PEER REVIEWED

Veterinary Related Issues Associated with Feeding Free Fatty Acids to Dairy Cows Pre- and Postpartum

R. M. Thornsberry[1], *DVM, MBA;* **A. F. Kertz**[2], *PhD*
[1]*Mid America Veterinary Consulting, P. O. Box 818, Richland, MO 65556, 573-257-0723, email cowman@dishmail.net*
[2]*ANDHIL LLC, 9909 Manchester Road, #366, St. Louis, MO 63122-1915, 314-821-2911, email andhil@swbell.net, www.andhil.com*

Abstract

In confined dairy production units, reproductive efficiency is a major concern for the attending veterinarian. Many parameters influence reproductive performance of lactating dairy cows. While attending veterinarians do not necessarily formulate dry cow, transition, and lactating cow diets, they could influence how these diets are formulated and fed. With increasing costs of commodity grains and traditional starch sources, producers are seeking economical means to supply dairy production animals with dietary energy. Indirect fat supplementation, in the form of grain and oil seeds, and some forages such as corn silage, are usual sources of dietary fat. In addition to these common feed sources of lipid-based energy, the dairy practitioner has alternatives to provide free fatty acids to a dairy production animal diet. Dairy veterinarians must understand how provision of free fatty acids can influence reproductive performance and health.

Key words: dairy cows, free fatty acids, linoleic to linolenic acid ratio, retained placenta, ketosis, body condition

Résumé

Dans les fermes laitières à stabulation entravée, l'efficacité reproductive est un sujet d'inquiétude important pour le vétérinaire traitant. Plusieurs paramètres influencent la performance reproductive chez les vaches laitières en lactation. Bien que les vétérinaires traitants ne formulent pas nécessairement le régime alimentaire des vaches taries, en transition ou en lactation, ils peuvent influencer la formulation et la distribution de l'alimentation. En considérant les coûts accrus des denrées à base de grains et de sources traditionnelles d'amidon, les producteurs recherchent des moyens économiques de fournir de l'énergie alimentaire aux vaches laitières. Le supplément indirect en gras, par l'entremise de grains et de graines oléagineuses, de même que par certains fourrages comme l'ensilage de maïs sont des sources bien connues de gras alimentaire. En plus de ces sources habituelles d'énergie lipidique associées au fourrage, le producteur laitier a aussi accès à des alternatives afin d'ajouter des acides gras libres dans le régime alimentaire des animaux de production laitière. Le vétérinaire praticien doit comprendre comment l'apport en acides gras libres peut influencer la performance reproductive et la santé.

Introduction

This review examines the basic biochemistry associated with the biological concept of providing an alternative fat source to dairy cattle, other than that provided by grains, grain by-products, and oil seeds. Energy is a limiting factor in lactating dairy cow diets. Dietary fat can provide 2.25 times the energy as an equal amount of carbohydrate. The metabolism and digestion of dietary fat are different from that of carbohydrates, providing a biochemical pathway of energy production that does not interfere with carbohydrate digestion or metabolism. Fat provides an additional energy source without the complications of lactic acid production that can occur when additional carbohydrates are incorporated into the diet. All fats are not created equal, and come from different sources and cause different effects and outcomes for cows consuming them.

Fat Classifications

Fats are classified as fully saturated (SAT) if all four covalent bonds on each carbon atom are occupied with a single carbon-to-carbon bond, hydrogen, or some other compound such as a methyl or carboxyl unit (Figure 1). Fats are classified as unsaturated (UNS) if at least two carbon-to-carbon double covalent bonds occur. If more than one of these double carbon-to-carbon covalent bonds is present in a fat molecule, it is referred to as a polyunsaturated fatty acid (PUFA; Figure 2).

Nomenclature of Fatty Acid Families

There are different families of fatty acids (FA) in food and feed items. Biochemists and nutritionists refer to these groups as 1) omega-3; 2) omega-6; 3) omega-7; and 4) omega-9 FAs. A fat molecule is made up of a series of carbon-to-carbon bonds with a methyl group on one end and a carboxyl group on the other end. Although fats are not soluble in water, the carboxyl end is slightly polar, and somewhat soluble in water. Most FAs are in the form of triglycerides (TGs), a series of three individual FAs bonded to a glycerol molecule (Figures 3 and 4). This TG bonding must be broken before a free fatty acid (FFA) is available for absorption from the small intestine. Highly saturated TGs have high melting points which can reduce their digestibility by 50%[79] since they are poorly hydrolyzed in the rumen.[63] Similarly in another study,[23] a mostly saturated TG had 26% lower digestibility than a similar degree of saturated FFA source. Greasiness of TG, such as lard or tallow, can reduce dry matter intake (DMI) most when applied last or on the forage portion[22] of a total mixed ration.

The omega family classification system numbers carbon atoms in sequence, starting from the methyl end. The omega-7 family of FAs is synthesized from palmitic acid, a fully saturated FA identified as C16:0. The omega-9 FAs are synthesized from stearic acid, C18:0, via oleic acid (C18:1). These two families are not considered essential FAs for the diets of dairy cattle.

The omega-3 and omega-6 FAs are essential since they are not synthesized *in vivo* by metabolic pathways. Linoleic acid (C18:2) belongs to the omega-6 family, while linolenic acid (C18:3) belongs to the omega-3 family. Since chemists describe the formula of a FA starting from the methyl end, the location of the first double carbon-to-carbon bond determines the group classification. Linoleic acid has two double carbon-to-carbon bonds, and the first double bond occurs between carbons 6 and 7. Linoleic acid is classified as a PUFA of the omega-6 family. Since the first double carbon-to-carbon bond in linolenic acid occurs between carbons 3 and 4, linolenic acid is classified as a PUFA of the omega-3 family (Figure 5). Feeding more linolenic acid to dairy cows has resulted in milk production with higher levels of omega-3 FAs.[45]

Published information demonstrates that dairy cows fed supplemental fat may experience improved energy balance and begin to cycle earlier than unsupplemented cows. This is postulated to be the result of enhanced follicular growth and development.[30]

Reproductive Physiology Relative to Fatty Acids

It is also necessary to understand basic reproductive physiology when determining what types and how much supplemental fat to feed pre- and postpartum dairy cows. A thorough evaluation of the reproductive

Saturated fats		
Formula	Common name	Melting point
$CH_3(CH_2)_{10}CO_2H$	lauric acid	45°C
$CH_3(CH_2)_{12}CO_2H$	myristic acid	55°C
$CH_3(CH_2)_{14}CO_2H$	palmitic acid	63°C
$CH_3(CH_2)_{16}CO_2H$	stearic acid	69°C
$CH_3(CH_2)_{18}CO_2H$	arachidic acid	76°C

Figure 1. Common saturated free fatty acids. (Adapted from Gunstone FD, Harwood JL, Dijkstra DJ: *The Lipid Handbook*, ed 3. New York, Chapman and Hall, 2007 and Lewkowltsch J: *Chemical Technology and Analysis of Oils, Fats, and Waxes*, ed 6, (3 volumes). London, Macmillion, 1922.)

