# Effects of feed in the dry period on fertility of dairy cows post partum

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#### SUMMARY

It has been hypothesized that the decrease in fertility of dairy cows is related to the increase in milk yield and associated with fatty liver and increased vulnerability for both infection and metabolic diseases. To elucidate the mechanisms behind this complex of health and reproductive problems an Animal Model, based on overfeeding in the dry period, has been developed. In an experiment carried out in 1996 sixteen animals were fed 119 MJ/d net energy to lactation (NEL) (experimental group) and another sixteen just 49 MJ/d NEL (control group) in the last 8 weeks before parturition. During lactation the cows from both groups were given the lactation ration ad libitum. After parturition both groups differed significantly in level and duration of the NEB, in weight loss, increased concentrations of NEFAs and bHBB in the blood and TAG in the liver. Although the insulin level was lower in the experimental animals during the first week, the difference was not significant. The number of ovulations within the first 100 days pp was significant lower in the experimental group and was related to depth and duration of the NEB and the weight loss: more NEB leads to less ovulations. The interval partus-1e ovulation was also significant different but related to the TAG level in the liver: more TAG leads to a longer interval. The experimental group with the fatty liver suffered more incidenses of metabolic diseases. It has been concluded that the Model leads to a severe NEB, to Fatty Liver and to more incidences of diseases. Both the NEB and the fatty liver influence the fertility of the cows pp and it can not be excluded that some diseases have an effect too. More studies are planned to unravel the factors that affect the fertility of high yielding cows.

# INTRODUCTION

It is well documented that the fertility of dairy cows is decreasing. It has been hypothesized that this is related to the increase in milk yield and associated with fatty liver and increased vulnerability for both infection and metabolic diseases (1 - 5).

The energy requirement for milk production is high. High yielding cows of today nearly all inevitably have lack of sufficient energy during the first weeks after parturition: they are in a negative energy balance (NEB). The amplitude of the NEB depends on milk yield (6) and on appetite around calving (7). One of the consequences of a NEB is a low level of insulin. Insulin is well known as a hormone that is active in the hypothalamic region influencing the release of Gn-RH and LH (8). Insulin is also known as a factor that influences the ovarian activity by inducing receptors for LH in the granulosa cells (9) and thereby modulating the ovulation rate. By this way NEB influences fertility (10).

Moreover, a low insulin level induces the release of growth hormone (GH). GH induces lipolysis, resulting in a substantial increase of the blood non-esterified fatty acids (NEFAs) concentration (11). During deep NEB, the plasma NEFA concentration increases more than normal. The liver uses NEFAs for the synthesis of cholesterol-esters (CEs) phospholipids (PLs) and tri-acyl glycerol (TAG), the components of very-low-density-lipoproteins (VLDLs), or for the formation of ketone bodies: ketogenesis. In principle, NEFAs are toxic and very high hepatic concentrations, as occur during the deep NEB, force the

liver to detoxicate these also by esterification to TAG. In this way hepatic TAG synthesis increases extra and far more than that of phospholipids, cholesterol esters and apoproteins, all components necessary for the synthesis of VLDLs (12). As a result TAG is accumulated in the liver: hepatic lipidosis. When the NEB is severe and the insulin level low, the glucose concentration in the blood is under pressure. The liver is the most prominent organ that can synthesize glucose by gluconeogenesis. Shortness in propionic acid, the normal substrate for gluconeogenesis forces the liver to change over to the use of the carbohydrate backbone of glucogenic amino acids, provided by proteolysis. Thus the NEB enhances the synthesis of TAG in the liver from NEFAs as well as it can diminish the availability of amino acids, necessary for the hepatic apoprotein synthesis the carrier of TAG for transport to the udder for milk. As a consequence the release of TAG in VLDLs is hampered (13) and TAG is accumulated in the liver. Ketone bodies reduce the immune competence (14).

