Blood Parameters Modification at Different Ruminal Acidosis Conditions

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Summary

This study evaluated the reliability of various blood parameters to assess the ruminal acidosis in cattle. Six whole heifers were fed three experimental rations in a 3 x 3 Latin square design. The diets had different starch levels: high (HS), medium (MS) or low (CT). Ruminal pH values were continuously measured using wireless sensors. To evaluate the severity of ruminal acidosis, the amount of time per day that the pH was below 5.8, 5.5 and 5.0 was recorded. Blood samples were analyzed for complete blood count, venous blood gas and biochemical profile at 8:00 and 12:00 h. The data were analyzed according to a mixed model. Feeding on CT, MS and HS led to significant differences in DMI (7.7 vs. 6.9 vs. 5.1 kg/d; P < 0.01) which modified the amount of time per day that the pH was below 5.0 (0 vs. 12 vs. 92 min; P < 0.10). Feeding MS and HS diets led to inflammation as indicated by the significant increment of white blood cells when compared to the CT ones and to blood concentration due to the osmotic pressure at ruminal level. Furthermore a significant decrease of bicarbonate level, CO_2 partial pressure and oxyhemoglobin was observed as consequence of the activation of metabolic processes aimed to prevent metabolic acidosis. No differences were observed on blood sampling time, suggesting that one daily blood sample was enough to evaluate the metabolic variations related to ruminal acidosis.

Key words

dairy cattle, starch level, ruminal acidosis, blood parameters

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Aim

Ruminal acidosis is a metabolic disease in cattle fed high starchy concentrate diets and it has been defined as a disorder associated with low ruminal pH (Plaizier et al., 2009). The drop of ruminal pH is not only influenced by the amount of starch ingested, but also by the starch source (e.g., maize, wheat or barley) and its particle size (De Nardi et al., 2013). Although clinical signs are not always obvious, ruminal acidosis affects dry matter intake (DMI) and productions, can cause laminitis and other disorders resulting in substantial economic losses in dairy farming (Corato et al., 2005; Calsamiglia et al., 2012). Although several parameters were investigated to help in the diagnosis of ruminal acidosis (Gozho et al., 2007; Enemark et al., 2008; Marchesini et al., 2011) only few authors have extensively investigated the effect of ruminal acidosis and sampling time on blood variables (Brown et al., 2000; Marchesini et al., 2013).

The aims of this study were to identify the blood variables change in heifers affected by different levels of ruminal acidosis and to evaluate the variation of these parameters at different sampling times.

Materials and methods

Animals and Experimental Design - According to a 3 x 3 Latin square arrangement, six crossbred Valdostana x Belgian Blue non-pregnant heifers were randomly assigned to 1 of 3 dietary treatments. Each experimental period lasted 5 days and it was followed by a 2 weeks washout period during which the animals were fed the control diet (CT) *ad libitum*.

Dietary treatment - During the experimental period, heifers received one of three total mixed rations (TMR) characterized by different starch levels (% DM): control (CT) as low starch diet (17.3%), medium starch (MS) for SARA (33.4%) and high starch (HS) to induce acute ruminal acidosis (42.8%). The animals were individually restricted and fed three times a day at 8:00, 12:00 and 18.00 h. Heifers were offered 10 kg of their ration at each meal, and the feed that was not consumed was removed and weighed.

Acidosis Challenge Model - Each experimental period was preceded by 3 baseline days (pre-challenge days d-3, -2, -1) in which the heifers had access to the CT TMR three times a day. On the day before the challenge (restricted feeding day, d0), the feed was restricted to two meals a day (8:00 and 12:00 h) with a consequent reduction of DMI. On d1, the HS, MS and CT diets were fed to induce acute acidosis or subacute acidosis or to maintain the physiological ruminal pH, respectively. On the following three days (d2, d3 and d4), all the animals were fed the CT diet three times a day.

Ruminal pH - The ruminal pH of each heifer was measured every 10 min during the entire trial using KB1001 wireless sensors (Kahne Limited, Auckland, New Zealand). As reported by other authors (Dohme et al., 2008; Marchesini et al., 2013), to measure the severity of ruminal acidosis, the amount of time per day that the pH was below three ruminal pH thresholds (pH < 5.0; 5.0 ≤ pH < 5.5 and 5.5 ≤ pH < 5.8) was determined for each heifer.

Blood collection and analysis - Blood samples (20 mL) were taken from the jugular vein from each animal at 8:00 and 12:00 h on each experimental day immediately before the meal. The blood was analyzed for complete blood cell count (Cell Dyn 3500, Abbott Laboratories. Abbott Park, Illinois, USA), blood gas analysis (Synthesis 15, IL Instrumentation Laboratory SpA, Milano, Italy), urea and glucose (Roche Diagnostics, Indianapolis, IN, USA).

