Revista Brasileira de Zootecnia
© 2010 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.39, p.478-490, 2010 (supl. especial)

Scientific progress in ruminant production in the 1st decade of the XXI century

Geraldo Tadeu dos Santos[1], Daniele Cristina da Silva-Kazama[2], Ricardo Kazama[3], Helene V. Petit[4]

[1] Departamento de Zootecnia, Universidade Estadual de Maringá (UEM), Maringá-PR, Bolsista Produtividade do CNPq.
[2] Pós-Doutoranda do Programa de Pós-Graduação em Zootecnia, UEM, Maringá, PR – Bolsista do CNPq – Edital MCT/CNPq/CT-Agronegócio/ MAPA-SDC No. 40/2008 – Proc. No. 505.855/2008-9.
[3] Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina (UFSC), Florianópolis-SC.
[4] Agriculture & Agri-Food Canada, CRDVLP, Sherbrooke, QC, Canada.

ABSTRACT - Scientific advances in nutrition of dairy cows in the first decade of the XXI century have occurred. This paper will review the most common additives fed to transition cows to decrease the incidence of metabolic disorders, which will be discussed separately with emphasis on their mechanisms of action, utilization and efficiency. Some changes on protein in the 2001 updated version of the Nutrient Requirements of Dairy Cattle to reach better precision of the nitrogen and amino acid requirements of lactating cows also are presented. Many of the advances in nutritional manipulation of milk fat concentration are related to fat supplementation, then the relationship between the action of rumen microbes on biohydrogenation of dietary polyunsaturated fatty acids and milk fatty acid profile are discussed as well as the main factors identified as being responsible for milk fat depression.

Key Words: additives, biohydrogenation, CLA, lipids, metabolic disorders, protein

Avanços científicos na produção de ruminantes na 1ª década do século XXI

RESUMO - Avanços científicos em nutrição de vacas leiteiras têm ocorrido na primeira década do século XXI. Desta forma, este artigo abordará os principais aditivos utilizados na alimentação de vacas leiteiras no período de transição, empregados com o objetivo de diminuir a incidência de distúrbios metabólicos, na qual serão discutidos separadamente com ênfase em seus mecanismos de ação, utilização e eficácia. A partir da versão atualizada do NRC de 2001 serão abordadas algumas modificações na utilização de proteína com intuito de alcançar de forma precisa as exigências em nitrogênio e aminoácidos para vacas leiteiras. Além disso, observa-se que os principais avanços em manipular a gordura do leite estão relacionados com a suplementação lipídica na dieta, então, discutir-se-ão estudos sobre manipulação do perfil de ácidos graxos do leite na qual dependem da ação dos microrganismos ruminais sobre os ácidos graxos poli-insaturados da dieta, e também, os principais fatores relacionados à diminuição da gordura do leite.

Palavras-chave: aditivos, biohidrogenação, CLA, distúrbios metabólicos, lipídios, proteína

Introduction

This paper will review the major scientific advances in nutrition of dairy cows in the first decade of the XXI century. Although many factors influence the performance of cows, the focus of this paper is to present the main research areas studied over the last ten years. Moreover, new areas of research in cow nutrition that have attracted attention over the last few years also are presented. The most common additives fed to transition cows to decrease the incidence of metabolic disorders are presented and discussed separately with emphasis on their mechanisms of action, utilization and efficiency. Changes were brought by the 2001 updated version of the Nutrient Requirements of Dairy Cattle (NRC, 2001). Better precision of the nitrogen (N) and amino acid (AA) requirements of lactating cows has provided improved guidelines for establishing concentration of crude protein (CP) in the diet of lactating cows. A mechanistic model has been developed from *in situ* data for calculating the rumen undegradable protein (RUP) content of feedstuffs, which is based on individual characteristics of each feed ingredient. As result, RUP concentration is now better defined for individual feed ingredients. Moreover, more emphasis is placed on the relationship between CP concentration of the diet and N excretion to avoid environmental pollution. Manipulation of milk fatty acid (FA) profile also is discussed with emphasis on the sources of fat required to meet the consumer's demand for

Corresponding author: gtsantos@uem.br

products resulting in better human health. Under the action of rumen microbes, biohydrogenation of polyunsaturated FA originating from the diet is very high. Therefore, the most common treatments that have been studied to prevent FA biohydrogenation are discussed. Many of the advances in nutritional manipulation of milk fat concentration are related to fat supplementation. Therefore, the main factors identified as being responsible for milk fat depression are presented.

Additives for dairy cows

In the last decade, several studies on the effects of additives on metabolism of the transition dairy cow and milk yield in early lactation have been published (Grünberg et al., 2009; Moreira et al., 2009; Ramos-Nieves et al., 2009). Ionophores and anionic salts are the most common additives used on dairy farms and the most evaluated in scientific trials. This paper will review the main additives that have been fed in the transition period (from three weeks prepartum until three weeks postpartum) in early lactation, and briefly, in mid and late lactation of dairy cows.

Antibiotics (Ionophores)

The main ionophores used in the nutrition of bovine are monensin (Rumensin®, Rumensin Controlled Release Capsule® (CRC), and Monensin®), lasalocid, salinomycin, and laidlomycin propionate. However, there are more than 120 ionophores available, but not all have license to be used in animal nutrition. Monensin is a carboxylic polyester antibiotic ionophore produced by natural fermentation of *Streptomyces cinnamonensis* and its utilization in dairy cow nutrition is approved in many countries such as Argentina, Australia, Brazil, Canada, New Zealand, South Africa, and USA (Odongo et al., 2007a). Administration of monensin is done through the diet, mixed in the minerals or the concentrate, or through a CRC (Mutsvangwa et al., 2002; Petersson-Wolfe et al., 2007).

Mechanism of action

Ionophores are liposolubles and their basic function is to create a transport flow of ions across cells. They depress or inhibit rumen microorganism growth, specially Gram (+) bacteria, and stimulate the development of bacteria that produce propionic acid, thus increasing glucose concentration in blood (Juchem et al., 2004; Zahra et al., 2006). Moreover, ionophores such as Rumensin CRC (300 mg/cow/day) may alter volatile FA (VFA) production in the rumen (Conti et al., 2008). The effects of ionophores on the metabolism of beef and dairy cattle are similar (McGuffey et al., 2001).

Apparent benefits of ionophores during the transition period of dairy cows are linked to lower loss of body condition as shown by lower concentration of non-esterified FA (NEFA) in blood and, consequently, decreased concentration of b-hydroxytutyrate (BHBA) in blood (McGuffey et al., 2001). Monensin also improves body condition (Melendez et al., 2004), energy status and decreases the incidence of some metabolic disorders such as ketosis, abomasum displacement, and placenta retention (Duffield et al., 2002; Duffield et al., 2003) when fed at doses ranging from 9 to 23 mg/kg of DM/day. Moreover, monensin supplementation decreases the incidence of milk fever as shown in Table 1 from data obtained from more than 2.300 cows in Québec, Ontario, and Prince Edward Island (Canada).

In early lactation dairy cows, monensin contributes to decrease ruminal biohydrogenation of long-chain FA (LCFA) (Jenkins et al., 2003; Benchaar et al., 2006; Odongo et al., 2007b). Consequently, production of short-chain FA (SCFA) in the mammary gland decreases, resulting in lower milk fat percentage (Gallardo et al., 2005; Da Silva et al., 2007) and higher concentration of conjugated linoleic acid (CLA) in milk fat (Duffield et al., 2008b). Treating cows with 2 monensin CRC, one given 30 d before the expected calving date and the other 60 d after calving for a daily dose of 335 mg/d, also contributes to decrease ruminal proteolysis and deamination of dietary protein (Ruiz et al., 2001; Melendez et al., 2004), and increase post-ruminal digestion of fiber (Osborne et al., 2004), milk production (27.7 vs. 26.6 kg/d) and milk protein yield (0.890 vs. 0.860 kg/d) with no effect on milk fat yield (0.959 kg/d) (Gallardo et al., 2005).

