THE EFFECT OF TREATMENT OF SOYBEAN MEAL ON THE AVAILABILITY OF AMINO ACIDS AND THE EFFICIENCY OF NITROGEN USAGE FOR MILK PRODUCTION IN DAIRY COWS

By

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ABSTRACT

Doctor of Philosophy **Animal Science**

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THE EFFECT OF TREATMENT OF SOYBEAN MEAL ON THE AVAILABILITY OF AMINO ACIDS, AND THE EFFICIENCY OF NITROGEN USAGE FOR MILK PRODUCTION IN DAIRY COWS

In the first of three experiments, four Holstein multiparous dairy cows, equipped with ruminal and duodenal cannulas, were used to determine the impact of different methods of treating soybean meal (SBM) on ruminal degradability and intestinal digestibility of CP and amino acids (AA). Solvent extracted SBM (SE), expeller SBM (EP), lignosulfonate SBM (LS) and heat and soy hulls SBM (HS) were incubated in the rumen, in nylon bags, for various intervals up to 48h. Additional samples incubated for 16 h were pre-treated with pepsin HCl, and then used for determination of intestinal digestibility either *in situ* (mobile bag technique) or *in vitro.* Treatment of SBM (EP, LS, HS) increased rumen undegradable protein (RUP), and AA availability in 30%. Estimates of intestinal digestibility of AA and CP were lower when measured *in vitro* than *in situ*. Among the treated SBM products, four EAA (Ile, Leu, Phe and Val) showed differences in availability, with values consistently lower for HS than for LS. The second experiment involved a comparative assessment of the availability of lysine in SE and rumen protected SBM products (EP and LS). Lysine availability was assessed either by the increment in plasma flux of ^{15} N lysine, the increment in intestinally digestible lysine (using chromic oxide as a digesta marker) or by the use of a "plasma lysine response curve" designed to predict intestinally available lysine. The plasma Lys flux was not different between the different SBM products. However, SE + 70 g.d^{-1} omasal infusion of Lys (SE70) increased Lys flux by exactly 70 g.d^{-1} above the SE treatment. No differences were observed on duodenal Lys flow, post-ruminal digestion and digestible Lys between

the SBM diets (SE, EP, LS). Irrespective of the method of assessment, lysine availability was not altered by the method of treating SBM. The third study evaluated the relative impact of supplying rumen-undegraded protein (RUP) versus altering the carbohydrate source on milk yield and efficiency N usage for milk production. Protein supplementation (SE and EP) increased DMI, whereas the inclusion of beet pulp (BP) replacing 50% of high moisture shelled corn decreased DMI. The results of this research suggest that, under normal conditions of feeding, supplying rumen protected SBM to enhance the supply of intestinally available essential AA does not improve lactation performance; providing beet pulp as a source of readily fermentable fibre also failed to affect milk yield or efficiency of N usage for milk production by dairy cattle.

RÉSUMÉ

Doctorat **Science Animale**

Sylvia I. Borucki Castro

L' EFFET DU TRAITEMENT DE TOURTEAU DE SOYA SUR LA DISPONIBILITÉ EN ACIDES AMINÉS, ET SUR L'EFFICIENCE D'UTILIZATION DE L'AZOTE CHEZ LES VACHES LAITIÈRES

Dans le premier essai, quatre vaches laitières Holstein équipées avec des cannules ruminales et duodénales ont été utilisées pour déterminer l' impact de différentes méthodes de traitement du tourteau de soja (SBM) sur la dégradabilité ruminale et la digestion intestinale des protéines brutes (CP) et des acides aminés (AA). Les extraits au solvant de SBM (SE), le tourteau de soja « expellé » (EP), le lignosulfonate (LS), et le SBM traité par la chaleur et les écailles de soja (HS) ont été incubés dans des sachets de nylon pendant 48, 24, 16, 8, 4, 2 et 0 heures. Des sacs supplémentaires ont été incubés pendant 16 heures, immergés dans une solution de pepsine-HCl, et utilisés pour déterminer la digestibilité intestinale *in situ* (sachet mobile) ou *in vitro.* Le traitement du TS (EP, LS, HS) a augmenté la fraction non dégradable dans le rumen (RUP) et la disponibilité de AA 30%. Les estimations de la digestibilité intestinale des CP et des AA étaient plus basses avec la méthode *in vitro* comparativement à la méthode *in situ.* Dans le deuxième essai, l'augmentation des valeurs de flux corporel de Lys plasmatique (Lys flux) a été comparée avec l'augmentation du flux intestinal et de la digestibilité apparente de Lys, ainsi qu'avec les augmentations post-ruminales en utilisant des infusions omasales de Lys. Le flux de Lys plasmatique ne montre pas de différences entre les différents produits de SBM. Néanmoins, SE + 70 $g.j^{-1}$ d'infusion omasale de Lys (SE70) a augmenté le flux de Lys plasmatique de 70 $g.j^{-1}$ en comparaison au traitement SE. Aucune différence n'a été observée pour le flux duodenal de Lys, la digestion post-ruminale et la Lys digestible entre les diètes SE, EP et LS.

