Udder edema and association with some serum biochemical measurands and dietary factors in first calving cows

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(Received 25 Mar 2015; revised version 13 Jun 2015; accepted 7 Jul 2015)

Summary

The aims of this study were to determine some major biochemical alterations observed in first calving cows with udder edema during the periparturient period and to detect some associations between dietary factors and the disease. For that, the concentrations of some electrolytes (Na^+, K^+, Cl, Ca^{2+}, P and Mg^{2+}), lipid (triglycerides and cholesterol) markers and lipoproteins (HDL, LDL and VLDL) and total proteins were measured in serum samples collected from 70 first calving cows (35 with udder edema and 35 healthy ones) whereas the percentages of dry matter and crude proteins and the electrolyte (Na^+, K^+, Cl, Ca^{2+}, Mg^{2+}, P and sulphates) amounts in feed rations were determined in parallel. The total protein, the calcium and the phosphorus as well as the concentrations of lipid markers and lipoproteins (HDL and LDL) were significantly decreased in first calving cows with udder edema compared to the healthy ones and these biochemical alterations were correlated with a reduced dry matter content and an electrolyte desequilibrium mainly involving Na^+ and Cl^- in feed rations distributed to the cows with udder edema. To our knowledge the mechanism(s) of physiologic udder edema is uncertain and the obtained results suggest that a transient liver dysfunction (decreased total protein and LDL) probably linked to a feed ration deficient in dry matter may be involved in the aetiology of the udder edema in first calving cows.

Key words: Biochemical measurands, First calving cows, Physiologic edema, Udder edema

Introduction

Udder edema is a periparturient disorder characterized by excessive accumulation of fluids in the intercellular tissue spaces of the mammary gland. The highly vascular nature of the bovine mammary gland makes the tissue more prone to developing localized edema (Ghodasara *et al*., 2012). In addition, the developing bovine mammary gland undergoes extensive growth and physical changes during late gestation that likely contributes to the edema development (Zeitlin and Eshraghi, 2002; Melendez *et al*., 2006; Bacic *et al*., 2007). Udder edema causes management problems such as difficulty with milking machine attachment, increased risk for teat and udder injuries and mastitis, and may also reduce milk production (Al-Ani and Vestweber, 1986; Goldberg *et al*., 1992; Bacic *et al*., 2007).

The etiology of udder edema is unclear and may be associated with reduced mammary blood flow and increased intravenous pressure (Al-Ani and Vestweber, 1986; Melendez *et al*., 2006). But some investigators have also shown that early milking and prepartum milking of heifers may reduce udder edema and the incidence of clinical and subclinical mastitis (Waage *et al*., 2001; Bowers *et al*., 2006; Compton and McDougall, 2008).

Udder edema appears in two forms: physiologic (acute) and chronic. Physiologic (acute) edema is usually not painful and occurs within two different clinical stages: at first, a gradual congestion appears under the skin, meanwhile the udder becomes turgid and fills out with colostrum. In the next stage, pitting edema develops symmetrically. Udder edema can become a chronic condition and persist throughout the lactation period (Al-Ani and Vestweber, 1986).

Although the exact mechanism for developing udder edema in heifers is unknown, there have been numerous studies investigating the risk factors related to this condition; one of which being the nutrition regimen. Predisposing causes for udder edema or the increasing severity of the situation include genetics (being a heifer), prepartum concentrations of hormones in plasma such as some steroids, obesity, lack of exercise during the precalving period, excessive energy intake, excessive sodium or potassium intakes, increasing age at first calving, the gender of offspring (having a male calf), calving season (especially winter), increasing heifer height at parturition and higher milk yield (Emery *et al*., 1968; Randall *et al*., 1974; Malven *et al*., 1983; Norgaard *et al*., 2005; Melendez *et al*., 2006). Also, oxidative stress of mammary tissue resulting in reactive oxygen metabolites production may play a role in the formation of udder edema (Bacic *et al*., 2007).