Table 1 - Effect of monensin (Rumensin CRC® containing 32 g of monensin) supplementation between week 2 and 4 precalving on some metabolic disorders in periparturient cows (number of occurrence and percentage)

Item	Without monensin	With monensin CRC	Results
Number of cows	1159	1168	
Placenta retention	120 (10.3%)	92 (7.9%)	⇓23% (P = 0.05)
Ketosis	29 (2.5%)	17 (1.5%)	⇓41% (P = 0.08)
Mastitis	136 (11.6)	135 (11.1)	⇓4.5 (P = *0,99*)
Milk fever	97 (8.3%)	90 (7.8%)	⇓7% (P = 0.63)
Abomasum displacement	69 (5.9%)	42 (3.6%)	⇓39% (P = 0.01)

Adapted from Duffield et al. (2002).

In a meta-analysis of the impact of monensin on cow performance, Duffield et al. (2008a) reported that monensin supplemented from day 21 to 60 before calving as topdress or CRC with doses ranging from 254 and 320 mg/d decreases the incidence of ketosis, abomasum displacement and mastitis although there was no effect on the incidence of milk fever, laminitis, dystocia, placenta retention and metritis. Monensin has no effect on dry matter intake (DMI) of dairy cows during the transition period and in early lactation (Ruiz et al., 2001; Eifert et al., 2005; Fairfield, et al., 2007). However, Duffield et al. (2008b) observed that monensin depressed DMI (0.3 kg/d) and increased milk production (0.7 kg/d), thus improving milk production efficiency (2.5%). Martineau (2004) also observed a slight decline in DMI (2.5%) and an improvement in milk production (2.8%) of dairy cows in mid lactation fed ionophores but milk percentages of fat and protein decreased by 0.13% and 0.03%, respectively (Table 2). Nevertheless, the effects of ionophore supplementation on milk yield are inconsistent. Some studies have shown positive effects of ionophores on milk yield and milk protein yield (McGuffey et al., 2001) but others reported no significant effects (Juchem et al., 2004; Zahra et al., 2006; Fairfield et al., 2007; Chung et al., 2008; Conti et al., 2008).

Monensin supplementation decreased concentrations of SCFA and stearic acid in milk fat and increased that of CLA by 22% (Duffield et al., 2008b). Odongo et al. (2007b) administered monensin (24 mg/kg of DM) for a 6 month period in the diet of dairy cows and they observed an increase of 5 % of total monounsaturated FA, 19 % of omega-6 LCFA, 16 % of omega-3 LCFA, 7 % of *cis*-18:1 FA, 43 % of CLA, 19 % of docosahexaenoic acid (DHA, 22:6), and 13 % of docosapentaenoic acid (DPA, 22:5) in milk fat when compared to cows fed a control diet (without monensin).

Table 2 - Effects of dietary ionophore (monensin + lasalocid) supplementation on intake, milk yield and milk composition of dairy cows

Item	Without monensin	With monensin	Results
Dry matter intake (kg/day)	20.3	19.8	⇓ 2.5 %
Milk yield (kg/day)	27.8	28.6	⇑ 2.8 %
Milk fat (%)	3.94	3.76	⇓ 4.8 %
Milk fat yield (g/day)	1095	1075	⇓ 1.9 %
Milk protein (%)	3.20	3.17	⇓ 0.9 %
Milk protein yield (g/day)	890	907	⇑ 1.9 %

Adapted from Martineau (2004).

A function also attributed to ionophores is the reduction of methane production as shown for cows fed monensin at a dose of 24 mg/kg of DM (Martineau et al., 2007; Odongo et al., 2007a). Methane may contribute to ozone layer destruction, and a reduction in methane production may be good for the environment. However, studies with dairy cows on pasture receiving a monensin CRC did not show any effect on methane production when fed at doses of 240 mg/day/cow (Grainger et al., 2008).

Some byproducts from feed processing containing oil or pectin, such as cottonseed hulls, citric pulp, and soybean hulls are incorporated in the diet of cows during the transition period, which could be a good strategy to improve farm profitability and reduce methane emission. Bacteria that degrade pectin are Gram (-) and resistant to monensin (Melendez et al., 2004). Pectin degradation does not produce lactic acid as does starch degradation. Therefore, monensin may affect pH, and the molar proportions of VFA and concentrations of lactate in the rumen, and concentrations of blood metabolites of cows during the transition period (Melendez et al., 2004).

There is an agreement in the literature that supplementation of monensin and other ionophores in the diet of dairy cows during the transition period may decrease the incidence of ketosis and abomasum displacement and milk fat percentage, and increase LCFA concentration in milk fat. However, other effects attributed to monensin are inconsistent.

Utilization and efficiency

Ionophores are recommended to minimize problems during the transition period of dairy cows. Utilization of ionophores has shown cost benefits for farmers although some studies have reported inconsistent results.

Anionic salts

Anionic salts are minerals with a negative charge supplied mainly by chlorine and sulfur used for the prevention of milk fever occurring early after calving of dairy cows. They are characterized by a bitter taste which may affect ingestion of anionic diets by cows. However, it is possible to mask the bitter taste of the anionic diets through the addition of molasses.

Anionic salts act on calcium metabolism (Lean et al., 2006; Charbonneau et al., 2008; Penner et al., 2008; Ramos-Nieves et al., 2009) with the objective to improve calcium homeostasis in periparturient dairy cows. Penner et al. (2008) fed timothy hays (*Phleum pratense* L.) with high or low cation-anion difference to dairy cows from 30 d before calving to evaluate the effects of hays differing in dietary cation-anion difference (DCAD) on the capability of cows to maintain calcium homeostasis around parturition. They observed that timothy hay with low compared to high DCAD decreased urinary pH and blood bicarbonate concentration at 2 mM. They concluded that timothy hay with low cation-anion difference was effective to improve calcium homeostasis with no interference on DMI. Therefore, utilization of grass hay with low DCAD is another alternative to diminish the severity of hypocalcemia in dairy cows (Charbonneau et al., 2008; Penner et al., 2008).

Urinary pH monitoring is another way to observe the efficiency of anionic salts administration. Adequate amounts of anionic salts should result in urinary pH values between 6.0 and 7.0 (6 h postfeeding) for most breed of cows and values between 5.5 and 6.0 for Jersey cows as they are more vulnerable to hypocalcemia (Herdt, 2000). A great problem with anionic salts utilization is their low palatability, mainly for ammonium chloride, which may decrease DMI and then augment the risk of fatty liver and ketosis in the postpartum period (Ortolani, 2002).

Anion function

To better understand the utilization of anionic salts in the diet of the transition dairy cow, one must understand some metabolic conditions that regulate calcemia in the bovine. Three elements act on the maintenance of calcemia. A first hormone is calcitonin that suppresses bone resorption through diminution of osteoclastic activity, and a second one is parathyroid hormone (PTH) that has the opposite effect (hypercalcemia). A third element is 1,25-dihydrocholecalciferol, an active metabolite of vitamin D with similar functions to PTH (Blood and Radostits, 1991). Diets with anionic salts or acidifier agents promote metabolic acidosis that may improve action of PTH and 1,25-dihydrocolecalciferol (Penner et al., 2008). The DCAD may be inadequate if cows have acidosis or metabolic alkalosis, and the balance may be calculated using the following equation proposed by Block (1984): DCAD (mEq/kg) = $[(Na^{+}) + (K^{+}) - (Cl^{-}) + (S^{--})]$.

Forages rich in cations (potassium) such as grass silage and hay will favor metabolic alkalosis, thus contributing to higher milk fever incidence (Charbonneau et al., 2008). Some researchers have fed forages with low DCAD to reduce urinary pH and blood concentration of bicarbonate (Charbonneau et al., 2008), which facilitates administration of anion sources that are normally refused by cows. A negative DCAD is desirable for cows during the dry period to increase bone calcium mobilization and blood calcium concentration, thus preventing clinical and sub-clinical signs of milk fever. Chlorine based products are more efficient than sulfur based products to meet desirable negative charges in the diet (-100 to -150 meq/kg DM) (Cavalieri & Santos, 2001) but as chlorine based products are expensive, nutritionists have been using a mixture of different sources.

Utilization and efficiency

Recommended dosage may vary according to the product being used. First, it is necessary to analyze mineral composition (potassium, sodium, chlorine, and sulfur) of forages and concentrates fed to dairy cows during the transition period and then to calculate the DCAD of diets for transition cows. Urinary pH monitoring of transition cows allows verification of the dietary DCAD, with normal values ranging from 6.0 to 6.5.

