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Can ruminal inoculum from slaughtered cattle replace inoculum from cannulated cattle for feed evaluation research?

O inóculo ruminal de bovinos abatidos pode substituir o inóculo de bovinos canulados em pesquisas de avaliação de alimentos?

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Abstract

The objective was to test the hypothesis that ruminal inoculum obtained from slaughtered cattle can replace inoculum from cannulated cattle in trials evaluating animal feedstuffs through *in vitro* gas production and digestibility. Five adult Holstein × Zebu steers with ruminal cannula were used to collect and compare rumen liquid from *in vivo* and slaughtered animals. *In vitro* dry matter digestibility (IVDMD), *in vitro* neutral detergent fiber digestibility (IVNDFD) and ammoniacal nitrogen concentrations did not differ between inoculum sources (slaughtered × cannulated) for all byproducts and levels tested. Total *in vitro* gas production in the ruminal inoculum of cannulated animals was greater ($P < 0.001$) than slaughtered cattle for different levels of licuri cake. However, the greatest total concentrations of *in vitro* gases for slaughtered animals were observed when evaluating different levels of crude glycerin ($P < 0.001$). No differences were observed for diets containing castor bean meal ($P > 0.05$). Thus, the ruminal inoculum obtained from the ruminal contents of slaughtered cattle can replace the use of fistulated animals and is a viable alternative to digestibility analysis. This approach is ethically more correct because it alleviates the suffering of animals by avoiding an invasive procedure.
Key words: Ammoniac nitrogen. Cannula. Digestibility. pH. Ruminal fermentation.

Resumo

O objetivo foi testar a hipótese de que o inóculo ruminal obtido a partir de bovino abatido pode substituir o inóculo do bovino canulado em ensaios que avaliam os alimentos para ruminantes através da produção

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de gás *in vitro* e digestibilidade. Foram utilizados cinco novilhos adultos Holstein × Zebu com cânula ruminal para coleta e comparação de líquido ruminal provenientes destes animais *in vivo* e de animais abatidos. A digestibilidade *in vitro* da matéria seca (DIVMS) e da fibra em detergente neutro *in vitro* (DIVFDN) e as concentrações de nitrogênio amoniacal não diferiram entre fontes de inóculo (abatidos × canulados) para todos os subprodutos e níveis testados. A produção total de gás *in vitro* no inóculo ruminal de animais canulados foi maior ($P < 0,001$) comparado ao inóculo de animal abatido para os diferentes níveis de torta de licuri. No entanto, as maiores concentrações totais de gás *in vitro* foram observadas para animais abatidos quando o co-produto utilizado foram níveis de glicerina bruta ($P < 0,001$). Não foram observadas diferenças nas dietas contendo farelo de mamona ($P > 0,05$). Assim, o inóculo ruminal obtido a partir do conteúdo ruminal de bovino abatido pode substituir o uso de animais fistulados, sendo uma alternativa viável para a realização da análise de digestibilidade. Esta abordagem é eticamente mais correta porque alivia o sofrimento dos animais evitando um procedimento invasivo.
Palavras-chave: Cânula. Digestibilidade. Fermentação ruminal. Nitrogênio amoniacal. pH.

Introduction

The use of animals in scientific research has been a constant practice permeating the history of science, from ancient times to the present (PAIXÃO; SCHRAMM, 1999; PACHECO et al., 2012). Along with the use of animals for various purposes, ethical concerns and questions about the merits of these experiments have grown, as has increasing demands on society for the care and welfare of animals. Although it is a relatively old practice (dating back to the 1920s), the fistulation process (surgically opening a canal between an organ and the outside) and cannulation (inserting a valve into the cavity) of one of the stomach compartments (rumen) of cattle still causes outrage among the public (THYFAULT et al., 1975; CHAUDHRY; MOHAMED, 2012; PACHECO et al., 2012). After all, why would anyone perform this procedure? Does the animal feel pain? Is its life at risk?