Incidence and severity of udder edema are greater in

pregnant heifers than in dairy cows and tend to be more severe in older rather than in young heifers. In other words, individual conditions may predispose heifers to udder edema (Emery *et al*., 1968; Melendez *et al*., 2006; Bacic *et al*., 2007; Radostits *et al*., 2007; Ghodasara *et al*., 2012). The objectives of this study were to determine and compare some serum components between heifers with udder edema and healthy ones to investigate their relation with dietary factors.

Materials and Methods

Animals

This study was conducted on a total number of 70 first calving heifers (35 healthy and 35 with udder edema) kept in dairy farms. In the first stage, calving time was determined using the date of insemination, ultrasonography and rectal palpation. At calving, the rate of udder edema was recorded. Total feed ration was sampled and whole blood samples were simultaneously collected from the jugular vein of both affected and non affected heifers. Blood samples were centrifuged at 1500 g, and sera were carefully harvested and stored at -20°C until used.

Udder edema was diagnosed by observation, palpating and pitting appearence of udder tissue. Several subjective scoring systems have been developed for evaluating udder edema (Dentine and McDaniel, 1983; Tucker *et al*., 1992; Melendez *et al*., 2006). In the current study, a score of 1 to 4 was assigned to each animal according to the edema extension as described below:

Score 1: Edema just covering the udder

Score 2: Edema extending to the subcutaneous spaces around the udder and navel (Fig. 1)

Score 3: Midline fluid accumulation extending to the brisket

Score 4: Severe edema with marked fluid accumulation in the vulva, mammary gland and extended to the brisket

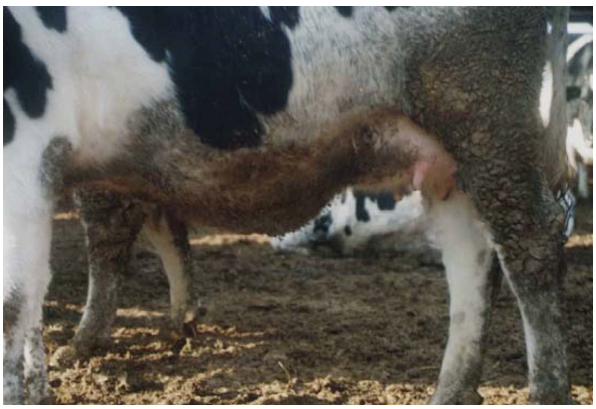

Fig. 1: Score 2 edema: extension of subcutanous edema to the navel area

Biochemical analysis

The serum total protein concentrations were determined using an auto analyzer utilizing a biuret reaction as previously described (Burtis and Ashwood, 1994). After dilution, sera were analyzed for Na^+, K^+, and Ca^{2+} using an atomic absorption spectrophotometer (Shimadzu AA, 670, Japan). Serum Cl^- concentrations were determined using an autoanalyzer according to the method described by Randall *et al*. (1974). Serum phosphorus and magnesium concentrations were measured by colorimetric and flame photometric methods, respectively (Burtis and Ashwood, 1994). Triglycerides, cholesterol, HDL, LDL, and VLDL concentrations were measured using an autoanalyzer (Perkin-Elmer, USA) (Burtis and Ashwood, 1994).

Total feed ration was collected and sent to the laboratory for determining crude protein (CP) and dry matter (Goldberg *et al*., 1992) as well as the Ca, P, Mg, K, Cl, Na, and sulphate contents. The CP analysis was performed according to the macro-Kjeldahl method as described earlier and calculated as percentage of N × 6.25 (Conklin-Brittain *et al*., 2004). Na^+, K^+, Ca^{2+}, and Mg^{2+} were measured by atomic absorption spectrophotometry (model No. 3030, Perkin-Elmer, Norwalk, CT) following acid digestion. Phosphorus was determined colorimetrically using a Beckman DU-60 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) following acid digestion. Chloride was extracted using a combination of acetic acid and nitric acid and was determined by chloridometer (Haake Buchler Instruments, Inc., Saddle Brook) (Chan *et al*., 2006). To determine DM, the food sample was weighed and dried in an oven for 24 h at 105°C. Then, the sample was weighed again, and after calculating the reduced weight, the DM percentage was recorded.

