

Effect of addition barley on the degradability kinetics, net energy, *in vitro* dry matter digestibility and metabolizable energy of potato plant silage by gas test technique

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ABSTRACT

This experiment was conducted to investigate effect of adding different levels (0, 2, 4 and 6%) of barley on potato plant silage degradability were studied by *In vitro* gas producing technique. Gas production test with mixtures of filtered rumen liquid of three Holstein male cattle rumen in times of 2, 4, 6, 8, 16, 24, 48, 72 and 96 hours were performed. Chemical compositions for dry matter, crude protein, NDF were 27, 10.16, and 34%, respectively. The results showed that gas volume at 16h incubation (for different treatments), were 33.6^d, 36.6^c, 40.6^b and 42.6^a ml/200mg DM for potato plant silage. The gas production from soluble fraction ($a_{4\%}$), and from insoluble fraction ($b_{4\%}$), rate constant of gas production during incubation ($c_{4\%}$) and the potential gas production ($a + b_{4\%}$) contents of potato plant silage were 20.9^a (ml/200mg DM), 54.2^a (ml/200mg DM), 0.046^a (ml/h) and 67.4^a (ml/200mg DM), while for level control were 10.6^d (ml/200mg DM), 47.3^c (ml/200 mg DM), 0.04^b (ml/h) and 57.9^d (ml/200 mg DM). This study demonstrated that the potato silage barley (PSBa), have the potential to use as out se of forage for ruminal nutrition.

Keywords: Potato Plant Silage, Crude Protein, Gas Production Technique, Rumen Liquid.

INTRODUCTION

Feeding by-products from the crop and food processing industries to livestock is a practice as old as the domestication of animals by humans. Increased disposal costs in many parts of the world have increased interest in utilization of potato by-product feedstuff as alternative feeds for ruminants. It has two important advantages these being to diminish dependence of livestock on grains that can be consumed by humans, and to eliminate the need for costly waste management programs. This second advantage has become important in recent years, as the world human population and the amount of crop and food by-product has increased, particularly in developed countries. Potato farm residue is a valuable, high energy by-product that can be partly replaced with forage section in cow's diet without adverse effect on milk efficiency or composition. Leiva et al. [11] reported that potato by-product feedstuff can be used as a high energy feed in ruminant rations to support growth and lactation, with fewer negative effects on rumen fermentation than starch rich feeds. A large amount of the citrus by-product feedstuff is suitable for inclusion in ruminant diets because of the ability of ruminants to ferment high fiber feeds in the rumen.

Potato production in world was 330 million tons/years; also, this amount in Iran is 21.4 million tons/years that although, Khorasan state is 4% of production total in Iran.

Feeding ruminant in intensive production systems, particularly for dairy production, requires supplies of high levels energy and protein. Ruminant animals are therefore fed on diets rich in starch and high quality protein, which are fermented very rapidly. It's well known which the rapid degradation of starch tends to cause ruminal acidosis. The

rapid breakdown of dietary protein to ammonia increases nitrogenous excretions rather than contributing directly to the animals nutrient requirements.

In order to delay ruminal protein degradation, dietary protein was denatured with treatment by formaldehyde or more controversially, antibiotics were used to suppress the bacterial populations responsible for the rapid protein fermentation. But the use of such compounds has been criticized, as they may leave harmful residues in the food chain and promote the spreading of resistance genes.

Accordingly there is greater interest in using plants the use of potato plant in livestock diets had been examined for lactating dairy cows, sheep and other ruminant. General observations arising from these researches are that large quantities of waste potato can be consumed by ruminant's animals and degraded in the rumen. The objective of this study was to evaluate the potential of PSBa degradability on fermentation pattern by the *In vitro* gas production test.

MATERIAL AND METHODS

The fresh Plant Potatoes was collected from potato farm in Khorasan of Iran and it was chopped (4 to 5 cm) and used as treated with grounded barley at 2, 4, and 6% of fresh plant potatoes. Chemical composition of utilized fresh plant potatoes is shown in Table 1. Silos were stored in the dark at ambient temperatures (20°C) and opened after 45 days of ensiling. The contents of each opened silo were thoroughly mixed and samples were taken for chemical analysis.

