, respectively. *B2* fraction was calculated through the following formula:

B2= CP-(A+B1+B3+C)

C fraction was obtained as the ADIN and *B3* fraction calculated through the difference between NDIN and ADIN. Metabolizable Protein (MP), Rumen Degraded Protein (RDP), Undegraded Dietary Protein (UDP), Digestible Undegraded Protein (DUP) and Effective Rumen Degradable Protein (ERDP), Quickly Degraded Protein (QDP) and Slowly Degraded Protein (SDP) were estimated according to AFRC (1992) equations, using *in situ* CP degradation data.

In situ Disappearance

The degradability of samples was determined through nylon bag technique, using three rumen fistulated sheep (57±9 Kg BW) as described by Vanzant *et al.* (1998). The sheep were fed twice daily on a 60% hay and 40% concentrate (Table 1); 10% above maintenance requirements.

Throughout the experimental period Dacron bags of 40-45 µm pore size were filled with 3 g of sample, in duplicate, and incubated for 4, 8, 12, 24, 48, 72 and 96 hours in the rumen of each sheep. Following incubation, bags were removed from the rumen and rinsed with cold tap water, until the rinsing water clear. DM, OM, CP, N*DF* and NDFom degradation data were fitted to the exponential model of Ørskov and McDonald (1979) in which:

$$P= a+b \ (1-e^{-ct})$$

Where, *p* is rumen disappearance at time *t*, *a* the rapidly soluble fraction, *b* the potentially degradable (fermentable) fraction, and *c* the constant rate of degradation of the *b* (percentage per hour).The kinetic parameters were estimated using a NEWAY program from Rowett Research Institute, Aberdeen, UK. The effective degradability (P) of *DM* and protein samples were calculated using the equation of Ørskov and McDonald (1979):

Table 1. Formulation of the diet used for nylon bag and for Tilley and Terry trials (g kg^{-1} DM basis).

Ingredient	Dietary percentage	Ingredient	Dietary percentage
Alfalfa hay	150.7	Wheat bran	60.0
Corn silage	266.7	Calcium-carbonate	8.0
Wheat straw	182.7	Min/Vitamin premix[a]	8.0
Barley grain	260.0	Salt	4.0
Canola meal	60.0		

[a] Each kg composed of: Vitamin A: 500,000 IU; Vitamin D3: 100,000 IU; Vitamin E: 100 mg; Ca: 190,000 mg; P: 90,000 mg; Na: 50,000 mg; Mg: 19, 000 mg; Fe: 3,000 mg; Cu: 300 mg; Mn: 2,000 mg; Zn: 3,000 mg; Co: 100 mg; I: 100 mg; Se: 1 mg, Antioxidant (B.H.T): 3,000 mg.

$$P= a+[(b \times c)/(c+k)]$$

Where, k is the rate of particulate outflow from the rumen of 0.05 h^{-1}.

In vitro Digestibility

In vitro digestibility of grass pea was determined according to Tilley and Terry (1963) procedure. Samples were incubated in triplicate with three jars containing only rumen liquor. DM and OM digestibility were corrected for the blank. Rumen liquor was obtained from one Varamini fistulated sheep that was fed with same diet as those assigned to nylon bag experiment (Table1). Half a mg of sample (milled through 1 mm sieve), was incubated with rumen fluid and pepsin in two steps. After 96 hours past, digested residue was filtered through Watman ashless 41-pore size filter paper, dried at 105°C overnight and ignited in muffle furnace at 525°C for 4.5 hours, to be used for a determination of DM and OM digestibility, respectively. Metabolizable Energy (ME) (MJ kg^{-1} DM) content was estimated using AFRC (1995) equation:

ME= 0.0157×DOMD

Where, *DOMD* is the rate in gram of digestible OM in kg of DM.

In vivo Digestibility Experiment

Three Varamini rams (83±9 kg BW) were taken as specimens to determine the apparent nutrient digestibility of grass pea. Digestibility trial included 10 days for adaptation and 7 days as collection period. Animals were kept in individual pens equipped with rubber mat bed. They were fed twice a day (0800 and 1600), with a sole diet which corresponded to 10% above maintenance requirements. CNCPS software (CNCPS, 2004) was made use of in computations. Fresh water and salt licking blocks were all times available. Total fecal collection and AIA, as an internal marker, were used to determine apparent digestibility of OM, CP, ADF, NDF and as well NDFom. Along with fecal samples, diet samples were also daily collected. Samples were stored at -20°C until were taken during the collection period. *AIA* was determined according to Van-Keulen and Young (1977) procedure.