Unsaturated fats		
Formula	Common name	Melting point
$CH_3(CH_2)_5CH{=}CH(CH_2)_7CO_2H$	palmitoleic acid	0°C
$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	oleic acid	13°C
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$	linoleic acid	-5°C
$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$	linolenic acid	-11°C
$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2CO_2H$	arachidonic acid	-49°C

Figure 2. Common unsaturated and polyunsaturated free fatty acids. (Adapted from Gunstone FD, Harwood JL, Dijkstra DJ: *The Lipid Handbook*, ed 3. New York, Chapman and Hall, 2007 and Lewkowltsch J: *Chemical Technology and Analysis of Oils, Fats, and Waxes*, ed 6, (3 volumes). London, Macmillion, 1922.)

physiology related to feeding specific FAs to dairy cows has been published.[11,12,13]

Prostaglandins synthesized from dietary fats are key hormonal components of animal reproductive physiology. Arachidonic acid (C20:4) is an omega-6 FA. Although arachidonic acid is considered essential, it can be synthesized by the process of acylation and deacylation from linoleic acid.[5] Arachidonic acid is incorporated into the phospholipid cell walls of cells lining the uterus, and is utilized by these cells to manufacture prostaglandin $F2_{\alpha}$ ($PGF2_{\alpha}$). $PGF2_{\alpha}$ is the primary reproductive prostaglandin whose function is to cause the destruction or lysis of the cells of the corpus luteum. The corpus luteum produces large amounts of progesterone, the hormone that maintains pregnancy. When cells of the corpus luteum are lysed or destroyed, progesterone levels drop, and the reproductive cycle begins again. If too much $PGF2_{\alpha}$ is produced or if not enough progesterone is produced, pregnancy cannot continue, the embryo dies, and the dairy cow absorbs this pregnancy and begins another reproductive cycle. If embryo loss occurs, the lactating dairy cow loses another 21 days of potential pregnancy.

By contrast, linolenic acid (C18:3) is metabolized into eicosapentaenoic acid (EPA; C20:5n-3), which along with docosahexaenoic acid (DHA; C22:6n-3) leads to reduced use of the similarly structured arachidonic acid.[9] Linolenic acid, EPA, and DHA produce prostaglandins of the three series, which are reproductively inactive. This competitive inhibition of arachidonic acid leads to lower levels of reproductively active $PGF2_{\alpha}$ being produced by the uterine cells, which could help in the maintenance of pregnancy (Figures 6 and 7).

Diets rich in linoleic acid (C18:2) will increase arachidonic acid concentration in the cell walls of uterine cells, which in turn can lead to more $PGF2_{\alpha}$ production by these cells. By contrast, diets rich in linolenic acid (C18:3), EPA, and DHA will lead to competitive inhibition of arachidonic acid, and less $PGF2_{\alpha}$ production.[40,44,50] This information has led to much interest in the linoleic to linolenic acid ratio in dairy cattle diets, which is basically an omega-6 to omega-3 ratio. A low linoleic to linolenic acid ratio should decrease $PGF2_{\alpha}$ production, as described by Bauman and Griinari.[8] In a review of 25 published studies with duodenal FA flow measurements,[41] only in a few cases did "rumen protection" by calcium salts or FA amides appreciably decrease ruminal biohydrogenation below 82% for linoleic and below 86% for linolenic and oleic FAs, as compared to "unprotected" sources of these FAs. This illustrates the difficulty of bypassing dietary unsaturated (UNS) FAs to the small intestine for absorption, and of quantitatively knowing what amount of these dietary UNS FAs survive ruminal biohydrogenation.

Dietary Polyunsaturated Fatty Acid (PUFA) Sources and Impact

The EPA and DHA are found in fish oil and fish meal, but fish oil is unpalatable and may considerably decrease DMI. Cows fed fish oil could have increased incidence of displaced abomasum due to a decrease in DMI when first introduced immediately following calving.[11,12,13] Improved protection of EPA and DHA in the rumen to allow feeding of fish meal or fish oil containing

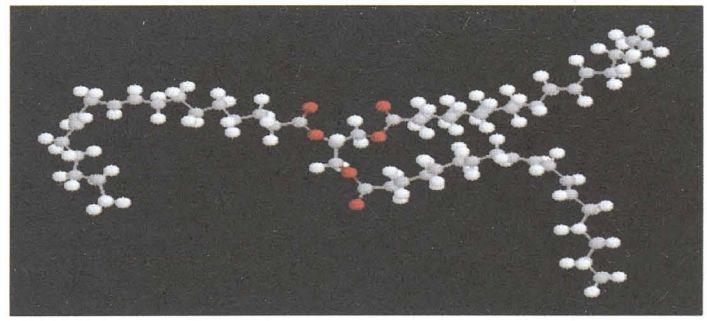

Figure 3. Trilinoleic ester of glycerol—three-dimensional chemical diagram. (www.brevardbiodiesel.org/iv.html, used with permission)

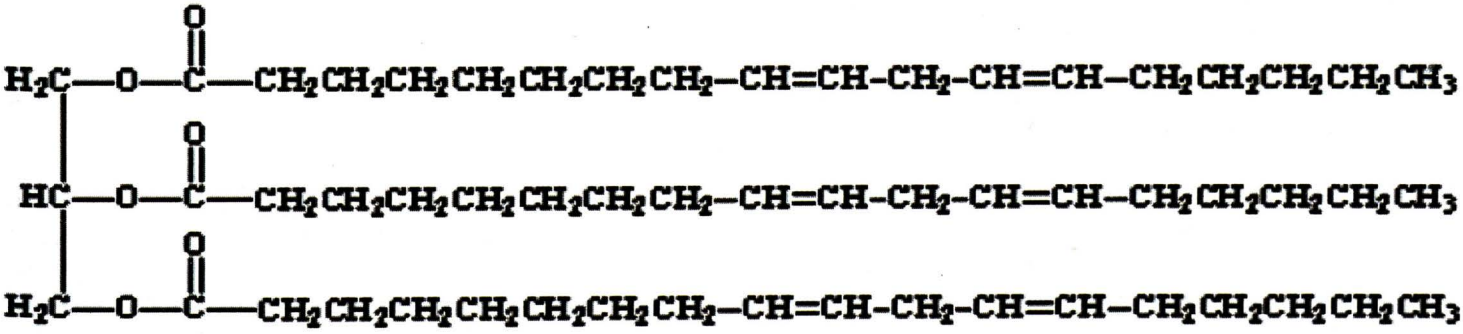

Figure 4. Trilinoleic ester of glycerol—two-dimensional chemical diagram. (www.brevardbiodiesel.org/iv.html, used with permission)

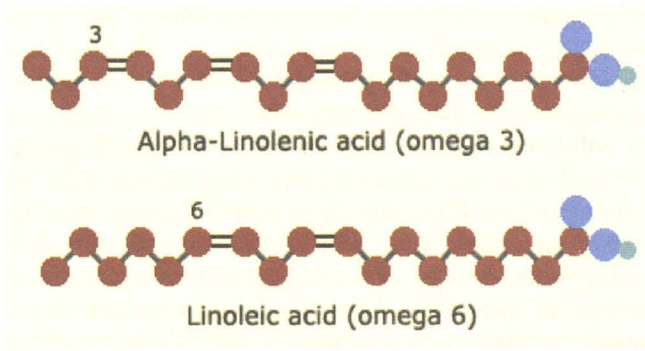

Figure 5. Chemical structure differences between linolenic and linoleic acids. (www.siberiantigernaturals.com/omega3.htm)

