Chemical composition and nutrient degradability in elephant grass silage inoculated with *Streptococcus bovis* isolated from the rumen

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ABSTRACT

The objective of the present study was to assess the chemical and bromatological composition and *in situ* degradability of elephant grass silages inoculated with *Streptococcus bovis* isolated from cattle rumen. A complete randomized design was used with four treatments and six replications: elephant grass silage, elephant grass silage inoculated with 10^6 CFU/g *Streptococcus bovis* JB1 strains; elephant grass silage inoculated with 10^6 CFU/g *Streptococcus bovis* HC5 strains; elephant grass silage inoculated with 10^6 CFU/g *Enterococcus faecium* with six replications each. The pH and ammoniacal nitrogen values were lower (P<0.05) for the silages inoculated with *Streptococcus bovis* JB1 and HC5, respectively. The silage inoculated with *Streptococcus bovis* had a higher crude protein content (P<0.05) and there were no differences for the fiber contents in the silage. The (a)soluble fraction degradability, especially in the silages inoculated with *Streptococcus bovis* JB1 and HC5, had higher values, 30.77 and 29.97%, for dry matter and 31.01 and 36.66% for crude protein, respectively. Inoculation with *Streptococcus bovis* improved the fermentation profile, protein value and rumen degradability of the nutrients.

Key words: digestion, microorganisms, nutritive value, ruminant.

INTRODUCTION

Feeding cattle in feed lots in Brazil are based on supplying good quality bulk. However, climate seasonality affects yield and nutritive value over the year, concentrating forage production in the months with greater rainfall and higher temperatures. One of the ways to minimize the effects of seasonality in production consists of conserving these forages when their nutritive value is high.

Elephant grass (*Pennisetum purpureum,* Schum) is considered one of the most important tropical forages because of its high potential for biomass production, easy adaptation to diverse ecosystems and good acceptance by the animals. It is widely used to feed herds in the forms of pasture and especially as silage, because of its high soluble carbohydrate content that favors good fermentation (Patrizi et al. 2004).

During the ensiling process, the fresh forage placed in the silo is transformed until the mass is completely stabilized and acquires the characteristics

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of silage. The main objective of the ensiling process is to reach pH values sufficient to inhibit the growth of undesirable microorganisms and enzymatic cataballistic activity of the ensilaged plant.

Lactic fermentation must predominate in the anaerobic conditions so that forage plants can be conserved as silage. Several factors can interfere in the fermentation quality, including microorganisms that lead to secondary fermentation, soluble carbohydrate content, dry matter content, compacting and speed in closing the silo (McDonald et al. 1991).

It is expected in good quality silage that the homofermentative lactic bacteria become dominant as quickly as possible and promote - because of low pH and reduced oxi reduction potential - inhibition of enterobacteria and bacteria of the *Listeria, Bacillus, Clostridium* genera and the heterfermentative lactic bacteria themselves. According to Muck (1996), an ideal fermentation profile is that where the maximum lactic acid is produced because lactic acid fermentation does not result in losses from gas formation and secondary metabolites.

In this context, *Streptococcus bovis* is a lactic bacteria isolated from the rumen, with characteristics that enable its use as inoculant in silage. The main characteristic of this species is its specific growth speed, 30% faster than other species of lactic acid used as inoculant for silage, suggesting that it can act as a culture starter in the fermentation process and promoting a fast fall in the silage pH (Jones et al. 1992). Accelerated fall in the pH can favor the growth of the lactic bacteria in detriment to the enterobacteria, a necessary condition for fermentation to take place correctly.

In this reality, microbial inoculation has been indicated as an alternative to improve the fermentation profile and nutritional value of silages of some forage plants, although the results reported have varied considerably (Bolsen et al. 1992). Most microbial inoculants contain strains of homofermentative lactic bacteria, whose purpose is to quickly stimulate lactic fermentation, reduce pH and inhibit the action of undesirable bacteria, especially proteolytic bacteria (Filya et al. 2000). Hence, a great variety of additives has been recommended to guarantee the best nutritional quality in the silage. The objective of the present study was to assess the chemical and bromatological composition and *in situ* degradability of nutrients in elephant grass silages inoculated with *Streptococcus bovis* isolated from the rumen.

