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Influence of Different Amounts of Molasses and Microbiologically Treated Potato Aerial Part on *in vitro* Gas Production

Research Article

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ABSTRACT

This project aimed to study the effects of *in vitro* gas production when varying amounts of molasses- and microbial additive-treated aerial part of the potato are added in place of alfalfa hay in a total mixed ration (TMR). The experiment used three treatments and three replicates in a completely randomized design. 1) control (100% alfalfa hay in the fodder part of the diet), 2) T50 (50% alfalfa hay, 50% processed aerial part of potato in the fodder part of the diet) and 3) T100 (100% processed aerial part of potato in the fodder part of the diet) were the treatments. To collect rumen fluid, two fistulated male sheep were used. After incubation, the *in vitro* gas production amount was measured at 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 hours. After two hours of incubation, no difference was apparent between the experimental treatments. Treatments T50 and T100 showed no significant difference in the 4th to 12th hour of incubation, but both showed a significant difference from treatment control ($P \leq 0.05$). Furthermore, all three treatments (control, T50 and T100) showed a highly significant difference ($P \leq 0.001$) in *in vitro* gas production from the 16th to the 96th hour of incubation. Treatments control, T50, and T100 produced 52.67, 61.6, and 68.53 ml of *in vitro* gas per 300 mg dry matter, respectively, based on the total volume of *in vitro* gas production. On this basis, lambs fed TMR instead of alfalfa hay can to utilize all of the processed aerial part of potato.

KEY WORDS alfalfa hay, *in vitro* gas production, microbial additive, molasses, processed potato aerial part.

INTRODUCTION

Agricultural waste must be used to feed livestock as fodder production has its limits. In addition, enriched crop residues must be used for animal feed, as many countries lack sufficient animal feed supplies (Najafyar *et al.* 2011; Havekes *et al.* 2019). Feeds containing unprocessed agricultural residues reduce feed consumption because weight gain due to the presence and distribution of lignin, which slows the fermentation rate and increases bulk. Several restrictions as livestock feed result in a reduced importance and nutritional value of these materials (Van Soest *et al.* 1991). The influence of these factors on the decreasing digestibility of certain agricultural products was greater than that of lignin. An example of this are glycoalkaloids from the nightshade family (Alibes, 1984).

After rice and wheat, potatoes (*Solanum tuberosum*) are the third most consumed crop in the world. Currently grown in 100 different countries, potatoes are a crop with great versatility that can be raised in a variety of environments. In comparison to maize (7.5M), rice (7.4M), wheat (3M), and soybean (2.8M), potato crops can yield 9.2M calories per acre, which was more valuable. As one of the most important glycol-alkaloids in potatoes, solanine was found in both the tuber and the above-ground part of the plant after harvest. Therefore, potatoes with leaves and stalks cannot be used in the livestock ration in the recommended quantities. This was just one of the by-

products that can be mentioned (Jadhav *et al*. 1981). Annual global potato production was estimated at over 105.8 million tons, including the above-ground potato parts (FAOSTAT, 2020). The surface part of the potatoes was rarely used. Reports are indicating that the concentration of solanine in the aerial part of the potato decreases when this part was made into silage (Parfitt *et al.* 1982).

A by-product of the sugar industry was molasses. Sugar production produces two types of molasses: sugar beet molasses, which was obtained from sugar beets, and sugar cane molasses, which was obtained from sugar cane (McDonald *et al.* 2011). The nutrient composition changes depending on the starting substrate. With a dry matter content of about 75%, molasses was high in sugar but low in fat and fiber (NRC, 2007). In particular, the many minerals contained in molasses include potassium, sulfur and sodium (NRC, 2007). Ruminants can obtain inexpensive carbohydrate sources from molasses (Karalazos and Swan, 1977). In tropical countries where sugarcane was grown in large quantities and conventional grains become more expensive, molasses may be added to the diet of ruminants (El Khidir and Vestergaard Thomsen, 1982). Molasses can be added to a dietary supplement as an easily fermentable energy source because grazing in tropical and subtropical regions was typically of low quality (McDonald *et al.* 2011). The water-soluble carbohydrates in molasses provide rumen microorganisms with an easily fermentable source of carbohydrates, which was beneficial when feeding animals low-quality feed.