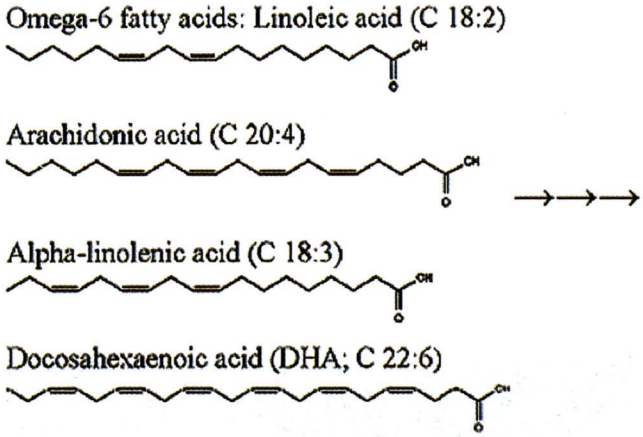

Figure 6. Enzyme action on dietary fats: production of arachidonic acid from omega-6 fatty acids; omega-3 fatty acids yield, instead, eicosapentaenoic acid (EPA), which is further metabolized to form docosahexaenoic acid (DHA). (from Dharmamanda S: Reducing Inflammation with Diet and Supplements. www.itmonline.org/arts.lox.htm, 2003, p 1, used with permission.)

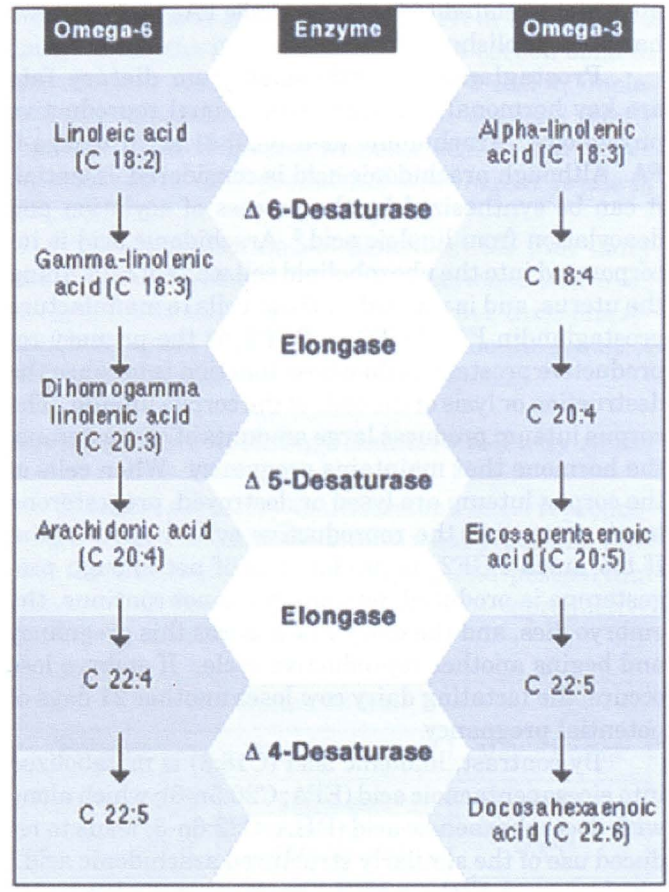

Figure 7. The basic dietary omega-6 fatty acid (linoleic acid) and its product arachidonic acid; the basic plant-based omega-3 fatty acid (linolenic acid) and its product of metabolism, docosahexaenoic acid (DHA), which, like eicosapentaenoic acid (EPA), is obtained directly from fish oil. The molecules are labeled according to the number of carbons and number of double bonds in the chain. (from Dharmamanda S: Reducing Inflammation with Diet and Supplements. www.itmonline.org/arts.lox.htm, 2003, p 1, used with permission.)

EPA and DHA in sufficient amounts to prevent reduction in DMI, but still have the benefit of reduced $PGF2_{\alpha}$ production, is needed.[11,12,13] Feeding calcium salts of fish oil offered no protection against ruminal biohydrogenation of EPA and DHA beyond that observed with unprotected fish oil.[18]

Feeding flaxseed, an oilseed relatively high in linolenic acid, is an alternative since linolenic acid can be synthesized by biochemical pathways into EPA. However, no practical method is available for protecting linolenic acid in flaxseed from rumen microorganisms that biohydrogenate linolenic acid into another more saturated FA. If a method were discovered to protect linolenic acid in the rumen and this method would allow delivery of unchanged linolenic acid to the small intestine, linolenic acid could be converted to EPA. This would decrease $PGF2_{\alpha}$ production at critical times of reproductive necessity.

A recent literature review conducted by Ambrose and Kastelic[3] has demonstrated and summarized the potential effects of PUFAs on dairy cattle reproduction. Five key points were highlighted:

1. PUFAs such as linoleic (omega-6) (C:18:2n-6), α-linolenic (omega-3) (C:18:3n-3), eicosapentaenoic (omega-3) (C:20:5n-3), and docosahexaenoic (omega-3) (C:22:6n-3) acids can affect reproduction and fertility.

2. Linoleic acid is found mainly in oilseeds, whereas linolenic acid is found predominantly in forages and in some oilseeds (flaxseed); EPA and DHA are high in fish oils.

3. Dairy cows fed diets high in EPA and DHA (supplementation with menhaden fish meal) or linolenic acid (supplementation with flaxseed) during early pregnancy had reduced $PGF2_{\alpha}$ production and increased pregnancy rates.

4. Feeding diets high in linolenic acid during the dry period may increase the incidence of placental retention due to inhibition of $PGF2_{\alpha}$ production.[44]

5. Dietary supplementation of select PUFAs during the postpartum period can potentially improve fertility in dairy cows, but more research is essential.

Metabolism and Impact of trans Fatty Acids

Another important aspect of incorporating dietary fat into lactating cow diets is the effect of *trans* FAs. *Cis* and *trans* isomers of the same chemical organic compound refers to the actual geometric shape of a particular organic chemical compound. This geometric shape can greatly alter the biochemical function and action of a specific isomer. Fatty acid isomers are no different. *Trans* FAs refer to isomeric UNS FAs containing one or more double carbon-to-carbon bonds in the *trans* configuration.[62] Similar changes to PUFAs occur in the rumen and in industrial processes, both yielding higher levels of *trans* fatty acids.[46,61] Since most FAs in plants are in the *cis* form, they must first be converted to the *trans* form through a progression of isomerase- and hydrolyase-mediated steps before ruminal biohydrogenation can occur (Figure 8). The term *cis* refers to hydrogen atoms being on the same side of a double bond, while *trans* refers to hydrogen atoms being on the opposite side of a double bond.

Vaccenic acid (C:18:1 Δ1-*trans*) is the major product of rumen action to produce *trans* FAs. It is the *trans*-10 *cis*-12 C18:2 isomer that appears to cause the most milk fat depression.[29]

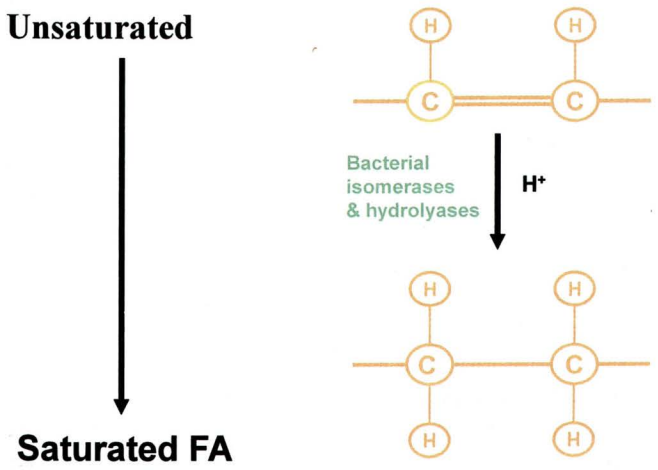

Figure 8. Initial steps of isomerastion and hydrolyation before ruminal biohydrogenation. (Created by Dr. Adam Lock, Michigan State University, used with permission.)

