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Effect of forage type and forage to concentrate ratio on ruminal fermentation in single-flow continuous-culture and Rusitec fermenters

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Abstract. Eight single-flow continuous-culture (SFCC) and 8 Rusitec fermenters were used to compare the ruminal fermentation of 4 diets with forage:concentrate ratios (F:C) of 70:30 or 30:70 and alfalfa or grass hay as forage. Four rumen fistulated goats and 4 rumen fistulated sheep were used as donors for SFCC and Rusitec fermenters, respectively. There were no differences ($P>0.05$) between fermentation systems (FS) in the daily production of ammonia-N and molar proportions of propionate and isobutyrate, but the other fermentation variables were affected ($P<0.05$) by the FS. Dilution rate, solids retention time, dry matter digestibility, pH and molar proportions of butyrate, isovalerate and valerate were lower ($P\leq0.005$) in SFCC than in Rusitec fermenters, whereas digestibility of neutral-detergent fibre and acid-detergent fibre, daily production of volatile fatty acids, molar proportion of acetate and acetate:propionate ratio were lower ($P<0.001$) in Rusitec compared with SFCC fermenters. Interactions ($P<0.05$) FS x F:C and FS x type of forage were detected for most of the fermentation variables, indicating differences between SFCC and Rusitec fermenters in detecting the effects of F:C ratio and type of forage on ruminal fermentation parameters.

Keywords. Single-flow continuous-culture fermenters – Rusitec – Forage:concentrate ratio – Ruminal fermentation.

Effet du type de forage et du rapport forage : concentré sur la fermentation ruminale dans un fermenteur à flux simple continu et dans un fermenteur Rusitec

Résumé. *Huit fermenteurs à flux simple continu (SFCC) et 8 Rusitec ont été utilisés pour comparer la fermentation ruminale de 4 régimes avec des rapports fourrage: concentré (F: C) de 70 : 30 ou 30 : 70,et deux types de fourrages, le foin de luzerne et le foin de graminées. Quatre chèvres et quatre moutons munis de canules ruminales ont été utilisés comme donneurs d´inoculum pour le SFCC et le Rusitec, respectivement. Il n'y avait pas de différences (P>0,05) entre les systèmes de fermentation (FS) concernant la production quotidienne d´azote ammoniacal et les proportions molaires des acides propionique et isobutyrique, mais les autres variables de la fermentation ont été affectées (P<0,05) par le FS. Le taux de dilution, la durée de rétention des solides, la digestibilité de la matière sèche, le pH et la proportion molaire des acides butyrique, valérique et isovalerique ont été plus faibles (P≤0,005) dans les SFCC par rapport au Rusitec, tandis que la digestibilité des parois totales (NDF) et de la lignocellulose (ADF), la production quotidienne d'acides gras volatils, la proportion molaire de l'acétate et le rapport acétate: propionate étaient plus faibles (P<0,001) dans le Rusitec que dans les SFCC. Des interactions (P<0,05) FS x F :C et FS x type de fourrage ont été détectées pour la plupart des variables de fermentation, indiquant des différences entre les SFCC et le Rusitec pour détecter les effets du rapport F : C et du type de fourrage sur les paramètres de fermentation ruminal.*

Mots-clés. *Fermenteur á flux simple continu – Rusitec – Rapport fourrage:concentré – Fermentation ruminale.*

I – Introduction

Many types of artificial rumen apparatus have been described in the literature, but two of the most widely used are the continuous-flow fermenters (Hoover *et al.,* 1976; Miettinen and Setälä, 1989) and the semi-continuous flow Rusitec (Czerkawski and Breckenridge, 1977). The two systems have several functional differences, such as the dilution rate, the solids retention time, the amount of feed delivered daily, the frequency of feeding and others, that can affect the fermentation pattern. Although both types of fermenters are used in many laboratories, there is no standardization among systems**,** and direct comparisons between them are limited (Carro *et al.*, 2009). The aim of the present work was to compare ruminal fermentation of diets differing in forage:concentrate (F:C) ratio and forage type (FOR) in single-flow continuous-culture (SFCC) and in Rusitec fermenters.

II – Materials and methods

1. Apparatus, diet and experimental procedure

Four total mixed diets were studied according to a 2 x 2 factorial arrangement of treatments. The diets had F:C ratios [dry matter (DM) basis] of 70:30 (H) or 30:70 (L) with either alfalfa hay (A) or grass hay (G) as forage, and were designated as HA, LA, HG and LG. The concentrate was based on barley, gluten feed, wheat middlings, soybean meal, palmkern meal, wheat, corn and mineral-vitamin premix in the proportions of 215, 204, 200, 135, 115, 50, 50 and 31 g/kg, respectively (fresh matter basis). Chemical composition of diets is given in Table 1.

