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In vitro forage digestibility under suboptimal microbial inoculum and culture media pH conditions

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Abstract. In order to study the effects of the diet fed to animals from which rumen liquor was obtained (inoculum) and culture media pH conditions on *in vitro* forage dry matter digestibility (IVD), a factorial design experiment was carried out (3 diets and 3 pH conditions). Diets varied in forage to concentrate ratio: 100% forage (F100), 50% forage (F50) and 20% forage (F20). Rumen liquor from animals eating these diets was used to inoculate batch cultures to determine IVD (ANKOM procedure). pH was decreased by means of either: (i) adding barley or grass hay into culture media (10 g/l of media); or (ii) replacing control buffer (bicarbonate) with any of two McIlvaine (MI) solutions, 1000 ml containing 180 (MI180) or 530 (MI530) ml of citric acid. Four forages of different cell wall characteristics were used in digestibility assays. Diet affected pH of the ruminal inoculum (6.8, 6.3 and 4.9 for F100, F50 and F20, respectively). Bicarbonate solution buffered initial pH of culture media (7.0, 6.9 and 6.7 for F100, F50 and F20, respectively). Digestibility was significantly affected ($P < 0.001$) by the diet fed to donor animals, decreasing as concentrate increased. MI180 and MI530 treatments led to a lower initial pH (6.5 and 5.5 for MI180 and MI530, respectively). The addition of barley or hay lowered final pH (6.8, 6.1 and 6.5 for control, barley and hay treatments, respectively). Barley decreased digestibility of straw and beet pulp, especially with F20. With MI buffers there was a linear decrease in digestibility of grass hay, straw and beet pulp. This decline was greater with buffer MI530 (lower pH) and high proportions of concentrate in the diet. In conclusion rumen pH and the type of diet fed to donor animals have an important effect on forage digestibility, probably due to changes in the ruminal microbial population.

Keywords. Rumen pH – Forage digestibility – Ruminal inoculum.

Digestibilité* in vitro *des fourrages dans des conditions suboptimales d'inoculum microbien et de milieux de culture : Conditions du pH

Résumé. *Afin d'étudier les effets du régime alimentaire pour les animaux donneurs de jus de rumen et du pH du milieu de culture sur la digestibilité* in vitro *de la matière sèche des fourrages, on a conduit une expérience selon un plan factoriel (3 régimes et 3 conditions de pH). Les rations avaient différentes proportions fourrage:concentré : uniquement le fourrage (F100), fourrage 50% (F50) et fourrage 20% (F20). Les jus de rumen des animaux recevant les trois rations ont été utilisés pour inoculer les systèmes de fermentation pour déterminer la digestibilité* in vitro *(procédé d'ANKOM). On a réduit les niveaux de pH au moyen de : (i) l'addition d'orge ou de foin d'herbe dans les milieux de culture (10 g/l de milieu) ; ou (ii) en remplaçant le tampon de contrôle (bicarbonate) par deux solutions de McIlvaine (MI), contenant 180 (MI180) ou 530 (MI530) ml d'acide citrique dans 1 litre de tampon. Quatre fourrages avec des parois cellulaires de différentes caractéristiques ont été utilisés dans des essais de digestibilité. Le régime haut ou bas en aliment concentré a affecté significativement le pH de l'inoculum (6,8, 6,3 et 4,9 pour F100, F50 et F20, respectivement). Le tampon du témoin a amorti le pH initial des milieux de culture (7,0, 6,9 et 6,7 pour F100, F50 et F20, respectivement). La digestibilité a été sensiblement affectée ($P < 0,001$) par la ration, étant diminuée à mesure que la proportion de concentré augmentait. Les traitements MI180 et MI530 ont mené à un pH initial inférieur (6,5 et 5,5 pour MI180 et MI530, respectivement). L'addition de l'orge ou du foin a diminué le pH final (6,8, 6,1 et 6,5 pour des traitements contrôle, orge et foin, respectivement). L'orge a diminué la digestibilité de la paille et des pulpes de betterave, particulièrement avec F20. Avec le tampon MI il y a eu une diminution linéaire de la digestibilité du foin d'herbe, de la paille et des pulpes de betterave, mais elle a été plus forte avec MI530 (pH inférieur) et les proportions élevées de concentré dans la ration. En conclusion, le pH du rumen et la ration distribuée aux*

animaux affectent la digestibilité du fourrage probablement suite à la perturbation de la population microbienne du rumen.

