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Effect of Addition Molasses on the Degradability Kinetics, Net Energy for Lactation, In Vitro Dry Matter Digestibility and Metabolizable Energy of Potato Plant Silage in Ruminant Animals

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Authors' contributions

This work was carried out in collaboration between all authors. Author HRP designed the study, wrote the protocol and wrote the manuscript. Author AV performed the statistical analysis, and author AAN in managed the analyses of the data and financial support. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The purpose of this research is to use green potatoes for animal feed, Which is considered as a waste at the farm level.
This study was carried out to determine the chemical composition and estimation of effects four levels (0, 2, 4 and 6%) of Molasses on potato silage Males (PSM) degradability were studied by In Vitro gas producing techniques. Fermentation of PSM samples were carried out with rumen fluid obtained from three mature canulated steers (BW=550). The amount of gas production for PSM samples at 2, 4, 6, 8, 16, 24, 48, 72 and 96 hours were measured. The results showed that the DM, Ash, crude protein (CP), natural detergent fiber (NDF) and Non fiber carbohydrate (NFC) were 27, 3.5, 10.2, 35 and 48.8 percent, respectively. Calculated amounts of In Vitro dry matter digestibility (DMD4 (M6%)), organic matter digestibility (OMDM6%), metabolizable energy (MEC), short chain fatty acid

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(SCFAM6%) and net energy for lactation (NEl (M6%)) contents of PSM were 892.1a (%), 65a g/kg DM, 11.1a MJ/kg DM, 1.1a mmol and 6.9a MJ/kg DM respectively. The gas production from soluble fraction (aM6%), and from insoluble fraction (bM6%), rate constant of gas production during incubation (CM6%) and the potential gas production (a+ bM6%) contents of potato plant silage were 15.5a (ml/200mg DM), 60.6c (ml/200mg DM), 0.03b (ml/h) and 76.1b (ml/200mg DM), while for level control were 10.6d (ml/200mg DM), 47.3c (ml/200 mg DM), 0.04b (ml/h) and 57.9d (ml/200 mg DM). This investigation demonstrated that the PSM, have the potential to enhance ruminal fermentation efficiency, milk production efficiency in dairy cattle and a promising methane mitigating agent.

Keywords: Potato silage males; NFC; short chain fatty acid; in vitro.

1. INTRODUCTION

In ruminants, nutrient inputs are first subjected to fermentative digestion by ruminal microorganisms. The microbial fermentation products eventually become available as energy (Volatile Fatty Acids) and protein (microbial cells) for animal tissue metabolism. Provision protein requirements of lactating cows and fattening calves with high growth rate use of slowly degradable protein in the rumen, but digestible in the small intestine is essential. Ruminal fermentation of hexoses and amino acids is accompanied with losses of energy and amino nitrogen, respectively [1].

About, 8-12% of digestible energy ingested by ruminants is lost in the rumen as methane, and from 75-85% of the nitrogen consumed by dairy cows is excreted in feces and urine [2].

Chumpawadee et al. [3] found that nutritive value of ruminant feeds is determined by the concentration of their chemical composition, as well as rate and extent of digestion in the rumen.

Three common methods including*; In Situ*, *In Vivo* and *In Vitro* techniques have been used in order to evaluate the nutritive value of feedstuffs. The nylon bag (*In Situ*) technique provides a useful tool for the initial estimation of feedstuffs in the rumen. It's an efficient method for measuring rate and extent of digestion in the rumen [4,5].

The *In Vitro* gas production technique developed by Menke et al. [6] is a useful tool for the rapid screening of feeds to assess their potential as energy sources for ruminant animals, assuming that the volume of gas produced reflect the end result of the fermentation of the substrate to short chain fatty acids (SCFA), microbial biomass and the neutralization of the SCFA [7].

This technique has been used by Blummel and Ørskov [8], to determine gas production at several incubation times, and the values obtained could describe the pattern of fermentation of feed by using the model of Larbi et al. [9].