Digestibility and absorption in turn determine how effective the food was, for example in muscle growth or milk synthesis. However, *in vivo* studies (on live animals) to assess feed digestibility are costly, time-consuming and require large quantities of feed. Such studies are unsuitable for rapid and routine feed evaluations by commercial laboratories that provide feed manufacturers and feed manufacturers with feed data. Biological techniques called "*in vitro* techniques," which are performed outside the animal system but mimic the digestive process, can also be used to estimate the digestibility of feedstuffs. *In vitro* methods are typically based on the quantification of the products or residues of fermentation. The former weighs the amount of unfermented residue left after *in vitro* incubation of a feed with rumen fluid. Anaerobic fermentation products are measured using more modern techniques. Anaerobic microbes ferment rumens to produce microbial mass, gases (carbon dioxide [CO_2] and methane [CH_4]), and short-chain fatty acids (SCFA). As a measure of the acids produced during fermentation, the amount of gas produced was proportional to the amount of acid produced. The degree and rate of feed digestion are predicted by measuring the amount of gas produced during incubation.

Studies examining the effects of replacing alfalfa hay with the above-ground part of the potato are insufficient. With this motivation, the goal of the current study was to determine how *in vitro* gas production would change if alfalfa replaced the above-ground potato parts processed with molasses and microbial additives at zero, 50%, and 100% concentrations.

MATERIALS AND METHODS

Chemical compounds

The AOAC (2005) and Van Soest *et al*. (1991) methods were used to measure the chemical composition of the materials tested in the study.

Aerial part of potato and its processing

The above-ground part of the potato, which belongs to the modified variety Jilli, was harvested from fields in the Sarab region of Iran. The microbial additive used in this study was manufactured by Gol Sahand Company, a knowledge-based company based in Khosrowshahr, Iran. where the bacterium *Lactiplantibacillus pentosus* was used. The company that produces microbial additives recommended mixing 1000 kg of air-dried potato aerial parts with 500 kg of molasses, 1000 kg of water and 300 kg of microbial additive. The resulting mixture was then stored at room temperature for 40 days after being sealed in Dark nylon bags under anaerobic conditions.

Animals

Rumen liquor samples were obtained from the two adult (16 months old) Afshar male sheep (weight 45.8±3 kg) which were kept in metabolic cages and fed on a diet containing 60% alfalfa hay and 40% commercial concentrate at maintenance level (NRC, 2007). Diet offered to the animals twice daily at 9.00 AM and 4.00 PM in equal sized meals. The animals had access to fresh water and mineral lick *ad libitum*. Rumen fluid was collected 2 h after the morning feeding.

Measurement of *in vitro* gas production

To measure gas production, used the method of Fedorak and Hrudey (1983). In this method, the amount of gas production was represented by the amount of liquid displacement in the U-shaped test tube attached to the jars containing the feed sample and rumen fluid (Fedorak and Hrudey, 1983).

Treatments

The three treatments used in the experiments were: 1) control (100% alfalfa hay in the fodder part of the diet), 2) T50 (50% alfalfa hay, 50% processed aerial part of potato in the

fodder part of the diet) and 3) T100 (100% processed aerial part of potato in the fodder part of the diet) shown in Table 1.

Determination of the components of gas production

The components of *in vitro* gas production were determined using the MacDonald (1979). To reconcile the *in vitro* gas production data, equation 1 was applied.

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

Where:

P: gas production rate at time t.

a and *b*: gas production rates of the soluble and insoluble parts, respectively.

c: gas production rate per hour or the gas production rate, respectively.

e: Neprin constant number (2.718).

Determination of metabolizable energy

The amount of metabolizable energy was estimated for the feed materials using Equation 2, which took into account the amount of gas produced (as reported in the report by Getachew *et al.* (2004) and Menk and Steingass (1988).

Equation 2:

ME (kcal/kg DM)= (2.2+(0.136×GP)+(0.057×CP)+(0.0029×CF)) × 238

The variables in the above equation are: GP: gas produced in 24 hours (mL), CP: crude protein (percentage), CF: crude fiber (percentage) and CA: crude ash (percentage).

Estimating the amount of digestibility of dry matter

For this reason, the amount of gas produced was based on Khazaal *et al.* (1995) report was used. Equation 3 was applied as follows accordingly:

Equation 3:

DMD (g/kg DM)= -17 + (14.9×a) + (10.9×b) + (1559×c)

The above equation values are: a: soluble part gas production (mL), b: non-soluble part gas production (mL), c: gas production rate (mL/h), and CP: crude protein (percentage).

The amount of digestibility of organic matter

For this purpose, using the amount of gas produced, raw ash and raw protein according to the report of Menk and Steingass (1988), equation 4 was used.

Equation 4: DOM (g/kg DM)= 14.88 + (0.889×GP) + (0.45×CP) + (0.0951×CA)

The variables in the above equation are: GP: gas produced in 24 hours (mL), CP: crude protein (percentage), CF: crude fiber (percentage), and CA: crude ash (percentage).

Estimating the production of short-chain fatty acids

For this purpose, using the amount of gas produced according to the report of Getachew *et al.* (2002), equation 5 was used.

Equation 5: SCFA (mmol/dL)= - 0.00425 + (0.0222×GP)

The variables of the above equation are: GP: gas produced in 24 hours (mL).