Mots-clés. *pH du rumen – Fourrage – Digestibilité – Inoculum de jus de rumen.*

I – Introduction

Since the energy supply from roughages to high milk producing ruminants is limited even with roughages of high digestibility (Kaufmann, 1976), feeding systems based on high concentrate diets have become common for ruminants in intensive systems. As a result, starchy diets rich in cereals are considered a factor responsible for rumen acidosis, a common problem in dairy herds (Krause and Oetzel, 2006). Acidosis is also a problem in feedlot steers or lambs due to the market conditions where the cost per megacalorie of dietary ingredients favours feeding high concentrate diets (Brown *et al*., 2006). Additionally, the move towards high concentrate diets is stimulated because rich concentrate diets are easier to manipulate.

Ruminal pH drops when ruminants consume high amounts of rapidly fermentable (non-fibre) carbohydrates, the main constituents of high grain diets. *In vivo*, low ruminal pH can produce a decrease in fibre digestion (Miller and Muntifering, 1985). In contrast, when *in vitro* fermentation systems are used to determine digestibility, pH in the incubation medium is usually adjusted to near 7.0. Thus, in order to mimic *in vivo* pH conditions some buffer systems, allowing low pH, have been evaluated (Elving *et al*., 1956; Grant and Mertens, 1992a). In addition, it is well known that the type of diet fed to donor animals from which rumen fluid that is used as inoculum for *in vitro* studies is obtained may affect the microbial population, and thus the fermentation pattern in *in vitro* cultures (Ottou and Doreau, 1996). Our experiment was designed to investigate the effects of the type of diet fed to animals that are used as donors of rumen liquor and culture media pH conditions on forage dry matter digestibility *in vitro*.

II – Materials and methods

Three mature rumen cannulated Brown Swiss steers were individually housed and fed a diet consisting of grass hay and concentrate. Three diets were formulated by changing the forage to concentrate ratio: 100% forage (F100), 50% forage (F50) and 20% forage (F20) on a dry matter (DM) basis. Each diet was fed at maintenance to the three animals during a 14-d feeding period. On the last day of each period ruminal fluid was taken to inoculate bottles for the *in vitro* digestibility ANKOM procedure (Mabjeesh *et al*., 2000), and then diet fed to the animals was changed. Water was freely available at all times.

Rumen digesta was collected 4 hours after morning feeding from the reticulum near the reticuloomasal orifice. The digesta from each animal was filtered through several layers of gauze cloth, mixed on a volume basis for each animal and kept in pre-warmed thermos until use (within approximately 30 min).

Apart from the control treatment (C, bicarbonate buffer, no fermentable substrate added to the incubation medium), culture medium pH conditions were decreased by different means. In Experiment 1, pH in the incubation medium was changed by adding either finely ground barley (B) or grass hay (H) directly (10 g/l of medium) into the fermentation vessel. In Experiment 2, bicarbonate buffer [with 4 g of $(NH_4)HCO_3$ and 35 g of $NaHCO_3$/l of distilled water] was used in the control cultures, whereas in the treatment cultures the bicarbonate buffer was replaced with the same volume of any of two McIlvaine (MI) solutions (mixture 0.1 M citric acid and 0.2 M Na_2HPO_4, resulting in lower pH as more citric acid is included in the mixture), containing either 180 ml of citric acid solution and 820 ml of Na_2HPO_4 solution (buffer MI180) or 530 ml of citric acid solution and 470 ml of Na_2HPO_4 solution (buffer MI530). Culture medium contained buffer solution (as described above), macro- and micro-minerals solutions, resazurin and reducing agent (Menke and Steingass,

1988). Just before incubation, culture medium (800 ml/l) and rumen inoculum (200 ml/l) were mixed under anaerobic conditions.

