



## Manejo de la vaca en transición para maximizar la salud y reproducción

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The period from three weeks before to three weeks after parturition in dairy cows, also known as the transition period, is characterized by significant changes in hormonal profile, feed intake, nutrient requirements, metabolism, and energy balance. These changes are known to dramatically affect immune function. In this manuscript we will discuss the physiological changes that affect immune function and we will discuss situations that accentuate immune suppression and predispose cows to health disorders. We will also evaluate how to improve health of transition cows through management in order to reduce health disorders and improve reproductive efficiency.

### **Peripartum Changes Associated with Immune Suppression and Disease**

*Peripartum hormonal changes and immune function:* In the last weeks of gestation, significant changes in concentrations of cortisol, progesterone, estradiol, prostaglandin  $F_{2\alpha}$ , and prolactin occur (Stevenson, 2007). These changes in hormone concentrations occur mainly in response to increased production of monoamine oxidase by the fetus, an enzyme that breaks-down serotonin. The reduction in serotonin concentrations results in increases in corticotropic releasing factor and adrenocorticotrophic hormone concentrations in fetal circulation. Consequently, cortisol secretion by the fetus' adrenal gland increases. Cortisol up-regulates the expression of 17- $\alpha$  hydroxylase, an enzyme that increases secretion of estradiol in the placenta in detriment of progesterone production. Simultaneously, an increase in prolactin and prostaglandin  $F_{2\alpha}$  concentrations is observed. These changes are important for onset of colostrum production and preparation for parturition (Akers, 2002).

Although increases in concentration of estradiol and prostaglandin  $F_{2\alpha}$  in uterus increase blood flow to the uterus and theoretically the influx of immune cells, cortisol suppresses immune response because it down regulates the neutrophil expression of L-selectin and CD18, adhesion molecules involved in the trafficking of neutrophils from the endothelium to the site of infection (Burton and Kehrl, 1995a; Burton et al., 1995b; Burton et al., 2005).

Cortisol is also produced in adverse conditions (e.g. transport, overstocking) that results in stress and circulating concentrations of cortisol has been used as an indicator of stress (Nanda et al., 1990). Therefore, conditions during the prepartum period that increase stress are expected to increase cortisol concentrations and consequently further suppress immune function of peripartum cows.



Homeorhetic adaptations during the peripartum period and immune function: At the same time that dramatic hormonal changes are occurring, feed intake in the last 14 d before parturition decreases by approximately 50%, reaching its nadir on the day before parturition (Grummer et al., 2004). Although feed intake starts to increase immediately after parturition, it is not sufficient to meet nutrient requirements for the rapidly increasing milk yield. Thus, cows suffer from negative energy balance for up to 8 to 12 weeks after parturition and must utilize body energy reserves to meet nutrient requirements for milk production.

Therefore, during the transition period cows go from a state of homeostasis to a state of homeorhesis, "orchestrated or coordinated changes in metabolism of body tissues necessary to support a dominant physiological state (Bauman and Currie, 1980)." For peripartum cows, increasing milk production is the dominant physiological state as the utilization of nutrients by the mammary gland of high producing dairy cows exceeds that of the rest of the body in the first trimester of lactation (Bauman, 2000). Some of the homeorhetic changes observed in the peripartum dairy cows are discussed below.

Before the decrease in feed intake prepartum starts, cows have low circulating concentrations of growth hormone (GH) and high circulating concentrations of insulin and insulin-like growth factor-I (IGF-I). Once feed intake starts to decrease and negative energy balance occurs, GH concentration increases and insulin and IGF-I concentrations decrease indicating a decoupling of the somatotropic-IGF-I axis because the liver, under the influence of GH, is the main source of circulating IGF-I (Rhoads et al., 2004; Lucy, 2008). This occurs because during negative energy balance the expression of GH receptor (GHR), particularly GHR1 $\alpha$ , is decreased (McCarthy et al., 2009). As cows return to positive energy balance hepatic expression of GHR1 $\alpha$  increases and hepatic IGF-I production starts to increase (Lucy, 2008).

Insulin-like growth factor-I is a fundamental factor that stimulates growth, differentiation, and functionality of several different cell types. For example, IGF-I is likely to affect innate immunity of peripartum cows because it regulates functionality (i.e. superoxide anion production, oxidative burst, and degranulation) of neutrophils, the primary defense line against infections (i.e. metritis and mastitis). Further, circulating concentrations of neutrophils and production of antibodies (i.e. IgG, IgM, and IgA) are significantly increased in GH-deficient humans and mice after GH-induced increase in concentrations of IGF-I (Kimata and Yoshida, 1994; Ibanez et al., 2005; Sohmiya et al., 2005). Pigs that were treated with IGF-I stimulating compounds and were subjected to simultaneous weaning and transport had greater count and concentrations of neutrophil in the blood than non-treated pigs (Kojima et al., 2008). Thus, exacerbation of negative energy balance during the peripartum period is likely to affect innate and humoral immunity because cows would be exposed to extended periods of time with reduced IGF-I concentration.

Energy sources, liver function, and resulting metabolites: Ruminants have evolved to substitute glucose by volatile fatty acids (i.e. propionate, butyrate, and acetate) and their



derivative ketoacids as respiratory and lipogenic fuels (Bauman and Currie, 1980). Nonetheless, glucose remains essential for normal brain and liver function and for production of lactose in the mammary gland, being the latter the most important osmotic solute of milk production. During early lactation and negative energy balance, insulin-dependent uptake of glucose by tissues other than the mammary gland (i.e. muscle and adipose tissue) is reduced, in part because of increased GH concentrations, assuring that glucose is available for production of copious amounts of lactose and milk (Bauman, 2000; Lucy, 2008). In situations in which cows are exposed to severe and prolonged negative energy balance large amounts of body reserves (i.e. glycogen, lipids, and amino acids) are mobilized to provide the necessary substrate for milk production (Grummer et al., 2004). A consequence of extreme adipose tissue mobilization during the peripartum period is the increasing circulating concentration of non-esterified fatty acids (NEFA), which predisposes cows to hepatic lipidosis (Grummer et al., 2004). Consequently, concentrations of ketone bodies [e.g. beta-hydroxy butyrate (BHBA)] may also increase because of compromised liver function and incomplete oxidation of NEFA (Grummer et al., 2004).