Some complications may occur after implantation of permanent cannulas, such as peritonitis and leakage of ruminal contents, which may even lead to death (HARRISON, 1995). Nevertheless, the use of rumen cannula is widespread in the scientific community because the number of animals required is reduced and such techniques reduce costs and provide a reliable method for assessing feed (BERCHIELLI et al., 2005). Additionally, the procedure allows analysis of the digestibility of feed and enables the collection of rumen material for *in vitro* digestibility trials and *in vivo* analysis of small samples through *in situ* digestibility (DAMIRAN et al., 2008). However, the leakage of large amounts of rumen contents can lead to dehydration and malnutrition (GROVUM, 1989). The *in vitro* fermentation technique proposed by Tilley and Terry (1963) has been widely used to estimate *in vivo* digestibility, with numerous studies around the world applying the technique to assess the nutritional value of ingredients used in diets for production animals. Nonetheless, this technique utilizes ruminal inoculum, which can lead to variation in results.

The constant handling of an animal affects its welfare, which is a violation of animal care laws (MUTIMURA et al., 2013) and is considered unacceptable by many people and animal protection agencies (CHAUDHRY; MOHAMED, 2012; PÔSSAS et al., 2015). Therefore, many experiments have used alternative sources of ruminal inoculum, such as sheep feces (VÁRADYOVÁ et al., 2005), buffalo feces (CUTRIGNELLI et al., 2005), and buffalo ruminal fluid and sheep ruminal fluid (CALABRÒ et al., 2005), and some have applied different methods of ruminal inoculum conservation (DENEK et al., 2010; HERVÁS et al., 2005). Although *in vitro* approaches depend on a source of ruminal inoculum (AMMAR et al., 2008), the use of fistulated animals must be phased out and fistulation replaced with other techniques that do

not cause suffering to animals (CHAUDHRY; MOHAMED, 2012). Accordingly, we hypothesized that the use of ruminal inoculum obtained from slaughtered cattle can replace fistulated animals for use in analyses of gas production, digestibility and parameters of ruminal fermentation for *in vitro* trials. Thus, the aim of this trial was to compare ruminal inoculum obtained from the rumen of slaughtered and cannulated cattle for use in determining *in vitro* digestibility and ruminal fermentation parameters of feeds.

Materials and Methods

Animals and experimental design

This study was conducted at the Federal University of Bahia, in São Gonçalo dos Campos, Bahia, located at 12° 25′ S and 38° 58′ W and carried out in strict accordance with the recommendations in the Guide for the National Council for the Control of Animal Experimentation (CONCEA) and was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Bahia, Bahia State, Brazil (Protocol Number: 17-2014).

Five cattle (n = 5) with ruminal cannula and an average body weight of approximately 320 ± 9.4 kg were used. At the beginning of the adaption period, the cattle were treated for internal and external parasites (Ranger LA, Ivermectin®, dose of 200 mcg kg^{-1}, 1 mL per 50 kg of body weight (BW), 3.5%, Salvador, Brazil). The cattle were housed in individual stalls (3 × 6 m^2) with feeders and drinkers.

The experiment was a completely randomized design comparing two sources of ruminal inoculum: ruminal inoculum obtained from cannulated cattle and ruminal inoculum obtained from cattle healthy slaughtered for marketing. To compare the two sources of ruminal inoculum, sixteen different diets representative of conventional ruminant diets (using different proportion of concentrate: forage) containing byproducts from the biodiesel production chain (crude glycerin, licuri cake and castor bean meal) in the concentrate and Tifton-85 (*Cynodon* sp.) grass hay as forage (Table 1).

Table 1. Chemical composition of the ingredients used in the formulation of the diets.

Chemical composition (g kg^{-1}, DM)	Tifton-85 hay	Ground corn	Soybean meal	Licuri cake	Castor bean meal	Crude glycerin
Dry matter (g kg^{-1}, as fed)	880	891	893	930	899	764
Ash	73.0	14.3	75.4	51.2	82.0	139
Crude protein	87.3	80.3	490	302	381	-
Ether extract	13.0	42.0	16.0	73.0	2.00	283
$_{ap}$Neutral detergent fiber[b]	690	114	128	333	528	-
Acid detergent fiber	331	26	68.3	144	345	-
Acid detergent lignin	40.2	5.32	11.1	57.2	229	-
Cellulose	291	21.2	57.4	87.2	116	-
Hemicellulose	358	88.2	61.3	190	183	-
Non-fiber carbohydrates	138	751	291	242	7.06	577
Total digestive nutrients[b]	614	898	797	772	473	716

[a]Corrected for ash and protein.
[b]Estimated per second NRC (2001).

