**บทความพิเศษ**

# Endotoxins and Release of Prostaglandin F2a In Ruminants

*Suneerat Aiumlamai* [1], *Hans Kindahl* [2], *Gunnar Fredriksson* [2] *and Lars-Erik Edqvist* [2]

Endotoxins are lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, consisting of a polysaccharide chain (O-antigen), a core polysaccharide region and lipid A. The O-antigen polysaccharide defines the serological specificity of the organisms. The lipid A region has the major toxic activity of endotoxins. The biochemical structure of lipid A from different Gram-negative bacteria is almost identical. The structure of endotoxin and the biological effects of different types of bacteria are similar (Smith, 1986). Infections with Gram-negative bacteria are frequent in animals and constitute an important component of diseases such as enteritis, mastitis and endometritis. Endotoxins are very potent biological compounds in stimulating prostaglandin release. Prostaglandin is one of the endotoxin-induced products of soluble mediators from macrophages and mononuclear cells (Morrison and Ryan, 1987). Injection of endotoxin elicits rapid and pronounced prostaglandin synthesis and release in cattle, goats, pigs and horses. Since prostaglandin F2a acts luteolytically in most domestic animals, response to endotoxin also involves lysis of the corpus luteum with subsequent alterations of sexual cycles and abortion in female animals (Kindahl et al., 1990). It is believed that fever is mediated by an endogenous pyrogen, interleukin, a protein which is released from the reticuloendothelial cells and acts on the temperature regulating centers of the central nervous system (Dinarello, 1983; van Miert, 1987). Other clinical signs caused by endotoxins in the ruminants involve depression, inhibition of ruminal contractions and intestinal motility. Clinically evident hypocalcaemia accompanies both experimentally induced endotoxaemia and peracute infection with *Escherichia coli* post partum. Serum calcium concentrations showed a direct decrease in cows and goats injected with *E. coli or Salmonella typhimurium* endotoxin (Fredriksoon, 1984; van Miert, 1987).

Many disease syndromes such as ruminal acidosis, ruminal stasis and parturient paresis have been discussed to be linked to affection from endotoxin. These diseases show some clinical and blood biochemical changes similar to what is

[1] *On leave from Dairy Farming Promotion Organization of Thailand.*

[2] *Depts. of Obstetrics & Gynaecology and Clinical Chemistry, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

found in endotoxaemia (van Miert, 1987; Lohuis et al., 1988; Blood and Radostitis, 1989a,b). Endotoxin from the gastro-intestinal tract has been discussed as a source of the endotoxin induced pathophysiological changes in these disease syndromes.

Endotoxins are present in large quantities in the intestine as a result of bacterial death and release during active growth. Small amounts of LPS are regularly absorbed but are rapidly detoxified by the liver which stands as a barrier to bacterial toxins arised in the gastro-intestinal tract. It has been demonstrated that endotoxin can be resorbed from the gastro-intestinal tract of normal and shocked animals as well as from ligated segments of intestine into which endotoxin has been injected and during colic in horses (Olofsson et al., 1985; Fessler et al., 1989). These findings supported the possibility of resorption of endotoxin from the gastro-intestinal tract in the above mentioned diseases.

## Experimentally induced endotoxaemia

An endotoxin from *Salmonella typhimurium* was given to male goats (Aiumlamai et al., 1990) and heifers (Table 1=A) (Aiumlamai and Kindahl, 1990) in dose of 1 ug/kg, intravenously. The goats did not show clinical signs, but the heifers showed obvious signs of endotoxaemia. Rectal body temperature (BT) increased and ruminal contractions (RC) decreased. A rapid increase of 15-ketodihydro-PGF2a (PG) occurred one to three hours post-injection and the goats showed a biphasic release pattern of PGF2a. Calcium levels (Ca) and bile acids levels (BA) were decreased in the goats (Figure 1) as well as in heifers and there was a decrease in levels of zinc (Zn) and iron (Fe) in heifers. In the latter, the liver enzyme, glutamate dehydrogenase (GLDH) activities in serum showed a dramatic increase. A remarkable leukopenia (indicated by total white blood cells (WBC)) was observed both in polymorphonuclear (PMN) and mononuclear cells (MN) followed by a tendency of leukocytosis after 36 h in the heifers. No significant changes in sodium (Na) and poteasium (K) concentrations were found. Endotoxin levels were detected immediately after injection of endotoxin in two out of six goats. Interestingly, the elevations in blood endotoxin levels seen about three hours after the injection are observed simultaneously with low levels of bile acids; an indication of ruminal stasis. Thus, accumulation of endotoxin may occur in the gastro-intestinal tract during the stasis and then be resorbed to the blood circulation later. This amount of endotoxin might complicate and result in a more severe endotoxaemic state.