In addition, the application of models permits description of the fermentation kinetics of the soluble and readily degradable fraction of the feed and the more slowly degradable fraction to be described [10].

Ruminal fermentation of amino acids result in losses of energy and amino nitrogen, respectively [1].

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- products as alternative feed stuff for ruminants. The by-product has the advantages reducing dependence of livestock on grains that can be consumed by humans, and to eliminate the need for costly waste management programs. The 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 partly replace the forage component in the cow's diet without adverse effect on milk efficiency with yield 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. Similarly 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 the world was 330 million tons/year; in Iran potato production is 21.4 million tons/year. With production in, Khorasan state being 4% of production total in Iran.

Feeding ruminants in intensive production systems, particularly for dairy production, requires supplies high of levels energy and protein. Ruminant animals are therefore fed on diets rich in starch and high quality protein, which are fermented very rapidly. However 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 can be denatured by treatment by form aldehyde, antibiotics have been used to suppress the bacterial populations responsible for the rapid protein fermentation [10]. However But the use of such compounds has been criticized, as they may leave harmful residues in the food chain and promote the spread of resistance genes. The observations arising from these researches the that large quantities of waste potato can be fed to ruminant animals and adequately degraded in the rumen. The objective of this study was to evaluate the potential of PSM degradability and fermentation pattern by the *In Vitro* gas production test.

2. MATERIALS AND METHODS

2.1 Silage Preparation and Sampling

The experiment was arranged as a completely randomized design with 3 replications. The fresh Plant Potatoes material was collected from Khorasan state Iran, the material was manually chopped (4-5cm lengths) as treatments the material were treated with Molasses at 2, 4, and 6% fresh the treatments were made in triplicate and ensiling was done in micro silos in laboratory plant potatoes. Silos were stored in the dark at ambient temperatures (20-22ºC) and opened after 45 days of ensiling. The contents of each opened silo were thoroughly mixed and samples were collected for DM determination and chemical analysis.

2.2 Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105^{o}C overnight and Ash by igniting the samples in muffle furnace at 600^{o}C for 8h and Nitrogen (N) content was measured by the Kjeldahl method [12].

Crude protein (CP) was calculated as N×6.25 [13].

Non-Fibrous Carbohydrate (NFC) is calculated using the equation of [14], NFC=100 – (NDF + CP + EE + Ash). All chemical analyses were carried out in triplicate.

2.3 In vitro Gas Production

Fermentation of PSM samples were carried out with rumen fluid was obtained from three mature canulated steers (BW=550) fed twice daily by a ration containing alfalfa (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of [15] as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100ml in the absence and presence of level 0, 2, 4 and 6% (PSM). The syringes were pre-warmed at 39ºC before injecting 30ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39ºC. The syringes were gently shaken 30min after the start of incubation and every hour for the first 10h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 16, 24, 48, 72 and 96 hours after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for PSM samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model of [16].

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

Where P, is the gas production at time t. also, a, gas production from soluble fraction (ml/200mg DM), b, the gas production from insoluble fraction (ml/200mg DM), c, the gas production rate constant (ml/h), a+ b the potential gas production (ml/200mg DM) and t is the incubation time (hours).

The metabolizable energy (MJ/kg DM) content of PSM was calculated using equations of [6,15,17] as follows: for all feeds.

ME (MJ/kg DM) = 0.016 DOMD for forage feeds
ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CF2 for concentrate feeds
ME (MJ/kg DM) = 1.06 + 0.157 GP + 0.084 CP + 0.22 CF - 0.081 CA

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 [18,19].
Where, Gas is 24 h net gas production (ml/200mg DM).
SCFA (m mol) = 0.0222 × GP – 0.00425

The organic matter digestibility was calculated using equations of [6] as follows: OMD (g/kg DM) = (%) 14.88 + 0.889 GP + 0.45 CP +XA

Where:

GP = about 24 h net gas production (ml /200mg-1)
CP = Crude protein (%)
XA = Ash content (%)
NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA -0.36 [20].