Association among feed intake, metabolites concentrations, immune function, and health peripartum: Amount of feed intake is inversely associated with plasma NEFA concentrations, and the latter affects neutrophil function (Klucinski et al., 1988; Rukkwamsuk et al., 1999; Hammon et al., 2006). Hammon et al. (2006) demonstrated that cows that had reduced feed intake during the prepartum period had reduced neutrophil activity (phagocytosis and oxidative burst) during the peripartum and were more likely to develop metritis postpartum. This seems to be a consequence of the onset of colostrum/milk production and the simultaneous insufficient feed intake peripartum because cows that were mastectomized 4 months before parturition had greater expression of L-selectin prepartum, greater leukocyte count postpartum, and greater neutrophil killing activity postpartum than cows with intact mammary glands (Kimura et al., 1999).

Compromised immune function due to altered metabolic status predisposes cows to infectious diseases (i.e. metritis, endometritis, and mastitis). Postpartum hepatic lipidosis has been associated with increased length of bacterial shedding from mastitic cows (Hill et al., 1985) and prepartum increase in fat mobilization and serum lipoprotein metabolism resulted in increased risk of metritis and retained fetal membranes (Kaneene et al., 1997). In a recent large study, Ospina et al. (2010) demonstrated that increasing prepartum and postpartum NEFA plasma concentrations were associated with increased risk of retained fetal membranes, metritis, clinical ketosis, and displacement of abomasum. Accentuated negative energy balance accompanied by increased BHBA plasma concentrations during early postpartum also has been associated with increased risk of peripartum diseases (Erb and Grohn, 1988; Grohn et al., 1989; Correa et al., 1993). For example, higher milk acetone concentrations were associated with increased risk of endometritis (Reist et al.; 2003) and increasing BHBA plasma concentration was associated with increased risk of metritis and displacement of abomasum (Ospina et al., 2010).



The selection of cows for high milk yield has resulted in significant homeorhetic alterations that predispose them to immune suppression and more diseases postpartum. Managerial inadequacies that increase and prolong the negative energy balance during the peripartum transform the normal homeorhetic changes into metabolic diseases (i.e. excessively elevated fat mobilization, hepatic lipidosis, and ketosis) further suppressing immune function of dairy cows and predisposing them to health disorders, and compromised productive, reproductive, and economic performances.

### **Housing Strategies**

Cows are social animals and as such are highly susceptible to social interactions and hierarchical order. Once housed within a group, dominant cows display physical and non-physical aggressive behavior towards submissive cows. Situations that exacerbate these deleterious interactions among dominant and submissive cows have the potential to affect health and performance. Although group performance is the most common used parameter to evaluate management and protocols, often evaluation of averages masks the poor performance of subordinate cows in particular. Therefore, management should be focused to provide all cows with sufficient feed, water, and resting space to minimize the expression of subordinate behaviors.

Separation of Prepartum Heifers and Cows: Smaller cows are in general more submissive than larger cows. Consequently, when prepartum heifers are housed together with mature cows they are more likely to expressive submissive behavior. In a study in which prepartum heifers were housed with mature cows during the prepartum or were housed alone, heifers housed with mature cows had reduced feed intake and reduced resting time during the prepartum and reduced milk yield compared with heifers housed alone (Table 1).

Table 1. Performance of primiparous when grouped separately from multiparous cows.		
Item	Multipar. + Primip.	Primiparous Only
Eating time, min/d	184	205
Eating bouts / d	5.9	6.4
Concentrate intake, kg/d	10.1	11.6
Silage intake, kg/d	7.7	8.6
Lying time, min/d	424	461
Resting periods/d	5.3	6.3
Milk yield, kg/130d	2,383	2,590
Milk fat, %	3.92	3.97
Adapted from Grant and Albright (1995)		



Therefore, we recommend that primiparous cows be housed separately from mature cows from at least 21 d before to 21 d after calving. If this is not possible, prepartum and postpartum pens should have a stocking density of < 80%.

*Stocking density prepartum and its effects on behavior, feed intake, and immune function:* Situations of limited space or access to feed exacerbate aggressive and submissive behaviors. Two small but elegant studies conducted in research facilities of the University of British Columbia in Canada demonstrated the effects of overstocking of prepartum cows on behavior and feed intake. According to one of these studies, cows housed in pens in which the ratio of cows to feeding bin was 2:1 had altered behavior compared with cows housed in pens with cow to feeding bin ratio of 1:1 (Hosseinkhani et al., 2008). Similarly, the second study demonstrated that cows housed in pens with 30 cm/cow of feed bunk space had altered behavior compared with cows housed in pens with 60 cm/cow of feed bunk space (Proudfoot et al., 2009). These altered behaviors included increased rate of feed intake, fewer meals per day, increased feed sorting, decreased overall feed intake, increased standing time, and increased rate of displacement from the feeding area (Hosseinkhani et al., 2008; Proudfoot et al., 2009). The consequences of stocking density for dominant and submissive cows are likely to be distinct. Dominant cows are predisposed to ruminal acidosis when they have increased rate of feed intake, fewer meals per day, and increased feed sorting. On the other hand, submissive cows are more likely to have metabolic diseases such as hepatic lipidosis and ketosis because of reduced feed intake and to develop lameness because of increased standing time and displacement rate. Therefore, overstocking of pens of prepartum cows, a common problem in dairy operations of all sizes, predisposes all cows to inadequate nutrient intake prepartum and consequently compromised immune function. Because cows have allelomimetic behavior, characterized by cows doing the same activity at the same time, it is fundamental during the prepartum period to assure that space is available for all cows to eat at the same time without the expression of aggressive and submissive behaviors.

