

Rumen Fermentation Parameters and Rumen Papillae Characteristics in Finishing Bulls as Affected by Nonfibrous Carbohydrate Level and Lipid Source of the Diet

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Abstract: The aim of this study was to evaluate the relationship between rumen fermentation parameters and rumen papillae characteristics in finishing Holstein bulls fed three levels of Nonfibrous Carbohydrates (NFC) and two lipid sources. Thirty-four male Holstein bulls were randomly assigned to six different concentrates with three levels of NFC: 450 (low), 550 (medium) and 650 g per kg of DM (high) and two lipid sources (whole canola seed vs whole linseed). Corn meal was the main source of NFC in all treatments. Concentrates were isonitrogenous and isocaloric. Animals (initial weight = 300±25.5 kg) were fed barley straw and concentrate *Ad libitum* until slaughter weight (443±12.5 kg). Animal body weight was recorded every two weeks and feed consumption weekly. To study rumen fermentation parameters, a rumenocentesis was performed monthly. At slaughterhouse, samples from the rumen wall mucosa of the ventral floor of the cranial sac were collected. After separating rumen papillae individually, a digital vision technique was used to estimate papillae numbers per cm², average papillae surface area (mm²) and mucosa surface area (mm²/cm²). Daily concentrate and straw intakes were greater in animals fed canola seed than in animals fed linseed. In contrast, lipid source did not affect rumen parameters and papillae characteristics. Daily concentrate intake was lower in animals fed low NFC (6.93 kg DM/d) than in those fed medium NFC (7.21 kg DM/d) and high NFC (7.15 kg DM/d). Rumen molar concentrations (mM) of Volatile Fatty Acid (VFA) decreased significantly in animals fed the low NFC diet and consequently, rumen pH tended to be greater in these animals (6.53) compared with those the fed medium NFC (6.24) or the high NFC (6.28) levels. Animals fed the medium and the high NFC levels had greater propionate and *n*-butyrate rumen molar concentrations and tended to have a greater average papillae surface area and mucosa surface area than those fed the low NFC level. Regression equations showed a quadratic relationship between average papillae surface area and rumen propionate molar concentration ($R^2 = 0.18$). Number of papillae ($R^2=0.30$) and mucosa surface area ($R^2=0.22$) increased quadratically with rumen propionate molar concentration.

Key words: Rumen, papillae, volatile fatty acids, nonfibrous carbohydrates, oilseed

INTRODUCTION

Carbohydrates (CHO), typically divided in fiber and nonfibrous carbohydrates (NFC), are the primary energy source for ruminants. Both types of CHO are attacked, digested and fermented to Volatile Fatty Acids (VFA) by microorganisms in the rumen. Volatile fatty acids can provide up to 70 percent of the energy needs of the animal^[1]. Volatile fatty acids are passively absorbed through the reticulo-rumen wall and metabolized by tissues^[2]. If the reticulo-rumen wall capacity to absorb VFA is reduced, VFA can accumulate in the rumen inducing an increase of osmolality and a decrease rumen pH, which may compromise the performance and health of the ruminant^[2]. The capacity of the reticulo-rumen mucosa to absorb VFA depends on its rumen papillae, which

distribution, size and number are closely related to VFA production and profile^[3-5]. Propionate and butyrate molar concentrations in the rumen have been reported to increase the size and number of rumen papillae, enhancing the capacity of reticulo-rumen mucosa to absorb VFA^[4-7]. The effect of NFC on rumen fermentation parameters and rumen papillae characteristics has been studied in transition cows and growing calves^[7-12]. In contrast, few data are available describing the relationship between concentrate NFC levels, rumen fermentation and papillae characteristics in finishing bulls fed concentrate and straw *Ad libitum*. High-energy diets fed to finishing beef are often high in NFC making them susceptible to rumen acidosis^[2]. Oilseeds are an attractive alternative energy source^[13], because allow to increase the energy density of diets without adverse effects on microbial growth^[14].