Dans le troisième et dernier essai, huit vaches Holstein multipares en début de lactation, ont été utilisées pour déterminer l'impact de produits de SBM protegé ou l'utilisation d'une autre source d'énergie, sur la production et l' efficacité de production de lait. La supplémentation avec SE et EP a augmenté la quantité de matière sèche ingérée (DMI), tandis qu'avec la pulpe de betterave (BP) la DMI a diminuée. Les résultats indiquent que sur des conditions normales d'alimentation, la supplémentation avec des produits de SBM protégé pour augmenter la disponibilité intestinale d'acides aminés essentiels n'a pas améliore la production. La supplémentation avec la pulpe de betterave n'a pas augmente l'efficacité d'utilisation de l'azote pour la production laitière et n'avez pas réduit l'excrétion de l'azote dans l'environnement.

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Finally my special thanks to my loving husband and children, for their time, unconditional patience, love and support.

CONTRIBUTIONS TO KNOWLEDGE

According to the judgment by the author, this research resulted in the following original contributions to the scientific literature dealing with dairy cattle nutrition:

1. The first study where ^{15}N lysine flux has been used to determine lysine availability in dairy cattle.
2. The first study where in vitro intestinal digestibility of individual AA was determined and compared with the mobile bag technique.
3. Determination of kinetics of rumen degradation of individual essential amino acids in rumen protected soybean meal products.
4. Quantification of the availability of essential AA in rumen protected soybean meal products for dairy cattle.
5. Development of a new technique to determine the concentration of amino acids in feeds and digesta using the gas chromatography mass spectrometry.
6. The demonstration that *in vitro* techniques cannot replace the *in situ* method for determining intestinal digestibility of amino acid in rumen protected soybean meal products.
7. An evaluation of the efficacy of supplying RUP versus changing the source of energy in altering lactation performance and N excretion in manure.
8. Generation of knowledge regarding intestinally available amino acids that would be useful for improvement of computerized models of metabolizable amino acid systems for the nutrition of dairy cattle.
9. Generation of information for the development of accurate and integrated models to evaluate the nutrient partition in dairy cattle between the animal and its environment.

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EXPERIMENT 2

CONTRIBUTION OF CO- AUTHORS TO MANUSCRIPTS FOR PUBLICATION

The main body of this thesis consists of three manuscripts. The first manuscript (Section IV) has been submitted to the Journal of Dairy Science and is under review. The second and third manuscripts are soon to be submitted to the Journal of Dairy Science. Dr. Leroy Phillip, Dr. Robert Berthiaume, Dr. Hélène Lapierre and Dr. Phill Jardon, are co-authors on all manuscripts that form part of this thesis. Dr. L.E. Phillip (student's supervisor), Dr. Robert Berthiaume (student's co-supervisor) and Dr. Hélène Lapierre (student's advisory committee member) were co-investigators of the project, and assisted with the planning and conduction of the research and the preparation of the manuscripts. Dr. Phil Jardon kindly provided the soybean meal products to be studied, financial support and contributed to the revision of the first manuscript.

1. INTRODUCTION

a) Challenges facing the Canadian Dairy Industry

The Canadian dairy industry is confronted with many challenges that result in public and consumer pressures forcing dairy farmers to engage in production practices that result in minimal damage to the environment. It also faces enormous challenges that arise from market globalization. To remain globally competitive, the Canadian dairy industry must maintain markets, adjust to the restriction on the use of animal products in diet formulation, and be sensitive to public concerns about animal welfare. Additional consumer concerns relate to the healthfulness of dairy products, which are viewed as contributing to chronic diseases as a result of high fat intake. All of these issues represent threats to the profitability of dairy farming and to the sustainability of the Canadian dairy industry. Dairy farmers must, therefore, adopt appropriate yet cost effective feeding strategies to improve product quality, sustainability and farm profitability. The dairy-processing sector must also play a role in maintaining the viability of the dairy industry by continuing to introduce to the markets value-added products of high quality.

