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Investigation of the Possible Role of Endotoxin, TXA2, PGI2 and PGE2 in Experimentally Induced Rumen Acidosis in Cattle

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Haubro Andersen, P. and N. Jarløv: Investigation of the possible role of endotoxin, TXA2, PGI2 and PGE2 in experimentally induced rumen acidosis in cattle. Acta vet. scand. 1990, 31, 27-38. - Rumen acidosis was induced experimentally with 70 g barley/kg b.w. in 2 rumen fistulated cows. The cows were followed for 80 h after the grain engorgement. Endotoxin was monitored in cell-free ruminal fluid and peripheral plasma together with inflammation mediators TXA2, PGI2 and PGE2 and several clinical and clinical-chemical parameters. The results do not support the theory of systemical endotoxemia due to a large increase in rumen endotoxin concentration in cattle suffering from rumen acidosis. However, both clinical and clinical-chemical data suggest that an endotoxicosis developed, but the levels of inflammation mediators TXB2, FGI2 and PGE2 were not significantly elevated in the peripheral circulation. An absorbtion of endotoxins and synthesis of inflammation mediators are therefore suggested to take place prehepatically.

Limulus amoebocyte lysate test; hypozincaemia; cortisol, stress-response; rumen endotoxin.

Introduction

Roughage is the natural diet of the ruminant. However, the almost non-physiologically high milk production of the modern dairy cow is only maintained by rations containing various mixtures of grain, proteins and fat (concentrates). Epidemiological investigations have associated high concentrate feeding regimes with production diseases such as abomasal displacement, the fatty liver syndrome, laminitis and the low-fat milk syndrome (*Coppock* 1972, *Dougherty* 1976, *Engvall* 1980, *Hesselholt et al.* 1982, *Gerloff et al.* 1986). Rumen acidosis, defined as a digestive disturbance with a non-physiological depression of the pH of the rumen contents, has often been proposed to play a central role in the pathogenesis of production diseases, as this clinical disease complex is initiated by rations rich in readily digestible carbohydrates and/or low in crude fiber level (*Dirksen* 1970). The acidotic rumen environment has been investigated by several authors in attempts to determine one or more toxic factors responsible for the clinical symptoms. Particular attention has been paid to the accumulation of D(-) lactic acid, ethanol, tyramine, histamine and endotoxin (*Dougherty & Cello* 1949, *Turner & Hodgetts* 1955, *Allison et al.* 1964, *Ahrens* 1967, *Dunlop* 1972, *Koers et al.* 1976). Of these single factors, only the experimental administration of endotoxin seems to elicit all the typical clinical signs of acute rumen acidosis.

Although the presence of large amounts of

endotoxin in both normal and acidotic ruminal fluid has been demonstrated (*Nagaraja et al.* 1978a, *McManus et al.* 1978), no convincing evidence has been given for the development of an endotoxemic condition in grain engorged cattle (*Dougherty* 1975, *Andersen* 1985). A hypothetical reason for this may be that any endotoxin absorbed to the portal circulation is cleared rapidly by the liver and that the clinical signs usually related to endotoxaemia are a result of release of local inflammation mediators, e.g. arachidonic acid metabolites as thromboxane A2 (TXA2), prostacycline (PGI2) and prostaglandine E2 (PGE2) in the hepatic and prehepatic tissues and circulatory system. Investigations on experimental endotoxemia in horses, pigs, dogs, primates and cattle have described the pathophysiological role of the arachidonic acid metabolites TXA2, PGI2 and PGE2 and elucidated their firm relationship to the development of the clinical syndrome endotoxicosis (*Bottoms et al.* 1982, *Bottoms et al.* 1983, *Harris et al.* 1980, *Schrauwen et al.* 1983, *Jarløv et al.* 1988). Administration of endotoxin induces increased synthesis of TXA2, PGI2 and PGE2, and the increases in plasma concentration of these metabolites have been shown to persist for several hours after endotoxin clearance from the systemic circulation (*Andersen et al.* 1988, *Jarløv et al.* 1988). As the biological half-lives of the arachidonic acid metabolites are short, their synthesis have to continue post challenge. An increase in plasma concentration of these mediators in grain engorged cattle might therefore indicate that an endotoxemic state has occurred, despite a possible lack of demonstration of endotoxemia by the Limulus assay. The aim of the present work has therefore been to investigate if peripheral endotoxemia may be detected in the experimental rumen acidosis and to determine if this disease is accompanyed by systemic increases of plasma concentrations of the inflammation mediators TXA2, PGI2 and PGE2.

