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A NEW APPROACH TO REPRODUCTION, NUTRITION AND IMMUNE RESPONSE RELATIONSHIP IN DAIRY COWS

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SUMMARY – Speaking in terms of general relationship between nutrition and reproduction, many different aspects are more or less involved according to geographical areas, species, production systems, technological levels etc. They are deficiency conditions: energy, proteins, vitamins and minerals, but also some excesses (few minerals) or toxic substances like micotoxins or plant compounds (i.e. oestrogen-like). Their relevance is different in the intensive systems for an easier making of appropriate diets. Nevertheless intensification does not reduce the nutritional risks for livestock reproduction for several reasons, namely a suspected higher susceptibility to the usual stresses and a new one: the metabolic stress. The latter is particularly relevant when early lactation and new pregnancy are close (dairy cows, does and, in some extent, sows). The negative energy balance is the main cause of metabolic stress, but the oxidative stress as well as the disease stress (pro-inflammatory cytokines) seem to be of great relevance. In dairy cows, inflammatory phenomena around calving – then an immune response in spite of clinical symptoms can be missing – are significantly related to a lower pregnancy rate, while milk yield and BCS are also reduced. The apparent paradox could be justified by pro-inflammatory cytokines which modify liver synthesis and seem to impair energy balance (increasing expenditure and reducing feed intake).

INTRODUCTION

Fertility is the result of so many factors that it is not surprising the definition of Fromageot (1978): reproduction can be considered a “luxury” function. In fact, many papers have demonstrated how many and different are the causes of reduced fertility. Among them, nutrition has a relevant role (Wolter, 1973; Bertoni, 1990; Ferguson, 1991): deficiency (and sometime excess) of either energy, protein, fiber, macro and trace elements, vitamins; as well as toxic effects from natural compounds (i.e. phytoestrogens, nitrates etc.), from conservation spoilage (mycotoxins etc.), from environment or farming pollution (heavy metals, pesticides etc.).

Nevertheless, the real risks from each potential cause can be different according to many factors of which the breeding system is of major importance. In fact, from a nutritional point of view, the intensive breeding systems tend to reduce many of the above causes for either the better satisfaction of the nutrient requirements, the better conservation systems of feeds and for a more accurate selection of the diet ingredients. Therefore, if it is understandable that trace elements, particularly when supplemented in organic state, can improve dairy cows fertility (Boland, 2003), in practice we agree that a proper supplementation of minerals and vitamins can be “easily” achieved in the intensive farms (Schaver and Howard, 1993). This could explain why not always, trace element and vitamin supplements, improve the reproductive efficiency of intensively bred dairy cows (Jukola et al., 1996).

For these reasons, vitamins, minerals and toxins, will not be considered here; for similar reasons – supposed to be possible a proper management in the intensive dairy farms – many other causes of low fertility will not be considered. In particular, we are referring to the general management (Ouweltjes et al., 1996; De Vries and Risco, 2005), to climate conditions and particularly the heat stress (Jordan, 2003), to any other stress factor (Bono, 2003), to the breeding system and so on. We will not consider them, but we remind them with a Lucy’s (2001) statement: “...declining fertility is probably a combination of a variety of physiological and management factors that have an additive effect...”.

MILK YIELD AND FERTILITY

As suggested before, intensive dairy farms have several peculiarities, some of them can improve the management of cows and then can reduce – if properly applied – the reproduction problems: more

hygiene and prophylaxis, better possibility to inseminate at right time, protection against environmental adversities, good feed quality and proper diet formulation, constant availability of feeds and water etc. Nevertheless, intensive dairy systems can be also cause of reproductive impairments: too high ratio between cows and labourers, unsuitable buildings and equipments and first of all the continuously rising of milk yield level; the latter making more difficult the full satisfaction of energy and protein requirements, particularly in the early stages of lactation (Veerkamp et al., 2003).

According to the more recent reviews regarding dairy cow fertility, the close negative relationship between genetic merit increase and fertility reduction have been underlined (Butler, 1998; Webb et al., 1999; Santos, 2001; Lucy, 2001; Lucy, 2003; Webb et al., 2004). A possible explanation could be a genetic antagonism between milk yield and fertility, more likely for a pleiotropic gene effect (Veerkamp et al., 2003) that, modifying some hormones, induces changes in energy partitioning with a consequent reduction of metabolic fuel availability for reproductive organs. Nevertheless, Hansen (2000) suggests that genetic control of fertility is low, while environmental factors are so many and important. An indirect confirmation of the low genetic importance can be seen in the good fertility of heifers with a very high genetic merit for milk yield (Hansen et al., 1983; Lucy, 2003). The low genetic control does not exclude the possibility of a profitable, despite slow, improvement of fertility by a proper selection scheme (Gonzales-Recio and Alenda, 2005); however, it also would mean a great importance of management practices – namely proper nutrition to cover energy deficiency – as suggested by Ouweltjes et al. (1996), Webb et al. (1999), Santos (2001), Inskeep (2004).

Therefore it is now essential to better understand whether high milk yield can really explain the decline of fertility and whether the effect can be avoided (or reduced). For this purpose it appears noteworthy to consider that a similar behaviour of fertility reduction has been observed in USA, (Fig.1 by Butler, 1998), and in UK as showed in Fig.2 by Royal et al. (2000). Unfortunately, the milk yield change in USA ranged from 6000 to 9000 kg, while in UK it ranged from 4500 to 6000 kg. These data seem to suggest that milk yield per se is not the main cause of fertility reduction; in fact, the average yielding dairy breeds, also suffer for some fertility problems (Harman et al., 1996a and 1996b). From the above comparison, it appears interesting the strong decline, since the end of '80s, in both cases. It can be therefore suggested that some other factors, like growing number of cows per herd, declining milk prices and therefore less skilled manpower and animal care etc. might be recognized as cause of lower fertility. Moreover, some interest could be recognized to the possibility of a gradual change of the diets during last decades – of course in the intensive farms – with an increase, of omega-6 fatty acids, as suggested by Petit et al. (2002).

A further useful comparison can be made between USA Holstein herds with different levels of milk yield. Stevenson (1999) has described the 1999's data of Dairy Records Management System (USA) comparing herds within a range of less than 6800 and more than 11350 kg of milk per lactation. He also compared them with similar data of 1989 where the range of milk yield was within 5700 and 9300 kg. Further data of an analogous comparison, showed by Call and Stevenson (1985) and regarding 5880 herds from 3600 to 9000 kg of milk yield, have been also utilized. In Fig.3 and 4 milk yield and days open mean values, corresponding to the herd groups, are respectively showed. Two aspects appear of great interest:

- the days open difference between high and low yielding herds in each of the three sets of data was not so relevant, particularly in the past (ranges were 118-133 days in 1983, 126-142 days in 1989 and 151-195 days in 1999); but interestingly, the situation was always worse in the low yielding herds;
- the difference between the low and high yielding cows became important only recently: 1983 it was small, while 1999 it was considerable (the absolute values were also very high). Moreover, still considering a similar milk yield close to 8000-8500 kg, the days open were 121 in 1983, 126 in 1989 (very similar) and 162 in 1999 (a big rise in the last ten years).

These data again confirm that the reproductive success is not only affected by milk yield, but other factors could be important, namely the management. Despite lack of inflammation, it appears likely that herds with the lowest milk performances – at least in part – were badly managed; low genetic merit

cows would have lower milk yield, but more acceptable fertility. A new possible cause of reduced fertility could be a too quick genetic improvement, respect to the general management adaptation of dairy farms, particularly in the last 20 years.

It can be therefore concluded that high yielding dairy cows are per se fertile and that the genetic antagonism between milk yield and reproduction is not the major factor of low fertility. Nevertheless, as much as the milk yield is higher, it become more and more difficult, particularly in the not well managed farms, to satisfy the energy (and protein?) requirements of cows which are genetically partitioning nutrients toward mammary gland (Verkaamp et al., 2003). Thus they become more susceptible to metabolic stress and consequently to metabolic and/or to infectious diseases as well as to reproductive problems (Ward and Parker, 1999; Knight et al., 1999; Garnsworthy and Webb, 1999). What is suggested is therefore a higher risk for cows with unsatisfactory nutrition supply, for the efficiency of reproductive apparatus, but also for that of the immune system (Ferguson, 1991; Schukken et al., 1999). This can explain the higher frequency of infectious problems regarding the reproductive apparatus (Heuer et al., 1999), but also of other diseases, infectious or metabolic troubles with more or less dramatic consequences, like liver triglyceride accumulation impaired fertility and culling.

These concepts fit very well with the recent review of Drakley (2006) who, discussing about the production metabolic diseases, pointed out: “given the exquisite matching of nutrient demands and supply in healthy high-producing dairy cows, it is reasonable to expect that stressors which place additional nutrient or adaptive demands on the cow may disrupt her ability to maintain homeostasis. Thus, the conceptual view that high-producing cows are more susceptible to environmental insults that in turn disturb homeostasis and causes production disease is more attractive than the notion that production disease is caused by high production per se”.

Likely, if this is the case we can expect – besides a certain number of high yielding farms with lower problems – that a certain number of cows could have satisfactory performance both for milk yield and fertility, despite their farms are not in optimal conditions. In our recent experiment (Bertoni et al., data not published), 120 cows owning to 3 high yielding dairy herds with an acceptable management, have been monitored (blood sampling, milk yield, health problems, BCS, reproduction events etc.), from the end of pregnancy and till next pregnancy. The non pregnant or the cows with problems 2-3 months after calving have been excluded, while the remaining 77 cows have been retrospectively divided in 4 equal groups (separately for each herd). The main data of milk and reproductive indices are been showed in fig.5 and tab.1. From them it appears that:

- the best 25% of cows (UP) had both higher milk yield and better fertility (53% pregnant at 1st insemination and 1.6 services per pregnancy);
- the worst 25% (LO) had both lower milk yield and less satisfactory fertility (37% pregnant at 1st insemination and 2.0 services per pregnancy);
- the intermediates (INUP and INLO) had good milk yield, but the worst fertility.

