EFFECT OF DIETARY FACTORS ON DIGESTIVE CAPACITY OF RUMINAL DIGESTA OF DAIRY COWS ESTIMATED THROUGH NYLON-BAG TECHNIQUE

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Abstract:
Using Total Mixed Rations (TMRs) for high yielding dairy cattle prevent ruminal fermentation peaks that would have negative effect on intake level and milk fat concentration. In this framework, the use of TMR is beneficial, because it provides an optimal balance of nutrients to ruminal microorganisms to stabilize ruminal diurnal fluctuation of short chain fatty acids concentration and pH. This work aimed to study the effects of feeding TMR and the amount of concentrate on TMRs on digestive capacity of ruminal digesta through nylon-bag technique. The digestive capacity of cows' digesta fed TMRs and separate ingredients with two different concentrate level (22% and 43%) for concentrate mixture and maize silage was tested by incubating samples of them for 24 hours into the rumen of three fistulated dairy cows. The differences on ruminal degradability of dry matter and neutral detergent fibres of concentrate and maize silage were not tested to be consequence of different feeding strategies or different levels of concentrate in the diet. However, among cows fed 43% concentrate, those who fed TMR tend to have higher fermentation rate of dry matter and neutral detergent fibres with origin from maize silage that can be result of more stabilized ruminal conditions created by mixed ration.

Keywords: ruminal digestive capacity; TMR; dairy cows.

1. Introduction

Total Mixed Rations (TMRs) have been widely used in large cattle farms because of their benefits in nutrition of ruminant animals. For high yielding dairy cattle which require high concentrate level in the ration, TMRs have been known to give benefits by increasing intake, improving fiber digestion and increasing milk yield [18; 1]. Based on the physiological point of view, many authors recommend the use of TMR for high producing dairy cows because they avoid feeding at once large amounts of concentrate, thus preventing ruminal fermentation peaks that would have negative effect on intake level and milk fat concentration [9]. As a result of more stabilized conditions in the rumen, the crude fibre concentration in the ration could be reduced up to 18% or 16% of the ration's DM [3; 11]. In this framework, the use of total mixed rations is beneficial, because it provides an optimal balance of nutrients to ruminal microorganisms to stabilize ruminal fluctuation [2].

The effect of feeding Total Mixed Rations on ruminal environment can be evaluated through the estimation of the ability of rumen content to digest different feedstuffs, as there exist an impact of the interaction diet x substrate ($P < 0.01$) on nutrients degradability [12]. Nylon or synthetic fiber bag technique has been used over years to estimate the feed degradation in the rumen [16; 7; 6; 13; 8]. The nylon-bag technique of ruminal digestive capacity estimation has the advantage of being closer to *in vivo* techniques. The technique includes the incubation of feedstuffs in nylon-bags into the rumen through fistula [7]. Degradability of a particular nutrient is calculated from the difference of the amount of nutrient in the nylon-bag before and after its incubation in the rumen for a given time (24 to 72 hours). Dry matter disappearance has been the most common measurement for digestion studies, but neutral detergent extraction has given more repeatable and biologically relevant results for *in sacco* digestibility [12]. A major problem has been the integrity of nylon-begs as an analytical filter. Studies have shown that lignified matter can enter and accumulate in bags causing low results [14; 15]. So, the control of the ratio sample weight to surface area of the bag is essential. Small pore sizes retard the entry of microorganisms and thus inhibit optimum fermentation while large ones permit the transit of lignified particles [17]. Besides this, bags in the rumen are continuously agitated and compressed by ruminal contents during contractions of the rumen. According to the authors [12] this king of physical action and pressure is necessary to remove the material blocking the pores of the bags or force gas through the pores.

The objective of the work presented in this paper was to study the effects of feeding TMRs and the amount of concentrate on TMRs on digestive capacity of digesta through nylon-bag technique.

Abbreviations: ADF (Acid Detergent Fiber); ADL (Acid Detergent Lignin); CELL (Cellulose); CF (Crude Fiber); CL (Crude Lipids); CP (Crude Protein); DM (Dry Matter); HC (Hemicellulose); HFT (Hohenheimer Futter Test or Gas-Test); NDF (Neutral Detergent Fiber); NE_L (Net Energy of Lactation); NFC (Non Fiber Carbohydrates); NfE (Nitrogen Free Extracts); OM (Organic Matter); SI (Separate Ingredients Feeding); TMR (Total Mixed Ration).