Materials and methods

Animals

Two adult, non-lactating Jersey cows (body weight 340 and 300 kg) were rumen fistulated. The experiment was performed after a 4 week recovery period, where the cows were maintained on a hay diet. Immidiately before the start of the experiment, the cows were equipped with an indwelling 16G catheter in the left jugular vein.

Experimental design

During a control period from 0.00 to 24.00 hours, baseline clinical and clinical-chemical parameters (rectal temperature, pulse, respiration rate, ruminal movements and pH, standard base excess (SBE), packed cell volume (PCV), lactate, leukocyte and thrombocyte counts, cortisol and zinc plasma concentrations) were determined together with endotoxin in plasma and ruminal fluid and plasma TXA2, PGI2 and PGE2.

Immidiately after the control period (hours 0.00), a rumen acidosis was induced with 70 g/kg b.w. of ground barley (*Dougherty et al.* 1975). The cows were offered the barley as well as water ad libitum in order to imitate a spontaneously occurring grain engorgement. Any remanining barley was administered through the rumen fistula 30 min after feeding. Clinical monitoring and sampling of blood were continued from 0.00 to 80.00 hours according to the sampling charts given in Tables 1-4 and Figs. 2-6. Rumen fluid for pH and endotoxin determination was sampled at -24.0, -23.5, -22.0, -21.0, -19.0, -15.5, -16.0, -14.0, -12.0, -10.0 (control), 0.0, 0.5, 2.0, 3.0 and 4.5 hours, every hour from 6.0 to 15.0 hours and at 18.0, 21.0 (day 1) 24.0, 28.0, 32.0, 36.0 (day 2), 48.0, 56.0 (day

3), 72.0 and 80.0 hours (day 4). The rumen endotoxin results are given as means + standard deviation per day.

Sampling for the Limulus Amoebocyte Lysate (LAL) assay

Blood samples for endotoxin determinations were drawn in sterile, pyrogen free 10 ml syringes under sterile precautions. The samples were stabilized with 10 i.e. of heparin (DAK 100 i.e./ml) and centrifuged immidiately. Plasma was diluted 1 + 2 with sterile pyrogen free water (Atlas, Sussex, UK), heat extracted for 5 min at 100°C and stored at -22°C until testing.

Rumen fluid for endotoxin determination was obtained directly from the ventral sac of rumen after stirring of the content with the arm. The fluid was centrifugated immeidiately at 3000 r.p.m. for 10 min and the supernatant was filtered through 0.2 nm sterile non-pyrogenic filters (Millipore) and stored in glass tubes below -20°C. Before testing, appropiate dilutions were made with water (Atlas, Sussex, UK) and pH was determined in a sample from each dilution to exclude pH-interference with the LAL assay. As the ruminal fluid in general was diluted 10^3-10^6 times before testing, interference with other compounds in the ruminal fluid was considered to be of little relevance.

LAL-assay

The LAL assay was performed as a regular tube test (following the directions for use by the manufacturer) combined with rocket immunoelectrophoresis of the incubate, using a specific antibody to coagulogen to determine the reaction between endotoxin and LAL. The method records the last step in the coagulation mechanism of the lysate and improves the sensitivity and reproducibility several times (*Bæk* 1983). The electrophoresis was run with antibody 6.5 µl/ml in the whole gel with 10 V/cm for at least 5 h. The plates were stained with Coomassie brilliant blue and the rockets measured. If the rockets were either too high or too low, an appropriate dilution of the sample was tested. The mean of 2 replicates of each sample was compared to a standard curve produced with standard control endotoxin. Limulus Amoebocyte Lysate (Pyrotell lot #99-58-401) and standard control endotoxin (*E. coli* 0113: H10:K neg. lot # 36) was obtained from Ass. of Cape Cod, Inc. MA, USA, and the sensitivity with the described method was determined to be 0.03 EU/ml in our laboratory. Coagulogen antibody (0886) was obtained from DakoPatts, Copenhagen, Denmark. Laboratory glassware, syringes, needles, catheters and other supplies were either of commercial non-pyrogenic type or depyrogenised in dry heat at 250°C for at least 6 h.

