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Influence of temperature and humidity on rumen pH and fatty acids in dairy cows

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Abstract

The aim of this study was to investigate the variations of rumen pH and fatty acids (acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid and total fatty acids) in 245 early lactating dairy cows under different temperature and humidity conditions. The animals were divided into six groups and rumen fluid was collected by rumenocentesis on 22 dairy cows in April (Group A), 33 in May (Group B), 43 in June (Group C), 48 in July (Group D), 36 in September (Group E) and 60 in October (Group F). One-way analysis of variance (ANOVA), followed by the Bonferroni's test, showed a significant effect of environmental variations on all studied parameters ($P<0.0001$). Changes in studied parameters can be explained in relation to the microbial population and shift in the optima for rumen conditions associated with variations of environmental conditions. We can affirm that the microbial assemblages that underlie energy and protein supply to wild ruminant are evident especially in relation to temperature and humidity conditions.

Key words

Dairy cows, Early lactation, Fatty acids, Rumen pH , Rumen acidosis.

Introduction

Subacute rumen acidosis (SARA) is one of the most important metabolic disorders affecting dairy cows, adversely influencing rumen fermentation, animal welfare, productivity and farm profitability (Krause and Oetzel, 2005). SARA, characterized by abnormally low rumen pH, is generally induced by excessive consumption of dietary nonstructural carbohydrates (NFC) resulting in excessive production of volatile fatty acids and lactic acid (Duffield *et al*., 2004). Dietary and feeding management factors that influence feed sorting behavior result in cows consuming a diet with an effective increase in dietary NFC content coupled with decreased consumption of physically effective fiber, thus predisposing to the abnormal rumen fermentation patterns leading to the acidosis condition. In contrast to acute lactic rumen acidosis, the subclinical form should be regarded as herd rather than an individual problem (Enemark, 2008). The onset of SARA is marked by the intake of a high energy diet , while the rumen environment is not yet adapted to ferment and later absorb the arising short-chain fatty acids adequately enough to keep the rumen pH within physiological borders (Kleen *et al*., 2003). Fermentation of non-structural carbohydrates, such as starches and sugars, leads to production of large quantities of fatty acids and lactate that can accumulate in the rumen and subsequently decrease

rumen pH (Keunen *et al*., 2003). In fact, according to Nordlund and Garrett (1994), SARA can be defined as a condition characterized by rumen pH below 5.8, with an increased total concentration of short chain fatty acids, ratio between acetic acid, propionic acid and butyric acid that has been shifted towards propionic acid and butyric acid, and an elevated concentration of lactic acid in the rumen fluid that does not exceed 5-10 m mol l^{-1} (Enemark *et al*., 2002; Morgante *et al*., 2007). It has been demonstrated that in early lactating dairy cows, the use of diets with high levels of rapidly fermentable carbohydrates and/or marginal (often deficient) levels of physically effective fiber causes the onset of SARA (NRC, 2001). Also, There is a strong evidence that day length controls changes in fatty acids in seasonal breeds of some ruminants through hypothalamic, pituitary and peripheral hormones (Barry *et al*., 1991). However, no neurohormone has been convincingly shown to exert any effect on seasonal changes of fatty acids (Barry *et al*., 1991). It seems that the annual rhythm of fatty acids, with a peak food intake that occurs in late spring to early summer and the lowest intake during midwinter is the result of an increase in metabolic demand.

In light of the above, the present study aimed was to investigate the variations of rumen pH and fatty acids and the concentrations of acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid of rumen fluid in early lactating dairy cows under different temperature and humidity conditions from April to October.

Materials and Methods

Study area : The study was carried out in an intensive Italian dairy herd, located in North Italy (45° 38' N; 10° 87' E) at an altitude of 68 m asl. The study was carried out from April to October 2009 on 245 multiparous Holstein dairy cows. Selected cows showed no clinical signs of disease, they had high average farm milk production (about 10.000 kg per year) and the body condition score (BCS), evaluated according the procedure of Edmonson *et al*. (1989) was 3.00 ± 0.25.

All cows were in the first period of lactation (between 7 and 90 days in milk) and they were fed the same diet for the study duration. Cows were housed in free stalls, fed a total mixed ration (TMR), and had received a steam-up diet in the final part of the dry period. Feed were analyzed by near infrared spectroscopy (NIRS) using NIRS 5000 (Foss NIRSystem) and an in house calibration. Dietary cation/anion balance (DCAB) was calculated based on mineral analysis determined by atomic absorption methods. Thermal and hygrometric records (environmental temperature and humidity) were carried out daily for the whole study by means of a data logger (Gemini, UK).

Experimental design and assays : The rumen fluid was collected on 22 dairy cows in April (Group A), 33 in May (Group B), 43 in June (Group C), 48 in July (Group D), 36 in September (Group E) and 60 in October (Group F). The rumen fluid was collected by rumenocentesis as described by Morgante *et al*. (2007), without sedation, using a 13 ga 105 mm needle. The time of sampling was between 4 and 6 hr post total mixed ration (TMR) distribution as recommended previously. Rumen pH was determined immediately after sampling using a portable pH meter (Piccolo, Hanna Instruments, Leighton Buzzard, Bedfordshire, UK).

