Pathophysiology of the Liver in High Yielding Dairy Cows and Its Consequences for Health and Production

H. J. Breukink, Th. Wensing
Department of Large Animal Medicine and Nutrition
Faculty Vet. Med., University of Utrecht
Utrecht, the Netherlands

Summary

A brief summary is given regarding the pathophysiological consequences of negative energy balance (NEB) and hepatic lipidosis. The relationship between hepatic lipidosis and NEB in early lactation is shown. The consequences of overconditioning in relation to metabolic stress after parturition is discussed. It is concluded that although not all aspects of these relations have been shown, careful monitoring of the amount of energy provided during late lactation, dry period and early postpartum can prevent the metabolic disturbances that have great impact on health, reproduction and welfare of the high producing dairy cow.

Introduction

The liver is the most important organ in the maintenance of the metabolic homeostasis in animals. This includes a number of metabolic processes regarding dietary amino acids, carbohydrates, lipids and vitamins. The liver is also involved in the synthesis of serum proteins, biotransformation of circulating metabolites, the detoxification and excretion of poisonous (waste) products and of poisonous xenobiotics.

The liver is vulnerable to a wide variety of metabolic, toxic, microbial and circulatory insults. If the resistance against such insults is overcome, the resulting failure of liver function can have far-reaching consequences. As in most organs, the liver can be diseased long before failure of function becomes apparent. The over-capacity of the bovine liver function is about 50% and clinical-chemical liver function tests stay normal as long as 20-25% of the liver function remains.

In the cow, pathological changes can result from metabolic disturbances, disturbances in rumen fermentation, intoxications and infection with viruses, bacteria and parasites such as the liver fluke. In the high yielding cow hepatic lipidosis is by far the most frequent cause of liver failure.[49] Although it is often shown that fat droplets can be observed in all dairy cows early *post partum*, it is clear that a definable clinical syndrome exists in association with considerable fat accumulation in the hepatocyte.[14] Whether this hepatic lipidosis is the cause of a number of diseases seen at parturition[9,12,13,29,30,37] or is secondary to a specific problem will be discussed in this paper.

Triacylglycerol (TAG) and Lipoprotein Synthesis in the Liver During Pregnancy and Early Lactation

During late pregnancy and lactation, large amounts of glucose are needed, but, because only small amounts are absorbed from the intestine, ruminants have to rely on other metabolic fuels and maintain a constant state of gluconeogenesis. When the cow experiences a period of negative energy balance (NEB), changes occur in the activity of a number of hormones (insulin, glucagon, growth hormone) which activate lipase in the adipose tissues and induce changes in gluconeogenesis and ketogenesis. The activation of hormone sensitive lipase in the adipose tissues stimulates fatty acid mobilization (lipolysis). Tissue fat is converted to free fatty acids (FFA) (former name: nonesterified free fatty acids NEFA) and glycerol. In the liver, glycerol can be used to produce glucose or can be recombined with FFA to form triacyl glycerols that are either deposited in the hepatocytes or released in the blood as very low density lipoproteins (VLDL).[8] The FFA can also be degraded through beta-oxidation and converted to acetyl-CoA. Acetyl-CoA combines with oxaloacetate and enters the Krebs cycle for the production of ATP. Here fat metabolism competes with gluconeogenesis because both reaction chains need oxaloacetate. If there is not enough oxaloacetate available because of a lack of sufficient glucogenic precursors such as propionate, acetate, glycerol or amino acids or because of a large demand for glucose, the acetyl-CoA cannot be introduced into the Krebs cycle and is then converted to ketone bodies. These ketone bodies form an important energy source during fasting, lactation or pregnancy, but can disturb metabolic processes when their concentration exceeds certain levels.[27] High concentrations of ketone bodies reduce feed intake, decrease mobilization of fatty acids

From the Israel Journal of Veterinary Medicine, Vol. 52, No. 2-3, 1997, p 66-72.