Through the combination of NEB, negative protein balance and hepatic lipidosis, milk yield is thought to influence reproduction. Our group is interested in the elucidation of the mechanisms behind the well known complex of health and reproductive problems of the high producing dairy cow around parturition. For that study we developed an Animal Model: the cow with induced fatty liver. This animal model is, based on overfeeding in the dry period. The so induced overcondition results in loss of appetite before and during parturition and induces a deep NEB post partum (pp), an intense lipolysis, fatty liver (FL) and the associated problems. The more overconditioned ante partum, the less appetite and the greater the chance for a severe NEB (15,16) and development of a fatty liver.

# MATERIALS AND METHODS

Thirty-two multiparous (2nd and 3rd lactation) dairy cows (selected on lactation value (90 to 110 % of the mean milk production on the farm, being 9000 ltr = 100%) entered the experiment at the onset of the dry period, 8 wk before the estimated calving date. The cows were paired for lactation value and randomly allotted to one of the two dietary treatments. During the first 7 wk of the dry period, the control group was fed according to their energy requirements (17). Feed intake was restricted to 14 kg of dry matter per day of a totally mixed ration composed of 30 % grass silage, 10% maize silage, and 60 % straw. Extra minerals were provided. In anticipation of lactation, the cows of this group were provided ad libitum with a ration designed to sustain lactation (see further) during the last wk of the dry period. The group treated to develop fatty liver syndrome, the fatty liver inducing treatment (FLI treatment) was provided ad libitum with this lactation ration during the whole dry period. On dry matter basis, this ration consisted of 20 % grass silage, 35 % maize silage, 6 % sugar beet pulp, 6 % extracted soy, and 33 % of concentrates. Per kilogram of dry matter, it delivered 6.7 MJ net energy for lactation (NEL) and 94 g of digestible protein in the intestine according to current Dutch feed evaluation systems (18,19). Thus the average intake of NEL during the dry period was 49 MJ/d in the control group and 119 MJ/d in the FLI-treated group. During lactation, cows from both groups were given the lactation ration ad libitum. Thus, differences in energy or protein intake during lactation are due to differences in feed intake only. Energy balance during the dry period and during lactation was calculated as the difference of energy intake and the energy requirements for maintenance and milk production (17).

The animals were housed in a loose housing system. Oestrusbehavior was observed 4 times per day during 15 minutes each. The animals were inseminated in the second standing heat.

Liver biopsies were taken on 8 days ante partum, between the 7th and 13th day post partum, between the 20th and 25th day and between the 35th and 42th day post partum. The biopsies were analysed for TAG.

Blood samples were taken twice weekly, early in the morning before the first feeding. In these samples the concentrations of NEFAs, glucose, insulin,  $\beta$ HBB and P4 have

been measured.

The onset of ovarian activity is determined by measuring the P4 profiles. A high P4 level (larger than 1 ng/ml) lasting for at least 7 days was interpreted as and corpus luteum after an ovulation. The % of animals that showed heat was calculated over the endocrinological heats (progesterone lower than 1 ng/ml) and the open heats (standing heat).

### **RESULTS**

The FLI treatment resulted in almost 100 kg weight gain during the 8 week dry period and in loss of appetite around calving.

After parturition both groups differed significantly in level and duration of the NEB (fig.1) and by that in weight loss, in the blood concentrations of NEFAs and  $\beta$ HBB and in the hepatic TAG concentration in the liver. Although the insulin level was lower in the FLI animals during the first week, the difference was not statistically significant.

The patterns of P4 indicated that persistent CLs on the one hand and ovarian inactivity during a long time (up to 140 days pp) are the main problems. The number of ovulations within the first 100 days pp was significantly lower in cows that had - a deeper NEB nadir, - lost more weight within the first 4 weeks pp and - needed more days to restore the energy balance. Deeper NEB was found to lead to less ovulations.

When all cows are taken together, the interval partus-1e ovulation was also significantly different and positively related to the hepatic TAG level. Higher hepatic TAG content leads to a longer interval.

The FLI treated group suffered more incidenses of different metabolic diseases (ketosis and milkfever).

Standing heat was seen in only 46% of all endocrinological (silent) heats (low P4 and new ovulation). Based on the duration of the experiment and the normal cycle length of 21 days in total 152 heats were expected but only 32% detected.