Palatability Trial

Six Varamini ewes (45±5 kg) were taken for an evaluation of palatability of grass pea and its comparison with control (afalfa). The ewes were kept in individual pens bedded on rubber mat, while being fed twice a day (4:00 PM and 8:00PM). Fresh water and salt licking blocks were all times available. The ratio of the two forages (grass pea and alfalfa) were calculated through CNCPS, while each meal covering 55% of maintenance energy requirement, where test plant *vs.* control were each providing exactly half of the *ME*. Palatability test started with the morning of the first feeding day and ended after the morning of day 10. The first 3 days were considered as the preliminary period. The two meals were offered in two

identical troughs. The allocation of the two different forages was switched between troughs in the evening meal, to avoid any association of place, forage type and day-time by the animals. To evaluate the palatability of the grass pea in comparison with alfalfa as the control forage (Ctr), the intakes of the two diets were assessed by weighing the boxes at a fixed time (t) after the start of feeding. Based on preliminary tests, *t* was set at 10 minutes after feeding. This period was approximately equivalent to the time needed by the sheep to consume about half of the total feed. At last, the Palatability Index (PI) was calculated according to Ben Salem *et al*. (1994). The *PI* relates the quantity of test plant consumed to that eaten of Ctr (alfalfa) and is calculated based upon the following formula:

PI (t)= [ITT (t)/ICtr (t)]×100

Where, *ITT* (t) is the intake of test plant (grass pea) eaten after time t per total intake of test plant eaten after half a day; *ICtr* (t) intake of control (alfalfa) eaten after time (t) per total intake of control eaten after half a day , thus accounting for the differences in total intake.

Statistical Analysis

Means of estimated ME of grass pea, based on different methods, OMD contents determined by different methods and as well the digestibility coefficients related to different methods were compared using GLM procedure of SAS 9.1. Duncan's multiple range test, was conducted for a comparison of ME contents *vs*. digestibility coefficients of grass pea as based on different methods. All the statements of significance were based on the probability level of 0.05. The following model was employed to determine the effects of methods:

$Yi= \mu + Ti + ei$

Where, μ is the overall mean, Tj the fixed effect of determination method (*in vitro* digestibility, gas production and *in vivo* digestibility), and while ei representing the residual errors.

RESULTS

Chemical Composition

Chemical composition of GPHP is given in Table 2. Experimental plant contained a high level of ODAP (11.8 g kg^{-1} DM) but low CT content (0. 2 g kg^{-1} DM). Condensed tannin constituted less than 2% of total tannins. The experimental plant showed a high concentration of CP (232.4 g kg^{-1} DM). A high most portion of total nitrogen was available (94% of total nitrogen) and ADIN content was 2.2 (g kg^{-1} DM). *RFV* index of grass pea hay was 153.43, with regard to NDF, ADF and CP contents, experimental forage had high quality. As shown in Table 3, the concentration of Fe and Zn were high and low in GPHP, respectively.

Table 2. Chemical composition (g kg^{-1} DM) of GPHP.[a]

Composition		Composition		Composition	
DM	953.7	NDFom	383.3	CP	232.4
OM	908.3	Hemicelluloses	96.5	Total nitrogen	37.2
Ash	87.5	TP	13.4	NDIN	4.2
EE	43.0	TT	12.6	ADIN	2.2
ADF	300.6	CT	0.2	Available nitrogen	35.0
NDF	397.0	β-ODAP	11.8	RFV	153.43

[a] Reported values are means of triplicate and in the case of phenolics, compounds' mean of 10 replicates. NDIN: Neutral Detergent Insoluble Nitrogen; ADIN: Acid Detergent Insoluble Nitrogen; TP: Total Phenolic Compounds; TT: Total Tannins; CT: Condensed Tannins; β-ODAP: β-N-Oxalyl –L-α, β-DiAminoPropionic acid, RFV: Relative Forage Value.