The dairy industry ranks fourth in the Canadian agricultural sector following grains, red meat and horticulture (CDIC, 2005). Dairy products are worth $ 11.5 billion at the retail level, representing 15.9% of Canadian sales in the food and beverage sector. Dairy systems in Canada are based on intensive farming and this has led to several environmental concerns across the country (McRae and Smith, 2000). According to Statistics Canada (2001) Canadian livestock excrete approximately 783 million kilograms of N in their manure, and ruminants are responsible for more than 68 % of the manure N excreted into the environment. Quebec and Ontario account for 81% of Canadian dairy farms (CDIC, 2005), and therefore these provinces also have the highest levels (60

kg/ha) of residual N (McRae and Smith, 2000). As this residual N accumulates in soils, the risk of N run-off and volatilization increases, contributing to water and air pollution. Therefore, there is a pressing need to improve the efficiency of N usage in dairy cattle, as a means of reducing environmental pollution from manure N.

b) Improving amino acid nutrition of dairy cattle

Strategies to improve the efficiency N usage in dairy cattle include reducing the rumen degradability and improving the intestinal supply and bioavailability of metabolizable essential amino acids. Van Horn et al., (1996) have emphasized that the type of feedstuff and its digestibility are important factors that determine efficiency of N usage by dairy cattle. According to Castillo et al. (2000) up to 72% of the N consumed by dairy cattle could be excreted in feces and urine. Thus, current feeding practices in the dairy industry could lead to enormous inefficiencies in N usage for milk production. Management practices, such as the balance between the animal's protein requirements and the correct choice and amount of supplement, need to be considered when designing environmentally sustainable feeding programmes for dairy cattle.

One of the main objectives in feeding high-producing dairy cattle is to formulate a diet with adequate amounts of rumen degradable protein (RDP) to maximize microbial protein synthesis, while supplying the required quantities of rumen undegradable protein (RUP) to enhance the flow of absorbable essential amino acids (Schwab, 1995). A practical approach to increasing the intestinal supply of RUP is to include in the diet a source of ruminally protected proteins. Rumen protection can be defined as a process that supplies a nutrient in a form that prevents its degradation by ruminal microorganisms. Effective rumen protection should ultimately ensure that a nutrient not only escapes ruminal degradation and flows to the duodenum, but that it can be absorbed, hence "available", in

the small intestine. An available amino acid (AA) has been defined as an AA, which is digested and absorbed, and capable of being used for protein synthesis by the animal (Batterham, 1992).

c) Soybean meal as a protein supplement for dairy cattle

Soybean meal is the most commonly used protein supplement in diets for dairy and beef cattle in Canada and the US (Statistics Canada, 2003; USDA-NASS, 2004). Soybean meal is conventionally produced by solvent extraction or mechanical expression (expeller process) of the oil in soybean, an important agricultural commodity worldwide (ASA, 2005). The manufacturing process used to produce soybean meal has a profound influence on its RUP content. Soybean meal has a high protein content (50%), and compared with other plant protein supplements, it has a high content of essential amino acids (47.5% of crude protein; NRC, 2001). Over the last 25 years, due to an increase in protein demands for higher-producing lactating dairy cows, much research has been devoted to improving the quality of SBM protein for dairy cattle by reducing protein degradation in the rumen (O'Mara et al., 1997a; Gonzalez et al., 2001; Ceresnáková et al., 2002). Over the next 10 years, there is likely to be even more global interest in the use of SBM to improve the amino nutrition of high producing dairy cows, because of North American and European restrictions on the use of animal protein in diets for ruminants (Hasha, 2002; FDA, 2004).

d) Improving systems for amino acid nutrition in dairy cattle

Substantial progress has been made in computerized modelling of requirements and dietary supply of amino acids in dairy production, and current models recognize the importance of supplying lysine and other essential amino at the level of the small intestine. With the emergence of updated models for protein nutrition of dairy cattle (NRC 2001; Rulquin et. al., 2001b; CNCPS, 2003) and as a consequence of public pressure to

reduce environmental pollution from livestock, there is now general acceptance in the dairy industry that diet formulation must be based on metabolizable amino acids (Bequette et al., 2003; Berthiaume et al., 2003). This concept places emphasis not only on the amount and profile of essential amino acids (EAA) delivered to the small intestine from microbial protein and RUP, but also on the extent to which the EAA are absorbed (NRC 2001). In order to improve existing diet-formulation models, additional knowledge is required concerning intestinal availability of individual amino acids in common feeds, and the impact on metabolizable AA of the various methods of protecting SBM from rumen degradation.