MATERIALS AND METHODS

The experiment was carried out at the Department of Animal Science at the Universidade Federal de Viçosa, located in the municipality of Viçosa-MG, Brazil. Viçosa is situated at 20° and 45' latitude south, 42° and 51' longitude west and at 657 m altitude, with 1,341 mm mean annual rainfall, 86% of which falls from October to April. An established elephant grass meadow was used of approximately 1.5 ha. After a standardizing cut, it was fertilized with nitrogen and potassium in the form of ammonia sulfate and potassium chloride, respectively. The grass was harvested and ensilaged 65 days after the standardizing cut.

A completely randomized experimental design was used with four treatments: elephant grass silage, elephant grass silage inoculated with 10^6 CFU/g *Streptococcus bovis* JB1 strainy, elephant grass silage inoculated with 10^6 CFU/g *Streptococcus bovis* HC5 strain, elephant grass silage inoculated with 10^6 CFU/g *Enterococcus faecium* with six replications of each treatment.

The grass was cut using a big knife and chopped in a stationary forage machine. It was then ensilaged for ensiled in experimental 15 L silos, equipped with a Bunsen valve, for gas escape. Three kg sand were placed at the bottom of the silos to capture the effluents, separated from the forage by cotton fabric. The material was compacted manually, placing approximately 9 kg fresh forage in each silo. The silos were opened 60 days after closing.

To prepare the inoculant, the cultures were grown in MRS culture medium (De Man, Rogosa and Sharpe) and submitted to three successive evaluations prior to the day of ensiling. They were later cultured in solid MRS to count the microbial populations. Based on the result of the bacterial concentration of each inoculate, the dilution necessary for each inoculate to reach 10^6 CFU/g fresh forage was determined, based on previous counting in Agar MRS culture medium. Before ensiling, the cultures were again activated and diluted in distilled water at ensiling to reach the pre-established concentrations.

When the silos were opened sub-samples of approximately 25 g were collected to analyze the pH and 100 mL water were added and after resting for two hours, the pH was read using a potentiometer. In another 25 g sub-sample, 200 mL were added of a H_2SO_4, 0.2 N solution and allowed to rest for 48 hours and then filtered through Whatman 54 filter paper. This filtrate was stored in a refrigerator for later ammoniacal nitrogen analysis (Bolsen et al. 1992).

To assess the bromatological composition, fresh matter samples were collected before ensiling (Table I) and after opening the silos. These samples were pre-dried for 72 hours in a forced air ventilation chamber at 65°C and then ground in a Willey-type grinder. The contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to methodology reported by Silva and Queiroz (2002).

For the *in situ* incubation, three steers were used, fistulated in the rumen, whit 400 kg mean weight, kept on *Brachiaria decumbens* pasture.

TABLE I
Contents of dry matter (DM), crude protein (CP) neutral fiber detergent (NFD), acid detergent fiber (ADF), in elephant grass before ensilage.

Treatment	DM %	CP % DM	NDF % DM	ADF % DM
Elephant grass	26.38	5.51	72.01	41.25

The silage samples were ground in a 5 mm sieve. The ground foods were weighed in nylon bags to supply about 10 to 20 mg sample/cm² N/A useful area of the bags (Nocek 1988), in duplicate for each food, that represented one replication in each animal. The bags were inserted in the rumen at the same time and removed 0, 2, 4, 8, 16, 24, 48, 72, 96 and 144 hours after incubation of the bulk, according to the NRC (2001).