2.4 Statistical Analysis

Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS [21]. Multiple comparison tests used Duncan's multiple-range test. Significance between individual means was identified using the Duncan's multiple range tests. Mean differences were considered significant at ($P<0.01$). Standard errors of means (SEM) were calculated from the residual mean square in the analysis of variance. All data obtained from three replicates n=3.

3. RESULT AND DISCUSION

Chemical composition of utilized fresh plant potato and silage is shown in Table 1. The effect of incubating the substrate *In Vitro* during 0, 2, 4, 6, 8, 16, 24, 48, 72 and 96 hours with different levels of Molasses (0, 2, 4 and 6%) on gas production and the parameters estimated from gas production is shown in Table 2.

The results showed that gas volume at 2h incubation (for 200mg samples), were 7.6^{d} and 8.6^{c} ml/200mg DM for PSM with 0 and 2% Molasses, also, 9.6^{b} and 10.6^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1 ml/30ml buffered rumen fluid), respectively. Gas volume at 4h incubation (for 200mg dry samples), were 17^{d} and 18^{c} ml/200mg DM for PSM with 0 and 2% Molasses, also, 22^{b} and 26^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 6h incubation (for 200mg dry samples), were 26^{d} and 27^{c} ml/200mg DM for PSM with 0 and 2% Molasses, although, 31.01^{b} and 36.01^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 8h incubation (for 200mg dry samples), were 28.6^{d} and 31.6^{c} ml/200mg DM for PSM with 0 and 2% Molasses, also, 34.6^{b} and 40.6^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 16h incubation (for 200mg dry samples), were 33.6^{d} and 34.6^{c} ml/200mg DM for PSM with 0 and 2% Molasses, although, 38.6^{b} and 43.6^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 24h incubation (for 200mg dry samples), were 39.01^{d} and 44^{c} ml/200mg DM for PPS with 0 and 2% Molasses, also, 46.03^{b} and 52^{a} ml/200mg DM for PSM with 4 and 6% Molasses (1ml/30 ml buffered rumen fluid), respectively. Gas volume at 48h incubation (for 200mg dry samples), were 46.6^{d} and 47.6^{c} ml/200mg DM for PSM with 0 and 2% Molasses, although, 52.6^{b} and 55.6^{a}ml/200mg DM for PSM with 4 and 6% Molasses (1ml/30 ml buffered rumen fluid), respectively.

Table 1. Chemical composition and fermentation properties of green part of plant before and after ensiling (DM basis)

Item	DM%	CP%	EE%	NDF%	ADF%	Ash%	NFC%	pH	Ca%	P%	CF%	CE%	Mn%	Mg%	Lactat (mMol)
M_C	27.1	10.1	2.5	33	27	3.5	48.8	4.2	4.7	0.15	13.7	3280	0.03	4	1.9
$M_{2\%}$	27.8	9.3	2.4	30.01	22.5	3.8	54.5	4.9	3.7	0.16	10.3	3814	0.1	4	3.7
$M_{4\%}$	28.3	8.4	2.6	28.1	21.5	4.2	56.7	4.3	4.4	0.14	9.5	3867	0.007	4	4.7
$M_{6\%}$	29.07	8.1	2.3	27.07	20.5	4.8	57.7	4.1	4.1	0.16	8.7	3858	0.02	4	5.03

DM, Dry matter; CP, Crude protein; EE, Ether extract; NDF, neutral detergent fiber; ADF, Acid detergent fiber; CF, Crude fiber; GE, Gross energy.
$M_{2\%}$= added 2% Molasses; $M_{4\%}$= added 4% Molasses; $M_{6\%}$= added 6% Molasses