Table 3. Mineral contents of *L. sativus* (DM basis).

Mineral (g kg^{-1})		Mineral (mg kg^{-1})	
Ca	4.7	Mn	290.8
P	1.5	Zn	11.8
Mg	2.0	Fe	143.3
		Cu	1.8

Gas Production and Estimated Parameters

Data related to gas production during the fermentation period are given in Table 4. The cumulative volume of gas production increased with increasing time of incubation. Gas production after 96 hours of incubation amounted to 33.39±1.1. Estimated OMD and DMI contents were recorded as 481.8±18.6 (g kg^{-1} DM) and 4.82±0.223 (kg day^{-1}), respectively.

CNCPS Fractioning of Protein and Metabolizable Protein System

Table 5 shows *A, B1, B2, B3* and *C* fractions of protein in grass pea hay equal to 107.8±10.8, 266.5±26.7, 511.6±51.2, 54.5±5.5 and 59.5±5.9 (g kg^{-1} CP), respectively. The highest fraction of GPHP crude protein was *B2* that is potentially degradable in rumen. According to

Metabolizable protein system results, rumen degradable protein (828.3±11.4, g kg^{-1} CP) was higher than the undegradable one (171.7±11.4, g kg^{-1} CP) in the experimental plant.

In situ Disappearance

Table 6 shows the kinetics of rumen degradation parameters and as well the effective degradability of DM, OM, CP, NDF and NDFom obtained through *in situ*. Disappearance of all nutrients raised, as incubation time increases. CP degradation was (more intensively but less extensively) from 72.04±2.38 to 90.92±1.24 as compared with the degradation of DM, OM, NDF and NDFom. The rumen degradation of CP as regards GPHP was characterized by (i) high values of the soluble fraction (0.74±0.00 g g^{-1}), (ii) high values of the fractional degradation rate (0.103±0.03 h^{-1}), and (iii) high effective degradability at any of the rumen flow rates (from 0.79±0.01 to 0.84±0.01 g g^{-1}). The effective degradability of NDF and NDFom had low values at all of the rumen flow rates (0.02, 0.05 and 0.08 h^{-1}).

In vitro Digestibility and OMD Comparison

The levels of DM, OM and OM in DM

Table 4. Gas production and estimated parameters of *L. sativus* hay incubated with rumen fluid.[a]

IT	GP(ml)	Estimated parameters	
4	2.58±0.4	*a* (g g^{-1})	0.05±0.004
8	8.84±0.9	*b* (g g^{-1})	0.33 ±0.01
12	15.04±1.4	*a+b* (g g^{-1})	0.38±0.014
24	25.12±1.3	*c* (h^{-1})	0.059±0.00
48	30.44±1.1	Lag time	2.33±0.061
72	32.42±1.1	OMD	481.8±18.6
96	33.39±1.1	DMI	4.82±0.223

[a] Reported values are means of triplicates±standard deviations. IT: Incubation Time (h); GP: Gas Production*; a*: Gas production from the immediately soluble fraction (ml); *b*: Gas production from the insoluble fraction (ml); *a+b*: Potential gas production; *c*: Gas production rate constant for the insoluble fraction (ml); OMD: Organic Matter Digestibility (g kg^{-1} DM); *OMD%= 14.88+0.889GP24+0.45 CP +0.0651ash*, DMI: Dry Matter Intake (kg day^{-1}).

Table 5. Fractioning of grass pea's protein through CNCPS and Metabolizable protein system (g kg^{-1} CP).

CNCPS[a]		Metabolizable protein[b]	
A	107.8±10.8	QDP	740.0±0.00
B1	266.5±26.7	SDP	88.3±11.4
B2	511.6±51.2	RDP	828.3±11.4
B3	54.5±5.5	ERDP	680.3±11.4
Total B	832.7±83.3	UDP	171.7±11.4
C	59.5±5.9	DUP	101.0±10.3
		MP	534.7±3.0

[a] Reported values are means of triplicates±standard deviations. A: Soluble Protein Fraction; B1, B2 and B3: Potentially Ruminal Degradable Fractions, C: Undegradable and indigestible protein fraction (unavailable).
[b] Fractional passage rate of 0.05h^{-1} was used for calculation of SDP. QDP: Quickly Degraded Protein; SDP: Slowly Degraded Protein; RDP: Rumen Degraded Protein; ERDP: Effective Rumen Degradable Protein; UDP: Undegraded Dietary Protein; DUP: Digestible Undegraded Protein, MP: Metabolizable Protein.