A study conducted in Italy evaluated the humoral immunity and productive performance of dairy ewes that were housed in high or low stocking density conditions from late gestation to mid-lactation (Carporese et al., 2009). Ewes that were housed in high stocking density conditions had reduced anti-ovalbumin IgG concentration in response to an ovalbumin challenge compared with ewes housed in low stocking density conditions (Carporese et al., 2009). Further, ewes that were housed in high stocking density conditions tended to have greater number of aggressive interactions and had reduced milk yield and increased milk somatic cell count (Carporese et al., 2009).

Current recommendations indicate that stocking density during the prepartum should be 1 cow per stall and at least 76 cm of linear feed bunk space per cow. Even in herds in which prepartum cows are housed in good pasture conditions, prepartum cows should have sufficient access to feed bunk to assure that the whole group is ingesting the proper amount of feed and



nutrients. An issue that is often overlooked in overstocked and non-overstocked conditions is the amount of water and access to water available to prepartum and postpartum cows. In general, we recommend that a minimum 10 cm of linear water trough space is available per cow and at least 2 water troughs per group to assure that cows have sufficient access to water.

Regrouping frequency and its effects on behavior, feed intake, and milk yield: Another situation commonly observed in dairy operations that may pose a risk to the health of peripartum cows is frequent regrouping during the prepartum period. Regrouping of dairy cows is used in dairy operations to maintain homogenous groups in terms of gestation stage to optimize nutritional management. Thus, in many dairy operations cows are housed as a group from approximately 230 to 250 d of gestation in so called “dry cow pens” and as another group from 251 d of gestation to parturition in so called “close-up cow pens”. Every week, cows from the dry-cow pen are moved to the close-up cow pen, which results in weekly disruption of social interactions and for many cows disruption of social interactions in the last days before parturition. Constant regrouping of cows changes the hierarchical order among them, forcing cows to reestablish social relationships through physical and nonphysical interactions and exacerbating aggressive and submissive behaviors (von Keyserlingk et al., 2008). Also, in small studies conducted in Canada it has been demonstrated that on the days following regrouping cows have reduced feeding time, greater rate of displacement from the feed bunk and stalls, and reduced milk yield (von Keyserlingk et al., 2008). Although the question has not yet been definitively answered, cows may require 3 to 14 days after regrouping to reestablish social stability to pre-regrouping levels (Grant and Albright, 1995). Therefore, in situations in which cows are regrouped every week during the prepartum period, they likely undergo constant stressful social interactions that further suppresses innate and humoral immunity and compromise metabolic status, resulting in significantly higher risk of postpartum diseases.

Heat Abatement: Exposure of cows to heat stress during the prepartum period results in smaller calf birth weight (31 vs. 44 kg) and reduced milk yield throughout the lactation (7.5 kg/d less milk; do Amaral et al., 2009). In a study conducted in CA, prepartum cows (last 28 d before calving) were offered shade and fans in addition to sprinkler for heat abatement (Urdaz et al., 2006). Fans were 91.4 cm diameter with air speed of 317.2m<sup>3</sup>/min and placed 2.4 m high, 6.1 m apart, and in 30° angles. Shades were 3.9 m (front) and 3.4 m (back) high and provided 95% shade. Sprinklers were 1.7 m high and 1.5 m apart, and provided 1.4-1.8 l of water per minute. Providing shade and fans resulted in reduced exposure of cows to heat stress from 60% to 48% of the time spent in the prepartum pen. Cows that had shades and fans in addition to sprinkler produced 84 kg more milk in the first 60 d postpartum (2408 vs. 2324 kg), which resulted in 95% profit per year over each dollar spent.

Therefore, heat abatement should be a goal for prepartum as well as postpartum cows to assure improved health and productivity.



## **Transition Cow, Energy Sources, Fat and Reproduction**

Lipogenic nutrients are those that can be metabolized and form 2 C compounds favoring the production of acetyl-CoA, whereas glucogenic nutrients are those that once metabolized form 3 C compounds and favor the production of oxaloacetate. Metabolism of carbohydrates and protein in the rumen results in the formation of volatile fatty acids (VFA) of which acetate and butyrate are considered lipogenic and propionate is considered a glucogenic. Fats that are not digested in the rumen are another source of dietary lipogenic nutrients.

Early lactation cows have reduced concentrations of insulin, which diverts glucose utilization from muscle and adipose tissue to mammary gland for production of lactose and milk. Therefore, during early lactation, the NEB results in shortage of glucogenic precursors and in increase of lipogenic precursors, particularly NEFA from body fat mobilization. The excessive mobilization of body fat, and production of acetyl-CoA precursors, if not met by sufficient amounts of oxaloacetate precursors, results in production of ketones, acetone, acetoacetate, and  $\beta$ -hydroxybutyrate (BHBA), resulting eventually in ketosis. Excess of lipogenic precursors may also result in esterification and storage of fatty acids and triacylglycerides (TAG) in the liver, resulting eventually in fatty liver. As mentioned above, high concentrations of NEFA have been correlated with reduced oocyte competence. Further, increased and longer NEB has been correlated with anovular condition and reduced fertility.

Therefore, researchers have attempted to improve fertility of lactating dairy cows by shifting diet composition to a more glucogenic than lipogenic diet, such that increased absorption of propionate would increase production of glucose and increase concentrations of insulin, diverting glucose utilization from mammary gland and reducing mobilization of body energy reserves.

Cows fed 3.4% fat and 26.7% starch (DM basis) from 3 weeks pre-partum to 9 weeks postpartum had similar milk yield, DMI, and metabolizable energy (ME) intake compared with cows fed diets containing 5.4% fat and 9.5% starch (van Knegsel et al., 2007a). However, the former had smaller milk fat ( $1.68 \pm 0.03$  vs.  $1.90 \pm 0.02$  kg/d), smaller partitioning of energy to milk production ( $256.8 \pm 2.9$  vs.  $280.2 \pm 4.3$  kcal/(kg<sup>0.75</sup> d)), and reduced body fat mobilization ( $332$  vs.  $558$  g/d) (van Knegsel et al., 2007a). In a follow-up study, cows were fed extremes of glucogenic (3.1% fat and 26.6% starch) and lipogenic (5% fat and 10.4% starch) diets or an intermediate (4.1% fat and 17.9% starch) diet from 3 weeks pre-partum to 9 weeks postpartum (van Knegsel et al., 2007b). There were no differences in body weight and BCS changes, DMI, net energy (NE) intake, milk yield and milk protein yield. Cows fed the more glucogenic diets, however, had smaller milk fat yield, less calculated NEB and earlier return to positive energy balance, smaller NEFA, BHBA, and liver TAG (Figure 1; van Knegsel et al., 2007b). Furthermore, cows fed the glucogenic diet ( $4.20 \pm 0.39$   $\mu$ IU/ml) had greater insulin concentration than cows fed the intermediate ( $2.78 \pm 0.21$   $\mu$ IU/ml) or lipogenic ( $2.86 \pm 0.27$   $\mu$ IU/ml) diets (van Knegsel et al., 2007b). In this same study, the interval from calving to first rise in P4 concentration,