Table 1. Dry matter content (g/kg; DM) and chemical composition (g/kg DM) of the experimental diets

Diets†	Dry matter	Organic matter	Crude protein	Neutral-detergent fibre	Acid-detergent fibre
HA	927	913	168	426	270
LA	925	913	177	374	189
HG	925	927	121	499	239
LG	924	919	160	401	176

† HA: 70:30 alfalfa hay:concentrate; LA: 30:70 alfalfa hay:concentrate; HG: 70:30 grass hay:concentrate; LG: 30:70 grass hay:concentrate.

Eight SFCC (Mietinen and Setälä, 1989) and 8 Rusitec (Czerkawski and Breckenridge, 1977) fermenters were used in two identical incubation runs. Both systems were inoculated with rumen liquor from 4 rumen-cannulated goats and 4 rumen-cannulated sheep, respectively. One animal of each species received each diet for 15 days before starting the *in vitro* trials. Each incubation run consisted of 8 days for diet adaptation and 3 days for sampling. On the first day of each incubation run, ruminal contents from each goat and sheep were collected, strained through two layers of cheesecloth, and transferred to the corresponding fermenters within 30 minutes after collection. The flow through the fermenters was maintained by continuous infusion of artificial saliva (pH=8.4) at a rate of 960 ml/d (4.21 % h^{-1}) in SFCC and of 740 ml/d (5.14% h^{-1}) in Rusitec. Each fermenter received daily 30 g of DM of the corresponding diet. The SFCC fermenters were fed twice daily (8:00 and 14:00 h) and the Rusitec fermenters were fed once. The diet was supplied directly to the SFCC, while in Rusitec fermenters it was supplied into 2 nylon bags (100 µm pore size), one containing forage and the other containing concentrate; the incubation time was 48 h for forage and 24 h for concentrate. The flux of CO_2 was continuous in the SFCC fermenters, while Rusitec fermenters were flushed with 2 l of CO_2 both before and after feeding. The over-

flow from SFCC fermenters was collected into flasks maintained at 4ºC, and liquid effluent from Rusitec fermenters was collected in flasks containing H_2SO_4 (20 ml per 100 ml). The weight and volume of effluents were recorded daily in all fermenters.

On each sampling day, the pH of fermenter´s content was determined immediately before the morning feeding and the following samples were taken from the effluents: 1 ml that was added to 1 ml of deproteinizing solution (100 g of metaphosphoric acid and 0.6 g of crotonic acid per litre) for volatile fatty acids (VFA) analysis, and 5 ml which were stored at -20ºC for NH_3-N analysis. In the SFCC fermenters, DM, neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) degradability (DMD, NDFD and ADFD, respectively) was calculated as the difference between the intake and the amounts of each fraction in the effluents, and the DM output was corrected for the DM in the artificial saliva. In Rusitec fermenters, the nylon bags collected daily from each fermenter were washed twice with 40 ml of fermenters' fluid, and then washed in the cold rinse cycle (20 min) of a washing machine. The DMD was calculated from the weight lost after oven drying at 60ºC for 48 h, and residues were analyzed for NDF and ADF to determine NDFD and ADFD.

2. Analytical procedures and statistical analyses

Procedures for determination of DM, ash, NDF, N, VFA and ammonia-N have been reported by Cantalapiedra-Híjar *et al.* (2009). Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The effects of fermentation system (FS), F:C ratio, FOR, period, and the interactions FS x F:C, FS x FOR, and F:C X FOR were considered fixed, and the effect of fermenter within FS was considered random. When a significant effect of treatment ($P<0.05$) was detected, differences among means were tested using the Tukey's multiple comparison test.

III – Results and discussion

In this study we decided to run both *in vitro* systems under the conditions that are usually utilized in each of the two participant laboratories, as there is currently no standardization among different types of fermenters; there were, therefore, various functional differences between SFCC and Rusitec fermenters. As shown in Table 2, dilution rate and solids retention time were lower ($P<0.001$) in SFCC than in Rusitec fermenters. In SFCC fermenters the solids retention time is determined by the outflow rate of the fermenters content, whereas in Rusitec a fixed incubation time of 48 h is generally used. In the present study we decided to incubate forage for 48 h and concentrate for 24 h, as this approach has been shown to improve the simulation of *in vivo* ruminal fermentation in Rusitec fermenters (Martínez *et al*., 2009).