However, no data are available about the effect of oilseed supplementation on rumen papillae characteristics. The aim of this study was to evaluate the relationship between rumen fermentation parameters and rumen papillae characteristics in finishing Holstein bulls fed three different levels of nonfibrous carbohydrates and two oilseed sources.

MATERIALS AND METHODS

Animals, housing and diets: Thirty-four male Holstein bulls were used in a finishing trial following a complete randomized block design. Animals were blocked into three body weight groups (274.2±11 kg, 295.2±9.9 kg; 329.4±14.6 kg) and randomly assigned to one of six dietary treatments. Treatment arrangement followed a 3x2 factorial design, with three NFC levels: 450, 550 and 650 g per kg of DM and two lipid sources: whole canola seed or whole linseed. The diets were isonitrogenous and isocaloric (Table 1), with corn meal being the main source of NFC in all treatments. Animals were housed in outdoor paved, fed barley straw and concentrate *ad libitum* until slaughter weight of 443±12.48 kg. The current study was conducted in the IRTA experimental farm (Prat de Llobregat, Spain) in accordance with the principles and specific guidelines of the IRTA Animal Care Committee.

Measurements and sample collection: Body weight was recorded every two weeks and concentrate and barley straw consumption weekly. Feed samples were analyzed for DM (24 hrs at 103°C), ash (4 h at 550°C), CP by Kjeldahl method^[15], NDF according to Van Soest *et al.*^[16] using sodium sulfite and alpha amylase and fat by Soxhlet with previous acid hydrolysis^[15].

Rumenocentesis was performed monthly 4 h after feeding during three consecutive separate day to avoid differences due to sampling time within day. Rumenocentesis was conducted with a 14 cm 14 gauge needle inserted into the ventral sac approximately 15 to 20 cm caudo-ventral to costo-condral junction of the last rib. Rumen fluid pH was measured immediately. Based on Jounay^[17], 1 mL⁻¹ of a solution containing a 0.2% (wt/wt) mercuric chloride, to inhibit microbial growth, 2% (vol/vol) orthophosphoric acid, and 0.2% (wt/wt) 4-methylvaleric (internal standard) in distilled water was added to 4-mL⁻¹ of rumen fluid subsample and frozen. Volatile fatty acids were analyzed with a polyethylene glycol TPA treated capillary column 25 m (0.25 mm diameter, 0.25 µm film thickness, BP21, SGE, Europe Ltd., UK) by gas chromatography using a GC model Carlo Erba Instruments chromatograph (CE 5300 Ht, Italy).

Animals were transported to slaughterhouse when achieving their slaughterweight (443±12.48 kg). At the slaughterhouse, a 10x10 cm section of rumen wall mucosa was excised from the ventral floor of the cranial sac, washed with 0.9% (wt/vol) NaCl solution and kept with 10% formalin solution until subsequent analysis. A 3.4x3.4 cm² subsection of the excised rumen mucosa, each papilla was cut with a dissection Metzemaum scissors and transferred onto a black surface area with a graded scale and photographed. Photographs were analyzed with artificial vision (Carnoy 2.1 for MacOS X) and total number of papillae per cm² and the average papillae surface area (mm²) were estimated. Mucosa surface area per cm² was calculated multiplying the total number of papillae per cm² and their average papillae surface area (mm²).

Statistical analyses: The data for performance and rumen were analyzed with an analysis of variance with repeated measures^[18]. The statistical model included bull as a random effect and block (initial body weight), level of NFC, lipid source, time, the interaction between NFC level and lipid source and the interaction of main factors with time as fixed effects. The model also included the quadratic effect of NFC. For each analyzed variable, animal nested within the interaction between NFC level and lipid source (the error term) was subjected to three variance-covariance structures: compound symmetry, unstructured and autoregressive order one. The variance-covariance matrix that yielded the smallest Schwarz's Bayesian criterion was considered to be the most desirable structure. Rumen papillae data were analyzed as described below without time effect (as there were no repeated measures). Differences among treatment means were assessed by a Tukey's test.