Feeding high levels of grain-based FAs high in linoleic acid results in considerable production of *trans* FAs within the rumen, which in turn reduces milk fat production by a complex biochemical pathway. This has contributed to milk fat depression in dairies utilizing high levels of distiller's grains, a byproduct of the ethanol production industry, or in combination with other dietary UNS FA sources.[42] Distiller's grains are high in corn oil and linoleic acid. Feeding fats already in a *trans* configuration does not mean PUFAs will not be further altered by rumen microorganisms and modify their biochemical and/or physiological effects. It has been demonstrated that nearly all PUFAs are altered by biohydrogenation to some extent in the rumen (Figure 8).[41] The only way to prevent this process is to feed a higher level of already-saturated free fatty acids (SFFAs).[66] Milk fat depression caused by *trans-10 cis-12* C18:2 formation in the rumen is reported in the literature. As little as 3 grams of this isomer formed daily in the rumen is sufficient to reduce milk fat production by 25% or more.[8,38,47,52] This is a more minor side pathway as shown in Figure 9.

Although SFFAs may undergo some biochemical alterations in the rumen, a recent study with ileum-cannulated cows indicated that SFFAs are not converted into unsaturated fats, nor are they converted into *trans* FAs.[37] Another study has demonstrated that plasma prostaglandin metabolite concentration was greater in multiparous cows fed a *trans* FA supplemented diet than those receiving an isocaloric control diet enriched with a saturated fat.[66] In primiparous cows, no difference in prostaglandin metabolites was discovered. Although increased levels of prostaglandin metabolites may benefit a multiparous cow by stimulating more motility and increased inflammatory response in the uterus post-calving, it would not benefit the dairy cow that is recently bred and attempting embryo implantation. *Trans* FAs in excess are not beneficial and excess linoleic acid in the postpartum cow could lead to increased levels of $PGF2_{\alpha}$ and reduced reproductive efficiency, as well as increased amounts of rumen alteration to *trans* FAs.[66] The linoleic to linolenic FA ratio in a dairy cow diet is important to lactating dairy cow health.[44]

Three Major Transition Cow Health Events

Hypocalcemia, ketosis, and retained fetal membranes are clinical challenges for veterinarians providing professional services to modern dairy production units. These three major health-altering events may be controlled in some respects by having influence over the diet fed to the dairy cow during the dry cow period and the first 150 days of lactation. The practicing veterinarian

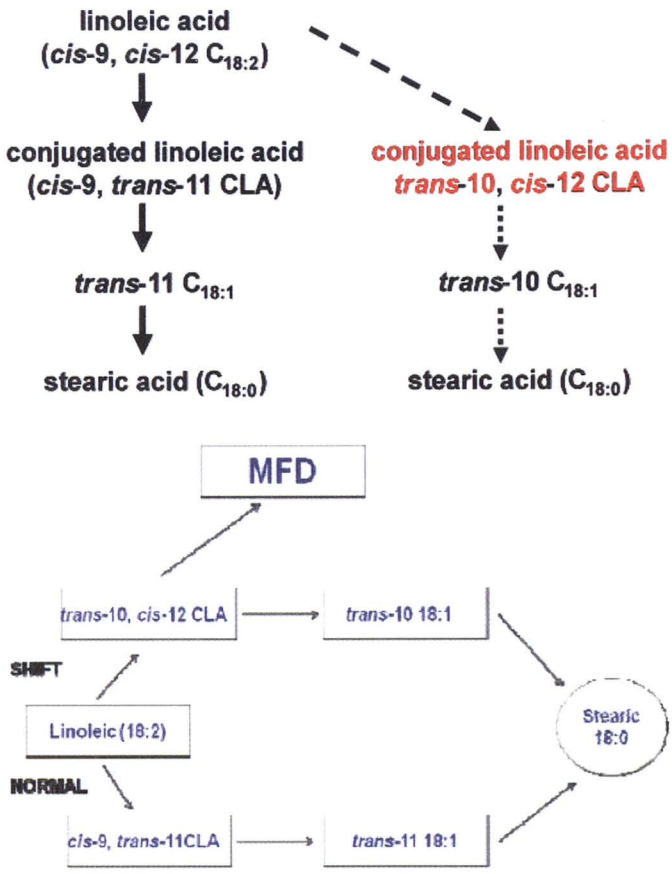

Figure 9. Major and minor pathways of ruminal biohydrogenation of linoleic acid. (top figure: from Bauman DL, Griinari JM: Regulation and nutritional manipulation of milk fat: low-fat syndrome. *Liv Prod Sci* 70:15-29, 2001. bottom figure: from Jenkins TC, Klein CM, Mechor GD: Managing milk fat depression: interactions of ionophores, fat supplements, and other risk factors. *FL Ruminant Nutr Conf, Gainesville*, pp 1-11, 2009, used with permission.)

may not have control over the nutritional management of these production periods, but he or she should influence the dietary management of the dairy cow to prevent nutritionally related disease conditions.

The dairy cow has a complex endocrine system designed to maintain and nurture a fetus. Progesterone is produced by the ovarian corpus luteum. This hormone feeds back on the entire endocrine system to prevent routine reproductive cycling, the production of $PGF2_{\alpha}$, and the loss of pregnancy. It is understood that progesterone is immunosuppressive.[28,33,34,35,53,55] Immunosuppression is necessary to prevent overreaction of the maternal immune system, which in turn may recognize the different genetic structure of the fetus as foreign. This recognition could result in rejection of the fetus and subsequent abortion prior to full-term development.

While progesterone immunosuppression is recognized as normal and beneficial, the resulting immunosuppression appears to be greatest during the last two months of pregnancy in the dairy cow. If this period of production is coupled with increased stress and avoidable stressor events, increased levels of cortisol, another immunosuppressive hormone released from the adrenal cortex, will add to the immunosuppressive activity of progesterone. The resulting severe immunosuppression can in turn lead to increased incidence of retained placenta, hypocalcemia (milk fever), and metabolic imbalances such as ketosis. It has also been demonstrated that lactation itself is a stressor, especially in high producing dairy cows.[27] An association has been developed between metabolic disease and subsequent development of reproductive disorders.[7,27,56,60,70,76] In Israeli dairies studied,[57] there was a very strong association between parturient hypocalcemia and retained placenta. For instance, the odds ratio (multiplicative increases in occurrence) found that a milk fever cow was 3.2 times more likely to retain her placenta than a cow that had not had milk fever. Hypocalcemia also greatly increased the risk of mastitis.[21] Strong links between hypocalcemia and the increased incidence of endometritis have also been demonstrated.[81] A large scale Swedish study[24] involving 772 herds found the risk of ovulatory dysfunction was increased in cows that had suffered ketosis. Another study conducted in 1985[56] reported that 80% of cows with ketonuria developed metritis. These studies suggest metabolic diseases impair immune function, predisposing the dairy cow to uterine infection and mastitis. Retained placenta has been identified as a risk factor for development of ketosis.[25,27,51]