Statistical Analysis - Sample distribution normality was assessed with the Shapiro-Wilk test. Those variables that showed W < 0.95 were log-transformed (natural logarithm) to meet parametric assumptions. However, data of normalized variables are presented in the results section as raw LSmeans. The associated P-values were calculated from the transformed data with the following model. The blood pH, count, gas analysis, and plasma hematological profile data were analyzed using a mixed procedure with a CS (compound symmetry) structure. The linear model included fixed effect of dietary treatment, period, day, daily time, and their interactions. Heifer was considered a random effect and day a repeated measure. In the case of DMI, data were analyzed with the same model but without the daily time effect and its interactions. The degrees of freedom of D effect were used in the two orthogonal contrasts: *D1*, CT vs. (MS and HS)/2; *D2*, MS vs. HS. The average amount of time for each heifer with a pH below the three established pH thresholds (< 5.0; between 5.0 and 5.5; between 5.5 and 5.8) were not normally distributed, thus they were tested using the non-parametric Kruskal-Wallis criteria to discriminate among the dietary treatments. Significance was declared at P < 0.05, however a trend was considered to exist if 0.05 ≤ P < 0.10. All of the statistical analyses were performed using SAS (2008; release 9.2).

Results and discussion

DMI was significantly affected by the treatment resulting the highest in CT-fed heifers (7.7, 6.9 and 5.1 kg/d DM; P < 0.01 both in *D1* and *D2*). Figure 1 shows the ruminal pH drop caused by the rapid fermentation due to both the high amount of starch ingested and the small (0.5 mm) mean maize particle size (Plaizier et al., 2009). As expected, the ruminal pH of the animals fed the CT diet tended to spend the lowest (P < 0.10) time between 5.0 and 5.5 and never registered values lower than 5.0 (Figure 1).
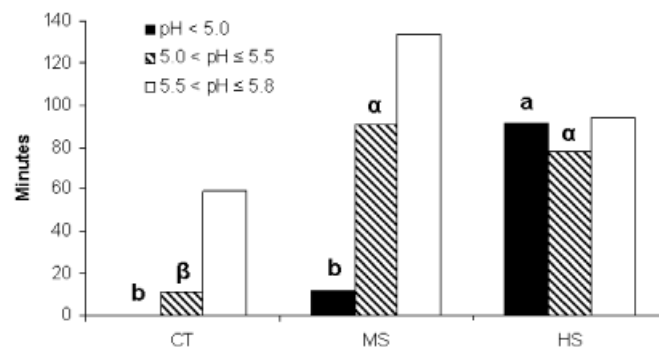
Figure 1. Mean time spent daily below three ruminal pH thresholds ([a, b] Means of time per day below pH 5.0 with different superscripts are different (P < 0.05); [α, β] Means of time between pH 5.0 and 5.5 with different superscripts are different (P < 0.10))

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Table 1. Effect of dietary treatment (D, n = 18) and daily time (T, n = 36) on blood pH, count, gas and hematological profile

| Daily Time	Dietary treatment						SEM	Probability		
	CT		MS		HS			D1	D2	T
	8	12	8	12	8	12				
pH	7.39	7.39	7.39	7.39	7.39	7.39	0.004	ns	ns	ns
Red blood cells (10^{12}/L)	8.0	7.9	7.6	7.7	8.2	8.0	0.16	ns	*	ns
White blood cells (10^9/L)	8.5	9.0	9.8	10.1	9.5	9.7	0.42	**	ns	ns
§Neutrophils (10^9/L)	2.9	3.1	3.8	4.0	3.8	3.9	0.28	**	ns	ns
Lymphocytes (10^9/L)	4.6	4.8	5.0	5.2	4.8	4.9	0.34	ns	ns	ns
Monocytes (10^9/L)	0.84	0.93	0.82	0.82	0.72	0.75	0.068	**	*	ns
Basophils (10^9/L)	0.09	0.10	0.08	0.09	0.07	0.07	0.010	*	ns	ns
§Eosinophils (10^9/L)	0.04	0.04	0.06	0.06	0.13	0.11	0.028	†	†	ns
Hemoglobin (g/dL)	11.1	11.0	10.7	10.8	11.4	11.2	0.16	ns	*	ns
Hematocrit (%)	33.8	32.9	32.8	32.7	34.1	33.3	0.51	ns	*	ns
Platelets (K/μL)	503	519	477	516	601	607	42.8	†	**	ns
pCO_2 (mmHg)	52.0	51.0	50.4	50.8	50.4	49.6	0.42	*	ns	ns
pO_2 (mmHg)	62.1	61.1	71.3	67.8	67.4	64.6	4.26	ns	ns	ns
HCO_3^- (mmol/L)	32.1	31.4	31.2	31.0	30.6	30.3	0.27	**	†	ns
Oxyhemoglobin (%)	87.2	85.5	87.7	88.1	85.4	83.6	1.24	ns	*	ns
Reduced hemoglobin (%)	10.9	11.8	11.1	10.2	13.7	13.7	0.92	ns	*	ns
sO2m (%)	89.4	88.3	90.0	90.9	87.0	85.8	1.12	ns	*	ns
Urea (mmol/L)	5.32	5.57	4.28	4.24	3.98	3.93	0.122	**	**	ns
Glucose (mmol/L)	4.34	4.34	4.37	4.41	4.32	4.36	0.08	ns	ns	ns

CT, control diet; MS, medium starch diet; HS, high starch diet. $D1$: orthogonal contrast CT vs (MS + HS)/2; $D2$: orthogonal contrast MS vs HS. The interaction DxT was never significant. **: $P < 0.01$; *: $P < 0.05$; †: $P < 0.10$. §Data are presented as raw LSmeans, and the associated P-values are given by statistical analysis of the log-transformed data. pCO_2, partial pressure of CO_2; pO_2, partial pressure of O_2; HCO_3^-, bicarbonate level; sO2m, measured oxygen saturation.