Milk fever is costly to farmers because of lower milk yield and higher incidence of health problems. Low levels of calcium in blood in the postpartum period will affect placenta expulsion due to low uterine contractility. In the same way, lower contraction of sphincter smooth muscle may contribute to higher incidence of mastitis. Therefore, it is highly recommended to prevent milk fever during the transition period, especially by administration of anionic diets to cows producing more than 28 kg of milk at lactation peak and to cows from the Jersey breed. At the same time, it is important to care about administration of calcium in the diet. It is recommended to provide around 150 g of calcium per cow daily (Cavalieri & Santos, 2001).

Protein

The XXI century began with publication of an updated edition NRC (2001) that intended to approach the cow's requirements for desired yields of milk and milk protein with minimum amounts of CP in the diet. Thus, it can be assumed that the goals of ruminant protein nutrition are to optimize utilization efficiency of dietary CP which depends on protein and non-protein nitrogen (NPN) concentrations of the diet. These two fractions of N will provide the types and amounts of rumen degradable protein (RDP) for synthesis of microbial crude protein (MCP) in the rumen, and of RUP that will provide AA for absorption in the small intestine by the host.

Some differences between the updated edition (NRC, 2001) and the previous one (NRC, 1989) are found in terminology. To avoid the implication that all proteins are absorbed, the term metabolizable protein (MP) replaces absorbed protein, and also, the terms degradable intake of CP (DIP) and undegradable intake of CP (UIP) are replaced with RDP and RUP, respectively. A constant digestibility of 80% was used for RUP of all feeds by NRC (1989) while RUP digestibility is considered to be dependent on feed type in NRC (2001).

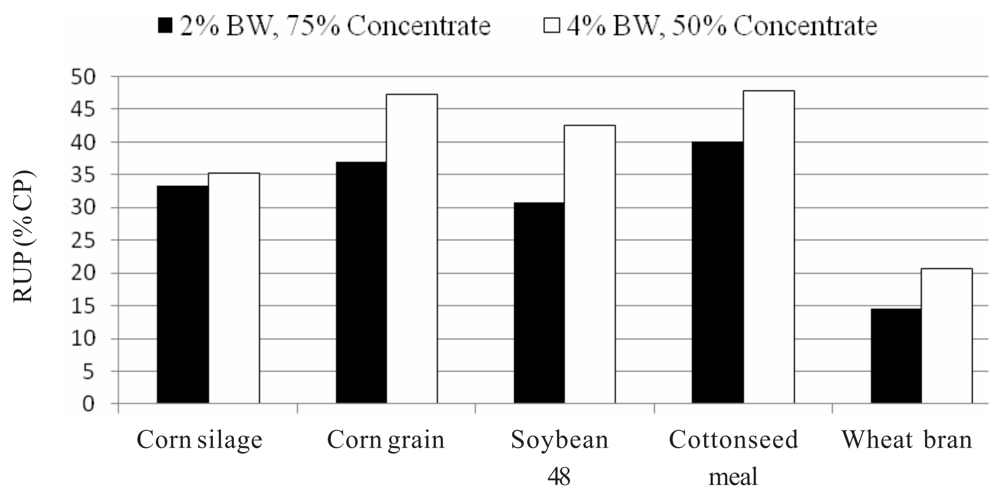
There is an effort to calculate more accurate values of RUP in the current edition instead of using constant values of RUP in the former one. A mechanistic model developed from *in situ* data is used for calculating the RUP content of feedstuffs, which is based on how rapidly the protein degrades in the rumen, related on rate of digestion (Kd) of feedstuff and its properties, and how rapidly the feed passes through the rumen, related on rate of passage (Kp) according to DMI and percentages of concentrate and neutral detergent fiber (NDF) in the DM (Figure 1).

The effect of RDP from NPN sources such as urea and from true protein sources on N efficiency and excretion by dairy cows has been evaluated. An adequate diet formulation to not exceed RDP content will contribute to optimize N efficiency utilization by dairy cows and to reduce N excretion and environmental pollution. For example, high producing Holstein cows fed a diet with 16.5% CP and 1.9% (DM basis) urea had higher concentration of urinary N, milk urea nitrogen (MUN), blood urea N (BUN) and lower N efficiency than those fed true protein sources, which would increase the potential for N pollution (Brito & Broderick, 2007). Moreover, Broderick & Reynal (2009) offered increasing levels of urea (0, 0.41, 0.84, and 1.31% of DM) to dairy cows fed a diet containing 16.1% CP (DM basis) and they observed a linear increase in excretion of urinary and fecal N, concentrations of MUN, BUN, and ruminal ammoniaN.

In the first decade of the XXI century, *Journal of Dairy Science* has published more than 176 articles on protein for dairy cow nutrition. Authors have evaluated the efficacy of N and AA utilization by dairy cows in a large number of published articles as demonstrated in Figure 2. Moreover, other studies have been looking at synchronization of RDP with that of soluble carbohydrates to improve synthesis of MCP, milk urea nitrogen (MUN) concentration, and the need to reduce N excretion by cows to decrease environmental pollution and feeding costs.

According to Broderick (2003), concentration of dietary CP above 16.7% (DM basis) has no benefit in terms of milk yield or milk components although 18% CP (DM basis) is commonly reported in the diet of high producing dairy cows. Law et al. (2009) assumed that in early lactation, dietary protein concentration above 17.3% has beneficial effects on milk yield and that better N efficiency can be achieved by feeding diets with lesser dietary CP levels (14.4–17.3%) without realizing detrimental effects on milk production of late lactation cows. Briefly, some studies indicate that improved N efficiency is reached when dairy cows producing 35-40 kg/day of milk are fed diets with 16.6 to 17% CP (% DM) (Frank & Swensson, 2002; Olmos Colmenero & Broderick, 2006).

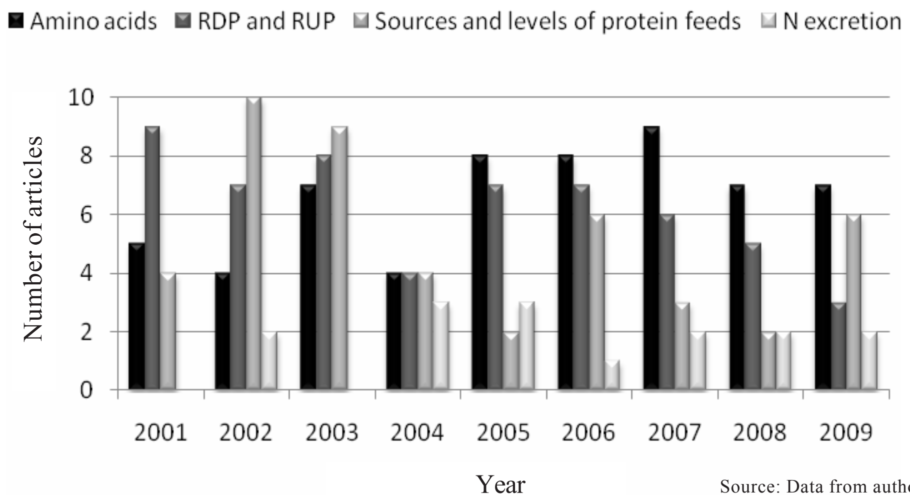
Attempts to diminish CP concentration in the diet of dairy cows is desirable for the purpose of enhancing N utilization as showed by Kalscheur et al. (2006) in Figure 3. These authors related that N efficiency declined as RDP in



Source: Data from NRC (2001)

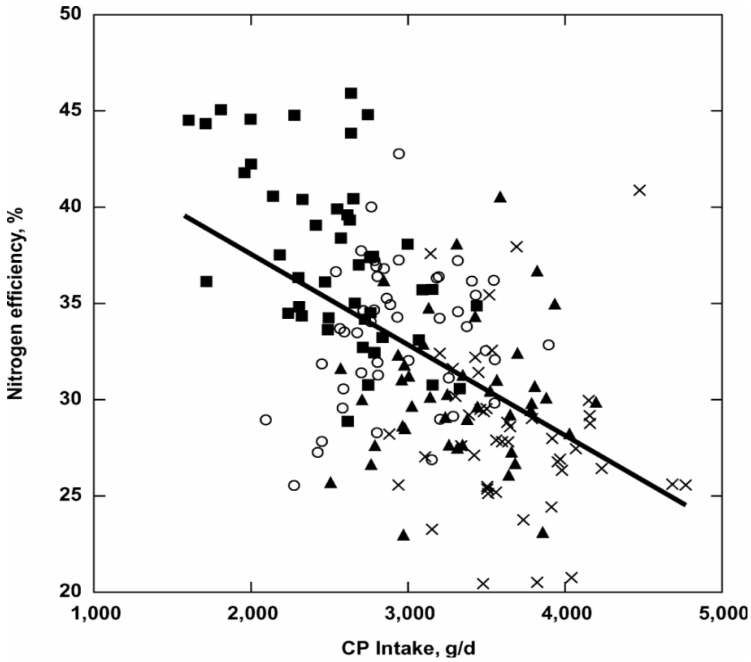
Figure 1 - Rumen undegradable protein (RUP) content of feeds according to dry matter intake (% BW) and percentage of concentrate in the diet.