Table 2. Gas production in different time rumen and treatments

Incubation time	M_C	$M_{2\%}$	$M_{4\%}$	$M_{6\%}$	Pr>F	SEM
2	7.6^{d}	8.6^{c}	9.6^{b}	10.6^{a}	<0.0001	0.33
4	17^{d}	18^{c}	22^{b}	26^{a}	<0.0001	1.07
6	26^{d}	27^{c}	31.01^{b}	36.01^{a}	<0.0001	1.18
8	28.6^{d}	31.6^{c}	34.6^{b}	40.6^{a}	<0.0001	1.33
16	33.6^{d}	34.6^{c}	38.6^{b}	43.6^{a}	<0.0001	1.18
24	39.01^{d}	44^{c}	46.03^{b}	52^{a}	<0.0001	1.4
48	46.6^{d}	47.6^{c}	52.6^{b}	55.6^{a}	<0.0001	1.7
72	52.8^{d}	54^{c}	58^{b}	69^{a}	<0.0001	1.9
96	62.6^{d}	67.6^{c}	71.6^{b}	76.6^{a}	<0.0001	1.55

$M_{2\%}$= added 2% Molasses; $M_{4\%}$= added 4% Molasses; $M_{6\%}$= added 6% Molasses

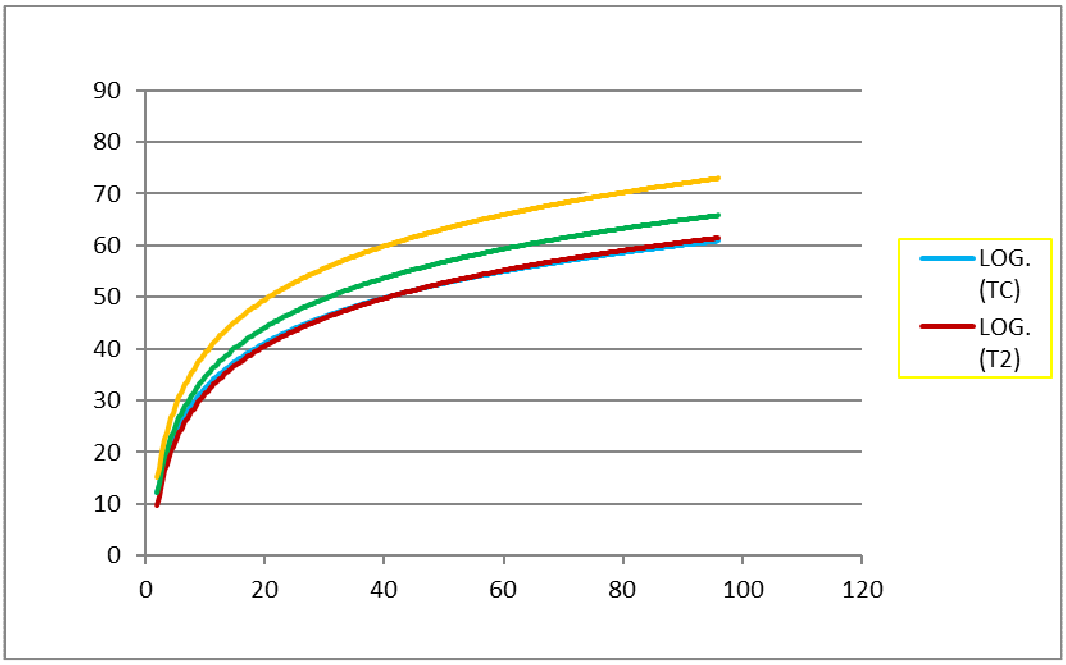

Fig. 1. *InVitro* gas production volumes of PSM **treated by Molasses at different incubation time**

Based on the above Fig. 1 the cumulative volume of gas production increased with increasing time of incubation. Although there're other models available to describe the kinetics of gas production, the Ørskov and McDonald [23]; Mirzaei-Aghsaghali et al. [22,10]; Maheri-Sis et al. [19] was chosen because the relationship of its parameters with Intake, Digestibility and Degradation characteristic of forages and concentrate feedstuffs had been documented. Sommart et al. [23] 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 *Invitro* system [19,22,10]. Gas volumes also have shown a close relationship with feed intake [24] and growth rate in cattle [8,19,22,10].