TXA2, PGI2 and PGE2

Plasma concentrations of the stable hydrolysis products TXB2, 6-keto PGF and bicyclic PGE2 were determined by use of commercial RIA Kits (Amersham TRK code 780, 790 and 800) and managed as described by the manufacturer. The measured concentrations indirectly reflect the concentrations of TXA2, PGI2 and PGE2, respectively (*Granstrøm et al.* 1976, *Demers & Derck* 1980, *Fitzpatrick* 1980). To prevent spontaneous mediator release, blood samples were collected in tubes containing EDTA and indomethacin. Before each collection, approximately 100 ml blood were allowed to flush the catheter in order to minimize catheter-and trauma-induced arachidonic acid metabolism. Immediately after collection the blood samples were centrifuged, and the plasma samples were stored below -30°C. Prior to the RIA, plasma extraction was performed at SepPak C-18 columns (Waters) as described elsewhere (*Powell* 1980).

Clinical-chemical blood analysis

Thrombocyte counts were determined by means of phase contrast microscopy (*Housted* 1976). Plasma zinc concentrations were measured by atomic absorbtion spectrophotometry (*Makino & Takahara* 1981). SBE was determined on an automatic blood gas analyzer (ABL 4, Radiometer, Denmark). Plasma cortisol concentrations were determined by a RIA kit (Farmos, Finland). Leukocyte counts, glucose, lactate and PCV were determined according to routine clinical-chemical methods.

Results

Clinical symptoms

Four hours after the experimental overfeeding, the first clinical symptoms appeared: rumen motility was slow and irregular, and rumen pH decreased from above 7 to below 6 in both cases. The rumen pH decreased continuously and was below 5 approximately 15 h after the feeding (Fig. 1). At this time, the normal appearance of the rumen contents (stratified with a green-brown colour and an aromatic smell) was changed to a uniform grey-yellowish sour smelling soup. Both cows developed diarrhoea and signs of dehydration and shock (pulse rate, PCV and capillary refill time increased and the animals were acidaemic and lactaemic (Tables 1, 2, 3 and 4). Twenty hours after feeding both cows were depressed and unable to stand up. The clinical picture did not change significantly for the subsequent 24 h. Forty-four hours after the overfeeding, weak rumen movements were detected, the cows were able to stand up and the restitution began. Onset of fever (temperature increases above 1°C) was modest and only noted for cow B between 11 and 14 h after feeding.

Endotoxin determinations

Endotoxin was not detected in any of the plasma samples. The ruminal endotoxin concentration, which averaged 1500-1600

Table 1. Cow A. Key clinical and clinical-chemical parameters.

Hours	Rectal Temp. °C	Pulse rate beats/min.	Respiration rate resp./min.	PCV %	Venous pH	SBE mmol/l
-23.5	38.2	56	23	25	7.402	4.7
-16.5	38.0	55	26	25	7.374	2.4
-10.0	38.0	55	23		7.351	1.0
0.5	38.2	60	36	30	7.385	2.5
2.0		60	44	28	7.363	0.7
4.5	38.6	60	31	32	7.360	0.5
7.0	38.6	60	24	33	7.293	-4.4
11.0	38.5	54	30	37	7.245	-6.9
12.0					7.215	-7.5
13.0	38.9	60	24	36	7.214	-9.1
14.0	38.8	72			7.181	-9.0
18.0	38.3	68	30	40	7.124	-13.0
21.0	38.2	76	32	41	7.062	-17.2
24.0				45	7.014	-17.7
28.0	36.3	44	18	48	7.033	-18.4
36.0	37.0	85	23	45	7.096	-17.6
48.0	38.4	88	32	40	7.312	- 7.0
72.0	38.5	60	28	40	7.301	- 3.8
80.0				38	7.386	2.1

Table 2. Cow B. Key clinical and clinical-chemical parameters.

Hours	Rectal Temp. °C	Pulse rate beats/min.	Respiration rate resp./min.	PCV %	Venous pH	SBE mmol/l
-23.5	38.0	53	18	28	7.416	6.0
-16.5	38.6	55	23	26	7.376	3.5
-10.0	38.1	50	28		7.302	- 1.7
0.5	38.5	60	42	30	7.344	0.5
2.0	38.2	60	42	28	7.320	- 0.2
4.5	38.2	60	24	32	7.376	1.1
7.0	38.5	60	32	29	7.356	0.4
11.0	39.1	54	24	28	7.282	- 2.6
12.0					7.263	- 3.1
13.0	39.7	60	27	30	7.253	- 5.7
14.0	39.9	76	26	30	7.226	- 8.0
18.0	37.8	72	33	40	7.162	-11.4
21.0	37.6	72	32	41	7.144	-14.1
24.	38.1	90	32	45	7.133	-15.4
28.0	37.3	64	16	50	7.173	-13.2
36.0	37.1	92	32	45	7.198	-11.1
48.0	37.9	96	32	40	7.215	- 9.3
72.0	37.0	60	28	40	7.335	1.8
80.0				36	7.303	- 3.2

Table 3. Cow A. Plasma glucose, lactate and cortisol concentrations.