RUP (%CP) = B*[Kp/(Kd+Kp)] + C; where B: fraction B (%CP); C: fraction C (%CP); Kd: rate of degradation of the B fraction (%/h); Kp: rate of passage from the rumen (%/h). Kp of concentrates = $2.904 + 1.375X_1 - 0.020X_2$; where X_1: DMI (% BW); X_2: concentrate (% of diet DM).



Source: Data from authors (2010)

Figure 2 - Relative distribution of crude protein related articles published in the Journal of Dairy Science from 2001 through 2009, subdivided in amino acids, rumen degraded and undegraded protein (RDP and RUP), sources and levels of protein feeds, and nitrogen (N) excretion themes.

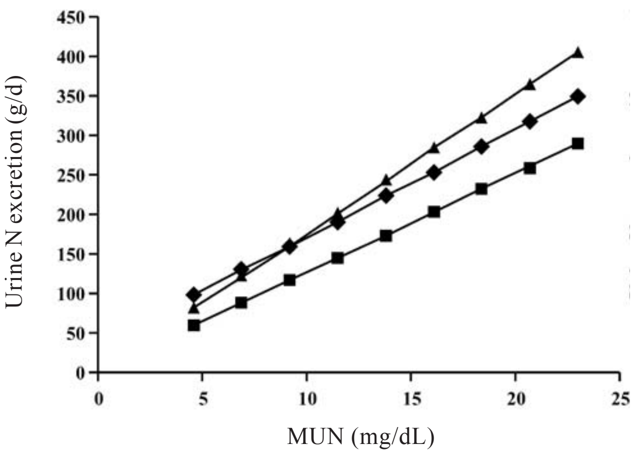


Source: Kalscheur et al. (2006)

Figure 3 - Effect of rumen degradable protein (RDP) intake (g/d) on efficiency of conversion of feed N to milk N (%) for cows fed diet RDP concentrations of 6.8 (■), 8.2 (o), 9.6 (▲), and 11.0 (×) % of DM.

the diet of dairy cows increased (6.8, 8.2, 9.6, and 11.0 % of RDP in DM basis) and that N efficiency decreased as CP intake increased within treatments with the lowest dietary RDP concentration. Moreover, MUN concentration increased linearly from cows fed the lowest concentration of RDP to cows fed the highest one.

Assessment of MUN concentration analyzed in bulk tank or individual milk samples has been associated to urinary N excretion as shown in Figure 4 by Nousiainen et al. (2004). According to Kohn et al. (2002), urinary N excretion may be estimated by the following mathematical equation: 0.026 * BW (kg) * MUN (mg/dL). Moreover, MUN



Source: Nousiainen et al. (2004)

Figure 4 - Comparison of the predictions of urinary N excretion based on milk urea nitrogen (MUN) according to (◆) Nousiainen et al. (2004), (■) Jonker et al. (1998), (▲) Kauffman & St-Pierre (2001).

concentration is considered a great tool to identify overfeeding or underfeeding of CP in diet of dairy cows. Then, monitoring MUN could help to reduce excessive N in manure of dairy cows, monitor protein status of dairy cow diets and improve farm profitability.

Considerable effort has been extended to evaluate the synergism of rumen protein degradability with different sources of carbohydrates (CHO) for optimization of MCP synthesis. High-energy diets stimulate MCP synthesis, which increases the supply of MP for the animal (Cadorniga & Satter, 1993) but may increase also RDP requirements. However, attention must be considered to not over feed protein, which may result in excessive urinary N excretion and increase environmental pollution (urine and feces).

Studies on AA have increased since 2001 to bring more information about its usage in dairy diets for the purpose to augment N efficiency, milk and milk protein yield, and also to diminish N excretion. Among 20 AA, 10 AA are considered essentials i.e., that they are not synthesized by the animal. The only exceptions are arginine and histidine (HIS) that are synthesized by the animal but not in sufficient levels for high production. The two AA that have been mainly considered in research trials are lysine (LYS) and methionine (MET). According to NRC (2001), MET is the first limiting AA for milk production and its requirement increases when cows are fed small amounts of corn, high forage diets or RUP provided by soybean products, animal-derived proteins or associations of both. The second limiting AA is LYS and its requirement increases when corn and feeds of corn origin provide most RUP in diets of dairy cows.

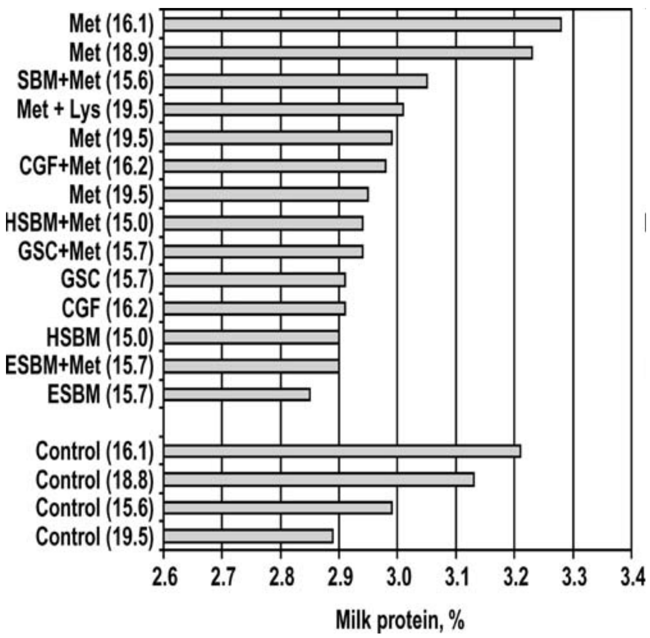
Considerations about these two AA are related by NRC (2001): (1) content of protein in milk is more responsive than milk yield to supplemental LYS and MET, particularly in post-peak lactation cows; (2) increases in milk protein percentage are independent of milk yield; (3) casein is the most influenced milk protein fraction; (4) increases in milk protein production to increased supplies of either LYS or MET in MP are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements; (5) milk yield responses to LYS and MET are more common in cows during early lactation than in mid or late lactation cows; (6) production responses to increased supplies of LYS and MET in MP typically are greater when CP in diet DM approximates normal levels (14 to 18%) than when it is lower or higher.

Research trials have related that increasing amounts of CP in the diet significantly increased MUN and urinary and fecal N excretion, and lower N efficiency (Leonardi et al., 2003; Broderick et al., 2008; Broderick et al., 2009). Leonardi et al. (2003) reported an increase in milk protein concentration (3.17 to 3.26%) when cows received rumen protected MET (RPM, 0.07 g/100g of DM) in a soybean-based diet independent of CP level (16.1% or 18.8%, DM basis). Broderick et al. (2009) observed a trend for higher milk true protein concentration when cows received a supplement of RPM (9 g/cow/day) in diets with 15.8% and 17.1% of CP with or without supplemental RUP from expeller soybean meal.

Changes in milk protein yield in several studies have been extensively related to supplemental AA (mainly MET) or considerable changes in amount or source of protein in the diet of dairy cows although as shown in a review from Jenkins & McGuire (2006), only modest changes in protein content of milk were reported (Figure 5). A small range of milk protein concentration from 2.85 to 3.27% occurred when protein content in the diet varied from 15.0 to 19.5% with many sources of dietary protein and rumen-protected AA.

According to Broderick et al. (2009), dairy cows fed a diet with 15.8% CP (% DM) with supplemental RPM showed similar milk yield and milk components to those fed 17.1% CP (%DM) without RPM. Moreover, a diet with RPM decreased CP content of the diet and consequently, decreased MUN and urinary N excretion by dairy cows, thus reducing potential environment pollution.

