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Effect of Different Levels of Milk Thistle (*Silybum Marianum*) in Diets Containing Cereal Grains with Different Ruminal Degradation Rate on Rumen Bacteria of Khuzestan Buffalo

Research Article

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ABSTRACT

The objective of this study was to investigate the effects of diets containing different levels of milk thistle (0, 100 and 200 g/kg dry matter) and grains (barley and corn) with different rumen degradation rates on rumen bacteria and whole rumen microorganisms (WRM) of Khuzestan buffalo. The gas production (GP) technique, two steps digestion and specific bacteria culture medium methods were used for this purpose. The rumen fluid was taken from two fistulated buffaloes. The results of GP potential of experimental diets by WRM were not significantly different, however in both basal diets GP increased by increasing the level of milk thistle (P>0.05). The highest amount of GP in diet based on barley and corn was for diets containing 200 and 100 g milk thistle, respectively. Rate of GP by WRM was significantly different (P<0.05), so that in both basal diets at the level of 100 g milk thistle had the highest GP rates. Potential and rate of GP of diets by buffalo rumen bacteria was not significantly different (P>0.05). In two-steps digestion method using of different levels of milk thistle in diets (based on barley and corn) had no negative effect on the digestibility of nutrients by WRM (P>0.05). In barley-based diet adding milk thistle was numerically increased dry matter and neutral detergent fiber (NDF) digestibility compared with the control (P>0.05), while in the corn-based diet dry matter and NDF digestibility were reduced (P>0.05). The digestibility of dry matter by bacteria in corn-based diet was significantly reduced compared with the control (P<0.05). Nutrient digestibility of experimental diets by bacteria in the specific bacteria culture did not be affected by milk thistle in diets (P>0.05). Therefore, results provided here suggest that milk thistle could be used up to 20% of buffalo's diet without any negative effect on digestion and fermentation characteristics by WRM and bacteria.

KEY WORDS bacteria, barley grain, corn grain, whole rumen microorganisms.

INTRODUCTION

Milk thistle (*Silybum marianum*) or Maritighal is an annual grass with medicinal characteristics and is grown in different parts of Iran. The medicinal properties of milk thistle are due to presence of silymarin in its seed which is primarily consists of an isomeric mixture of 6 phenolic compounds, silydianin, silychristin, diastereoisomers of silybin (silybin A and B), and diastereoisomers of isosilybin (isosilybin A and B) (Lee *et al.* 2007). Silymarin has been used to treat liver disorders such as unwell of alcoholic and various hepatitis (Carmen Tamayo, 2007; Gazak *et al.* 2007). It also inhibits chemically induced carcinogenesis and shows direct anti-carcinogenic activity against several human carcinoma cells. The silymarin has antidiabetic, hypolipidaemic, anti-inflammatory, cardioprotective, neurotrophic and neuroprotective effects (Kren and Walterova, 2005; Abascal *et al.* 2003). The seed of this plant also contains betaine, tri-methyl glycine and a large amount of oil that play an important role in anti-inflammatory and anti-hepatitis effects of the extract (Hadolin *et al.* 2001).

In was reported that by using milk thistle no adverse effects have been found but nitrate poisoning maybe occurred (Macadam, 1966). Nitrate is not always toxic to animals but it is converted to nitrite and then to ammonia, by rumen microorganisms when animals consume feed containing nitrate. When high levels of nitrites accumulate in the gastrointestinal tract, they are absorbed into the bloodstream and it changes haemoglobin to methaemoglobin (Robson, 2007). If enough energy (such as grain) is available in the rumen, nitrite easily converted into ammonia, and ammonia ultimately can be used for microbial protein synthesis. Since the converting of nitrate to nitrite and ammonia is an endergonic process, therefore the degradation rate of carbohydrate source may influence on ruminants which consumed the plants containing nitrates, such as milk thistle (Kamra, 2005).

Rumen microorganisms are comprised predominantly of cellulolytic bacterial populations, anaerobic fungi and protozoa. In recent years, it shown the rumen cellulolytic bacteria have a primary role in fiber digestion and so animal performance (Denmen and McSweeney, 2006).

