IJAS Iranian Journal of Applied Animal Science

The Comparison of *in vitro* Digestibility of Wheat Straw by Rumen Anaerobic Fungi of Khuzestan Buffalo and Holstein Cattle

Research Article

F. Shakarami[1], M. Chaji[1*], M. Eslami[1], T. Mohammadabadi[1] and M. Bojarpour[1]

[1] Department of Animal Science, Ramin Agricultural and Natural Resources University, Mollasani, Ahvaz, Iran

Received on: 26 Apr 2014
Revised on: 3 Jun 2014
Accepted on: 15 Jun 2014
Online Published on: Jun 2015

*Correspondence E-mail: chaji@ramin.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This study was conducted to compare digestibility of wheat straw (WS) by fungi and whole rumen microorganisms (WRM). Dry matter (DM), neutral and acid detergent fiber (NDF and ADF) digestibility of WS were compared with *in vitro* digestion (IVD), gas production (GP) and specific rumen anaerobic fungi culture (SRAFC). Dry matter, NDF and ADF digestibility of WS by WRM of buffalo (60.80, 49.93 and 17.45%, respectively) were more than cattle (53.00, 38.63 and 10.62%, respectively) (P<0.05). Regardless the type of microorganisms, digestibility of DM (P>0.05), NDF and ADF by buffalo (51.03, 44.41 and 12.09%) was more than cattle (48.04, 36.34 and 8.76) (P<0.05). Potential of GP (B) by fungi and WRM in cattle was more than buffalo (P<0.05). Rate of GP (C) for WRM and fungi of cattle was less than buffalo (P<0.05). Regardless the type of microorganisms, C in buffalo was more than cattle (P<0.05) and was vice versa for B (P<0.05). Regardless the type of animal species, WRM had higher digestibility and B than fungi (P>0.05), but rate of GP of them was same. In SRAFC, DM digestibility of WS by fungi of buffalo at days 3 and 12 was more than cattle (P<0.05). The number of fungi in cattle rumen was more than buffalo (P<0.05). The potential of fungi and WRM of buffalo were more than cattle. Therefore, the results were shown the advantage and supremacy of buffalo in usage the low quality roughages.

KEY WORDS gas production, rumen fungi enumeration, rumen microorganism, specific rumen anaerobic fungi culture.

INTRODUCTION

A lot of wheat straw is produced and used in the feeding of livestock. Among the limiting factors the nutritional value of agricultural byproducts are high lignin content and low quantity of easy accessible carbohydrates (Osorio and Cruz, 1990).

In ruminants due to having special digestive system, feeds exposed to microbial fermentation, before digestive enzymes of animal. Since the main limitation for increasing the animal productions is deficiency of feedstuffs in developing country, production of livestock with ability to consume the low quality and fibrous materials is necessary. Buffalo has a high capacity to use the low quality fibrous feedstuffs and roughage. Advantage of rumen metabolism and function of buffalo than the cattle, especially in terms of the activity of cellulolytic microorganisms, is considered (Bahatia *et al.* 2004).

The anaerobic rumen fungi have the necessary enzymes (mostly extra cellular) for degradation of plants cell wall. The fungi digest about 70% of cellulose in rumen, and their cellulase and xylanase activity are more than other cellulolytic microorganisms like bacteria and protozoa in rumen (Lee *et al.* 2004). Before rumen bacteria could degrade the forage, they must be attached and colonized on the plant tissues. Cuticle that covers the aerial parts of all plants, prevented from colonization of rumen bacteria. The rumen anaerobic fungi have ability to penetrate to the cuticle layer

for access to plant tissue. The fungi penetrate into the plant tissue and produced a wide range of high activity extracellular enzymes, so they have ability to degrade nearly more than 34% of plant tissue lignin (Krause *et al.* 2003). The chitinase activity of rumen anaerobic fungi, make them able to degrade hard section of cell wall of plants, including sclerenchyma and vesicular tissue.

There was significant difference in number of buffalo and cattle rumen bacteria, fungi and protozoa (Bahatia *et al.* 2004). The anaerobic fungi in the rumen of buffalo were significantly higher than cattle (Wanapat *et al.* 2009). Experiments showed that in diets based on ryegrass, numbers of rumen fungi in cattle is greater than buffalo (Kumar *et al.* 2002); as well diets containing oats-concentrate (with 27.2% cellulose) resulted to stimulate rumen cellulase microbial activity of cattle than buffalo (Kumar *et al.* 2002). Due to the low rumen passage rate of buffalo in comparison to cattle, digestibility and efficiency of low quality forage in buffaloes is higher than dairy cow (Bahatia *et al.* 2004). In the most parts of the world, there is the restriction of feed resources, and ruminants were fed with low quality by-products.