For measurement of pH, 50g samples of silage from each treatment were diluted with 450 ml sterile deionized water and blended for 2min, strained through four layers of cheesecloth, and pH determined immediately by a pH meter (Model 691, Metrohm). The DM content of silage samples was determined with drying in a forced-air oven at 60°C for 48h. Samples were ground to pass a 2 mm sieve. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed by the method of Clovis et al [5]. Crude protein (CP) was calculated as $N \times 6.25$ with Kjeldahl technique, ether extract (EE), Ash, was determined according to AOAC [1]. The quality of different silages was determined by estimating the flieg point data. Flieg point was calculated using the following formula [6]:

$$\text{Flieg point} = 220 + (2 \times \text{dry matter\%} - 15) - 40 \times \text{pH}$$

The contents of each silo were thoroughly mixed and 1kg of silage samples were transferred into separate 1L containers (3 containers per treatment). Each container was embedded with 2 thermometers in the lower and mid layers of the silage mass to record the temperature every 15min. The containers were each covered by a double layer of cheesecloth and stored at ambient temperature (20°C, 7 days). Ambient temperature was also simultaneously measured at 15minute intervals during this period [3].

In vitro gas production was carried out using the method as described with Menke and Steingass [14]. Samples (200mg) were weighed into 100ml calibrated glass syringes. Buffered mineral solution was prepared and placed in a water bath at 39°C under continuous flushing by CO₂. Rumen fluid was collected after the morning feeding from three Holstein male cattle, strained through four layers of cheesecloth, and flushed by CO₂. The syringe was then filled by 30ml of medium consisting of 10ml rumen fluid and 20ml buffer solution. All handling was under continuous flushing by CO₂. The syringes were placed in a water bath at 38.6°C. Gas production was measured at 2, 4, 6, 8, 16, 24, 48, 72, and 96h. Syringes were gently shaken after each recording. Rate and extent of gas production were determined for each treatment with fitting gas production data to nonlinear equation $Y = b(1 - e^{-ct})$ [17], thus, Y is the volume of gas produced at time t, b is the potential gas production (ml/g DM), and c is the fractional rate of gas production. Parameters b and c were estimated with an iterative least squares method using a non-linear regression procedure of the statistical analysis systems [20]. Organic matter digestibility (OMD) was estimated using 16h gas production as well as the CP and ash contents of the feeds as described with Menke and Steingass [14].

Short chain fatty acids were predicted according to the method of Getachew et al. [7].

$$\text{ME (MJ/kg DM)} = 0.016 \text{ DOMD for forage feeds}$$

Where:

GP = the 24 h net gas production (ml/200 mg-1)

CP = Crude protein

Short chain fatty acid (SCFA) is calculated using the equation of [12 and 13].

Where, Gas is 24 h net gas production (ml/200mg DM).

$$\text{SCFA (m mol)} = 0.0222 \times \text{GP} - 0.00425$$

The organic matter digestibility was calculated using equations of [14] as follows:

$$\text{OMD (g/kg DM)} = (\%) 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + \text{XA}$$

Where:

GP = about 24 h net gas production (ml /200mg-1)

CP = Crude protein (%)

XA = Ash content (%)

$$\text{NEL (MJ/kg DM)} = 0.115 \times \text{GP} + 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{CA} - 0.36$$

The data were analyzed in a completely randomized design using the MIXED procedure of SAS [20]. Comparison of means was performed according to the Duncan's Multiple Range Test.

The statically model used, was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where, μ = the common mean, T_i = the effect of treatments and e_{ij} = the random error. Levels of significance of liner and quadratic contrasts are presented.

RESULTS AND DISCUSSION

Chemical composition and fermentation properties

The chemical composition and fermentation properties of experimental silages also, Incubation different time, other items and degradation parameters are given in Tables 1, 2, 3, 4 and 5. Experimental treatments hadn't significant effect on DM of trails silages ($P > 0.05$). Data showed that CP concentrations were significantly different among treatments, that by increasing wheat bran in silage, CP concentration linearly and increased too ($P < 0.05$). Ammonia nitrogen concentration, EE, ash, and pH were similar among treatments ($P > 0.05$). The NDF concentration indicated a positive liner relationship ($P < 0.05$) by increasing barley in potatoes plant silage. The ADF concentration decreased linearly ($P < 0.05$) with increasing PSBa.