Managing Cow Comfort and Stress

Cow comfort is a major management issue in today's confinement dairy operations. Most veterinarians recognize the essential nature of individual lactating cow comfort, and its importance for maximizing milk production and reproductive efficiency. It is just as important to manage individual cow comfort in the dry cow pen, and extremely important to manage individual cow comfort in the up-close and postpartum pens. For optimal reproductive management, practitioners should ensure their dairy clients are properly managing transition cows, which in turn can improve breeding management programs.[64]

The usual chain of events for a dairy cow nearing the end of the dry period produces stress. The fetus is experiencing very rapid growth in the last 60 days of gestation. The mammary gland is growing, repairing, and preparing for lactation, producing large amounts of colostrum. These normal functions require considerable protein and energy. Unfortunately, at this time, DMI

can decrease as much as 20-30%. These stressful events cause the cow's adrenal cortex glands to produce higher levels of cortisol, the stress hormone, which along with high blood levels of progesterone can cause severe immunosuppression. If dry cows are mismanaged at this period by overcrowding, under-feeding, exposing them to heat stress, or by making excessive pen or group changes, the stress response is exacerbated, resulting in weight loss, fat mobilization, and ketosis. Overconditioned cows are at a much higher risk for ketosis. Thus, in addition to the ketogenic effects of increasing levels of cortisol, a metabolic risk has been created.

A direct link between blood cortisol levels, ketosis, and the increased incidence of retained placenta has been determined in some studies,[54] with retained placenta being directly related to immune status before calving. Additionally, immunity can be negatively affected by decreases in energy intake.[59,77] Conversely, cows experiencing calving difficulty, hypocalcemia, twinning, or retained placenta have higher risks for ketosis and uterine infection after calving.[27]

Studies suggest retained fetal membranes are caused by an impaired immune response,[31] and that neutrophil function determines whether or not a cow will develop retained placenta.[49] At parturition, the immune system must recognize fetal membranes as foreign tissue and mount an immune response to have normal or natural release of the placental tissues from maternal tissues. Cows that are stressed, nutritionally mismanaged, and improperly handled will have a depressed immune function, and are at risk for retained fetal membranes. Cows that develop retained placenta have reduced levels of antioxidants in their blood.[17] Veterinarians recognize that a storm of retained placentas needs immediate medical attention and possible nutritional management intervention. Selenium and vitamin E levels, the calcium to phosphorus ratio in dry cow and fresh cow diets, as well as the total calcium and phosphorus levels must be fully evaluated by the attending veterinarian.[6,16,70,82] All fresh dairy cows are likely in a negative protein and energy balance shortly after calving. It is common for a fresh cow to lose 30 to 50 lb (14 to 23 kg) of body protein during the first two weeks of lactation, which has been shown to support amino acid and glucose requirements for milk production.[1,4,14,59,65]

Managing Ketosis and Energy Needs During The Transition Period

The ketogenic effects of abrupt fat mobilization to meet additional energy needs associated with reduced DMI at calving, and negative energy balances associated with early lactation, is detrimental to not only hepatic function (fatty liver syndrome), but also immune system and specific immune cell functions.[58] Recent research has discovered that neutrophil activity in fresh cows was significantly reduced, and this reduced activity was evident the day of calving, even before lactation and before bacterial exposure to the uterus.[32] This may explain why 20 to 30% of fresh cows will develop clinical metritis.[48] Endometritis is actually present in 40 to 50% of cows examined four weeks post-calving.[48] Leukocytes from cows with endometritis are significantly less phagocytic than those from cows without endometritis.[48]

This information should lead a practitioner to becoming intimately involved in the nutritional and animal husbandry management of the dry cow and the early lactation cow. To be successful at attaining a reasonable goal for reproductive efficiency and to avoid metabolic disorders in a modern dairy, veterinarians must be focused on reducing stress at critical points when immunosuppression is known to exist. The challenges are to improve feed intake, to improve energy balance, and to make a high energy diet palatable, especially when supplemented with fat. At the critical period around calving, veterinarians must help ensure palatable and properly balanced rations are provided, and must promote animal husbandry practices to ensure clean maternity pens, clean obstetrical equipment, clean housing and stalls, and cool, uncrowded feeding areas with at least 30 linear inches (76 cm) of bunk space per cow. This approach involves the following areas:

Provide for high energy diets in lower volumes consumed at, near, and following calving by adding SFFAs to the diet for consumption at a rate of 0.5 to 1 lb (0.23 to 0.45 kg) per cow per day. This process should commence at least two weeks prior to calving and should extend through the first 150 days of lactation when cows are in negative energy balance and proceeding to replenish body condition.

Reduce dietary potassium intake in dry cow rations and insure that dietary magnesium levels are at least 0.4%.[63] Since dietary potassium levels are not always known, use higher magnesium levels since potassium decreases magnesium absorption.[78]

Use anionic-based ration management to control the incidence and severity of hypocalcemia, while monitoring the calcium to phosphorus ratios[16] and levels in the dry cow and early lactation diets. This is also directly related to dietary potassium and magnesium levels, as noted in 2 above.

Closely monitor the linoleic to linolenic acid ratios in both dry cow and early lactation rations, but especially for dry cow and close-up rations.[43]

Maintain adequate effective fiber in dry cow and fresh cow rations fed to prevent displaced abomasums.[75]

Avoid overfeeding dry cows as fat cows develop ketosis much faster, have a higher incidence of ketosis, and develop a greater incidence of ketosis that is nonresponsive or poorly responsive to treatment.[69]

All of these practices are necessary to prevent severe immunosuppression in dairy cows at the critical time of late dry cow and early lactation management.

Types and Characteristics of Major Fat Supplements

Several types of fat supplements are available for feeding to dairy cows: triglycerides (TGs) which can be liquid, dependent on melting point; calcium salts of fatty acids (CSFA), typically comprised of palm fatty acid distillate which has about 50% UNS FAs and contains about 82% fat; and mostly saturated free fatty acids (SFFAs) which are about 85% saturated and contain 98% fat. The latter provide the dairy cow with a net energy of lactation that is 20% greater than CSFA simply based on fat content. The level of UNS FAs has been shown to be directly related to DMI decrease.[2] This information was incorporated into a linear relationship by the 2001 Dairy NRC,[63] which established that for each one percentage unit that CSFAs were incorporated into a diet, DMI was reduced by 2.5%. Feeding higher levels of corn silage or distiller's grains, or other grain-based products high in linoleic acid would exacerbate the DMI decrease. Feeding SFFAs can provide many of the benefits of feeding higher levels of omega-3 FAs without the DMI suppression associated with fish oils.