2. Material and Methods

The study was carried out at the Institute of Animal Nutrition, University of Hohenheim, Stuttgart. The diets were formulated by combining two factors, each with two levels. The factors studied were: feeding system (total mixed ration [TMR] vs. ration with separate ingredients [SI]) and concentrate level in the ration (22% vs. 43%). Two of the diets were provided as TMR consisting of different forage to concentrate (F/C) ratios: 78,1 /21,9 and 57,5 /42,5 (DM basis), indicated respectively as TMR-22 and TMR-43. The two other diets (SI) forage and concentrate were fed separately and the targeted F/C ratios were 77,5 /22,5 and 57,1 /42,9 (DM basis), indicated as SI-22 and SI-43.

Table 1. DM, nutrient (Mean±SD) and energy content (mean) for concentrate mixures and maize silage (n=24)*

	Concentrate mixtures		*Maize silage*
	1	2	
DM (%)	91,3 ± 0,3	91,8 ± 0,2	33,0 ± 2,6
Content (% of DM)			
OM	92,4 ± 0,3	92,1 ± 0,1	94,4 ± 0,2
CP	23,3 ± 0,7	36,4 ± 1,3	8,8 ± 0,5
CL	3,2 ± 0,2	3,2 ± 0,2	2,5 ± 0,3
CF	6,6 ± 0,1	7,2 ± 0,7	24,5 ± 1,0
NFC	41,9 ± 1,5	28,2 ± 3,5	28,9 ± 4,2
NDF	24,1 ± 0,8	24,4 ± 2,3	54,2 ± 4,7
ADF	8,7 ± 0,2	9,4 ± 1,0	28,2 ± 1,3
ADL	1,8 ± 0,2	1,8 ± 0,2	2,5 ± 0,1
HC	17,0 ± 2,5	15,4 ± 1,7	28,2 ± 3,9
CELL	7,0 ± 0,2	7,6 ± 0,9	25,7 ± 1,3
NfE	59,4 ± 0,7	45,4 ± 1,9	58,6 ± 0,7
NFC/NDF	1,74 ± 0,1	1,17 ± 0,3	0,54 ± 0,1
NE_L** (MJ/kg DM)	**7,3**	**7,3**	**6,1**

*3 cows x 4 treatments x 2 different days of analysis (end of the adaptation period and end of the period of experimental measurements)

** *Estimated with HFT* (Hohenheimer Futter Test or Gas-Test)

DM (Dry Matter); OM (Organic Matter); CP (Crude Protein); CL (Crude Lipids); CF (Crude Fiber); NFC (Non Fiber Carbohydrates); NDF (Neutral Detergent Fiber); ADF (Acid Detergent Fiber); ADL (Acid Detergent Lignin); HC (Hemicellulose); CELL (Cellulose); NfE (Nitrogen Free Extracts); NE_L (Net Energy of Lactation).

The dietary forage consisted of whole plant maize silage (MS). Two mixtures of concentrates were offered respectively with the high and low concentrate diets. DM, nutrient and energy content for concentrate mixures and maize silage are shown in Table 1.

The four treatments were tested on three multiparous Holstein cows fitted with ruminal fistula and BW 652 ± 48,5 kg at the beginning of the experiment and in the late lactation phase, in an experiment designed as Latin square. The first 14 days of each treatment were for dietary adaptation, after which the ruminal digestive capacity was measured. Cows were housed and fed individually in tie stalls.

Samples of feeds were taken every second day of the experiment and were used to form combined samples for each feed stuff. Feed analyses were performed in combined samples two times during each treatment.