Published research dealing with kinetics of ruminal degradation and intestinal availability of individual AA from rumen protected SBM products is quite limited (Ipharraguerre and Clark, 2005). In addition, the research that has been done to assess the effectiveness of rumen- protected protein and amino acids have often been conducted with dairy cattle fitted with ruminal and intestinal cannulas. These experimental approaches may not be readily acceptable by a public concerned with animal welfare. Consequently, progress in advancing the metabolizable AA system in dairy cattle nutrition may depend on the development of non-invasive approaches to assess the quality of the protein in ruminant feeds.

Although an increase in RUP supply may be an effective strategy to optimize the flow of metabolizable AA in dairy cattle, it is well recognized that responses in milk production to increased RUP supply have been variable (Ipharraguerre and Clark, 2005). Such inconsistency in milk production responses have led some researchers to suggest that the approach of synchronizing ruminal energy and protein supply to enhance microbial protein synthesis may be an alternative strategy to increase

lactation performance and the efficiency of N usage in dairy cattle (BØrsting et al., 2003; Broderick, 2005).

This research addresses ways to maximize the feeding value of SBM, a plant protein supplement, and to reduce environmental pollution by optimizing amino acid nutrition; the research also responds to animal welfare concerns by investigating alternatives to the use of surgically prepared animals for feed quality evaluation. Knowledge from this research will aid the dairy industry in adapting to some of the threats to its profitability and viability.

2. LITERATURE REVIEW

2. 1. Protein and amino acid nutrition of dairy cattle

The concept of metabolizable protein and amino acids

In dairy cows, ruminally synthesized microbial protein, ruminally undegraded feed protein, and to a lesser extent endogenous protein all contribute to metabolizable protein. Metabolizable protein (MP) is defined as the true protein that is digested postruminally, yielding absorbable amino acids (AA) in the intestine (NRC, 2001). Amino acids and not protein per se, are the required nutrients. Absorbed AA are used principally as building blocks for the synthesis of proteins, and are vital for maintenance, growth, reproduction and lactation of dairy cattle.

An ideal pattern of absorbed AA exists for each of these physiologic functions. However, in ruminant animals, this pattern is more difficult to assess due to the presence of rumen microorganisms, which transform the AA ingested into microbial protein or energy. Therefore, the objectives in protein nutrition of dairy cattle are to provide adequate amounts of rumen degradable protein (RDP) to ensure maximal synthesis of microbial protein, and to supply rumen undegradable protein (RUP) to optimize the profile of absorbable AA.

A practical way to increase the supply of RUP for dairy cattle is to include ruminally protected proteins in the diet. Rumen protection involves processing the feedstuff, or the nutrient in such a manner that the nutrient flows unchanged to the abomasum, yet is available for absorption in the small intestine. Several sources of rumen protected (bypass) proteins are available; however, accurate information on the kinetics of ruminal protein degradation is required in order to reliably estimate how much of this protected protein is readily digested in the small intestine.

Although it is desirable to formulate diets to meet AA needs of dairy cattle, such diet formulation have not been widely adopted in the industry, because of the limited knowledge of the amount of AA that can ultimately be absorbed from commercial feedstuffs (Bequette et al., 2003). The development of models to improve AA nutrition of dairy cattle has stimulated research into metabolizable AA for dairy cattle.

Models for predicting metabolizable amino acids in dairy cattle

To advance understanding of AA nutrition in dairy cattle, Rulquin et al. (2001 b) developed an AA submodel, which uses the PDI system (intestinally-digestible protein; INRA, 1989) to predict flows of protein fractions and, using the AADI system (intestinally-digestible AA), to predict the amount of AA in duodenal protein. The AA submodel of Rulquin et al. (2001 b) uses estimates of ruminal degradation of the feed protein and intestinal digestion of the undegraded protein to predict the ruminal degradation and intestinal digestibility of the constituent AA in the feed protein. This approach assumes that AA composition of undegraded protein is identical to that of the original feed. The model is corrected by an equation of adjustment based on experimental abomasal and duodenal AA data to improve accuracy. An additional limitation of this model is that it does not account for protein derived from endogenous sources.

The AA model of NRC (2001) contrasts from that of Rulquin et al. (2001), in that there is no need to assign AA values to ruminally synthesized microbial protein and endogenous protein. This model is based on a multivariate regression method, where the duodenal flows of each EAA, compiled from 57 published studies, form the basis of the prediction model. In the NRC (2001) system, the intestinal availability of AA is calculated based on its concentration in the original feed, with the same assumption that ruminal degradability and intestinal digestibility of the AA

are identical to that of the CP in the original feed.