Tannins are phenolic compounds, which are able to precipitate proteins, alkaloids and gelatin. High concentrations of the tannins in fodder plants inhibit gastrointestinal bacteria and reduce ruminant performance for usage the nitrogen and protein sources (Smith *et al.* 2005).

In an early experiments on nutritional value of milk thistle in sheep, both positive and negative effects on fiber and other nutrients digestibility were observed. Also, in a gas production (GP) experiment, milk thistle showed extremely negative effect on the digestion and fermentation activities of microorganisms (Mojadam, 2012). It is likely that some of these results may be due to the effects of active ingredients of *Silybum marianum* on rumen microorganisms, including bacteria. In Khuzestan province, milk thistle is being consumed by animals such as sheep, camels and cows, but there is no information about its effects on rumen microorganisms. Therefore, the aim of the present experiment was to investigate the effects of diets containing *Silybum marianum* and grains with different degradation rates on rumen bacteria of Khuzestan buffalos.

MATERIALS AND METHODS

This experiment was carried out at Ramin Agricultural and Natural Resources University of Khuzestan. Whole milk thistle plant (leaves, stems and flowers) was collected from Mollasani. After drying and powdering, its dried powder was added to the basal diet containing corn or barley (fast fermentation rate than corn) grains in 3 levels of 0, 100 and 200 g/kg DM as top dress.

Experimental animal

Rumen fluid was taken before morning feeding from 2 fistulated water buffalo (live weight around 450 kg). Buffalo diets were prepared based on the standard requirements (NRC, 2001) and included 50% forage (30% alfalfa hay and 20% wheat straw) and 50% concentrate (36.9% barley or corn, 12% wheat bran, 0.03% fish meal [for corn base diet only], 0.3% salt, 0.7% calcium carbonate, 0.1% mineral and vitamin supplement). The diets were offered to the animals about 6 kg per day on DM base. The effect of milk thistle on bacteria and whole rumen microorganisms (WRM) of the buffalo was studied using the following techniques.

Gas production by WRM of buffalo

Gas production of the experimental treatments were measured in 100 mL glass syringes containing 300 mg of ground sample, 20 mL of artificial saliva and 10 mL of rumen liquid (Menke and Stingass, 1988). Artificial saliva was supplied by McDougall method (McDougall, 1948). Rumen fluid was collected through the rumen fistula of 2 buffalo that were fed at maintenance level for 6 weeks before the start of experiment. Collected rumen fluid was strained through 4 layers of cheese cloth and mixed with artificial saliva.

Gas production by rumen bacteria of buffalo

The method used for determining GP of rumen bacteria was the same that used for WRM of rumen, but for isolation of rumen bacteria, after collection and strain of liquor, rumen fluid was centrifuged (1000 g, 10 min) for removing protozoa. Then bacteria were isolated from non-protozoa strained rumen fluid using antifungal agent, benomyle (500 mg/L of medium culture; Sigma-Aldrich Co., Taufkirchen, Germany) and metalaxyle (10 mg/L; Sigma-Aldrich Co., Taufkirchen, Germany) (Mohammadabadi *et al.* 2012). The GP on 39 degrees of centigrade was measured at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Cumulative GP data were analyzed with the exponential equation (Orskov and McDonald, 1979).

Two steps digestion

Rumen fluid was obtained from buffalos and purified by the methods described in the previous section (Mohammadabadi *et al.* 2012). Then, *in vitro* digestibility of experimental treatments by whole microorganisms and buffalo rumen bacteria were measured in the 100 mL test tubes containing 0.5 g sample, 40 mL artificial saliva (McDougall, 1948) and 10 mL of rumen fluid (1/4 ratio). Digestibility of dry matter and neutral detergent fiber (NDF) was calculated based on the differences of raw material and material remaining at end of incubation (Tilly and Terry, 1963). Neutral detergent fiber was measured by Van Soest *et al.* (1991) method.