and, as a consequence, the NEB perpetuates.[14,15,44] There is a metabolic link between gluconeogenesis and ketogenesis but the way this link operates is still a matter of discussion and research.[47] The increase of corticosteroid concentrations before calving and decrease of thyroid hormone concentration after calving result in amino acid mobilization (proteolysis) and provides a substrate for gluconeogenesis.[53] Hepatic lipidosis is the result of a situation where the rate of hepatic TAG formation exceeds the formation and release of VLDL into the circulation.[15] The liver and the adipose tissues are used to store fat as reserve fuel. In the cow the energy balance is mostly positive during the latter part of lactation and during the dry period, this can result in fat storage at this time. This is an important feature because after parturition a period of NEB is to be expected. In the weeks after calving the amount of fat in the liver returns to normal.[14,17] Cows with a strong positive energy balance during the dry period accumulate more fat in the liver and adipose tissues. When cows enter the dry period in a good condition this effect is strengthened.[44]

After parturition, NEB develops because lactation starts and uses more energy than pregnancy did. It takes some time before the energy intake reaches the level necessary for energy equilibrium. In the high producing dairy cow the negative energy balance starts before calving because of diminished appetite and the onset of the colostrum production.[3] After parturition these cows experience a more pronounced energy deficit because of the high milk yield. Cows in a good or fat condition tend to start with a higher production than cows in a normal condition. In any case the liver is confronted with large amounts of FFA, coming from the adipose tissue and resulting in deposition of TAG in the liver cells that already have fat stored. Studies at Utrecht[15,44,45] showed that diacylglycerol acyl transferase (DGAT) activity increased in the liver cell after parturition. DGAT stimulates the conversion of fatty acids into TAG. Also the intra hepatic phosphatidate phosphohydrolase (PAP) activity increased about 10-fold. PAP also stimulates TAG synthesis.[15,44,45] The rise in PAP activity is probably needed to control the high cellular concentration of fatty acids which can become cytotoxic.[15] In a number of cases it is shown that in particular the secretion of VLDL is insufficient to keep up with the hepatic production of TAG. In the lactating cow the demand for VLDL production is very high as VLDL have to provide the udder with the major part of the lipids necessary for milk fat production.[28,46]

In the case of liver failure (as caused by fatty infiltration) or an inadequate supply of proteins, hepatic synthesis of apolipoprotein A can become inadequate. This decreases the liver's ability to produce and secrete VLDL. As a consequence the accumulation of the TAG in the liver is continued.[28,46] If the production of apolipoproteins was inhibited by ethionine administration, hepatic lipidosis was induced.[46] Recently, new evidence was obtained about the impact of adequate protein intake on the ability of the periparturient cow to cope with the metabolic situation at calving.[7,53]

It has been shown that a low protein diet in the dry period increased the incidence of hepatic lipidosis.[19] Recently it was hypothesized that the hormones responsible for activation of hormone-sensitive lipase and the inhibition of lipogenesis and glycogen synthesis also inhibit the hepatic production of VLDL while intra-hepatic TAG production continues. This seems redundant and certainly a waste of energy under NEB circumstances.

Consequences of Hepatic Lipid

The temporary accumulation of fat in the liver is a normal physiological process. All high producing dairy cows have a moderate degree of hepatic lipidosis after the dry period and accumulate fat in the liver during the first few weeks following parturition. The accumulation of fat in the liver peaks at about 2 weeks post partum, thereafter it returns to normal. It is clear that under extreme situations this may lead to the so called fat cow syndrome.[30] These cows are depressed, anorectic, lose weight rapidly and become week. A number of concurrent diseases such as metritis, retained placenta, milk fever, mastitis and abomasal displacement may aggravate the clinical picture just as it occurs frequently in cows with hepatic lipidosis.[9,12,13,16,33,34,52]

It may be expected that also in cows with less severe hepatic lipidosis the liver function is impaired. In cows with mild hepatic lipidosis the disappearance time of endotoxins was found to be increased substantially.[1,2,20] However, in most cases, hepatic lipidosis is a reversible condition and thanks to the over-capacity of the liver the negative effect of this temporary hepatic lipidosis is restricted. Cows fasted and refed turned to normal liver fat content within 18 days of refeeding.[3] When a large number of cows experiencing hepatic lipidosis was followed, it was found that at six months after calving, liver fat content had returned to normal values (less than 15%).[35] The function of liver cells that continue to accumulate fat may eventually become impaired.[49,52] It was found that in fasting cows the surface area of the rough endoplasmic reticulum and the number of mitochondria per unit volume decreased.[37] The plasma activity of enzymes that are used to evaluate the condition of the liver such as lactic dehydrogenase (LDH), alkaline phosphatase (AP), aspartate aminotransferase (AST) and gamma glutamyl trans peptidase (gamma-GT) were higher in cows where severe hepatic lipidosis was induced than in normal cows in the same lactation stage.[34,44,49]