The cows (all multiparous) of the same herd have obviously received the same management (included nutrition because they were fed a TMR) and the 4 groups contained almost the same number of cows from each herd. Which was the difference between groups? Simply some blood indexes of inflammatory phenomena (i.e. negative acute phase proteins produced by liver: albumin, lipoproteins and Retinol Binding Protein) observed in the first month of lactation. Therefore it seems that:

- the best group had few and modest inflammations;
- the worst group had the highest frequency of serious inflammatory conditions (and contemporary a higher Negative Energy Balance (NEB) has been observed: lower BCS).

It can be therefore concluded that high milk yield is an important predisposing factor to fertility reduction; nevertheless, the high variability among dairy farms (Stevenson, 1999), as well as within the same farm (Bertoni et al., data not published), suggests the possibility of improvement. The most promising aspects seem those regarding the effects of NEB (Veerkamp et al., 2003) on both oestrus resumption after calving (Butler, 1998) and conception rate, as well as the effects of “diseases” in the early lactation (Roche et al., 2000).

ENERGY BALANCE AND REPRODUCTION

It is generally well accepted that a shortage of energy, mainly before calving in beef cows (Hess et al., 2005) and mainly after calving in the dairy cows (Butler, 1998), impairs reproduction. For dairy cows, the major interest for long time has been devoted to the early oestrus resumption which appears to have a strong relationship with the following pregnancy (Fig.6). A prolonged anestrus after calving seems justified by the negative effect of lower insulin levels – of course linked to a deeper NEB but also to genetic traits (Bonczek, 1986) – both on the follicle development and ovulation. This effect has been showed by Beam and Butler (1997), Landau et al., (2000) and Gong et al. (2002). Furthermore, NEB would also compromise the blood levels of IGF-1 (which is reduced, despite higher GH, for the lower presence of GH receptors into liver, Lucy et al., 2001 and Veerkamp et al., 2003), useful for growth of small follicles and for granulosa cell proliferation. Low energy availability seems as well to compromise LH stimulation – because GnRH pulse frequency is reduced – as well as ovarian responsiveness to LH (Butler, 2005). The importance of insulin on reproduction has been confirmed by Opsomer et al. (1999) that suggest an increase of cystic ovarian diseases in cows affected by insulin resistance (i.e. a peripheric insensitivity to the hormone).

Nevertheless, this “acute” effect of NEB is obviously characterized by a shortage of some metabolic fuels (i.e. glucose and aminoacids), drained by mammary gland for its syntheses, and by an increase of others (i.e. NEFA, β OHB). Therefore, besides the above hormone changes, the glucose shortage could be the primary cause of lower reproduction activity as suggested by Wade et al. (1996), Schneider and Wade (2000), Veerkamp et al. (2003) and partly confirmed by Francisco et al. (2003) as well as Butler et al. (2006). Moreover, the rise of NEFA and keton bodies could exert a negative (toxic) effect on follicles and oocytes (Kruip et al., 1999).

It is very likely that both mechanisms could be involved; nevertheless, NEB seems to exert an effect on reproduction in a more chronic way (i.e. with consequences occurring far from the NEB), in fact:

- poor BCS at calving due to a previous energy deficiency, no matter if post calving diet is appropriate, is associated (at least in beef cows) to a delayed oestrus resumption (Hess et al., 2005);
- long term energy deficiency (before or after calving) affects oocyte quality with ensuing negative effects on early embryonic development and subsequent foetal loss (Walters et al., 2002).

In summary, from these literature contributions, it appears that the issue of energy deficiency and reproduction cannot be limited to post-calving anestrus and to the insulin influence on follicle development. Moreover, if BCS is so important, it can be supposed that any cause of its reduction can impair fertility.

Oestrus resumption, oocyte quality and fertility - The relevance of NEB (i.e. low insulin) for the post calving oestrus resumption and the assumption that early oestrus always means good fertility, are therefore argued. Namely, the effects of NEB on the anestrus length are important according to Call and Stevenson (1985), Butler and Smith (1989), Butler et al., (2006), but they are not so important according to Villa-Godoy et al. (1988), Harrison et al. (1990) and Spicer et al. (1990).

With concern to oestrus resumption and following fertility, we like to recall our experience (Bertoni et al., 1999). The data of 40 cows of our research barn were retrospectively divided in 2 groups, pregnant and not pregnant, and again in 2 groups according to their genetic merit. According to the plasma progesterone (P_4) changes, it appears that only the high yielding pregnant cows had an early resumption of oestrus (1,9 cycles within 60 DIM vs 1,0 cycles), nevertheless they had a lower fertility (171 vs 125 days open). The two groups of cows, low and high genetic merit, had an average peak of 34 and 45 kg/d of milk, their health status was normal and BCS after calving was slightly reduced in low, but significantly reduced in high yielding group. Furthermore the real genetic origin of the milk yield difference has been confirmed by the insulin and GH pattern of changes in the first 3 months of lactation (Fig.7); these hormones represent in fact the major endocrine changes observed in the highly selected cows (Bonczek, 1986; Veerkamp et al., 2003). Not all the 40 cows were utilized for hormone evaluation and therefore a relationship with reproduction is not possible; however it appears noteworthy that, despite insulin was lower in high yielding cows see also the patten after main meals in

Fig.8, oestrus resumption was not compromised. On the contrary, the fertility was reduced in this kind of cows which were characterized by a strong NEB (higher loss of BCS, low glucose and high NEFA/BOHB).

This suggests that early oestrus resumption is important, but not sufficient to guarantee the reproductive capacity; furthermore that the role of insulin seems arguable because oestrus resumption was independent. For insulin it is noteworthy its reduction after calving also in cows of low genetic merit, despite acceptable glucose values, while insulin of high yielding cows remains lower in the first 2-3 months of lactation (Fig.8) despite glucose returned to acceptable values. This, of course, does not exclude a role of insulin in the reproductive function, while it confirms insulin as part of the homeostatic mechanisms; in fact it declines before calving (Fig.8), when glucose is still high.

A further mechanism – independent from insulin – of NEB to impair reproductive efficiency, i.e. the quality of oocytes, has been recently recognized by Garnsworthy (2006, personal communication) who has observed that a diet which ensures “high” glucose-insulin values have a favourable effect on follicle growth and resumption of oestrus cycle, but not on oocytes quality and pregnancy maintenance. It seems confirmed that this resumption does not guarantee the pregnancy at time of insemination. Similar results have been obtained by Horan et al. (2005), while Britt (1994) has suggested a different response to insemination: there is a good conception rate if insemination occurs very early (around 50 DIM), but low conception rate if it is delayed since 80-90 DIM despite early oestrus resumption occurred in both cases.

The following hypothesis seems therefore to arise: the regular (or not too delayed) resumption of oestrus is important for fertility, but not less important is the good quality of oocytes. Both aspects are affected by energy nutrition, nevertheless times and ways could be different:

- a) early resumption of oestrus seems mainly affected by the “acute” energy deficiency: low “fuels”, low insulin, and low IGF-1 impair follicle growth and GnRH response, consequently low estrogens, low LH and low progesterone (Butler, 2005). Nevertheless, it has been previously reviewed that BCS at calving can be also important; this particularly in the beef cows (Hess et al., 2005), but also in dairy cows because Markusfeld et al. (1997) have observed a delay of ovarian activity onset in cows with low BCS at calving. As recently reviewed by Veerkamp et al. (2003) and by Butler (2005), besides the difference in the oocyte quality, a possible explanation of the low BCS effects on reproduction could be linked to leptin. This hormone is produced primarily by adipose cells, therefore its blood concentrations are positively correlated to the size of the fat depots and then to the nutritional status. The receptors of leptin have been identified in the hypothalamus and bovine ovarian granulosa cells, suggesting a permissive threshold of its blood level for pubertal or postpartum reproductive activity (Barash et al., 1996; Chilliard et al., 2005);
- b) the quality of oocytes seems mainly affected by the “chronic” energy deficiency. In fact, according to Britt (1992), the exposure of oocytes to adverse conditions, such as NEB, during the initial growing and developing phases (60-80 d before full maturation of follicles), results in altered or impaired development of mature oocytes and embryos. Not always but some experiments have confirmed the Britt’s hypothesis: Kruip et al. (1996) and Walters et al. (2002), as well as Sakaguchi et al. (2004) who have obtained greater pregnancy rates at the first detected oestrus after calving (possibly because the released oocytes were matured during dry period, generally with acceptable energy availability). The possibility of an effect on oocyte quality of nutritional status, occurring during last pregnancy period, has been also suggested by Hess et al. (2005) who, citing Hunter (1991) and Krisher (2004), have however pointed out an effect of pre-calving nutrition both on follicle development and oocyte quality in the subsequent breeding season. The consequences of low BCS, thus of a NEB occurring a long time before the evaluation of oocyte quality, have been showed by Snijders et al. (2000). They slaughtered 98 cows in late lactation (23-24 kg/d of milk) to evaluate the oocytes (number and blastocysts formed after in vitro maturation and fertilization). The number was similar, but cleavage and blastocyst formation rates were lower for oocytes from cows with a lower BCS. The same hypothesis has been partly confirmed by Walter et al. (2002): oocyte quality was reduced in the

3rd month of lactation of cows that immediately after calving showed higher NEFA and greater BCS losses. Nevertheless, the same cows, with greater NEB, had lower oocyte quality also in the first month of lactation. This is noteworthy because suggests that the quality of oocyte can be negatively affected both by long term energy availability (2-3 months in advance) and by short term availability, namely during first month of lactation, itself (the 2-3 months before calving were supposed to be good).

It is therefore clearly showed that NEB, “acute” or “chronic”, impairs both oestrus resumption and oocyte quality; about the mechanism, we do not have direct experience but our data on hormone changes in the early lactation make sense of the Veerkamp et al. (2003) conclusions: “the reduced metabolic fuel availability, rather than direct effects of hormone concentrations on reproduction, seems to cause poorer fertility, i.e. poorer oestrus behaviour, poorer oocyte quality and lower progesterone values during the luteal phase”. Particularly for oocyte quality, it appears noteworthy that not only available energy was showed to be important; in fact, according to Garnsworthy (2006, personal communication), a proper lactation diet (with a balanced supply of starch and fat) was needed to improve oocyte quality and conception rate of high yielding cows. This could suggests that other nutritional (and non nutritional ?) factors – besides energy – can affect the resumption of oestrus and/or the quality of oocytes (i.e. the omega-6/omega-3 ratio, which will be illustrated later).