Additional research also is needed to obtain more accurate predictive models to determine AA requirements for high-producing dairy cows and to further investigate other essential AA that may limit or co-limit milk yield and N efficiency. According to Lapierre et al. (2006), determining



Source: Jenkins & McGuire (2006)

Figure 5 - Samples of data from published studies showing the extent to which milk protein percentage varies with amount and type of dietary protein. Diet protein percentage is shown in parenthesis following source of protein, which includes the amino acids methionine and lysine, soybean meal (SBM), corn gluten feed (CGF), heated soybean meal (HSBM), ground shelled corn (GSC), and extended soybean meal (ESBM)

and recognizing the influence of gut metabolism on each measurement (duodenal flow, apparent small intestinal disappearance, portal absorption) of available AA to peripheral tissues should provide additional information to create more accurate mathematical models for dairy cows AA requirements.

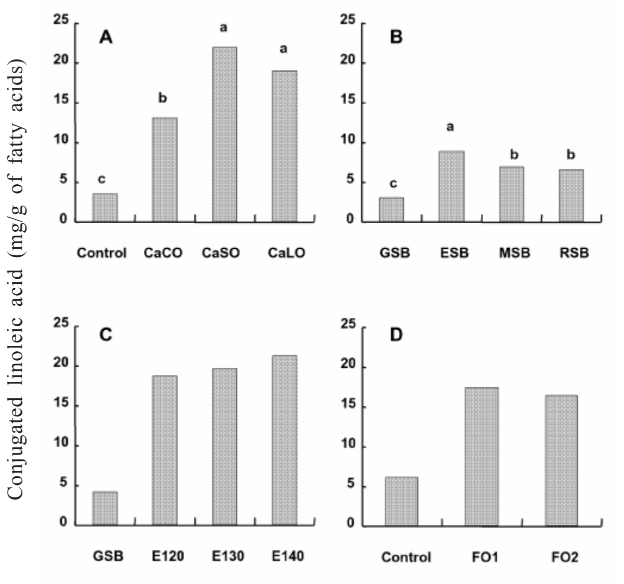
Lipids

The use of fat in ruminant nutrition, mainly for lactating animals, has the objective to increase energy density of the diet. Beyond this goal, fat has been used to change composition of the product, e.g., milk, in order to increase the incorporation of beneficial compounds for better human health. In the last decade, many new concepts about fat utilization in ruminant feeding have arisen. The release of an updated version of NRC (2001) brought important information about biohydrogenation of FA by rumen microbes, which can reach up to 90% for polyunsaturated FA that are not protected or 40% for those that are fed as calcium salts.

From 2001 to 2009, a total of 220 papers have been published in the main Brazilian national and international journals on the topic of "fat" for ruminant production, and emphasis was mostly on dairy cattle. The *Journal of Dairy Science* is the major source of these papers with a number of publications exceeding the number of the sum of all papers published in *Animal Feed Science and Technology, Journal of Animal Science, Brazilian Journal of Animal Science* (Revista Brasileira de Zootecnia). The emphasis on feeding fat to animals was to change composition of the product (e.g., milk and meat) for better human health. Because biohydrogenation of polyunsaturated FA by rumen microbes is high, treatment of fat sources to protect them was the target of many papers. Extensive work on treatments such as extrusion, micronization, grinding, and protection as rumen-inert calcium salts, and supplementation with products that alter ruminal fermentation (as ionophores) occurred. Moreover, sources of oilseeds, fish oil, tallow, and synthetic fat also were compared with the expectation to improve composition of the final product. Other research also has been carried out on modification of milk composition to increase the proportion of CLA in milk fat with comparison between cows on pasture and those fed a total mixed ration (TMR).

Many studies to increase the proportion of CLA in milk fat occurred during the last 10 years. In the early 2000s, Chouinard et al. (2001) tested many fat treatments (Figure 6) and concluded that calcium salts of soybean oil and linseed oil were more efficient to increase c9t11 CLA in milk fat than a control diet or a diet with calcium salts of canola oil. Moreover, extruded soybeans were better than micronized, roasted or ground soybeans to increase c9t11 CLA in milk fat. Extruded soybeans result in higher concentration of c9t11 CLA than a control diet (Chouinard et al., 2001; Neves et al., 2007) and temperature of extrusion (120ºC until 140ºC) has no effect on c9t11 CLA concentration of milk fat. Moreover, fish oil supplementation from 0 to 200 ml/d increased concentration of CLA in milk fat linearly and there was a quadratic effect when providing 400 mL/d of fish oil.

Pasture also has an important role on milk fat composition. According to White et al. (2001), the isomer c9t11 CLA is 83% higher when animals are fed on pasture compared to when they are fed a TMR. Moreover, many papers reported that pasture and fresh grass are more efficient to increase CLA and polyunsaturated FA proportions in milk fat than TMR or silage (Elgersma et al., 2004; Dewhurst et al., 2006). Access to a TMR and a mixed grass/clover pasture for 8 h daily also increases concentrations of 18:3*n*-3, t11c15-18:2, c9t11 CLA, and t11-18:1 in milk fat (Loor et al., 2003). On the other hand,



Source: Chouinard et al. (2001)

Figure 6 - Effect of dietary fat supplements on the conjugated linoleic acid content of milk fat. Panel A compares Ca salts of fatty acids from canola oil (CaCO), soybean oil (CaSO), and linseed oil (CaLO). Panel B compares processing methods of full fat soybeans from control (ground; GSB), extruded (ESB), micronized (MSB), and roasted (RSB) soybeans. Panel C compares ground soybeans (GSB) with full fat soybeans extruded at 120ºC (E120), 130ºC (E130) and 140ºC (E140). Panel D compares two levels of fish oil, 200 ml/d (FO1) and 400 mL/d (FO2).

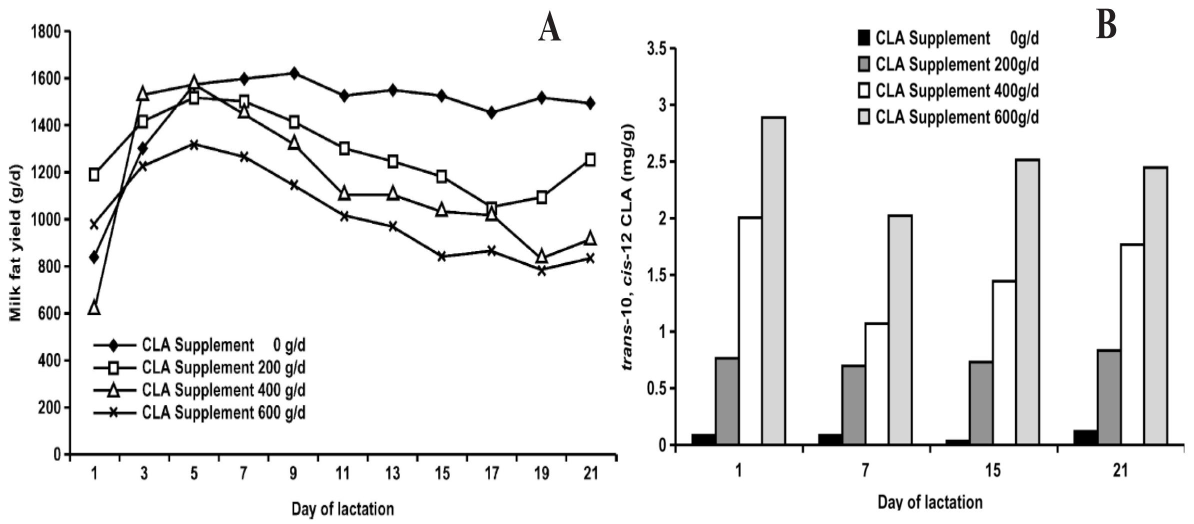
changes in herbage mass or daily herbage allowance has no effect on milk FA composition of grazing dairy cows (Palladino et al., 2009).

