

Venous and arterial ammonia in dairy cows with fatty liver and hepatic failure

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ABSTRACT: In the present study we aimed to compare the arterial and venous plasma ammonia in dairy cows with fatty liver and hepatic failure. 75 Holstein-Friesian dairy cows were used in the investigation. Clinical examinations revealed liver failure in 14 cows (group LF). Diagnosis of hepatic failure was based on the clinical signs of hepatic encephalopathy, including anorexia, depressed consciousness, ataxia, somnolence, and coma, and on venous plasma ammonia $>35 \mu\text{mol/l}$. The other cows were divided according to their liver triglyceride content (wet tissue) into the following groups: low triglycerides (LT) $<30 \text{ mg/g}$ ($n = 24$), medium triglycerides (MT) $30\text{--}100 \text{ mg/g}$ ($n = 31$), and high triglycerides (HT) $>100 \text{ mg/g}$ ($n = 6$). Both arterial and venous plasma ammonia levels were highest in cows with hepatic failure (93.0 ± 44.9 and $70.4 \pm 34.1 \mu\text{mol/l}$, respectively). We observed that arterial ammonia was significantly higher than venous ammonia ($P < 0.01$) only in the dairy cows suffering from liver failure, implying a higher informative value of arterial ammonia in detection of liver function damage in cattle demonstrating signs of hepatic encephalopathy.

Keywords: dairy cows; blood ammonia; fatty liver; liver failure; hepatic encephalopathy

A depressed energy intake associated with high milk production in dairy cows, characteristic of the postparturient period, triggers an increased fat mobilisation, with fatty liver as a frequent consequence (Stober and Scholz, 1991). Accumulation of fat in the liver occurs when the rate of hepatic fatty-acid esterification exceeds the capacity of the liver to secrete the re-established triglycerides as a part of very low density lipoproteins. Fatty liver syndrome of periparturient dairy cows is a very common condition and is often associated with impaired health, fertility, and production (Reid, 1980). Frequently, cows suffering from fatty liver can develop hepatic failure (Rehage, 1996). However, it was shown that this development does not exclusively depend on the rate of simple triglyceride accumulation in the liver (Meier, 1992). Both necrotic and degenerative changes can be involved in this process. The economic significance of liver diseases in dairy cows, related to lipid mobilisation, has led to the development and establishment of numerous diagnostic methods for detection of liver damage. In the last decade, blood ammonia determination was introduced into the laboratory diagnostics of liver dysfunctions in cattle (Rehage, 1996).

Ammonia is produced primarily in the gastrointestinal tract by bacterial degradation of amines, amino acids, and purines, by the action of bacterial and intestinal urease on urea, and by the catabolism of glutamine by enterocytes (Dimski, 1994). The main source of blood ammonia in dairy cows is bacterial activity in the rumen and catabolism of amino acids in the tissue. Ammonia produced in the rumen then diffuses readily into the portal circulation from which it is efficiently extracted and converted to urea by the liver or glutamine by the liver and other organs, including muscles and brain (Fraser and Arieff, 1985; Haussinger, 1990). These two systems work in a tight correlation, so that any ammonia escaping from ureagenesis can be subsequently removed by glutamine synthetase (Zhu et al., 2000). However, when there is liver dysfunction or portosystemic shunting of blood, ammonia is inefficiently metabolised, and high systemic ammonia concentrations may result (Taboada and Dimski, 1995). As the ammonia is a neurotoxin that exerts direct toxic effects on inhibitory and excitatory neurotransmission in the brain, its analysis is particularly useful in cases when signs of hepatic encephalopathy have been noted (Rehage, 1996).

The determination of blood ammonia levels thus provides a useful index of hepatic function.

The objective of the present study was to establish whether there exists any difference between arterial and venous plasma ammonia in dairy cows with fatty liver and hepatic failure.

