www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of methanolic extract of *Viscum album* on *in vitro* fermentation and digestibility of soybean meal

Niasati M, Palizdar* MH, Pourelmi MR and Pasha Chalandari H.

Department of Animal Science, Islamic Azad University- Chalous Branch, Mazandaran, Iran

Abstract

The objective of this study was to determine the effect of different levels of methanolic extract of *Viscum album* on fermentation pattern of soybean meal in *in vitro* gas production technique. Completely randomized experimental design with four treatments namely 0, 0.2, 0.4 and 0.6 ml of *Viscum album* and three replicates for each treatment were used. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation time using a mixture of filtered rumen liquor of three fistulated Talleshi native steers. Chemical analysis showed that the soybean meal contained 43.58, 2.74 and 6.32 percent of crude protein, crude fat and crude ash respectively. The results showed that gas production decreased at 8, 12 and 24 h of incubation for treatments of 0.2, 0.4 and 0.6 ml respectively, and gas production increased exponentially with incubation time. There was a significant difference between treatments in potential of gas production (a+b) and gas production from insoluble fraction (b) at the test level ($P<0.05$). The results also showed that the effect of *Viscum album* extract on organic matter digestibility (OMD), metabolizable energy (ME) and net energy for lactation (NEL) was not significant between treatments. *Viscum album* extract fairly reduced degradability of soybean meal and decreased gas production in the rumen with no effect on the rate and extent of fermentable organic matter.

Keywords: Fermentation; gas production technique; soybean meal; *Viscum album*

To cite this article: Niasati M, MH Palizdar, MR Pourelmi and HP Chalandari, 2014. Effect of methanolic extract of Viscum album on in vitro fermentation and digestibility of soybean meal. Res. Opin. Anim. Vet. Sci., 4(7): 411-415.

Introduction

One of the major challenges in ruminant nutrition is to modify fermentation of protein supplements efficiently through the use of rumen fermentation modulators. Accordingly finding food sources that have beneficial effects on ruminal fermentation of proteins seems to be necessary. On the other hand, during fermentation process, about 12 percent of gross energy intake would be lost due to conversion into methane (CH_4) gas (Holter and Young, 1992; Johnson and Johnson, 1996). Rumen fermentation modulators can reduce the fermentation of protein meals to supply more bypass protein as well as reducing energy loss through reducing methane gas production. Gas production has a close relationship with fermentation and digestibility of organic substances in rumen (Moss et al., 2000). Antibiotics and ionophores were used previously as rumen fermentation modulators in order to decrease ammonia and methane production in rumen but because of some complications, their usage has been stopped in Europe since 2006; consequently, today researchers paid their attention to plant extracts as an alternative (Castillejos et al., 2006).

Viscum album as a semi-parasitic plant grows in north of Iran especially in Mazandaran providence as well as Europe and North Africa (Mills, 1994). It is common in tropical regions of Iran as well as north and it can be found on tree's crown such as *Carpinus betulus*, *Ulmus minor* and *Parrotia persica* (Kartoolinejad et al., 2007). Anti nutrient factors extracting from *Viscum album* are tannin, lectin, thionine and viscotoxins (Pryme et al., 2006) which may have great impact on rumen fermentation.

***Corresponding author:** Palizdar MH, Department of Animal Science, Islamic Azad University- Chalous Branch, Mazandaran, Iran; E-mail: mh_paliz@yahoo.com

Flavonoids, polysaccharides, phytosterol, hydrocarbons, vitamins and long chain fatty acid are the important constituents of *Viscum album* having medical features (Delior man et al., 2001). All these substances might alter the fermentation pattern of food in the rumen of ruminants.

Viscum album has a high percentage of tannin (2-8%) and lectin (1-5%) that affect rumen's microbes; hence it can be used to decrease fermentation of protein meals in rumen to enhance rumen un-degradable protein from rumen. To achieve more rumen undegradable protein, tannin could be useful (McSweeney et al., 1999). There is evidence that plant extracts like thyme and fennel can be used to modify the fermentation pattern of proteins in the rumen *in vitro* (Mirzadeh Ahari et al., 2011; Rezaei et al., 2011; Salamatazar et al., 2011 a&b; Salamatazar et al., 2012a&b). The aim of this research was to determine the effect of *Viscum album* methanolic extract on the fermentation kinetics of soybean meal through *in vitro* gas production technique.