The choice of dietary fat to supplement in order to improve milk FA profile depends on the type of FA to increase in milk fat (e.g., increase CLA or polyunsaturated FA concentrations or decrease the omega 6:3 ratio). For example, fish oil is more efficient to increase CLA in milk fat than soybean oil (Qiu et al., 2004). However, c9t11 and t10c12 CLA concentrations are greater when feeding sunflower oil than when feeding linseed oil or fish oil (Loor et al., 2004). On the other hand, linseed oil is better than soybean oil to decrease the omega 6:3 ratio (Petit, 2002). In general, oils rich in linoleic acid are more effective in enhancing contents and yield of c18:1 (trans vaccenic - VA) and CLA in milk fat than oils containing linolenic acid (Bu et al., 2007). Or-Rashid et al. (2008) concluded that algae meal could be used to increase concentration of *trans*-18:1 isomers in rumen contents which serve as precursors for CLA synthesis in the mammary gland of dairy cows.

Many protection methods were studied and proved to be efficient to protect FA against ruminal biohydrogenation. Protection methods like calcium salts (Lundy et al., 2004), extrusion (Neves et al., 2007), and grinding (Da Silva et al., 2007) of fat sources improve milk FA profile for better human health. Furthemore, monensin supplementation was successful in decreasing ruminal biohydrogenation of FA (Bell et al., 2006; Eifert et al., 2006; Da Silva et al., 2007). However, the use of essential oils such as cinnamaldehyde, condensed tannins, and saponins have a low potential to decrease ruminal biohydrogenation and modify milk FA profile when these extracts are used at practical feeding rates in dairy cow diets (Benchaar & Chouinard, 2009).

Some studies were conducted to investigate the effect of supplemental fat on DMI. There was a negative effect of fat supplementation on DMI and the decrease was more important with diets rich in concentrate than with diets rich in forage (Ueda et al., 2003). Moreover, high ether extract concentration in the diet (7.5% of DM) had no effect on DMI (Gonthier et al., 2004). The detrimental effect of fat on DMI seems to be related to the source of fat. For example, unsaturated free FA more potently suppresses DMI than an equivalent amount of unsaturated triglycerides (Litherland et al., 2005). Moreover, linoleic acid, linolenic acid, and oleic acid have all suppressive effects on DMI of dairy cows (Drackley et al., 2007).

One of the most cited papers in *Journal of Dairy Science* is entitled "*trans*-10, *cis*-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows" (Baumgard et al., 2002), which relates the role of CLA in milk fat depression and is one of the major breakthroughs on the theories of milk fat depression in dairy cattle. Baumgard et al (2002) related that t10c12 and not c9t11 is the FA responsible for milk fat depression. Moreover, they observed that the depression was a result of a decrease in mRNA production of key-enzymes involved in fat production, i.e., a decrease rate of acetate incorporation into FA which decreases *de novo* synthesis and triglycerides production in mammary gland. This led to the publication of many papers relating the effect of t10c12 on milk fat depression (Giesy et al., 2002; Mackle et al., 2003; Gervais et al., 2009). Moore et al. (2004) related that the depression in milk fat concentration was linearly correlated with the level of t10c12 supplementation (Figure 7).



Source: Moore et al. (2004).

Figure 7 - Temporal pattern of milk fat yield (A) and temporal appearance (B) of trans-10, cis-12 CLA (mg/g) in milk fat from cows fed increasing amounts of a rumen-protected conjugated linoleic acid (CLA) supplement during early lactation.

Supplementation with amide and encapsulated t10c12 CLA to provide 10 g/cow/day of t10c12 CLA depressed milk fat concentration by 21 and 22%, respectively (Perfield et al., 2004). However, Piperova et al. (2004) reported that both t10c12 and t10-18:1 were involved in milk fat depression. According to Loor et al. (2005), increases in ruminal t11-18:1 may represent an effective strategy to maintain c9t11 CLA synthesis in the mammary gland. Furthermore, they hypothesized that besides t10c12 CLA, other biohydrogenation intermediates may be involved in milk fat depression. In a review paper on milk fat depression, Kadegowda et al. (2008) related that among *trans*-18:1 isomers, t10-18:1 was the most negatively correlated to milk fat percentage and that not only t10c12, but also t7c9 CLA may be potentially involved in milk fat depression. On the other hand, it was discovered recently that the addition of vitamin E to the diet partially prevented the depression in milk fat associated with the addition of oils to the dairy cow diet (Bell et al., 2006; Pottier et al., 2006).

Offer (2002) showed that a depression in milk fat proportion of c9t11 CLA could be achieved if the grass was simply cut and fed after a short period of wilting. This led Lee et al. (2007) to investigate the effects of green odor FA oxidation products (FAOP) from cut grass on lipid metabolism and microbial ecology using *in vitro* incubations of rumen microorganisms. They related that FAOP can alter FA biohydrogenation in the rumen, increasing it with changes in the microbial population that were detected through DNA and branched- and odd-chain FA profiling approaches.

In conclusion, there has been extensive research on feeding fat to dairy cows to improve composition of the final product although the source of fat to feed depends on the expected aim: increase concentrations of CLA, polyunsaturated FA, or omega 3 FA, or decrease the omega 6:3 FA ratio. Many sources of fat and methods have been employed to achieve these purposes.

Future Directions

Manipulation of product composition from animal origin will continue to be highly regarded in the future. Consumers' demand for high quality products resulting in better human health will remain of great interest. However, this will have to be reached by looking at ways that are compatible with preserving the environment and animal welfare. This means that emphasis will be placed on the study and development of innovative models of sustainable industry capable of optimizing production, processing and distribution of milk and dairy products and meeting the needs of lucrative domestic and export market segments. Another issue that is central to concerns is the establishment of directives supporting environmental protection through sustainable development throughout the entire dairy chain from farm to plate. Animal health and welfare are important aspects for sustainable development of the dairy industry. In fact, North American consumers have been increasingly calling for animal welfare regulations. Several American states now have adopted animal welfare laws through public referendum votes. Recent research covering all types of food shows that the two ethical issues best understood by the public are: animal welfare and food origin (traceability). All these aspects will have to be considered when defining new product and technology innovation strategies.

References

BAUMGARD, L.H.E.; MATITASHVILI, B.A.; CORL, D.A. et al. *Trans*-10, *cis*-12 Conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. **Journal of Dairy Science**, v.85, p.2155-2163, 2002.

BELL, J.A.; GRIINARI, J.M.; KENNELLY, J.J. Effect of safflower oil, flaxseed oil, monensin, and vitamin e on concentration of conjugated linoleic acid in bovine milk fat. **Journal of Dairy Science**, v.89, p.733-748, 2006.

BENCHAAR, C.; CHOUINARD, P.Y. *Short communication*: Assessment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. **Journal of Dairy Science**, v.92, p.3392-3396, 2009.

BENCHAAR, C.; PETIT, H.V.; BERTHIAUME, R. et al. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk produtuction, and milk composition in dairy cows. **Journal of Dairy Science**, v.91, p.4352-4364, 2006.

BLOCK, E. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. **Journal of Dairy Science**, v.67, n.12, p.2939- 2948, 1984.

BLOOD, D.C.; RADOSTITS, O.M. **Clínica veterinária**. 7.ed. Rio de Janeiro: Guanabara Koogan, p.927-936, 1991.

BRITO, A.F.; BRODERICK, G.A. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. **Journal of Dairy Science**, v.90, p.1816-1827, 2007.

BRODERICK, G.A. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. **Journal of Dairy Science**, v.86, p.1370-1381, 2003.

BRODERICK, G.A; REYNAL, S.M. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. **Journal of Dairy Science**, v.92, p.2822-2834, 2009.

BRODERICK, G.A; STEVENSON, M.J.; PATTON, R.A. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. **Journal of Dairy Science**, v.92, p.2719-2728, 2009.

BRODERICK, G.A.; STEVENSON, M.J.; PATTON, R.A. et al. Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. **Journal of Dairy Science**, v.91, p.1092-1102, 2008.

BU, D.P.; WANG, J.Q.; DHIMAN, T.R. et al. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. **Journal of Dairy Science**, v.90, p. 998-1007, 2007.

CAVALIERI, F.L.B.; SANTOS, G.T. [2001]. **Balanço catiônico-aniônico em vacas leiteiras no pré-parto**. Disponível em: <http://www.nupel.uem.br/balanco.pdf> Acesso em: 25/3/2010.

CHARBONNEAU, E.; CHOUINARD, P.Y.; TREMBLAY, G.F. et al. Hay to reduce dietary cátion-anion difference for dry dairy cows. **Journal of Dairy Science**, v.91, p.1585-1596, 2008.