Limitations of Feeding Whole Cottonseed

Cottonseed is extensively utilized in diets for lactating dairy cows as a source of protein, fat, and fiber. Attempting to obtain fat energy from whole cottonseed has a possible detrimental effect.[15,73,83] Cottonseed contains gossypol, a potentially toxic polyphenolic compound produced by the pigment glands of the cotton plant. Cows fed diets with significant levels of gossypol experienced reduced conception rates and experienced greater fetal losses.[19,67,72,73] When more energy is required to maximize production, it is tempting for dairy producers to simply increase the cottonseed content of the lactating cow diet. When lactating dairy cow recipients received embryos from heifers fed 12 g per day of free gossypol, the embryos produced reduced pregnancy rates at 28 and 42 days of gestation.[26] Lactating dairy cows fed diets high in free gossypol exhibited increases in measured plasma gossypol, reduced conception rates, and increased pregnancy losses.[72] Gossypol can also cause bulls to become sterile.[15,19] This is an important issue for herds commingling bulls with lactating cows.

The fat contained in whole cottonseed is in the form of cottonseed oil. Unsaturated FAs are the predominate FAs found in cottonseed oil. Although the oil fat is in a TG chemical configuration, the TGs must first be hydrolyzed in the rumen, and the resulting UNS FAs are further biohydrogenated by rumen organisms. Fifty percent of FFAs released by TG hydrolysis are linoleic acid, making whole cottonseed a significant contributor to the total linoleic acid contained in the diet of dairy cows. The linoleic acid released from TG hydrolysis contributes very significant amounts of linoleic acid to the linoleic side of the linoleic to linolenic acid ratio in the diet of the dairy cow.[71] Gossypol's effect on reproductive parameters in the dairy cow are significant enough that researchers have questioned the use of whole cottonseed as an energy source in early lactation cow diets and in donor cow diets.[74]

Effects of Supplemental Fatty Acids in Free Form or as Calcium Salts

Saturated FFAs do not contain gossypol, and can be recommended to increase energy levels in the late dry cow diet and early lactation diet without any of the detrimental effects associated with PUFAs or whole cottonseed intake. Palm FA distillate is also much higher in linoleic acid than are SFFAs, which can lead to greater production of prostaglandin $F2_{\alpha}$ and possible negative effects on pregnancy, as discussed previously.

Calcium salts of FAs of palm distillate are known to depress DMI when supplied in the diet of dairy cattle, which should be avoided in the late dry period and early lactation period.[20] These two periods of production are already plagued with DMI limitations. Adding CSFAs could exacerbate this problem at a very critical time. SFFAs are palatable, adding a dense source of energy at a time of stress and endocrine-induced depressed DMI, without negatively influencing DMI itself. Michigan State University scientists[36,37] demonstrated that as dietary UNS FA levels increase relative to SFFAs , cows decreased DMI through the mechanism of greater cholecystokinin (CCK) plasma levels leading to decreased rumination and meal size,[68] increased non-essential FA (NEFA) levels from body condition mobilization in an attempt to maintain milk production, and decreased milk fat due to incomplete ruminal biohydrogenation of PUFAs. This resulted in more *trans*-10 *cis*-12 C18:2 production and significantly decreased C18:3 duodenal flow, even though dietary intake of C18:3 significantly increased with increased levels of dietary PUFAs. About 30% PUFAs were contained in the CSFA product which is intended to deliver more PUFA to the small intestine (but the opposite actually occurred), and to consequently enhance reproduction. Fat sources incorporating more PUFAs will further decrease DMI and milk fat than more conventional CSFA sources. It is critical that supplemental fat sources do not decrease DMI even in a low volume, high-energy ration commonly fed to dairy cattle during the late dry period. When utilizing unpalatable anionic sources in the late dry cow diet to

control hypocalcemia, the practitioner will want to use a highly palatable fat source like SFFAs to provide the additional energy needed, without the detrimental DMI depression associated with CSFAs, and without adding any appreciable amount of linoleic acid to the dry cow diet. While it is commonly assumed that supplemental fat reduces DMI in early lactation, most studies have been done with CSFA which do reduce DMI because of their UNS FA content, as already noted.

Early lactation studies with a treated tallow,[39] which may have had reduced digestibility depending on the degree of saturated TGs and high melting point, and a SFFA supplemental fat source[80] did not reduce DMI compared to non-fat-supplemented control diets. Over a 15-week period in the latter trial, cows averaged 52 lb (23.6 kg) DMI and 99 lb (45 kg) milk on the fat-supplemented treatment with 60% forage, and 54 lb (24.5 kg) DMI and 107 lb (48.6 kg) milk on the fat-supplemented treatment with 40% forage.

Impact of SFFA Supplementation on Fat Soluble Vitamins

Some veterinarians and nutritionists may be concerned about fat soluble vitamin availability and absorption in diets containing SFFAs. Recent research has determined that vitamin E absorption and status is unaffected by adding a fat supplement of SFFAs to the diets of transition cows.[6]

Conclusions

Veterinarians typically do not formulate dry cow and early lactation diets, but they can influence ration composition when health and reproductive efficiency is critical. Two very recent studies define the importance of body condition for postpartum dairy cows, the significance and importance of maintaining body condition, and the effect loss of body condition may have on health and welfare of lactating dairy cows under the supervision of food animal veterinarians.[10,77]

These studies point out a direct correlation between postpartum body condition of dairy cows and increased somatic cell counts and lameness scores. Feeding a fat supplement to up-close dairy cows and early lactation cows is an important tool for the veterinarian to consider to provide additional dietary energy to keep body condition from being lost too quickly after calving, which may impact the health of the early lactation cow. Although there are many sources of dietary fat to consider, careful study of the literature and practical applications on the dairy farm readily illustrate the medical and nutraceutical benefits of adding SFFAs to the diets of dry and early lactation dairy cows.

Acknowledgements

No external funds were used to support this study.