The rumen fluid, conserved in tests-tube maintained to -20°C, was subordinated to the determination of the short chain fatty acids by means of high performance liquid chromatography (HPLC), following the method of Martillotti *et al*. (1987). The rumen liquid of defrosted samples was homogenized, centrifuged and filtered. The solution standard of calibration in amount of 5 μl was analyzed and, after calibration of the instrument, the concentration of the fat acids expressed in mg ml^{-1}, was calculated applying the following formula:

weight of acid = [(area champion × weight of the standard)/area of the standard] × n° dilutions

Subsequently, the concentrations of the fatty acids, calculated in mg ml^{-1}, were converted in m mol l^{-1}: m mol l^{-1} = (mg ml^{-1} × 1000)/P.M

All results were expressed as means ± SD. Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov's test) and one-way measures analysis of variance (ANOVA) was used to determine the statistical differences between mean values of the studied parameters. A *P value* <0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post-hoc comparison. Data were analyzed using STATISTICA 7.0 (Stat Soft Inc.) software package.

Results and Discussion

Table 1 shows the chemical composition of diets consumed by dairy cows during steaming-up and subsequent early lactation. The value of neutral detergent fiber (NDF), acid detergent fiber (ADF), non fiber carbohydrates (NFC), starch and crude protein, were same in all herds, with values within the normal range for each period and this chemical composition represent the normal condition in Italian dairy farm.

Table- 1: Mean chemical composition (%) of total mixed rations fed to dairy cows during steaming-up and early lactation

Chemical composition of diet (%)	Steaming-up	Early lactation
Crude protein	13.37	16.59
Ethreal extract	4.30	6.01
Ash	7.38	7.42
NDF	40.77	30.17
NFC	34.17	38.81
DMD	59.02	68.48
ADF	24.82	20.37
Starch	14.74	28.46
DCAB	35.49	49.39

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; NFC: Non-fiber carbohydrates; DCAB: Dietary cation-anion balance; DMD: Dry Matter degradable

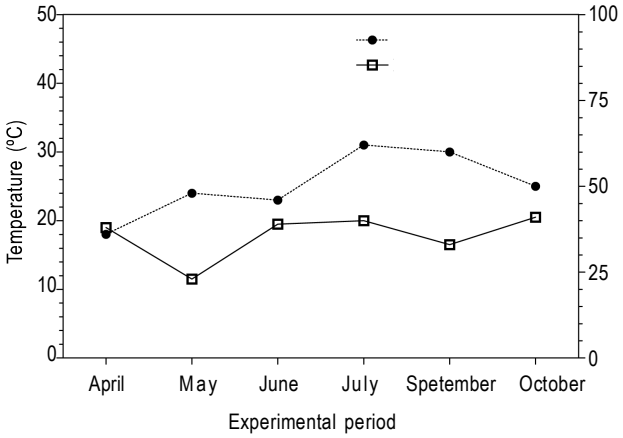

Fig. 1: Environmental temperature (°C) and relative humidity (%) during the experimental period considered

During the trial, mean temperature-humidity values averaged 18 and 31°C and 23 and 41(%), respectively (Fig. 1). Table 2 shows the mean values (±SD) of all studied parameters: rumen pH, and acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid expressed both in m mol l^{-1} and in %, and total fatty acids in m mol l^{-1}.

One–way measures analysis of variance (ANOVA) showed statistically significant differences between considered groups on all parameters with P-values ranging between 0.0001 and 0.02. The results of ANOVA (F-value, P-value and description of effects) of rumen pH, acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid and total fatty acids are shown in Table 3.

The most important short chain fatty acids are acetic, propionic and butyric acid and their concentrations depend on the

Table- 2: Average values of rumen pH and acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid and total fatty acids, expressed in m mol l^{-1} and in % (in brackets)