The following were determined for the silages: the degradations of the dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber ADF) (NRC 2001). To estimate the DM and CP kinetic parameters, the first order asymptotic model was used $Deg(t) = a + b(1 - e^{-ct})$, proposed by Orskov and McDonald (1979), where Deg(t) represents the degradability or disappearance of the constituent (DM, CP, NDF or ADF) of the food, expressed in percentage; "a" is the water soluble fraction of the food at time zero; "b" is the water insoluble fraction, but potentially degradable in the rumen in a certain time; "c" is the degradation rate of the potentially degradable fraction in the rumen (b); "t" is the incubation time (hours).

In the NDF degradability estimates, the model was used proposed by Waldo et al. (1972), $R(t) = D(e^{-ct}) + I$, where R(t) is the incubation residue in time t (hours); D is the NDF fraction potentially degradable in the rumen; C is the degradation rate of the fraction D; "I" is the non-degradable fraction of the NDF.

The degradation curves of the DM, CP, NDF and ADF of the foods assessed for each animal used were fitted to the respective models, using the Marquardt regression procedure of the SAEG software (1999), which gives the estimates of the analyzed parameters.

The data obtained were submitted to analysis of variance and the mean values of the treatments were compared by the Tukey's test at the level of 5% significance, using the SAEG program, 8.0 version (Universidade Federal de Viçosa 1999).

RESULTS AND DISCUSSION

Lower pH and N-NH$_3$ (P<0.05) values were observed in the silages inoculated with *Streptococcus bovis* JB1 and HC5 (Table I). These results probably occurred because of the greater lactic bacteria growth in these silages, bearing in mind that lactic acid production is largely responsible for reducing pH and N-NH$_3$ in silages (Muck 1996) because reduced pH reflects quick lactic acid fermentation, guaranteeing better nutritional quality of the silage and greater crude protein CP content according to Table II. The pH values observed in the inoculated silages were in the ideal range (3.8 and 4.2) for good quality silage (McDonald et al. 1991). Another fact that may explain the higher CP contents in silages inoculated with *Streptococcus bovis* strains HC5 and JB1 is their common ability to synthesize protein from ammonia (Mantovani et al. 2002). These results were close to those by Bonelli (2011) who observed lower pH values in silages inoculated with *Streptococcus bovis* JB1 and HC5 with addition of 10% wheat meal, that was 3.86 and 3.93, respectively, in Tanzania grass silage (*Panicum maximum*).

Higher dry matter (DM) values were observed for silage inoculated with microbial additives compared to silage without inoculant (P<0.05) (Table I), that may be associated with maintaining homfermentative fermentation, where the DM losses are lower. Oliveira et al. (2007) reported that inoculation with both *Streptococcus bovis* strains resulted in greater dry matter recovery from the silages, with values of 20, 28, 21.42 and 21.46% for the control, and treatments with *Streptococcus bovis* HC5 and *Streptococcus bovis* JB1 inoculation, respectively. According to Muck (1996) lactic acid bacteria predominance in silage results in minimal dry matter losses because these bacteria transform sugars into lactic acid without producing secondary metabolites or gases.

The highest crude protein contents (P<0.05) were observed in the silages treated with *Streptococcus*

bovis and HC5 and JB1 (Table II). This may have been associated to the fact that the *Streptococcus bovis* HC5 species releases (bovicine HC5) in the bacteriocin medium that inhibits growth of proteolytic bacteria, such as the enterbacteria or clostridia, and thus decreases the protein nitrogen losses from the inoculated silages. Another fact that may explain the greater CP contents in the silages inoculated with *Streptococcus bovis* HC5 and JB1 strains may reflect the common capacity of all the *Streptococcus bovis* strains to synthesize protein from ammonia (Mantovani et al. 2002).

Similar results to those reported by Bonelli (2011) in Tanzania grass silage inoculated with *Streptococcus bovis* that registered values close to two percentage points compared to be silage without inoculate. However, Clavero (2001) did not obtain the same effect on CP with the use of two microbial additives in millet cultivars and elephant grass cv Mott, respectively, when using lactic acid bacteria. According to McDonald et al. (1991) low CP contents in silage can result from the action of plant proteolytic enzymes, because protein hydrolysis can raise soluble nitrogen contents to over 50% of the total nitrogen, contributing to increase in nitrogen losses through lixiviation in forages with high moisture content.