## Endotoxin-related diseases in ruminants

The ruminal microflora contains Gram-negative and Gram-positive bacteria. Dying and disintegrating Gram-negative bacteria during acidosis are a potential source of endotoxins. Experimentally induced ruminal acidosis by overfeeding of oats (Table 1=B) (Aiumlamai et al., 1991a) and induced ruminal stasis by methscopolamine injections (Table 1=C) (Aiumlamai and Kindahl, 1991) were carried out in calves to

investigate the possibility of resorption of endotoxins from Gram-negative bacteria residing in the gastro-intestinal tract. The animals showed signs of ruminal acidosis and stasis in the first study and very clear signs of ruminal stasis in the second study. General clinical signs and changes in blood biochemical parameters were similar to what is found in experimental endotoxaemia. However, being less dramatic the changes in many relevant parameters such as an increase in prostaglandin F2a metabolite levels, body temperature, endotoxins, glutamate dehydrogenase and a decrease in iron, zinc and calcium indicating exposure of endotoxins. The results from the present studies provide evidence that ruminal acidosis/stasis is linked to resorption of endotoxins from the gastro-intestinal tract causing endotoxaemia/endotoxicosis.

Cows which had a previous history of parturient paresis were followed with clinical and blood biochemical changes two weeks before and after parturition (Aiumlamia et al., 1991b). Endotoxin levels during two days before and after parturition were evaluated. Three out of six animals (Table 1=D) showed signs of paresis and two animals were treated. The clinical and general changes of all parameters in the present study supported that during parturient paresis and parturition, there is a resorption of endotoxins from the gastro-intestinal tract : ruminal stasis, an increase in body temperature, prostaglandin metabolite levels, endotoxin levels and a decrease in calcium, zinc, iron and bile acids. The changes were more pronounced in paretic animals than in the non-paretic ones. The results suggest that endotoxins from the gastro-intestinal tract can be involved in paresis and influence the outcome of the disease.

## Conclusions

The general changes of most relevant parameters in group B (ruminal acidosis), C (ruminal stasis) and D (parturient paresis) resembled the changes found after i.v. injection with endotoxin (group A). Endotoxin was detected in serum during the period that the animals showed clinical signs in group B, C and D. The blood biochemical parameters, including also the prostaglandin metabolite, can be used to determine the effect of endotoxin in the ruminants. The changes of clinical and blood biochemical parameters taken together indicate that an endotoxaemic state had occurred.

(These studies are part of a Ph.D. thesis "Studies on effects of inhibition of prostaglandin biosynthesis, and on prostaglandins and endotoxin-related diseases in ruminants" presented by Suneerat Aiumlamai at the Swedish University of Agricultural Sciences).

## Acknowledgements

This study was supported by grants from the Swedish Council for Forestry and Agricultural Research. The Dairy Farming Promotion Organization of Thailand is gratefully acknowledged for permitting the leave of absence of Suneerat Aiumlamai.