indicative of resumption of ovarian cycles, was shorter for cows fed glucogenic diets ( $20.4 \pm 0.9$  d) compared with cows fed intermediate ( $24.4 \pm 2.9$  d) and lipogenic diets ( $26.4 \pm 2.2$  d), but there were no effects on length of luteal phase or estrous cycle (van Knegsel et al., 2007b). Probably as a result of increased cholesterol precursors offered in the intermediate and lipogenic diets, mean concentrations of P4 during the first postpartum luteal phase were greater for cows fed intermediate or lipogenic diets compared with cows fed glucogenic diets ( $31.4 \pm 2.4$ ,  $27.0 \pm 3.0$ , and  $22.5 \pm 1.4$  ng/mL, respectively). From these experiments, it is possible to suggest that feeding diets more conducive to production of glucogenic nutrients may improve fertility through the following mechanisms: 1. increased glucose production and increased insulin and IGF-I concentrations; 2. reduced milk fat yield, improved energy balance, and reduced NEFA concentrations; and, 3. early resumption of normal ovarian cycles and improved oocyte quality.

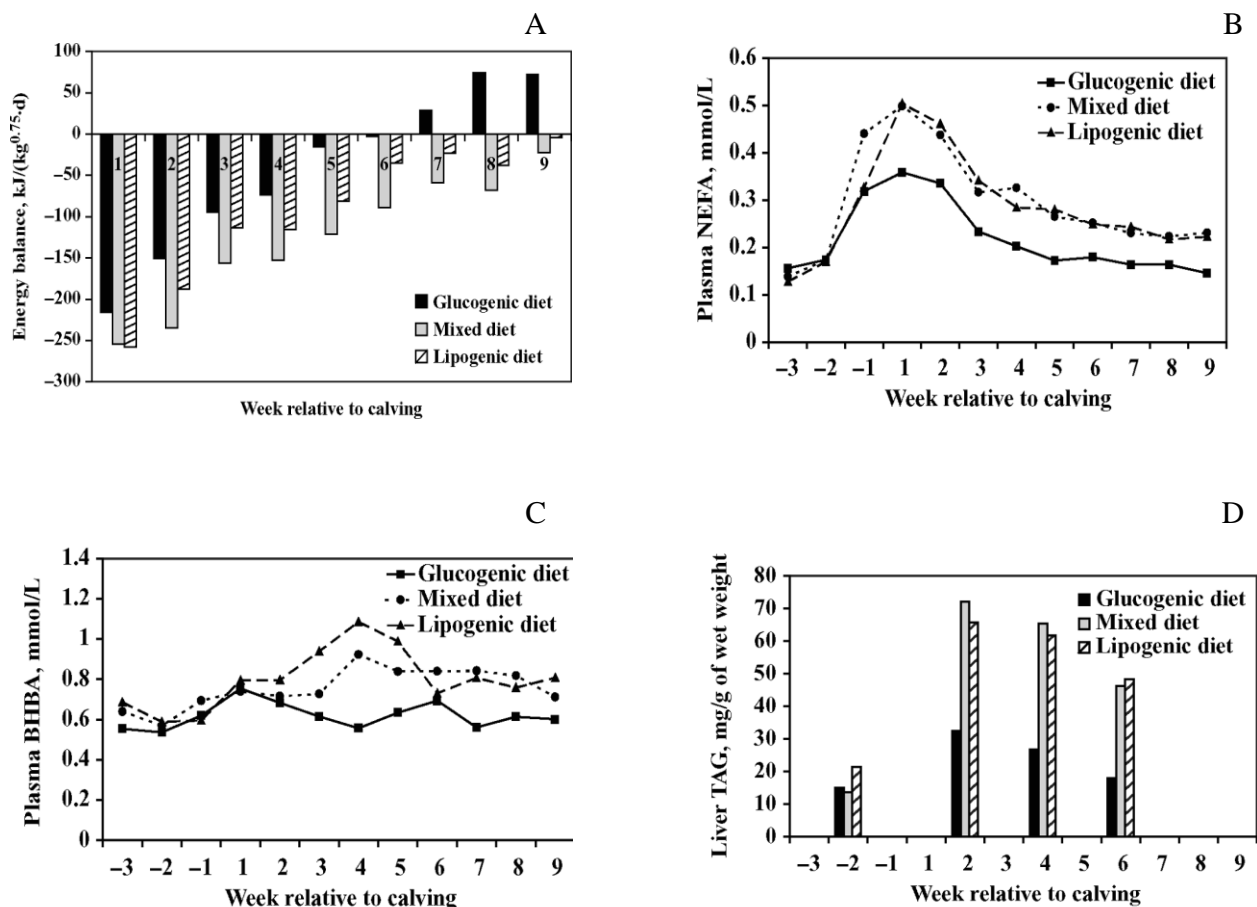


Figure 1. Effects of glucogenic, mixed, or lipogenic diets on calculated energy balance (panel A), plasma NEFA concentration (panel B), plasma BHBA concentration (panel C), and liver triacylglycerides (panel D). From van Knegsel et al. (2007b).