The rumen fungi have direct and indirect role in digestion of cell wall (to facilitate access to the cell wall for bacteria and protozoa through weaken the lingo cellulosic bounds of them), and exist conflicting results regarding the activity of fungal populations in cattle and buffalo (depending on their food and habitat). Therefore, the present experiment was designed to compare the activity of rumen anaerobic fungi of Holstein cattle and Khuzestan buffalo in digestion of WS as typical roughage.

MATERIALS AND METHODS

Animal, diet and sample preparation

The experiment was conducted by 5 Holstein steer (weight 430±4 kg, 18±2 months old) and 5 male buffalo (weight 420±5 kg, 18±2 months old) which fed similar diet. The diet was formulated based on NRC (1996). The diet was composed from: alfalfa hay, wheat straw, sugarcane pith, soybean meal, barley grain, wheat bran, corn grain, urea, minerals and vitamins additives. The animals were fed near *ad libitum* (10% less that), two meals per day within 4 months. The animals had free access to water during the experiment. The WS was oven dried (60 ˚C, 48h) and milled (through 1 mm screen, Tecator, Sweden).

In vitro digestibility

The test was conducted three times, for each run the rumen fluid was collected from all animals before morning feeding via stomach tube, combined and filtered through four layers of cheese cloth, stored in plastic bottles (filled by CO_2) with tight stopper, kept that in a flask with warm water, and transported to the laboratory. The *in vitro* digestibility of WS was measured by the two-step method (Tilley and Terry, 1963). Briefly, 4 tubes contained 0.5 g WS were incubated in rumen fluid for 48 h and then for 48 h in a pepsin-HCl solution. IVD was calculated from difference between the amount of the nutrients at initial and end of incubation for samples. The NDF and ADF were measured Van Soest *et al.* (1991).

IVD-by rumen fungi

For isolation fungi from whole rumen liquor, rumen fluid was collected before morning feeding via stomach tube from all steers, filtered centrifuged (10 min at 1000 rpm), and mixed by 1:4 ratio to McDougall (1948), buffer then 2 mL antibiotics (penicillin, streptomycin and chloramphenicol, each 0.1 mL/L) was added to the culture medium (Davies *et al.* 1993). The other steps of experiment were operated like above.

Gas production

GPwas analyzed in triplicate by the Menkeand Steingass (1988) technique, using 100 mL glass syringes (model Fortuna; Haberle Laborte chnik, Germany) filled with 300 mg of the WS sample, 20 mL of artificial saliva (McDougall, 1948) and 10 mL of rumen fluid. Rumen liquied sampled and prepared ast mention before. Syringes were incubated at 39 ˚C and GP was measured after 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. The potential (B) and rate of GP (C) were fitted by the exponential equation:

$$Y= B(1-e^{-Ct})$$

Where:
B: gas production from the fermentable fraction (mL).
C: gas production rate constant C (mL/h).
t: incubation time (h).
Y: gas produced at time t.

To measure the GP by anaerobic rumen fungi, isolation was done like the procedure described in IVD Section.

Preparation anaerobic fungi culture

The SRAFC was prepared based on Davies *et al.* (1993) and including: saline solution 1 (D-potassium hydrogen phosphate) and 2 (potassium hydrogen phosphate, ammonium sulfate, sodium chloride and calcium chloride), centrifuged rumen fluid (15000 rpm, 30 min), yeast extract, trypticase peptone, glucose, cellobiose, sodium bicarbonate, cysteine HCl and Resazurin (0.1%/L of culture medium). The culture mediums transferred into glass serum bottle, under anaerobic condition, then autoclaved for 15 min at 120 ˚C. Isolated rumen fungi prepared as inoculants (For

preparing rumen fungi inoculants, WS was incubated in the rumen of fistulated steers, and used as the carbon source for growth of the rumen anaerobic fungi., Rezaeian *et al.* 2005), were cultured in glass serum bottle containing SRAFC, experimental samples (WS, three replicates for each) and antibiotics (penicillin, streptomycin and chloramphenicol, each of 0.1 mg/L) and for purifying cultures three subculture was done. The glass serum bottles were incubated at 39 ˚C for periods of 3, 9 and 12 days (three replicates for each time). On days 3, 9, 12 replicates removed, glass content was filtered and dried (60 ˚C, 48 h). The disappearance of DM by fungi was calculated for each time.