Also, mixed-effects linear regression analyses were conducted between rumen papillae characteristics and rumen fermentation parameters considering bull as a random effect. The stepwise multivariate regression procedure was also performed to analyze the relationship between rumen papillae characteristics and rumen VFA. The explanatory variables were progressively introduced, the best predictor being first to enter the regression equation. Variables associated to a p-value smaller than or equal to 0.10 were included. Variables were excluded if their p-value exceeded 0.05 after later variables had been introduced.

RESULTS AND DISCUSSION

Animal intake: Daily concentrate intake increased quadratically ($p < 0.01$) with NFC level, being 7.02, 7.39,

Table 1: Ingredient and chemical composition of experimental concentrates

	Treatment*					
	CL	LL	CM	LM	CH	LH
Ingredient,% of DM						
Corn meal	41.4	44.1	57.9	56.0	75.4	77
Linseed	-	18.0	-	11.2	-	3.6
Canola seed	16.1	-	10.0	-	2.8	-
Beet pulp	10.0	14.3	12.8	14.3	2.1	2.3
Wheat co-product	9.0	9.5	2.9	2.9	2.4	-
Sunflower meal	5.6	4.6	3.0	0.1	-	0.4
Soybean meal 48%	-	-	5.7	7.5	10.2	10.3
Corn gluten feed	2.1	2.1	2.1	2.1	2.4	2.1
Molasses	1.9	2.0	1.0	2.0	1.4	1.6
Oat meal	9.8	1.2	0.7	-	0.6	-
Salt	1.20	1.22	1.22	1.22	0.64	0.65
Bicalcium phosphate	1.00	1.00	1.00	1.00	0.42	0.43
Calcium carbonate	0.71	0.71	0.71	0.71	0.7	0.71
Sodium bicarbonate	0.70	0.70	0.48	0.48	0.42	0.43
Magnesium oxide	0.29	0.29	0.29	0.29	0.28	0.29
Vitamin premix†	0.21	0.21	0.21	0.21	0.21	0.21
Nutrient,						
UFV/kg‡	1.1	1.1	1.1	1.1	1.1	1.1
CP, % of DM	13.9	14.5	14.4	13.9	14.3	13.8
EE, % of DM	11.6	11.1	9.0	8.8	5.2	5.4
FND, % of DM	22.7	21.4	15.6	16.5	11.2	10.8
Ash, % of DM	7.6	7.9	6.6	7.3	5.1	4.9

*CL = canola seed, low NFC content; LL= linseed, low NFC content; CM = canola seed, medium NFC content; LM = linseed, medium NFC content; CH = canola seed, high NFC content and LH = linseed, high NFC content All treatments contained 100 UI kg⁻¹ of Vit E. †Vitamin premix consisted of vitamin A =10 000 UI kg⁻¹, vitamin D3 = 2 000 UI kg⁻¹ and vitamin E50 = 100 mg kg⁻¹. ‡UFV= Unit for maintenance and meat production^[19]

Table 2: Rumen fermentation parameters in Holstein bulls fed concentrates containing two different lipid sources and three different levels of NFC