Diets and chemical analysis

The diets contained a mean of 102 g kg^{-1} protein and 337 Mcal of digestible energy kg^{-1} DM, with a proportion of 400 g kg^{-1} forage (Tifton-85 hay - *Cynodon* sp.) and 600 g kg^{-1} concentrate (ground corn, soybean meal, urea, and mineral mix). Feeding was performed twice daily, at 09h00 and 17h00. The crude glycerin used contained 266 g kg^{-1} of water, 36 g kg^{-1} of ash, 434 g kg^{-1} of glycerol and 26 g kg^{-1} of alcohol. The castor bean meal was detoxified by autoclaving with 10 g CaO 1000 g^{-1} castor bean meal for 30 minutes. The composition of the diets and the inclusion levels of the byproducts in the diets are presented in Table 2.

Table 2. Proportional inclusion levels and chemical composition of the experimental diets (g kg^{-1} DM) with byproducts.

Byproducts	Crude glycerin (g kg^{-1})				Licuri cake(g kg^{-1})				Castor bean (g kg^{-1})			
Inclusion levels	0	70	140	210	0	70	140	210	0	70	140	210
Ingredients proportion (g kg^{-1} DM)												
Ground corn	255	167	83	0	498	448	408	368	272	300	280	185
Soybean meal	230	248	262	275	80	60	30	0	213	178	65	90
Byproducts	0	70	140	210	0	70	140	210	0	7	140	210
Urea	0	0	0	0	12	12	12	12	0	0	0	0
Mineral mix[a]	15	15	15	15	10	10	10	10	15	15	15	15
Tifton-85 hay	500	500	500	500	400	400	400	400	500	500	500	500
Chemical composition (g kg^{-1} DM)												
Dry matter, as fed	901	874	849	810	899	900	898	898	900	898	900	904
Ash	64	64	66	58	45	45	43	48	60	61	62	66
Crude protein	162	184	183	193	163	160	159	150	174	174	175	163
Ether extract	10.0	22.0	31.0	42.0	8.00	16	16	22	9.00	7.00	7.00	5.00
$_{ap}$NDF[b]	471	446	465	462	383	422	426	432	477	470	527	532
ADF[c]	196	213	220	225	153	175	179	183	200	232	260	291
ADL[d]	27	25	27	27	16	24	25	28	22	57	78	100
Cellulose	169	188	193	198	137	151	155	156	177	175	183	191
Hemicellulose	275	233	245	238	230	247	247	248	277	239	266	242
NFC[e]	293	284	255	245	401	358	356	349	281	288	230	234
TDN[f]	696	734	751	717	728	735	733	728	701	640	601	568

[a]Recommended levels (per kg in active elements): calcium (max) 220.00 g; (min) 209,00 g; phosphorus 163.00 g; sulfur 12.00 g; magnesium 12.50 g; copper 3,500,00 mg; cobalt 310.00 mg; iron 1,960.00 mg; iodine 280.00 mg; manganese 3,640.00 mg; selenium, 32.00 mg; zinc 9,000.00 mg; and fluorine (max) 1,630.00 mg.
[b]Neutral detergent fiber corrected for ash and protein.
[c]ADF = acid detergent fiber.
[d]ADL= acid detergent lignin.
[e]NFC: Non-fiber carbohydrates.
[f]Total digestible nutrients estimated per second, NRC (2001).

The samples of feeds (n= 3) used to compose the experimental diets were dried in a forced-air oven at 55°C for 72 h. All feeds were then processed in a Wiley knife mill using 1-mm sieves and analyzed according to the Association of Official Methods of Analysis (AOAC, 1990) for dry matter (DM, method 967.03), ash (method 942.05), crude protein (CP, method 981.10), and ether extract (EE, method 920.29). The content of neutral detergent fiber (NDF) was determined according to Van Soest et