It is important to note that feeding diets high in glucogenic precursors, particularly propionate, may result in suppressed DMI. This would be explained by the hepatic oxidation theory according to which excessive hepatic oxidation would cause stimulation of afferent nerves in the liver (vagal nerve) and suppression of DMI (Allen et al., 2009). This is particularly important for transition cows that weeks before calving start to mobilize fat, which results in a state of hyperlipidemia, increased concentrations of NEFA, increased TAG in the liver and reduced gluconeogenesis, and increased hepatic oxidation and reduced DMI (Allen et al., 2009). On the other hand, diets that increase lactate concentrations increase DMI because of lesser effect of lactate on hepatic oxidation, particularly during meals, because of the longer interval from feed intake to lactate absorption in the small intestine and because liver extraction of lactate from the blood is slower than that of propionate, resulting in slower and lesser hepatic oxidation (Allen et al., 2009). Furthermore, continued feeding of diets containing elevated amounts of glucogenic nutrients up to first AI and thereafter, however, may have negative results on fertility, particularly because of increased catabolism of steroidal hormones. Increased energy intake and the resulting increased insulin concentrations have been suggested to increase blood flow through the liver, which may elicit greater catabolism of steroidal hormones (i.e. progesterone and estradiol), which are critical for establishment and maintenance of pregnancy (Vasconcelos et al., 2003).

Feeding supplemental fats in diets of ruminants was thought to be a method to increase feed efficiency by increasing energy density of diets, because diets of ruminants fed no supplemental fats have approximately 2% fat (DM basis). Therefore, feeding of supplemental fats to early lactation cows was thought to be an appropriate approach to minimize or at least reduce NEB. However, cows fed supplemental fats usually do not have improved energy balance either because of reduced DMI or increased milk yield (Santos et al., 2008). Even so, initial studies demonstrated that despite the lack of effect of fat feeding on energy balance, cows fed supplemental fats had improved reproductive efficiency, leading to the suggestion that feeding certain long chain fatty acids (**LCFA**) improved fertility by altering fatty acid (**FA**) substrates and not energy balance. As with many other nutrients, there are essential and non-essential FA. In mammals the lack of desaturase enzymes beyond the 9<sup>th</sup> carbon of the acyl chain does not allow for the production of n-6 and n-3 fatty acids, which are therefore considered essential fatty acids. Lipids offered in the diet are critical sources of energy, but are also essential part of cell membrane physical and functional structures. Cell membrane functions are regulated in part by fatty acid composition (i.e. increased unsaturation of membranes results in reduced fusion point altering fluidity of membranes). Mattos et al. (2000) demonstrated that unsaturation level and position of double bonds have a marked effect on function of cell membranes, which may partly explain their effects on reproduction. One of the major impediments to the understanding of the mechanisms by which supplemental fats may alter fertility, aside from potential improvement in energy balance, is the fact that nearly 70% of C18:2 n-6 and 85% of C18:3 n-3 fed to lactating dairy cows are biohydrogenated in the rumen



when fed as unprotected oils or as Ca salts of long chain fatty acids (**Ca-LCFA**), respectively (Juchem, 2007). Therefore, lipolysis of triacylglycerols and biohydrogenation of unsaturated FA in the rumen and the lack of data to determine the needs of reproductive organs for FA complicates the understanding of the role of FA on fertility.

Among other things, FA are precursors to eicosanoids (i.e. prostaglandins, thromboxanes, leukotrienes, etc.). Therefore, uterine synthesis of prostaglandin (**PG**)  $F_{2\alpha}$  is regulated in part by the availability of substrate (arachidonic acid – AA; C20:4 n-6) of which C18:2 n-6 is a precursor. There is evidence to support that feeding greater levels of C18:3 n-3 through fish meal or Ca-LCFA results in shift of endometrial cells membrane fatty acid composition with greater incorporation of eicosapentaenoic (EPA) and total n-3 FA compared with AA (Santos et al., 2008). The ability to manipulate production of  $PGF_{2\alpha}$  by the endometrium through different feeding strategies is of extreme importance because higher concentrations of  $PGF_{2\alpha}$  around parturition may result in improved uterine health and reduced concentrations of  $PGF_{2\alpha}$  during the estrous cycle, particularly close to the period of maternal recognition of pregnancy, may significantly improve reproductive performance.

In an attempt to improve uterine health through increased production of  $PGF_{2\alpha}$  early postpartum, researchers have fed cows supplemental fat prepartum. Cullens et al. (2004) observed that cows fed Ca-LCFA rich in n-6 FA had reduced incidence of retained placenta, metritis, and mastitis. When Juchem et al. (2007) supplemented cows Ca-LCFA of palm oil or a blend of C18:2 n-6 and trans-octadecenoic reduced incidence of puerperal metritis was observed (8.8 vs. 15.1%). Finally, although cows fed diets rich in C18:2 n-6 had improved immune function, no benefits were observed on incidences of retained placenta and metritis (Santos et al., 2008). Therefore, benefits of supplementing C18:2 n-6 rich FA in diets of prepartum cows on incidence of clinical disease are limited.

Replacement of corn with Ca-LCFA in the postpartum diet increased the number of medium and large follicles and the size of the largest follicle of lactating dairy cows (Lucy et al., 1993). However, it appears that not only fat feeding has an effect on follicular growth, but type of fat fed plays a role as well, because cows supplemented with increasing amounts of polyunsaturated FA (PUFA) had larger follicles compared with cows fed more monounsaturated FA (Santos et al., 2008). Although it has been observed that cows that ovulate sooner after calving also have earlier emergence and growth of larger follicles, studies in which cows supplemented with Ca-LCFA of palm and fish oils postpartum or Ca-LCFA of palm oil (Juchem et al., 2007) or a blend of C18:2 n-6 and trans-octadecenoic FA pre- and postpartum (Juchem et al., 2007) no differences in incidence of anovular condition were observed. Similarly, when cows were supplemented with Ca-LCFA of palm oil or safflower oil from 2 wk prepartum up to 4 wk postpartum and thereafter fed supplemental Ca-LCFA of palm oil or fish oil no differences in proportion of anovular cows were observed (Silvestre, unpublished data; Santos et al., 2008).