Table 1. Summary of clinical and blood biochemical parameters seen after endotoxin injection (A), induced ruminal acidosis (B), methscopolamine induced ruminal stasis (C) and parturient paresis (D).

| Parameters | A | B | C | D |
|---|---|---|---|---|
| Clin. signs | +++ | ++ | ++ | +++ |
| BT | ++ | + | + | +/- |
| RC | - | - | -- | - |
| Endotoxin | N | + | + | + |
| PG | +++ | + | + | +++ |
| Ca | -- | - | -- | -- |
| Zm | -- | - | -- | -- |
| Fe | -- | - | -- | -- |
| Bile acids | - | + | -/+ | - |
| GLDH | +++ | - | -/+ | 0 |
| Na | 0 | 0 | 0 | 0 |
| K | 0 | 0 | - | 0 |
| WBC | -- | + | + | + |
| MN | -- | 0 | - | 0 |
| PMN | -- | + | + | + |

0 = no significant changes

+ = increased levels

- = decreased levels

N = not analysed

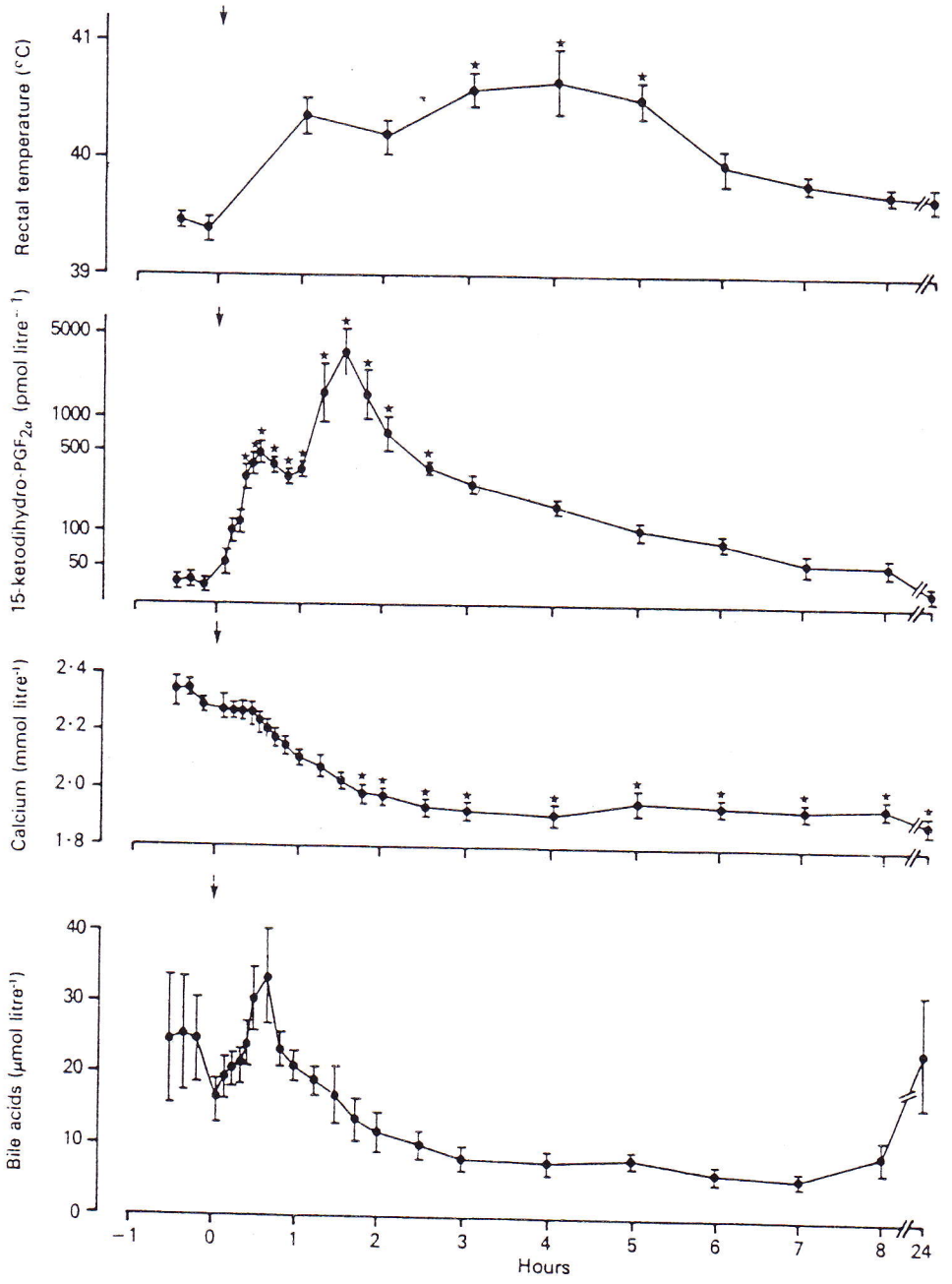


FIGURE 1 Show rectae body temperuture, 15 ketodihydro PGF22 levels, calcium levels and bile acuds levels during endotoxaemic induction