Enumeration of rumen fungi

Dilution and enumeration of fungi was performed by MPN methods (Theodorou *et al.* 1990). Briefly, the dilution solution was saline solution 1 and 2 that explained above. By obtained rumen fluid from the animals under study, the dilution solution was prepared to dilution of 10^{-1} to 10^{-5}. Then, with serial inoculated 0.5 ml from each dilution into medium culture of anaerobic fungi (as mentioned above), five tubes were prepared for each dilution and incubated at 39 ˚C for 12 days. After this time, the pH of the samples (Metrohm model 726, Switzerland) was measured, and turbidity (visually) and pH changes were indexes of fungi growth characteristics. By comparing observations with MPN tables and using existing software fungi were counted (Dehority, 2003).

Ammonia nitrogen assay

To measure ammonia nitrogen, the liquid of medium cultures was mixed with HCl (0.2 N), then used phenol-hypochlorite method (Broderick and Kang, 1980).

Statistical analysis

The IVD, GP (hours 2-96) and SRAFC (days 3, 9, 12) data statistically analyzed by SAS (1996) with a split-plot designs (animals and microorganisms as main plots and subplots, respectively) and compared of means done by Duncan's multiple range test (P<0.05). Statistical model included:

$$Y_{ijk}= (\mu+A_i+\delta_{ik}+B_j+A)_{(ij)} + e_{ijk}$$

Where:

Y_{ijk}: observed value.

μ: population mean.

A_i: effect of animal (cow or buffalo).

B_j: effect of treatment (WRM or fungi).

$(AB)_{ij}$: interaction effects of treatment in animals.

δ_{ik}: main plot error.

e_{ijk}: residual error.

RESULTS AND DISCUSSION

Digestibility of WS-by WRM

The digestibility of DM, NDF and ADF by WRM in buffalo were more than cattle (P<0.05). Regardless the type of microorganisms, digestibility of DM (P>0.05), NDF and ADF by buffalo were more than cattle (P<0.05).

Digestibility of WS-by rumen fungi

Dry matter and ADF digestibility of WS by rumen fungi of cattle and buffalo did not differ (P>0.05), but NDF digestibility in buffalo was more than cattle (P<0.05). Regardless the type of animal, the digestibility of DM, NDF (P<0.05) and ADF (P>0.05) by WRM was higher than fungi (P<0.05). However, the digestibility of DM, NDF and ADF of WS by rumen fungi was about 74.74, 36.82 and 48.50% of the WRM.

Gas production parameters

Potential of GP (B) of WS by WRM, was a significant difference between cattle and buffalo (P<0.05). GP rate (C) of WS in buffalo was more than cattle (P<0.05). Regardless the type of microorganisms, the B of WS in cattle was greater than buffalo (P<0.05), but C was higher in buffalo than cattle (P<0.05). Potential of GP by rumen fungi of cattle was greater than buffalo (P<0.05), but the rate of GP in buffalo was more (P>0.05). Regardless the type of animal species, potential of GP by WRM was more than fungi (P<0.05). Proportion of B and C by rumen fungi, respectively was 69.93 and 95.23% of WRM.

Digestibility and fermentation parameters

Digestibility of DM by rumen fungi of buffalo and cattle in buffalo on days 3 and 12 was higher than cattle (P<0.05). The highest DM digestibility in cattle and buffalo was on the 12^{th} day of culture (Table 3). The concentration of ammonia nitrogen of medium containing WS (Table 4) in the day 6, 9 and 12, had no difference between buffalo and cattle (P>0.05), but was higher on third day in buffalo. In both cattle and buffalo ammonia nitrogen concentration was greater with increasing duration of incubation until the ninth day, and from the ninth and twelfth days stopped. Fungi medium culture pH (Table 3) at all cultural times between buffalo and cattle were similar (P>0.05). It was observed that pH of medium culture containing bovine rumen fungi on the 12^{th} day was the lower than other days.

Number of rumen fungi

The concentration of rumen fungi in cattle was more than buffalo (P<0.05).the fungi concentration per ml rumen fluid of buffalo and cattle was 2×10^3 and 2.7×10^3, respectively (Table 5).

Digestibility of WS-by WRM

Agrees with our results, digestibility of DM, NDF and cellulose of WS and Berseem grass by WRM of buffalo were reported more than cattle (Tewatia and Bhatia, 1998). Also, the digestibility of DM, NDF and ADF of WS by WRM in buffalo was higher than Holstein cattle (Jabbari *et al.* 2011). Differences in nutrients digestibility between cattle and buffalo in the present experiments may be was attributed to variety of factors, including differences in density and type of rumen microorganisms (Bahatia *et al.* 2004) and physiological differences of buffalo and cattle (Wanapat, 2001). There are large cellulolytic populations including bacteria, anaerobic fungi and protozoa in rumen (Chen and Wang, 2008). Therefore, in the present experiments, one reason for the higher digestibility of nutrients in buffalo may be attributed to the role of their protozoa. Stated that about 30 to 40 % of total microbial fiber digestion in the rumen is done by protozoa; 34% of rumen cellulase activity is related to protozoa (Bauchop and Clarke, 1976).