	Treatments						s.e.	Significance ^l		
	CL	LL	CM	LM	CH	LH		LS	NFC	LSxNFC
pH	6.45 ^a	6.60 ^a	6.06 ^b	6.43 ^b	6.38 ^b	6.18 ^b	0.12	*†	‡	
Total VFA, mM	70.6 ^b	64.2 ^b	83.4 ^{ab}	77.6 ^{ab}	73.0 ^a	91.3 ^a	4.65	††	‡	
Individual VFA, mM										
Acetate	45.1 ^b	39.2 ^b	54.1 ^a	48.5 ^a	41.9 ^{ab}	51.2 ^{ab}	2.47	*†	*	
Propionate	16.2 ^b	14.9 ^b	20.5 ^{ab}	18.8 ^{ab}	21.1 ^a	28.6 ^a	2.08	**		
<i>iso</i> -Butyrate	0.6	0.7	0.7	0.5	0.6	0.8	0.03			‡
<i>n</i> -Butyrate	6.7 ^b	6.4 ^b	10.1 ^a	8.2 ^a	7.3 ^{ab}	8.6 ^{ab}	0.55	**†		
<i>iso</i> -Valerate	1.1	1.3	2.1	1.2	1.5	2.1	0.28			
<i>n</i> -Valerate	0.8 ^b	0.9 ^b	1.0 ^{ab}	0.9 ^{ab}	0.9 ^a	1.2 ^a	0.08		‡	
Individual VFA, mol/100 mol										
Acetate	63.4 ^a	60.9 ^a	61.9 ^a	63.4 ^a	57.3 ^b	54.7 ^b	0.90	***		
Propionate	23.2 ^b	24.0 ^b	23.4 ^b	23.1 ^b	28.1 ^a	31.2 ^a	1.06	**		
<i>iso</i> -Butyrate	0.8 ^a	1.1 ^a	0.8 ^b	0.7 ^b	1.0 ^a	0.9 ^a	0.04	‡	**†	‡
<i>n</i> -Butyrate	9.8	10.2	10.8	10.0	9.9	9.6	0.65			
<i>iso</i> -Valerate	1.6	2.3	1.9	1.7	2.4	2.3	0.21			
<i>n</i> -Valerate	1.2 ^a	1.5 ^a	1.2 ^b	1.1 ^b	1.3 ^a	1.3 ^a	0.06		**†	

[§]CL = canola seed, low NFC content; LL = linseed, low NFC content; CM = canola seed, medium NFC content; LM = linseed, medium NFC content; CH = canola seed, high NFC content and LH = linseed, high NFC content ^lLS = lipid source effect; NFC= NFC level effect; LS x NFC= interaction between lipid source and NFC level [†]Quadratic NFC effect [‡]Approaching significance (p<0.1). ^{a,b,c}In a row, means with the same superscript are not significantly different (p>0.05)

7.19±0.13 kg DM/d for low, medium and high NFC levels, respectively. Lower concentrate consumption in the low NFC treatment may be due to the higher NDF content. Neutral detergent fibre is related to filling effects in the rumen and as a consequence it may depress feed consumption^[20]. Concentrate intake (p<0.01) was greater in animals fed canola seed than in those fed linseed. Few studies are available in the literature comparing the effect of different oilseed sources on ruminant intake. Reported differences

comparing different levels or different treatments of only one oilseed source in intake among studies^[13,21,22] can be attributed to oilseed used, hypothesis supported by differences in intake detected in the present study between canola seed and linseed, and/or to the remaining concentrate ingredients and oilseed inclusion level^[23,24]. Barley straw intake (p<0.01) was also greater in animals fed canola seed (1.25±0.02) than in those fed linseed (1.12±0.03), but it was not affected by NFC

Table 3: Rumen papillae characteristics in Holstein bulls fed concentrates containing two different lipid sources and three different levels of NFC

	Treatments						s.e.	Significance ^c		
	CL	LL	CM	LM	CH	LH		LS	NFC	LSxNFC
Papillae number per cm ²	38.0	39.3	54.2	51.1	38.5	55.8	5.51			
Average papillae surface area, mm ²	2.6	2.3	4.1	4.9	3.9	4.5	0.74			
Mucosa surface area ^f , mm ² /cm ²	102.2	86.2	244.8	245.3	150.3	304.3	47.57		††	

[§]CL = canola seed, low NFC content; LL = linseed, low NFC content; CM = canola seed, medium NFC content; LM = linseed, medium NFC content; CH = canola seed, high NFC content and LH = linseed, high NFC content. ¹LS = lipid source effect; NFC = NFC level effect; LSxNFC = interaction between lipid source and NFC level. [†]Quadratic NFC effect. ^{††}Approaching significance (p<0.1). ^fMucosa surface area per cm² was calculated multiplying total number of papillae per cm² and their average surface area (mm²)

level. The decrease of NFC levels in current study was counterbalanced by an increase in dietary fat.