Increased fat feeding has consistently resulted in increased concentrations of progesterone. Fat supplementation increases concentration of cholesterol in plasma and follicular fluid, which is a precursor for progesterone synthesis in the ovarian cells. Because progesterone concentration before and after ovulation is critical for fertility of dairy cows, it could be expected that increases in progesterone concentration resulting from supplemental fat in diets of lactating dairy cows would improve fertility.

As mentioned above, changes in fatty acids profile fed in the diet may alter cell membrane FA composition. Developmental capacity of oocytes collected transvaginally from cows fed different amounts of Ca-LCFA (200 vs. 800 g/d) was assessed in vitro (Fouladi-Nashta et al., 2007). Although cleavage rate was slightly smaller for cows supplemented with more fat ( $66.6 \pm 2.1$  vs.  $72.0 \pm 2.2\%$ ), blastocyst/IVF production ( $19.4 \pm 1.8$  vs.  $27.4 \pm 2.2\%$ ) and blastocyst/cleaved oocyte production ( $29.1 \pm 2.5$  vs.  $38.0 \pm 2.8\%$ ) were greater for cows supplemented higher levels of fat. However, from this experiment it was not possible to determine if any specific FA was responsible for better quality oocytes. Supplementation of superovulated cows with unsaturated n-6 and n-3 FA resulted in better embryo development compared with cows fed saturated fats, but there were no differences in fertilization and development rates between cows fed diets rich in n-6 or n-3 FA (Thangavelu et al., 2007). Cerri et al. (2009) demonstrated that non-superovulated lactating dairy cows fed diets rich in C18:2 n-6 and trans-octadecenoic FA produced embryos with greater proportion of live cells and of better quality compared with cows fed diets rich in Ca-LCFA of palm oil. Similarly, embryos from gilts fed diets rich in C18:2 n-6 had greater number of nuclei after cryopreservation compared with embryos from gilts fed diets with reduce fat concentration (Kojima et al., 1996). Therefore, it is possible to suggest that diet FA composition may affect embryo quality.

As mentioned above, altering diet composition of FA may alter cell membrane FA composition. Cows fed n-3 FA rich diets had attenuated production of PGF metabolites (**PGFM**) either after estrogen/oxytocin challenges or after parturition, because of increased concentrations of EPA and docosahexaenoic acid (**DHA**) in endometrial cells (Mattos et al., 2002; Mattos et al., 2004). On the other hand, feeding diets rich in n-6 FA increased PGFM after an oxytocin challenge, probably as a result of increased concentration of AA in endometrial cell membranes (Robinson et al., 2002; Petiti et al., 2004). Increasing n-3 FA in diets inhibits PGF<sub>2 $\alpha$</sub>  secretion because of reduced availability of AA, increased concentration of FA that compete with AA for PG synthase-2 (i.e. EPA and DHA), or by inhibiting PG synthase-2 activity (Mattos et al., 2002; Bilby et al., 2006).

Finally, effects of supplemental fat on fertility have been summarized by Santos et al. (2008). In general it can be concluded that when supplemental fat results in improved energy balance improvements in reproductive performance are expected; feeding n-6 FA rich diets pre- and postpartum may improve fertility by improving uterine environment, fertilization rate, and embryo quality; and, feeding diets rich in n-3 FA may improve fertility by reducing



pregnancy loss because of inhibition of  $\text{PGF}_{2\alpha}$  secretion around the time of maternal recognition of pregnancy.

### **Body Condition Score, Energy and Reproduction**

Body condition scoring is an indirect method to assess body reserves, particularly fat, and change in BCS can indicate energy balance of cattle. Although in the 1980's body condition score (BCS) was from a 1 to 9 scale, more recently a scale of 1 to 5 with increments of 0.25 unit has been used for dairy cattle (Ferguson et al., 1994). The amount of energy provided by the loss of 0.25 BCS unit in a 1,320 lb cow is between 86.5 to 99.8 Mcal of  $\text{NE}_L$  and the amount of energy required to gain 0.25 BCS unit is between 107.8 and 117 Mcal of  $\text{NE}_L$ , depending on initial BCS. Therefore, a 1,320 lb cow with BCS = 4 at calving that loses 1 unit of BCS during the first 60 d of lactation would have mobilized enough energy to produce approximately 1,145 lb of 4% fat-corrected milk. Because of insufficient DMI during early lactation, it is not uncommon for cows to lose more than 0.75 unit of BCS from calving to 60 DIM.

Body condition at calving and body condition loss after calving: Santos et al. (2009) demonstrated that cows with BCS < 3.0 at calving were less likely to be cyclic at 65 DIM than those cows with BCS = 3.0 – 3.5 or those with BCS > 3.75 (Figure 2). Similarly, cows with BCS < 3.0 at 65 DIM were less likely to be cyclic at 65 DIM than those cows with BCS = 3.0 – 3.5 or those with BCS > 3.75 (Figure 2). Further, cows that lost 1 unit or more of BCS from calving to 65 DIM were less likely to be cyclic at 65 DIM than those that lost between 0.25 and 0.75 unit or those that did not lose BCS in the same period (Figure 2). Consequently, BCS at calving and at 65 DIM and BCS change from calving to 65 DIM affected fertility of lactating dairy cows (Santos et al., 2009; Figure 2).