The contents of the fibrous fraction consti-tuents and ether extract were not influenced by inoculation (P<0.05) although some authors have reported reduction in the NDF fraction, due to the possible acid hydrolysis of the hemicellulose as a consequence of the reduced pH in the medium from fermentation carried out by lactic bacteria (Muck 1996, Penteado et al. 2006). Similar results to those of the present experiment were reported by Bonelli (2011) in Tanzania grass silage inoculated with *Streptococcus bovis*, who observed values of 71.43, 70.90 and 71.76 for the neutral detergent fiber values and 38.34, 37.17 and 38.454 acid detergent fiber values, in the respective silages without inoculant, inoculated with *Streptococcus*

bovis JB1 and inoculated with *Streptococcus bovis* HC5. This showed that the inoculant studied did not affect the fiber quality in the silage.

Considering that the "a" factor represents the portion of the plant that is readily available for rumen microorganisms, the microbial inoculant

TABLE II

Contents of pH, ammoniacal nitrogen (N-NH₃), dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in elephant grass silages without inoculant (control) and inoculated with *Enterococcus* (*Enterococcus faecium*), JB1 (*Streptococcus bovis* JB1) and HC5 (*Streptococcus bovis* HC5).

Treatment	pH	NH3 (mg/dL)	DM (%)	CP (%MS)	EE (%MS)	NDF (%MS)	ADF (%MS)
Control	4.32a	11.44a	25.53b	6.23b	3.02a	69.74a	38.75a
Enterococcus	4.19b	11.09b	27.12a	6.30b	2.99a	69.37a	37.30a
Streptococcus bovis JB1	3.99c	10.54c	28.12a	6.98a	2.95a	71.71a	36.88a
Streptococcus bovis HC5	4.04c	10.68c	26.93a	7.08a	3.06a	68.09a	37.57a
CV (%)	2.02	2.12	3.87	3.58	5.78	6.76	5.97

Means followed by the same lowercase letter in the column do not differ statistically by the Tukey's test at the level of 5% significance.

may have contributed to the increase in this fraction in the silage, especially in the silages inoculated with *Streptococcus bovis* JB1 and HC5, because these inoculants resulted in the highest values, 30.77 and 29.97%, respectively, for DM and 37.01 and 36.66 for crude protein, respectively (Table III), due to the good quality of the silages studied (Table I). These results corroborated Henriques et al. (2004), who reported superiority of the "a" fraction of the dry matter and crude protein in elephant grass silage inoculated with microbial additive. However, Tosi et al. (1999) observed "a" fraction values of 34.7% when assessing elephant grass silage without inoculant, a value higher than that in the present experiment. In spite of the higher "a" fraction values, the insoluble potentially degradable "b" fraction of both the dry matter and the crude protein was lower in silage inoculated with *Streptococcus bovis*. The degradation rate of the potentially degradable crude protein fraction ranged from 2 to 8% per hour (NRC 1985). In spite of the variations in the "a" and "b" fractions, the crude protein degradation rates in % per hour were constant between 4 and 5% per hour, and

were similar for the dry matter, whose "c" value remained between 3 and 4% per hour.

The greater degradability of the "a" fraction of the dry matter and crude protein was probably due to *Streptococcus bovis* inclusion, that reduced the moisture content of the grass, contributing to reduced pH and N-NH₃ (Table II) favoring lactic acid bacteria growth in detriment to the enterobacteria, increasing lactic acid formation and reducing dry matter and crude protein losses.

For NDF and ADF degradability, the greatest values of the potentially degradable insoluble "a" fraction were observed in the inoculated silages (Table IV). For the ADF, the highest values of the potentially degradable insoluble "b" fraction were observed in the inoculated silages, with mean values over 4 percentage points higher compared to the silage without inoculant. ADF degradation is closely linked to food digestibility and, therefore, its use or degradation will be greater or lesser, according to its composition, because the lignin present in ADF is not used (Silva and Queiroz 2002).