Rumen fermentation parameters: Rumen total VFA concentration tended to be lower (p = 0.06) in the low NFC (67.39 mmol L⁻¹) level than in the medium (80.72 mmol L⁻¹) or high (82.15 mmol L⁻¹) NFC level (Table 2). This tendency was most likely due to the lower concentrate and straw consumption in bulls receiving the low NFC concentrate. Accordingly, average rumen pH was greater (p<0.05) when animals were fed low NFC (6.53) treatment than when fed medium NFC (6.24) or high (6.28) NFC treatments. In fact, a negative correlation between rumen total VFA concentration and rumen pH (r = -0.80; p<0.001) was found. Interactions between NFC levels and lipid source were observed in pH (p=0.06) and total VFA concentration (p = 0.09). At the high NFC level, bulls fed linseed had lower pH than those fed colza, in contrast at the medium and low NFC level bulls fed linseed had a greater pH than animals fed colza. The pH of animals fed linseed decreased linearly with NFC level, whereas in animals fed colza pH followed a quadratic tendency with the medium NFC level having the lowest rumen pH. Although all bulls were fed high amounts of high fermentable CHO, no signs of rumen acidosis were registered and none of the treatments had a rumen pH value below 5.6 indicative of subclinical rumen acidosis. Molar concentration of acetate in animals fed linseed increased linearly with NFC level, whereas in animals fed colza followed a quadratic pattern with the medium NFC level resulting in the greatest acetate concentration (p<0.05). As expected, propionate molar concentration increased linearly (p<0.01) with NFC level. Molar *n*-butyrate (p<0.05) and *n*-valerate (p = 0.09) concentrations were lower in animals receiving the low NFC than in those receiving the medium NFC or high NFC levels. An interaction (p=0.07) between NFC level and lipid source was found in *iso*-butyrate molar concentrations.

Molar proportion of acetate was greater (p<0.001) in the medium NFC treatment in contrast to the

Table 4: Results of the stepwise multiple regression analysis between rumen papillae characteristics and rumen volatile fatty acids

	β [†]	s.e.(β)	R ^{2†}	p-value
Papillae number per cm ²				
Propionate, mM	2.16	0.62	0.28	**
<i>n</i> -valerate, mM	-31.02	16.87	0.36	†
Average papillae surface area, mm ²				
Propionate, mM	0.18	0.06	0.12	*
<i>iso</i> -butyrate, mM	-5.65	2.83	0.13	*
<i>n</i> -butyrate, mM	0.48	0.20	0.08	†
Mucosa surface area [§] , mm ² /cm ²				
Propionate, mM	12.16	3.57	0.20	**
<i>iso</i> -butyrate, mM	-322.88	182.65	0.08	†

[†]β = regression coefficient; R² = proportion of variability explained by each variable in the model. [†]Approaching significance (p<0.1). [§]Mucosa surface area per cm² was calculated multiplying total number of papillae per cm² and their average

low or high NFC treatments, whereas molar proportion of propionate was greatest (p<0.001) in the high NFC treatment. A tendency (p = 0.07) in *iso*-butyrate rumen molar proportion in the interaction between NFC level and lipid source was found. Rumen molar proportions of *iso*-butyrate and *n*-valerate were greater (p<0.01) in bulls fed medium the NFC treatment than bulls fed low and high NFC treatment.

Rumen papillae characteristics: Papillae numbers per cm² and average papillae surface area were not affected by treatments (Table 3). Zitnan *et al.*^[4] reported that papillae numbers and average papillae surface area were greater in bulls fed concentrate-rich diets than in bulls kept on pasture. Reynolds *et al.*^[12] found that papillae numbers tended to be greater in cows fed high levels of NFC and protein levels compared with those fed low level of NFC and protein levels, but in contrast to Zitnan *et al.*^[4], who found that the average papillae surface area decreased when cows received high levels of NFC and protein. These differences may be attributed to dietary concentrate to forage ratio and rumen tissue sampling technique^[11].