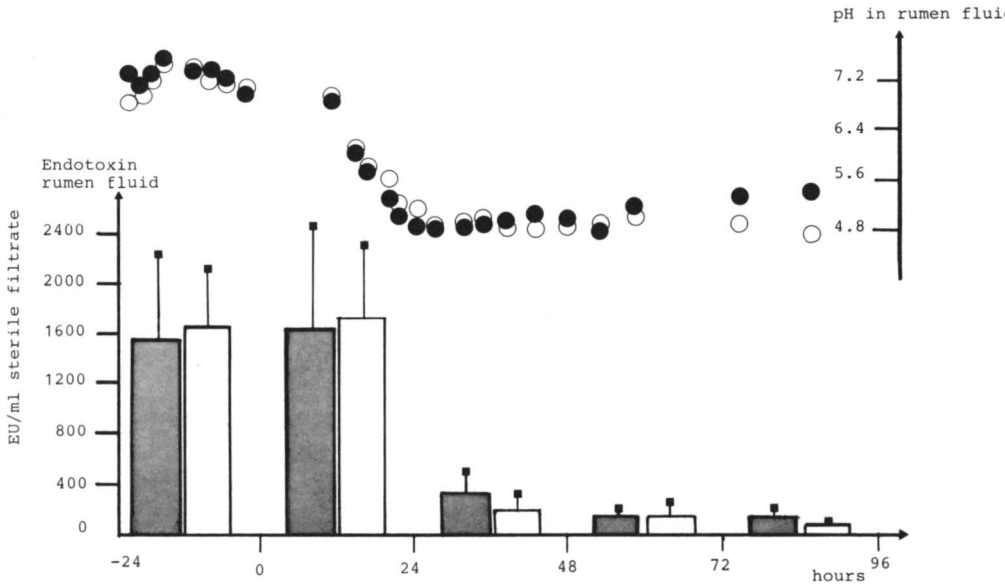
Hours	Glucose mmol/l	Lactate mmol/l	Cortisol ng/ml
-23.5	3.63	1.13	-
-21.0	3.96	0.83	<3.63
-16.5	3.91	0.72	<3.63
-14.0	3.64	0.52	<3.63
-10.0	3.75	0.37	<3.63
0.5	3.91	0.54	-
3.0	3.78	1.27	<3.63
6.0	4.13	0.63	<3.63
8.0	4.12	0.83	<3.63
11.0	3.88	0.92	9.36
15.0	3.57	0.73	7.92
24.0	3.03	1.27	9.81
32.0	2.70	2.88	38.16
48.0	3.94	2.00	31.32
56.0	3.78	1.51	18.36
72.0	4.47	1.38	11.16
80.0	4.42	0.87	18.36

-: not analyzed.

Table 4. Cow B. Plasma glucose, lactate and cortisol concentrations.

Hours	Glucose mmol/l	Lactate mmol/l	Cortisol ng/ml
-23.5	3.73	0.75	<3.63
-21.0	3.75	0.55	<3.63
-16.5	3.69	0.54	<8.34
-14.0	3.84	0.52	4.68
-10.0	3.90	0.35	3.96
0.5	3.86	0.81	<3.63
3.0	4.31	0.61	4.68
6.0	4.31	0.61	<3.63
8.0	4.14	0.77	<3.63
11.0	4.48	1.03	30.60
15.0	3.92	1.51	22.32
24.0	3.66	2.17	23.40
32.0	4.00	1.72	19.44
48.0	4.44	1.58	30.96
56.0	4.64	0.90	26.94
72.0	4.82	1.50	27.00
80.0	4.40	2.19	9.36

-: not analyzed.



Legend for Fig. 1
Alterations in rumen pH and endotoxin concentration.
● Rumen pH cow A
□ Rumen endotoxin concentration cow A
○ Rumen pH cow B
□ Rumen endotoxin concentration cow B

EU/ml of sterile filtrate in the observation period, did not change significantly day 1 after the grain engorgement. On days 2-4 after the overfeeding, the concentrations had diminished to less than 25% of the control values (Fig. 1).