Specific rumen bacteria culture medium

The experimental treatments were cultured in specific culture medium of rumen bacteria (Galdwell and Bryant, 1966). The culturing glasses, containing 1 g sample, were sterile by autoclave for 15 min at 120 ˚C, and then, 25 mL of a mixture of centrifuged rumen fluid of buffalos with cellobiose, sodium sulfide, cysteine-HCL, sodium carbonate (Merck Co., Darmstadt, Germany), fungicides (Benomyl and metalaxyl; Sigma-Aldrich Co., Taufkirchen, Germany), trypticase and yeast extract (Merck Co., Darmstadt, Germany) was added to them. After that, 3 mL from sucrose solution was added to each glass. In order to create anaerobic conditions, carbon dioxide injected to test tubes. Then, 4 mL of rumen fluid was added to each bottle. The culturing glasses were incubated at a temperature of 39 ˚C for 96 h. Six replicates for each treatment were considered, after drying and weighing, the disappearance of DM and NDF samples of bacteria were calculated.

Statistical analysis

Data was analysis by split-plot design (main plot was basal diet containing barley or corn and subplot diets include different value of milk thistle), using the General Linear Model procedure of SAS (2004) Duncan's multiple range tests was used to compare the significant means (Mohammadabadi *et al.* 2012).

RESULTS AND DISCUSSION

Gas production by WRM

The potential of GP of experimental diets (Table 1) did not show significant differences (P>0.05). Numerically, in diets based on barley and corn grain, diet containing 200 and 100 g milk thistle had the highest potential of GP, respectively. Compared with the control, GP rate of experimental diets was significantly different (P<0.05). In both basal diet, the highest rate of GP was for diet containing 100 g milk thistle. In total, the highest GP rate was belonged to barley diet containing 100 g milk thistle.

Gas production by rumen bacteria

The trends of results related to potential of GP of experimental diets by rumen bacteria were the same as WRM (Table 1).

The potential and rate of GP of experimental diets by rumen bacteria of buffalo was not significantly different (Table 1). In diets based on barley and corn, the highest potential of GP was for diets containing 100 and 200 g milk thistle, respectively. Therefore, adding milk thistle to either barley or corn based diets, had no significant effect on GP by rumen bacteria and WRM (P>0.05). However, GP increased numerically as the amount of milk thistle was increased in the diets.

Regardless the level of milk thistle (Table 2), GP potential by WRM for the corn-based diet was higher than barley. Rate of GP by WRM in barley-based diets was significantly higher than diets containing corn (P<0.05). The basal diets had no significant effects on the potential and the rate of GP by rumen bacteria (P>0.05).

Regardless the type of basal diet (Table 3), potential of GP by WRM for the different levels of milk thistle was similar (P>0.05), but GP rate was significantly different (P<0.05). Adding milk thistle to the diets significantly increased GP rate, and the 100 g milk thistle had the highest GP rate (P<0.05), which was not significant compared with the diet containing 200 g milk thistle.

The potential and GP rate of buffalo rumen bacteria for different levels of milk thistle were not significant (P>0.05). Compared with the control diet, 100 g milk thistle increased the potential of GP (P>0.05).

Digestibility of experimental diets

WRM

The milk thistle inclusion in diets (based on barley and corn) had no significant effect on nutrients digestibility (Table 4) (P>0.05). In barley-based diets, increasing milk thistle increased numerically DM digestibility compared with the control group, but had no significant effect on NDF digestibility (P>0.05). In diets based on corn grain, , adding milk thistle to diet caused to slightly decrease in DM and NDF digestibilities compared with the control diet. Diet without milk thistle showed the highest DM and NDF digestibilities (P>0.05). Within corn-based diets (Table 4), the control had the highest DM and NDF digestibilities (P>0.05).

Bacteria

DM digestibility (Table 4) was higher in barley-based diets with milk thistle compared to the control, but there was no significant difference between 2 levels of milk thistle (P>0.05). In diet based on corn grain, milk thistle decreased DM digestibility significantly compared to the control diet (P<0.05), and there was significant difference between 2 levels of milk thistle. Increasing the amounts of milk thistle in both diets, had no significant impact on NDF digestibility by bacteria (P>0.05).