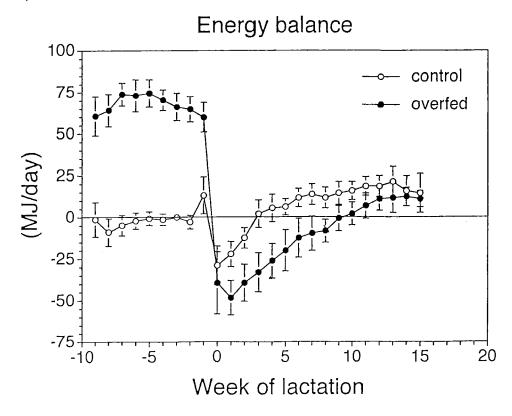


Fig. 1. The energy balance of dairy cows ante and post partum in the Animal Model.

## **DISCUSSION**

It can be concluded that all animals in this experiment face a negative energy balance. In the FLI treated group the NEB was deeper: in this respect the animal Model is usefull.

Ovarian inactivity during a long time can be explained by the hypothesis that extreme weight loss reduces the level of leptin and insulin and enhances the level of NPY resulting in a block on Gn-RH and LH pulsatility. The ovarian inactivity was most prominent in the animals that developed a high TAG concentration in the liver. Most of them were overconditioned ante partum too but not all. As expected, there was a strong positive correlation between the blood NEFAs concentration and the hepatic TAG concentration of individual cows. The relationship between hepatic TAG concentration and the interval partus-1e ovulation can be explained through the high blood NEFA concentration. This interpretation is in accordance with the statement of Senatore et al. (20): "the more NEB, the longer the interval partus-1st ovulation". However, the way the high blood NEFA concentration affects the ovary, the follicles and the granulosa cells has still to be elucidated. It might be via a direct effect on the ovary that leads to a lower P4 production in the corpora lutea of the first 4 to 5 cycles (21).

The relationship between NEB and number of ovulations within the first 100 days pp can be explained by the difference in blood insulin level in the first week pp: an effect on the LH-pulsatility. This should be proven by monitoring the LH-pulsatility in each individual animal. In a pilot study performed with 6 control and 4 FLI treated cows in which LH pulsatility was measured every 14 days during 8 hours, every 10 minutes a sample for 8 hours, no LH pulsatility patterns were found that could be interpreted as an indicator for onset of ovarian activity pp (22). Although hard to quantify stress might have been involved. Therefore, apart from an adequate animal model, we need a stressless way of blood sampling in order to prevent the possible effect of cortisol that, as is well documented, can affect the results substantially.

Corpus luteum persistence is most prominent for the first CL pp. It can be explained by the hypothesis that some animals ovulated so quickly after parturition that the uterus was not involuted entirely. In this respect it has been hypothesized that the uterus has not developed adequately receptors for oxytocin, can not react on that ligand and will not secrete PGF2 $\alpha$  on time. The corpus luteum persists. In one case the 1e CL pp persisted over 140 days and was thereafter treated.

It can be concluded from this experiment that the animal model leads to a severe NEB, to the development of hepatic lipidosis and to more incidences of diseases. Both the NEB and the fatty liver influence the fertility of the cows pp and it can not be excluded that some diseases have an effect too. In connection herewith, special attention has to be paid to the possible role of decreased immuno competence, frequently observed in cows with NEB and hepatic lipidosis. The increased vulnerability for diseases might be related to the low blood concentration of glutamine pp. High yielding cows show a very low glutamine level pp (23). Glutamine is thought to be an important energy source for the cells of the immune system.

The low percentage of standing heats as observed could be explained on the base of the hypothesis that a change in the ratio progesterone/oestradiol as an effect of NEFAs on the granulosa cells has occurred.

More studies should follow to unravel the main factors that affect reproduction of high yielding cows.