Parameters	Experimental Groups					
	Group A	Group B	Group C	Group D	Group E	Group F
Rumen pH	5.90 ± 0.07	6.02 ± 0.05	5.84 ± 0.03	6.08 ± 0.05	6.04 ± 0.08	5.95 ± 0.04
Acetic acid	58.30 ± 2.34 (61.48 ± 0.73)	49.64 ± 1.90 (59.07 ± 0.71)	57.80 ± 1.61 (56.39 ± 1.05)	67.22 ± 1.94 (53.88 ± 0.74)	78.53 ± 2.44 (53.83 ± 0.79)	67.31 ± 1.72 (50.84 ± 0.54)
Propionic acid	20.04 ± 1.39 (23.03 ± 0.73)	20.66 ± 0.82 (24.76 ± 0.66)	25.93 1.30 (25.99 ± 0.75)	29.52 ± 1.27 (27.55 ± 0.68)	31.45 ± 1.49 (26.85 ± 0.60)	33.26 ± 0.99 (29.80 ± 0.61)
Iso-butyric acid	0.63 ± 0.03 (0.67 ± 0.02)	0.48 ± 0.02 (0.65 ± 0.02)	0.48 ± 0.02 (0.52 ± 0.02)	0.43 ± 0.01 (0.47 ± 0.01)	0.50 ± 0.02 (0.48 ± 0.01)	0.38 ± 0.01 (0.40 ± 0.02)
N-butyric acid	10.53 ± 0.53 (11.07 ± 0.26)	9.64 ± 0.39 (11.50 ± 0.26)	11.53 ± 0.39 (12.24 ± 0.27)	12.10 ± 0.47 (13.13 ± 0.39)	14.85 ± 0.64 (14.33 ± 0.34)	13.31 ± 0.44 (13.63 ± 0.23)
Iso-valerianic acid	1.42 ± 0.08 (1.49 ± 0.04)	1.40 ± 0.09 (1.68 ± 0.10)	1.31 ± 0.11 (1.45 ± 0.11)	1.47 ± 0.08 (1.79 ± 0.09)	1.56 ± 0.09 (1.70 ± 0.09)	1.44 ± 0.07 (1.70 ± 0.08)
N-valerianic acid	1.52 ± 0.08 (1.59 ± 0.04)	1.42 ± 0.06 (1.73 ± 0.08)	2.12 ± 0.17 (2.37 ± 0.19)	1.97 ± 0.12 (2.35 ± 0.11)	1.96 ± 0.11 (2.17 ± 0.08)	2.14 ± 0.07 (2.52 ± 0.08)
Caproic acid	0.60 ± 0.04 (0.65 ± 0.05)	0.47 ± 0.03 (0.60 ± 0.05)	0.71 ± 0.05 (0.83 ± 0.07)	0.44 ± 0.05 (0.57 ± 0.06)	0.38 ± 0.04 (0.43 ± 0.04)	0.33 ± 0.04 (0.43 ± 0.05)
Total fatty acids	95.06 ± 3.99	83.79 ± 2.80	101.20 ± 3.34	116.10 ± 3.47	125.20 ± 4.89	130.00 ± 2.98

Table- 3: Results of ANOVA: F-value, P-value and description of effects of rumen pH, acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valerianic acid, n-valerianic, caproic acid and total fatty acids

Parameters	F-value (df, error)	P-value	Description of effect
Rumen pH	2.61 (5,236)	0.02	Group D *Vs* Group C
Acetic acid	22.47 (5,236)	0.0001	Group E *Vs* Groups A,B,C,D; Group D *Vs* Groups B,C; Group F *Vs* Groups C,E
Propionic acid	15.83 (5,236)	0.0001	Group D *Vs* Groups A,B; Group E *Vs* Groups A,B,C; Group F *Vs* Groups A,B,C
Iso-butyric acid	10.10 (5,236)	0.0001	Groups B, C, D, E *Vs* Group A; Group F *Vs* Groups A,B,C, E
N-butyric acid	12.72 (5,236)	0.0001	Group E *Vs* Groups A,B,C,D; Group F *Vs* Groups A,B
Iso-valerianic acid	6.51 (5,236)	0.0001	Group C *Vs* Groups A,B; Group F *Vs* Groups A,B
N-valerianic acid	5.96 (5,236)	0.0001	Group C *Vs* Groups A,B; Groups D,E *Vs* Group B; Group F *Vs* Groups A,B
Caproic acid	8.70 (5,236)	0.0001	Group C *Vs* Groups B; Groups D,E *Vs* Group C; Group F *Vs* Groups A,C
Total fatty acids	24.18 (5,236)	0.0001	Group C *Vs* Group B; Group D *Vs* Groups A,B,C; Groups E,F *Vs* Groups A,B,C,D

df : degrees of freedom

source of carbohydrates, pre-treatment of feed and feeding portions. However, the exposure to heat stress modifies the digestibility of NDF and ADF in the total tract and tends to change the digestibility of fatty acids in calves (Bunting *et al*., 1996). As observed in our study the higher temperature values recorded in summer period affect the concentrations of fatty acids. Previously, it has been demonstrated that changes in rumen conditions were related to seasonal pattern (Crater *et al*., 2007). So probably, the seasonal changes that accompany changes in rumen pH could be associated with changes in the microbial population and shift in the optima for rumen conditions and substrate availability (Crater *et al*., 2007). The rumen may be considered an allostatic system where observed changes shift conditions to new set points reflecting the activities of the microbes. Unstable or variable conditions within the rumen may be associated with differences in the number and activity of bacteria (Barboza *et al*., 2006). The seasonal changes in food intake and mucosal exchange alter the allostatic set points around rumen pH. Allostatic set points of rumen conditions can be selected for different phylotypes of bacteria with varying substrate affinities and metabolic pathways (Crater *et al*., 2007). Changes in the microbial population and shift in the optima for rumen conditions and substrate availability could be associated with seasonal variations in minor fermentation acids that accompany changes in food intake (Crater *et al*., 2007). Although shift in microbial diversity have not yet been related, specifically, to natural changes in food intake that accompany seasonal changes, modification in food intake drive allostatic set points that may further influence the homeostatic range as diet and substrate diversity change with season.

In conclusion we can affirm that the microbial assemblages that underlie energy and protein supply to wild ruminant are evident especially in relation to temperature and humidity conditions.

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