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Effects of Adding Different Levels of Lactobacillus Inoculant to Alfalfa Silage Ensiled With Orange Pulp on *In Vitro* Gas Production and DM Digestibility

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Abstract

This study was conducted to study the effects of supplementation alfalfa silage with orange pulp and difference of Lactobacillus buchneri on in vitro dry matter digestibility and gas production. wilted alfalfa with no additive (control), wilted Alfalfa and orange pulp (1750 g wilted Alfalfa mixed with 750 g fresh orange pulp) treated with LAB for final application rates of 0, 2.5, 5 and 7.5 g LAB inoculant/ton of wilted alfalfa and orange pulp (LAB0, LAB1, LAB2, LAB3, respectively). Alfalfa hay harvested at flowering stage and after 24 hours wilted and mixed orange pomace with ratio of 2100 g and 760 g, respectively, and was ensiled for 90 days. The data were analyzed in a completely randomized design with three replications. After 24 h incubation, treatments AO (alfalfa + orange pulp) and CON (without additive) had the highest and lowest in vitro gas production ($p<0.05$) and adding orange pulp and molasses increased gas production. Adding inoculant decreased in vitro DM digestibility. Results showed that ensiling alfalfa with orange pulp and molasses can improved silage quality and increased gas production and in vitro DM digestibility.

Keywords: Alfalfa silage; *In vitro* gas production; *Lactobacillus buchneri*; Orange pulp.

1. Introduction

Alfalfa is a forage crop with high nutritive value and is often a major component of diets for high-producing dairy cows [1]. It has a high buffering capacity, low water soluble carbohydrates (WSC) content and is rich in highly degradable crude protein [2]. As a result, it is more difficult to quickly reduce the silage pH, minimize clostridia growth, proteolysis and heterolytic fermentation, and to improve silage palatability compared to maize silage [3]. Growing up feeds cost values in many parts of the world have increased attending in utilization of citrus by-product feedstuffs as specific feeds for ruminants. One of the citrus by-products that produced exceedingly is orange pulp and its cost is partly low compared to its nutritive value. According to the FAO [4] the annual rate of world production of citrus fruits is about 106 million tons that the orange fruits represented the 63 % of the world citrus production. Due to the perishable property of these products, it would be convenient to develop methods of preservation that would enable these by-products to be utilized for longer periods of time [5]. According to statistics, Iran is one of 10 countries in the world's main producer of citrus pulp [6] that if the waste is not properly disposed of in the environment, can in the long term become an environmental problem. Because of the properties of surplus perishable fruits produced in highly productive seasons in different countries, as well as waste from processing plants, citrus products, an effective solution for the efficient use of these products in animal feed can be ensile. In addition, pectin also as a source of easily digestible carbohydrates are found at high levels in citrus pulp, which can be added to the waste of valuable forage quality alfalfa silage in the silo of the increase was derived.

One of the main concerns in the preparation of a good silage is the rapid decrease in silage pH in the shortest time. Hay pH at harvest time is between 6 and 7 and after the incubation period with proper fermentation, pH can be equal to or less than 4, which this reduction in pH is due to production of lactic acid and other organic acids by bacteria. Accelerate the reduction of pH by adding lactic acid bacteria in food is very important to minimize depreciation. Recent studies have shown that inoculation with *Lactobacillus buchneri* inhibits yeast growth and reduces the susceptibility to aerobic spoilage of various ensiled forages [7].

This study was conducted to study the effects of supplementation alfalfa silage with orange pulp and different of *Lactobacillus buchneri* on *in vitro* dry matter digestibility and gas production.

2. Materials and Methods

Chemical composition of wilted Alfalfa and orange pulp before ensiling is given in Table 1. The chemical compositions feeds were determined using the methods recommended by AOAC [8]. Determinations of N were conducted using the Kjeldahl method in an automated Kjel foss apparatus (Foss Electric, Copenhagen, Denmark).

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Neutral-detergent fiber (NDF) and Acid-detergent fiber (ADF) were determined by the detergent procedures of Van, *et al.* [9].