References

1. Agnew RE, Yan T, McCaughey WJ, McEvoy JD, Patterson DC, Porter MG, Steen RWJ: Relationships between urea dilution measurements and body weight and composition of lactating dairy cows. *J Dairy Sci* 88:2476-2486, 2005.
2. Allen MS: Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J Dairy Sci* 83:1598-1624, 2000.
3. Ambrose DJ, Kastelic JP: Dietary fatty acids and dairy cow fertility. *Advances in Dairy Tech* 15:35-47, 2003.
4. Andrew SM, Erdman RA, Waldo DR: Prediction of body composition of dairy cows at three physiological stages from deuterium oxide and urea dilution. *J Dairy Sci* 78:1083-1095, 1995.
5. Ashes JR, Fleck E, Scott TW: Dietary manipulation of membrane lipids and its implications for their role in the production of second messengers. Ruminant Physiology: Digestion, metabolism, growth, and reproduction. *Proc 8th Int Symp on Ruminant Physiology* pp 373-386, 1995.
6. Baldi A, Savoini G, Pinotti L, Monfardini E, Cheli F, Dell Orto V: Effects of vitamin E and different energy sources on vitamin E status, milk quality, and reproduction in transition cows. *J Am Vet Med Assoc* 47:599-608, 2000.
7. Bamouin J, Chassagne M: An aetiological hypothesis for the nutrition induced association between retained placenta and milk fever in the dairy cows. *Ann Rech Vet* 22:331-343, 1991.
8. Bauman DL, Griinari JM: Regulation and nutritional manipulation of milk fat: low-fat syndrome. *Liv Prod Sci* 70:15-29, 2001.
9. Bereziat G: Voies metaboliques et regulations de la biosynthese des prostaglandins et thromboxanes. *Rev Fr Coups Gras* 25:463-473, 1978.
10. Bicalho RC, Caixeta LS, Machado VS: Lameness in dairy cattle: a debilitating disease or a disease of debilitated cattle? A cross-sectional study of lameness prevalence and thickness of the digital cushion. *Proc Am Assoc Bov Pract Conf* 42:173, 2009.
11. Bilby TR, Sozzi A, Lopez MM, Silvestre F, Ealy AD, Staples CR, Thatcher WW: Pregnancy, bST and omega-3 fatty acids in lactating dairy cows: I. Ovarian, conceptus and growth hormone – IGF system response. *J Dairy Sci* 89: 3360-3374, 2006.
12. Bilby TR, Guzelogu A, MacLaren LA, Staples CR, Thatcher WW: Pregnancy, bST and omega-3 fatty acids in lactating dairy cows: II. Gene expression related to maintenance of pregnancy. *J Dairy Sci* 89:3375-3385, 2006.
13. Bilby TR, Jenkins T, Staples CR, Thatcher WW: Pregnancy, bST and omega-3 fatty acids in lactating dairy cows: III. Fatty acid distribution. *J Dairy Sci* 89:3386-3399, 2006.
14. Botts RL, Hemken RW, Bull LS: Protein reserves in the lactating dairy cow. *J Dairy Sci* 62:433-440, 1979.
15. Brocas C, Rivera RM, Paula-Lopes FF, McDowell LR, Calhoun MC, Staples CR, Wilkinson NS, Boning AJ, Chenoweth PJ, Hansen PJ: Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos. *Biol Reprod* 57:901-907, 1997.
16. Brozos CN, Kiossis E, Georgiadis MP, Piperelis S, Boscos C: The effect of chloride ammonium, vitamin E, and selenium supplementation throughout the dry period on the prevention of retained fetal membranes, reproductive performance, and milk yield of dairy cows. *Livestock Sci* 124:210-215, 2009.
17. Brzezinska Slebodzinska E, Miller JK, Quigley JD, Moore JR, Madsen FC: Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. *J Dairy Sci* 30:202-207, 1994.
18. Castaneda-Gutierrez E, de Veth MJ, Lock AL, Dwyer DA, Murphy KD, Bauman DE: Effect of supplementation with calcium salts of fish oil on n-3 fatty acids in milk fat. *J Dairy Sci* 90:4149-4156, 2007.

19. Chase CC Jr, Bastidas P, Ruttle JL, Long CR, Randel RD: Growth and reproductive development in Brahman bulls fed diets containing gossypol. *J Anim Sci* 72:445-452, 1994.
20. Choi Bryung-Ryul, Palmquist DL: High fat diets increase plasma cholecystokinin and pancreatic polypeptide and decrease plasma insulin and feed intake in lactating cows. *J Nutr* 126:2913-2919, 1996.
21. Curtis CR, Erb HN, Sniffin CJ, Smith RD, Powers PA, Smith MC, White ME, Hillman RB, Pearson EJ: Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *J Am Vet Med Assoc* 185:559-561, 1983.
22. Drackley JK, Grum DE, McCoy GC, Klusmeyer TH: Comparison of three methods for incorporation of liquid fat into diets for lactating dairy cows. *J Dairy Sci* 77:1386-1398, 1994.
23. Elliott JP, Overton TR, Drackley JK: Digestibility and effects of three forms of mostly saturated fatty acids. *J Dairy Sci* 77:789-798, 1994.
24. Emanuelson U, Oltenscu PA, Grohn YT: Nonlinear mixed model analyses of five production disorders of dairy cattle. *J Dairy Sci* 76:2765-2772, 1993.
25. Foldi J, Kulcsar M, Pecsi A, Huyghe B, de Sa C, Lohuis JA, Cox P, Huszenicza G: Bacterial complications of postpartum uterine involution in cattle. *Anim Reprod Sci* 96:265-281, 2006.
26. Galvao KN, Santos JEP, Coscioni AC, Juchem SA, Chebel RC, Sischo WM, Villasenor M: Embryo survival from gossypol-fed heifers after transfer to lactating cows treated with human chorionic gonadotrophin. *J Dairy Sci* 89:2056-2064, 2006.
27. Goff JP, Kimura K, Hammon DS: Transition cow nutrition: Effects on immune function and postpartum health. *Proc Dairy Cattle Repro Council Annual Meeting and Conv*, Nov. 6-8, 2006, Denver, CO. pp 1-8.
28. Gottshall LL, Hansen PJ: Regulation of leukocyte subpopulations in sheep endometrium by progesterone. *Immunol* 76:636-641, 1992.
29. Griinari JM, Dwyer DA, McGuire MA, Bauman DE, Palmquist DL, Nurmeia KVV: *Trans*-octadecenoic acids and milk fat depression in lactating dairy cows. *J Dairy Sci* 81:1251-1261, 1998.
30. Grummer RR, Carroll DJ: Effects of dietary fat on metabolism disorders and reproductive performance of dairy cattle. *J Anim Sci* 69:3838-3852, 1991.
31. Gunnink JW: Prepartum leukocytic activity and retained placenta. *Vet Quarterly* 6:52-54, 1984.
32. Hammons DS, Evjen IM, Dhiman TR, Goff JP, Walters JL: Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet Immunol Immunopathol* 113:21-29, 2006.
33. Hansen PJ, Bazer FW, Segerson EC: Skin graph survival in the uterine lumen of ewes treated with progesterone. *Am J Reprod Immunol Microbiol* 12:48-54, 1986.
34. Hansen PJ: Temporal relationship between progesterone and uterine lymphocyte-inhibitory activity in ewes. *Vet Rec* 131:371-372, 1992.
35. Hansen PJ: Regulation of uterine immune function by progesterone-lessons from the sheep. *J Reprod Immunol* 40:63-79, 1998.
36. Harvatine KJ, Allen MS: The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. *J Dairy Sci* 88:4018-4027, 2005.
37. Harvatine KJ, Allen MS: Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. *J Nutr* 136:677-685, 2006.
38. Harvatine KJ, Bauman DE: Update on milk fat: cellular mechanism for inhibition of milk fat synthesis by rumen bio-hydrogenation intermediates. *Proc Cornell Nutr Conf* pp 67-74, 2006.
39. Hoffman PC, Grummer RR, Shaver RD, Broderick GA, Drendel TR: Feeding supplemental fat and undegraded intake protein to early lactation dairy cows. *J Dairy Sci* 74:3468-3474, 1991.
40. Holman RT: Nutritional and biochemical evidence of acyl interaction with respect to essential polyunsaturated fatty acids. *Prog Lipid* 25:29-39, 1986.
41. Jenkins TC, Bridges WC Jr: Protection of fatty acids against ruminal biohydrogenation in cattle. *Eur J Lipid Sci Technol* 109 778-789, 2007.
42. Jenkins TC, Klein CM, Mechor GD: Managing milk fat depression: interactions of ionophores, fat supplements, and other risk factors. *FL Ruminant Nutr Conf, Gainesville*, pp 1-11, 2009.
43. Kankofer M, Kemp B, Taverine MAM: The relationship between linoleic/linolenic acid ratio in the diet of periparturient cows, the activity of phospholipase A_2, superoxide dismutase, glutathione peroxidase and thiobarbituric acid reactive substances in placental tissues at calving. *Revue Med Vet* 151:1147-1152, 2000.
44. Kemp B, Soede NM, Kankofer M, Bevers M, Taverne MAM, Wensing Th, Noordhuizen JPTM: Influence of linoleic/linolenic acid ratio in the diet of periparturient cattle on plasma concentrations of $PGF2_{\alpha}$ metabolite and placental expulsion rate. *Therio* 49:571-580, 1998.
45. Kennelly JJ: The fatty acid composition of milk fat as influenced by feeding oilseeds. *Anim Feed Sci Technol* 60:137-152, 1996.
46. Kepler CR, Hirons KP, McNeill JJ, Tove SB: Intermediates and products of the bio-hydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J Biol Chem* 241:1350-1354, 1966.
47. Kim YJ, Liu RH, Rychlik JL, Russell JB: The enrichment of a ruminal bacterium (*Megasphaera elsdenii* YJ-4) that produces *trans*-10, *cis*-12 isomer of conjugated linoleic acid (CLA). *J Appl Microbiol* 92:976-982, 2001.
48. Kim IH, Na KJ, Yang MP: Immune responses during the peripartum period in dairy cows with postpartum endometritis. *J Reprod Dev* 51:757-764, 2005.
49. Kimura K, Goff JP, Kehrli ME Jr, Reinhardt TA: Decreased neutrophil functions as a cause of retained placenta in dairy cattle. *J Dairy Sci* 85:544-550, 2002.
50. Leat WMF, Northrop CA: Supplementation with linolenic acid of a diet deficient in essential fatty acids results in impaired parturition in rats. *J Physiol* 290:37P, 1979.
51. Lewis GS: Uterine health and disorders. *J Dairy Sci* 80:984-994, 1997.
52. Lock AL, Overton TR, Harvatine KJ, Geisy J, Bauman DE: Milk fat depression: impact on dietary components and their interaction during rumen fermentation. *Proc Cornell Nutr Conf* pp 75-86, 2006.
53. Low BG, Hansen PJ: Actions of steroids and prostaglandins secreted by the placenta and uterus of the cow and ewe on lymphocyte proliferation in vitro. *Am J Reprod Immunol* 18:71-75, 1988.
54. Lyimo ZC, Nielen J, Ouweltjes W, Kruip TAM, Eerdenburg FJCM van: Relation between estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Therio* 53:1783-1795, 2000.
55. Majewski AC, Hansen PJ: Progesterone inhibits rejection of senogeneic transplants in the sheep uterus. *Horm Res* 58:128-135, 2002.
56. Markusfeld O: Relationship between overfeeding, metritis, and ketosis in high yielding dairy cows. *Vet Rec* 116:489-491, 1985.
57. Markusfeld O: Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationship among traits. *J Dairy Sci* 70:158-166, 1987.
58. Mashek DG, Bertics SJ, Grummer RR: Effects of intravenous triacylglycerol emulsions on hepatic metabolism and blood metabolites in fasted dairy cows. *J Dairy Sci* 88:100-109, 2005.
59. McKeown NA, Patton RS: Composition for the treatment or prevention of an energy imbalance in ruminants. *U.S. Patent No. 527494* Filed on 09-13-1995.
60. Moore K, Thatcher WW: Major advances associated with reproduction in dairy cattle. *J Dairy Sci* 89:1254-1266, 2006.
61. Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willet WC, Rimm EB: Dietary intake of *trans* fatty acids and systemic inflammation in women. *Am J Clin Nutr* 79:606-612, 2004.
62. Mozaffarian D, Rimm EB, King IB, Lawler RL, McDonald GB, Levy WC: *Trans* fatty acids and systemic inflammation in heart failure. *Am J Clin Nutr* 80:1521-1525, 2004.
63. National Research Council (NRC) Requirements of Dairy Cattle. 2001. 7th ed. National Academy Press, Washington, DC. 2001.
64. Overton MW: Transition cow management to improve reproduction. *Proc 43rd Annual Dairy Cattle Day*, University of California at Tulare. March 26, 2003, pp 53-56.
65. Paquay R, de Baere R, Lousse A: The insensible loss of weight in dairy cows. *Z Tierphysiol Tierernahr Futtermittelkd* 30:202-207, 1972.
66. Radriquez-Sallaberry C, Caldari-Torres C, Collante W, Staples CR, Badinga L: Plasma prostaglandin and cytokine concentration in periparturient Holstein cows fed diets enriched in saturated or *trans* fatty acids. *J Dairy Sci* 90:5446-5452, 2007.