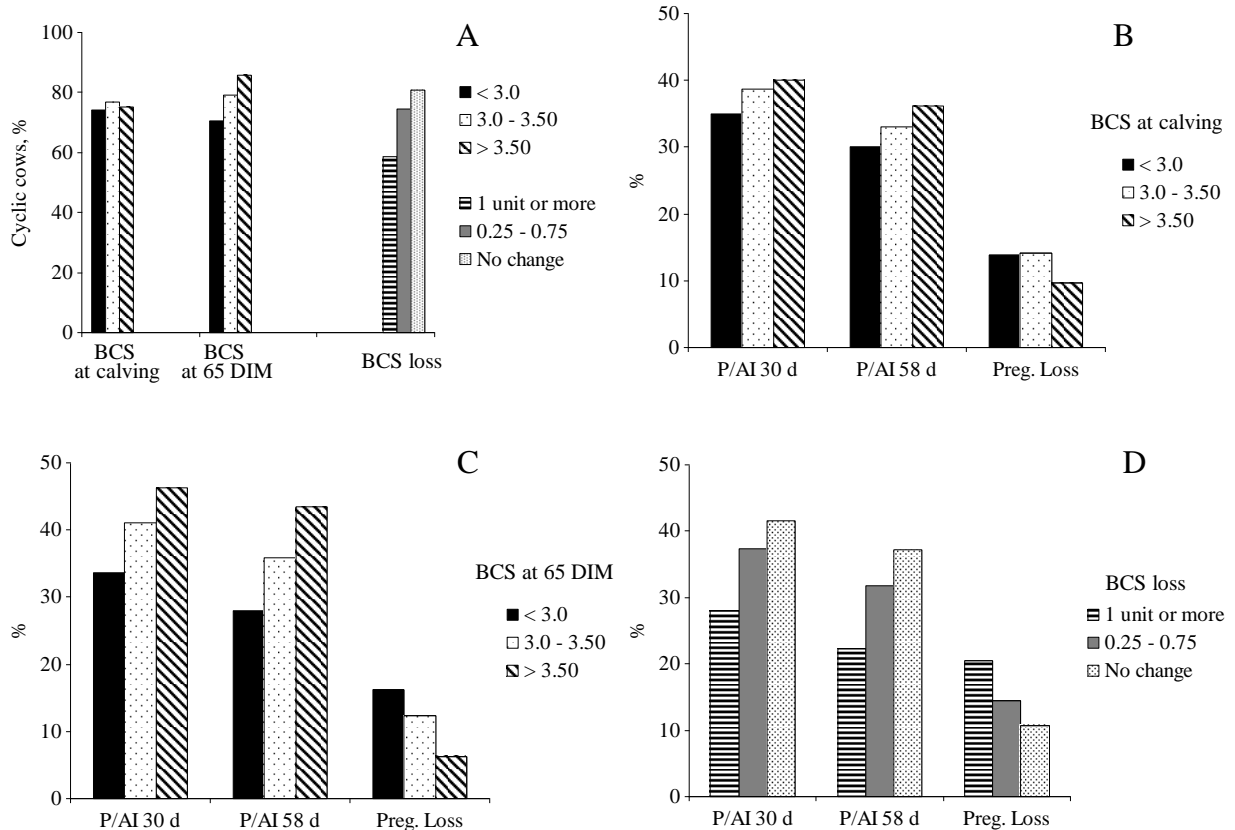


Figure 2. Effect of BCS at calving and at approximately 65 DIM and BCS change from calving to approximately 65 DIM on incidence of cyclic cows and on pregnancies per AI. From Santos et al. (2009).

**Changes in body condition score during the dry-period:** Change in BCS during the dry period also had a significant impact on fertility. A total of 8,982 lactations from 6,583 mature Holstein cows were evaluated (Mendonça and Chebel, 2011). Cows received a BCS at dry-off and at calving and were classified into 3 groups: no change (NC), gained (G), or lost (L) BCS during the dry period. Dry period lasted approximately 55 d. Proportion of cows pregnant at 90 and 150 DIM was smallest for cows that lost BCS (31.6 and 59.3%) followed by cows that gained BCS (33.9 and 62.5%) and cows that had no change in BCS (35.1 and 63.9%) during the dry period (Table 2; Mendonça and Chebel, 2011). Further, the median interval from calving to conception was nearly 6 and 8 d shorter for cows that gained BCS and cows that had no change in BCS, respectively, compared with cows that lost BCS (Table 2; Mendonça and Chebel, 2011). This reduced in fertility of cows that lost BCS during the dry period could in part be explained by increased incidence of retained fetal membranes, metritis, and post-parturient diseases in cows that lost BCS during the dry period (Table 2; Mendonça and Chebel, 2011).



Table 2. Incidence of postpartum diseases and reproductive performance according to BCS change during the dry-period.

	No Change	Lost	Gained
Lactations evaluated	2972	2951	1109
RFM, %	3.4 <sup>a</sup>	5.5 <sup>b</sup>	3.0 <sup>a</sup>
Metritis, %	15.9 <sup>a</sup>	23.7 <sup>b</sup>	11.8 <sup>c</sup>
Postpartum treatment, %	25.0 <sup>a</sup>	34.2 <sup>b</sup>	23.1 <sup>a</sup>
% Pregnant 90 DIM	35.1 <sup>a</sup>	31.6 <sup>b</sup>	33.9 <sup>a,b</sup>
% Pregnant 150 DIM	63.9 <sup>a</sup>	59.3 <sup>b</sup>	62.5 <sup>a</sup>
Median days open	118 <sup>a</sup>	126 <sup>b</sup>	120 <sup>a</sup>

a,b,c Values with different superscript differ ( $P < 0.05$ )

Average BCS at dry off was  $3.49 \pm 0.01$ ,  $3.3 \pm 0.01$ , and  $3.77 \pm 0.01$  and at calving  $3.49 \pm 0.01$ ,  $3.6 \pm 0.01$ ,  $3.41 \pm 0.01$  for NC, G, and L cows, respectively. There was a strong correlation ( $P < 0.01$ ;  $r^2 = 0.96$ ) between BCS at dry-off and proportion of cows losing BCS during the dry period (Figure 3). This is in agreement with data from Hayirli et al. (2002) that demonstrated that obese cows had smaller DMI throughout the dry period and steeper decrease in DMI one week before calving than cows with low or moderate BCS. As expected, extended interval from calving to conception resulted in greater BCS at dry-off (Figure 4), indicating that aggressive reproductive programs to assure conception early in lactation are critical steps to control BCS at dry-off, BCS change during the dry period, incidence of diseases, milk yield, and reproductive performance in the subsequent lactation.