Whole fourth cut Alfalfa was harvested at 35 dry matter and wilted for 24h at room temperature. The wilted Alfalfa and fresh Orang pulp was chopped manually to approximately 2cm theoretical length of cut. Experimental treatment was wilted Alfalfa with no additive (as control treatment), wilted Alfalfa (1750g) mixed with fresh orange pulp (750g) and treated with LAB for final application rate of 0, 2.5, 5 and 7.5 g LAB inoculant/ton (LAB 0, LAB 1, LAB 2 and LAB 3 respectively). Inoculant were dissolved in distilled water (recommended by factory) and sprayed uniformly onto the treatment and for control treatment sprayed of distilled water. Experimental treatments were ensiled in triplicate laboratory mini silos for 90d at ambient temperature (15 to 18^{o}c) in a closed barn.

Table 1. The chemical composition of wilted alfalfa and orange pulp silage after 90 d (g kg^{-1} of DM)

Item	Treatments[1]					SEM
	CON	LB0	LB1	LB2	LB3	
DM	33.03	30.2	33.93	30.93	31.03	0.923
pH	4.5^{a}	4.34^{b}	4.33^{b}	4.26^{b}	4.24^{b}	0.043
NDF	29.56	31.36	31.03	29.83	30.03	0.658
ADF	20.96^{b}	23.23^{a}	19.46b^{c}	20.13^{b}	20.46^{b}	0.327
CP	17.75^{a}	16.87^{b}	17.06^{b}	16.31^{c}	16.18^{c}	0.95
QI[2]	90.86	91.53	99.4	96.2	97.46	-

[1]Treatments; wilted alfalfa with no additive (CON), wilted alfalfa (1750g) mixed with fresh orange pulp (750g) and treated with LAB for final application rate 0, 2.5, 5 and 7.5 g LAB inoculant/ton (LB0, LB1, LB2 and LB3, respectively).

[2]Quality index: 220+ (2×DM%-15)-40×pH.

Within a row, means followed by different letters differ (P<0.05).

2.1. In Vitro Trial

The amount of *in vitro* DM digestibility and gas production of treatments was measured in serum bottles according to the method of Fedorak and Hrudey [10]. Firstly, 300 mg of finely-ground silage (1 mm screen size) were weighed into 50 mL sterile serum bottles. A 20 mL mixture of rumen fluid and artificial buffer at a ratio of 1:2 [11], was added to each bottle and kept under continuous CO_2 flow. The rumen fluid was obtained 2 h after the morning feeding from two rumen fistulated sheep fed a total mixed ration of 600 g concentrate and 400 g lucerne hay/kg DM. The rumen content was filtered through four layers of cheesecloth to extract the filtrate to a warm flask containing CO_2, before being transfer to the laboratory. To avoid microbial heat shock, the bottles were warmed up to 39°C for 30 min before and while adding the mixture of rumen fluid and buffer to the sample under CO_2. The bottles were tightly capped and placed in an incubator at 39°C, shaking at 120 rounds per min. For each batch in the *in vitro* study three blank bottles, containing only the rumen fluid preparations without any sample were used to adjust the results for DM originating from the rumen fluid. The amount of DM digestibility of treatments was recorded at 2, 4, 8, 12 and 24 h post-incubation and Gas production was measured in each vial after 2, 4, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h of incubation using a water displacement apparatus [10].

3. Analytic Method

Data obtained was subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS [12] and treatment means were compared by the Duncan test.

4. Results and Discussion

Effects of treatments on *in vitro* gas production are given in Table 2. There were significant differences among treatments (p<0.05). After 12 h incubation, treatments LB0 with LB1 and CON (without additive) had the highest and lowest *in vitro* gas production (p<0.05) and adding orange pulp increased gas production. At the 24 h incubation supplemented treatments with orange pulp and bacterial additive had the highest gas production and. Supplementation treatments with orange pulp and inoculant had significant effect on gas production and increased gas production volume (p<0.05).

The effect of treatment on *in vitro* DM digestibility are given in Table 3. At the 2 h of incubation control treatment had lowest *in vitro* DM digestibility among treatments and adding orange pulp increased digestibility (P<0.05). After 24 h incubation, LB0 and CON respectively had highest and lowest *in vitro* DM digestibility among treatments (P<0.05). Supplementation silage with orange pulp and bacterial inoculant increased *in vitro* DM digestibility in all incubation times. There is highly relationship between *in situ* and *in vitro* DM digestibility in this study.