Thrombocyte, leukocyte and Zinc determinations

Thrombocyte counts decreased significantly at 4.5 and 11 h in cow A and B, respectively (Figs. 2 and 3). Both cows showed an initial leukophilia at 24 h followed by a decrease to the baseline range or slightly below at 48 h. Plasma Zinc concentrations showed a slight decrease at 15 h in both cows, but returned to baseline values at 24 h and kept on decreasing until 56 h where a manifest hypozincaemia developed, which persisted throughout the experiment.

TXB2, 6-keto PGF and bicyclic PGE2 determinations

In cow A no alterations in plasma TXB2 concentrations were detected. However, plasma 6-keto PGF and bicyclic PGE2 concentrations showed a moderate increase at 25 h with peak values of 92 pg/100 µl and 65 pg/100 µl, respectively compared to baseline values. (Fig. 4).

Plasma TXB2, 6-keto PGF and bicyclic PGE2 concentrations in cow B were not markedly increased during the experimental period when compared to baseline values. (Fig. 5).

Cortisol, lactate and glucose

Plasma cortisol concentrations in cow A showed a slight increase at the 12th hour followed by a markedly increase at 32 h with peak values of 38 ng/ml which persisted to

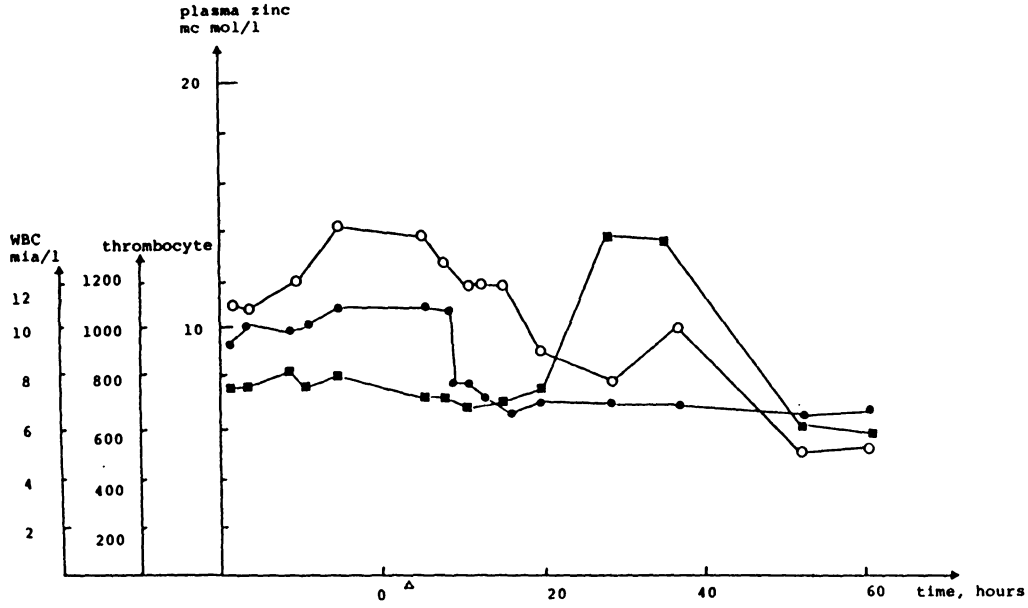


Figure 2. Cow A. Plasma zinc concentration ○ , thrombocyte count ● and leukocyte count □.