CHOUINARD, P.Y.; CORNEAU, L.; BUTLER, W.R. et al. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. **Journal of Dairy Science**, v.84, p.680-690, 2001.

CHUNG Y.H.; PICKETT, M.M.; CASSIDY, T.W. et al. Effects of prepartum dietary carbohydrate source and monensin on periparturient metabolism and lactation in multiparous cows. **Journal of Dairy Science**, v.91, p.2744-2758, 2008.

CONTI, R.M.C.; SALLES, M.S.V.; SCHALCH, E. Efeitos da administração de monensina por meio de cápsulas de liberação controlada no desempenho de vacas Holandesas no início da lactação. **Revista Brasileira de Zootecnia**, v.37, p.890-895, 2008.

DA SILVA, D.C.; SANTOS, G.T.; BRANCO, A.F. et al. Production performance and milk composition of dairy cows fed whole or ground flaxseed with or without monensin. **Journal of Dairy Science**, v.90, p.2928-2936, 2007.

DEWHURST, R.J.; SHINGFIELD, K.J.; LEE, M. R.F. et al. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. **Animal Feed Science and Technology**, v.131, p.168-206, 2006.

DRACKLEY, J.K.; OVERTON, T.R.; ORTIZ-GONZALEZ, G. et al. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasum of lactating dairy cows. **Journal of Dairy Science**, v.90, p.5165-5175, 2007.

DUFFIELD, T.F., BAGG, R., KELTON, D. A field study of dietary interaction with monensin on milk fat percentage in lactating dairy cattle. **Journal of Dairy Science**, v.86, p.4161-4166, 2003.

DUFFIELD, T.F.; BAGG, R.; DESCOTEAUX, L. et al. Prepartum monensin for the reduction of energy associated disease in postpartum dairy cows. **Journal of Dairy Science**, v.85, p.397-405, 2002.

DUFFIELD, T.F.; ROBIEE, A.R.; LEAN, I.J. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 3. Health and reproduction. **Journal of Dairy Science**, v.91, p.2328-2341, 2008a.

DUFFIELD, T.F.; ROBIEE, A.R.; LEAN, I.J. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. **Journal of Dairy Science**, v.91, p.1347-1360, 2008b.

EIFERT, E.C.; LANA, R.P.; LANNA, D.P.D. et al. Efeitos do fornecimento de monensina e óleo de soja na dieta sobre o desempenho de vacas leiteiras na fase inicial da lactação. **Revista Brasileira de Zootecnia**, v.34, p.2123-2132, 2005

EIFERT, E.C.; LANA, R.P.; LANNA, D.P.D. et al. Perfil de ácidos graxos do leite de vacas alimentadas com óleo de soja e monensina no início da lactação. **Revista Brasileira de Zootecnia**, v.35, n.1, p.219-228, 2006.

ELGERSMA, A.; ELLEN, G.; VAN DER HORST, H. et al. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. **Animal Feed Science and Technology**, v.117, p.13-27, 2004.

FAIRFIELD, A.M.; PLAIZIER, J.C.; DUFFIELD, T. et al. Effects of prepartum administration of a monensin controlled release capsule on rúmen pH, feed intake, and milk production of transition dairy cows. **Journal of Dairy Science,** v.90, p.937-945, 2007.

FRANK, B.; SWENSSON, C. Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. **Journal of Dairy Science**, v.85, p.1828-1838, 2002.

GALLARDO, M.R.; CASTILLO, A.R.; BARGO, F. et al. Monensin for lactating dairy cows grazing mixed-alfafa pasture and supplemented with partial mixed ration. **Journal of Dairy Science**, v.88, p.644-652, 2005.

GERVAIS, R.; MCFADDEN, J.W.; LENGI, A.J.B. et al. Effects of intravenous infusion of trans-10, cis-12 18:2 on mammary lipid metabolism in lactating dairy cows. **Journal of Dairy Science**, v.92, p.5167-5177, 2009.

GIESY, J.G.; MCGUIRE, M.A.; SHAFII, B. et al. Effect of dose of calcium salts of conjugated linoleic acid (CLA) on percentage and fatty acid content of milk fat in midlactation holstein cows. **Journal of Dairy Science**, v.85, p.2023-2029, 2002.

GONTHIER, C.; MUSTAFA, A.F.; BERTHIAUME, R. et al. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. **Journal of Dairy Science**, v.87, p.1854-1863, 2004.

GRAINGER, C.; AULDIST, M.J.; CLARKE, T. et al. Use of monensin controlled-release capsules to reduce methane emissions and improve milk production of dairy cows offered pasture supplemented with grain. **Journal of Dairy Science**, v.91, p.1159-1165, 2008.

GRÜNBERG, W.; STAUFENBIEL, R.; CONSTABLE, P.D. et al. Liver phosphorus content in Holstein-Freisian cows during the transition period. **Journal of Dairy Science**, v.92, p.2106-2117, 2009.

HERDT, T.H. Metabolic diseases of ruminant livestock. **The Veterinary Clinics of North America – Food Animal Practice**, v.4, p.213-439, 1988.

JENKINS, T.C.; FELLNER, V.; MCGUFFEY, R.K. Monensin by fat interactions on tyrans Fatty acids in cultures of mixed ruminal microorganisms grown in continous fermentors fed corno or barley. **Journal of Dairy Science**, v.86, p.324-330, 2003.

JENKINS, T.C.; McGUIRE, M.A. Major advances in nutrition: Impact on milk composition. **Journal of Dairy Science**, v.89, p.1302-1310, 2006.

JONKER, J.S.; KOHN, R.A.; ERDMAN, R.A. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. **Journal of Dairy Science**, v.81, p.2681-2692, 1998.

JUCHEM, S.O.; SANTOS, F.A.P.; IMAIZUMI, H. et al. Production and blood parameters of Holstein cows treated prepartum with sodium monensin or propylene glycol. **Journal of Dairy Science**, v.87, p.680-689, 2004.

KADEGOWDA, A.K.G.; PIPEROVA, L.S.; ERDMAN, R.A. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. **Journal of Dairy Science**, v.91, p.749-759, 2008.

KALSCHEUR, K.F.; BALDWIN, R.L; GLENN, B.P. et al. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. **Journal of Dairy Science**, v.89, p.249-259, 2006.

KAUFFMAN, A.J.; St-PIERRE, N. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. **Journal of Dairy Science**, v.84, p.2284-2294, 2001.

KOHN, R.A.; KALSCHEUR, K.F.; RUSSEK-COHEN, E. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. **Journal of Dairy Science**, v.85, p.227-233, 2002.

LAPIERRE, H.; PACHECO, D.; BERTHIAUME, R. et al. What is the true supply of amino acids for a dairy cow. **Journal of Dairy Science**, v.89, p.E1-E14, 2006 (suppl.).

LAW, R.A.; YOUNG, F.J.; PATTERSON, D.C. et al. Effect of dietary protein content on animal production and blood metabolities of dairy cows during lactation. **Journal of Dairy Science**, v.92, p.1001-1012, 2009.

LEAN, I.J.; DEGARIS, P.J.; McNEIL, D.M.eMeta-analysis and dietary cation anion difference theory resisited. **Journal of Dairy Science**, v.89, p.669-684, 2006.

LEE, M.R.F.; HUWS, S.A.; SCOLLAN, N.D. et al. Effects of fatty acid oxidation products (green odor) on rumen bacterial populations and lipid metabolism in vitro. **Journal of Dairy Science**, v.90, p.3874-3882, 2007.

LEONARDI, C.; STEVENSON, M.; ARMENTANO, L.E. Effect of two levels of curde protein and methionine supplementation on performance of dairy cows. **Journal of Dairy Science**, v.86, p.4033-4042, 2003.

LITHERLAND, N.B.; THIRE, S.; BEAULIEU, A.D. et al. Dry matter intake is decreased more by abomasal infusion of unsaturated free fatty acids than by unsaturated triglycerides. **Journal of Dairy Science**, v.88, p.632-643, 2005.

LOOR, F.D.; SORIANO, X.; LIN, J.H. et al. grazing allowance after the morning or afternoon milking for lactating cows fed a total mixed ration (TMR) enhances *trans11*-18:1 and *cis9, trans11*-18:2 (rumenic acid) in milk fat to different extents. **Animal Feed Science and Technology**, v.109, p.105-119, 2003.