Causes of negative energy balance and BCS loss - At least in the intensive breeding system, the real unavoidable (or better really difficult to be matched) nutritional cause of low fertility might be the negative energy balance in the first months of lactation. Now the question is: the high yield and the obvious capacity limits of the digestive apparatus are the only cause of the energy deficiency? The most easy response can be taken from Lucy's (2003) conclusions: “cows selected for high milk production partition nutrients toward lactation. The partitioning of nutrients leads to cows with less adipose tissue mass (lower body condition) and greater infertility”. It is obviously true, but it appears an over simplification because it does not explain the results of Stevenson (1999): herds with higher milk yield have showed better reproductive performances, as well as our aforementioned results: inflammation free cows produce more milk and are more fertile. Furthermore, Lopez et al. (2005), have recently suggested that high incidence of a prolonged anovulatory period is not associated to the milk production but to the lower BCS. Similar, with respect to BCS, is the suggestion of Markusfeld et al. (1997): “cows that lost more BCS during the dry period suffered more from inactive ovaries after calving”.

In other words, the real discriminating factor – at least when speaking about the early lactation period – seems the BCS level at breeding time (Fig.9) as suggested by Santos et al. (2001): “cows that lose BCS during the breeding period, or have a low BCS during insemination have reduced conception rate and are less likely to respond to oestrus synchronization. The result seems to be the same, but for the second case (low absolute value of BCS) a further explanation could be the too low level of lipid reserves which appears per se important (Friggens, 2003). The new question is: why the reduction of BCS can be, at least in part, independent from the level of milk yield? Of course excluding the influence of pre-calving conditions on BCS level. Again, the answer is not easy, in fact the generally accepted reasons of serious NEB of high yielding cows in the transition period are: 1) the quick rise of milk yield; 2) the less quick rise of Dry Matter Intake (DMI) because the digestive apparatus needs to adapt to the needs imposed by milk yield. Unfortunately, two more reasons might impair the NEB: 1) the possibility that DMI could be lower than usual and justified in a manner not related to milk yield or to the bulkiness of the diet (total fiber and its physical efficacy); 2) the possibility of an increased maintenance cost.

The first is not new, Villa-Godoy et al. (1988) showed clearly that the variations in NEB were explained largely by intake of energy (appetite) and to a lesser extent by milk yield (lower DMI than expected did justify the worse NEB). Similarly, Staples et al. (1990) have showed that anestrous cows ate less feed, produced less milk and lost more body weight, resulting in a more negative energy status than cycling cows (both groups were of the similar genetic merit and were receiving the same standardized husbandry). The real problem seems therefore a reduced DMI, respect to the expected values and then not simply justified by the gradual increase of digestive capacity. Thus it appears interesting what

observed by Butler et al. (2006): a voluntary reduction of appetite was the cause of lower energy balance and longer anestrus after calving, milk yield was in fact similar to the ovulating cows, but the lower DMI began before calving. So they have recalled the Grummer (1995) suggestions: “DMI during late pregnancy plays a role in “programming” susceptibility to periparturient metabolic disorders”; in other words, the periparturient metabolic disorders – linked to the reduced DMI in late pregnancy – could be the origin of the abnormally lower DMI in the transition period.

We generally agree with this hypothesis, except that we suggest a more wide range of DMI reducing causes: i.e. any inflammatory condition occurring immediately before, during and after calving. In fact, our results have showed that the reduction of DM intake observed in dry period is responsible of a lower intake after calving, but the first one is contemporary to a subclinical acute phase response (inflammation) as showed in Fig.10 and 11. In this case, a group of cows have lost appetite before calving and a second has lost appetite “at calving”, both associated to inflammatory phenomena and both with negative consequences on the early lactation DMI. That agree quite well with Drackley et al. (2005): liver lipidosis and low DMI after calving are more linked to the changes of DMI occurring at the end of dry period (and much less to absolute values). Nevertheless, from the previous Fig.10, it can be observed an acute phase response, immediately after calving, also in the group without an apparent DMI reduction. Unfortunately nobody can exclude a negative effect of this phenomenon, but it is difficult to confirm it because the optimal growth of DMI after calving, is not known. These results are noteworthy because there is an high frequency of inflammations in the transition period (Cappa et al., 1989; Sordillo et al., 1995). They can be justified by more or less serious clinical symptoms (followed by lower milk yield and other problems), but also by some subclinical situations not easily revealed by milk yield, or by other negative consequences. In fact, in our experiences, the high frequency of inflammatory phenomena is based on blood indices of the pro-inflammatory cytokines activity. It is therefore noteworthy because these cytokines are responsible of some anorexia, of increased catabolism and often of some fever (Fig.12); thus, lowering the input and increasing the output of energy, they can worsen NEB and therefore they can accentuate the BCS loss.

Of course, the negative effects will be more or less important according to the seriousness of the illness or the subclinical inflammations; however, their effects will concern milk yield, liver functions, digestive capacity and many others included reproduction.

Health conditions, energy balance and fertility - In the last few years, a growing attention has been paid to the health aspects to find the causes of a reduced pregnancy rate. This not only for the obvious lower fertility observed in animals with reproductive disorders (Ouweltjes et al., 1996; Labernia et al., 1999; Rajala and Grohn, 1998), but also for the negative effects on reproduction of both peripartum metabolic diseases (Markusfeld et al., 1997; Beaudeau et al., 2000; Fleisher et al., 2001; Sogstad et al., 2006) and infectious diseases like mastitis (Barker et al., 1998; Schrick et al., 2001) as well as any other kind of health problem around calving (Opsomer et al., 2000). Then, the statement of Roche et al. (2000) i.e. “high reproductive efficiency in the dairy cow requires a disease free transition period...”, must be reminded and carefully applied. The same concept has been expressed by Lucy (2001): “epidemiological studies suggest that disease parameters (e.g. ketosis, mastitis, retained placenta and cystic ovary) have a greater effect on herd fertility compared with non-disease parameters (e.g. milk production and BCS)”.

Thus it appears interesting if the incidence of diseases in dairy herds was increased, almost contemporary to the reduction of fertility. According to Müller et al. (1999), from 1970 to 1996 the percentage of cullings, as result of disease, more than doubled from 13,4% to 27,4%. These data have been obtained from German Black Pied cattle that in the same period had an increase of milk yield from 4670 to 7020 kg. The same authors have remembered the relationship between milk yield increase and physiological changes in fitness-related traits which can justify more diseases and less fertility; so their suggestion to select cows not only for performance but rather for a balance of performance and non productive traits (longevity, resistance to specific diseases, fertility etc.). The above data confirm that high yielding cows are more susceptible both to infectious diseases, namely mastitis, and to metabolic stress, moreover they are less tolerant to small management mistakes (Ward and Parker, 1999; Knight et al., 1999; Garnsworthy and Webb, 1999; Heuer et al., 1999). This susceptibility is

further increased in a period of reduced immunological capacity as the transition period (Mallard et al., 1998). In fact, in periparturient cows has been experienced more rapid bacterial growth, higher peak bacterial concentration, higher fever, and equal or greater proinflammatory cytokine concentrations in foremilk than did midlactation cows (Shuster et al., 1996). Nevertheless we like to point out the agreement with the conclusions of Ingvarlsen et al. (2003): there is not an inevitable association between increased milk production and poorer health. The high milk yield (besides an unsuitable diet and a lower DMI) is responsible of an excessive reserve mobilization which impairs the immune system (some endocrine changes and nutrient depletion) and increases the disease risk. However a proper feeding strategy can help to alleviate these risks (Ingvarlsen et al., 2003).

This suggests that “health” conditions around calving are essential to get the best results, not only for milk yield, but for fertility as well; therefore both traits can cohabit together when cows are in good conditions. To similar conclusions arrived Distl et al. (1989) that in double purpose cows suggest: “the milk production of cows can be increased without the risk of altering profit by higher occurrences of metabolic, udder and some fertility disorders, if management and feeding practices are adjusted for high-yielding cows”. Quite similar to the suggestions of Aeberhard et al. (2001) for high yielding cows: “results of this study demonstrate that cows yielding ≥ 45 kg ECM/day could be maintained with no more serious problems than the cows with yields of around 35 kg ECM/day, if husbandry, management and feeding were adequate”.

Noteworthy is anyhow the observation that nutrition is important for the health status of cows, particularly of the high genetic merit and during transition period. This in fact confirm the aforementioned statement of an indirect effect of nutrition on reproduction by means of the compromised health status. Nevertheless we have also previously showed that disease can interfere with nutrition status (Calder and Jackson, 2000), particularly for the inflammatory consequences, mediated by pro-inflammatory cytokines that reduce DMI and increase the metabolic cost.

Cytokines are part of a counterregulatory system that play a critical role – in case of immune system activation – in preventing the host from mounting an excessive defence response (Kapcala, 1999), which would be dangerous. There are pro-inflammatory cytokines (IL-1, IL-6 and TNF α) which promote a local and a systemic response to help the defence system (Cousins, 1985; Elsasser et al., 1997; Dinarello, 1997; Gruys et al., 1999), but also anti-inflammatory cytokines like IL-4 and IL-10 which depress the formers to avoid unneeded and dangerous effects (Grimble, 2001). The pro-inflammatory cytokines are responsible of a sequence of events that involve several organs and tissues, as hypothalamus, liver, reproductive apparatus (Elsasser et al., 1997). In addition, cytokines increase body temperature, induce anorexia, increase catabolism (mainly from adipose and muscle tissues), determining several endocrine and metabolic changes (Elsasser et al., 1995). It seems noteworthy to point out some effects that can be particularly pernicious in the peripartum period: anorexia, catabolic conditions and fever, adipose mobilization and – not included in the Elsasser’s statement – a great change of liver activity. All together, these effects can increase the risk of many metabolic diseases (namely ketosis and liver lipodosis), but also infectious.