Table 2. Intake levels, nutrients and energy intake (Mean ± SD)

	n	*Diets*			
		SI-22	SI-43	TMR-22	TMR-43
Intake level (kg DM/d)	12*	15,9 ± 0,8	16,3 ± 0,9	16,4 ± 0,9	15,7 ± 1,0
Intake level of MS (kg DM/d)	12	12,3 ± 0,9	9,4 ± 0,7	12,8 ± 1,0	9,5 ± 0,7
Concentrate intake level (kg DM/d)	12	3,6 ± 0,2	7,0 ± 0,3	3,6 ± 0,2	7,0 ± 0,3
Proportion of concentrate in the ration (%)	12	22,5 ± 1,7	42,9 ± 1,9	21,9 ± 5,1	42,5 ± 3,6
NE_L*** (MJ/kg DM)	12	6,4	6,6	6,2	6,7
Content (% of DM)					
CP	24**	15,0 ± 0,6	15,3 ± 0,4	13,9 ± 1,3	14,0 ± 0,5
CL	24	2,6 ± 0,2	2,8 ± 0,2	2,1 ± 0,2	2,4 ± 0,1
CF	24	20,6 ± 1,0	16,7 ± 0,6	21,3 ± 1,8	18,1 ± 1,1
NFC	24	28,7 ± 2,7	34,4 ± 1,8	29,5 ± 1,7	36,1 ± 1,3
NDF	24	47,5 ± 3,4	41,1 ± 2,0	48,6 ± 2,9	41,3 ± 2,0
CELL	24	21,7 ± 1,1	17,7 ± 0,9	23,5 ± 2,4	19,9 ± 1,7
NFC/NDF		0,6 ± 0,1	0,8 ± 0,1	0,6 ± 0,1	0,8 ± 0,1

*3 cows x 4 treatments
**3 cows x 4 treatments x 2 different days of analysis (end of the adaptation period and end of the period of experimental measurements)
*** *Estimated with HFT* (Hohenheimer Futter Test or Gas-Test)
SI (Separate Ingredients Feeding); TMR (Total Mixed Ration); DM (Dry Matter); NE_L (Net Energy of Lactation); CP (Crude Protein); CL (Crude Lipids); CF (Crude Fiber); NFC (Non Fiber Carbohydrates); NDF (Neutral Detergent Fiber); CELL (Cellulose)

In Table 2 the DM intake, nutrients and energy intake for the four diets (treatments) are presented. Total Mixed Rations were mixed every day before offering them to the cows. All diets were offered *semi ad libitum.* During the experiment the daily ration was offered in two meals, 50% each, at 8.00 AM and 4.00 PM.

Digestive capacity of cows' digesta fed TMR and SI with two different level of concentrate (22% and 43%), for concentrate mixture 1 and maize silage (Table 1) was tested by incubating samples of them into rumen through rumen fistula. DM and NDF degradability were tested for both concentrate and maize silage samples. The samples were incubated in rumen for 24 hours in nylon-bags (Polyester Monofilament, ANKOM rumen sampling bags, Bar Diamond) (7). Nylon-bags size were 5 x 10 cm and the size of the pores were 53 micron. Before incubation concentrate and maize silage were dried and grinded in 2 mm particle sizes. The same samples were incubated in three cows, each with four parallels. The weight of incubated samples were 7,0 g for concentrate and 6,5 g for maize silage.

Before incubation the nylon-bags were washed in washing machine for 90 min in 60^0C, dried and weighed. The nylon-bags, after filling with concentrate and maize silage samples, were closed and fastened in a heavy cylinder as shown in Figure 1. The samples were introduced into the ventral sac of the rumen through the fistula (Figure 2). After 24 hours of incubation the samples in nylon-bags were taken out of rumen and rinsed out several times with cold water in order to stop further fermentation processes. Nylon-bags were further rinsed out in washing machine 3 times of 12 min with cold water and than, after dried for 48 hours in 60^0C, were weighed. The amount of digested sample was estimated as the difference of its amount before and after the incubation. The amount of digested DM was calculated separately for each parallel and the mean value was calculated. The amount of fermented NDF of concentrate and maize silage was estimated from the sample composed by mixing the parallels of each animal, since NDF analysis requests a relatively high amount of the sample.

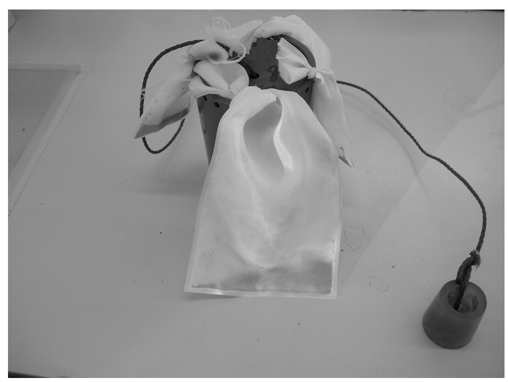

Figure 1. Samples in nylon-bags fasted in a heavy cylinder.