In buffalo that fed WS and concentrate, removes protozoa resulted to diminish digestibility of structural carbohydrates in the rumen and whole digestive tract (Chaudhary *et al.* 1995). In a study on similar diet, number of protozoa per ml rumen fluid of buffaloes was more than cattle (Jabbari *et al.* 2011). In another study digestibility of DM, NDF and ADF of steam treated sugarcane pith and WS by rumen protozoa of Khuzestan buffalo was higher than cattle (Jabbari *et al.* 2012). The population of cellulolytic bacteria in rumen of buffalo compared with cattle has been reported three times (Singh *et al.* 1992; Singh *et al.* 2003). Wanapat (2001) also found that number of cellulolytic bacteria, cellulose digestibility and rumen ammonia concentration in the rumen of buffalo was higher than cattle. Thus, one reason for better digestion of cellulose by buffalo is more cellulolytic bacteria and higher concentration of ammonia in the rumen.

It seems that the *Bacteroid succinogenes, Ruminococcus albus* and *Ruminococcous flavefaciens* be dominant cellulolytic bacteria of rumen (Bryant and Small, 1960; Bryant, 1973), which actively led to the breakdown the plant tissues, and their population in buffalo reported more than cattle (Singh *et al.* 2003). The higher digestibility of WS in buffalo than cattle is unlikely can be attributed to the quantity of their rumen fungi (Table 5), because number of fungi in buffalo rumen is less than cattle, which this reality confirmed by others. For example on oats-concentrate diet, researchers stated that the population of rumen fungi in cattle was higher than buffalo (Kumar *et al.* 2002). But perhaps it can be attributed to the quality of their cellulase activity, because it has been suggested that compared with dominant rumen cellulolytic bacteria and protozoa, the activity of fungal cellulase and xylanase enzymes are more (Lee *et al.* 2004).

The cellulase activity of isolated rumen fungi of buffalo was higher than cattle (Samanta *et al.* 1999). More than 70% *in vivo* cellulose digestion is made by rumen fungi (Akin and Borneman, 1990). However, the population of the rumen fungi is significantly affected by diet. Mansouri *et al.* (2005) reported that the consumption of alfalfa hay, sugarcane hay and WS had no significant effect on rumen fungi populations of Holstein and Sistani cattle, but the most fungal zoospore density was observed with the consumption of WS; since the fungal zoospore per ml rumen fluid, when intake of WS, was more than sugarcane hay and alfalfa hay. Dey *et al.* (2004) reported that anaerobic fungi in the cross bred calves, causing increase of growth rate, feed efficiency and nutrients digestibility. Another reason for the difference in digestion between buffalo and cattle can be related to the body size of them, however in the present experiment the animals had approximately the same weight. In this case, Hungate (1966) showed that rumen fermentation per unit of rumen contents weight increases, and with body size of ruminant decrease, which might be associated with the energy needs of the animal.

Digestibility of WS-by rumen fungi

The DM and ADF digestibility of WS by rumen fungi of cattle and buffalo did not differ, but NDF digestibility in buffalo was more than cattle ($P<0.05$). This difference might be due to the higher fungal cellulase activity of buffalo as compared to cattle (Samanta *et al.* 1999). The diets rich of roughage like diets based on WS or silages which have long retention time in the rumen, leading to the development of anaerobic fungi (Hobson and Stewart, 1997). Investigators reported that direct feeding of *neocallimastix* resulted to increase the nutritional value of diets based on WS (Sehgal *et al.* 2008). It is reported that the digestibility of DM, NDF and ADF of WS by rumen fungi in cattle is more than buffalo (Kumar *et al.* 2002), which agrees with the results of this experiment in terms of digestible DM, but is opposite for ADF and NDF digestibility (Table 1). The higher DM digestibility (non-significantly) of WS by rumen fungi in cattle than buffalo can be due to differences in active rumen fungi strains. Because comparing the different active enzymes of anaerobic fungi in domestic and wild animals shown *Promises* species that isolated from bull, increased nutrients digestibility and growth of buffalo calves (Paul *et al.* 2004). Dayanand *et al.* (2007) studied biodegradation of WS (treated with urea) by different species of *Promises* and found that *Promises* increased the digestibility of nutrients and volatile fatty acids production (Dayanand *et al.* 2007).