The aforementioned reduction of dry matter intake induced by cytokines (Johnson and Finck, 2001), was also hypothesized by Ingvarlsen and Andersen (2000) to explain the lower DMI of periparturient cows and was confirmed by us in the last part of dry period (Trevisi et al., 2002) as previously showed. Nevertheless, the negative effects of pro-inflammatory cytokines on energy balance are not restricted to the reduced DMI. The catabolic conditions and particularly the rise of body T $^{\circ}$ (fever), due to secretion of thyroid and adrenal hormones to rise metabolic rate, to peripheral vasoconstriction and to shivering, increase the energy wastage. According to Klasing (2000), there is an increase of resting energy expenditure, an increase of futile cycles and of course a decrease of feed conversion efficiency; in case of early lactating cows, this means a worsening of the energy balance and higher risks for health and fertility. Our unpublished results (Trevisi et al.) have confirmed that cows affected by inflammatory conditions – still without marked clinical symptoms – after calving, show a lower energy efficiency.

The pro-inflammatory cytokines can therefore impair the NEB both by lowering DMI and increasing maintenance cost; further negative effects in the transition period the increase of adipose mobilization and the modified liver activity. These latter aspects can be discussed together for the well known

interactions; nevertheless, for the specific metabolism problems of released fatty acids, the papers of Drackley (1999) and Drackley et al. (2005) can be very useful. With concern to liver, the released cytokine are responsible of a peculiar phenomenon; they in fact promote the synthesis of several plasma proteins (Cousins, 1985; Elsasser et al., 1997; Gruys et al., 1999; Peterson et al., 2004; Gruys et al., 2005): the positive acute phase proteins (+APP: i.e. haptoglobin, ceruloplasmin, C-reactive protein). Unfortunately, this increased activity partly compete with the production of the usual liver proteins, which are more or less reduced (Wan et al., 1989). Several proteins are affected by this competition (i.e. albumins, enzymes, “carriers” of vitamins and hormones, lipoproteins) and are called negative acute phase proteins (-APP). Unfortunately, they are involved in many liver functions which can be impaired, despite liver remains active or maybe more active. This apparent paradox of liver – active to produce +APP and less active to produce –APP – becomes particularly important in the transition period, namely when it is overcharged because protein and fat storages are mobilized to cover the nutrient deficiency while the liver becomes responsible of the gluconeogenesis from aminoacids and of the fatty acid metabolism (Drackley, 1999; Drackley et al., 2005). In fact, a lower fatty acid oxidation and/or a lower Apo-lipoprotein (-APP) synthesis can contribute to a worsening of liver lipidosis (Bertoni, 1990; Murthy et al., 1997; Lippi et al., 1998; Katoh, 2002; Bertoni et al., 2006).

In conclusion, pro-inflammatory cytokines could represent an important part of the linkage between any kind of disease occurring at calving time and the impairment of reproduction efficiency, particularly by worsening NEB and disturbing liver functions. The cytokines can also interfere directly with reproductive function in some different ways (Fig.13), but many of them are less likely in the transition period. In particular, we are referring to the negative effects of an inopportune release of LH (Braden et al., 1998), an inopportune release of progesterone from adrenals (Trevisi et al., 1996) or a large release of PGF_{2α} (Fredriksson et al., 1985).

FERTILITY AND DIET PROTEINS

The second major nutritional cause of lower fertility has been considered the protein excess (or the level of blood urea above 19.0 mg/100 ml of milk) (Butler et al., 1996; Butler, 1998). Nevertheless, in a previous review on nutrition and fertility in dairy cows, one of us (Bertoni, 1990) has showed that there were papers suggesting that fertility can be either negatively affected (Jordan and Swanson, 1979) or without consequences (Howard et al., 1987) by a very high protein intake (19-20% d.m.). Because, according to Visek (1984), the high NH₃ was suggested to be responsible of this reproductive impairment, the excess of rumen degradable protein might be the real cause. This hypothesis has been confirmed by Folman et al. (1981): the same protein content (16%) gave much better fertility results when soybean was treated to reduce rumen degradability (formaldehyde).

Afterwards, many papers were in favour of a reduction of fertility in dairy cows fed excesses of proteins, particularly in case of high blood urea level (Butler et al., 1996; Garcia-Bojilil et al., 1998; Rajala-Schultz et al., 2001); on the contrary, other papers have excluded this negative effect of urea (Gustafsson and Carlsson, 1993; Godden et al., 2001). According to Godden et al. (2001), there are many possible explanations to this disagreement:

- only values extremely high (or low) of urea could be cause of reduced fertility;
- high urea values could impair reproduction performance only when associated to some kind of disease (Ferguson et al., 1993; Barton et al., 1996);
- the negative effect of high urea levels could occur only in case of a wrong protein: energy ratio.

Our experience does not exclude the first two points, but it suggests that the last one would be the most like case and particularly important would be the high degradability of proteins, together with a low availability of fermentable energy. They induce a high release of NH₃ into rumen, as showed by Ferguson and Chalupa (1989) and by Gustafsson and Carlsson (1993); thus, the mechanism would not be urea and its negative effect at uterus level, as suggested by Butler (1998), but the increase of NH₃ in blood which depresses insulin (Sinclair et al., 2000a), but also impairs the quality of oocytes and their capacity to develop (Sinclair et al., 2000b). Furthermore, a negative effect on oocyte and not on the

uterus environment of protein excess, has been demonstrated by Gath et al. (1999) because good embryos transferred in heifers with low or high urea levels conceived in similar proportion.

To conclude this topic, we agree with Santos (2001): “although there is no clear relationship between protein intake and reproduction, cows fed diets that result in high urea nitrogen concentrations in blood might have reduced conception or pregnancy rates”. Nevertheless we add that milk urea values above 19 mg/100 ml of urea-N (risky according to Butler et al., 1996) occurs when crude proteins of the diets are above requirements and moreover the ratio between degradable proteins and fermentable energy is not appropriate for optimal cow performance; all aspects well known and quite easy to be managed according to NRC (2001) or other proteins systems. Therefore the well managed intensive dairy farms have all the required tools to cover protein needs for good milk yield and satisfactory fertility.

HOW TO PREVENT DISEASES, NEB AND FERTILITY DEPRESSION

If energy balance – no mind to its origin – is the most important nutritional cause of impaired reproductive performance (proteins are important as well, but in the intensive farms they are an accessible task), it appears noteworthy to suggest the ways to reduce its seriousness and extension after calving. Nevertheless, as showed before, it is not a pure feeding problem and anyhow NRC (2001) can help about the dietary management of far-off dry, close-up dry and early lactation cows. On the contrary, nutrition and health become a whole for the extremely important interactions among themselves and in relationship with high milk yield. Referring to Knight et al. (1999) a metabolic load can be caused by “the burden imposed by the synthesis and secretion of milk” and a metabolic load which cannot be sustained for the insufficiency of nutrients, such that some energetic processes must be down regulated, causes a metabolic stress (the aforementioned inflammatory conditions can be part of this stress) which can impair reproductive and immune systems.

It seems therefore quite clear that nutritional, environmental and management strategies to reduce postpartum disorders (any kind), to minimize the extent and duration of NEB and then the length of postpartum anestrus, are critical for a successful reproduction (Jordan et al., 2002). A similar statement comes from Rhodes et al. (2003), but of great interest is the suggestion of Stevenson (2001) and concerning the great importance of DMI and its relationship with health: “balanced nutrition and health of the transition cow are keys to maximal DMI after calving. Maximal DMI ensure that milk yield, onset of oestrus cycles, and initiation of pregnancy can occur in a timely manner, if the programmed breeding protocols and good detection of oestrus are in place”.

Nevertheless, a further concept must be clearly recalled: the narrow relationship between energy balance and health; i.e. energy deficiency is responsible of disease occurrence which in turn have a negative effect on energy balance (Calder and Jackson, 2000). Therefore, the relationship between nutrition, health and fertility is quite complex and needs a special emphasis, but taking into account that we are not speaking about sick animals, but about cows in a crucial situation so that a small added nuisance can alter the unstable equilibrium. This mean that for prevention of health disorders and for the mantainement of an acceptable NEB, any needed attention must be paid to the care of dry period and particularly of transition period.

With concern to this, Goff and Horst (1997) stated:” the transition from the pregnant, nonlactating state to the nonpregnant, lactating state is too often a disastrous experience for the cow... The well-being and profitability of the cow could be greatly enhanced by understanding those factors that account for the high disease incidence in periparturient cows”. The above factors accounting for an high disease incidence can be obviously considered for a proper prevention: the general management, the prophylactic tools (hygienic practices, vaccinations, feet trimming, etc.) and of course the nutrition. In fact, according to Dann et al. (2005), nutrition during the dry period may affect susceptibility of cows to metabolic and infectious diseases during the periparturient period; in turn, the latter it can affect the DMI after calving as showed for each disease by Bareille et al. (2003). In other words it seems that the greatest part of the suggested practices are aimed to reduce the risk of diseases or health disorders: infections, metabolic diseases, lameness, stresses like oxidative stress, metabolic stress,

micotoxines and other toxins, psychological stress etc; all them could be, more or less, a cause of the cytokine release (Fig.13) which have negative effects on DMI and energy needs.

Going back to the nutrition strategies to manage the transition (or dry) period and to obtain an acceptable negative EB, it appears inconsistent the attempt to simply modify the energy content of the diet to increase DMI before calving. In fact, Grummer (1995) suggested an increase of nutrient density during dry period, but this strategy was not fruitful and could cause an increase of fatty liver after calving, no mind whether the cows were too fat or not (Van den Top et al., 1996). Furthermore, it has been recently demonstrated (Dann et al., 2006) that cows overfed during dry period have a greater negative energy balance during the first 10 days of lactation (and higher risk of health problems).

They, as well as Van de Top et al. (1996), were not able to explain the results, but our suspicion is that the pro-inflammatory cytokines could be involved. Again this means that the real target of transition is to minimize the release of cytokines and therefore to reduce the incidence of diseases and health disorders with any kind of tools. Drackley et al. (2005) suggested that: “deficiencies in management that individually would not have major impact on dairy cow health and productivity (i.e., “subclinical stressors”) may sum to result in pronounced negative effects. For example, one might envision that the multiple stressors of overcrowding first-lactation cows with mature cows in the pre-calving pen of an uncooled barn during heat stress conditions might be disastrous for the younger cows”. This is exactly the best way of thinking, but many other examples can be given (i.e. unsuitable rest conditions, inadequate trimming strategy, poor hygiene or lack of proper vaccinations etc.); thus a cumulative effect of many stressors can occur and overcome some critical threshold. Again according to Drackley et al. (2005), “this concept may explain why “outbreaks” of metabolic disorders often follow a period of excessive rain and mud, or are more frequent during summer heat stress. It also may explain why producers struggling with management during farm expansion, or producers operating under generally poor management, have greater problems with disease in periparturient cows”.