evaluation effect of tree doses clove methanolic extract (0, 0.5 and 1ml) on degradability, of Soybean meal and report gas volume at 48h incubation (for 200mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a+ b) and rate constant of gas production (c) of Soybean meal were 71.240, 1.767, 70.880, 72.647 ml/200mg DM and 0.100 ml/h, gas volume at 48h incubation (for 200mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a+ b) and rate constant of gas production (c) of clove methanolic extract (1ml) were 22.717, 8.914, 19.516, 28.429 ml/ 200mg DM and 0.051 ml/h, respectively. Gas volume at 72 and 96h incubation (for 200mg dry samples), of Soybean meal were 72.24 and 74.360 ml/200mg DM, while for clove methanolic extract (1ml) were 25.383 and 29.130 ml/200mg DM, respectively.

calculated amounts of *In vitro* dry degradability (DMD), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) of PSBa, percentages (0, 2, 4 and 6% of barley) are presented in Table 4.

Table1: Chemical composition of leaflets green part of plant before ensiling (DM basis %)

Item	DM	CP	EE	NDF	ADF	CF	GE(Kcal/Kg)	Ash	pH	Ca	P	Mn	Mg	Lactat (mMol)
	27.1	10.1	2.2	33.87	27.1	13.77	3260	3.59	6	4.79	0.15	0.03	3.9	1.27

DM, Dry matter; CP, Crude protein; EE, Ether extract; NDF, neutral detergent fiber; ADF, Acid detergent fiber; CF, Crude fiber; GE, Gross energy.

Table2: Chemical composition and fermentation properties of different treatments

Item	Treatments		
	B _{2%}	B _{4%}	B _{6%}
DM%	28.17	28.97	29.97
CP%	11	12	13
EE	3.11	3.02	3.16
NDF%	39.07	40.05	41.97
ADF%	31.10	31.77	32.20
CF%	14.40	15.23	15.76
CE (Kcal/Kg)	2898	3193	3858
Ca %	4.28	4.8	4.59
P%	0.18	0.17	0.18
Ash%	3.72	3.97	4.07
pH	5.30	5.05	4.77
Mg %	4.01	4.01	4.01
Mn %	0.03	0.03	0.03
Lactate(mMol)	1.96	2.25	3.34
Quality	Very Good	Very Good	Very Good

B_{2%}= added 2% ground barley

B_{4%}= added 4% ground barley

B_{6%}= added 6% ground barley

Table3: Gas production in different time rumen and treatment

Item	Treatments				SEM	Pr>F
	B _C	B _{2%}	B _{4%}	B _{6%}		
2 h	7.6 ^d	8.6 ^c	9.6 ^b	10.6 ^a	0.32	<0.0001
4 h	17 ^d	24 ^c	27 ^b	29 ^a	1.13	<0.0001
6 h	26 ^d	30.01 ^c	34 ^b	36 ^a	1.15	<0.0001
8 h	28.6 ^d	34.6 ^c	38.6 ^b	40.6 ^a	1.38	<0.0001
16 h	33.6 ^d	36.6 ^c	40.6 ^b	42.6 ^a	1.05	<0.0001
24 h	39.01 ^d	42 ^c	46 ^b	49 ^a	1.14	<0.0001
48 h	46.6 ^c	43.6 ^d	48.6 ^b	50.6 ^a	0.7	<0.0001
72 h	52 ^d	55 ^c	62 ^b	63 ^a	1.39	<0.0001
96 h	62.6 ^d	66.6 ^c	74.6 ^b	77.6 ^a	1.81	<0.0001