As mentioned before, the bovine liver has a clear over-capacity for a greater part of its functions but fatty infiltration substantially affects at least some of them. There are indications that the total capacity of the liver as a detoxifying organ is diminished. This becomes a problem in situations where endotoxins circulate during periods when hepatic lipidosis exists. In a number of diseases in the early postpartum period, directly or indirectly related to NEB, endotoxins may be produced. It has been suggested that portal endotoxemia, as found in rumen acidosis, can be of significance in the clinical picture of rumen acidosis. Since in cows fed high grain diets, short term rumen acidosis does occur, occasionally portal endotoxemia may become systemic endotoxemia, if hepatic lipidosis exists.[1,2,20] As mentioned, in cows with mild or moderate fatty liver, the plasma disappearance time of endotoxin was 14-16 times longer, while cows with severe fatty liver were unable to clear an injected dose of endotoxin at all.[2,20] These findings suggest yet another cascade of processes resulting in a higher incidence of infectious and metabolic diseases[12,13,14,18,50,52] may be the process behind laminitis, sole erosions and ulcers and in inflammatory processes such as endometritis and mastitis. Subclinical endotoxicosis may therefore become a clinical problem in cows suffering from hepatic lipidosis. It was found that the clinical signs of endotoxicosis are induced by eicosanoids such as thromboxane A_2, proscyclins and prostaglandins. Treatment with non-steroid, anti-inflammatory drugs (NSAID), suppressing eicosanoid synthesis alleviated clinical signs of endotoxicosis.[20] Eicosanoids may also be responsible for the motility disorders of abomasal smooth muscle during abomasal displacement due to its effect on the neurally mediated reflexes. Recently it has been suggested that the decreased contractility of the mantal muscle of the abomasum is caused by a loss of cholinergic excitatory and an increase of nitrooxergic inhibitory tone.[11]

A Danish epidemiological study concluded that milk yield and body weight were positively associated with the incidence of sole ulcers. Since grain engorgement may be the result of a feeding practice aimed at an increase of energy input in early lactation, this may result in clinical problems such as laminitis and sole ulcers in cows that experience liver failure because of hepatic lipidosis. Endotoxicosis worsened by hepatic lipidosis may also be involved in the pathogenesis of those syndromes where cows with otherwise mild problems such as retained placenta or endometritis do not respond to therapy or become chronically ill and die.[1,2]

Belgian researchers have suggested that the glycogen content of the liver cell is important in protecting the cell against pathologic lipidosis. Severe hepatic lipidosis was always associated with liver glycogen depletion. In livers that have been infiltrated by fat for a long period, a range of chronic changes can be found.[33,34]

Spontaneous ketosis occurs mostly near the peak of lactation (3-4 weeks postpartum). Mild ketosis, immediately post partum, definitely indicates fatty infiltration of the liver. Cows with fatty liver often show an unfavorable outcome of clinical conditions that in most cases do not cause serious problems in normal cows. Cows with metritis and/or retained placenta for instance may die despite intensive antibiotic treatment, and ketonuria does not respond to glucose and corticosteroid therapy.[21,25,27]

A severe fatty liver greatly increases morbidity and mortality of common periparturient diseases.

