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Use of *in Vitro* Gas Production Technique to Compare Nutritive Value of Quackgrass and Alfalfa for Ruminants

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Abstract: The objective of this study, was to assess the nutritive value of two forage species include Alfalfa (ALF) and Quackgrass (QCK) grown in Iran by using the chemical composition, *in vitro* gas production kinetics, Organic Matter Digestibility (OMD) and Metabolisable Energy (ME) contents. Rrumen mixed microbe inoculums were taken from two fistulated Gezel rams. Samples of forages were incubated with rumen fluid to determine gas production. Gas productions were measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. No significant differences were found between Organic Matter (OM), Ether Extract (EE), ash and Acid Detergent fiber (ADL) contents of experimental forages, although the differences for Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Neutral Detergent Fiber (NDF), ADF and Gross Energy (GE) were significant ($p<0.01$). Gas production at all incubation times and gas production constants (a, b, c and a + b) were significantly ($p<0.01$) higher in ALF hay. The OMD and ME for ALF and QCK hays were 71.2, 43.45% and 10.96, 6.58 MJ per Kg DM, respectively. Under the climatic conditions of the 2005 growing season, the nutritive value of ALF hay was higher to that of QCK hay, because of lower NDF, greater cell content, OMD and ME.

Key words: Alfalfa, quackgrass, gas production, nutritive value, digestibility

INTRODUCTION

In the most part of Iran, perennial legumes especially Alfalfa and grasses are the primary forage in terms of providing energy, protein and minerals. However, there are reports that show the high rate of alfalfa infestation as high as 78% with Quackgrass. Quackgrass (*Agropyron repens L.)* is a perennial weed that competes aggressively with cereal and forage crops. Graminicides can be used to control Quackgrass in alfalfa stands but not in established forage grass stands due to lack of selectivity. Thus, producers renovate their fields because of Quackgrass infestation, although Quackgrass has been used in ruminant feeding, information on its nutritive value is limited. Many palatable hybrid crosses of QCK and other species has been developed and planted for livestock. Feeding trials in Minnesota showed that a QCK biotype was as palatable as alfalfa (*Medicago Sativa*) in cattle. The QCK has been rated fair in energy value and poor in protein value. However, food value studies in Minnesota showed that QCK had as much crud protein as alfalfa during May (Dutt *et al*., 1979; Marten *et al*., 1987; Christen *et al.*, 1990). In recent years, the accurate and precise determination of forage nutrition value is becoming more important. The gas production technique is rapid, reliable and cost effective method for determining the nutritive value of forages (Chenost *et al*., 2001).

The aim of this study, was to determine the chemical composition and relative nutritive value of Quackgrass (QCK) and Alfalfa (ALF) using gas production technique.

MATERIALS AND METHODS

Forages: Two forage species, alfalfa (ALF; Hamedani variety) and Quackgrass (QCK) (*Agropyron repens L.*) were used in the experiment. Samples of the 2 forages were collected near Miandoab and Karaj, Iran in 2005 and evaluated at the laboratories of Animal Science Research Institute in Karaj. Forages, at harvested, were estimated to be at late maturity. Samples were collected, air-dried and ground (1 mm screen) for chemical analysis and *in vitro* gas production (Kamalak *et al*., 2005).

Chemical analysis: Dry Matter (DM) was determined by drying the samples at 105°C overnight and ash by

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igniting the samples in muffle furnace at 525°C for 8 h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as N×6.25. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid-Detergent Lignin (ADL) and Acid Insoluble Ash (AIA) were determined by procedures outlined by Goering and VanSoest (1979) with modifications described by VanSoest *et al*. (1991), sulfite was omitted from NDF analysis. Hemicellulose and cellulose were calculated as (NDF-ADF) and (ADF-ADL-AIA), respectively (Andrighetto *et al*., 1993). Gross Energy (GE) was measured in an adiabatic bomb calorimeter.

***In vitro* gas production:** Fermentation of ALF and QCK hays samples were carried out with rumen fluid obtained from 2 fistulated Gezel rams (1.5 year old, avg initial BW 55 kg) fed twice daily with a diet containing hay (60%) and concentrate (40%) following the method described by Menke and Steingass (1988). Approximately 200 mg hay samples were weighed into the glass syringes of 100 mL. The fluid-buffer mixture (30 mL) was transferred into the glass syringes of 100 mL. The glass syringes containing hay samples and rumen fluid-buffer mixture were incubated at 39°C. The syringes were gently shaken 30 min after the start of incubation. The gas production was determined after 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. All samples were incubated in triplicate with 3 syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for hay samples were determined by subtracting the volume of gas produced in the blanks. Gas production data were fitted to the model of Ørskov and McDonald (1979).