On average, the lower values of the "I" undegradable fraction and the "c" degradation

TABLE III

Estimates of rumen degradability parameters of dry matter (DM), crude protein (CP) of the elephant grass silages in the treatments: control (elephant grass) *Enterococcus* (*Enterococcus faecium*), JB1 (*Streptococcus bovis* JB1) and HC5 (*Streptococcus bovis* HC5).

Silage	Parameters			r^2
	DM Degradability			
	a	b	c	
Control	26.99	47.45	0.0292	0.97
Enterococcus	27.48	47.19	0.0325	0.96
Streptococcus bovis JB1	30.77	46.99	0.0328	0.96
Streptococcus bovis HC5	29.97	43.76	0.0363	0.99
	CP Degradability			
	a	b	c	
Control	35.12	48.22	0.0468	0.97
Enterococcus	35.99	48.30	0.0471	0.97
Streptococcus bovis JB1	37.01	46.77	0.0443	0.97
Streptococcus bovis HC5	36.66	46.67	0.0454	0.98

a = soluble fraction (%); b = potentially degradable insoluble fraction (%); I = undegradable fraction (%); c = fractional degradation rate (h^{-1}) (% per hour); r^2 = coefficient of determination.

TABLE IV

Estimates of the rumen degradable parameters neutral detergent fiber (NDF), acid detergent fiber (ADF) of the elephant grass silages in the following treatments: control (elephant grass) *Enterococcus* (*Enterococcus faecium*), JB1 (*Streptococcus bovis* JB1) and HC5 (*Streptococcus bovis* HC5).

Silage	Parameters			r^2
	NDF Degradability			
	b	I	c	
Control	63.10	36.90	0.0307	0.98
Enterococcus	64.48	35.52	0.0286	0.98
Streptococcus bovis JB1	64.98	35.02	0.0300	0.97
Streptococcus bovis HC5	65.88	34.12	0.0293	0.98
	AFD Degradability			
	b	I	c	
Controle	62.84	37.16	0.0271	0.97
Enterococcus	68.51	32.39	0.0266	0.97
Streptococcus bovis JB1	67.61	31.49	0.0219	0.97
Streptococcus bovis HC5	69.22	30.78	0.0225	0.98

a = soluble fraction (%); b = potentially degradable insoluble fraction (%); I = undegradable fraction (%); c = fractional degradation rate (h^{-1}) (% per hour); r^2 = coefficient of determination.

fractional rate occurred in the inoculated silages, that is, for the "I" and "c" fraction the response to NDF and ADF was inversely proportional to the "b" potentially degradable insoluble fraction. According to Chesson et al. (1985), the variation in fraction (I) is due to the natural selectivity of the rumen bacteria for different types of substrates. This observation is in line with the fact that using microbial inoculate enhanced the silage use, and consequently favored the microbial population in the rumen, thus influencing silage degradation.

Table V shows the potential and effective degradable rates of the dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the silages. The effective degradablity was estimated considering passage rates of 2, 5 and 8% per hour. Measuring the degradability in the rumen, without considering the passage rate, can overestimate the extension of the degradation, because the food particles are

subject to passage to the next compartment before they are completely degraded.

The dry matter potential degradability values presented fairly similar responses of around 75%, although the elephant grass silage inoculated with *Streptococcus bovis* HC5 presented potential degradability 2.5 percentage points higher compared to the silage without inoculant (Table V). These results were similar to those observed by Bonelli (2011) in Tanzania grass silage inoculate with *Streptococcus bovis*.