The DMD was lower ($P<0.001$) in SFCC than in Rusitec fermenters, but NDFD and ADFD were greater ($P<0.001$) in SFCC (Table 2) in comparison with Rusitec. The lower solid retention times in SFCC compared with those in Rusitec fermenters may partly explain the lower DMD values obtained in SFCC, as the extent of ruminal digestion depends on feed digestion rate and retention time in the rumen. The greater fibre digestibility found in SFCC compared with Rusitec is in agreement with previous works (Carro *et al.*, 2009), and it would indicate that microbial populations in SFCC had greater fibrolytic activity than those in Rusitec. Both FS x F:C and FS x FOR interactions ($P<0.001$) were observed for NDFD and ADFD, indicating that the two types of fermenters detected different effects of F:C and FOR on ruminal fermentation. In the SFCC fermenters, the L diets had greater ($P<0.05$) NDFD and ADFD compared with the H diets. In Rusitec, there were no differences ($P>0.05$) between H and L diets when alfalfa was the forage, but the LG diet had lower ($P<0.05$) NDFD and ADFD compared with HG. These results might indicate that different microbial populations were established in the two types of fermenters.

Table 2. Dilution rate, solids retention time (SRT) and apparent digestibility of dry matter (DMD), neutral-detergent fibre (NDFD) and acid-detergent fibre (ADFD) in single-flow continuous culture (SFCC) and Rusitec fermenters fed diets with forage:concentrate (FC) ratios of 70:30 (H) or 30:70 (L) and alfalfa hay (A) or grass hay (G) as forage (FOR)

Item	FS†	Diet				SEM††	Statistical effect (*P* =)				
		HA	LA	HG	LG		FS	F:C	FOR	FS x F:C	FS x FOR
Dilution rate, % h^{-1}	SFCC	4.21	4.24	4.13	4.39	0.096	<0.001	0.13	0.95	0.14	0.25
	Rusitec	5.13	5.18	5.09	5.15						
SRT, h	SFCC	23.8	23.6	24.3	22.8	0.24	<0.001	<0.001	0.67	<0.001	0.67
	Rusitec	40.1^{b}	31.2^{a}	40.8^{b}	31.2^{a}						
DMD	SFCC	42.8^{b}	48.1^{c}	38.6^{a}	48.1^{c}	0.80	<0.001	<0.001	<0.001	<0.001	0.31
	Rusitec	71.3^{b}	70.7^{b}	67.4^{a}	68.2^{a}						
NDFD	SFCC	51.3^{a}	57.9^{b}	49.6^{a}	57.2^{b}	0.67	<0.001	0.005	<0.001	<0.001	<0.001
	Rusitec	36.0^{a}	34.7^{a}	44.8^{c}	38.0^{b}						
ADFD	SFCC	48.4^{a}	55.2^{c}	51.2^{b}	55.7^{c}	0.80	<0.001	0.16	<0.001	<0.001	<0.001
	Rusitec	25.3^{a}	26.3^{a}	39.3^{c}	30.4^{b}						

$^{a, b, c}$ Within a row, means with unlike superscripts differ (*P*<0.05).

† FS: fermentation system.

†† Standard error of the mean.

One factor that may have affected the results was the different origin of the ruminal inocula, as ruminal fluid from goats was used to inoculate the SFCC fermenters and fluid from sheep was used for Rusitec. However, most studies reported in the literature have concluded that differences between sheep and goats in rumen degradability and total tract digestibility are negligible when they are fed medium or good quality diets (Molina-Alcaide *et al.*, 2000, 2003). In addition, various studies have reported a lack of differences between sheep and goat ruminal fluid used as source of inoculum to determine the *in vitro* digestibility and fermentation kinetics of different feeds (Martín-García *et al.*, 2003; Ammar *et al.*, 2008) or as inoculum for ruminal fermenters (Moumen *et al.*, 2007; Molina-Alcaide *et al.*, 2009). Because the animals from both species received the same diets, it seems improbable that the observed differences between the two types of fermenters can be attributed to differences in the ruminal inocula. Moreover, it has been shown that changes in composition of microbial populations occur over the incubation period in fermenters (Slyter and Putnam, 1967).

The pH values before feeding were greater (*P*<0.05) in Rusitec than in SFCC fermenters for HA and LG diets, but no differences (*P*>0.05) were detected for the LA and HG diets (Table 3). Both types of fermenters detected the same differences between diets in HN_3-N production (Table 3), with the HG diet showing the lowest values and the HA diet the greatest ones. The daily VFA production was greater (*P*<0.001) in SFFC compared with Rusitec fermenters. As fermenters were supplied daily with the same amount of diet and dilution rate and solids retention time were lower in SFCC fermenters than in Rusitec, the greater VFA production observed in SFCC might have been due to greater microbial concentration and/or activity as it has been reported previously (Carro *et al.*, 2009).