Table 6. *In situ* degradability of DM, OM, CP, NDF and NDFom, and the estimated parameters of *L. sativus* hay.[a]

	Chemical constituents				
IT	DM	OM	CP	NDF	NDFom
4	37.77±2.7	36.48±2.88	72.04±2.38	8.75±2.74	14.79±3.31
8	42.16±3.4	41.17±4.28	72.21±2.16	17.12±5.98	21.23±3.79
12	54.88±3.1	53.91±2.78	84.93±0.70	30.20±6.16	35.77±6.65
24	59.10±3.2	58.96±2.56	83.38±3.20	36.94±3.83	41.05±4.02
48	61.16±1.2	61.72±2.05	85.80±2.10	39.71±1.70	44.71±1.26
72	59.27±3.6	60.72±5.48	85.44±4.03	38.15±3.02	42.76±1.59
96	66.49±1.4	67.56±1.98	90.92±1.24	45.68±1.73	49.72±2.10
	Estimated parameters				
a (g g^{-1})	0.37±0.0	0.34±0.00	0.74±0.00	0.12±0.00	0.15±0.00
b (g g^{-1})	0.25±0.02	0.29±0.03	0.13±0.02	0.30±0.02	0.30±0.01
c (h^{-1})	0.108±0.0	0.096±0.01	0.103±0.03	0.105±0.02	0.109±0.02
	Effective degradability (ED) (g g^{-1})				
k= 0.02 (h^{-1})	0.57±0.02	0.57±0.03	0.84±0.01	0.34±0.03	0.39±0.03
k= 0.05 (h^{-1})	0.51±0.02	0.50±0.02	0.81±0.01	0.27±0.03	0.32±0.03
k= 0.08 (h^{-1})	0.48±0.02	0.46±0.02	0.79±0.01	0.23±0.03	0.27±0.03

[a] Reported values are means of triplicates±standard deviations. IT: Incubation Time (h); a: The dry matter soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable; b: The portion of insoluble nutrient which is potentially degradable by micro-organisms; c: The degradation rate of fraction b per hour, k= Rumen outflow rate (h^{-1}).

digestibility were recorded as 891.4, 843.8 and 766.4 (g kg^{-1} DM), respectively (unpublished data). A comparison of OMD by different methods is presented in Table 7. Based upon these results, there is no significant difference observed between OMD obtained through *in vitro* digestibility trial *vs.* AIA. In *in vivo* digestibility trial, AIA method showed the higher OM digestibility (789.2 g kg^{-1} DM) as compared with total fecal method (699.6 g kg^{-1} DM).

Estimated OMD by *in vitro* gas production revealed the lowest level (481.8 g kg^{-1} DM).

In vivo Digestibility Experiment and Palatability

Apparent digestibility coefficients for OM, CP, CF, ADF, NDF, and NDFom by internal marker *vs.* total fecal are given in Table 8. There were no significant

Table 7. Comparison of OMD by different methods.[a]

Method	OMD (g Kg^{-1} of DM)
In vitro digestibility	772.8[a]
Total fecal	699.6[b]
AIA	789.2[a]
Gas production	481.8[c]
SE	9.09
Sig.	***

[a] OMD: Organic Matter Digestibility, *OMD%= 14.88+0.889 GP24+0.45 CP+0.0651ash*; SE: Standard Error of mean, Sig: Significance level; *** *P< 0.001.*

differences observed between nutrient digestibility obtained by the two methods except for OM and CF. In both situations' digestibility as based on internal marker was higher than that based on total fecal collection. Palatability of grass pea was 87% in comparison with that of alfalfa.

Estimation of ME through Different Methods

Comparisons of the estimated ME by *in vitro vs. in vivo* methods are presented in Table 9. Among the different methods employed for an estimation of *ME* through gas production, the first formula resulted in the highest figure (8.52 MJ kg^{-1} DM). No significant differences were observed between the two *in vivo* methods for the estimation of ME. AIA was an in vivo method that confirmed results obtained through chemical composition trials.