LOOR, J.J.; FERLAY, A.; OLLIER, A. et al. High-concentrate diets and polyunsaturated oils alter trans and conjugated isomers in bovine rumen, blood, and milk. **Journal of Dairy Science**, v.88, p.3986-3999, 2005.

LOOR, J.J.; UEDA, K.; FERLAY, A. et al. Short Communication: Diurnal profiles of conjugated linoleic acids and trans fatty acids in ruminal fluid from cows fed a high concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. **Journal of Dairy Science**, v.87, p.2468-2471, 2004.

LUNDY, F.P.; BLOCK, E.; BRIDGES, W.C. et al. Ruminal biohydrogenation in holstein cows fed soybean fatty acids as amides or calcium salts. **Journal of Dairy Science**, v.87, p.1038-1046, 2004.

MACKLE, T.R.; KAY, J.K.; AULDIST, M.J. et al. Effects of abomasal infusion of conjugated linoleic acid on milk fat concentration and yield from pasture-fed dairy cows. **Journal of Dairy Science**, v.86, p.644-652, 2003.

MARTINEAU, R.L. Ionophore monensin: un nouvel additif alimentaire en production laitière. **Le Produducteur de Lait Quebecois**, v.12, p.39-41, 2004.

MARTINEAU, R.; BENCHAAR, C.; PETIT, H.V. et al. Effects of lasalocid or monensin supplementation on digestion, ruminal fermentation, blood metabolites, and milk production of lactationg dairy cows. **Journal of Dairy Science**, v.90, p.5714-5725, 2007.

McGUFFEY, R.K.; RICHARDSON, L.F.; WILKINSON, J.I.D. Ionophores for dairy cattle: current status and future outlook. **Journal of Dairy Science**, v.84, p.E194-E203, 2001.

MELENDEZ, P.; GOFF, J.P.; RISCO, C.A. et al. Effect of a monensin controlled-release capsule on rumen and blood metabolites in Florida Holstein transition cows. **Journal of Dairy Science**, v.87, p.4182-4189, 2004.

MOORE, C.E.; HAFLIGER, H.C.; MENDIVIL, O.B. et al. Increasing amounts of conjugated linoleic acid (CLA) progressively reduces milk fat synthesis immediately postpartum. **Journal of Dairy Science**, v.87, p.1886-1895, 2004.

MOREIRA, V.R.; ZERINUE, L.K.; WILLIAMS, C.C. et al. Influence of calcium and phosphorus feeding on markers of boné metabolism in transition cows. **Journal of Dairy Science**, v.92, p.5189-5198, 2009.

MUTSVANGWA, T.; WALTON, J.P.; PLAIZIER, J.C. et al. Effects of a monensin controlled-release capsule or premix on attenuation of sbacute ruminal acidosis in dairy cows. **Journal of Dairy Science**, v.85, p.3454-3461, 2002.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of dairy cattle**. 6.rev.ed. Washington, D.C.: National Academy Press, 1989.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of dairy cattle**. 7.rev.ed. Washington, D.C.: National Academy Press, 2001.

NEVES, C.A.; SANTOS, G.T.; MATSUSHITA, M. et al. Intake, whole tract digestibility, milk production, and milk composition of Holstein cows fed extruded soybeans treated with or without lignosulfonate. **Animal Feed Science and Technology**, v.134, p.32-44, 2007.

NOUSIAINEN, J.; SHINGFIELD, K.J.; HUHTANEN, P. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. **Journal of Dairy Science**, v.87, p.386-398, 2004.

ODONGO, N.E.; BAGG, R.; VESSIE, G. et al. Long-term effects of feeding monensin on methane production in lactating dairy cows. **Journal of Dairy Science**, v.90, p.1781-1788, 2007a.

ODONGO, N.E.; OR-RASHID, M.M.; BAGG, R. et al. Long-term effects of feeding monensin on milk fatty acid composition in lactating dairy cows. **Journal of Dairy Science**, v.90, p.5126-5133, 2007b.

OFFER, N.W. Effect of cutting and ensiling grass on levels of CLA in bovine milk. In THE INTERNATIONAL SILAGE CONFERENCE, 13., 2002, Auchincruive, Scotland. **Proceedings...** Auchincruive: [s/n], 2002. p.16-17.

OLMOS COLMENERO, J.J.; BRODERICK, G.A. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. **Journal of Dairy Science**, v.89, p.1635-1643, 2006.

OR-RASHID, M.M.; KRAMER, J.K.G.; WOOD, M.A. et al. Supplemental algal meal alters the ruminal *trans*-18:1 fatty acid and conjugated linoleic acid composition in cattle. **Journal of Animal Science**, v.86, p.187-196, 2008.

ORTOLANI, E.L. Diagnóstico de doenças nutricionais e metabólicas por meio de exame de urina em ruminantes. In: GONZÁLEZ, H.F. et al. (Eds.) **Avaliação metabólico-nutricional de vacas leiteiras por meio de fluídos corporais**. Porto Alegre, 2002. p.18-26.

OSBORNE, J.K.; MUTSVANGWA, T.; ALZAHAL, O. et al. Effects of monensin on ruminal forage degradability and total tract diet digestibility in lactating dairy cows during grain-induced subacute ruminal acidosis. **Journal of Dairy Science**, v.87, p.1832-1839, 2004.

PALLADINO, R.A.; O'DONOVAN, M.; MURPHY, J.J. et al. Fatty acid intake and milk fatty acid composition of Holstein dairy cows under different grazing strategies: Herbage mass and daily herbage allowance. **Journal of Dairy Science**, v.92, p.5212-5223, 2009.

PENNER, G.B.; TREMBLAY, G.F.; DOW, T. et al. Timothy hay with a low dietary cátion-anion difference improves calcium homeostasis in periparturient holstein cows. **Journal of Dairy Science**, v.91, p.1959-1968, 2008.

PERFIELD, J.W.; LOCK, A.L.; PFEIFFER, A.M. et al. Effects of amide-protected and lipid-encapsulated conjugated linoleic acid (CLA) supplements on milk fat synthesis. **Journal of Dairy Science**, v.87, p.3010-3016, 2004.

PETERSSON-WOLFE, C.S.; LESLIE, K.E.; OSBORNE, T. et al. Effect of monensin delivery method on dry matter intake, body condition score, and metabolic parameters in transition dairy cows. **Journal of Dairy Science**, v.90, p.1870-1879, 2007.

PETIT, H.V. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. **Journal of Dairy Science**, v.85, p.1482-1490, 2002.

PIPEROVA, L.S.; MOALLEM, U.B.; TETER, B. et al. Changes in milk fat in response to dietary supplementation with calcium salts of *trans*-18:1 or conjugated linoleic fatty acids in lactating dairy cows. **Journal of Dairy Science**, v.87, p.3836-3844, 2004.

POTTIER, J.; FOCANT, M.; DEBIER, C. et al. Effect of dietary vitamin e on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. **Journal of Dairy Science**, v.89, p.685-692, 2006.

QIU, X.; EASTRIDGE, M.L.; FIRKINS, J.L. Effects of dry matter intake, addition of buffer, and source of fat on duodenal flow and concentration of conjugated linoleic acid and *trans*-11 $c_{18:1}$ in milk. **Journal of Dairy Science**, v.87, p.4278-4286, 2004.

RAMOS-NIEVES, J.M.; THERING, B.J.; WALDRON, M.R. et al. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. **Journal of Dairy Science**, v.92, p.5677-5691, 2009.

RUIZ, R.; ALBRECHT, G.L.; TEDESCHI, L.O. Effect of monensin on the performance and nitrogen utilization of lactating dairy cows consuming fresh forage. **Journal of Dairy Science,** v.84, p.1717-1727, 2001.

UEDA, K.; FERLAY, A.; CHABROT, J. et al. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage:concentrate ratios. **Journal of Dairy Science**, v.86, p.3999-4007, 2003.

WHITE, S.L.; BERTRAND, J.A.; WADE, M.R. et al. Comparison of fatty acid content of milk from jersey and holstein cows consuming pasture or a total mixed ration. **Journal of Dairy Science**, v.84, p.2295-2301, 2001.

ZAHRA, L.C.; DUFFIELD, T.F.; LESLIE, K.E. et al. Effects of rúmen-protected choline and monensin on milk production and metabolismo of periparturient dairy cows. **Journal of Dairy Science**, v.89, p.4808-4818, 2006.