Statistical analysis

The General Linear Model (GLM) procedure and SAS software version 9.2 (SAS, 2009) were used to analyze the data from the gas production test in a completely randomized design with three treatments and three replicates. The Duncan test was used to compare the average at a five percent error level. Consequently, the data were fitted with the subsequent model (equation 6).

Formula 6:

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

Where:

Y_{ij}: value of each observation.

T_i: treatment effect.

μ: was the mean of the whole experiment.

eij: test error.

RESULTS AND DISCUSSION

Chemical composition of potato aerial part, processed potato aerial part and alfalfa hay were repoted in table 1. Compared to those reported by Salehi *et al.* (2008) given the values (14.1 and 30.18 respectively), the aerial part of potato examined contained less crude protein (CP) and more neutral detergent insoluble fiber (NDF) by Salehi *et al.* (2008).

The hydrolysis of cell wall components during the fermentation process was the reason for the decrease in NDF that processing has caused, as Table 2 illustrates (Noordar, 2012).

Table 3 shows that there was no noticeable change in the *in vitro* gas production capacity of the experimental treatments after 2 h of incubation.

Table 1 Components and composition experimental treatments (percentage of ration dry matter)

Composition of feed ingredients	Control	T50	T100
Processed aerial part of potato	0	20	40
Dry alfalfa hay	40	20	0
Corn seeds	24.3	24.3	24.3
Barley seed	24.2	24.2	24.2
Wheat bran	7.68	7.68	7.68
Slow-release urea	0.82	0.82	0.82
Buffer	1.02	1.02	1.02
Mineral supplement	1.80	1.80	1.80
Salt	0.18	0.18	0.18
Nutrients			
Metabolizable energy (Mcal/kg)	2.76	2.76	2.76
Crude protein (%)	13.5	13.5	13.5
Calcium (%)	0.75	0.75	0.75
Phosphorus (%)	0.3	0.3	0.3

Control: 100% alfalfa hay; T50: 50% alfalfa hay, 50% processed aerial part of potato and T100: 100% processed aerial part of potato.

Table 2 Chemical composition of potato aerial part, processed potato aerial part and alfalfa hay (% DM)

Item	DM	CP	NDF
Alfalfa hay	92.3±0.6	12.71±1.2	47.35±3.9
Processed aerial part of potato	44.91±0.4	12.99±1.1	34.23±2.4
Aerial part of potato	92.83±0.8	13.45±0.9	48.94±2.1

DM**:** dry matter; CP: crud protein and NDF: neutral detergent insoluble fiber.

Table 3 Statistical analysis of *in vitro* gas production values of experimental treatments (mL/300 mg of DM)

Incubation Time	Control	T50	T100	P-value	SEM
2	9.47^{a}	9.46^{a}	9.27^{a}	0.3944	0.06455
4	15.13^{b}	19.20^{a}	20.67^{a}	0.0231	0.97866
6	21.27^{b}	26.00^{a}	27.93^{a}	0.0320	1.19861
8	26.07^{b}	31.87^{a}	33.20^{a}	0.0316	1.3239
12	31.67^{b}	37.47^{a}	42.13^{a}	0.0069	1.68197
16	36.60^{c}	43.47^{b}	49.13^{a}	0.0037	1.97062
24	40.07^{c}	47.93^{b}	53.93^{a}	0.002	2.1479
36	46.07^{c}	53.73^{b}	59.87^{a}	0.0032	2.16062
48	49.00^{c}	57.33^{b}	64.00^{a}	0.0026	2.33487
72	51.33^{c}	59.93^{b}	66.73^{a}	0.0013	2.35985
96	52.67^{c}	61.60^{b}	68.53^{a}	0.0019	2.45198

Control: 100% alfalfa hay; T50: 50% alfalfa hay, 50% processed aerial part of potato and T100: 100% processed aerial part of potato.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Treatments T50 and T100 were not significantly different from observation 4 to 12 hours of incubation, but the *in vitro* gas producers of both treatments were significantly (P<0.001) greater than treatment control, in the incubation period of 24 to 96 hours, the values of *in vitro* gas production of treatment T50 were significantly (P<0.001) higher than those of treatment control and the values of treatment T100 were significantly (P<0.001) higher than those of treatment T50. The current results confirmed the data of Noordar (2012), that ensiling and processing the above-ground part of the potato with molasses significantly increased the digestibility of dry matter.

It seems that the reason for the highly significant increase (P<0.001) in *in vitro* gas production values in treatments T50 and T100 compared to treatment control may be due to decreased in lignin content, an increase in the digestibility of the cell wall and the release of soluble sugars in the rumen and increase the growth of amylolytic bacteria.