Materials and Methods

Experimental design and treatments

This research was conducted in a completely randomized design consisting of four treatments which were further divided into 3 triplicates. Experiment treatments were 1) Soybean meal without adding any extract to the rumen fluid, 2) rumen fluid containing soybean meal plus 0.2 ml of *Viscum album* extract 3) rumen fluid containing soybean meal plus 0.4 ml of *Viscum album* extract 4) rumen fluid containing soybean meal plus 0.6 ml of *Viscum album* extract. The *Viscum album* collected from the top of Alder trees in the north of Iran from Mazandaran province were dried at room temperature and milled subsequently. In order to produce methanolic extract of *Viscum album*, approximately 100 g of dried and milled *Viscum album* (leaf and stem) was added to 1000 ml of methanol and mixed gently for 24 h at room temperature. Afterward the mixture of solvent and extract was filtered to obtain the initial extracts. The initial extract was distilled off and the solvent was evaporated at 60°C for one h. When the whole solvent was evaporated, the final extract was achieved after 6 to 7 h.

In vitro gas production technique

To investigate gas production, 200 mg of soybean meal samples were weighed and placed in 100 ml graduated glass syringes. Buffer mineral solution was prepared and placed in a water bath at 39°C under continuous flushing with CO_2. The rumen fluid was collected after the morning feeding from two ruminally fistulated, Iranian Taleshi steers fed on a total mixed ration consisting of approximately 40 percent chopped alfalfa hay and 60 percent concentrate (barley, corn grain, beet sugar pulp, soybean meal and wheat bran). Rumen fluid was pumped with a manually operated vacuum pump from the rumen into pre-warmed thermos flasks. The rumen fluid from the two cows was mixed and filtered through four layers of cheesecloth and flushed with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in a water bath at 39°C and mixed. Rumen fluid was added to the buffered mineral solution with constant stirring, while maintained in a water bath at 39°C. About 30 ml of buffered rumen fluid was dispensed into syringes containing the samples. *Viscum album* extracts at different levels (0, 0.2, 0.4 and 0.6 ml) was injected using insulin syringes. All handling was conducted under continuous flushing with CO_2. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, the initial volume was recorded and the syringes were affixed to a rotary shaker platform (Lab-line instruments Inc Melors dark, USA), set at 120 rpm in an incubator at 39°C (Palizdar et al., 2012).

Incubation was completed in triplicate with readings of gas production (GP) after incubation for 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h for samples. Kinetics of total GP was calculated for each treatment (Ørskov and McDonald, 1979). Differences in the composition and activity of rumen fluid inoculum were controlled by parallel measurements with incubation of buffered ruminal fluid without substrate (Blank test). Cumulative GP data were fitted to the exponential equation:

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

Where, Y is the gas produced at "t" time, "a" the GP from the immediately soluble fraction (ml), "b" the GP from the insoluble fraction (ml), "c" the GP rate constant for "b" and "t" is the time of incubation (h). The metabolizable energy (ME), net energy for lactation (NEL) contents and organic matter digestibility (OMD) were calculated using equations of Menke and Steingass (1988) as:

$$ME\ (MJ/kg\ DM) = 0.157\times GP + 0.0084\times CP + 0.022\times EE - 0.0081\times XA + 1.06$$

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

$$OMD\ (g/100\ g\ DM) = 0.9991 \times GP + 0.0595 \times CP + 0.0181 \times XA + 9$$

Where, CP is crude protein in g/100 g DM, XA ash in g/100 g DM, EE is ether extract in g/100 g DM and GP is the net gas production (ml) from 200 mg after 24 h of incubation.

Statistical analysis

Data were subjected to analysis of variance in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS, 9.1, 2005). Means were compared using the least square method. Mean differences were considered significant at P<0.05 (5% test level of significance). Standard errors of means were calculated from the residual mean square in the analysis of variance.