Statistical analysis

Values were reported as mean ± SEM. ANOVA was used to compare differences between healthy and affected heifers. Pearson correlation test was also used to assess the correlation between measurands at confidence of 5%.

Results

The majority (80%) of the affected heifers (n=35) exhibited a mild to moderate udder edema (eleven, i.e. 31.43% with an edema just covering the udder and 17, i.e. 48.57% with lesions around the udder and navel and extended to the subcutaneous spaces). Twenty animals had an important fluid accumulation extending to the brisket (score 3) but no cow showed edema with score 4 (Table 1).

Table 1: Frequency and severity of the udder edema in the affected heifers (n=35)

	Score 1	Score 2	Score 3	Score 4
Number of cases	11	17	7	0
Frequency (%)	31.43	48.57	20.00	0.00

Score 1: Edema just covering the udder, score 2: Edema extending to the subcutaneous spaces around the udder and navel, score 3: Midline fluid accumulation extending to the brisket, and score 4: Severe edema with marked fluid accumulation in the vulva, mammary gland and extended to the brisket

As shown in Table 2, the serum Ca^{2+} and P concentrations were markedly decreased in heifers with udder edema compared to the healthy cows (P<0.02 and P<0.001, respectively) whereas the Mg and electrolyte (Na^+, K^+ and Cl^-) concentrations were not significantly altered. In the same way, the triglycerides (P<0.05), cholesterol (P=0.001) and lipoprotein concentrations (except for the VLDL) (P<0.001 for HDL and P<0.05 for LDL) as well as the total protein (P<0.01) were also dramatically lowered in the affected heifers compared to the healthy controls.

The chemical analysis of the feed rations given to heifers with udder edema or to healthy heifers was summarized in Table 3. It was observed that the DM percentages (P<0.05) and the food Cl (P=0.05) and Na (P<0.02) contents were significantly lower in feed rations distributed to the affected animals than in those given to healthy heifers.

The correlations between some feed ration and serum measurands in heifers with udder edema were presented in Table 4.

Significant correlations were observed between serum calcium and feed ration potassium (r=-0.522; P=0.026), serum triglycerides and feed ration calcium (r=-0.629; P=0.004), serum cholesterol and feed ration dry matter (DM) (r=+0.660; P=0.002), serum HDL and feed ration protein (r=+0.567; P=0.014), and serum total protein and feed ration calcium (r=-0.458; P=0.05).

The correlations between some serum measurands in heifers with udder edema were presented in Table 5.

Table 2: Comparison of biochemical measurands determined in sera from heifers with udder edema (n=35) and from healthy heifers (n=35)

Measurands	Healthy heifers (n=35)	Affected heifers (n=35)	P-value
Na^+ (mmol/L)	139.23 ± 2.71	141.62 ± 2.89	NS
K^+ (mmol/L)	4.35 ± 0.31	4.41 ± 0.47	NS
Cl^- (mmol/L)	102.46 ± 2.75	103.14 ± 3.27	NS
Ca^{2+} (mmol/L)	2.46 ± 0.05	2.30 ± 0.05	<0.02
P (mmol/L)	2.26 ± 0.07	1.86 ± 0.07	<0.001
Mg^{2+} (mmol/L)	0.93 ± 0.03	0.98 ± 0.02	NS
TG (mg/L)	287.7 ± 11.3	260.6 ± 9.0	<0.05
Cholesterol (mmol/L)	4.24 ± 0.35	3.28 ± 0.15	0.001
HDL (mg/L)	902.5 ± 53.7	733.2 ± 21.7	<0.001
LDL (mg/L)	666.4 ± 100.9	475.2 ± 48.5	<0.05
VLDL (g/L)	157.9 ± 14.1	141.7 ± 6.4	NS
Total protein (g/L)	78.7 ± 2.9	71.3 ± 1.2	<0.01