Table 4 shows that there was no apparent difference between the experimental treatments in terms of *in vitro* gas production values for section a. The results obtained may indicate that there was no significant difference between the experimental treatments in terms of the water-soluble fraction of dry matter digestibility.

Table 4 Statistical analysis of *in vitro* gas production parameters of experimental treatments (mL/300 mg of DM)

Parameters[2]	Control	T50	T100	P-value	SEM
a	4.49[a]	3.96[a]	2.36[a]	0.1987	0.49775
b	46.75[c]	55.37[b]	63.75[a]	0.0024	2.63873
c	0.0698[b]	0.0771[b]	0.0793[a]	0.2871	0.002446

Control: 100% alfalfa hay; T50: 50% alfalfa hay, 50% processed aerial part of potato and T100: 100% processed aerial part of potato.
a: gas production of the soluble portion; b: gas production of insoluble but fermentable portion and c: constant rate of gas production (mL/h).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Statistical analysis of metabolizable energy (ME), dry matter digestibility (DMD), digestibility of organic matter (DOM) and short-chain fatty acids (SCFA) values

Item	Control	T50	T100	P-value	SEM
ME (kcal/kg DM)	1485.64[c]	1636.07[b]	1760.16[a]	0.0036	43.1108
DOM (%)	42.34[c]	46.77[b]	50.52[a]	0.0023	1.26850
DMD (%)	66.84[c]	76.57[b]	83.66[a]	0.0021	2.61078
SCFA (mmol/dL)	5.34[c]	6.38[b]	7.22[a]	0.0037	0.29088

Control: 100% alfalfa hay; T50: 50% alfalfa hay, 50% processed aerial part of potato and T100: 100% processed aerial part of potato.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

However, as the proportion of the above-ground portion of the processed potato increased from zero to fifty percent and from fifty to one hundred percent, the amount of gas produced in the laboratory by the water-soluble portion of the dry matter also decreased numerically. Given that the concentrated fraction was the main component in each of the three experimental treatments, the above result makes sense.

The *in vitro* gas production of part b (potentially degradable) was significantly different among the experimental treatments (P<0.001), and the *in vitro* gas production values for treatments Control, T50, and T100 were 46.75, 55.37, and 63.75, respectively. This finding suggests that processing the aerial part of the potato with molasses and microbial additive increased the degradability of part b (potentially degradable) in treatments T50 (50 percent of the processed aerial part of potato) and T100 (100 percent of the processed aerial part of potato) significantly increased (P<0.001) compared to treatment Control (with 100 percent alfalfa hay).

Furthermore, although there was no statistically significant difference between experimental treatments, part c (constant gas production rate) was highest in experimental treatments T100, T50, and Control (0.0793, 0.0771, and 0.0698, respectively). It seems likely that the increase in the constant *in vitro* gas production rate (section c) in treatments T50 and T100 compared to treatment control was caused by: a decrease in the amount of lignin, an increase in cell wall digestibility, the release of soluble sugars in the rumen, and a growth of amylolytic bacteria.

As shown in Table 5, the ME, DMD, DOM, and SCFA values of treatment *T50* are significantly (P≤0.001) higher than the ME, DMD, DOM, and SCFA values of treatment Control and treatment T100 were significantly (P<0.001) higher than that of treatment T50.

According to the above results, the application of molasses and a microbial additive increased the nutritional value of the aboveground part of the potato in terms of ME, DMD, DOM and SCFA. The results also show that ME, DMD, DOM and SCFA values increased significantly (P<0.05) when the proportion of aboveground fraction (from zero to fifty percent) and feed fraction (from fifty to one hundred percent) of processed potatoes in the diet was increased. The use of molasses in processing and increasing the digestibility of the potato cell wall contents in the above-ground part appears to be the cause of the significant increase in ME, DMD, DOM and SCFA values in treatments T50 and T100 compared to treatment Control microbial additives lead to the breakdown of the lignin. Furthermore, the ensiling step of processing offsets the deleterious effects of the low pH of solanine (Parfitt *et al.* 1982). The results we obtained were consistent with those of Behzadifard (2011), who found that processing the aerial part of potatoes with urea and molasses significantly increased the concentrations of ME, DMD, DOM and SCFA (P≤0.05).

CONCLUSION

Due to the limitations in forage production in many parts of the world, the use of agricultural waste, such as the aerial parts of potatoes after harvesting, can be utilized as part of livestock feed. The results of a recent study indicate that processing, along with the addition of molasses and microbial additives to the aerial parts of potatoes, increases their nutritional value and *in vitro* gas production. Overall, it can

be stated that the aerial parts of potato plants have nutritional value, but to optimize their use and increase their efficiency in animal feed, processing is necessary. In this regard, the addition of microbial additives and molasses can be considered.

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