Four forages with different cell wall characteristics were incubated: lucerne hay, grass hay, straw and previously washed sugar beet pulp NDF. For incubation, forages were ground to pass through a 1 mm screen and dried at 60℃ for 48 h. Then, 500 mg of each sample were weighed into porous synthetic fibre bags (F57; 5 × 5.5 cm^2, ANKOM Technology Corporation), which were then heat-sealed and incubated in a 4 l digestion vessel (20 bags per vessel) for 24 h at 39℃. After that, bags were oven dried and weighed to determine DM disappearance. One incubation vessel was used for each experimental treatment, and then in each vessel five bags were incubated with each of the forages studied. Dry matter digestibility data were analyzed by ANOVA following a factorial design with three diets fed to donor animals (F100, F50 and F20) and three treatments used to affect incubation pH: (i) treatments C, B and H in Experiment 1; or (ii) treatments C, MI180 and Ml530 in Experiment 2. In addition, the pH of each vessel was measured at inoculation (initial) and after 24 h (final) of incubation.

III – Results and discussion

The type of diet fed to donor animals had a marked effect on the inoculum pH, which was noticeably lower with diet F20 (4.9) than with the other two diets (Table 1). This lower pH with a concentrate diet was in agreement with Krause and Oetzel (2006) who pointed out that ruminal pH is driven by the amount of fermentable carbohydrate in each meal. The addition of the bicarbonate solution buffered pH in the culture medium resulting in initial values close to 7.

Table 1. pH of culture medium upon the addition of a fermentable substrate (barley or grass hay)

Treatments	Incubation time	Diet of donor animals		
		F100	F50	F20
Rumen fluid	Before	6.8	6.3	4.9
Control	Initial	7.0	6.9	6.7
	Final	6.8	6.8	6.7
Barley	Initial	7.0	7.0	6.7
	Final	6.4	6.2	5.8
Grass hay	Initial	7.1	7.0	6.8
	Final	6.5	6.5	6.5

Addition of both barley and hay to the incubation medium had an effect on final pH, which was more noticeable with barley (Table 1) because of its higher content of rapidly fermentable carbohydrates. These results are in line with those presented by Mould *et al*. (2005) who found a decline in culture medium pH from 6.7 to 6.0 after 24 h of incubation as a result of adding 1.0 g ground wheat in 100 ml of Theodorou's culture medium.

In contrast, when the diet was switched from F100 to F20, M180 and M530 buffers caused a drop in initial pH of 0.6 and 0.8, respectively (Table 2). Grant and Mertens (1992b) observed that addition of bicarbonate was essential to keep proper fermentation conditions, and to prevent a sharp and irreversible drop in pH. Despite the donor animal diet, MI530 maintained a relatively stable pH after 24 hours of incubation, while some pH decline was observed with MI180 (between 0.4 and 0.6 units). It has to be pointed out that in our fermentation systems 1000 ml of culture medium contained only 190 ml of buffer (bicarbonate in the control, or MI180 or Ml530 buffers), whereas Grant and Mertens (1992b) used 800 ml of buffer per 1000 ml culture medium, and also incubated a greater relative amount of substrate dry matter (12.5 g/l *vs* 5.0 g/l).

Table 2. pH of culture medium with different buffer solutions

Treatments	Incubation time	Diet of donor animals		
		F100	F50	F20
Control	Initial	7.0	6.9	6.7
	Final	6.8	6.8	6.7
MI180	Initial	6.7	6.6	6.1
	Final	6.2	6.0	5.7
MI530	Initial	5.8	5.5	5.0
	Final	5.7	5.3	5.3

For the control buffer, forage digestibility was significantly affected ($P < 0.001$) by the diet fed to donor animals. Forage digestibility decreased as the proportion of concentrate in the diet increased (Tables 3 and 4). The decrease in digestibility was greater for straw, grass hay and beep pulp than for lucerne hay. Tejido *et al*. (2002) also found that the use of rumen liquor from animals fed a diet with 80% concentrate resulted in lower ($P < 0.05$) *in vitro* DM digestibility for six forages (oat straw and five hays) compared to rumen liquor from animals fed a diet with only 20% concentrate.