Results and Discussion

Effect of *Viscum album* extracts on gas production was significant during whole incubation time (P<0.05) except at 24h (Table 1). Also the results showed that using 0.2 ml of *Viscum album* extracts decreased gas production after 24h of incubation although in other times, gas production was increased. In addition, the level of 0.4 ml of *Viscum album* extract decreased gas production until 12 h of incubation. In the current study, increasing gas production in the final hours of incubation may be the result of degradation of residual substrates that rumen bacteria could not ferment. Decrease gas production at the primary hours of incubation may also be due to phenolic substances and anti-nutrients such as tannin and lectin in *Viscum album* extracts that can affect rumen microorganism functions (McSweeney et al., 1999). Furthermore, tannin could make complex with proteins so preventing the enzyme activity (Frutos et al., 2004). It has been shown that tannin can affect gas production in the rumen (Makkar et al., 1997b). In agreement with the current study, Salamatazar et al. (2012a) reported that addition of 0.15ml of thyme extract decreased gas production from soybean meal in all of the hours of incubation. In addition Rezaei et al. (2011) reported that addition of 0.5 and 1ml of fennel extract increased gas production from soybean meal at 2, 12, 24, 48, 72 and 96 h. Using 1 ml of fennel extract decreased gas production as well at 2 and 4 h of incubation, and at other h, gas production increased. Rezaei et al. (2011) also showed that addition of 0.5 and 1 ml carnation (clove) extract to soybean meal decreased gas production at all interval of incubation that is not consistent to the current study. It has been reported that decreased gas production observed during incubation could be the result of thymol and carvacrol in thyme and also anethole in fennel extract (Salamatazar et al., 2012b; Rezaei et al., 2011).

The amount of organic matter digestibility, metabolizable energy and net energy for lactation were not affected significantly (P>0.05) by treatments (Table 2). Metabolizable energy (11.14 Mj/kg dry matter) and net energy for lactation (6.98 Mj/kg dry matter) values for soybean meal was not according to Salamatazar et al. (2011 b,c&d) for NEL. The discrepancy may be the result of differences in soybean meal varieties used in the experiments. Inconsistent to our results, Palizdar et al. (2012) used soybean meal in an *in vitro* gas production study and reported 84.75% organic matter digestibility. The results of the current study confirmed previous study for soybean meal metabolizable energy and organic matter digestibility (Rezaei et al., 2011).

It has been suggested that adding 0.5 and 1 ml of thyme and clove to soybean meal incubated in an *in vitro* gas production syringes, organic matter digestibility and metabolisale energy decreased which are according to our result (Halimi Shabestari et al., 2011). This could be due to anti-nutrient substances such as tannin that make complex with proteins which are escaped from rumen (Bae et al., 1993; Halimi Shabestari et al., 2011).

The results of fermentation kinetic showed (Table 2) the effect of *Viscum album* extract on gas production potential (a+b) and the gas production from insoluble fraction (b) was significant (P<0.01) but the gas production rate was not affected by treatments (P>0.05). There was a significant difference between treatment including *Viscum album* extract as compared with control treatment in the potential of gas production (ml/200 mg DM) and these treatments had higher (a+b) compared to the control treatment. Insoluble but fermentable fraction (b) showed similar results in which treatments containing *Viscum album* extract had higher (b) fraction compared to the control. Experimental treatments had no significant effect on gas production rate.

Table 1: Effect of *Viscum album* extract on gas production during incubation of soybean meal

	Incubation time (h)								
Treatment	2	4	6	8	12	24	48	72	96
1	9.89^{a}	23.44^{a}	30.03^{a}	38.02^{a}	46.35^{a}	61.79	74.47^{b}	77.07^{c}	78.64^{b}
2	3.12^{c}	13.18^{c}	18.20^{b}	24.44^{b}	30.34^{b}	56.34	88.22^{a}	92.87^{b}	95.64^{a}
3	4.33^{b}	16.08^{bc}	23.87^{ab}	31.47^{ab}	40.64^{ab}	65.56	88.78^{a}	96.28^{ab}	99.56^{a}
4	4.68^{b}	18.72^{ab}	28.25^{a}	36.91^{a}	47.15^{a}	71.25	93.92^{a}	97.15^{a}	99.67^{a}
SEM	0.539	2.68	3.72	4.97	5.53	7.83	4.35	1.91	2.07
P-value	<0.01	<0.01	<0.019	<0.035	<0.021	<0.2	<0.01	<0.01	<0.01

1) Soybean meal without adding any extract to the rumen fluid, 2) addition of 0.2 ml of *Viscum album* extract to rumen fluid which contained soybean meal, 3) addition of 0.4 ml of *Viscum album* extract to rumen fluid including Soybean meal, 4) addition of 0.6 ml of *Viscum album* extract to rumen fluid which contained soybean meal