The Relation Between NEB and Hepatic Lipidosis Directly After Parturition

Research with cows with induced fatty liver *post partum* has provided evidence for the hypothesis that hepatic lipidosis should be a causal factor in the health and reproductive problems of the periparturient high producing cow needs reconsideration. Hepatic lipidosis, health problems and decreased fertility seem to be related to the severity of the negative energy balance after parturition. Whether hepatic lipidosis via liver failure initiates the decrease of immune competence and fertility or just contributes to it is still under investigation.[26,50,51] Cows with fatty liver have reduced breeding efficiency. The energy balance *post partum* is inversely related to the time of first ovulation and has a positive relationship with progesterone concentrations in milk.[4,6,7,39,53] The energy balance (blood glucose/insulin concentration) regulates ovarian function. It is postulated that insulin like growth factor-I (IGF-I) serves as the hormonal mediator for this regulation. IGF-I is a potent stimulator of bovine granulosa and luteal steroidogenesis. In addition, IGF-I concentrations in the blood of cattle are influenced by variations in protein or energy intake. The IGF-I concentration is reduced in lactating cows with high milk production.[48] An improvement of the energy balance (less severe NEB) is associated with increase in IGF-I concentration in serum and this increase is associated with an increased progesterone secretion during the first and second post partum oestrus cycle.[40,41] An increased milk production resulting in a (severe) NEB is associated with decreased IGF-I concentration and thus with a reduced ovarian activity. It has been shown repeatedly that high yielding cows need more inseminations per conception.[41,42] It has been hypothesized that oocytes and follicles that start to grow during the period of negative energy balance and hepatic lipidosis have less developmental potencies 80-100 days later.[5] Studies in the Netherlands support this hypothesis.[26] In cows with severe hepatic lipidosis due

to severe NEB, it was found that ovarial activity started late and most of the animals did not show any sign of oestrus before the 80th day of lactation. The cycles that occurred thereafter were irregular because of repeated occurrence of persistent corpora lutea. At 180 days post partum only 35% of the cows were pregnant. At that time, in the 48 cows in an experimental group, only 57 inseminations were carried out (because only that number of oestruses were seen). Not only the lack of oestruses shown or not detected despite intensive observation that caused this low percentage of pregnancies, but also the underdevelopment of the oocytes released from the ovaries (ovulated). Oocytes sampled after the 80th day post partum clearly lacked the potential for development.[26]

Insulin is one of the important hormones controlling nutrient metabolism. Plasma insulin is lower during lactation than during the dry period in dairy cows. Insulin responsiveness to glucose and tissue responsiveness to insulin is lower in late pregnancy than in non-pregnant, non-lactating cows. This may serve as a mechanism to protect the fetus from hypoglycaemia. At the start of the lactation, insulin responsiveness increases, thus increasing the sensitivity of glucose utilization.

The decrease in insulin responsiveness in late pregnancy is associated with a decrease in the number of insulin receptors in adipose tissues. This insulin resistance in late pregnancy may be caused by the joint action of progesterone, growth hormone, adrenergic hormones or glucagon.[26,53] Insulin seems to play a central role in the switch from lipogenesis at the beginning of pregnancy to lipolysis in late pregnancy and lactation.[31] It is shown that the decrease in insulin responsiveness (insulin insensitivity) in late pregnancy and early lactation inhibits ruminal emptying and abomasal contractions. Together with the frequently existing hypocalemia, this could be a factor in the pathogenesis of abomasal displacement.[22,31,33]

Consequently, much attention is drawn to the prevention of fatty liver *post partum* by adequate feeding and management of the dry cow. In fact all efforts should focus on the prevention of a severe NEB *post partum*. Based on experiences in daily practice and on the findings in experiments with cows with induced fatty liver post partum in the Netherlands, the ration given in the dry period, that should not exceed 5 weeks, equals 1.2 times the maintenance requirement.[44]

Results of recent studies however have demonstrated that treatment of a severe NEB diagnosed on the basis of a high hepatic concentration in a liver biopsy collected between days 7 and 13 post partum could be beneficial. It was found that first ovulation occurred in relation to the return of NEB towards neutral.[4,6,7,39] This means that a period from 10-14 days in which the NEB starts to return to neutral preceedes first ovulation. In connection here with supplementation of the high yielding cow in the first period after parturition with agents that directly improve the energy balance should be considered. Careful monitoring of the body condition[36] can be of assistance in feeding management during late lactation and during the dry period. Such practices can be guided by determining the TAG content in liver biopsies. However, treatment of fatty liver is still difficult, and all efforts should be directed towards prevention by careful regulation of the energy input in late lactation, the dry period and the first weeks post partum.

The Relationship Between Hepatic Lipidosis (Liver Failure) and Immune Competence

Input, throughput and output have been associated with disturbances of specific and non-specific resistance against disease. Various nutritional constituents or digestive products have been discriminated as having a negative impact on non-specific and specific defense mechanisms.