Table 9. Comparison of the ME estimated *in vitro* and *in vivo.*

Method	ME (MJ/kg DM)
Chemical composition [a]	10.76[b]
Tilley and Terry	12.03[a]
Total fecal collection	9.57[bc]
AIA	10.78[b]
Gas production [b]	8.52[c]
Gas production [c]	6.99[d]
Gas production [d]	6.86[d]
s.e.	0.145
Sig.	***

Means with differing superscripts are significantly different; ***P<0.001; s.e.: standard error of mean; Sig: significance level
[a] ME (MJ/kg DM) =15.33-0.0152MADF(gr/kg DM)
[b] ME (MJ/kg DM) = 2.2+0.136GP24+0.0057CP+0.00029CF^2
[c] ME =2.2+ 0.136GP24+0.057CP+ 0.0029CP^2
[d] OMD% =14.88+0.889 GP24 + 0.45CP +0.0651 ash
[e] DOMD %= OMD% × OM%
[f] ME(MJ/kg DM) = 0.0157 DOMD

DISCUSSION

Chemical Composition

The CP content of grass pea in the present study was higher than those reported by Tuna *et al.* (2004) (163.5 g kg^{-1} DM) and Poland *et al.* (2003) (182 g kg^{-1} DM). Poland *et al.* (2003) reported higher NDF and ADF values (486 and 363 g kg^{-1} DM) than those presented in the present study. CF content of

Table 8. Apparent digestibility of grass pea using AIA and total fecal collection (%).[a]

Method	Chemical constituents					
	OM	CP	CF	NDF	NDFom	ADF
Total fecal	69.9[b]	79.3	46.1[b]	65.0	65.0	61.2
AIA	78.9[a]	82.4	63.3[a]	70.9	71.8	70.7
SE	1.5	2.0	1.9	2.5	2.4	2.3
Sig.	*	NS	*	NS	NS	NS

[a] Means within the same column with differing superscripts are significantly different. AIA: Acid Insoluble Ash; SE: Standard Error of mean; Sig: Significance level, * *P< 0.05, NS: Non-Significant, P>0.05.

grass pea in the ongoing study was lower than that reported by Tuna *et al.* (2004), where it was 276.8 (g kg^{-1} DM). Differences in growing conditions, cultivars used and different vegetative stages at harvest may explain part or more of these differences. ADF is considered as a preparative residue for the determination of cellulose, lignin, Maillard products, silica, acid-insoluble ash and acid-detergent-insoluble N (ADIN) (Cherney, 2000; (c.f. Givens *et al.*, 2000c)). So different pretreatments such as drying, tannin deactivation and chemical treatments for enhancing digestibility can result in different ADF values due to reduced or enhanced levels of non-enzymatic browning products and products measured as ADIN (Condensed Tannin and protein complexes, as examples). In the present study more than 50 percent of N retained in grass pea cell wall, recovered in acid detergent residue as ADIN.

The experimental plant contained a high level of ODAP (11.8 g kg^{-1} DM), higher than the level reported by Hanbury *et al.* (1999) (0.4-7.6 g kg^{-1} DM). Recent studies have demonstrated that ODAP concentration can vary widely, however environmental conditions are not as important as genotype (Hanbury *et al.*, 1999). Nonetheless, stresses such as salinity and drought have been found to increase ODAP concentration but are little understood. Mineral contents of grass pea are given in Table 3. Zn and Fe contents of grass pea were respectively lower and higher than those reported in other literatures. According to Lambein *et al.* (1994), ODAP is hypothesized to function as a carrier molecule for zinc ions. Soils depleted in micronutrients or high in iron content may be responsible for a high level of neurotoxins. Therefore, the level of zinc in soil can be the cause of high level of ODAP in grass pea tested in this study.

CT content of grass pea (0.2 g kg^{-1} DM) was reported as lower than the level obtained by Deshpande and Campbell (1992). They found that CT of grass pea was approximately 1.2 g kg^{-1} DM. Differences in CT content can be attributed to difference in

variety, preservation, and extraction and as well to analysis method. Terrill *et al.* (1992) reported that only 30-35% of extractable condensed tannin could be extracted from low CT content plants. Therefore, it seems that low concentration of CT in GPHP can be attributed to this phenomenon.