Table 3. Effect of barley grain and grass hay on *in vitro* dry matter digestibility

	Diet of donor animals			Means	P<(±SE)
	F100	F50	F20		
Lucerne hay					
Control	0.48	0.48	0.45	0.47	
Barley	0.50	0.47	0.46	0.48	
Grass hay	0.49	0.49	0.48	0.49	
Means	0.49	0.48	0.46		0.028(±0.010)
P<(±SE)				0.290(±0.010)	
Grass hay					
Control	0.40	0.40	0.29	0.36[a]	
Barley	0.36	0.32	0.27	0.32[b]	
Grass hay	0.39	0.38	0.29	0.35[ab]	
Means	0.38[a]	0.37[a]	0.28[b]		0.000(±0.011)
P<(±SE)				0.001±(0.011)	
Straw					
Control	0.32	0.30	0.19	0.27[a]	
Barley	0.31	0.25	0.14	0.23[b]	
Grass hay	0.33	0.26	0.19	0.25[ab]	
Means	0.32[a]	0.27[b]	0.17[c]		0.000(±0.013)
P<(±SE)				0.018(0.013)	
Beet pulp					
Control	0.67	0.63	0.53	0.61[a]	
Barley	0.50	0.54	0.41	0.48[b]	
Grass hay	0.61	0.64	0.55	0.60[a]	
Means	0.59[a]	0.60[a]	0.50[b]		0.000(±0.017)
P<(±SE)				0.000(±0.017)	

Table 4. Effect of MI buffer on *in vitro* dry matter digestibility

	Diet of donor animals			Means	P<(±SE)
	F100	F50	F20		
Lucerne hay					
Control	0.48	0.48	0.45	0.47[a]	
MI180	0.41	0.43	0.45	0.43[b]	
MI530	0.43	0.39	0.40	0.41[b]	
Means	0.44	0.43	0.43		0.556(±0.008)
P<(±SE)				0.000(±0.008)	
Grass hay					
Control	0.40	0.40	0.29	0.36[a]	
MI180	0.33	0.30	0.29	0.30[b]	
MI530	0.28	0.23	0.25	0.25[c]	
Means	0.34[a]	0.31[b]	0.28[c]		0.000(±0.009)
P<(±SE)				0.000(±0.009)	
Straw					
Control	0.32	0.30	0.19	0.27[a]	
MI180	0.24	0.19	0.15	0.19[b]	
MI530	0.15	0.13	0.12	0.14[c]	
Means	0.24[a]	0.21[b]	0.16[c]		0.000(±0.009)
P<(±SE)				0.000(±0.009)	
Beet pulp					
Control	0.67	0.63	0.53	0.61[a]	
MI180	0.44	0.49	0.39	0.44[b]	
MI530	0.32	0.28	0.25	0.28[c]	
Means	0.47[a]	0.47[a]	0.39[b]		0.000(±00.15)
P<(±SE)				0.000(±0.015)	

The addition of barley caused a decrease in digestibility of straw and beet pulp, and this effect was more noticeable with the high concentrate inoculum (Table 3). This treatment may affect the activity of ruminal fibrolytic bacteria that digest plant cell walls. Cellulolytic activity in the rumen is influenced by predominant bacterial species, forage incubated and the environmental conditions under which ruminal digestion occurs (Grant and Weidner, 1992).

Lower culture medium pH induced by the addition of MI led to a linear decrease in digestibility of grass hay, straw and beet pulp (Table 4), indicating that cellulolytic activity was depressed as rumen pH declined. This effect was greater with MI530 (lower pH) than with MI180, and was also more noticeable as the proportion of concentrate in the diet of donor animals was higher, probably due to the type of microbial population that has been established on the rumen of animal fed a starchy diet. Mourino *et al.* (2001) found comparable results; the rate of cellulose digestion was decreased as pH was decreased from 6.86 to 6.02. In addition, Russell and Wilson (1996) reported that even a moderate decline in pH can have a negative impact on *in vitro* ruminal cellulose digestion, and observed that hay fermentation was drastically inhibited when the final pH was below 5.70.

IV – Conclusions

In conclusion, both the rumen pH of the culture media and the type of diet fed to animals used as donors of rumen fluid have an important effect on forage digestibility, probably due to changes in

the ruminal microbial population. Digestibility was substantially reduced in the case of more fibrous forages, and using inoculum from donor animals fed higher proportions of concentrate in the diet.

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