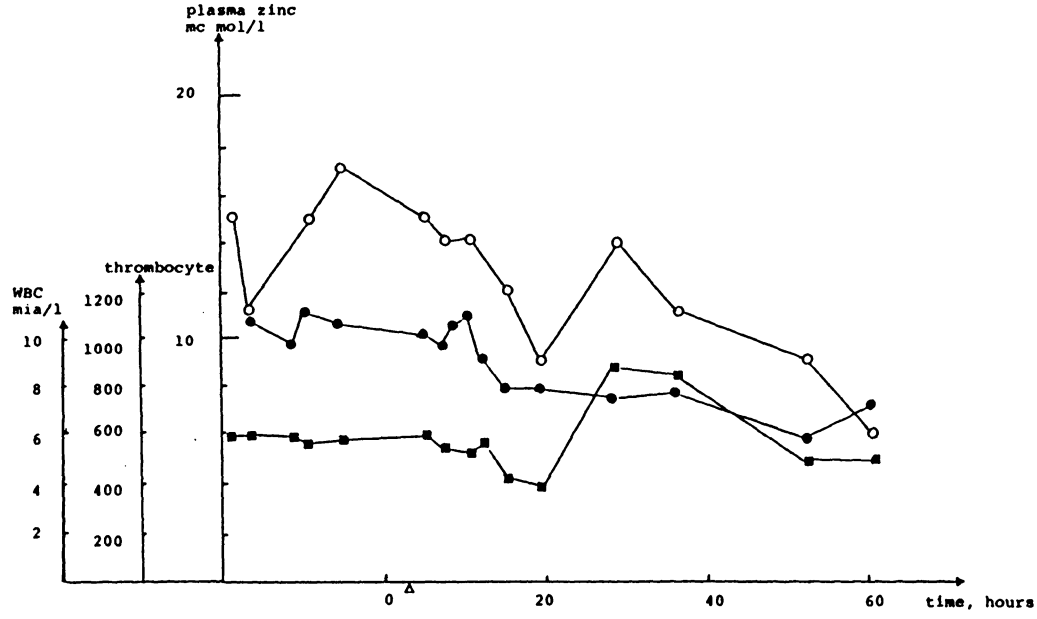


Figure 3. Cow B. Plasma zinc concentration ○ , thrombocyte count ● and leukocyte count □.

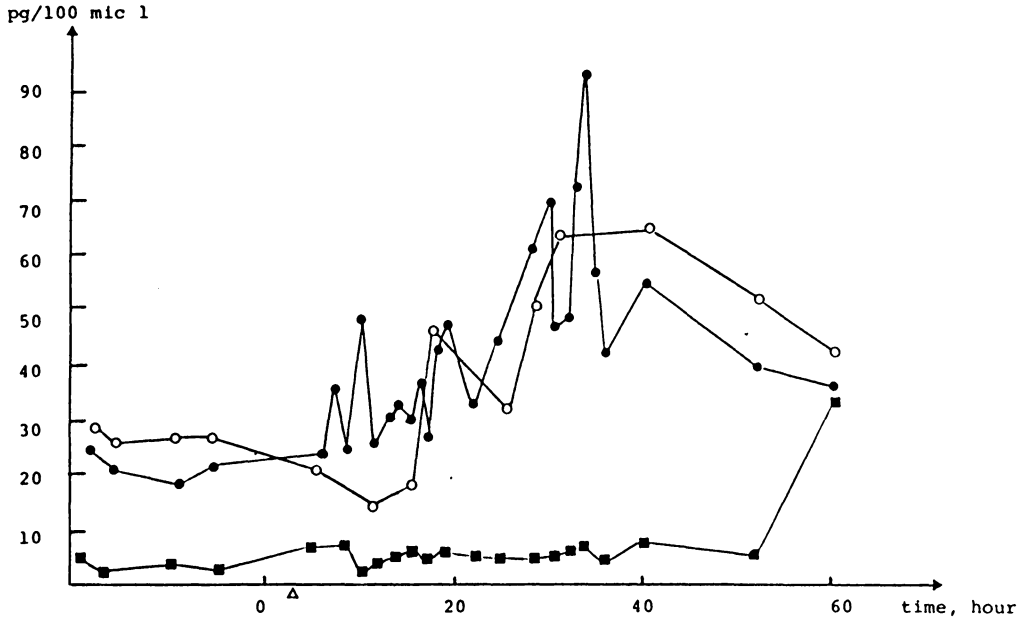


Figure 4. Cow A. Plasma TXB2 ■ , 6-keto PGF ● and bicyclic PGE 2 ○ concentrations.