B_{2%}= added 2% ground barley

B_{4%}= added 4% ground barley

B_{6%}= added 6% ground barley

Table4: other Items of different treatments

Item	Treatments				SEM	Pr>F
	B _C	B _{2%}	B _{4%}	B _{6%}		
GP	115 ^b	105 ^c	115 ^b	122.5 ^a	1.87	<0.0001
SCFA	1.01 ^b	0.93 ^c	1.01 ^b	1.08 ^a	0.01	<0.0001
ME	11.14 ^c	10.7 ^d	11.52 ^b	12.14 ^a	0.15	<0.0001
Intak ₄	66.18 ^a	54.77 ^d	56.31 ^c	63.98 ^b	1.46	<0.0001
Intak ₃	50.37 ^a	43.4 ^d	46.51 ^c	50.65 ^a	0.9	<0.0001
Intak ₂	65.09 ^a	52.01 ^d	54.01 ^c	62.28 ^b	1.57	<0.0001
Intak ₁	57.12 ^a	55.92 ^b	51.87 ^d	54.13 ^c	0.59	<0.0001
DMD ₄	739.27 ^d	769.4 ^c	923.77 ^a	852.3 ^b	21.78	<0.0001
DMD ₃	636.07 ^d	661.75 ^c	795.77 ^a	731.75 ^b	18.81	<0.0001
DMD ₂	664.45 ^d	669.03 ^c	814.6 ^a	749.9 ^b	17.05	<0.0001
DMD ₁	537.88 ^d	612.8 ^c	768.52 ^a	661.23 ^b	25.24	<0.0001
DOM ₁	60.7 ^c	57.52 ^d	61.53 ^b	64.54 ^b	0.75	<0.0001
DOM ₂	52.36 ^c	48.86 ^d	53.45 ^b	56.9 ^a	0.86	<0.0001
OMD	60.59 ^c	57.41 ^d	61.41 ^b	64.42 ^a	0.75	<0.0001
Net Energy	6.94 ^c	6.69 ^d	7.14 ^b	7.48 ^a	0.08	<0.0001

SCFA, short chain fatty acids; ME, metabolisable energy; Intake, Daily Intake; OMD, organic matter digestibility.

B_{2%}= added 2% ground barley

B_{4%}= added 4% ground barley

B_{6%}= added 6% ground barley

Table 5: Degradation parameters of different treatments

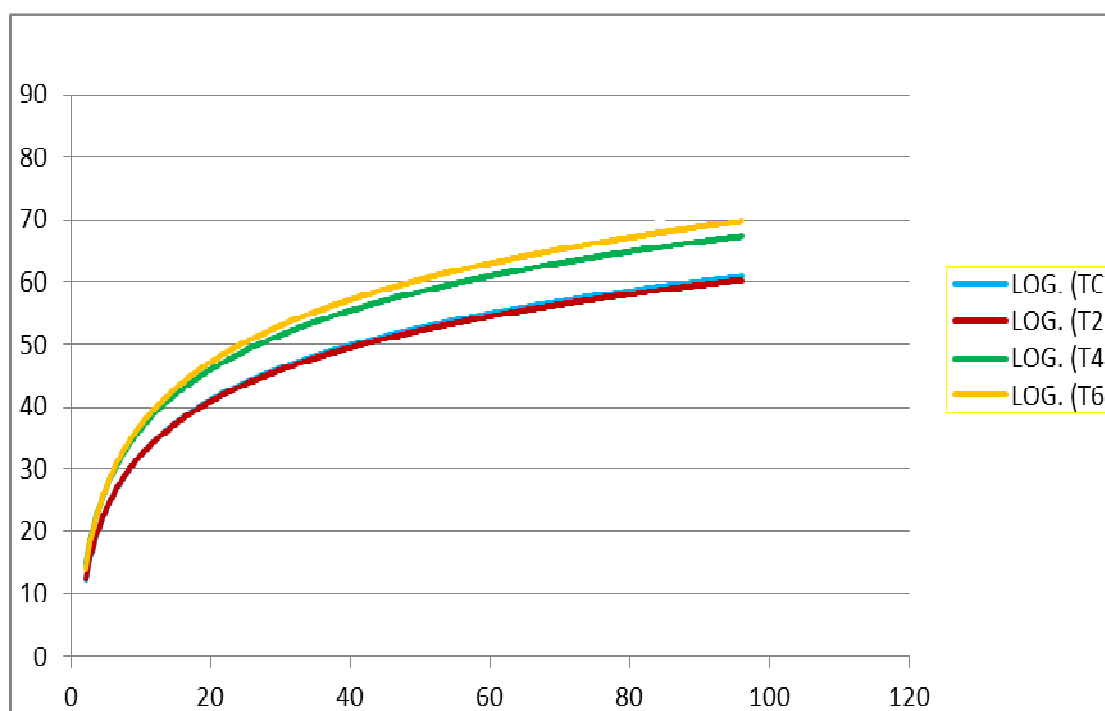
Degradation Parameter	Treatments				SEM	Pr>F
	B _C	B _{2%}	B _{4%}	B _{6%}		
a (mg/g)	10.6 ^d	15.8 ^b	20.9 ^a	15.3 ^c	1.1	<0.0001
b (mg/g)	47.3 ^c	45.4 ^d	54.2 ^a	52.7 ^b	1.06	<0.0001
c (h-1%)	0.04 ^b	0.03 ^c	0.02 ^d	0.04 ^a	0.004	<0.0001
Potential degradability (mg/g)	57.9 ^d	61.3 ^c	75.2 ^a	67.4 ^b	1.9	<0.0001