The soluble fraction (a) makes it easily attachable by ruminal microorganisms and leads to much gas production. The gas volumes at asymptote (b) have the advantage for predict feed intake Table 3.

The gas production for level control from soluble fraction (a), the gas production from insoluble fraction (b), rate constant of gas production during incubation (c) and the potential gas production (a+ b) contents of PSM were 10.6^{d} (ml/200mg DM), 47.3^{d} (ml/200mg DM), 0.04^{a} (ml/h) and 57.9^{d}(ml/200mg DM), while for level treated by 2% Molasses were 14.2^{b} (ml/200mg DM), 65.8^{a} (ml/200mg DM), 0.01^{d} (ml/h) and 80.1^{a} (ml/200mg DM).

Table 3. Degradation parameters of different treatments

Degradation parameter	$M_{C\%}$	$M_{2\%}$	$M_{4\%}$	$M_{6\%}$	Pr>F	SEM
a(mg/g)	10.6^{d}	14.2^{b}	12.2^{c}	15.5^{a}	<0.0001	0.5
b(mg/g)	47.3^{d}	65.8^{a}	62.1^{b}	60.6^{c}	<0.0001	2.1
Potential Degradability (a+b) (mg/g)	57.9^{d}	80.1^{a}	74.4^{c}	76.1^{b}	<0.0001	2. 5
c(ml/h)	0.04^{a}	0.01^{d}	0.02^{c}	0.03^{b}	<0.0001	0.002

$M_{2\%}$= added 2% Males; $M_{4\%}$= added 4% Males; $M_{6\%}$= added 6% Males

Table 4. OtherItems of different treatments

Item	$M_{C\%}$	$M_{2\%}$	$M_{4\%}$	$M_{6\%}$	Pr>F	SEM
GP	115^{b}	110^{c}	115^{b}	130^{a}	<0.0001	2.2
SCFA	1.01^{b}	0.9^{c}	1.01^{b}	1.1^{a}	<0.0001	0.02
ME	11.1^{a}	9.9^{c}	9.9^{c}	10.4^{b}	<0.0001	0.15
$Intake_4$	66.1^{a}	57.5^{d}	60.4^{c}	65.7^{b}	<0.0001	1.09
$Intake_3$	50.3^{b}	45.2^{d}	47.3^{c}	51.5^{a}	<0.0001	0.75
$Intake_2$	65^{a}	56.2^{d}	58.8^{c}	64^{b}	<0.0001	1.1
$Intake_1$	57.1^{a}	56^{b}	54.8^{c}	53.1^{d}	<0.0001	0.44
DMD_4	739.2^{d}	758.2^{c}	812.5^{b}	892.1^{a}	<0.0001	17.8
DMD_3	636^{d}	661.5^{c}	701.7^{b}	764.4^{a}	<0.0001	14.6
DMD_2	664.4^{d}	696.1^{c}	729^{b}	778.6^{a}	<0.0001	12.7
DMD_1	537.8^{d}	601.1^{c}	640.2^{b}	696.5^{a}	<0.0001	17.4
DOM_1	60.7^{b}	58.5^{d}	59.9^{c}	65.2^{a}	<0.0001	0.7
DOM_2	52.3^{b}	49.9^{d}	51.4^{c}	57.2^{a}	<0.0001	0.8
OMD	60.5^{b}	58.4^{d}	59.8^{c}	65^{a}	<0.0001	0.7
Net Energy	6.9^{a}	6.4^{b}	6.4^{b}	6.9^{a}	<0.0001	0.07

SCFA, short chain fatty acids; ME, metabolisable energy; Intake, Daily Intake; OMD, organic matter digestibility; $M_{2\%}$= added 2% Molasses
$M_{4\%}$= added 4% Molasses; $M_{6\%}$= added 6% Molasses