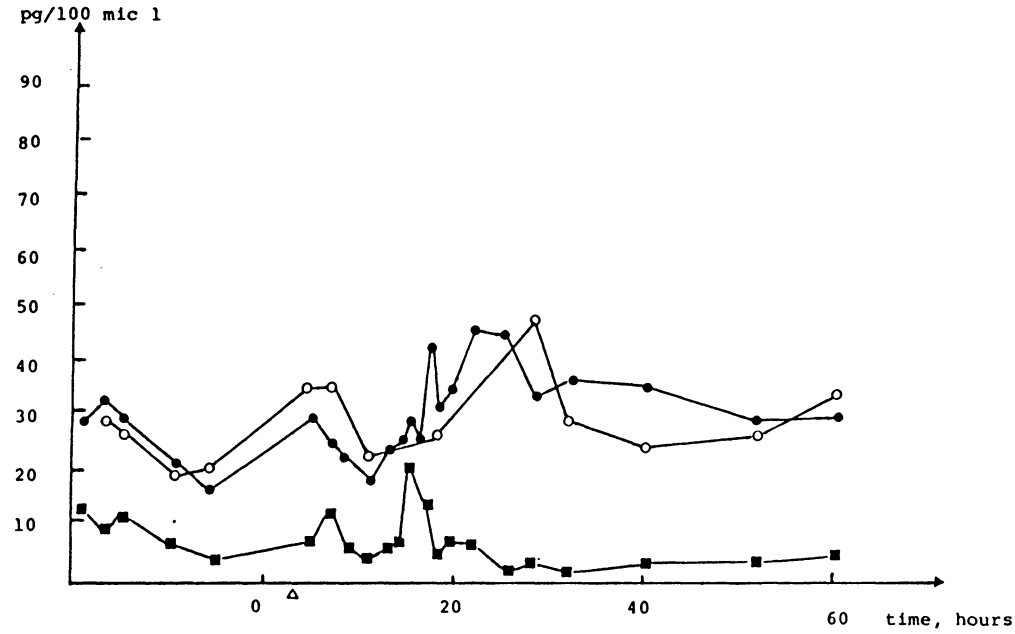


Figure 5. Cow B. Plasma TXB2 ■ , 6-keto PGF ● and bicyclic PGE 2 ○ concentrations.

48 h, after which a minor decrease began. In cow B a sudden increase in plasma cortisol concentrations was determined at the 12th hour with peak values on 30.9 ng/ml. The increase persisted until 72 h after engorgement.

Plasma glucose concentrations in both cows did not deviate significantly from baseline values.

At 11 h cow A and B showed an increase in plasma lactate concentrations with peak values on 2.88 mmol/l and 2.17 mmol/l, respectively. The lactaemia was persistent in both animals during the experimental period.

Discussion

The clinical and the clinical-chemical picture obtained in the present study is in accordance with the diagnostic criteria of acute rumen acidosis (*Dirksen* 1970, *Dunlop* 1976). Acute rumen acidosis does not develop under normal intensive feeding conditions, but is frequently diagnosed after over-eating or accidental shifts in rations. Anyway, the acute rumen acidosis is suitable as an experimental model for the more commonly occurring subclinical types of rumen acidosis. The subclinical rumen acidosis is characterized by low buffering capacity of the ruminal content and low pH values may be expected temporarily and/or locally. Various degrees of damage of the ruminal barrier are therefore common in both types of acidosis, ranging from local spots with superficial lesions to extensive areas with profound destruction of the ruminal epithelium (*Kay et al.* 1969, *Dirksen* 1970).

Intravenous administration of rumen bacterial endotoxin induces typical signs of endotoxicosis (*Nagaraja et al.* 1979) and the possible absorption of endotoxin from the gastro-intestinal tract is therefore of great interest.

Endotoxins are believed to escape through damaged epithelium to the portal circulation, but several studies have succeeded in detecting endotoxins in portal blood of apparently normal animals and humans (*Ravin et al.* 1960, *Gans & Matsumato* 1974, *Prytz et al.* 1976, *Andersen* 1985). While some authors believe that endotoxin is absorbed by passive diffusion (*Ravin et al.* 1960, *Gans & Matsumato 1974), Nolan et al.* (1977) showed that transepithelial transport of endotoxin in vitro occurred via a saturable carrier system, obeying Michaelis-Menton kinetics. The rumen endotoxin concentration is thought to be related to the total number of Gram negative bacteria in the rumen contents. High carbohydrate feeding results in an increased number of Gram negative bacteria (*Nagaraja et al.* 1978a) and large increases in toxicicty of ruminal fluid from high concentrate fed animals compared to hay fed animals have been reported (*Nagaraja et al.* 1978ab, *McManus et al.* 1978). This has led to the hypothesis that rumen acidosis is associated with a dramatic increase in ruminal endotoxin concentration (*Nagaraja et al.* 1978a, *Andersen* 1985). The present investigation does not support these observations. On the contrary, the endotoxin concentrations did not show any significant increases and even decreased to less than 25% of the pre-engorgement values in both animals 2-4 days after the overfeeding. This could be due to a dilution effect because of the high osmolarity of the ruminal contents concurrent to a compromized microbial activity, dominated by Gram positives (*Allison et al.* 1975). The endotoxin levels detected in ruminal fluid must be considered to be high enough to induce an endotoxic shock, if introduced systemically. Furthermore, it must be borne in mind that the LAL-assay was performed on sterile filtrate of ruminal fluid, and that the amount of biological active endotoxin in the crude ruminal fluid was

probably several times higher. It therefore seems unlikely that lethality of accidental overeating is caused by increases in ruminal endotoxin concentration.