Table 1 Digestibility of wheat straw that incubated by fungi and whole rumen microorganisms of cattle and buffalo

Animal		Microorganisms		Digestibility (%)		
Buffalo	Cattle	Whole	Fungi	DM	NDF	ADF
Buffalo	-	Whole	-	60.80[a]	49.93[a]	17.45[a]
Buffalo	-	-	Fungi	41.26[c]	38.89[b]	6.73[c]
-	Cattle	Whole	-	53.00[b]	38.63[b]	10.62[b]
-	Cattle	-	Fungi	43.80[c]	34.05[c]	6.89[c]
SEM				2.34	1.54	1.56
P-value				0.001	0.0005	0.0039
Regardless the type of animal species						
-	-	Whole	-	56.90[a]	44.28[a]	14.04[a]
-	-		Fungi	42.53[b]	36.47[b]	6.81[b]
SEM				1.64	1.11	1.11
P-value				0.0003	0.0010	0.0017
Regardless the type of microorganisms						
Buffalo	-	-	-	51.03	44.41[a]	12.09[a]
Cattle	-	-	-	48.40	36.34[b]	8.76[b]
SEM				1.64	1.11	1.11
P-value				0.29	0.0008	0.064

SEM: standard error of means; DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 2 Gas production parameters of wheat straw that incubated by fungi and whole rumen microorganisms of cattle and buffalo

Animals		Microorganisms		Parameters	
Buffalo	Cattle	Whole	Fungi	B (mL)	C (mL/h)
Buffalo	-	Whole	-	92.38±2.36[b]	0.028±0.0018[a]
Buffalo	-	-	Fungi	64.54±6.36[d]	0.028±0.0018[a]
-	Cattle	Whole	-	108.70±8.51[a]	0.013±0.0019[b]
-	Cattle	-	Fungi	76.09±5.96[c]	0.013±0.0019[b]
SEM				1.71	0.0006
P-value				0.0001	0.0001
Regardless the type of animal species					
-	-	Whole	-	100.54±5.44[a]	0.021±0.0018
-	-		Fungi	70.31±6.16[b]	0.020±0.0018
SEM				1.22	0.0004
P-value				0.0001	0.921
Regardless the type of microorganisms					
Buffalo	-	-	-	78.46±6.16[b]	0.0277±0.0018[a]
Cattle	-	-	-	92.39±5.44[a]	0.0135±0.0019[b]
SEM				1.21	0.0004
P-value				0.0001	0.0001

SEM: standard error of means; B: gas production from the fermentable fraction (mL for 96h); C: gas production rate constant (mL/h).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Gas production parameters of WS-by WRM and fungi

Potential of GP by WRM in cattle was higher than buffalo (P<0.05), and vice versa for GP rate (Table 2). In agrees to the present experimental results, Jabbari (2010) and Rafiei *et al.* (2013) reported that B by WRM in buffalo was significantly higher than cattle. The results of Jabbari (2010) on C were consistent with our experiment results. Higher B in cattle than buffalo might be due to further fermentation and degradation of feed by cattle rumen microorganisms (Agarwal *et al.* 1991) and different in their rumen microbial population. It is reported that steaming of sugarcane pith leads to a significant increase in the C and B, that most of this increment was related to the rumen bacteria (Chaji and mohammadabadi, 2011).

The rate of GP in buffalo was more than cattle, this means that more gas is produced in the early hours of incubation, or feed degradation rate in buffalo is faster. Since cattle rumen fungi in present experiment was higher and had more role in fiber digestion than buffalo, might the cause of this delay in cattle attributed to this item, since the colonization of fungi on feeds need longer period (Lowe, 1987), on the other hand, can be expressed that in buffalo other microorganisms than fungi have a greater role in digestion.

Dry matter digestibility and fermentation parameters

The highest dry matter digestibility in cattle and buffalo was on the 12th day of culture (Table 3), might be due to

Table 3 Dry matter digestibility of wheat straw that incubated in specific rumen anaerobic fungi culture contain rumen fluid of cattle and buffalo, and medium pH

Itemes	Incubation time (day)					
	3		9		12	
	pH	DMD (%)	pH	DMD (%)	pH	DMD (%)
Buffalo	7.02	26.63[a]	6.95	24.70	6.85	36.33[a]
Cattle	7.19	20.80[b]	7.07	22.26	6.86	33.53[b]
SEM	0.371	1.47	0.365	2.97	0.253	1.29
P-value	0.770	0.048	0.840	0.594	0.986	0.039

SEM: standard error of means and DMD: dry matter digestibility.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

increase of rhizoid growing which lead to break the physical structure of plant and softening of feed, and also may be due to the increased amount of fiber degrading enzymes (Dehority, 2003).