NS: Not significant, TG: Triglycerides, HDL: High density lipoproteins, LDL: Low density lipoproteins, and VLDL: Very low density lipoproteins. Results are expressed as mean ± SEM (standard error of the mean)

Table 3: Comparison of biochemical measurands determined in feed rations given to heifers with udder edema (n=35) and to healthy heifers (n=35)

Measurands	Healthy heifers (n=35)	Affected heifers (n=35)	P-value
Dry matter (%)	20.61 ± 1.32	17.82 ± 1.46	<0.05
Crude protein (%)	14.67 ± 1.06	14.30 ± 1.22	NS
P (%)	0.277 ± 0.018	0.230 ± 0.013	NS
K^+ (%)	0.817 ± 0.061	0.929 ± 0.070	NS
Ca^{2+} (%)	0.678 ± 0.038	0.607 ± 0.039	NS
Mg^{2+} (%)	0.496 ± 0.036	0.521 ± 0.047	NS
Cl^- (%)	0.727 ± 0.047	0.609 ± 0.058	=0.05
Na^+ (%)	0.135 ± 0.021	0.090 ± 0.010	<0.02
HSO_4^- (%)	0.725 ± 0.070	0.686 ± 0.078	NS

NS: Not significant. Results are expressed as mean ± SEM (standard error of the mean)

Table 4: Significant correlation between some ration and serum measurands in heifers with udder edema

Serum	Ration			
	Protein (%)	DM (%)	K^+ (%)	Ca^{2+} (%)
Ca^{2+} (mg/dl)	-	-	r=-0.522, P=0.026	-
Triglycerides (mg/dl)	-	-	-	r=-0.629, P=0.004
Cholesterol (mg/dl)	-	r=+0.66, P=0.002	-	-
HDL (mg/dl)	r=+0.567, P=0.014	-	-	-
Total protein (g/L)	-	-	-	r=-0.458, P=0.05

Table 5: Significant correlation between some serum measurands in heifers with udder edema

Measurands	P (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Mg^{2+} (mg/dl)	r=+0.37, P=0.031	-	-	-
Triglycerides (mg/dl)	-	-	-	r=+0.38, P=0.026
Cholesterol (mg/dl)	-	r=+0.386, P=0.024	r=+0.51, P=0.002	r=+0.93, P<0.00001

Significant correlations were observed between serum Mg and phosphorus (r=+0.37; P=0.031), serum triglycerides and LDL (r=+0.38; P=0.026), serum cholesterol and LDL (r=+0.930; P<0.00001), serum cholesterol and HDL (r=+0.510; P=0.002), and serum cholesterol and triglycerides (r=+0.386; P=0.024).

Discussion

The dry period is characterized by dramatic changes in the body status. These changes prepare cow for lactogenesis and parturition and also can result in the development of metabolic disorders such as fatty liver, ketosis, udder edema, milk fever, metritis, displacement of the abomasum, and rumen acidosis (Bacic *et al*., 2007). It was observed in the present study that the circulating concentrations of lipids (triglycerides and cholesterol) and of lipoproteins (HDL and LDL) as well as the proteinemia and the concentrations of some electrolytes (calcium and phosphorus) were significantly declined in heifers with udder edema compared to the healthy ones. To our knowledge the mechanism(s) of physiologic udder edema is uncertain and consequently, these measurands would be involved in the udder edema formation.

In agreement, previous studies have shown that in young and old animals, a decrease in the serum total protein and albumin concentrations occurred and in the final stages, edema in different organs can be observed due to the low concentrations of blood proteins (Radostits *et al*., 2007). Larson and Kendall (1957) have demonstrated that the proportions of some specific circulating proteins declined during the parturition and contributed to the development of hypoproteinemia. These authors have observed that around 14 weeks before parturition, the proteinemia in cattle started to increase, reaching a maximum around 4 weeks before parturition then started to decrease and reach minimal values at parturition (Larson and Kendall, 1957).