We did not detect endotoxin concentrations above the detection limit on 0.03 EU/ml plasma in the peripheral blood at any time during the experiment. If endotoxins were present below this level, they may be considered to be of no biological significance. Another possibility is that a significant endotoxemia is present in a short period between two sampling times. This possibility cannot be ruled out as plasma endotoxin clearance has been shown to be total in less than 15 min (*Andersen et al.* 1988). However, if the endotoxins escape the rumen at all, a continuous stream should be expected as the epithelial damages persist for many days. We therefore believe that if endotoxins do escape to the portal circulation, an immediate clearance by the normal liver will occur, preventing the endotoxins from entering the peripheral circulation. Some of the results obtained in this study are comparable to those observed in endotoxicosis such as thrombocytopenia and hypozincemia (*Andersen et al.* 1988).

It is therefore suggested that the portal endotoxemia may be of significance for the development of the clinical picture in rumen acidosis. The mecanism for this could be that inflammation mediators are synthesized either in the damaged ruminal wall, in the endotoxin triggered prehepatic endothelial cells and thrombocytes or in the endotoxin clearing cells in the liver.

We did not detect increases in plasma TXB2, 6kPGF or bicyclic PGE2 comparable to those obtained in experimental induced endotoxemia. As systemic endotoxemia was not detected, a post-hepatic synthesis of the mediators is unlikely. The fact that no arachidonic acid metabolites were detected might therefore be a consequence of the rapid bioinactivation rates for theese compounds and their metabolites in the liver and lungs.

However, the plasma concentrations of 6k-PGF and bicyclic PGE2 (Fig. 4) determined in cow A may indicate that some synthesis of TXB2, 6-keto PGF and bicyclic PGE2 had taken place. According to the general clinical appearance, cow A seemed to be more severely affected from the grain engorgement than cow B (Table 1 and 2). This difference could be an effect of an apparently more sufficient stress »defense« to the disease in cow B. As showed in Table 3 and 4, an increase in plasma cortisol concentration in cow B was manifest 11 h after feeding, compared to 32 h for cow A. These findings underline the significance of the endocrine metabolic stress response in limiting the effects of the disease induced through the natural blocking action of cortisol on the development of the arachidonic acid cascade (*Breazile* 1987). Chronic stress (due to disease, housing and management conditions etc.) with depletion of adrenal function, is therefore believed to aggrevate not only the outcome of rumen acidosis but also any condition associated with release of inflammation mediators.

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Sammendrag

Undersøgelse af endotoksin, TXA2, PGI2 og PGE2 ved eksperimentelt induceret vomacidose hos kvæg. To vom-fistulerede køer blev eksperimentelt påført vom-acidose ved indgift af 70 g byg/kg legemsvægt intraruminalt. Køerne observeredes i en periode på 80 timer efter induktionen, hvor endotoksin koncentrationen blev målt i cellefri vomvæske og perifert plasma. Koncentrationerne af betændelsesmediatorerne TXA2, PGI2 og PGE2 samt af adskillige klinisk-kemiske parametre blev ligeledes bestemt i perifert blod. Der kunne ikke påvises endotoksin i det perifere plasma, og endotoksin koncentrationen i vomindholdet steg ikke gennem forsøgsperioden, selvom det kliniske billede på vom-acidose var veludviklet. Resultaterne fra denne undersøgelse støtter således ikke teorien om systematisk endotoksinæmi som led i patogenesen hos grut-forgiftede køer. De kliniskkemiske parametre udviste imidlertid ændringer, der ses ved endotoksikose, medens ingen af betændelsesmediatorerne viste signifikante koncentrations-stigninger. Som ny arbejdshypotese vedrørende endotoksiners rolle ved udviklingen af vomacidose hos kvæg, foreslås derfor at både endotoksin absorbtion og syntese af betændelsesmediatorer finder sted præhepatisk.

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