67. Randel RD, Chase CC Jr, Wyse SJ: Effects of gossypol and cottonseed products on reproduction of mammals. *J Anim Sci* 70:1628-1638, 1992.
68. Relling AE, Reynolds CK: Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. *J Dairy Sci* 90:1506-1515, 2007.
69. Richards BF, Janovick NA, Moyes KM, Nikkhab A, Drackley JK: Preventing fatty liver through use of high straw low-energy diets during dry period. *Proc University of Illinois Dairy Days Report,* 2009, pp 30-35.
70. Roche JF: The effect of nutritional management of the dairy cow on reproductive efficiency. *Anim Reprod Sci* 96:282-296, 2006.
71. Santos JEP, Villasenor M, Depeters EJ, Robinson PH, Baldwin BC: Type of cottonseed and gossypol in diets of lactating dairy cows: Lactation, performance, and plasma gossypol. *J Dairy Sci* 85:1491-1501, 2002.
72. Santos JEP, Villasenor M, DePeters EJ, Robinson PH, Holmberg CH: Type of cottonseed and level of gossypol in diets of lactating dairy cows: plasma gossypol, health, and reproductive performance. *J Dairy Sci* 86:892-905, 2003.
73. Santos JEP: Embryo losses: Prevalence, timing, and associated causes. *Proc Dairy Cattle Repro Conf*, Nov 6-8, 2006, Denver, CO, pp 95-104.
74. Santos JEP, Cerri RLA, Sartori R: Nutritional management of the donor cow. *Therio* 69:88-97, 2008.
75. Shaver RD: Nutritional risk factors in the etiology of left displaced abomasums in dairy cows: a review. *J Dairy Sci* 80:2449-2453, 1997.
76. van Dorp RI, Martin SW, Shoukri MM, Noordhuizen JP, Dekkers JC: An epidemiologic study of disease in 32 registered Holstein dairy herds in British Columbia. *Can J Vet Res* 63:185-192, 1999.
77. Van Straten M, Friger M, Shpigel NY: Events of elevated somatic cell counts in high producing dairy cows are associated with daily body weight loss in early lactation. *J Dairy Sci* 92:4386-4394, 2009.
78. Weiss WP: Macromineral digestion by lactating dairy cows: factors affecting digestibility of magnesium. *J Dairy Sci* 87:2167-2171, 2004.
79. Weiss WP, Wyatt DJ: Digestible energy values of diets with different fat supplements when fed to lactating dairy cows. *J Dairy Sci* 87:1446-1454, 2004.
80. Weiss WP, Pinos-Rodríguez JM: Production responses of dairy cows when fed supplemental fat in low- and high-forage diets. *J Dairy Sci* 92:6144-6155, 2009.
81. Whiteford LC, Sheldon IM: Association between clinical hypocalcemia and postpartum endometritis. *Vet Rec* 157:202-203, 2005.
82. Wilde D: Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. *Anim Reprod Sci* 96:240-249, 2006.
83. Zirkle SM, Lin YC, Gwazdauskas FC, Canseco RS: Effect of gossypol on bovine embryo development during preimplantation period. *Therio* 30:575-582, 1988.