The concentration of ammonia nitrogen (Table 4) was higher on d 3 in buffalo. In both cattle and buffalo ammonia nitrogen concentration from the ninth and twelfth days stopped that represents the end of fermentation. Increased the amount of ammonia during the incubation time, indicating increased the rate of proliferation ofsporangium and proteolytic enzymes follow the increasing of fungi, so digestion more protein and consequently produced greater ammonia nitrogen. Having active proteases by rumen fungi is their unique properties as cellulose-degrading microorganisms, because the majority of cellulose degrading bacteria are not proteolytic (Bahatia *et al.* 2004). The researchers observed that *Neocallimastix frontalis* have the extra cellular proteolytic activity; their activity was lower compared with aerobic fungi, but was comparable with most proteolytic bacteria (Wallace and Joblin, 1985). In ruminants that are fed low-quality forage, critical levels of ammonia nitrogen to maintain proper microbial activity have been reported equal to 5-20 mg/100 mL of rumen fluid (Bryant and Small, 1960). When the levels of ammonia increased in the rumen of buffalo up to 13.60 to 34.40 mg/100 mL, digestibility and intake of WS, also bacteria and protozoa as well as the urinary purine bases increased (Wanapat and Pimpa, 1999). Therefore, ammonia nitrogen levels in the present experiments in both cattle and buffalo was in the proper range. Fungi medium culture pH (Table 3) containing bovine rumen fungi on the 12th day was the lower than other days. In fact, the depression of pH showed low production of fatty acids, specifically the production of acetic acid and methane, while a slight increment of propionic acid (Erfle *et al.* 1982). Decreased the pH might be due to being closed of medium culture, and is a common occurrence that this causes to stopping the growth of microorganisms and is called suicide.

Rumen fungi

The number of rumen fungi (Table 5) in cattle was more than buffalo (P<0.05).

In agrees with the results of the present experiments, Malakar and Walli (1995) and Kumar *et al.* (2002) reported that under similar feeding conditions, rumen fungi of cow was more than buffalo, but in study of Wanapat *et al.* (2009) number of fungi in the rumen of buffalo was higher than cattle.

Table 4 Rumen ammonia nitrogen (mg/100 mL) of specific rumen anaerobic fungi culture contain incubated wheat straw by rumen fluid of cattle and buffalo

Items	Incubation time (day)			
	3	6	9	12
Buffalo	16.62[a]	16.94	18.76	18.61
Cattle	15.95[b]	16.46	18.97	19.08
SEM	0.063	0.302	0.281	0.360
P-value	0.017	0.381	0.659	0.449

SEM: standard error of means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 Rumen fungal population of cow and buffalo

Item	Number of fungi/mL of rumen fluid		SEM
	Cattle	Buffalo	
Concentration	2.7×10^{3a}	2.0×10^{3b}	272

SEM: standard error of means.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Rumen fungi and microbial population is not always constant and uniform, depended on physiological factors such as age, breeding, feeding behavior, production level, animal health, nature and relations between different microbial populations, as well as environmental factors such as diet composition, nature of the dite frequency of feeding, dietary changes, seasonal changes, changes at duration of day and geographical factors, affect the ratio and density of different groups of rumen microorganisms (Russell, 1986). Since the composition of diet, amount and frequency of feeding was similar in the present experiments, probably differences between rumen anaerobic fungi of cattle and Khuzestan buffalo was due to geographical conditions and animal species.

CONCLUSION

Overall, the results showed the greater number of rumen fungi in cattle than buffalo however, despite of this difference, the potential of fungi and whole rumen microorgan-

isms of buffalo in the present experiments was greater or equal with cattle. So, results showed the superior of buffalo compared to the Holstein cattlefor using low-quality fiber materials. In buffalo might be other microorganisms except fungi had a greater role in digestion of roughage.

ACKNOWLEDGEMENT

The authors appreciate from Ramin Agriculture and Natural Resources University of Khuzestan for supporting the present experiment.

REFERENCES

Akin D.E. and Borneman W.S. (1990). Role of rumen fungi in fiber degradation. *J. Diary Sci.***73,** 3023-3032.

Agarwal N., Kewalramani N., Kamra D.N., Agarwal D.K. and Nath K. (1991). Hydrolytic enzymes of buffalo rumen comparison of cell free rumen fluid, bacterial and protozoal fractions. *Buffalo J.* **7,** 203-207.

Bahatia S.K., Kumar S. and Sangwan D.C. (2004). Advances in Buffalo-Cattle Nutrition and Rumen Ecosystem. International Book Distributing Co.

Bauchop T. and Clarke T.J. (1976). Attachment of the ciliate epidinium crawley to plant fragments in the sheep rumen. *Appl. Environ. Microbiol.* **32,** 417-422.

Broderick G.A. and Kang J.H. (1980). Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Diary Sci.* **63,** 64-75.