B_{2%}= added 2% ground barley

B_{4%}= added 4% ground barley

B_{6%}= added 6% ground barley

Fig1. In vitro gas production volume of PSBa at different incubation time



Chemical composition and fermentation properties

Data showed that DM (Table3) weren't affected with treatments. In this investigate, with increasing barley in silage, CP linearly increased, that might be due to higher CP concentration in PSBa (17.3% versus 6.9%) (NRC, 2001). An increase was observed in the NDF concentration of silages by increasing PSBa, but ADF concentration decreased. Perhaps, this resulted from more NDF and less ADF concentration in PSBa. The aerobic stability of experimental silages with increasing PSBa linearly decreased, as a result of raising soluble carbohydrate content in the silages. Also, carbohydrates in the silage might motivate the growth of fungi or molds. Subsequently, by this activity, the temperature of silage has been increased. Data showed that pH and ammonia nitrogen weren't affected by treatments because of enough soluble carbohydrate for silage fermentation in different treatments (Table 5).

Arbabi, et al. [2] reported that with adding sugar beet pulp to citrus pulp silage, DM of silages increased. They found that citrus pulp silage with 5% DM sugar beet pulp had lower ADF, higher NDF and CP concentration, compared with control group. Therefore, they suggested that the addition of sugar beet pulp to citrus pulp silage hadn't effect on silage pH.

Chaudhry and Naseer [4] added a mixture of poultry litter and corn forage to fresh citrus pulp and ensiled them. They concluded that composition of initial and ensiled mixtures were similar except that DM and CF contents decreased after ensiling. They showed that, the decrease in cell wall constituents after ensiling may be due to the action of bacterial enzymes in hydrolyzing cell wall components, particularly for the more digestible constituent of plant cell wall. In their research, inclusion of citrus pulp caused linear decrease in DM, CP, CF and ash contents of the mixtures, and they concluded that these decreases were mainly attributed to the difference in chemical composition of citrus pulp. Similar results have been showed that ensiled fresh citrus pulp by high dry matter agro-industrial waste [15]. Kordi, et al [9] reported that with adding of 6, 12 or 18 g barley grain/kg of citrus pulp silage, DM of silages significantly increased but ash concentration and pH were not affected. Also according to another

research, they indicated that aerobic stability of silages significantly decreased with increasing amounts of barley grain. In another study, [10] suggested that adding of 6, 12, or 18 g/kg molasses to citrus pulp silage, decreased CP, and significantly increased ash and pH. Thus, they showed that DM and aerobic stability weren't affected by different levels of molasses.

There was a positive correlation between NFC content of feeds and gas production, but feed CP, NH₃-N and NDF levels were negatively correlated with gas production [8 and 13]. Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of in vitro gas production volume [12].

This study suggested that the PSBa, have the potential to affect ruminal fermentation efficiency, and be a promising methane mitigating agent ((Tables 3 and 5).

Salamatazar et al [18] evaluation effects of addition three doses zataria multiflora water extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metabolizable energy and organic matter digestibility of sunflower meal and report the organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) contents of sunflower meal were 66.43 g/kg DM, 8.36 MJ/kg DM, 0.937 mmol and 4.533 MJ/kg DM respectively, while for zataria multiflora water extract (0.3 ml/30ml buffered rumen fluid) were 64.76 g/kg DM, 8.04 MJ/kg DM, 0.895 mmol and 4.664 MJ/kg DM respectively.

Salamatazar et al [19] evaluation effects of the study three doses zataria multiflora water extract (0, 0.15 and 0.3 ml/30ml buffered rumen fluid) on the short chain fatty acid, net energy, metabolizable energy and organic matter digestibility of canola meal using in vitro gas production technique and report amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) of canola meal (79.46 g/kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM, respectively) were high as compared to zataria multiflora water extract (0.3 ml/30 ml buffered rumen fluid) were (41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively). These results are in agreement with the findings of Salamatazar et al [18 and 19].

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