The skin and the mucous membranes are probably the most important parts of the body in the protection against pathogenic intruders. Surrounding these membranes are a number of factors and molecules that prevent multiplication of these pathogens. This system of defense is called the innate (non-specific) immune system. This innate system consists of two components, the humoral and cellular. The humoral component comprises complement, lysozyme, cytokines, acute phase proteins and interferons. The cellular components such as polymorphonuclear cells, macrophages and natural killer cells each have specific actions against invading microorganisms. Parts of the ingested pathogens are then presented at the Major Histocompatibility Complexes (MHC) on the surface of these cells and stimulate immunocompetent of the specific immune system to produce specific antibodies aimed at the pathogen.[18,21,25,36,37,50,52]

The innate and the acquired immune systems interact comprehensively. These interactions are initiated by soluble factors secreted by damaged cells (products of arachidonic acid) or by infected cells (cytokines). Other interactions are cell-to-cell interactions in which adhesion molecules play a role.[38]

The reactions of the immune system can be evaluated by measuring the amount of product released into the peripheral blood (tumor necrosis factor (TNF), the cytokines and the different classes of globulins). The reactive capacity of immunocompetent cells can be measured *in vitro* by stimulating the cells with substances such as pokeweed mitogen and lipopolysacharide.[38,50,51]

The tests available do not however provide full insight into the complicated immune system with its mul-

tiple interactions. They only provide information regarding a certain aspect at a given moment, and *in vitro* tests do not necessarily reflect *in vivo* activity. Malnutrition and overfeeding are known to have impact on the immune functions; this applies also for a dietary imbalance of vitamins and minerals.[17]

When it was found that a leucopenia accompanies ketosis and fatty liver, the effect of ketosis on the bovine immune system became a subject of intense study. Epidemiological studies confirmed that ketosis is interrelated with several infectious diseases in the dairy cows.[10,21,25,43,52] *In vitro* responses against phytohemagglutinin-P and pokeweed mitogen of lymphocytes from calves with induced ketonemia were suppressed when compared with normal calves.[25,43]

Studies have shown that high concentrations of ß-hydroxybutyrate and acetoacetate inhibit bovine lymphocyte proliferation *in vitro*. Other studies however resulted in divergent results. Recently only minimal effects of ketone concentrations mimicking those in the plasma of ketotic cows on lymphoproliferation were found. The immunomodulatory effects of ketones on lymphocytes need further investigations.[10]

The higher susceptibility of periparturient cows to intramammary infections and clinical mastitis may also be related to hypocalcemia which in this turn is closely correlated with hepatic lipidosis.[9,44] Epidemiological studies have indicated that cows with parturient hypocalcemia have a five to eight times greater chance of contracting coliform mastitis. Since immunosuppression, characterized by impaired lymphocyte and neutrophil function, was already present before calving, it was concluded that cortisol and 1:25 DHCC concentration increases were not the primary causes, but they may contribute to the magnitude and/or duration of post partum immunosuppression.[22]

The significant immunosuppression found at one week *prepartum* indicates that changes in late gestation adversely influence immune cell functions in the dairy cow. It is suggested that oestrogens are responsible for this effect. Plasma oestrogen levels at the end of gestation are more than 100 times higher than during oestrus and oestrogens have been associated with altered leukocyte and lymphocyte function.[22]

It was also found that bacterial ingestion by neutrophils was increased at parturition. It is suggested that this was associated with a decreased oxidative killing of the ingested bacteria. This is suggested to be a consequence of the negative energy and protein balance in early lactation.[52,53]

Other factors contributing to a higher incidence of mastitis include the increased exposure of the teat tips to environmental bacteria in cows that are recumbent for a certain period and the possible loss of teat sphincter muscle tone due to hypocalcaemia, also visible as increased spillage of milk by these cows.

It is unlikely that immunosuppression during the periparturient period is the result of changes in the concentration of a single entity, rather that several factors are involved and act in concert. This in turn may have profound effects on organ function and immune cell function.