Bryant M.P. and Small N. (1960). Observations on the ruminal microorganisms of isolated and inoculated calves. *J. Diary Sci.* **43,** 654-67.

Bryant M.P. (1973). Nutritional requirements of the predominant rumen cellulolytic bacteria. *Federation Proc.* **32(7),** 1809-1813.

Chaji M. and Mohammadabadi T. (2011). The investigation of *in vitro* fermentation of sugarcane pith treated with low temperature steam and sulfuric acid by isolated rumen microbial fractions. *Anim. Nutr. Feed Technol.* **11,** 185-193.

Chaudhary L.C., Srivastava A. and Singh K.K. (1995). Rumen fermentation pattern and digestion of structural carbohydrates in buffalo (*Bubalus bubalis*) calves as affected by ciliate protozoa. *Anim. Feed Sci. Technol.* **56,** 111-117.

Chen X.L. and Wang J.K. (2008). Effects of chemical treatments of rice straw on rumen fermentation characteristics, fibrolytic enzyme activities and populations of liquid and solid associated ruminal microbes *in vitro*. *Anim. Feed Sci. Technol.* **141,** 1-14.

Davies D.R., Theodorou M.K., Lawrence M.I. and Trinci A.P.J. (1993). Distribution of anaerobic fungi in the digestive tract of cattle and their survival in faeces. *J. Gen. Microbiol.* **139,** 59-64.

Dayanand T.L., Nagpal R., Puniya A.K., Sehgal J.P. and Singh K. (2007). Biodegradation of urea-NH3treated wheat straw using anaerobic rumen fungi. *J. Anim. Feed Sci.* **16,** 484-489.

Dehority B.A. (2003). Rumen Microbiology. London, UK. Nottingham University, Academic Press. UK.

Dey A., Sehgal J.P., Puniya A.K. and Singh K. (2004). Influence of anaerobic fungal culture (*Orpinomycessp*) administration on growth rate, ruminal fermentation and nutrient digestion in calves. *Asian-australs J. Anim. Sci.* **17,** 820-824.

Erfle J.D., Boila R.J., Teather R.M., Mahadevan S. and Sauer F.D. (1982). Effect of pH fermentation characteristics and protein degradation by rumen micro-organisms *in vitro*. *J. Diary Sci.* **65,** 1451-1464.

Hobson P.N. and Stewart C.S. (1997). The Rumen Microbial Ecosystem. London: Chapman and Hall.

Hungate R.E. (1966). The Rumen and Its Microbes. London: Academic Press. UK.

Jabbari S. (2010). The comparison digestibility of steam treated sugarcane pith and wheat straw by rumen microorganisms of cattle and buffalo in Khuzestan. MS Thesis. Khuzestan Ramin Agricultural and Natural Resources Univ., Ahvaz, Iran.

Jabbari S., Eslami M., Chaji M., Mohammadabadi T. and Bojarpour M. (2011). The Comparison of *in vitro* digestibility of wheat straw by rumen microorganism of khuzestani buffalo and hostein cow *in vitro* digestibility by Khuzestani buffalo. Pp. 266-268 in Proc. Int. Conf. Biol., Environ. Chem. Hongkong, China.

Jabbari S., Eslami M., Chaji M., Mohammadabadi T. and Bojarpour M. (2012). Acomparison between water buffalo (Khuzestani) and cow rumen fluids in terms ofthe *in vitro* digestibility of steam treated sugarcane pith. Pp. 405-406 in Proc. WCDS Adv. Dairy Technol., Univ. Alberta, Canada.

Krause D.O., Denman S.E., Mackie R.I., Morrison M., Rae A.L., Attwood G.T. and McSweency C.S. (2003). Opportunities to improve fiber degradation in the rumen: microbiology, ecology and genomics. *FEMS Microbiol. Rev.* **27,** 663-693.

Kumar S., Singh S. and Bhatia S.K. (2002). Microbial and biochemical changes in the rumen of cattle and buffalo fed oat hay concentrate diet. *Indian J. Anim. Nutr.* **19,**78-79.

Lee S.S., Choi C.K., Ahn B.H., Moon Y.H., Kim C.H. and Ha J.K. (2004). *In vitro* stimulation of rumen microbial fermentation by a rumen anaerobic fungal culture. *Anim. Feed Sci. Technol.* **115,** 215-226.

Lowe S.E. (1987). Cellulases and xylanase of an anaerobic rumen fungusgrown on wheat straw, wheat straw holocellulose, cellulose and xylan. *Appl. Environ. Microbiol.* **53,** 216-1223.