Also, for level 4% from soluble fraction (a), the gas production from insoluble fraction (b), rate constant of gas production during incubation (c) and the potential gas production (a+ b) contents of PSM were 12.2^{c} (ml/200mg DM), 62.1^{b} (ml/200mg DM), 0.02^{c} (ml/h) and 74.4^{c}(ml/200mg DM), while for level treated by 6% Males were 15.5^{a} (ml/200mg DM), 60.6^{c} (ml/200mg DM), 0.03^{b} (ml/h) and 76.1^{b} (ml/200mg DM).

Salamat Azar et al. [25] estimation effect of tree doses thyme methanolic extract (0, 0.15 and 0.3) on degradability kinetics, of Sunflower 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 Sunflower meal were 44.99, 3.60, 49.32, 52.92 ml/200 mg DM and 0.135 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 thyme methanolic extract (0.15 ml) were 29.91, 0.53, 36.25, 36.79 ml/200mg DM and 0.049 ml/h, respectively.

Cardozo et al. [26], in a continuous culture experiment, were the first to suggest that cinnamon oil (0.22mg/L of rumen fluid) modified the N metabolism of rumen microorganisms

by inhibiting peptidolysis, but the effects on VFA concentration were negligible (Calsamiglia et al, 2006; Calsamiglia et al, 2007).

Rezaei et al. [27] evaluation effect of tree doses clove methanolic extract (0, 0.5 and 1 ml) 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 *InVitro* dry degradability (DMD), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) of Potato Silage Molasses (PSM), percentages (0, 2, 4 and 6% Molasses) are presented in Table 4.

The *InVitro* dry degradability (DMD), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) of PSM, were significant (*p<0.0001*).

Calculated amounts of *InVitro* dry matter digestibility (DMD), organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) contents of $PSM_{Control}$ were 739.2^{d} (%), 60.5^{b} g/kg DM, 11.1^{a} MJ/kg DM, 1.01^{b}mmol and 6.9^{a} MJ/kg DM respectively. while, for $PSM_{2\%}$ were 758.2^{c} (%), 58.4^{d} g/kg DM, 9.9^{c} MJ/kg DM, 0.9^{c}mmol and 6.4^{b} MJ/kg DM, respectively. Also, DMD, OMD, ME, SCFA and NE_l of $PSM_{4\%}$ were 812.5^{b} (%), 59.8^{c} g/kg DM, 9.9^{c} MJ/kg DM, 1.01^{b}mmol and 6.4^{b} MJ/kg DM respectively. while, for $PSM_{6\%}$ were 892.1^{a} (%), 65^{a} g/kg DM, 10.4^{b} MJ/kg DM, 1.1^{a}mmol and 6.9^{a} MJ/kg DM, respectively.

Salamatazar et al. [25] evaluation the effect of thyme water extract on the net energy for lactation, short chain fatty acid, *In vitro* dry matter digestibility, metobolizable energy and organic matter digestibility of soybean meal for ruminant and report metobolizable energy of soybean meal was 10.62 (MJ/Kg DM) and metobolizable energy of thyme water extract (0.15 and 0.3 ml/30 ml buffered rumen fluid) were 10.6 and 10.21 (MJ/Kg DM), respectively. Salamatazar et al. [25] evaluation effect of tree doses thyme (zatariamultiflora) water extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metobolizable energy and organic matter digestibility of sunflower meal using *In Vitro* gas production technique and report metobolizable energy of sunflower meal was 8.36 (MJ/Kg DM) and metobolizable energy of zatariamultiflora water extract (0.15 and 0.3 ml/30 ml buffered rumen fluid) were 8.20 and 8.04 (MJ/Kg DM), respectively.

4. CONCLUSION

This study found that green fodder potatoes can be useful in ruminant feed and microbial protein appears to be effective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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