al. (1991) and included the residual ash; heat-stable alpha-amylase was not used. The acid detergent fiber (ADF) content was determined according to Robertson and Van Soest (1981). The acid detergent lignin content was determined according to AOAC (2002) method 973.18 in which the ADF residue was treated with 72% sulfuric acid. We also analyzed neutral detergent insoluble nitrogen (NDIN) and the acid detergent insoluble nitrogen (ADIN) according to Licitra et al. (1996). Increases in NDIP and ADIP contents were calculated by multiplying the NDIN and ADIN concentrations, respectively, by 6.25. Non-fiber carbohydrates (NFC) content was calculated according to (MERTENS, 1997) by the equation NFC = (100 - CP + EE + Ash + $_{ap}$NDF) in g kg^{-1}. The total digestible nutrient (TDN) contents were calculated according to estimation formulas of digestibility for each analytical fraction (NRC, 2001): DNFC = 0.98 × (NFC); DCP = CP × [1- (0.4 × ADIP/CP)]; DEE = EE - 1; DNDF = 0.75 × (NDF - Lignin) × [1 - (Lignin/NDF) × 0.667] - 7, where DNFC is the digestible non-fibrous carbohydrates, DCP indicates digestible crude protein, DEE is the digestible ether extract, DNDF is the digestible neutral detergent fiber, and NDF is the neutral detergent fiber.

Buffer, slaughter and rumen fluid preparation

A buffer solution at pH 6.8, constant temperature of 39°C and saturated with CO_2 was prepared with a mixture of Solution A and Solution B. Solution A (g L^{-1}) was composed of 10 g KH_2P0_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.1 g $CaCl_2 \cdot 2H_2O$ and 0.5 g urea. Solution B (g 100 mL^{-1}) was composed of 15.0 g Na_2CO and 1.0 g $Na_2S \cdot 9H_2O$. Solutions A and B were mixed at a ratio of 1:0.02 (A:B), i.e., 20 mL Solution B:1000 mL Solution A.

For the cannulated cattle (n= 5), the ruminal content was collected two hours before the morning feeding (07h00). Four liters of ruminal contents per animal were collected in preheated thermal bottles with water at 39°C; the ruminal contents were constantly purged with CO_2. The ruminal contents collected were composed of a solid fraction (collected from the 6 points of the rumen) and a liquid fraction (collected with the help of a vacuum pump, preheated and purged constantly with CO_2) of the same proportion; approximately two liters of each fraction were obtained. The ruminal content of the five animals was mixed, producing the ruminal inoculum for *in vitro* incubations.

The alternative inoculum source was collected from five (n= 5) different slaughtered cattle in a slaughterhouse; these cattle had been fasted from water and solids for 12 h. The origin of this group was unknown; therefore, the feeding and handling was unknown but assumed to include tropical grasses and mineral supplementation.

The cattle were slaughtered by captive bolt without electrical stimulation, exsanguinated; the hides were removed, and the animals were eviscerated. The ruminal contents of this group were collected from the gastrointestinal tract in the washing room between 7 to 15 minutes after stunning. Four liters of ruminal contents were collected into a 1.5-L preheated jug with water at 39°C, and the contents were purged constantly with CO_2.

After the ruminal contents were placed in thermal bottles (n= 3), the collected material was processed as ruminal inoculum. The collected material of all animals was homogenized in a 1:1 ratio (solid fraction:liquid fraction) for 10 seconds using a blender, pre-heated, and constantly purged with CO_2. The homogenate was then filtered through four layers of cotton fabric (gauze) and used for incubations. The ruminal content of the slaughtered cattle was mixed, producing ruminal inoculum for *in vitro* incubations.

In vitro incubations

The *in vitro* dry matter digestibility (IVDMD) of the diets was determined using an artificial

rumen (Ankom® Ruminal Fermenter, «Daisy-II Fermenter," Ankom Technológic Corp., USA) according to Holden (1999). A total of 0.5 g of each diet (n= 3) was placed into TNT-100 g m^{-1} bags, which were then cut and sealed (5.0 cm × 5.0 cm) according to Casali et al. (2008). Two bags without samples (white) were also used. The bags with samples were evenly distributed into jars (previously heated and purged with CO_2), with 26 bags jar^{-1} (24 with a sample and 2 white) for a total of 104 bags. Then, 1600 mL of buffer solution and 400 mL of ruminal inoculum were added under CO_2 to maintain anaerobic conditions. After this procedure, the jars remained in the DaisyII Fermenter artificial rumen at 39°C for 48 h. For white, they were used bags with samples using ingredients known as standards (soybean and corn) with IVDMD already known (control) to confirm proper digestion of the samples tested. The digestion of the standards was performed every three sessions of incubation of the experiment diets, to verify and correct possible variations or problems in the incubation. However, no variation or error were observed.