Challenge experiments using animals brought into conditions which reflect naturally occurring metabolic diseases are probably the best model for the evaluation of the effects on the immune system. Such experiments have been done and they strongly suggest impaired immune reactivity in cows suffering from hepatic lipidosis.[44,50,51] In cows with increased levels of hepatic TAG, an increased severity and prolonged duration of mastitis after an experimental intramammary *Escherichia coli* infection and a reduced influx of polymorphonuclear cells into the milk was observed.[18,37] In these cows however the phagocytic activity of these cells and their killing capacity for bacteria was unaffected. Cows with induced hepatic lipidosis showed a reduction of specific response for tetanus toxoid after vaccination. The cows in that study had also a reduced lymphocyte accumulation associated with skin allotransplants performed three days after parturition as compared to animals with low levels of hepatic fat.[50,51]

The results of these studies clearly indicate that in cows experiencing a moderate to severe hepatic lipidosis and/or NEB, at least part of the immune system is affected in such a way that immune responsiveness is reduced. This is in accordance with the observations that infectious diseases are more prevalent. As mentioned before, clear evidence is obtained recently that, apart from a negative energy balance, an inadequate protein intake, resulting in an increased demand for more intense mobilization of amino acids from body proteins (proteolysis), is involved in the complex of adaptations the cow has to experience around calving.[7,39,53] **There is ongoing research on the subject of the interactions between the post partum NEB, inadequate protein intake, hepatic lipidosis and their consequences for periparturient diseases and reproduction.**

References

1. Andersen, PH 1990. Aspects of bovine endotoxaemia of possible relevance to lesions in the ruminant digit. *Proc. VIth International Symposium in Diseases of the Ruminant Digit.* 2. Andersen, PH 1988. Pathophysiology of experimental bovine endotoxicosis I: Endotoxin *in vivo* clearance in healthy and hepatic lipidotic cattle. *Proceedings, XVth World Buiatrics Congress.* 2:1410-1415. 3. Bertis, SJ, Grummer, RR, Cadorniga-Valino, C and Stoddard, EE, 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. *J. Dairy Sci.* 75-1914. 4. Butler, WR and Smith RD 1989. Interrelationships between energy balance on post-partum reproduc-