Malakar D. and Walli T.K. (1995). Relative fibre degradation *in vitro* by bacteria and fungi using inoculum from cow and buffalo rumen. *Indian J. Diary Sci.* **48,** 295-301

Mansouri H., Nik-Khah A., Rezaeian M. and Mirhadi S.A. (2005). Comparison of microbial population in ruminal fluid of Sistani and Holstein cattle fed different roughages. *Pajouhesh and Sazandegi.* **72,** 66-73.

McDougall E.L. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* **43,** 99-106.

Menk K.H. and Stenigass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* **28,** 6-55.

NRC. (1996). Nutrient Requirements for Beef Cattle. 7th Ed. National Academy Press, Washington, DC, USA.

Osorio H. and Cruz D.L. (1990). Steam treated bagasse for fattening cattle. Effect of supplementation with Giricidia

sepium and urea / molasses. *Lives. Res. Rural Dev.* **2(2),** 77-91.

Paul S.S., Kamra D.N., Sastry V.R.B. and Agarwal N. (2004). Effect of administration of an anaerobic gut fungus isolated from wild blue bull to buffaloes on *in vivo* ruminal fermentation and digestion of nutrients. *Anim. Feed Sci. Technol.* **115,** 143-157.

Rafiei M., Chaji M., Mohammadabadi T. and Sari S. (2013). The comparison digestibility of steamtreated sugarcane pith by rumen bacteria or rumen microorganisms of Holstein cow and buffalo of Khuzestan. *J. Rumin. Res.* **1(1),** 53-75.

Rezaeian M., Beakes G.W. and Chaudhry A.S. (2005). Relative fibrolytic activities of anaerobic rumen fungi on untreated and sodium hydroxide treated barley straw in *in vitro* culture. *Anaerobe.* **11,** 163-175.

Russell J.B. (1986). Ecology of rumen microorganism: energy use. Pp. 256 in Aspect of Digestive Physiology in Ruminants. A. Dabson and M.J. Dobson, Eds. London, Comstock Publishing Association, UK.

Samanta A.K., Walli T.K., Batish V.K., Grover S., Rajput Y.S. and Mohanty A.K. (1999). Characterization of anaerobic fungi in rumen of riverine buffalo. *Indian J. Anim. Nutr*. **16(4),** 275-278.

SAS Institute. (1996). SAS®/STAT Software, Release 6.11. SAS Institute, Inc., Cary, NC. USA.

Sehgal J.P., Jit D., Puniya A.K. and Singh S. (2008). Influence of anaerobic fungal administration on growth, rumen fermentation and nutrient digestion in female buffalo calves. *J. Anim. Sci.* **17,** 510-518.

Singh S., Bhatia S.K. and Pradhan K. (2003). Relative ruminal ciliates distribution and physiology of bacteria isolated in buffalo and cattle fed wheat straw-preformed protein diets. *Indian J. Anim. Sci.* **73,** 663-664.

Singh S., Pradhan K., Bhatia S.K., Sangwan D.C. and Sagar V. (1992). Relative rumen microbial profile of cattle and buffalo fed wheat straw-concentrate diets. *Indian J. Anim. Sci.* **62,** 1197-1198.

Tewatia B.S. and Bhatia S.K. (1998). Comparative studies in rumen ammonia anabolizing enzymes, microbial and mineral profiles between buffalo and cattle fed fibrous diet. *Buffalo J.* **12,** 169-170.

Tilley J.M. and Terry R.A. (1963). A two staged technique for the *in vitro* digestion of forage crops. *J. Br. Grassl. Soc.* **10,** 104-111.

Theodorou M.K., Gill M., King-Spooner C. and Beever D.E. (1990). Enumeration of anaerobic chytridiomycetes as thallus-forming units: novel method for quantification of fibrolytic fungal populations from the digestive tract ecosystem. *Appl. Environ. Microbiol.* **56(4),** 1073-1078.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods of dietary fiber, neutral detergentfiber and nonstarch polysaccharides in relation to animal nutrition. *J. Diary Sci.* **74,** 3583-3597.

Wallace R.J. and Joblin K.N. (1985). Proteolytic activity of a rumen anaerobic fungus. *FEMS Microbiol. Lett.* **29,** 19-26.

Wanapat M. (2001). Swamp buffalo rumen ecology and its manipulation. Pp. 155-187 in 1th Natio. Workshop on Swamp Buffalo Develop. Thailand.

Wanapat M. and Pimpa O. (1999). Effect of ruminal NH3-N levels on ruminal fermentation purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian-australs J. Anim. Sci.* **12,** 904-907.

Wanapat M., Pilagun R. and Kongmun P. (2009). Rominal ecology of swamp buffalo as influenced by dietary source. *Anim. Feed Sci. Technol.* **151,** 205-214.