Table 2: Effect of different levels of *Viscum album* extract on soybean meal gas production parameters

	a+b	c	b
Treatment	ml	ml/h	ml
1	76.90[b]	0.077	76.04[b]
2	91.00[a]	0.047	92.09[a]
3	97.77[a]	0.046	99.31[a]
4	97.05[a]	0.055	99.12[a]
SEM	5.59	0.016	6.38
P-Value	<0.01	<0.139	<0.01

1) Soybean meal without adding any extract to the rumen fluid, 2) addition of 0.2 ml of *Viscum album* extract to rumen fluid which contained soybean meal, 3) addition of 0.4 ml of *Viscum album* extract to rumen fluid including Soybean meal, 4) addition of 0.6 ml of *Viscum album* extract to rumen fluid which contained soybean meal. a+b : potential of gas production, c: rate of gas production and b: gas production from insoluble but fermentable fraction. SEM: standard error of means.

Table 3: Effect of different levels of mistletoe extracts on organic matter digestibility, net energy lactation and metabolizable energy

	OMD	NEL	ME
Treatment	%	Mj/Kg DM	Mj/Kg DM
1	73.45	6.98	11.14
2	68.00	6.36	10.28
3	77.21	7.42	11.73
4	82.89	8.08	12.62
SEM	7.82	0/899	1.23
P-Value	<0.204	<0.202	<0.202

1) Soybean meal without adding any extract to the rumen fluid, 2) addition of 0.2 ml of *Viscum album* extract to rumen fluid which contained soybean meal, 3) addition of 0.4 ml of *Viscum album* extract to rumen fluid including Soyban meal, 4) addition of 0.6 ml of *Viscum album* extract to rumen fluid which contained soybean meal. OMD: organic matter digestibility, ME: metabolizable energy and NEL: net energy for lactation.

Inconsistent to our results, Rezaei et al. (2011) reported that adding 0.15 ml of thyme extract to the syringes containing soybean meal and buffered rumen liquor, gas production, the potential of gas production (ml/200 mg DM) as well as (b) fraction would decrease throughout the incubation times. Moreover, Salamatazar et al. (2012b) showed that adding 0.15 ml of thyme extract would decrease all gas production parameters compared to the control which is not consistent to our findings. Previously, Rezaei et al. (2011) reported that adding 0.5 and 1 ml of thyme extract decreased the potential of gas production (ml/200 mg DM) (a+b) and (b) fraction of soybean meal although gas production rate (c), which is not consistent to the current study.

Rezaei et al. (2011) reported that adding 1 and 0.5 ml of fennel extract to soybean meal, potential of gas production and (b) fraction increased compared with control treatment, however, gas production rate may decreased because of antioxidant, antimicrobial and antifungal substance in fennel. In the current study, gas production rate did not decrease in treatments containing *Viscum album* extract, despite the presence of inhibitors of fermentation in *Viscum album*.

Conclusions

It can be concluded that using *Viscum album* extract, the gas production of soybean meal could decrease in the rumen without any change on the rate and extent of fermentable organic matter.

References

Bae, H.D., Mcallister, T.A., anke, J.Y., Cheng, K.J. and Muir, A.D. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by fibrobacter succinogenes S85. *Applied and Environmental Microbiology*, 59: 2132-2138.

Castillejos, L., Calsamiglia, S. and Ferret, A. 2006. Effect of essential oils active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. *Journal of Dairy Science,* 89: 649-2658.

Delior man, D., Calisa, I. and Ergum, F. 2001. A New ancyclic monterpene glucoside from visscum album SSP Album-fitoterapia. Department of Animal and Poultry Science. College of Agriculture and Bioresources, University of Saskatchewan. Saskatoon. SK.S7N 5A8. Canada.

Frutos, P., Hervás, G., Giráldez, F. J. and Mantecón, A. R. 2004. Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research*, 2: 191-202.

Halimi Shabestari, A., Salamatdoustnobar, R., Maheri-sis, N., Gorbani, A., Mirzadeh Ahari, K., Noshadi, A., Samadi, H. and Salimi Nezhad, J. 2011. Evaluation effects of clove methanol extract on methane production in the *in vitro* condition. *Pakistan Journal of Nutrition*, 10: 1154-1157.

Holter, J.B. and Young A.J. 1992. Methane production in dry and lactating dairy cows. *Journal of Dairy Science,* 75: 2165-2175.

Johnson, K.A. and D.E. Johnson. 1995. Methane emissions from cattle. *Journal of American Science,* 73: 2483-2492.