Thus, first care is for that must cover all needs (energy, proteins, minerals and vitamins) without excesses in dry period; furthermore it must facilitate the diet passage, during the close-up period, with the objective to ensure the maximum of energy and proteins since early lactation. The latter means a special care to the balance between carbohydrates (fermentable and structural) and different nitrogen fractions, but noteworthy are also the contents of fiber (amount and physical efficacy) and of fats (content, chemical type and rumen protection). These aspects would be finalized to increase energy and protein contents of diet; nevertheless this requires caution to avoid digestive troubles on the contrary, to maximize energy and protein availability, seems better to stimulate a quick rise of DMI with a proper diet as well as with a reduction of health problems.

Modulation of the inflammatory response - To avoid diseases and health disorders is possible, despite not easy, but other causes of cytokine release, particularly trauma and the reiteration of the inflammation itself, have been described. A typical example is TNF- α which is both induced by and an activator of NF κ B and can lead to a partially self-perpetuating inflammatory process (Choudhury and Sanyal, 2004; cited by Shapiro and Bruck, 2005). Unfortunately there are evidences that multiple insults to the immune system tend to reduce the cellular immune response, for the effect of the anti-inflammatory cytokines, while the inflammatory response tends to remain high or to increase after new infections which can be facilitated by the above immune depression (Heyland et al., 2006). The best known mechanism of inflammatory reiteration is linked to the ROM, typical metabolites of inflammation, which activate the NF κ B and this the gene expression of pro-inflammatory cytokines and other pro-inflammatory proteins (Rimbach et al., 2002; Thuruham, 2004).

Despite some attempts to “defend” the cytokine effects, Ingvarlsen and Andersen (2000) and Varga (2004) who refer the results of Murray and Murray (1979) demonstrating the role of hypophagia in early host defence mechanisms against invading pathogens, the real risk of apparently small inflammations is their reiteration and “growth” which cause more damage than usefulness (Grimble, 2001). This means that pro-inflammatory cytokine release is always a potential risk, so they must be maintained at lower rate. Unfortunately it appears an impossible dream, particularly for dairy cows around calving, because pro-inflammatory cytokines can be released also by placenta as observed in pregnant women (Hauguel de Mouzon and Guerre-Millo, 2005) or during women labor (Simpson et al., 1998). This and the well

known phenomena of uterus re-absorption, can explain the high frequency of inflammatory phenomena observed in the peri-partum (Cappa et al., 1989; Alsemgeest et al., 1993; Sordillo et al., 1995) and not always accompanied by clinical symptoms (Bertoni et al., unpublished).

Therefore, if the avoidance of any rise of cytokines in the transition period might be impossible; it could be useful to minimize their effects or levels by a stop to the eicosanoid production and/or to the reiteration of inflammation. Anti-inflammatory drugs are well known and sodium salicylate has given good results (Fig.14 and Tab.2) when administered to dairy cows immediately after calving (Trevisi and Bertoni, 2006). It is furthermore noteworthy to point out that the inflammatory response to injuries and infections can be influenced in a great extent (Fig.15) by genetic (Howell et al., 2002) and nutritional factors (Grimble, 2001b).

This possibility, to modulate the inflammatory response by nutrients, is part of the immune-nutrition (O'Flaherty and Bouchier-Hayes, 1999; Suchner et al., 2000) and, besides anti-oxidants (any of them) and omega-3 fatty acids, it considers the use of some other fats (CLA), of some aminoacids (glutamine, arginine, cysteine, taurine), of some trace elements (Se), but future will likely add many others. Among these nutritional factors, able to modulate the inflammatory response, two examples are particularly interesting:

- anti-oxidants which suppress the activation of the NFkB (nuclear factor-kB) by ROM and therefore avoid its stimulation of pro-inflammatory cytokine gene expression;
- some fatty acids, namely unsaturated long chain omega-3, which compete with arachidonic acid omega-6 and direct the eicosanoid synthesis toward PGE₃ and LTB₅ series instead of PGE₂ and LTB₄; the first series being less potent inflammatory factors respect to the 2nd one (Grimble, 2001b; Calder, 2002).

The use of omega-3 deserves some more attention because they are been studied with success also to directly improve fertility. At breeding time they appear to reduce the PGF_{2α} level and then the embryonic losses (Mattos et al., 2002; Petit et al., 2002); this particularly in case of low luteal-phase progesterone level after mating (Wamsley et al., 2005). Similar positive results have been obtained with fish oil (Ambrose et al., 2006), very rich of omega-3, but the net inhibition of endometrial PGF_{2α} biosynthesis may vary according to the omega-6/omega-3 ratio (Caldari-Torres et al., 2006). Moreover, very recent data (Bilby et al., 2006a) suggest that calcium salts of fish oil (i.e. omega-3) modulate progesterone and estrogen receptor gene expression, as well as the expression of gene of PGF_{2α} synthase within the endometrial cells. The final result is favourable to the establishment and maintenance of pregnancy. From the same experiment, but different paper (Bilby et al., 2006b), the authors suggest that feeding fish oil modify the omega-6/omega-3 ratio of tissues in a manner that could improve immune function. An effect of omega-3 fatty acids on the immune system has been suggested since many years, but it remains under discussion for their supposed negative effects, at least at high dosages and if not well protected against peroxidation, on the immune response (Wu and Meydani, 1998; Calder, 1998). More recently this negative effect has been largely ignored, while an antinflammatory effect of these omega-3 is widely recognized in humans and laboratory animals (Calder, 2006) but few attempts with this aim are known for domestic animals (Klasing, 2000). It is however noteworthy what stated by Petit et al. (2002): the substantial changes of the diets since the end of 1970's, more fat and richer in omega-6, could contribute to explain the reduction of fertility observed, as pointed out before, particularly in cows with increasing levels of milk yield (Butler, 1998; Royal et al., 2000) and bred in more intensive farms where feeding is often "less natural". If true, the same change of omega-6/omega-3 ratio, could contribute to justify the increase of diseases and health disorders (Müller et al., 1999) in high yielding dairy cows or at least the disproportionate response to the inflammatory phenomena (Calder, 2006).

BLOOD LEVEL OF PROGESTERONE AND FERTILITY

Before to conclude, a short discussion of a specific topic: the progesterone (P₄) level and its relationship with fertility; it could be in fact linked to some nutritional aspects which are been previously emphasized. No doubt that a good level of blood P₄ is essential for fertility; it reduces the embryo mortality when pregnancy is running, but its concentration regulates follicular development by

negative-feedback control of pulse frequency of LH secretion (Inskip, 2004). There are situations characterized by low P_4 and consequently low fertility (Lucy, 2001). Still according to Lucy (2001), there are several potential mechanisms for decreased progesterone in high-producing cows. Progesterone concentrations in blood are influenced by rates of secretion, metabolism and clearance of progesterone. The rate of P_4 secretion has been hypothesized to be affected by the plasma cholesterol level, precursor for ovarian steroidogenesis, and therefore by fat supplementation which increase lipoproteins and then cholesterol (Carroll et al., 1990). We consider very unlikely the inadequate availability of cholesterol: 3-4 weeks after calving it is 1.5-2.0 times the pre-calving values. In fact, Staples et al. (1998) in their review on the influence of fat on reproduction, suggest that the higher value of P_4 in fat fed cows are likely justified by the lower clearance of P_4 (perhaps “protected” by higher levels of lipoproteins which carry the blood circulating P_4). Maybe likely could be the positive effect of omega-3 fatty acids which are known to inhibit the secretion of $PGF_{2\alpha}$ prolonging the corpus luteum life and therefore the P_4 production.

More recently the plasma level changes of P_4 have been attributed to the different liver steroid excretion as influenced by the increase of liver blood flow, which in turn would be the result of elevated DMI in high yielding dairy cows (Wiltbank et al., 2000; Sangsritavong et al., 2002). If true, it would be a classic paradox: cows require more energy (DMI), but higher DMI is associated with hormonal abnormalities. Luckily this relationship between DMI and plasma P_4 has not been observed by Rabiee et al., (2002a). Furthermore the high insulin level, also linked to high DMI, could substantially contribute, at least in vitro, to the reduction of P_4 clearance by the liver (Smith et al., 2006) with an improvement of P_4 level.

However this approach to the P_4 level looks like an oversimplification because, according to Santos (2001), cows with greater DMI would have lower progesterone concentration, but improving energy status by enhancing DMI has been demonstrated to increase peripheral concentrations of progesterone (Santos, 1996; Britt, 1994; Villa-Godoy et al., 1988) or, anyway, to benefit reproduction. In other words, to obtain a good level of P_4 it is essential to maintain a good ovary function and perhaps to modulate $PGF_{2\alpha}$ release for a better corpus luteum activity; to reduce liver excretion of P_4 appears a secondary problem, particularly if it would mean reduction of DMI.

CONCLUSIONS

Reproduction could be considered a “luxury” function and the female appears able to “sense” whether the environment is too harsh and risky for a successful reproductive cycle (Friggens, 2003). Therefore, almost all the environmental, managerial (namely nutritional) and sanitary aspects can – if not acceptable – interfere with fertility. Nevertheless, because our attention has been limited to nutrition and to intensive dairy farming, it appears unlikely – at least in principle – that proteins, minerals, vitamins and toxic factors could seriously impair fertility. On the contrary, intensive farming means high genetic merit cows for milk yield and this suggests two difficulties:

- for cow to partition nutrients to mammary gland without to fall down in the metabolic stress and its consequences;
- for herdsman to guarantee optimal conditions (environmental, managerial and sanitary) for so “sophisticated” animals and then to reduce any risk to for health and fertility.