Compared to CT, MS and HS treatments led to an increase of white blood cells (WBC) and platelets (Table 1). Among WBC, both neutrophils and eosinophils increased, whereas monocytes and basophils decreased. Dong et al. (2011) reported that the early hours following grain engorgement are characterized by the rapid growth of Gram-negative bacteria, which undergo cell lysis and lipopolysaccharides (LPS) release following a reduction in the ruminal pH. The translocation of LPS from the digestive tract to the bloodstream leads to the activation of the systemic immune response that is responsible of the increase of neutrophils and eosinophils. The increase of platelets might represent the response to the onset of ruminal lesions consequent to ruminal acidosis (Steele et al., 2009).

The drop in HCO_3^- level in the MS and HS diet is part of the buffering mechanism to contrast the incoming of metabolic acidosis as a result of ruminal acidosis (González et al., 2012),

whereas the reduction in the partial pressure of carbon dioxide (pCO_2) was due to the compensation of the metabolic acid–base imbalance by the respiratory tract, which lowers the pCO_2 to prevent the drop in blood pH through increased ventilation (Dehkordi and Dehkordi, 2011). Significantly lower HCO_3^- and higher platelets values were observed in the HS treatment when compared to the MS one (Table 1), because in the former the ruminal acidosis resulted more severe (Figure 1). The HS diet led also to significantly higher concentrations of red blood cells (RBC), hemoglobin (HGB) and hematocrit (HCT), due to the high ruminal osmotic pressure that pulls fluid from plasma into the rumen and concentrates the blood components during ruminal acidosis. Moreover, during the HS treatment, excess organic acids that accumulated in the rumen were absorbed into the bloodstream at the risk of overwhelming the bicarbonate buffering system (González et al., 2012). This caused a shift in
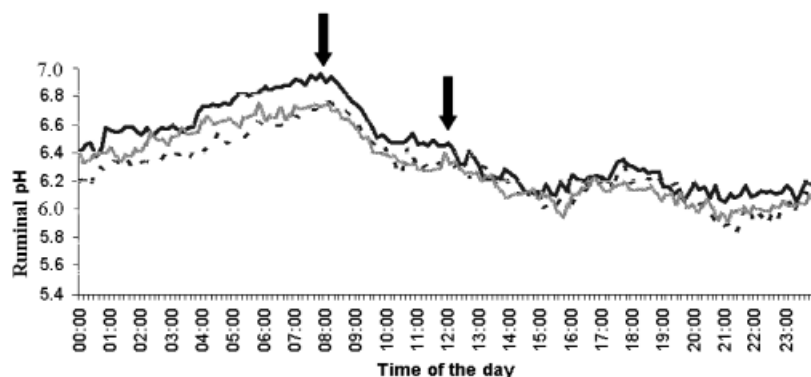
Figure 2. Mean ruminal pH trend at the time of blood collection (CT = control, black continuous; MS = medium starch, black dashed; HS = high starch, gray continuous. Arrows = time of blood collection and feeding (8:00 and 12:00 h))

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the oxyhaemoglobin (O_2Hb) dissociation curve (Jones, 2010) and the red blood cells released oxygen to the tissues more readily, which increased the reduced hemoglobin (RHb) and lowered the O_2Hb and the measured oxygen saturation (sO_2m). The urea levels decreased significantly from CT to HS, in line with the crude protein intake.

Sampling time had no effect on any considered blood variables (Table 1). This result could be explained by the fact that the second blood sampling occurred 4 h after the main meal of the day and, as reported in Figure 2, at 12:00 h the ruminal pH showed a reduction of only 0.4 units and had not reached its lowest value yet. The time interval between the main daily meal and the moment in which the ruminal pH reaches its lowest value (nadir pH) is variable and it is related to the feed distribution management. In this study the nadir pH was reached at 21:00 h, 13 h after the main daily meal.

Conclusions

The severity of ruminal acidosis, measured as the amount of time spent daily below three pH thresholds, affected many of the blood variables that are the expression both of the inflammation and the osmotic pressure at ruminal level and of the activation of metabolic processes aimed to prevent the metabolic acidosis. Variables like RBC, WBC, HCT, platelets, pCO2, HCO_3^-, O_2Hb and sO_2m should be taken into account to investigate on ruminal acidosis in cattle, even though additional studies are necessary to confirm their reliability. The blood sampling time did not affect the results suggesting that one blood daily sample is enough to identify the metabolic condition related to the ruminal acidosis.

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