Adding orange pulp with inoculant increased gas production. *In vitro* gas production is highly influenced by the availability of both N and fermentable carbohydrate content [13, 14]. Menke and Steingass [15] reported a strong correlation between *in vitro* gas production and organic matter degradability of feeds. Many researchers have successfully used this technique to assess the impact of digestibility of feeds through this relationship [16, 17], because gas production rates can indicate the rate of digestion in the rumen and thereby affect the rate of passage and dry matter intake. In the present study, gas production of additives treated silages were increased as compared with

the control silage, probably due to different additive treatments reduced loss of nutrients, and then increased gas production. This is consistent with the findings of Kozelov, *et al.* [18] and Li, *et al.* [19]. An increased gas production might be related to improve the silage quality [20], which would also determine the microbial access to fermentable carbohydrates in the rumen. The increased *in vitro* gas production by the adding of molasses agrees with previous reports on grass and cereal silages [21-23] and can be explained by the higher silage water soluble carbohydrate content and increased carbohydrate fermentation.

Muck, *et al.* [16] and Hashemzadeh-Cigari, *et al.* [23] showed that silages treated with inoculants generally produced less gas per unit of incubated DM than the control silages. Blümmel, *et al.* [24] reported that gas production was positively correlated with DM digestibility, but negatively correlated with microbial biomass yield. Based on these results, they suggested that forages that produce less gas should have better microbial biomass production. Recently, Muck, *et al.* [16], who conducted an *in vitro* study with alfalfa silage inoculated with one of 14 inoculants plus an uninoculated control, found that some inoculated alfalfa produced less, and some produced more, gas than did uninoculated controls, suggesting that effects of microbial silage inoculants on *in vitro*

fermentation of silage are not the same among inoculants. The kinetics of ruminal degradation by *in vitro* gas production technique potentially reflect in vivo digestibility of forages in ruminants [25].

In vitro DM digestibility was lower in silage with inoculant than without inoculant. Adding molasses increased *in vitro* DM digestibility. Furthermore, although there are some reports that adding molasses has no effect on DM digestibility [26, 27].

Table-2. Effects of treatments on *in vitro* gas production at various incubation times

Treatments[1]	Incubation times (h)											
	2	4	6	8	12	16	24	36	48	72	96	120
CON	33.96	73.60	121.29^{b}	150.49^{c}	188.29^{c}	200.82^{b}	213.98^{b}	224.33^{c}	233.95^{c}	243.45^{c}	252.27^{c}	257.68^{c}
LB0	36.24	81.05	138.26^{a}	173.79^{a}	217.15^{a}	233.83^{a}	251.44^{a}	268.55^{a}	283.35^{a}	296.85^{a}	306.12^{a}	311.59^{a}
LB1	34.08	76.35	128.41^{ab}	163.03^{ab}	205.83^{ab}	222.45^{a}	239.77^{a}	255.52^{ab}	269.64^{ab}	282.39^{ab}	291.60^{ab}	296.79^{ab}
LB2	34.08	75.87	127.10^{ab}	161.26^{cb}	204.12^{b}	220.68^{a}	237.32^{a}	252.67^{ab}	266.22^{b}	278.23^{b}	287.22^{b}	292.29^{b}
LB3	33.96	75.82	126.19^{ab}	159.96^{cb}	202.47^{b}	219.43^{a}	235.73^{a}	250.28b	263.72^{b}	275.84^{b}	284.60^{b}	289.67^{b}
SEM	1.11	2.69	4.00	3.78	4.13	5.13	5.53	5.61	5.60	5.80	5.84	5.73

[1]Treatments; wilted alfalfa with no additive (CON), wilted alfalfa (1750g) mixed with fresh orange pulp (750g) and treated with LAB for final application rate 0, 2.5, 5 and 7.5 g LAB inoculant/ton (LB0, LB1, LB2 and LB3, respectively).

Within a column, means followed by different letters differ (P<0.05).

Further studies [28, 29] have reported that diets with molasses have higher ruminal DM digestibility. For AO silage high content of ME, SCFA, DOMD, NE_L and MP can result from its high rate of gas production, extent of gas production at 24 h and its nutrient composition.