After 48 h, 40 mL of HCl (6N) and 8 g pepsin (Sigma 1:10000) were added. In an attempt to maintain the pH of the solution between 2.0 and 3.5, the pepsin was dissolved in 34 mL distilled H_2O at 35°C for 5 minutes (HOLDEN, 1999). The incubation was continued for an additional 24 h at 39°C, after which the bags were washed with tap water until the water ran clean. The bags were then pre-dried in forced-air ovens at 55°C for 12 h, dried in an oven at 105°C for 16 h and weighed. After drying, the bags with dry sample were analyzed to determine NDF content (VAN SOEST et al., 1991).

Finalized the *in vitro* dry matter digestibility assay, the DaisyII Fermenter incubator (39°C) was used to assess the parameters of ruminal fermentation. For each jar (caps were fitted with three track taps), 10 g of sample was weighed, and 1600 mL of buffer solution and 400 mL of ruminal inoculum were added. At 0, 2, 4, 6, and 8 h after the start of incubation, a sample of 20 mL of buffered ruminal fluid was collected; 10 mL of buffered ruminal fluid was stored in plastic pots, and the Kjeldahl method was used to measure ammoniac nitrogen (SOUZA et al., 2013).

Calculations and Statistical Analysis

In vitro dry matter digestibility (IVDMD) was calculated using the residue remaining after incubation with the following formula (ANKOM TECHNOLOGY, 2005):

$$IVDMD = 100 - W_3 \times [(W_1 \times W_4)/ W_2] \times 100$$

Where W_1 is the tare weight of the bag, W_2 is the weight of the dry sample, W_3 is the weight of the final bag, and W4 is the correction with the white bag. *In vitro* neutral detergent fiber digestibility (IVNDFD) was calculated with the residue after assessing NDF residue using the formula:

$$IVNDFD = 100 - W_7 \times [(W_5 \times W_8)/ W_6] \times 100$$

Where W_5 is the tare weight of the bag, W_6 is the weight of the NDF of the sample, W_7 is the final weight of the bag after the NDF, and W_8 is the correction with the white bag. Analysis of variance was performed on data, and when the difference was significant, Student's t-test at 5% probability was applied using SAS v. 9.1.3 (2008) statistical software (SAS® Inst. Inc., Cary, NC).

Results

Dry matter (IVDMD) and neutral detergent fiber digestibility (IVNDFD)

No difference in IVDMD and IVNDFD with respect to the source of ruminal inoculum (P >0.05) was observed for all byproducts and levels. The mean concentration of NH_3-N of the different byproducts and all levels incubated presented no differences (P >0.05) between ruminal inoculum from cannulated animals and slaughtered cattle (Table 3).

Table 3. Comparison between inoculum obtained from slaughtered cattle and cannulated cattle for *in vitro* digestibility variables.

Level	Crude glycerin		SEM[a]	P-value[b]	Licuri Cake		SEM[a]	P-value[b]	Castor bean meal		SEM[a]	P-value[b]
In vitro dry matter digestibility (IVDMD)												
0%	0.86	0.85	0.25	1.00	0.83	0.83	0.23	1.00	0.82	0.81	0.19	0.995
7%	0.85	0.84	0.17	1.00	0.83	0.82	0.33	0.96	0.79	0.78	0.15	1.000
14%	0.82	0.83	0.16	0.99	0.82	0.83	0.27	0.99	0.76	0.74	0.30	0.656
21%	0.81	0.83	0.12	1.00	0.83	0.83	0.30	0.19	0.71	0.70	0.24	0.983
In vitro neutral detergent fiber digestibility (IVNDFD)												
0%	0.65	0.63	0.55	0.996	0.67	0.68	0.50	1.00	0.65	0.63	0.36	1.000
7%	0.68	0.65	0.73	0.83	0.65	0.65	0.34	1.00	0.59	0.57	0.30	1.000
14%	0.60	0.63	0.58	0.98	0.65	0.67	0.31	0.99	0.58	0.55	0.52	0.925
21%	0.58	0.63	0.66	0.05	0.67	0.67	0.22	1.00	0.51	0.49	0.41	0.994
In vitro concentrations of ammoniacal nitrogen (NH_3-N)												
0%	164	122	14.6	0.84	130	111	13.6	0.99	117	120	11.4	1.000
7%	159	119	14.7	0.87	147	114	14.9	0.96	130	125	12.7	1.000
14%	147	132	13.3	1.00	160	105	16.4	0.62	152	141	14.9	1.000
21%	153	144	14.2	1.00	161	116	16.3	0.83	169	131	15.2	0.887