tive function in dairy cattle. *J. Dairy Sci.* 72:767. 5. Britt, JH 1991 Impacts of early post-partum metabolism on follicular development and fertility. *The Bovine Pract.* 24:39 6. Canfield RW and Butler WR 1991. Energy balance, first ovulation and effects of naloxone on LH secretion in early post-partum dairy cows. *J. Anim. Sci.* 69:740 7. Canfield RW, Sniffen, CJ and Butler, WR 1980. Effects of excess degraded protein on postpartum reproduction and energy balance in dairy cattle. *J. Dairy Sci.* 73:2342. 8. Dijk, S van, Wensing, Th. and Breukink, HJ 1983 Influence of dietary intake in the dry period and negative energy balance in early lactation on blood and liver metabolites, serum lipoproteins and serum enzyme activities in dairy cows. *Proc 5th Int. Conf. on Prod. Dis. in Farm Animals.* 9. Dijk, S. van, Wensing, Th, Wentink, GH and Jorna T 1988. Hepatic lipidosis in dairy cows related to health and fertility *Proc. VIIIth Int. Conf. Prod. Dis. Farm Animals* (New York), 289. 10. Franklin, ST, Young, JW and Nonnecke, BJ 1991. Effects of ketones, acetate, butyrate and glucose on bovine lymphocyte proliferation *J. Dairy Sci.* 74:2507. 11. Geishauser T. 1995 Untersuchungen zur Labmagenmotorik von Kühen mit Labmagenverlagerung. Habilitationsschrift, Giessen, Germany. 12. Gerloff, BJ, Herdt, TH and Emery, RS. Relationship of hepatic lipidosis to health and performance in dairy cattle. *J. A. Vet. M.A.* 188:845. 13. Grummer, RR, 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* 76:3882. 14. Herdt, TH 1988 Fuel homeostasis in the ruminant. *Vet. Clin. North Amer.* 4:213. 15. Herdt, TH, Wensing, T, Haagsman, HP, Golde, LMG van and Breukink, HJ. 1988. Hepatic triacylglycerol synthesis during a period of fatty liver development in sheep. *J. Anim. Sci.* 66: 1997. 16. Herdt, TH 1988. Fatty liver in dairy cows. *Vet. Clin. North Amer.* 4:269 (1988). 17. Hidiroghou, M and KE Harkin: Vitamins A, E and selenium blood levels in the fat cow syndrome. *Can. Vet. J.* 23:255. 18. Hill, AW, Reid, IM and Collins, RA 1985 Influence of liver fat on experimental *Escherichia coli* mastitis in periparturient cows. *Vet. Rec.* 117:549. 19. Holtenius, P and Hjort M. 1990. Studies on the pathogenesis of fatty liver in cows. *Bovine Pract.* 25:91. 20. Jarlov, N, Haubro Andersen, P and Hesselholt, M 1983. Pathophysiology of experimental bovine endotoxicosis II; investigations on endotoxin-induced synthesis of thromboxane A2, prostacycline and prostaglandins E2. *Proceedings XVth World Buiatrics Congress* 2:332-334. 21. Kandefer-Szerszen, M, Filar, J, Szuster-Ciesielska, A and Rzeski W. 1992. Suppression of interferon response of bovine leukocytes during clinical and subclinical ketosis in lactating cows. *Dtsch Tierärtl Wschr.* 99:40. 22. Kerli, ME and Goff, JP 1992. Periparturient hypocalcemia in cows: Effects on peripheral blood neutrophil and lymphocyte function. *J. Dairy Sci.* 72:1188. 23. Klucinski, W, Degorski, A, Miernik-Degorska, E, Targowski, S and Winnicka A 1988a. Effect of Ketone bodies on the mitogenic response of bovine milk lymphocytes. *J. Vet. Med.* A35:626. 24. Klucinski, W, Degorski, A, Miernik-Degorska, E, Targowski, S and Winnicka A. 1988b. Effect of ketone bodies on the phagocytic activity of bovine milk macrophages and polymorphonuclear leukocytes. *J. Vet. Med.* A35:632. 25. Kremer, WDJ, Noorhuizen-Stassen, EN, Grommers, FJ, Schukken, YH, Heeringa, R. Brand, and Burvenich, C 1993. Severity of experimental *Escherichia coli* mastitis in ketonemic and non-ketonemic dairy cows. *J. Dairy Sci.* 76:3428 (1993). 26. Kruip, Th. JHJ van den, Werf, AM and Wensing, Th 1995. Energy balance in early lactation of high producing dairy cows and its relation to reproduction and health and welfare. *Proc. Symp.* WIAS. Wageningen 29 September: utilization of local feed resources in dairy cattle. Prospectives of environmentally balanced production systems. Ed. Bruchem, J van der. 27. Lean, IJ, Bruss, ML, Troutt, HF Galland, JC, Farver, TB, Rostami, J, Holmberg, CA and Weaver, LD, 1994. Bovine ketosis and somatotrophin: risk factors for ketosis and effects of ketosis on health and production. *Res. Vet. Sci.* 57:200. 28. Marcos, E, Mazur, A, Cardot, P and Rayssiguier, Y. 1980. The effect of pregnancy and lactation on serum lipid and apolipoprotein B and A-I Levels in dairy cows. *J. Anim. Physiol. Nutr.* 64: 133. 29. Markusfeld, O 1993. Parturition disease complex of the high-yielding dairy cow. *Acta Vet. Scand.