Table 1 Effect of milk thistle on gas production parameters of the experimental diets by whole rumen microorganisms (WRM) and bacteria of buffalos

Basal diet	Milk thistle in diet, (g/kg DM)	WRM		Bacteria	
		b, (mL)	c, (mL/h)	b, (mL)	c, (mL/h)
Barley	0	70.53	0.039[ab]	39.99	0.041
	100	70.75	0.047[a]	38.17	0.048
	200	80.31	0.042[ab]	43.01	0.045
Corn	0	79.40	0.034[b]	44.58	0.046
	100	81.32	0.040[ab]	49.58	0.037
	200	76.21	0.037[b]	37.23	0.041
SEM		2.99	0.0024	3.010	0.0038
P-value		0.084	0.0377	0.104	0.387

b: potential of gas production and c: gas production rate.
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 2 Effect of the type of basal diet on gas production parameters by whole rumen microorganisms (WRM) and bacteria of buffalos (regardless the level of milk thistle)

Basal diet	WRM		Bacteria	
	b (mL)	c (mL/h)	b (mL)	c (mL/h)
Barley	73.89[b]	0.043[a]	40.39	0.045
Corn	79.25[a]	0.037[b]	43.80	0.041
SEM	1.727	0.0014	1.738	0.0022
P-value	0.049	0.0105	0.19	0.298

b: potential of gas production and c: gas production rate.
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 3 Effect of milk thistle on gas production parameters by whole rumen microorganisms (WRM) and bacteria of buffalos (regardless of the basal diet)

Level of milk thistle in diet, g/kg (DM)	WRM		Bacteria	
	b (mL)	c (mL/h)	b (mL)	c (mL/h)
0	75.03	0.037[b]	42.29	0.0433
100	76.09	0.043[a]	43.87	0.0426
200	78.60	0.039[ab]	40.12	0.0430
SEM	2.11	0.0017	2.13	0.0027
P-value	0.49	0.0422	0.48	0.983

b: potential of gas production and c: gas production rate.
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 4 Nutrients digestibility of the experimental diets by whole rumen microorganisms (WRM) and bacteria of buffalos

Basal diet	Level of milk thistle in diet, g/kg DM	WRM		Bacteria	
		Dry mater (%)	NDF (%)	Dry mater (%)	NDF (%)
Barley	0	67.51	79.10	66.72[b]	78.27
	100	73.84	79.15	69.31[b]	78.55
	200	71.80	78.74	70.42[b]	82.09
Corn	0	78.79	84.71	81.09[a]	84.09
	100	72.82	78.28	62.09[b]	75.47
	200	75.13	76.03	64.98[b]	78.69
SEM		3.50	3.21	2.66	1.98
P-value		0.43	0.28	0.024	0.16

NDF: neutral detergent fiber.
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.

In diet based on barley grain, diet containing 200 g milk thistle had the highest NDF digestibility; while in corn-based diets, use of milk thistle decreased numerically NDF digestibility (P>0.05). Regardless the levels of milk thistle in the diets (Table 5), the differences between nutrients digestibilities of barley- or corn- based diets by WRM and bacteria were not significant (P>0.05).

The DM and NDF digestibilities (Table 6) of the diets were also not affected by the levels of milk thistle (P>0.05). However, DM (P<0.05) and NDF digestibility by bacteria decreased significantly (P>0.05) with increasing the level of milk thistle in the diets (Table 6).