[a]Standard error of the mean.
[b]Means followed by lowercase letters differ significantly according to Student's t-test at 1% and 5% probabilities.

Gas production

Total *in vitro* gas production in ruminal inoculum was greater from cannulated animals (P <0.001) regarding to slaughtered cattle for licuri cake 0; 7 and 14% inclusion level diets, except for the inclusion level of 21% when slaughtered animals inoculum showed greater concentrations of gases *in vitro* (Table 4). However, the greatest total concentrations of gases *in vitro* for slaughtered than cannulated animals were observed when evaluating different levels of crude glycerin (P <0.001). No differences were observed for diets with inclusion levels of castor bean meal (P >0.05).

Table 4. Comparison between inoculum obtained from slaughtered cattle and cannulated cattle for total *in vitro* gas production (mL).

Byproduct	Inclusion level	Cannulated	Slaughtered	SEM[a]	*P*-value[b]
Crude glycerin	0%	12.8b	14.8a	0.27	< 0.001
	7%	10.2b	12.6a	0.26	< 0.001
	14%	9.20b	12.4a	0.36	< 0.001
	21%	8.60b	11.4a	0.26	< 0.001
Licuri Cake	0%	15.0a	12.7b	0.39	< 0.001
	7%	14.4a	13.0b	0.41	< 0.001
	14%	14.5a	11.9b	0.35	< 0.001
	21%	10.8b	11.7a	0.38	0.033

continue

continuation

Castor bean meal	0%	10.9	9.20	0.36	0.052
	7%	10.3	10.2	0.41	0.150
	14%	9.00	9.00	0.44	0.084
	21%	8.80a	7.10b	0.13	0.066

[a]Standard error of the mean.
[b]Means followed by lowercase letters differ significantly according to Student's t-test at 5% probabilities.

Discussion

The results for IVDMD, IVNDFD and N-NH_3 demonstrate that the use of ruminal inoculum from slaughtered animals is an interesting alternative to the use of cannulated animals because among all feed sources used, only one did not present similar results when comparing the two inoculum sources. In planning a research project, all ethical issues surrounding animal rights as well as the specific physiological characteristics of each species should be considered. The aim is to guarantee the welfare of animals during the experiment as a way to eliminate any discomfort that may be caused (CRISSIUMA; ALMEIDA, 2006).

In general, differences with respect to pH may be related to anaerobiosis, which is essential when isolating and culturing rumen microorganisms (HUNGATE, 1966). In addition, a decrease in both cellulolytic and amylolytic activities (LEEDLE; HESPELL, 1983) and a decline in ciliate protozoan abundance occur if an anaerobic environment is not sustained. Therefore, the inoculum should not be exposed to air, even short-term exposure; equally important, any increase in headspace pressure should not be so great as to force the CO_2 into the solution.

Decreased *in vitro* gas production was found for the use of inoculum source from slaughtered cattle, likely due to the presence of air during storage and the temperature making it difficult for the rumen microorganisms to degrade NDF.

Robinson et al. (1999) used the Ankom® end-point system to verify the effect of within-day delays of 0.5, 2.5, 4.5 and 6.5h between collection of rumen fluid from a cow and initiation of in vitro fermentation, as well as storage of rumen fluid for 48h at either −24°C or 6, 22 and 39°C, on IVNDFD at 48h was determined. The results of the authors demonstrated that this bulk in vitro procedure resulted in higher 48h digestion of NDF than those determined with a similar in sacco procedure, thereby suggesting that laboratories located some distance from the donor animal can utilize in vitro procedures to accurately estimate 48h digestion of NDF.