MATERIAL AND METHODS

Seventy-five Holstein-Friesian dairy cows (different lactation periods), admitted to the Clinic for Diseases of Cattle in Hannover, were used in the study. Sixty-one animals were referred to the Clinic because of the left displacement of the abomasum (LDA) and suspected hepatic disease. Fourteen lactating, non-pregnant healthy dairy cows, 3–5 months after parturition, were purchased for the surgical training of students (rumenotomy). Clinical examinations revealed liver failure in 14 cows (LF). The diagnosis of the hepatic failure was based on the clinical signs of hepatic encephalopathy including anorexia, depressed consciousness, ataxia, somnolence, and coma, and on the venous plasma ammonia $>35 \mu\text{mol/l}$ (Rehage, 1996). The intensive treatment of these cows started immediately after blood samplings and liver tissue biopsies (infusions of glucose, glucocorticoids, sodium propionate). The other cows were divided according their triglyceride liver content into the following groups: low triglycerides (LT) $<30 \text{ mg/g}$ ($n = 24$), medium triglycerides (MT) $30\text{--}100 \text{ mg/g}$ ($n = 31$), and high triglycerides (HT) $>100 \text{ mg/g}$ ($n = 6$).

A single blood sample for ammonia analysis, in cows with LDA on day 5 after omentopexy (clinically healthy and discharged from the clinic), and in other cows within 24 hours of admission, was drawn from the jugular vein and from the axillary artery (Nagy et al., 1998). At the same time, liver samples for triglyceride determination were

obtained by percutaneous needle biopsy (Scholz et al., 1989). The plasma concentrations of ammonia (Sigma) were measured on an automated analysing system (Cobas Mira®). The liver triglyceride contents were determined enzymatically in wet tissue (Bickhardt et al., 1988).

Statistical analysis was carried out by one-way analysis of variance. In cases where the ANOVA model revealed a significant group effect Student's *t*-test was performed in order to check differences between the groups. Pair *t*-test was used for assessment of differences between venous and arterial ammonia within the groups.

RESULTS

Dullness, depression and anorexia were the most common clinical signs observed in dairy cows suffering from hepatic encephalopathy. Six cows responded to the treatment and recovered, whereas eight animals in this group failed to recover and died within a week of the admission.

Biochemical examination of liver triglycerides (selection criterion) in cows without liver failure revealed liver lipidosis in 60.7% cases, i.e. 37 dairy cows had their liver triglyceride content higher than 30 mg/g wet tissue (Table 1). The ANOVA did not reveal a significant difference in hepatic triglycerides between the cows with hepatic failure and the group of cows with severe fatty liver ($>100 \text{ mg/g}$). However, there was a distinct variation in liver triglycerides among animals in the liver failure group (Table 1).

As venous plasma ammonia was a selection criterion for the diagnosis of liver failure, the highest venous ammonia levels ($70.4 \pm 34.1 \mu\text{mol/l}$) were found in the group with liver failure (Table 1). Moreover, venous ammonia in this group differed significantly from ammonia in the other three

Table 1. Concentrations of liver triglycerides and venous and arterial ammonia in cows with different liver triglyceride content and liver failure (mean \pm s)

Index	$<30 \text{ mg/g}$ ($n = 24$)	$30\text{--}100 \text{ mg/g}$ ($n = 31$)	$>100 \text{ mg/g}$ ($n = 6$)	Liver failure ($n = 14$)	ANOVA $P <$
Triglycerides (mg/kg)	14.0 ± 6.61^a	53.5 ± 17.2^b	117.0 ± 15.6^c	98.0 ± 45^c	0.01
Venous ammonia ($\mu\text{mol/l}$)	21.7 ± 8.04^a	27.3 ± 10.7^a	22.2 ± 21.9^a	70.4 ± 34.1^b	0.01
Arterial ammonia ($\mu\text{mol/l}$)	24.8 ± 8.70^a	30.8 ± 11.3^a	26.6 ± 8.46^a	$*93.0 \pm 44.9^b$	0.01

means with different superscripts within one row differ significantly ($P < 0.05$)

*venous ammonia differs significantly in the group from arterial ammonia ($P < 0.05$)

groups ($P < 0.01$). There were no significant differences in venous ammonia between animals with different liver triglycerides (group LT–HT).

A similar pattern of ammonia concentrations could also be observed in the plasma drawn from the axillary artery. We could not see any differences in arterial ammonia between triglyceride groups (LT–HT) but they all differed significantly from arterial ammonia in the group with liver failure (Table 1).

The difference between venous and arterial plasma was approximately 3–4 $\mu\text{mol/l}$ ($P > 0.05$) in the triglyceride groups (without clinical signs of liver failure) and 22.6 $\mu\text{mol/l}$ in the group with hepatic failure ($P < 0.05$).