As usual, two main ways can be followed to solve their kind of problems: genetics and husbandry; nevertheless it appears widely recognized that genetic selection will be useful in future, but management can be immediately crucial because – in this case – the problems are not inevitably associated to milk yield. With concern to the nutritional aspect, of major importance is the energy balance; in fact the requirements after calving can quickly rise (till 4 or more times of the maintenance), but DMI increases slowly and feed energy concentration have a narrow range of variability. The serious and prolonged negative energy balance appears therefore the cause of a great reserve mobilization with loss of the BCS; contemporary, and well correlated to BCS values, there is a prolonged anestrus and a lower quality of oocytes (and embryos). Some endocrine changes, part due to the genetic association to milk yield selection (i.e. insulin decline) and part to the NEB itself (i.e. lower IGF-1, lower leptin) but also to the reduced metabolic fuel availability (i.e. glucose), seem the responsible of reproductive failures. For

dairy cows the highest risk of NEB occurs during first weeks of lactation and this can compromise the immediate oestrus resumption. Nevertheless the oocytes can be also affected by this lack of nutrients, still occurring a long time before their complete maturation (60-80 days); thus the cows might have difficulty to conceive also in case of regular oestrus activity.

Several experiences have however showed a large variability of reproductive performance among the herds and within each herd; moreover there are data showing that fertility is more related to BCS changes and much less to the milk yield level. Also noteworthy appears the demonstration of a crucial role of DMI reduction as cause of a worse NEB; reduction that seems mainly affected by the “health” conditions (i.e. by pro-inflammatory cytokines which have anorexic and energy wasting effects) and much less by digestive volume and diet bulkiness. All these results suggest that there is room for a substantial improvement of reproductive performance if proper management conditions can be applied. Namely, to improve the energy balance in the 1st stage of lactation it is essential to maximize the rate of DMI rise; therefore it is suggested:

- any management tool useful to improve nutrition status before and after calving, but particularly to avoid any disease, health disorder, injury, trauma or stress which could trigger the release of cytokines. It appears noteworthy that this does not mean a high energy concentration before calving to get a higher DMI, but on the contrary a “low” energy supply in dry period followed by a proper close-up and early lactation diets (to provide needed nutrients and to optimize the digestive tract function);
- the reduction of inflammation effects – because it is impossible to avoid them – by a rapid therapy of “diseases” and, maybe better, by a prompt use of anti-inflammatory drugs;
- the preventive modulation of cytokine release – to avoid the self-perpetuation of inflammations after unusual causes of their release – with some immune-nutrition tools and mainly based on nutrients able to maximize the immune capacity, but also able to reduce the inflammatory response.

From this last point of view, the topic is quite new and need further work, particularly for domestic animals, despite some data suggest the promising positive effects of omega-3 fatty acids as anti-inflammatory compounds and well known in humans and lab animals. Furthermore it is noteworthy the suggestion of Petit et al. (2002): the diets are always more rich in omega-6 and poorer in omega-3, thus it could be a possible explanation of the sharp increase of reproductive failures of dairy cows in the last 3 decades. In addition, this could worsen the inflammation occurrence, also because the simplified, constant and monotonous diets with all-conserved feeds, could reduce the availability of other nutraceuticals (components of feeds that exert a beneficial effect upon health and metabolism, but are non nutrients). The importance and number of these nutraceuticals is growing quickly and the good supplier feeds are defined “functional”.

Only few words to suggest the possibility of their lack in the standardized TMR often utilized for dairy cows:

- Paterson et al. (2006) have reviewed papers showing the richness of salicylates (natural anti-inflammatory) in fruits and vegetables, sufficient in the “vegetarians” to prevent the human colon cancer;
- Mathers (2006) has recalled the better longevity of people with diets richer in plant foods (i.e. Mediterranean one), because plant-derived bioactive components of foods may protect the cells against genomic damage. The same effects were not properly reproduced with the prevention of oxidative damage (trace elements, vitamins etc.); thus, other non nutrient plant factors seem to have these attractive effects;
- Woodside et al. (2005) have confirmed the same Mathers’s statements: micronutrient supplementation quite often fails to reproduce the positive effects of any useful diet. For instance, the Mediterranean diet – rich of fruits and vegetables – was found to reduce – after two years – some indexes of inflammatory response (C-reactive protein, IL-6 and insulin resistance).

Going back to dairy cows, the questions are: what about a ruminant fed a variety of herbs? What about a ruminant fed a simplified diet? These could be new possibility that do not exclude the usual strategies to solve health and fertility problems; nevertheless their real usefulness must be evaluated a strict scientific approach.

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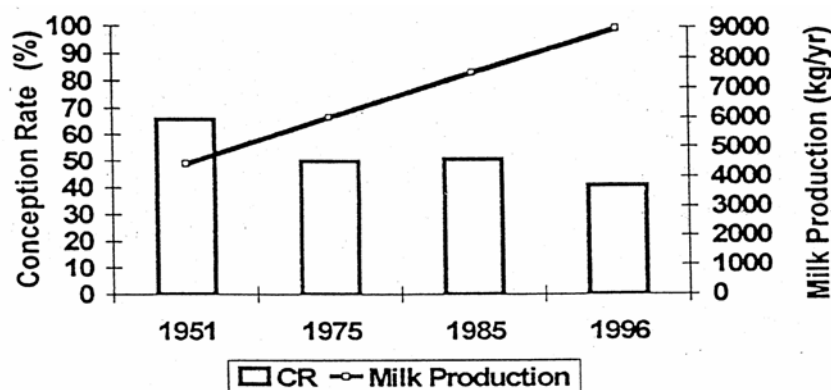


Fig.1: The inverse relationship between conception rate (CR) and annual milk production of Holstein dairy cows in New York (Butler, 1998).

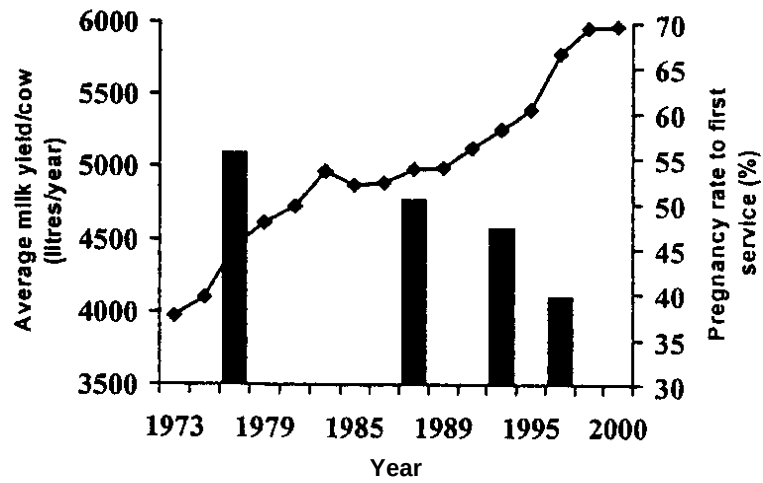


Fig.2: Declining fertility in UK dairy cows (Royal et al., 2000)

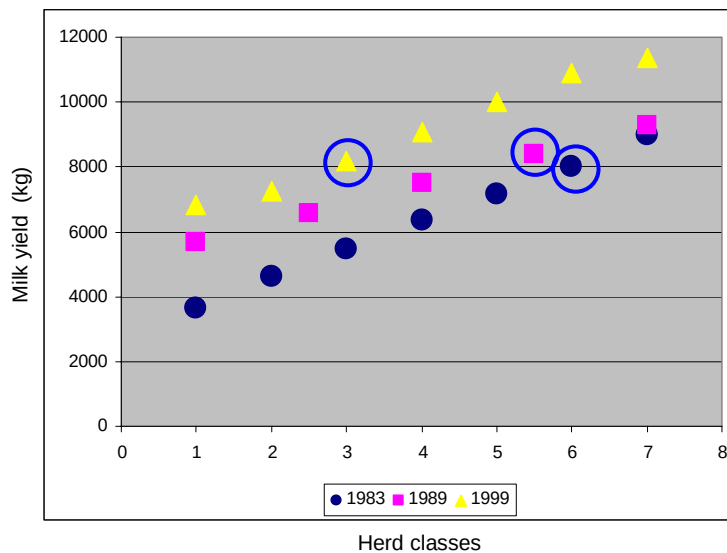


Fig.3: Ranking according to milk yield of some thousands USA Holstein farms in 1989 and 1999 (Stevenson, 1999) and 1983 (Call and Stevenson, 1983)

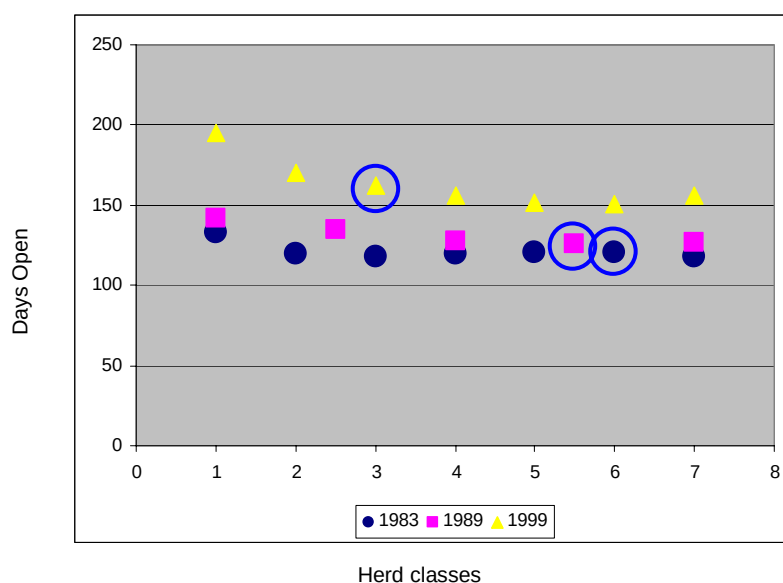


Fig.4: Ranking according to reproduction efficiency (Days open) of some thousands USA Holstein farms in 1989 and 1999 (Stevenson, 1999) and 1983 (Call and Stevenson, 1983)