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

Where:

a = The gas production from the immediately soluble fraction (mL).
b = The gas production from the immediately insoluble fraction (mL).
c = The gas production rate constant for the insoluble fraction (%/h).
a + b = Potential gas production (mL).
t = Incubation time (h).
Y = Gas production at time t.

The ME (MJ Kg^{-1} DM) contents of ALF and QCK hays samples were calculated using equation of Menke *et al*. (1979) as follows:

$$\text{ME (MJ Kg}^{-1}\text{ DM)} = 2.20 + 0.136\text{ GP} + 0.057\text{ CP}$$

Where:
GP = 24 h net Gas Production (mL 200 mg^{-1}).
CP = Crude Protein (%).

Organic Matter Digestibility (OMD) (%) of ALF and QCK hays samples were calculated using equation of Menke *et al*. (1979) as follows:

$$\text{OMD (\%)} = 14.88 + 0.889\text{ GP} + 0.45\text{ CP} + 0.0651\text{ XA}$$

Where:
GP = 24 h net Gas Production (mL 200 mg^{-1}).
CP = Crude Protein (%).
XA = Ash content (%).

In vitro gas production measurements were carried out in the laboratory of Animal Science Research Institute in Karaj.

Statistical analysis: All of the data were analyzed by using software of Statistical Analysis Systems (1985) and means were separated by independent-sample t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The chemical compositions of the two forage species are presented in Fig. 1 and Table 1. The quackgrass hay was significantly ($p<0.05$) higher in DM than ALF, but the CP and Cell Contents (CC) of QCK hay were less than one-third that of ALF hay ($p<0.01$). The chemical compositions of ALF and QCK hays used in this experiment were consistent with findings by Kamalak *et al*. (2005) and Christen *et al*. (1990), respectively. Crude protein for QCK was 8.9%, a value lower than that of 14.5% reported by Stoszek *et al*. (1979) for immature QCK and of 17.2% reported by Marten *et al*. (1987) for common QCK harvested at joint.

The EE and ash (which is an index of minerals contents) were similar ($p>0.05$) in ALF and QCK hays. The ALF Non-Fibrous Carbohydrate (NFC) content (29.44%) was significantly ($p<0.01$) higher than NFC of QCK (8.96%), whereas Nitrogen-Free Extract (NFE) content of QCK hay was significantly ($p<0.01$) higher than that of ALF hay. ADL was similar in both forages ($p>0.05$); ADF and NDF were significantly ($p<0.01$) higher in QCK. NDF value for QCK hay (69.5%) was similar than the value 66.4 found by Marten *et al*. (1987). The concentration of NDF and ADF for ALF hay were in agreement with the results of Coblentz *et al*. (1998). Stoszek *et al*. (1979) estimated that ADF content for immature QCK was 34.1%. This value for QCK was lower than the value

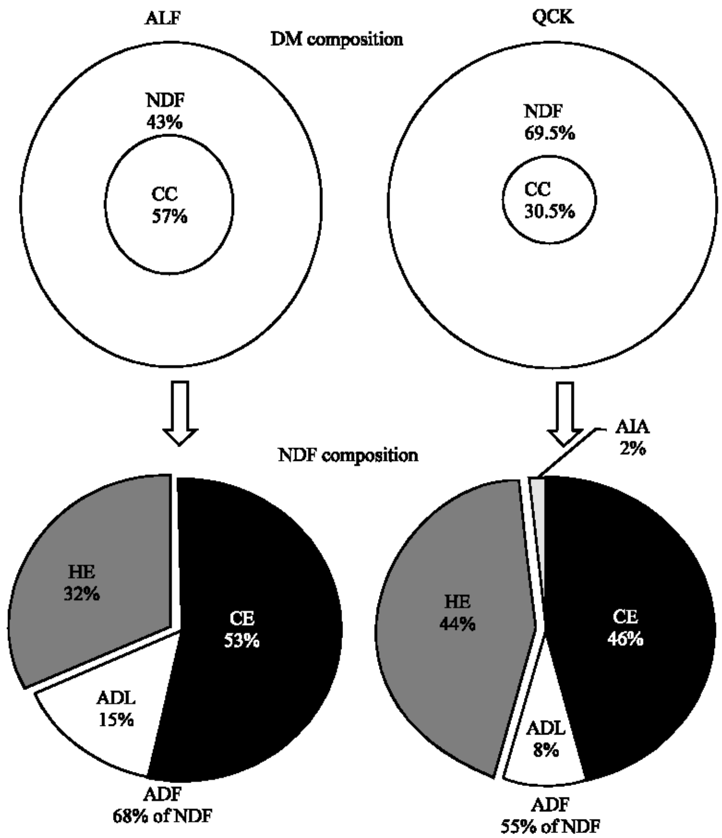

Fig. 1: Comparison of feed fractions of Quackgrass (QCK) and Alfalfa (ALF) hays. ADL = Acid-Detergent Lignin, CC = Cell Contents, CE = Cellulose, HE = Hemicellulose, AIA = Acid-insoluble ash