* indicates that mean values are significantly different ($P<0.05$).

## References


Aiumlamai, S., Fredriksson, G., Kindahl, H., Edqvist, L.-E., Kulander, L. and Eriksson, O. 1990. Endotoxin concentrations in te blood following intravenous injection and effect on prostaglandin F2a release, calcium and bile acids in goats. Research in Veterinary Science. 48: 190-195.

Aiumlamai, S. and Kindahl, H. 1990. Clinical and blood biochemical changes during induction of endotoxaemia in heifers. Acta veterinaria scandinavica. 31: 501-504.

Aiumlamai, S., Kindahl, H., Fredriksson, G., Edqvist, L.-E., Kulander, L. and Eriksson, O. 1991a. A role of endotoxins ? I. In induced ruminal acidosis in calves. (submitted).

Aiumlamai, S. and Kindahl, H. 1991. A role of endotoxins? II. In methscopolamine induced ruminal stasis in calves. (submitted).

Aiumlamai, S., Fredriksson, G., Kindahl, H. and Edqvist, L.-E. 1991b. A possible role of endotoxins in spontaneous paretic cows around parturition. (submited).

Blood, D.C. and Radostitis, O.M. 1989a. Acute carbohydrate engorgement of ruminants (rumen overload). In : Veterinary Medicine 7th ed., Bailliere Tindall, London, pp. 246-253.

Blood, D.C. and Radostitis, O.M. 1989b. Parturient paresis. In : Veterinary Medicine 7th ed., Bailliere Tindall, London, pp. 1104-1115.

Dinarello, C.A. 1983. Molecular mechanisms in endotoxin fever. Agents and Actions. 13: 470-486.

Fessler, J.F., Bottoms, G.D., Coppoc, G.I., Gimarc, S., Latshaw, H.S. and Noble, J.K. 1989. Plasma endotoxin concentrations in experimental and clinical equine subjects. Equine veterinary Journal. Suppl. 7: 24-28.

Fredriksson, G. 1984. Some reproductive and clinical aspects of endotoxins in cows with special emphasis on the role of prostaglandins. Acta veterinaria scandinavica. 25: 365-377.

Kindahl, H., Odensvik, K., Fredriksson, G., Cort, N., Daels, P., Hughes, J. and Stabenfeldt, G.H. 1990. Effects of endotoxins on prostaglandin biosynthesis and reproduction function in farm animals. Proc. 4th Congress of European Association for Veterinary Phamacology & Toxicology, Budapest, Aug. 28 - Sept. 2, 1988 (F. Simon, P. Lees & G. Semjen, eds.), pp. 205-212.

Lohuis, J.A.C.M., Verheijden, J.H.M., Burvenich, C. and van Miert, A.S.J.P.A.M. 1988. Pathophysiological effects of endotoxins in ruminants. The Veterinary Quartery. 10: 117-125.

Morrison, D.C. and Ryan, J.L. 1987. Endotoxins and disease mechanisms. Annual Review of Medicine. 38: 417-432.

Olofsson, P., Nylander, G. and Olsson, P. 1985. Endotoxintransport routes and kinetics in intestinal ischemia. Acta Chirurgica Scandinavica. 151: 636-639.

Smith, B.P. 1986. Understanding the role of endotoxin in Gramnegative septicemia. Journal of Veterinary Medicine. 81: 1148-1161.

van Miert, A.S.J.P.A.M. 1987. Fever, anorexia and forestomach hypomotility in ruminants. Veterinary Research Communication. 11: 407-422.