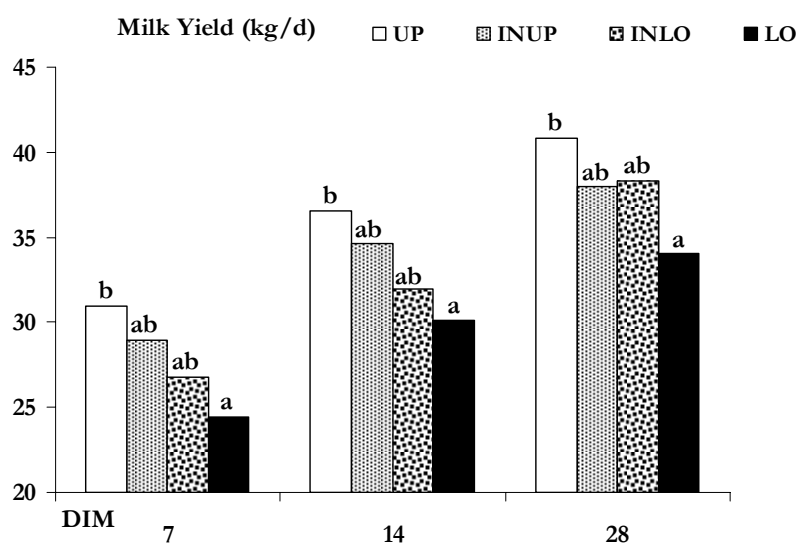


Fig.5: Average milk yield (kg/d) observed in the 1st month of lactation of cows within upper (UP), intermediate upper (INUP), intermediate lower (INLO) and lower (LO) quartiles of LAI (Liver Activity Index). Significant differences between groups are shown by different letters: (a,b,c) $P < 0.05$

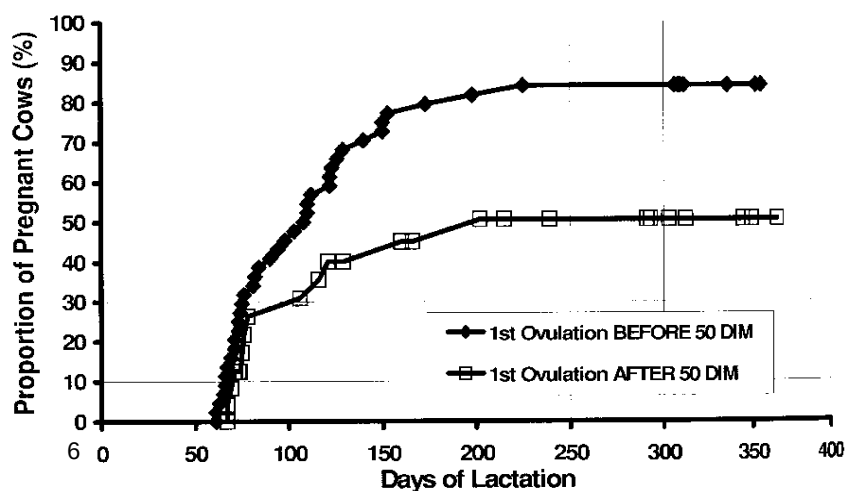


Fig.6: Survival curves ($P < 0.01$) for the proportion of cows becoming pregnant when first ovulation occurred before ($n=44$) or after ($n=25$) 50 days of lactation (DIM) (Butler, 2003).

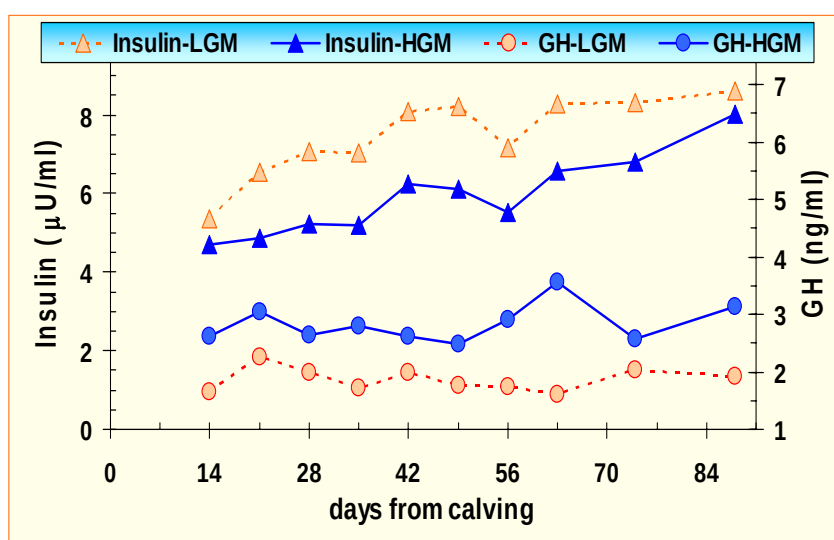


Fig.7: Pattern of changes of basal blood insulin and GH in low or high genetic merit dairy cows during the first 3 months of lactation

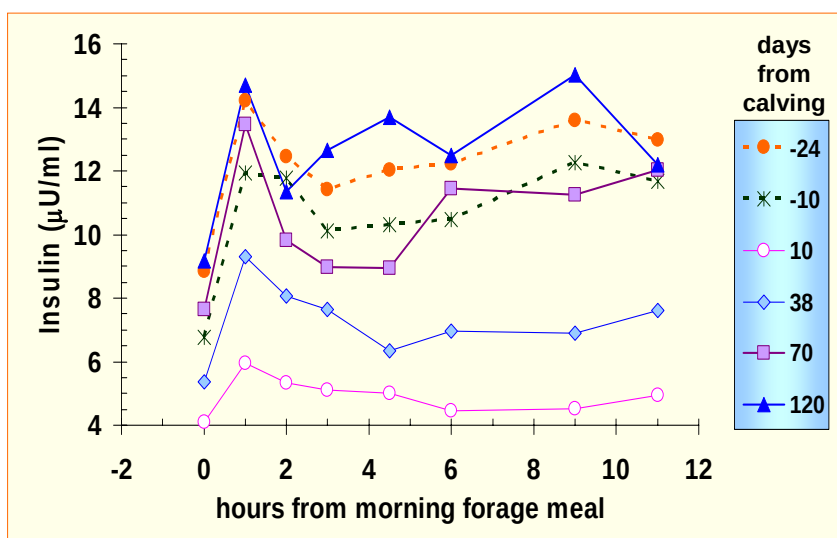


Fig.8: Daily pattern of changes of blood insulin in late pregnancy and different stages of lactation of high genetic merit dairy cows

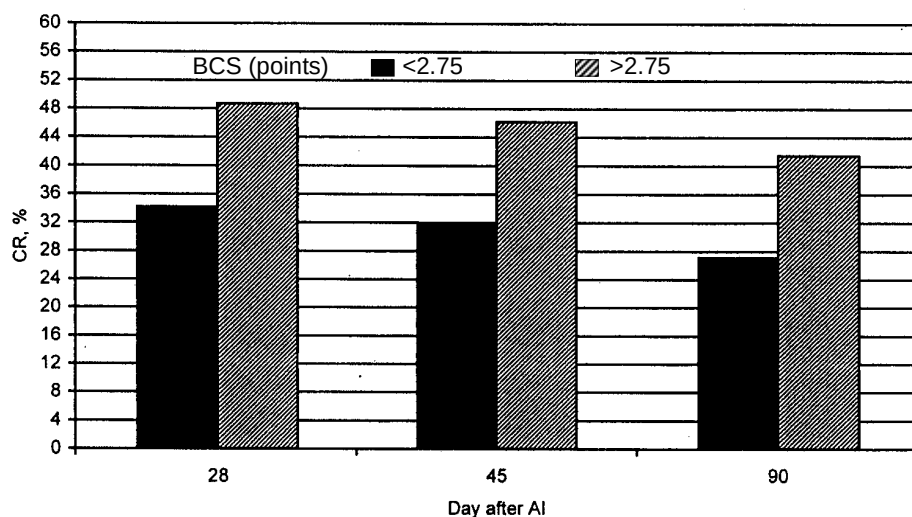


Fig.9: Effect of BCS at the time of AI on conception rate of dairy cows on d 28, 45 and 50 after AI (Santos et al., 2001)

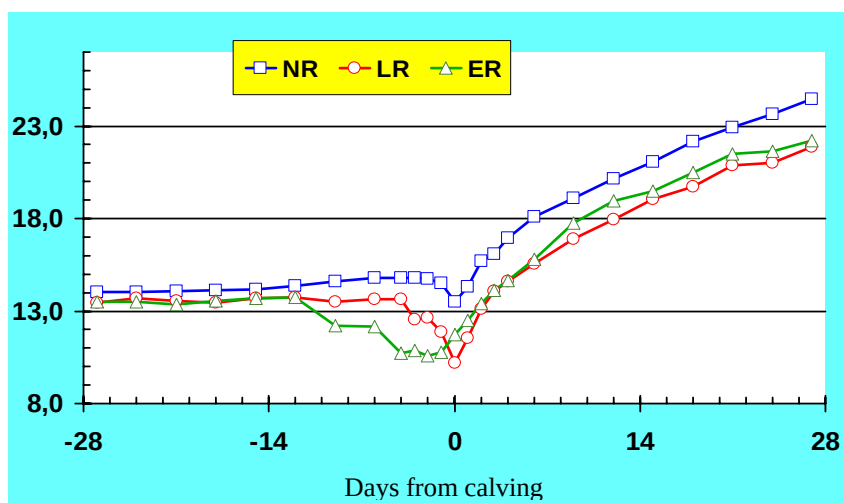


Fig.10: Pattern of changes of dry matter intake (kg) of dairy cows in the pre and post calving month retrospectively divided according to DMI reduction before calving: no (NR), late (LR) or early (ER) (Trevisi et al., 2002)