Concerns of animal welfare and the ethical issues of animal use in scientific experiments have prompted serious discussions between researchers and members of animal-protective societies. These debates intensified mainly at the beginning of nineteenth century when the number of entities and groups concerned with animal welfare increased substantially (RAYMUNDO; GOLDIM, 2002). Pressure from these groups on scientists, teaching and research institutions, and the rulers of a number of countries had created ethical criteria for publishing the results of animal research through editorial policies, thus revealing growing concern for animal rights in the scientific community (PAIXÃO; SCHRAMM, 1999), as well as the creation or update of laws regulating the use of animals in scientific experiments. Since Tilley and Terry (1963) developed the *in vitro* digestibility technique, millions of fistulated animals have been used for inoculum in research related to ruminant animal nutrition. Therefore, the possibility of using animal sources of inoculum that do not cause injury

to animals should be promoted and increasingly tested.

According to Rymer et al. (2005), the driving force has been either to reduce the use of surgically modified animals and/or to provide a more standardized inoculum than is possible with variable rumen fluid. However, as observed in this study, with the exception of crude glycerin, the use of inoculum from slaughtered animals reduced the *in vitro* production of gases, which may have occurred due to the type of feed the animals received, and consequently modified the pattern of liquid rumen microorganisms. When evaluating feces as inoculum in potential gas production, Mauricio et al. (2001) observed difference from ruminal liquid, with the former showing poorer fermentation capacity compared to ruminal liquid and gas volumes always being lower and often with substantially less volume. The authors concluded that fecal matter has potential as an alternative inoculum to rumen liquor for the *in vitro* gas production technique of Theodorou et al. (1994). However, the results obtained show a consistently longer lag phase when using the feces-based inoculum, in agreement with previous studies applying technique of the Menke et al. (1979).

Rumen contents harvested post-slaughter offer the benefit of no surgical preparation, though there is no possibility of examining animal effects (MOULD et al., 2005). In comparison to sampling a ruminally cannulated animal, which will have had access to feed, the practice of offering a minimal amount of feed and water prior to slaughter will negatively affect the microbial quality of the resulting rumen fluid inoculum (BORBA et al., 2001). Although the use of slaughterhouse waste allows sufficient material to be collected for a series of studies, thereby limiting variation among incubations conducted over time, this approach requires that the material be stored under conditions that will minimize the loss of microbial activity (MOULD et al., 2005).

The use of fistulated animals should be avoided, as these animals are often sick. Regardless, we believe that this discussion about the use of ruminal fistulas must be continued because despite the concern for establishing rules and parameters for evaluating the welfare of farm animals, little has been discussed in academic circles about the animals welfare used in scientific experiments and teaching. Although animals undergoing this procedure receive prophylactic antibiotics postoperatively in order to avoid infections, there may be surgical complications, discomfort, stress or clinical signs of discomfort or pain, proving the procedure if not used safely can be abusive about animal welfare preservation.

Even so, we are aware that the technique of fistulated animals is essential in studies of physiology and metabolism of rumen. From this, it is possible to obtain detailed knowledge of the digestive process and to carry out dietary assessments, feed passage rate, determinations of chemical parameters involved in digestion (pH, ammoniacal nitrogen, volatile fatty acids, among other utilities) and development of strategies for enteric methane mitigation. All these assessments provide key information for the sustainability of livestock. However, replacement of the rumen cannula with alternative ruminal liquid from slaughtered animals may increase animal welfare without prejudice to scientific research.

Conclusions

Ruminal inoculum obtained from the ruminal contents of slaughtered cattle can replace the use of ruminal fistula and is a viable alternative for digestibility analysis because the material was found to be efficient for almost all feed sources at all levels studied in this work; results were comparable to those obtained using inoculum from fistulated animals. Nonetheless, variations on *in vitro* gas analysis were observed using the ruminal contents

from slaughtered animals and fistulated animals. In addition, details of the slaughtered animal with respect to diet and nutrition are needed.

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