There were pronounced differences in the molar proportions of individual VFA between the two *in vitro* systems. The molar proportion of acetate and acetate:propionate ratio were lower (*P*<0.001) and the proportions of butyrate, isovalerate and valerate were greater (*P*<0.001) in Rusitec compared with SFCC fermenters, but no differences were observed in the proportions of propionate (*P*=0.60) and isobutyrate (*P*=0.30). Changes in molar proportions of individual VFA may reflect a shift in microbial species or alteration of microbial metabolism with changing culture conditions, which might have been due to differences in pH, dilution rate or solids retention time (Meng *et al.*,

1999). Carro *et al.* (2009) observed similar differences between the two types of fermenters when they were fed H and L diets. The observed FS x F:C and FS x FOR interactions (P=0.03 to <0.001) for most of the individual VFA indicate that the two types of fermenters detected different effects of F:C and type of forage on VFA pattern.

Table 3. Values of pH before feeding, daily production of NH_3-N and volatile fatty acids (VFA), molar proportions of individual VFA and acetate:propionate ratio (Ac/Pr) in single-flow continuous culture (SFCC) and Rusitec fermenters fed diets with forage:concentrate (FC) ratios of 70:30 (H) or 30:70 (L) and alfalfa hay (A) or grass hay (G) as forage (FOR)

Item	FS†	Diet				SEM††	Statistical effect (P =)				
		HA	LA	HG	LG		FS	F:C	FOR	FS x F:C	FS x FOR
pH	SFCC	6.48^{b}	6.47^{b}	6.56^{b}	6.19^{a}	0.039	0.005	<0.001	< 0.001	0.05	0.12
	Rusitec	6.85^{d}	6.43^{b}	6.56^{c}	6.35^{a}						
NH_3-N, mg/d	SFCC	284^{d}	196^{c}	19.1^{a}	67.9^{b}	8.56	0.06	<0.001	<0.001	0.12	0.006
	Rusitec	290^{d}	179^{c}	61.1^{a}	93.2^{b}						
Total VFA, mmol/d	SFCC	113^{b}	115^{b}	101^{a}	118^{b}	1.82	<0.001	0.12	<0.008	<0.001	0.70
	Rusitec	104^{b}	99^{a}	100^{ab}	96^{a}						
Molar proportions (mol/100 mol) of:											
Acetate	SFCC	68.5^{c}	63.3^{b}	64.5^{b}	60.7^{a}	0.71	<0.001	<0.001	0.02	0.41	0.001
	Rusitec	52.5^{b}	47.8^{a}	53.9^{b}	47.9^{a}						
Propionate	SFCC	17.2^{a}	22.1^{b}	21.1^{b}	25.5^{c}	0.72	0.60	<0.001	0.15	0.002	<0.001
	Rusitec	22.4^{ab}	23.2^{b}	20.1^{a}	21.1^{ab}						
Butyrate	SFCC	9.25^{a}	9.88^{ab}	11.1^{b}	9.30^{a}	0.385	<0.001	<0.001	0.001	<0.001	0.09
	Rusitec	15.4^{a}	19.6^{c}	18.0^{b}	20.2^{d}						
Isobutyrate	SFCC	0.96^{c}	0.97^{c}	0.69^{a}	0.79^{b}	0.030	0.30	0.07	<0.001	<0.001	0.01
	Rusitec	1.13^{c}	0.86^{b}	0.64^{a}	0.64^{a}						
Isovalerate	SFCC	1.56^{b}	1.56^{b}	1.00^{a}	1.53^{b}	0.136	<0.001	0.34	<0.001	0.002	<0.001
	Rusitec	2.58^{b}	1.40^{a}	3.18^{c}	3.43^{c}						
Valerate	SFCC	2.57^{b}	2.20^{ab}	1.61^{a}	2.20^{ab}	0.200	<0.001	<0.001	<0.001	<0.001	0.03
	Rusitec	5.97^{b}	7.15^{d}	3.98^{a}	6.81^{c}						
Ac/Pr mol/mol	SFCC	4.00^{c}	2.90^{b}	3.08^{b}	2.41^{a}	0.106	<0.001	<0.001	0.01	0.002	<0.001
	Rusitec	2.35^{ab}	2.06^{a}	2.66^{b}	2.27^{a}						

$^{a, b, c, d}$ Within a row, means with unlike superscripts differ (P<0.05).

† FS: fermentation system.

†† Standard error of the mean.

IV – Conclusions

The results show differences between Rusitec and SFCC fermenters in detecting the effects of F:C and FOR in the diet on most of the measured fermentation variables. Some of these discrepancies might be attributed to the observed differences in pH values, dilution rate and solids retention time, as the different conditions in the fermenters might have caused a selection of microbial populations. Studies identifying the changes in microbial populations through the incubation period in both types of fermenters are needed to understand the observed differences.

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