Surface area per cm² of rumen tended (p = 0.06) to be affected by NFC level. Bulls receiving the medium NFC concentrate had greater total surface area per cm² of rumen mucosa (245.1 mm²/cm²) than bulls receiving low

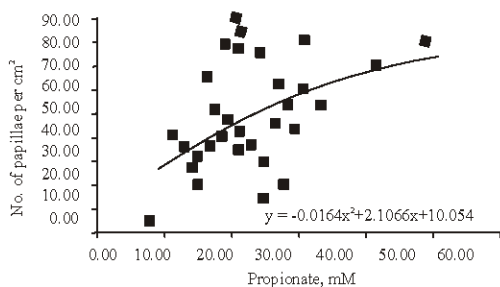


Fig. 1: Relationship between rumen propionate molar concentration and rumen papillae numbers in Holstein bulls

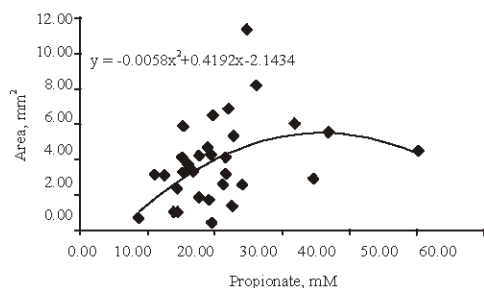


Fig. 2: Rumens mucosa surface area in relation to nonfibrous carbohydrates consumption in Holstein bulls

(94.2 mm²/cm²) or high (227.3 mm²/cm²) NFC levels as reported in the study of Lesmeister *et al.*^[11]. Values of surface area per cm² of rumen mucosa were smaller than those reported by Zitnan *et al.*^[4] who reported an average of 1044 and 1677 mm²/cm² in the ventral sac of the rumen from for extensively- and intensively-fed bulls, respectively. The effect of NFC levels on rumen surface area observed in the present study agree with different studies^[3,4,9,24] reporting that large amounts of NFC increase rumen VFA, mainly propionate and butyrate and enhance the development of rumen mucosa.

As expected, simple regressions indicated that average papillae surface area ($R^2=0.18$), papillae numbers per cm² ($R^2=0.30$, Fig. 1) and also mucosa surface area ($R^2=0.22$, Fig. 2) quadratically increased with rumen propionate molar concentration. These results are in agreement with previous studies^[3,4,9,24]. Total rumen VFA molar concentration was positively correlated ($R^2=0.25$, $p<0.01$) with rumen papillae numbers. To identify the most important individual VFA contributing to relationship with rumen papillae data a multivariate regression analysis progressively including all individual

VFA in the model was performed (Table 4). The number of papillae per cm² was affected by propionate ($R^2=0.28$) and tended ($p=0.08$) to be affected by *n*-valerate rumen molar concentration ($R^2=0.07$), while average papillae surface area (mm²) was affected ($p<0.001$) by rumen molar concentration of *iso*-butyrate ($R^2=0.13$), propionate ($R^2=0.12$) and *n*-butyrate ($R^2=0.08$). Mucosa surface area (mm²/cm²) was affected by molar concentration of propionate ($R^2=0.20$) and *iso*-butyrate ($R^2=0.08$).

Increasing proportion of butyric and propionic acid will result in increased blood flow through the rumen walls and this will stimulate mitosis of the cells, inducing rapid growth of the papillae^[24].

CONCLUSIONS

This study indicates that papillae numbers per cm² and average papillae surface area were not affected by NFC levels and lipid source. In contrast, mucosa surface area per cm² of rumen tended to be lower in bulls fed low NFC than in bulls fed medium and high NFC levels. Average papillae surface area, papillae numbers and mucosa surface area increased quadratically with rumen propionate molar concentrations.

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