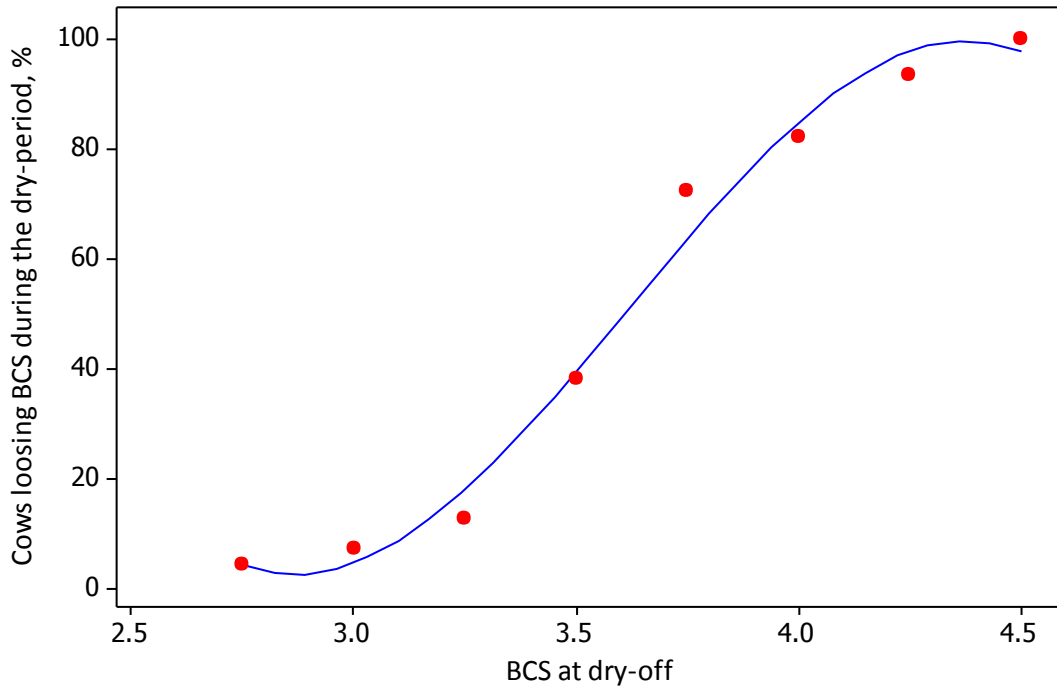


Figure 3. Correlation between BCS at dry-off and percentage of cows that lost BCS during the dry-period (Cows that lost BCS during the dry-period =  $2374 - (2119 \times \text{BCSD}) + (612 \times \text{BCSD}^2) - (56 \times \text{BCSD}^3)$ ;  $r^2 = 97.8\%$ ). Adapted from Mendonça and Chebel (2011).

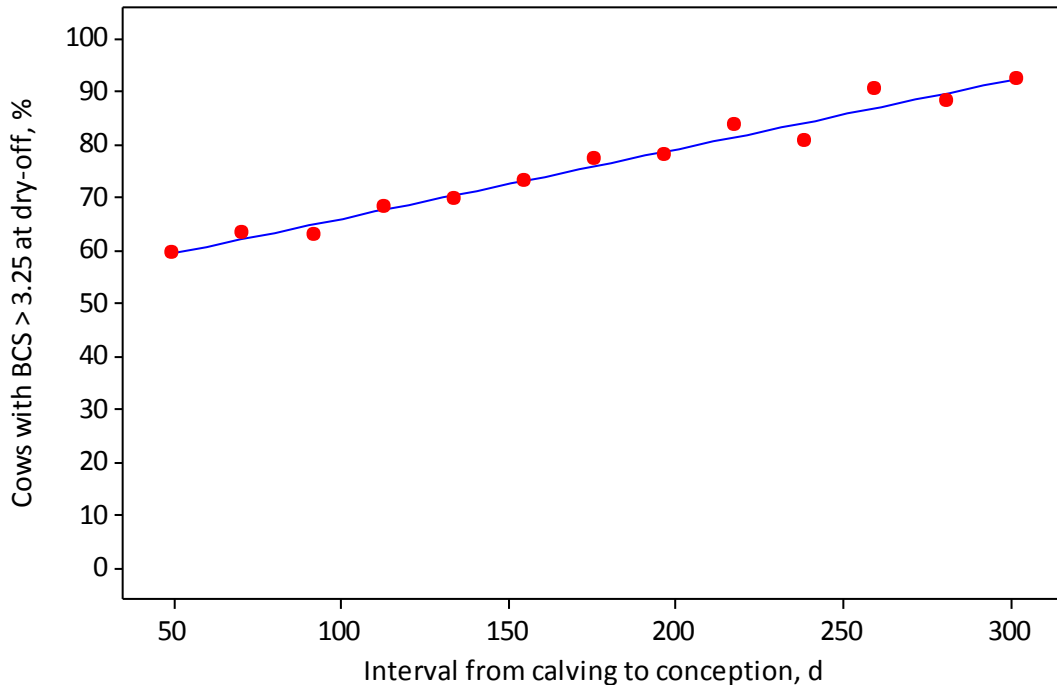


Figure 4. Correlation between interval from calving to conception (ICC) and percentage of cows with BCS at dry-off > 3.25 (Cows with BCS at dry-off > 3.25 = 52.8 + 0.1 x ICC;  $r^2 = 96.9\%$ ). Adapted from Mendonça and Chebel (2011).

### **Effects of diseases on performance and reproductive efficiency**

Cows exposed to conditions that limit feed intake prepartum either because of physical impediment (i.e. overstocking) or because of continued social disruption (i.e. regrouping), common problems observed during the prepartum period, are at greater risk of immune suppression and metabolic diseases peripartum and health disorders postpartum, which are expected to reduce productivity of dairy cows and profitability of dairy operations. Severe economic losses are estimated to result from the following diseases: retained fetal membrane, \$ 312/case (Laven and Peters, 1996); metritis, \$ 300/case (Guard, 1998); displacement of abomasum, \$ 340/case (Guard, 1998); and, mastitis, \$ 224/case (Steenefeld et al., 2011). These losses are the consequence of costs related to treatment, discarded milk, reduced milk yield, and increased culling. Because some of these references are nearly two decades old and because calculations did not account for negative effects of these diseases on reproductive performance, the costs associated with these diseases are much higher.





The effects of these diseases on reproductive efficiency are described in the companion manuscript presented on this proceeding entitled: “Causas de infertilidad en vacas de leche en lactación”.

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