As for DM, the fermentation level of NDF was calculated from the differences of its amount in samples before and after incubation.

Data analysis was carried out with PROC MIXED of SAS (1996) for Windows, Version 8.2, using a model considering the fixed effects of factors feeding strategy (TMR; SI); concentrate level in the diet, cow, treatment time, as well as their interactions. Treatment means were compared by a t-test and the differences were considered significant when P ≤ 0,05.

Figure 2 Ruminal fistula through which the nylon-bags were incubated

3. Results and Discussion

The results of the variance analysis on the effects of dietary factors on digestive capacity of ruminal digesta are presented in Table 3. The differences on ruminal degradability of DM and NDF of concentrate and maize silage after 24 hours of incubation were not evaluated to be consequence neither the different feeding strategies, nor the concentrate level in the ration.

Table 3. Ruminal *in sacco* degradability of DM and NDF of concentrate and maize silage (%) (LSM±SEM) (n=3).

Feeding strategy	*SI*		*TMR*		*Significant factors*
Conc. level	22%	43%	22%	43%	
Concentrate					
DM	89,1 ± 0,7	88,6 ± 0,8	87,9 ± 0,7	88,8 ± 0,8	ns
NDF	61,9 ± 1,8	60,6 ± 2,2	58,2 ± 1,8	61,7 ± 2,1	ns
Maize Silage					
DM	77,4 ± 1,9	74,5 ± 2,3	71,4 ± 1,9	76,2 ± 2,3	ns
NDF	60,8 ± 3,5	55,2 ± 4,2	49,3 ± 3,5	58,6 ± 4,2	ns

SI (Separate Ingredients Feeding); TMR (Total Mixed Ration); DM (Dry Matter); NDF (Neutral Detergent Fiber); ns (non significant effect)

However, among cows fed 43% concentrate, those who fed TMR tend to have higher fermentation rate of DM and NDF, especially those with origin from maize silage that can be explained with the more stable ruminal conditions created by mixed rations. An inverse effect, though not statistically significant, is expressed by the animals fed 22% concentrate. The 24 hours degradability of DM and NDF of concentrate and maize silage is numerically higher in cows fed separate ingredients compared with those fed TMR.

It is evident the low value of NDF degradability of maize silage in animals fed TMR-22 compared with those fed SI-22 that can be the result of the discrepancy between easy fermentable substrate (easy utilizable energy from ruminal microorganisms) and structural carbohydrates, since no impairment of ruminal conditions has been observed and CP level had almost no differences between different feeding variants (Table 2). The intake level did also not present significant differences. Other authors (1) similarly did not found any variation of *in sacco* degradability of concentrate and TMR between cows fed TMR and pasture + concentrate. Other researchers (5) have observed tendencies of increased microbial populations' growths in the rumen and fibrolytic enzyme activity in steers fed TMR compared with steers fed separate concentrate mixture and roughage. The same results are evidenced also in other studies (4; 10).

Numerical differences of ruminal degradability of DM and NDF of concentrate and maize silage as consequence of feeding with different concentrate levels in the diets are small and not significant. The effect of concentrate level in the diet was more evident in DM and NDF degradability of maize silage. It can be observed that by increasing the level of concentrate with around 21% in cows fed TMR there was a tendency of increasing ruminal digestibilities of DM and NDF, which were not evident when values of whole tract digestibility were evaluated (data not shown). It is likely that feeding TMR with 43% concentrate in DM, as the result of the more favorable proportion NFC/NDF in each portion of feed taken, create optimal conditions for microbial activity and advanced digestive capacity of the rumen. This is observed in numerically higher values of ruminal fermentability of DM and NDF of maize silage in cows fed TMR-43 compared with those fed SI-43.

4. Conclusions

TMR can be more favorable feeding system over SI feeding considering the tendency of a higher ruminal fermentation rate that was evidenced in cows fed TMR (concentrate + maize silage) with high level of concentrate in ration (43%) compared with those fed the same level of concentrate separately from maize silage.

Nylon-bag technique can be effectively used to provide detailed information on the effect of dietary factors on ruminal environment.

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