Within the periparturient period, many cows exhibit decreased dry matter intake (DMI), due to periparturient disease and loss of hepatic function (Shibano and Kawamura, 2006). In this way, it was reported that the amounts of hepatic lipids were increased whereas the hepatic output pathways were reduced leading to decreases in the circulating concentrations of total lipids, triglycerides, cholesterol and lipoproteins (Bobe *et al*., 2004; Radostits *et al*., 2007). Accordingly, the decreases in the serum triglycerides, cholesterol, HDL and LDL concentrations observed here would result from a transient impairment of the liver function during the periparturient period.

In the current study, significantly lower serum Ca^{++} and P concentrations were recorded in heifers with udder edema. However, to our knowledge, the electrolyte balance was not previously explored in cows with udder edema but may be directly affected by the diet regimens (Vigue, 1963). Investigation of dietary factors in the current study indicated that the amounts of DM, Cl, and Na in the feed rations for affected heifers were significantly lower than in feed rations distributed to healthy heifers (P<0.05). Nevertheless, the involvement of the dietary Na amount in the udder edema formation is highly controversial. Indeed, although Hemken *et al*. (1960), Randall *et al*. (1974) and Van der Kolk (1998) demonstrated that salt (sodium chloride) and water restriction reduced the severity of udder edema and that Johnson and Otterby (1981) and Sanders and Sanders (1981) showed that excessive intakes of sodium, potassium and grains in the prepartum period have been associated with the development of udder edema in dairy cattle, the excessive intake of nutrients such as sodium before parturition were not associated with increased udder edema in some other studies (Greenhalgh and Gardner, 1958; Schmidt and Schultz, 1959; Mashek and Beede, 2000).

Considering the dietary cation-anion differences (DCAD), Chan *et al*. (2006) showed that a dietary Ca content of 0.99% appeared to be adequate when prepartum cows were fed with an anionic diet in which the DCAD was -6 mEq/100 g of DM. In the present study, the DCAD in feed rations given to healthy heifers and to heifers with udder edema were -16.88 and -39.14, respectively and the desequilibrium between anions and cations in the diet was aggravated by the low Na and Cl amounts in the feed ration. Therefore, the dietary Ca and other cation supply would be markedly insufficient in diets given to heifers with udder edema. In agreement with that, the calcemia in heifers with udder edema was significantly lowered compared to the healthy animals. Shahzad *et al*. (2011) stated that feeding high anion and low anion diets prepartum can be a useful nutritional tool to minimize or prevent the incidence of milk fever and controlling udder edema in buffaloes.

Having better accuracy, the ratio K/(Ca + Mg) has to be considered. Indeed, Sanders and Sanders (1981) showed that potassium fertilization in order to improve alfalfa production can be the cause of increased udder edema. Nestor *et al*. (1988) have also confirmed that the addition of $KHCO_3$ to the diet increased the severity of udder edema, but they noted that the exact mechanism causing edema could not be determined. However, in the present study, the ratios K/(Ca + Mg) in the diets for healthy heifers and affected ones were 0.70 and 0.82, respectively. These results indicated that the dietary K supply was not in excess and that the dietary K was probably not involved in the formation of this disease.

Schmidt and Schultz (1959) noted that there were no statistically significant differences in the severity of udder edema in dairy cows fed with different levels of grains during the dry period for 2 years (Schmidt and Schultz, 1959). Greenhalgh and Gardner (1958) also have not recorded any noticable differences in the severity of udder edema among cows and heifers that were fed with basal roughage and those having had 4 kg concentrate in their diet. However, Hathaway *et al*. (1957), Hemken *et al*. (1960), and Johnson and Otterby (1981) observed a significant difference in the severity of udder edema among heifers that were fed with different amounts of concentrate before parturition. In the current

study, the low level of dietary DM in feed rations for affected heifers can be due to limited amounts of basal roughage, which can exacerbate the sensitivity of heifers to the formation of udder edema.

As a conclusion, based on our findings, the hypoproteinemia coupled to a low DM intake and to transient impairment in the liver function evidenced throughout low circulating lipid and lipoprotein concentrations may be directly involved in the udder edema formation in heifers.

Conflict of interest

The authors declare that they have no conflict of interest.

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