DISCUSSION

As suspected, the high proportion of cows with abomasal displacement involved in our investigation resulted in a relatively high percentage (67%) of animals with increased liver triglycerides. However, this liver lipidosis was not associated with any clinical signs. The left displacement of the abomasum is frequently associated with an increase in the hepatic fat content (Holtenius and Niskanen, 1985; Muylle et al., 1990; Mudron et al., 1997). The group of cows with clinical signs of hepatic encephalopathy showed an almost severe degree of liver fat accumulation, which is achieved when liver triglycerides are higher than 100 mg/g wet tissue (Gaal and Husveth, 1983; Bogin et al., 1988). The finding of the severe degree of liver fat accumulation in six clinically healthy animals indicates that simple liver lipidosis, even the most severe one, is only one of the factors contributing to the development of liver failure. We can agree with West (1997), who stated that the degree of fatty infiltration of the liver provided a valuable guide to prognosis. However, this is valid only for cows showing signs of hepatic encephalopathy as in plenty of dairy cows with the undisturbed health status liver triglycerides higher than 100 mg/g may be found. Thus, the separate determination of liver triglycerides does not provide complex information about the functional capability of the liver.

Increased ammonia concentrations in the plasma of the cows with liver failure demonstrate a strong inhibition of the urea synthesis in the liver, and thus hyperammonaemia can be used as a specific indicator of hepatic diseases. This phenomenon was observed in cattle several decades ago (Sherlock, 1968; Wolff et

al., 1972). Recently, in series of experiments on dairy cows with fatty liver and liver failure, venous plasma ammonia of 29 $\mu\text{mol/l}$ was postulated as the maximal physiological concentration for cattle (Rehage, 1996). However, it was shown that the venous plasma ammonia could reach even higher levels in cows around calving, with the maximum ($64.1 \pm 5.4 \mu\text{mol/l}$) seen in 16 hours after parturition (Zhu et al., 2000). As the high levels of plasma urea were found at the same time as that of ammonia, the authors postulated the capacity of ureagenesis might have reached its limit immediately after calving. The levels of venous plasma ammonia in their study oscillated around the value of 30 $\mu\text{mol/l}$ approximately a month before calving and the same period after it (33.4 ± 5.0 and $28.1 \pm 5.4 \mu\text{mol/l}$, respectively). Concerning the complex body vein system, the highest mean plasma concentrations of ammonia in cattle were found in the portal vein. Braun et al. (2000) demonstrated in nonlactating dairy cows 19.3 times higher mean concentrations of ammonia in portal than in jugular blood (290 $\mu\text{mol/l}$ and 15 $\mu\text{mol/l}$, respectively), which corresponded to physiological processes in the bovine gastrointestinal tract.

In our study, severe hepatic lipidosis (liver triglycerides above 100 mg/g) in dairy cows without clinical signs of hepatic encephalopathy was associated with neither increased mean venous nor arterial ammonia in the blood plasma. Significantly higher plasma values of both venous and arterial ammonia could only be recognised in animals with signs of health impairment, like dullness and depression. The fact that the only significant differences between venous and arterial ammonia were found in the cows with liver failure also shows that a simple accumulation of triglycerides in the liver is not always linked with a substantial suppression of liver functions. Rehage et al. (1999) did not see any significant differences in venous ammonia concentrations between dairy cows with mild, moderate, and severe fatty liver, but all these three groups differed in blood ammonia from the dairy cows with liver failure. Moreover, in contrast to venous ammonia values, they did not find any differences between the cows with liver failure and the cows with severe fatty liver in blood indices commonly used for diagnosis of liver damage, including AST, γGT , GLDH, total bilirubin, albumin, NEFA, β -hydroxybutyrate, cholesterol, and NEFA. The results of the present study demonstrate that the intensity of extrahepatic consumption of ammonia for glutamine synthesis in muscles and brain can strongly increase when

the blood ammonia is excessively elevated but is not sufficient to keep ammonia levels within the physiological range.

In conclusion, we observed that arterial ammonia was significantly higher than venous ammonia only in the dairy cows suffering from liver failure, implying a higher informative value of arterial ammonia in detection of liver function damage in cattle, especially if clinical signs of hepatic encephalopathy are recognised.

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