* 189:9. 30. Markusfeld, O, Nahari, N and Adler, N 1988. Traits associated with the fat cow syndrome in dairy cattle. A combined clinical, epidemiological and biochemical study of a multifactorial disease syndrome: *Isr. J. Vet. Med.* 44(3): 176. 31. McNamara, J: 1991. Regulation of adipose tissue metabolism in support of lactation *J. Dairy Sci.* 74:706. 32. Van Meirhaege, H, Deprez, P, van den Hende, C and Muylle, E 1988. Plasma glucose clearance and insulin response in cows with abomasal displacement. *J. Vet. Med. A.* 35:221. 33. Muyle, E, van den hende, C and Oyaart, W 1989. Spijsverteringsstoornissen en metabole afwijkingen bij hoogproduktieve runderen. Het impact op de genesis van ketonemie, leverve, vertraagde men lebmaagdilatatie (L.D.) Mededelingen. Sectie 3 I.W.O.N.L. Gent. 34. Muylle, E, van den Hende, C, Sustronck, B and Deprez, P 1990. Biochemical profiles in cows with abomasal displacement estimated by blood and liver parameters. *J. Vet. Med. A.* 37:259. 35. Pearson, EG and Maas, J 1996 Hepatic lipidosis in large animal internal medicine. Bradford P. Smith, editor. Mosby-Year Book Inc. 36. Perdon, O, Cheli, F, Senatore, E, Baroli, D and Rizzi, R 1993. Effect of body condition score at calving on performance, some blood parameters, and milk fatty acid composition in dairy cows. *J. Dairy Sci.* 76: 2528. 37. Reid, JM and Collins, RA 1980. The pathology of postparturient fatty liver in high-yielding dairy cows. *Invest. Cell. Pathol.* 3:237. 38. Roitt, JM 1991. *Essential Immunology,* 7th ed. Blackwel. 39. Senatore, EM, Butler, WR and Oltenacu, PA 1996. Relationships between energy balance and post partum ovarian activity and fertility in first lactation dairy cows. *Animal Science.* 62: 17-23. 40. Spicer, LJ, Tucher, WB and Adams G.D. 1990. Insulin-like growth factor-I in dairy cows: Relationships among energy balance, body condition, ovarian activity, oestrous behavior. *J. Dairy Sci.* 73: 929. 41. Spicer, LJ, Alpizar, E and Echternkamp, SE 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production *in vitro. J. Anim. Sci* 71: 1232-1241. 42. Staples, CR, Thatcher, WW and Clark, JH 1990. Relationships between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *J. Dairy Sci.* 73:938. 43. Targowski, SP, Klucinski, W, Littledike, ET and Hoy DA 1985. Suppresssion of mitogenic response of bovine lymphocytes during experimental ketosis in calves. *Am. J. Vet. Res.* 46:1378. 44. Top, AM van den 1995. Diet and lipid metabolism in ruminants with particular reference to fatty liver development. Thesis, Utrecht. 45. Top, AM van den, Geelen, MJH, Wensing, Th, Wentink, GH van 't Kooster, A and Th, Beijnen, AC 1995. Higher post partum hepatic triacylglycerol concentrations in dairy cows with free versus restricted access to feed during the dry period are associated with lower activities of hepatic glycerophosphate acyltransferase. *J. Dairy Sci.* 78:2208. 46. Uchida, E, Kotoh, N and Takahashi, K. 1992. Induction of fatty liver in cows by ethionine administration and concomittant diseases of serum apoliprotein B-100 and A-I concentrations. *Am. J. Vet. Res.* 53: 2035. 47. Veenhuizen, JJ, Drackley, JK, Richard, MJ, Sanderson, TP, Miller, LD and Young, JW. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J. Dairy Sci.* 74: 4238. 48. Vega, JR, Gibson, CA , Skaar, TC, Hadsell, DL and Baumrucker, CR. 1991. Insulin-like growth factor (IGF)-I and -II and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. *J. Anim. Sci.* 69:2538. 49. Wensing, Th, Kruip, Th, Müller, KE, Wentink, GH and van den Top 1996. Post partum fatty liver in high producing dairy cows in practice and in animal studies: the connection with health and reproduction - *Proc. VIIth Congress of the International Society for Animal Clinical Biochemistry,* University of Glasgow, 23. 50. Wentink, GH, Rutten, VPMG, van den Ingh, TSGAM, Hoek, A, Müller, KE and Wensing, Th 1996. Impaired specific immunoreactivity in cows with hepatic lipidosis. *Vet. Immunol. Immunopathol.* in press. 51. Wentink, GH, Rutten, VPMG and Wensing Th. 1996. Nutrition, metabolic diseases and immunity dairy cows. *Proc. 19th World Buiatrics Congress, Edinburgh,* 337. 52. West, HJ 1990. Effect on liver function of acetonaemia and the fat cow syndrome in cattle. *Res. Vet. Sci.* 48: 221. 53. Zurek, E, Foxcroft, GR and Kennedy, JP 1995. Metabolic status and interval of first ovulation in post partum dairy cows. *J. Dairy Sci.* 78:1909.