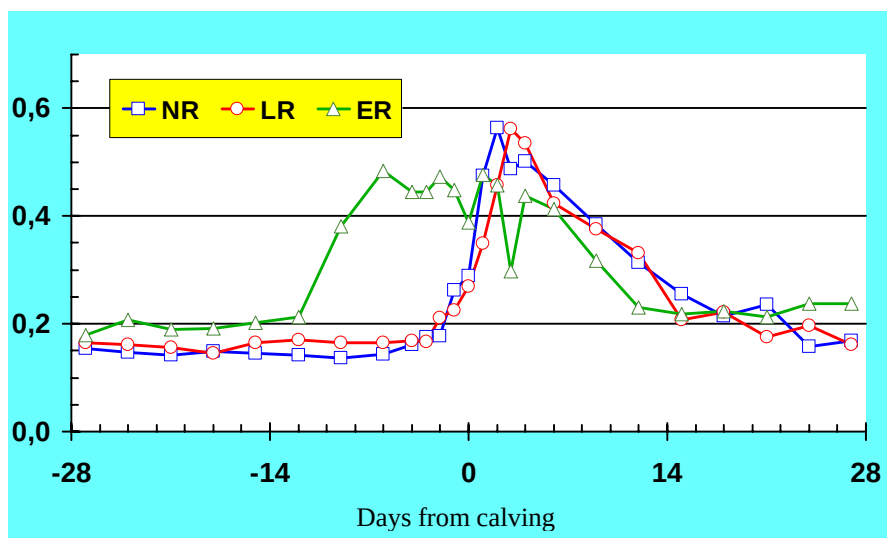


Fig.11: Pattern of changes of haptoglobin (g/l) of dairy cows in the pre and post calving month retrospectively divided according to DMI reduction before calving: no (NR), late (LR) or early (ER) (Trevisi et al., 2002)

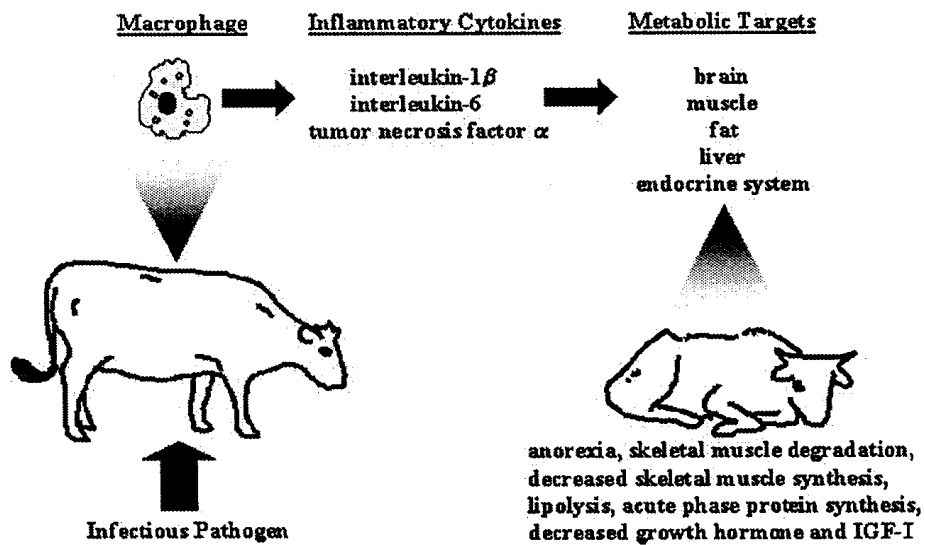


Fig.12: Pathogens activate immune cells, causing them to produce inflammatory cytokines. The cytokines mediate the immune response but also act on other systems and affect metabolism (Johnson and Finck, 2001)

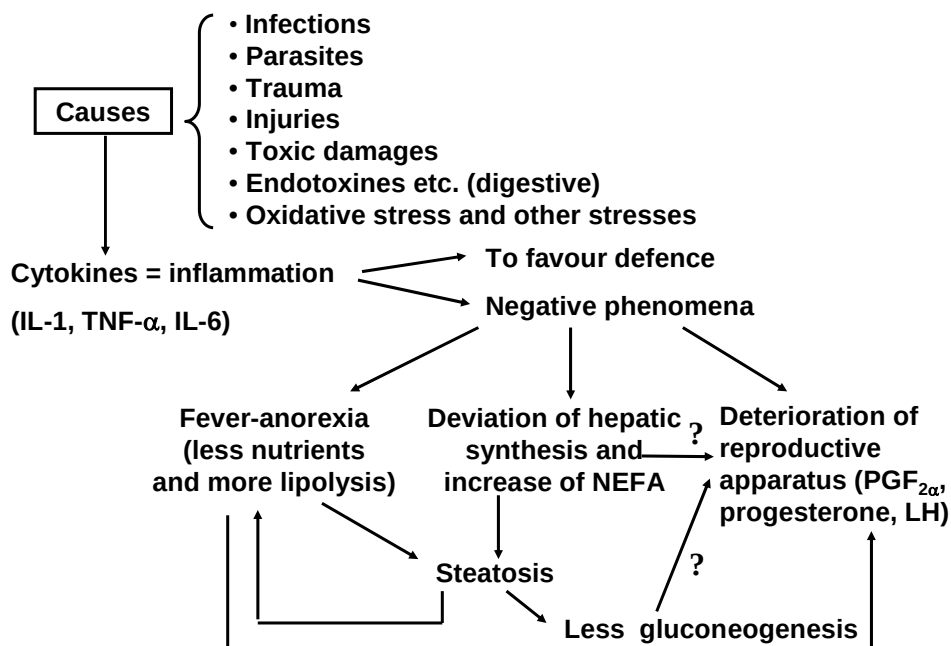


Fig.13: Causes of release of cytokines and possible mechanisms that compromise production and fertility (Bertoni, 2003).

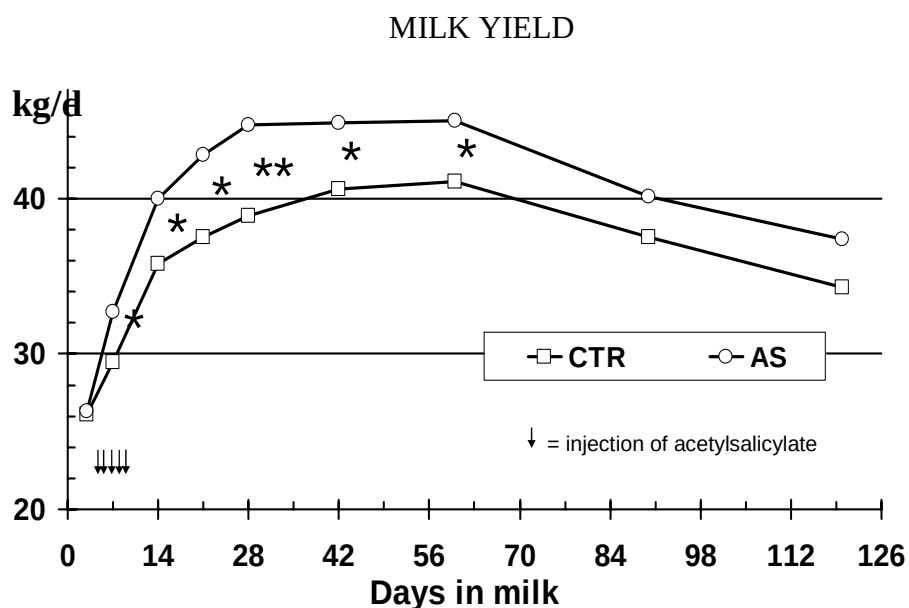


Fig.14: Pattern of changes of milk yield till 4th month of lactation in cows treated (AS) or not (CTR) with lysine acetylsalicylate in the first 5 days after calving (* $P < 0.05$; ** $P < 0.01$) (Trevisi and Bertoni, 2006).

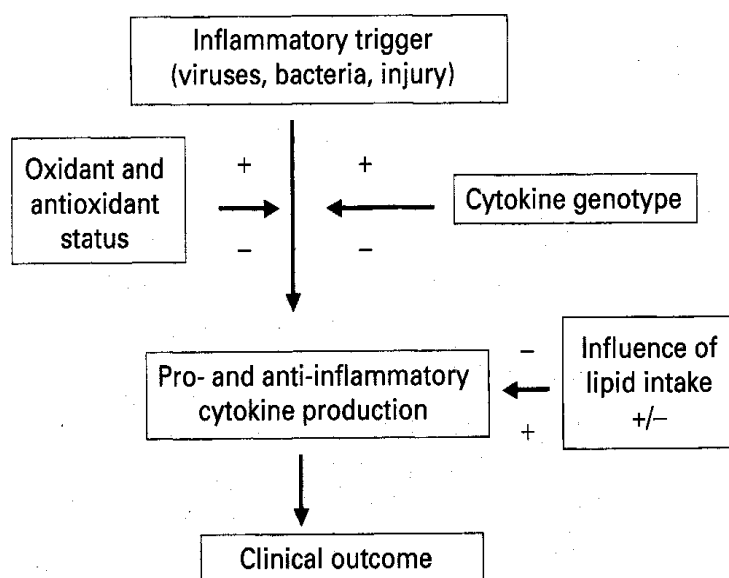


Fig.15: Summary of nutritional and genetic influences on cytokine production and clinical outcome. +, A stimulatory effect; -, an inhibitory influence (Grimble, 2001b).

Tab.1: Average values of main fertility indices observed in cows within upper (UP), intermediate upper (INUP), intermediate lower (INLO) and lower (LO) quartiles of LAI (Liver Activity Index).

Item	UP n = 19	INUP n = 20	INLO n = 19	LO n = 19
Services per pregnancy* (n°)	1.65±1.3 ^a	2.04±1.6 ^{ab}	2.68±1.5 ^b	2.01±1.5 ^{ab}
Days open* (DIM)	92.9±48 ^a	132.5±89 ^b	138.8±89 ^b	110.5±55 ^{ab}
Conception rate at 1 st service (%)	52.6	45.0	21.0	36.8
Repeat breeders (at least 3 services)	21.0	45.0	57.9	31.6

*after logarithmic transformation

Letters (a,b,c) show significant differences between groups (P<0.05).

Tab.2: Main fertility indices observed in cows treated (AS) or not (CTR) with Lysine acetylsalicylate in the 5 days after calving (Trevisi and Bertoni, 2006).

group		CTR	AS
cows	n°	22,0	23,0
culled cows	% of total	15,8	9,5
pregnant cows	% of total	86,4	91,3
pregnant at 1 st insemination	% of pregnant	21,1	52,4
repeat breeders	%	36,8	28,6
services per pregnancy (§)	n°	2,68	2,38
days open (§)	n°	131,8	106,3
Fertility Status Index (FSI) (@)	n°	12,6	61,8

LEGEND: (§) after logarithmic transformation; (@) Esslemont and Eddy (1977)