Table 5 Nutrients digestibility of the basal diets by whole rumen microorganisms (WRM) and bacteria of buffalos (regardless the level of milk thistle) using two-step digestion method

Basal diet	WRM		Bacteria	
	Dry mater (%)	NDF (%)	Dry mater (%)	NDF (%)
Barley	71.05	78.99	68.82	79.63
Corn	75.58	79.67	69.39	79.42
SEM	2.02	1.86	1.53	1.14
P-value	0.16	0.80	0.80	0.89

NDF: neutral detergent fiber.
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 6 Effect of milk thistle on nutrients digestibility by whole rumen microorganisms (WRM) and bacteria of buffalos (regardless of the basal diet)

Level of milk thistle in diet, g/kg DM	WRM		Bacteria	
	Dry mater (%)	NDF (%)	Dry mater (%)	NDF (%)
0	73.15	81.90	73.90[a]	81.18
100	73.33	78.71	65.70[b]	77.00
200	73.46	77.38	67.70[ab]	80.39
SEM	2.48	2.27	1.88	1.40
P-value	0.996	0.41	0.049	0.160

NDF: neutral detergent fiber.
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.

Nutrients digestibilities by specific bacteria culture method

In Table 7 the effect of milk thistle on digestibility of DM and NDF was not significant in 2 basal diets (P>0.05). Regardless the levels of milk thistle (Table 8) there was no significant difference between basal diets in digestibility of DM and NDF (P>0.05). As Table 9 demonstrated, regardless the type of basal diet, differences in nutrient digestibility of various levels of milk thistle was not significant (P>0.05). The DM and NDF digestibility decreased with increasing milk thistle (P>0.05), such that the zero level of milk thistle shown the highest digestibility (P>0.05).

GP by WRM and bacteria

Numeral increasing of GP (20 and 10% for barley- and corn- based diets, respectively) may be due to sugar compounds existing in milk thistle. Compatible with the results of the present experiment, Dong *et al.* (2010) in their study on goats, despite of a reduction in fermentative and microbial activities for high levels of phytogenic products, did not found any significant effect for phytogenic products on *in vitro* rumen fermentation and methane emission. Broudiscou *et al.* (2000) screened action of 13 plant extracts with high flavonoid content on fermentation and rumen microbes. Their result indicated that GP was increased by *L. officinalis* and *S. virgaurea* (from family of milk thistle). However, opposite to the results of the present study; a significant decrease of methane emission was reported by Sun *et al.* (2011) in evaluating the effects of *Cichorium intybus* on GP in sheep. In the present study may be the numerically reduced of GP (levels 20% in corn diet) was related with effects of tannin in milk thistle, tannins readily complex with carbohydrates and proteins (Hagerman *et al.* 1992).

Condensed tannin extracted from quebracho trees reduced methane emissions from cattle (Beauchemin *et al.* 2007) which is compatible with our results. They observed that feeding up to 2% of the dietary DM quebracho tannin extract failed to reduce enteric methane emissions from growing cattle.

Although, Carulla *et al.* (2005) reported that feeding *Acacia mearnsii* extract (2.5% of dry matter intake (DMI)) to sheep (as source of tannin) decreased methane per kilogram of DM intake by approximately 12%. In general, the addition of milk thistle to the diets in the current study did not have any negative effect on GP by WRM and bacteria of buffalo, perhaps due to the rapid adaptation of rumen microorganisms of buffalo to the tannin content of in milk thistle.

Maldar *et al.* (2010) reported, which habit of goats Alamut to oak leaf (rich source of tannin), reduce the negative impact of tannins and change conditions of rumen fermentation and digestibility of the byproduct was increased. Increasing the proportion of tannin-resistant bacteria in the rumen by adaptation protects ruminants from anti-nutritional effects (Smith *et al.* 2005). The higher GP potential in diets based on corn compared with the barley (Table 2) is probably due to the high fiber content in barley which is mostly hemicellulos; but non fiber carbohydrates of barley and corn were 53.9% and 60.6%, respectively (Parnian Khaje Dizaj, 2011). On the other hand, in feed sources with lower NDF potential of GP are high and increasing proportion of the lignin in cell wall reduced GP, and lead to reduction of digestibility (Sommart *et al.* 2000).