## **REFERENCES**

- 1. Gaines, W.L. (1927) Milk yield in recurrence of conception. J. Dairy Sci. 10: 117.
- 2. Butler, W.R., R.W. Everett and C.E. Coppck (1981) The relationships between energy balance, milk production and ovulation in post partum Holstein cows. J. Anim. Sci. 53: 742.
- 3. Lotthammer, K.H. (1982) Effect of improving energy intake during early lactation by corn-silage on metabolism and fertility of dairy cows. Factors influencing fertility in the postpartum cow:409.
- 4. Bagnato, A and P.A. Oltenacu (1994) Phenotypic evaluation of fertility traits and their association with milk production of Italian Freesian Cattle. J. Dairy Sci. 77: 874.
- 5. Gröhn, Y.T., H.N. Erb, C.E. McCulloch and H.S. Saloniemi (1989) Epidemiology of metabolic disorders in dairy cattle: Association among host characteristics, disease and production. J. Dairy Sci. 72: 1876.
- 6. Harrison, R.O., S.P. Ford, J.W. Young, A.J. Conley and A.E. Freeman (1990) Increased milk production versus reproductive and energic status of high producing dairy cows. J. Dairy Sci. 73: 2749.
- 7. Treacher et al. (1986) Effect of body condition at calving on the health and performance of dairy cows. Animal Production 43: 1, 1.
- 8. Cox, N.M., C.R. Barb, J.R. Kesner, R.R. Kraeling, I.A. Mataros and G.B. Rampacek (1989) Effects of intracerebroventricular (ICV) administration of insulin on luteinizing hormone (LH) in gilts. Proc. 3th Int. Conf. Pig Reprod. Nottingham. Abstract 7.
- 9. Poetsky, L. and M.F. Kalin (1987) The gonadotrophic function of insulin. Endocr. Rev. 8: 132.
- 10. Butler, W.R. and R.D. Smith (1989) Interrelationships between energy balance and post partum reproductive function in dairy cattle. J. Dairy Sci 72: 767.
- 11. Armentano et al. (1991) Effects of energy balance on hepatic capacity for oleate and propionate metabolism and triglyceride secretion. J. Dairy Sci 74:1, 132.
- 12. Herdt, T.H., T. Wensing, H.P. Haagsma, L.M.G. van Golde and H.J. Breukink. (1988) Hepatic triacylglycerol synthesis during a period of fatty liver development in sheep. J. Anim. Sci. 66: 1997.
- 13. Grummer, R.R. and D.J. Carroll (1991) Effects of dietary fat on metabolic disorders and reproductive performance in dairy cattle. J. Anim. Sci. 69: 3838.
- 14. Kremer et al. (1993) Severity of Experimental Escherichia coli Mastitis in ketonemic and nonketonemic dairy cows. J.Dairy Sci 76:3428.
- 15. Garnworthy. (1982) The effects of body condition at calving, food intake and performance in early lactation on blood composition of dairy cows given complete diets. Animal Production 35: 1, 121.
- 16. Britt, J.H. (1992) Influence of nutrition and weight loss on reproduction and early embryonic death in cattle. Proc. 25th Ann. Meeting Am. Assoc. Bovine Practioners, aug 31- Sept 4. pp. 143.
- 17. CVB; Centraal Veevoeder Bureau. Voedernormen landbouwhuisdieren en voederwaarde veevoeders. CVB-Report no. 18, Lelystad, August 1995.
- 18. Van Es, A.J.H. (1978) Feed evaluation for ruminants, I, the system in use from May 1977 onwards in The Netherlands. Livest. Prod. Sci. 5: 331.
- 19. Tamminga, S., W.M. van Straalen, A.P.J. Subnel, R.G.M. Meijer, A. Steg, C.J.G. Wever, M.C. Blok. (1994) The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40: 139.
- 20. Senatore et al. (1996) Relationships between energy balance and post partum ovarian activity and fertility in first lactation dairy cows. Animal science 62:1, 17.
- 21. Fonseca, F.A., J.H. Britt, B.T. Mcdaniel, J.C. Wilk and A.H. Rakes. (1983) Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus, estrous cycles, detection of estrus, conception rate and days open. J. Dairy Sci. 66: 1128.

- 22. Kruip, Th.A.M., R. Jelsma and P. Langendijk (1998) Not published.
- 23. Meijer, G.A.L., Meulen van der J., Bakker J.G.M., Koelen van der C.J., Vuuren van A.M. (1995) Free amino acids in plasma and muscle of high-yielding dairy cows in early lactation. J. Dairy Sci. 78:1131.