Table-3. Effect of treatments on in vitro DM digestibility (%)

Treatments[1]	Incubation times (h)				
	2	4	8	12	24
CON	12.44^{b}	22.05^{c}	30.77^{c}	37.55^{c}	52.44^{d}
LB0	18.66^{a}	28.50^{a}	37.22^{a}	44.88^{a}	62.55^{a}
LB1	18.77^{a}	28.61^{a}	36.88^{a}	44.77^{a}	61.77^{b}
LB2	18.77^{a}	27.61^{b}	35.00^{b}	43.11^{b}	59.44^{c}
LB3	18.55^{a}	27.77^{b}	34.77^{b}	42.77^{b}	59.44^{c}
SEM	0.09	0.15	0.15	0.11	0.22

1Treatments; wilted alfalfa with no additive (CON), wilted alfalfa (1750g) mixed with fresh orange pulp (750g) and treated with LAB for final application rate 0, 2.5, 5 and 7.5 g LAB inoculant/ton (LB0, LB1, LB2 and LB3, respectively).
Within a column, means followed by different letters differ (P<0.05).

5. Conclusions

Results showed that ensiling alfalfa with orange pulp can improved silage quality and increased gas production and *in vitro* DM digestibility.

Acknowledgements

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References

[1] Schmidt, R. J., Hu, W., Mills, J. A., and Kung, J. L., 2009. "The development of lactic acid bacteria and Lactobacillus buchneri and their effects on the fermentation of alfalfa silage." *J. Dairy Sci.*, vol. 92, pp. 5005-5010.

[2] NRC, 2001. *Nutrient requirements of beef cattle*. 7th ed. Washington D.C., USA: National Academy Press.