RFV index is employed to assess the quality of forage and is determined by ADF and NDF contents. High *RFV* index of GPHP (153.43) showed a high quality of grass pea as compared with alfalfa (142.4) (Mirzaei-Aghsaghali *et al.*, 2008). Forages with higher quality attain values greater than 100 (Canbolat *et al.*, 2006). Dairy producers handling large numbers of cattle and/or dairy cows often aim to 150 or greater *RFV* values (Mirzaei-Aghsaghali *et al.*, 2008).

Gas Production and Estimated Parameters

In vitro gas production is a rapid, simple and little time consuming method, closely correlated with *In Vitro* Digestibility (IVD) and forage quality. Therefore, this method has been successfully used to evaluate the Dry Matter (DM) degradability, Organic Matter Digestibility (OMD) or Metabolizable Energy (ME) of roughage hay (Lee *et al.*, 2000). In contrast with these points of view and according to a Cone *et al.* (1999) study, due to the accumulation of gas in syringe, the *in vitro* gas production is not a suitable method to estimate ME in the rich protein content plants. So another method is being recommended for an estimation of ME in grass pea. In the present study, low contents of OMD and ME were obtained through *in vitro* gas production as compared with other methods, as confirmed by Cone *et al.* (1999) results. *In vitro* gas production in GPHP was the same as that in alfalfa gas production reported by Taghizadeh *et al.* (2008) have.

CNCPS Fractioning of Protein and Metabolizable Protein System

High B2 and low C fractions can be attributed to low concentrations of CT in grass pea hay. On the other hand, tannin-protein complexes can be recovered in ADIN or C fraction (Sniffen *et al.,* 1992). Thus, the authors suggested that low concentrations of CT, following low tannin-protein complexes in protein fractions, reduce the influence of this compound, and may be insufficient to affect the protein fractions.

UDP measurement of feeds alone is not sufficient in evaluating nutritive value, because UDP may be partly indigestible in the small intestine (Haugen *et al.,* 2006). The presence of tannin-protein complexes in fiber fractions, both *in vitro* and *in vivo*, suggest that not all tannin-protein complexes would get dissociated in the post-rumen leading to higher availability of feed protein in the intestine (Makkar, 2003). The reversibility of the tannin-protein complex fraction would depend on the binding affinity of tannins to protein and other macromolecules. To the best of our knowledge, the present study is the first to report CNCPS protein fractions and MP system components of grass pea hay with seedpods, so no data was available to authors for a comparison of the obtained results.

In Situ Disappearance

There was no comparable data for disappearance of the nutrients in grass pea hay in literature. High disappearance of CP and soluble fraction (*a*), in grass pea hay can probably be attributed to the high concentration of CP *vs*. low concentration of NDIN and ADIN, based on CNCPS protein fractioning. According to Ørskov (2000), (c.f. Givens *et al*., 2000a), *in situ* technique is not ideal for the assessment of feeds containing ANFs, because the very small amount of ANFs among the sample contents in the bag will have either little or no effect on the environment of the rumen and so will not affect the sample's degradation characteristics. Here, *in vitro* techniques would be superior. Van Soest (1994) reported the tannins may include a group of cellulase inhibitors; reported in many wild plants browsed by ruminants. So the low degradation of cell wall could be explained by these effects of the tannins.

In Vitro Digestibility and OMD Comparison

Only few studies have attempted to determine the *in vitro* DM and OM digestibility in grass pea. Mlambo *et al.* (2008) have demonstrated that for high tanniferous materials, rapid passing of phenols through filter paper, leads to their take part in the digestible fraction, causing an overestimation of digestibility in *in vitro* trials. This effect can be further examined by tannin deactivation and a comparison of the results with those of *in vivo* experiments. The *in vivo* results (AIA, 789.2 g kg^{-1} DM) confirmed the *in vitro* OM digestibility (772.8 g kg^{-1} DM) ones. However, in the present study, estimated ME of grass pea, of low concentrations of CT, was higher than those in the other methods. So the estimated ME through this method must be compared with those of the other methods to have one of them suggested.