Table1: Chemical composition, lignification indices and Gross Energy (GE) of Alfalfa and Quackgrass hays

Component	ALF	QCK	SE	Sig.
DM, %	92.93	95	0.265	*
OM, %	89.66	88.7	0.245	NS
---(%DM)---				
CP	15.8	8.9	0.828	**
Crude fiber	29.2	34.3	0.508	**
Ether extract	1.33	1.44	0.193	NS
Ash	10.33	11.2	0.245	NS
NFC	29.44	8.96	0.677	**
NFE	43.3	66	0.790	**
Cell contents	56.9	30.5	0.307	**
NDF	43.1	69.5	0.307	**
ADF	29.4	38.3	0.323	**
Hemicellulose	13.7	31.1	0.322	**
Cellulose	22.9	32.6	0.686	**
ADL	6.3	5.7	0.296	NS
AIA	0.15	1.15	0.085	**
ADL/NDF	14.6	8.1	0.517	**
ADL/ADF	21.4	14.7	0.724	**
GE (Kcal Kg^{-1})	4219.0	4285.1	11.742	*

ALF = Alfalfa; QCK = Quackgrass; the data are mean value of three replicate; Non-fibrous Carbohydrate (NFC) is calculated using the equation of NRC (2001), NFC% = 100-(%NDF + % CP + % FAT + % Ash); NFE = Nitrogen-free extract; Acid-detergent lignin; AIA = Acid insoluble ash; ADL/NDF = Lignification index based on NDF; ADL/ADF = Lignification index based on ADF, SE = Standard Error, NS = Non-Significant, $^{*}p<0.05$, $^{**}p<0.01$

at 38.3% obtained in this experiment. NDF content was correlated positively with ADL and the ADL/ADF ratio, suggesting that as the plant matured lignification of cell walls increased ($r = 0.93$, $p<0.06$; $r = 0.95$, $p<0.05$, respectively). Hemicellulose (HE) was about two times higher in QCK than alfalfa. Cellulose (CE) content were 22.9 and 32.6% in ALF and QCK hays, respectively. The lignification index (Table 1), on either an NDF or an ADF basis (Van Soest, 1982) was significantly ($p<0.01$) higher in ALF hay (8.1 and 14.6% vs. 14.7 and 21.4%, respectively). The GE content of QCK hay was significantly ($p<0.05$) higher than that ALF hay.

Gas production data during the fermentation period are given in Table 2. The cumulative volume of gas production increased with increasing time of incubation. Gas production at 96 h incubation ranged between 52.4 and 73.1 mL per 200 mg of dry matter for QCK and ALF, respectively. Gas production from the fermentation of forages were measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h *in vitro* gas tests adapted to describe the kinetics of fermentation on the modified exponential model $y = a + b[(1-Exp(-ct)]$ (Ørskov and McDonald, 1979). Although,

Table 2: Organic matter digestibility, gas production (ml) and estimated parameters of QCK and ALF hays at different incubation times

Time (h)	2	4	6	8	12	24	48	72	96
QCK	4.35	6.4	7.7	9.75	15.1	32.2	44.7	49.5	52.4
ALF	18.4	27	45.02	50.3	54.8	64.4	69.8	72.2	73.1
SE	0.236	0.320	0.450	0.444	0.501	0.463	0.450	0.450	0.450
Sig.	**	**	**	**	**	**	**	**	**
Estimated parameters									
	a	b	(a + b)	c	OMD	ME			
QCK	-0.95	56.3	55.35	0.035	43.54	6.58			
ALF	0.9	68.7	69.6	0.137	71.2	10.96			
SE	0.328	0.630	0.907	0.0072	0.416	0.063			
Sig.	*	**	**	**	**	**			

a = the gas production from the immediately soluble fraction (ml); b = the gas production from the immediately insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction (%/h); (a + b) = potential gas production (mL); OMD: Organic matter digestibility (% of DM); ME: Metabolisable energy (MJ kg^{-1} DM). SE = Standard Error. ND = Non-Determined, $^{*}p<0.05$ $^{**}p<0.01$

there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages had been documented (Blummel and Ørskov, 1993). Cumulative gas production and estimated parameters in ALF hay were comparable with those reported by Kamalak *et al*. (2005).