Table 7 Nutrients digestibility of the experimental diets by rumen bacteria of buffalos after 96 h of incubation

Basal diet	Level of milk thistle in diet, g/kg DM	Nutrients	
		Dry mater (%)	NDF (%)
	0	42.60	22.97
Barley	100	40.51	21.47
	200	40.65	18.04
	0	43.58	22.60
Corn	100	43.04	18.47
	200	37.79	78.69
SEM		1.78	1.98
P-value		0.32	0.16

NDF: neutral detergent fiber.
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 8 Nutrients digestibility of the experimental diet by rumen bacteria of buffalos (regardless the level of milk thistle) after 96 h of incubation

Basal diet	Nutrients	
	Dry mater (%)	NDF (%)
Barley	41.26	20.83
Corn	41.47	20.78
SEM	1.029	1.007
P-value	0.80	0.97

NDF: neutral detergent fiber.
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 9 Effect of milk thistle on nutrients digestibility of experimental diets by rumen bacteria of buffalos after 96 h incubation (regardless of the basal diet)

Level of milk thistle in diet, g/kg DM	Nutrients	
	Dry mater (%)	NDF (%)
0	43.09	22.79
100	41.78	19.94
200	39.22	19.69
SEM	1.26	1.23
P-value	0.17	0.22

NDF: neutral detergent fiber.
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.

The higher GP rate of diets based on barley compared to diets based on corn perhaps was due to the difference between corn and barley starch degradation rate. The *in situ* experiments (Sadeghi and Shawrang, 2006; Sadeghi and Shawrang, 2008) shown that barley, unlike corn starch have a high effective degradability and its rumen degradability was faster and results in higher rates of GP from barley than corn grain (Herrera-Saldana *et al.* 1990; Nocek and Tamminga, 1991). Also, Khorasani *et al.* (2001) were examined the effects of replacing barley grain with corn on ruminal fermentation characteristics of Holstein dairy cows, and their results showed that barley starch is digested faster than corn starch. However, Garcia *et al.* (2000) reported that apparent digestibility of starch in the rumen of Holstein heifers was not different for rations with barley and corn. Perhaps one reason for slight decrease in GP by bacteria (level of 200 g milk thistle) was declines the population due to the presence of tannins in milk thistle, Zargari (1996) reported that various parts of milk thistle plant have tannin; and condensed tannins reduce methanogens variation (Tan *et al.* 2001). Feeding tannin-containing forages to ruminants may reduce methane emissions (Pinares-Patino *et al.* 2003).

Ruminal bacteria play a particularly important role in the biological degradation of dietary fiber because of their much larger biomass (Koike *et al.* 2003). Tannins are most effective against the fiber degrading bacteria. The reports have indicated that in animals fed tannin rich *Calliandra calothyrsus*, the population of *Ruminococcus* spp. and *Fibrobacter* spp. was reduced considerably, but fungi, protozoa and proteolytic bacteria were less affected by this diet (McSweeny *et al.* 2000). Sotohy *et al.* (1997) reported that the number of total bacteria in the rumen of goats decreased significantly when the animals were fed tannin-rich plant (*Acacia nilotica*), and this decrease had direct relation with the level of this feed in the diet. Disagree with above researches Brooker *et al.* (1994) isolated facultative anaerobe gram-positive bacteria (cellulolytic strains) from the rumen liquor of feral goats browsing on *Acacia* spp. leaves, which they could grow in a medium containing 2.5% tannic acid or condensed tannins. Also Odenyo and Osuji (1998) isolated three strains of tannin-tolerant bacteria from sheep, goat and an antelope and observed that the isolates could tolerate tannins up to 8 g/L of the medium. Therefore, in present experiment, adding milk thistle to diet had no negative effect on bacteria and WRM.

Nutrient digestibility of experimental diets
WRM

Within diets based on corn (Table 4), control diet had the highest DM and NDF digestibility. The numerical reduce of DM and NDF digestibility probably was because of unsaturated fatty acids found in milk thistle and corn seed. Milk thistle contains 28.9% oil and unsaturated fatty acid and linoleic acid is the predominant unsaturated fatty acid of its oil (Malekzadeh *et al.* 2011). As Palmquist and Jenkins (1980) stated, feeding unprotected fats, especially unsaturated, resulted to lower ruminal fiber degradability. Agree with the results of present experiment, Mojadam (2012) reported that DM and NDF digestibility of soybean meal and wheat straw, which were incubated by rumen fluid of sheep that fed with the diets containing milk thistle (barely and corn in basal diets) not affected by the treatments. Also Szumacher-Strabel *et al.* (2009) studied the effect of oils rich in linoleic acid (like oil of milk thistle) on *in vitro* rumen fermentation parameters of sheep, goats and dairy cows, there, unsaturated fatty acid does not disrupt ruminal fermentation; but Harvatine and Allen (2006) represented that using of fat sources in diet was effective on ruminal digestion.