In Vivo Digestibility Experiment and Palatability

The estimated digestibilities of nutrients, through AIA were higher than those through the total fecal method. However, the significance was only true for OM and CF digestibilities. Rymer (2000) reported adequate recoveries of AIA and close agreement between *in vivo* OMD and the predicted digestibility (c.f. Givens *et al.,* 2000b).

Palatability is defined as a result of the physical and chemical characteristics that evoke appetite (Baumont, 1996). In addition, ruminants do select feeds based on flavor

and color (Van Soest, 1994). As mentioned above, palatability of grass pea was 87% as compared with that of alfalfa. Therefore, it seems that the existence of an excess of wild plant components in *L. sativus* had negatively affected the palatability index, reducing the palatability of grass pea hay as compared with alfalfa hay. It should be mentioned that the chemical properties of the CT might be decisive in a limitation of CT content as regards palatability. Nevertheless, Poland *et al.* (2003) have demonstrated that, as their data suggested, no overt problems from feeding grass pea hay to sheep had arisen, and that grass pea hay is comparable with alfalfa hay to be used in gestating ewe diets.

Estimation of ME by Different Methods

As demonstrated before, estimated ME based upon gas production models resulted in the lowest figures among the methods. It seems that, accumulation of gas in the syringe, causes the *in vitro* gas production method to be introduced as an unsuitable one for an estimation of ME in rich protein containing plants (Cone *et al.*, 1999). It should be noted that, there is no significant difference observed between total fecal collection, as an index, and the first gas production model. So this model accompanied by chemical composition method can be used as superior to *in vitro* techniques.

CONCLUSIONS

In conclusion, a high CP content, and high digestibility of nutrients followed by low CT content, with a high ME, introduced *L. lathyrus* as a good alternative forage for ruminant nutrition, specially in dry and adverse environmental conditions. It seems that grass pea hay can be introduced as a practically effective replacement or alternative for alfalfa, as a rich protein plant, especially in arid regions. It is necessary to determine the nutritive value of grass pea at its different stages of growth and carry out more research as regards the effects of different ANFs in *L. lathyrus*, on performance of ruminants.

Abbreviations

ADF: Acid Detergent Fiber; ANFs: Anti-Nutritional Factors; β-ODAP: β-N-Oxalyl - L- α, β-DiAminoPropionic acid; CP: Crude Protein; CT: Condensed Tannin; DM: Dry Matter; DMI: Dry Matter Intake; ME: Metabolizable Energy; NDF: Neutral Detergent Fiber; OM: Organic Matter; OMD: Organic dry Matter Digestibility.

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ارزیابی ارزش غذایی علوفه خلر در تغذیه گوسفند و تعیین خوش خوراکی آن نسبت به یونجه

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چکیده

ارزش غذایی علوفه خلر به وسیله تعیین ترکیبات شیمیایی، حجم گاز تولیدی، بخش ها ی مختلف پروتئین در سیستم های CNCPS و AFRC ، انرژی قابل متابولیسم، میزان تجزیه پذیری در شکمبه گوسفندان فیستولادار با استفاده از روش کیسه های نایلونی و قابلیت هضم در شرایط برون تنی مورد مطالعه قرار گرفت. مقدار پروتئین خام، دیواره سلولی و دیواره سلولی بدون همی سلولز علوفه خلر به ترتیب ۲۳۲/۴ ، ۳۹۷ و ۳۰۰/۶ (گرم در کیلوگرم ماده خشک) بود. مقدار تانن متراکم و بتا اگزالیل دی آمینو پروپیونیک اسید هم به ترتیب ۰/۲ و ۱۱/۸ (گرم در کیلوگرم) بود. براساس روش های مختلف میزان انرژی قابل متابولیسم علوفه خلر بین ۶/۸۶ تا ۱۲/۰۳ (مگاژول در کیلوگرم ماده خشک) برآورد شد. مقدار پروتئین قابل متابولیسم علوفه خلر ۵۳۴/۷ (گرم در کیلوگرم پروتئین خام) بود. بالا بودن غلظت پروتئین خام و مقدار پروتئین قابل متابولیسم به همراه مقدار انرژی قابل متابولیسم بالا و خوشخوراکی مطلوب سبب می شود تا بتوان علوفه خلر را به عنوان یک علوفه جایگزین در شرایط خشکسالی و نا مطلوب آب و هوایی برای تغذیه گوسفند معرفی کرد.