[3] McDonald, P., Henderson, A. R., and Heren, S. J. E., 1991. *The biochemistry of silage*. 2nd ed. Bucks, UK: Chalcombe Publ.
[4] FAO, 2001. "Production yearbookw." Available: http://www.fao.org
[5] Aguilera, A., Perez-Gil, F., Grande, D., De, L. C. I., and Juarez, J., 1997. "Digestibility and corn stover fermentative characteristics of mango, Lemon silages with or without addition of molasses and urea." *Small Rumin. Res.*, vol. 26, pp. 87-91.
[6] Revuelta, L. D., Mosquera, L. D., and Cuba, M., F., 2008. "Ensiling potential of orange fruit wastes, Citrus sinensis." *Revista Ciencias Tecnicas Agropecuarias*, vol. 17, pp. 41-44.
[7] Driehuis, F. and Oude, E. S. J., 2000. "The impact of the quality of silage on animal health and food safety, A review." *Vet. Quarterly*, vol. 22, pp. 212-216.
[8] AOAC, 2000. *Official methods of analysis, association of official analytical chemists*. 17th ed. Arlington, Virginia, USA.
[9] Van, S. P. J., Robertson, J. B., and Lewis, B. A., 1991. "Methods for dietary fiber, Neutral detergent fiber, And non-starch polysaccharides in relation to animal nutrition." *J. Dairy Sci.*, vol. 74, pp. 3583–3597.
[10] Fedorak, P. M. and Hrudey, S. E., 1983. "A simple apparatus for measuring gas production by methanogenic cultuvesin serum bottles." *Environmental Technol. Let.*, vol. 4, pp. 425-435.
[11] McDougall, E. I., 1948. "The composition and output of sheep in saliva." *Biochem. J.*,, vol. 43, pp. 99-109.
[12] SAS, 2002. *Statistical analysis system version 9.1*. Cary, N.C., USA: SAS Institute Inc.
[13] Kondo, M., Kita, K., and Yokota, H. O., 2004. "Effects of tea leaf waste of green tea, Oolong tea, And black tea addition on sudangrass silage quality and in vitro gas production." *J. Sci. Food Agric.*, vol. 84, pp. 721–727.
[14] Nagadi, S. M., Herrero, M., and Jessop, N. S., 2000. "The influence of diet of the donor animal on the initial bacterial concentration of ruminal fluid and in vitro gas production degradability parameters." *Anim. Feed Sci. Technol.* , vol. 87, pp. 231–239.
[15] Menke, K. H. and Steingass, H., 1987. "Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid." *Anim. Res.*, vol. 28, pp. 7-12.
[16] Muck, R. E., Filya, I., and Contreras-Govea, F. E., 2007. "Inoculant effects on alfalfa silage, In vitro gas and volatile fatty acid production." *J. Dairy Sci.*, vol. 90, pp. 5115–5125.
[17] Negesse, T., Makkar, H. P. S., and Becker, K., 2009. "Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an in vitro gas method." *Anim. Feed Sci. Technol.*, vol. 154, pp. 204–217.
[18] Kozelov, L. K., Iliev, F., Hristov, A. N., Zaman, S., and McAllister, T. A., 2008. "Effect of fibrolytic enzymes and an inoculant on invitrodegradability and gas production of low-dry matter alfalfa silageq." *J. Sci. Food Agric.*, vol. 88, pp. 2568–2575.
[19] Li, M., Zi, X., Zhou, H., Hou, G., and Cai, Y., 2014. "Effects of sucrose, Glucose, Molasses and cellulase on fermentation quality and in vitro gas production of king grass silage." *Anim. Feed Sci. Technol.*, vol. 197, pp. 206–212.
[20] Hetta, M., Cone, J. W., Bernes, G., Gustavsson, A. M., and Martinsson, K., 2007. "Voluntary intake of silages in dairy cows depending on chemical composition and in vitro gas production characteristics." *Livestock Prod. Sci.*, vol. 106, pp. 47-56.
[21] Besharati, M., Shafipour, N., Abdi, E., and Nemati, Z., 2018. "Effects of supplementation alfalfa silage with molasses, Orange pulp and Lactobacillus buchneri on in vitro dry matter digestibility and gas production." *J. Bio. Biotech.*, vol. 6, pp. 43-47.
[22] Charmley, E., Winter, K. A., McRae, K. B., and Fillmore, S. A. E., 1996. "Effect of inoculation on silage quality and performance of steers fed grass and cereal silages either alone or in combination." *Can. J. Anim. Sci.*, vol. 76, pp. 571-577.
[23] Hashemzadeh-Cigari, F., Khorvash, M., Ghorbani, G. R., and Taghizadeh, A., 2011. "The effects of wilting, Molasses and inoculants on the fermentation quality and nutritive value of lucerne silage." *South African J. Anim Sci.*, vol. 41, pp. 377-388.
[24] Blümmel, M., Steingas, H., and Becker, K., 1997. "The relationship between in vitro gas production, In vitro microbial biomass yield and N incorporation and its implications for the prediction of voluntary feed intake of roughages." *Br. J. Nutr.*, vol. 77, pp. 911–921.
[25] Getachew, G., Robinson, P. H., DePeters, E. J., and Taylor, S. J., 2004. "Relationships between chemical composition, Dry matter degradation and in vitro gas production of several ruminant feeds." *Anim. Feed Sci. Technol.*, vol. 111, pp. 57-71.
[26] Granzin, B. C. and Dryden, G. M., 2005. "Monensin supplementation of lactating cows fed tropical grasses and cane molasses or grain." *Anim. Feed Sci. Technol.*, vol. 120, pp. 1–16.
[27] Wang, Z. S. and Goetsch, A. L., 1998. "Intake and digestion by Holstein steers consuming diets based on litter harvested after different numbers of broiler growing periods or with molasses addition before deep-stacking." *J. Anim Sci.*, vol. 76, pp. 880–887.
[28] Sahoo, B. and Walli, T. K., 2008. "Effects of formaldehyde treated mustard cake and molasses supplementation on nutrient utilization, Microbial protein supply and feed efficiency in growing kids." *Anim. Feed Sci. Technol.*, vol. 142, pp. 220–230.

[29] Shellito, S. M., Ward, M. A., Lardy, G. P., Bauer, M. L., and Caton, J. S., 2006. "Effects of concentrated separator by-product, Desugared molasses on intake, Ruminal fermentation, Digestion, And microbial efficiency in beef steers fed grass hay." *J. Anim. Sci.*, vol. 84, pp. 1535–1543.