Totally, adding milk thistle (as source of unsaturated fatty acid) to diet, particularly in higher levels and associated with diet contains corn, caused more decrease of nutrient digestibility; which one reason for that maybe was decrease digestibility of structural carbohydrates (White *et al.* 1992; Zinn, 1989; Zinn and Shen, 1996). Therefore, since in present experiment reduction of digestibility of DM and NDF was not significant, it can be said that the negative effects of tannin, unsaturated fatty acids and other compounds of milk thistle on digestibility were negligible.

Bacteria

Decline rumen digestibility of nutrients by bacterial in diets based on corn (Table 4) probably was due to the additive effect of unsaturated fatty acids of milk thistle and corn grain. Cellulolytic bacteria constitute 5% to 7% of the total bacterial population of rumen (Denmen and McSweeney, 2006). In beef cattle diets when fat added at the end of the finishing period, organic matter and fiber digestibility decreased.

This reduction was due to negative effect of fat on fiber digestibility in the rumen. The fats have directly inhibitory effects on fibrolytic bacteria and protozoa, also because of the physical coverage of the fibers and thus reduce rumen fermentation by microorganisms and reduce the absorption of cations, had a negative effect on digestibility (Lundy *et al.* 2004). Agrees with the present study, Harvatine and Allen (2006) reported that supplementation of saturated and unsaturated fatty acids reduced apparent digestibility of DM organic matter and NDF, because unsaturated fatty acids in oil with covering particles of fiber in rumen probably impaired cellulolytic bacteria function and ultimately reduce fiber digestion in the rumen, which is consistent with the findings of the present study.

The cell wall of barley is higher than that of corn (Parnian Khaje Dizaj, 2011), so probably, the higher digestibility (numerically) of corn diet (Table 5) was due to more cell wall of barley. Agrees with the results of present experiment, there was a trend for decreased total tract NDF digestibility when barley replaced by corn in diets of cattle (McCarthy *et al.* 1989; Overton *et al.* 1995).

Regardless the type of basal diet, DM and NDF digestibility by bacteria decreased with increasing level of milk thistle in diet (Table 6). The results were shown that negative effect of milk thistle on the bacteria was more than WRM, thus the hypothesis about interference of fat or oil on bacteria is supported. Totally, the digestibility results showed that using of milk thistle up to 20% in buffalo diets have not adverse effect on rumen bacteria and WRM.

Nutrients digestibility of experimental diets by rumen bacteria (with specific rumen bacteria culture method)

Probably the reason of numerical decrease in bacterial digestibility of the nutrients (Table 7) was unsaturated fatty acids (mainly linoleic acid) of milk thistle (Malekzadeh *et al.* 2011). Fatty acids are toxic to bacteria in pure culture experiments (Maczulak *et al.* 1981), but once in the rumen, fatty acids predominately are associated with feed particles their effects may satisfied (Harfoot, 1978).

Regardless the type of basal diet (Table 9), the milk thistle had no significant effect on DM and NDF digestibility. Thus, we may conclude from specific bacteria culture test that different levels of milk thistle have no negative effect on rumen bacteria functions.

CONCLUSION

In the present research the effects of using different levels of milk thistle in diet of buffalo on bacteria a whole rumen microorganism digestion function was investigated, and almost, the negative effects were not observed, even at most case the digestibility was improved. There was no significant difference between two basal diets in nutrient digestibility. Therefore, results suggest that milk thistle could be used up to 20% in diets of buffalo without any negative effect on digestion and fermentation characteristics of WRM and bacteria.

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