At all incubation times, cumulative gas productions (mL) of ALF hay, were significantly (p<0.01) higher than that of QCK hay. Therefore the estimated parameters (a, b, c and a + b) of ALF hay were significantly (p<0.01) higher than that of QCK hay due to low cell wall content. The OMD and ME contents of ALF hay were significantly (p<0.01) higher than that of QCK hay.

The QCK hay had the lowest value for a, intercept, while ALF hay had the highest a, intercept value. The value for a, intercept, in QCK hay was negative in this study. These data suggested that a lag phase due to delay in microbial colonization of the substrate may occur in the early stage of incubation. Several authors have also reported negative values with various substrates when using mathematical models to fit gas production kinetics. The soluble fraction makes it easily attachable by ruminal microorganisms and leads to much gas production (Blummel and Becker, 1997).

The gas volumes at asymptote (b) described the fermentation of the insoluble fraction and were lower in QCK hay than that of ALF hay. The gas volumes at asymptote have the advantage for predict feed intake. Blummel and Ørskov (1993) found that gas volume at asymptote could account for 88% of variance in intake.

Rate of gas production (c) expressed in %/h, were lower in QCK hay than that of ALF hay. High rate of gas production was observed in ALF hay, possibly influenced by carbohydrate fractions readily availability to the microbial population. Deaville and Givens (2001) have also reported that carbohydrate fraction could be affected to kinetics of gas production.

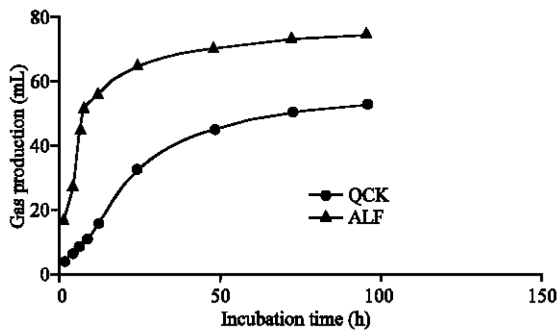

Fig. 2: Cumulative gas volume estimated by y = a + b [(1-Exp(-ct)] (mL/0.2 g DM subtrate) throughout 96 h (QCK = Quackgrass, ALF = Alfalfa)

well known that gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Getachew *et al*., 1998), whereas, protein fermentation dose not lead to much gas production (Khazaal *et al*., 1995). High potential extent of gas production was observed in ALF hay. It imply that their nutrients were high availabile in the rumen. The current finding agrees with in situ studies on perennial legumes and grasses (Hoffman *et al*., 1993). However, the potential extent of gas production in QCK hay was low, it possibly due to the carbohydrate fraction of QCK hay have a large proportion of cell walls (Table 1) with low fermentation and leading to low gas production.

Curve of cumulative gas production for forages are presented in Fig. 2. It can be see that gas production reached a plateau after 48 h fermentation, exception QCK hay. The reason for that, it possibly the carbohydrate fraction of QCK hay have a large proportion of cell walls and leading to difficulty attach by microorganisms. Cumulative gas volume at each sampling time was affected by variety of feedstuffs. These finding indicate that fraction of substrate and degradability of forages are difference. The gas produced is directly proportional to the rate at which substrate degraded (Dhanoa *et al*., 2000).

Potential extent of gas production (a + b) expressed in mL, were lower in QCK hay than that of ALF hay. It is Additionally, kinetics of gas production is depended on at relative proportions of soluble, insoluble but degraded and undegradable particles of the feed (Getachew *et al*., 1998).

Low OMD and ME were observed in QCK hay. The current findings agree with studies the nutritive value of pure QCK and timothy (Christen *et al*., 1990). The decrease in digestibility is due to increase in concentration of cell wall contents (Wilson *et al*., 1991), lignin content in mature plant (Morrison, 1980) and decrease in leaf/stem ratio (Hides *et al*., 1983). Menk *et al*. (1979) suggested that gas volume at 24 h after incubation has been relationship with metabolisable energy in feedstuffs. Sommart *et al*. (2000) reported that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart *et al*., 2000). Gas volumes also have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993).

CONCLUSION

It was concluded that there were significant variations in chemical composition, gas production characteristics, OMD and ME of 2 forages studied in this experiment. The results of this study demonstrated that kinetics of gas production of 2 forages differed among legumes and grasses. Although, the nutritive value of Quackgrass was lower than that of alfalfa however it seems that it could bee used as fair energy source in ruminant nutrition.

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