Kartoolinejad, D., Hosseini S.M., Mirnia, S.K. and Shayanmehr, F. 2007.The effect of mistletoe (Viscum album) on four nutrient elements Mg, Zn, Mn, Na and leaf area and weight of host trees in Hyrcanian forests. *Pajouhesh and Sazandegi*, 77: 47-52. (In Persian).

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*, 28: 7-55.

Makkar, H.P.S., Blummel, M. and Becker, K. 1997b. *In vitro* rumen apparent and true digestibilities of tannins-rich forages. *Animal Feed Science and Technology,* 67: 245-251.

McSweeney, C.S., Dalrymple, B.P., Gobius, K.S., Kennedy, P.M., Krause, D.O., Mackie, R.I. and Xue, G.P. 1999. The application of rumen biotechnology to improve the nutritive value of fibrous feedstuffs pre-and post ingestion. *Livestock Production Science*, 59: 265-283.

Mirzadeh Ahari, K., Salamatdoustnobar, R., Maheri-Sis, N., Gorbani, A., Halimi Shabestari, A., Noshadi, A., Samadi, H. and Salimi Nezhad, J. 2011. Effects of the Thyme extract on the ruminal methane production. *Pakistan Journal of Nutrition*, 10: 1146-1148.

Mills, S. 1994. The complete guide to modern Herbalism. 3rd edition. Thorsons, London.

Moss, A.R., Jouany, J.P. and Newbold, J. 2000. Methane production by ruminants: its contribution to global warming. *Annals of Zootechnology*, 49: 231-253.

Palizdar, M.H., Sadeghipanah, H., Amanlou, H., Mohammadian-tabrizi, H.R. and Mir Hadi, A. 2012. Effect of soybean meal coated whit fat on *in vitro* organic matter fermentation and Gas production. *Journal of Animal and Veterinary Advances*, 11: 171-180.

Pryme, I, F., Bardocz, S., Ousztai, A. and Ewen, S.W.B. 2006. Suppression of growth of tumor cell lines *in vitro* and tumors in vivo by mistletoe lectin. *The journal of Histology and Histopathology*, 21: 285-299.

Rezaei, N., Salamat doust-Nobar, R., Maheri Sis, N., Salamatazar, M., Namvari, M., Goli, S. and Aminipour, H. 2011. Effect of some plant extracts on degradability of soybean meal with gas product technique. *Annals of Biological Research,* 2: 637-641.

Salamatazar, M., Salamat Doust-Nobar, R., Maheri Sis, N, Noshadi, A., Najafyar, S., Khodaparast, B., Aminipour, H., Rezayi, N. and Kiyani Nahand, M. 2011c. Effect of thyme methanolic extract on the metobolizable energy of canola meal for ruminant. *Annals of Biological Research,* 2: 628-631.

Salamatazar, M., Salamat Doust-Nobar, R., Asadi, Y., Kiani Nahand, M., Najafyar, S., Bakhshali, Kh. and Aminipour, H. 2011d. Zataria Multiflora Extract could affect Metobolizable Energy and Organic Matter Digestibility of Canola Meal. *Journal of American Science,* 7: 131-134.

Salamatazar, M., Salamatdoust-Nobar, R., Asadi, Y., Maheri Sis, N., Najafyar, S., Aminipour, H., Khodaparast, B., Rezayi, N. and Maleki, M. 2011a. To study 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. *Annals of Biological Research,* 2: 197-205.

Salamatazar, M., Salamatdoustnobar, R., Asadi, Y., Maheri Sis, N., Najafyar, S., Aminipour, H., Khodaparast, B., Rezayi, N. and Maleki, M. 2011b. Effects of addition of Zataria multiflora methanolic extract on intake of dry matter and *in vitro* dry matter digestibility of soybean meal using *in vitro* gas production technique. *Annals of Biological Research,* 2: 252-259.

Salamatazar, M., Salamatdoustnobar, R., Maheri Sis, N., Aghdam Shahryar, H. and Asadi, Y. 2012a. Effects of Zataria multiflora extract as Rumen Modifiers Using *in vitro* Gas Production Technique. *Current Research Journal of Biological Sciences,* 4: 350-354.

Salamatazar, M., Salamat Doust-Nobar, R., Maheri Sis, N., Aghdam Shahryar, H. and Asadi, Y. 2012b. Effects of natural plant extract on ruminal fermentation using *in vitro* gas production technique. *Pakistan Journal of Nutrition,* 11: 561-565.