Lower results for potential dry matter degradability were observed by Cabral et al. (2005) when they assessed ruminal degradation of elephant grass silage and verified 64.9% dry matter potential degradability. The low potential dry matter degradability reported by these authors can be attributed to the more advanced stage of maturity of the elephant grass used (120

days growth) because tropical grasses, in spite of high productivity, accumulate over their growth cycle a high percentage of cell wall (NDF), a fraction with slow and incomplete digestion that occupies a lot of space in the gastric intestinal tract (Mertens 1996), causes variation in food digestion and affects intake (Van Soest 1994, Mertens 1996).

Similar results to those of the potential dry matter degradability were observed for the potential degradability of crude protein, neutral detergent fiber and acid detergent fiber that presented mean values close to 75, 65 and 68%, respectively (Table IV). This was in line with results by Bonelli (2011) in Tanzania grass silages inoculated with *Streptococcus bovis*.

TABLE V
Potential degradability (PD) and effective degradability (ED) of dry matter (DM) crude protein (CP) neutral detergent fiber (NDF) and acid detergent fiber (ADF) of elephant grass silages in the following treatments control (elephant grass) *Enterococcus* (*Enterococcus faecium*), JB1 (*Streptococcus bovis* JB1) and HC5 (*Streptococcus bovis* HC5).

Silage	Potential degradability	Effective degradability		
		Passage rate (% per h)		
		2	5	8
Dry Matter (%)				
Control	74.44	56.70	46.94	41.12
Enterococcus	74.53	57.86	46.07	41.79
Streptococcus bovis JB1	75.67	57.96	47.29	43.50
Streptococcus bovis HC5	76.96	57.58	48.11	42.54
Crude Protein (%)				
Control	73.21	58.33	48.27	43.49
Enterococcus	74.18	58.90	48.69	43.97
Streptococcus bovis JB1	75.41	59.19	49.66	44.81
Streptococcus bovis HC5	75.61	59.59	49.89	44.94
Neutral Detergent Fiber (%)				
Control	63.10	37.96	24.00	17.41
Enterococcus	64.48	38.24	23.47	16.99
Streptococcus bovis JB1	64.98	38.61	24.04	17.53
Streptococcus bovis HC5	65.88	39.89	25.06	18.27
Acid Detergent Fiber (%)				
Control	66.84	35.34	20.59	14.53
Enterococcus	67.61	36.43	22.34	15.19
Streptococcus bovis JB1	68.51	39.42	21.48	17.34
Streptococcus bovis HC5	69.22	36.65	24.08	16.11

CONCLUSION

Bacterial inoculation with *Streptococcus bovis* improved the fermentation profile, protein value and rumen degradability of the nutrients.

RESUMO

Objetivou-se avaliar a composição químico-bromatológica e a degradabilidade *in situ* de silagens de capim-elefante inoculadas com *Streptococcus bovis* isolado do rúmen bovino. O delineamento experimental foi o inteiramente casualizado, com quatro tratamentos e seis repetições: Silagem de capim-elefante; Silagem de capim-elefante inoculada com 10^6 CFU/g de estirpes de *Streptococcus bovis* JB1; Silagem de capim-elefante inoculada com 10^6 CFU/g de estirpes de *Streptococcus bovis* HC5; Silagem de capim-elefante inoculada com 10^6 CFU/g de estirpes de *Enterococcus faecium* com seis repetições. Os valores de pH e N-amoniacal foram menores (P<0,05) para as silagens inoculadas com *Streptococcus bovis* JB1 e HC5, respectivamente. A silagem inoculada com *Streptococcus bovis* apresentou maior teor (P<0,05) de proteína bruta e não houve diferenças para os teores de fibras nas silagens. A degradabilidade da fração solúvel (a), principalmente nas silagens inoculadas com o *Streptococcus bovis* JB1 e HC5, apresentou maiores valores, 30,77 e 29,97%, respectivamente, para a matéria seca e 37,01 e 36,66% para a proteína bruta, respectivamente. Conclui-se que a inoculação com o *Streptococcus bovis* melhora o perfil fermentativo, o valor proteico e a degradabilidade ruminal dos nutrientes.

Palavra Chave: digestão**,** microrganismos, valor nutritivo, ruminante.

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