In the case of the Cornell Net Carbohydrate and Protein System (CNCPS, 2003) the metabolizable amino acid supply is composed of that from the feed plus the rumen microorganisms. The AA derived from RUP in the duodenum is calculated by multiplying the AA content of the insoluble feed protein by the quantity of protein escaping ruminal degradation (Sniffen et al., 1992; O'Connor et al., 1993). A limitation with this approach is that, estimates of the AA content of the insoluble dietary protein, at the level of the duodenum, are available only for a limited number of common feeds. The microbial composition of EAA is estimated from cell wall and non-cell wall bacterial protein appearing at the duodenum. Experimentally measured coefficients are used to predict intestinal digestion of protein and these values are also used to estimate those of the intestinal digestibility of the AA (O'Connor et al., 1993). The model was tested against observed values, and model-predicted duodenal flows of individual EAA explained 81 to 90% of the variation observed. There were however, significant differences in some AA (Thr, Leu and Arg) indicating further improvements are necessary for the prediction of intestinal AA supply.

2. 2. Amino acid availability and its measurement

The concept of availability

The term "availability" could be defined as the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal (Forbes and Erdman, 1983; Sauberlich, 1987; Ammerman et al., 1995). Several other terms ("bioavailability", "availability", "biological availability", "bioactivity", "biopotency", and "bioefficiency") have been used interchangeably. Others authors rely on the demonstration of nutrient utilization within normal metabolic processes

to determine AA or nutrient availability (Fox et al., 1981; O'Dell, 1984; Batterham, 1992).

Various methods have been developed to determine the availability of amino acids in feedstuffs, but no single procedure has emerged as universally applicable; they all have advantages and disadvantages (Lewis and Bayley, 1995).

2. 2.1. *In vivo* methods to estimate AA availability

Digesta marker methods

The digestibility and availability of AA may be estimated indirectly, by incorporating an indigestible, digesta marker into the diet (or ruminal cannula). The marker should remain unaltered during its passage through the gut and it should be completely recoverable in the feces (Merchen, 1988; Rymer, 2000). Some markers, such as silicon and lignin could be partially absorbed or not completely recovered (Fahey and Jung, 1983; Minson, 1990). Chromic oxide offers certain advantages such as complete recovery (Merchen, 1988) and accurate methods of detection. It tends to travel as a suspension in digesta independent of that of either particulate or liquid phases (Merchen, 1988). According to Schneider and Flatt (1975) it cannot be used in powdered form or it will separate from the coarse feed and travel at a different rate. However, chromic oxide can be mixed with paper, pelleted or prepared in capsules (Luginbuhl et al. 1994; Dos Santos and Petit, 1996).

Proper selection of the marker is important and there should be reliable analytical methods for detection of the digesta marker. The digesta marker technique is a useful method for measuring AA availability but it requires representative sampling of the diet, feces and digesta. Frequent sampling is advisable because of possible sedimentation in the rumen and

sporadic transfers causing diurnal variation (Merchen, 1988; Rymer, 2000). Substances such as CrEDTA and CoEDTA, are ideal markers of the liquid phase of digesta (Merchen, 1988).

Bioassays

Determination of AA availability that involves measurements of animal growth is usually considered the ultimate standard against which other methods are evaluated (Lewis and Bayley, 1995). In growth assays, AA availability is measured by the capacity of a particular feed to provide a specific limiting AA and promote growth. The advantage of this method is that it shows the net effect of all components affecting availability (digestion, absorption and utilization) and shows its practical effect on performance. However, they are expensive and time consuming. Furthermore, they are subject to many sources of interference and inherent animal variation. Proper design and conduct of these assays are crucial considerations because these bioassays serve as '"absolute standards" (Littel et al., 1995).

In studies with soybean meal and cottonseed meal, Batterham et al. (1990) measured ileal digestibility of Lys and availability of Lys by "slope ratio growth assay". The results showed that both estimates were similar for soybean meal but the availability of the Lys in cottonseed meal was much lower than its ileal digestibility.

A quantitative test referred as "the calibration curve" was developed by Rulquin and Kowalczyk (2003) to assess AA availability. A calibration curve was constructed with measurements of plasma lysine as a function of graded amounts of lysine infused at the duodenum. After verifying the linearity of blood level response, the technique was applied to a commercial product. The technique yielded reliable results for availability of methionine but not for lysine.

The use of stable isotopes

Basic concepts

In ruminants, a number of studies have used the isotope dilution technique to investigate responses of AA fluxes to nutritional interventions (Bequette et al., 1996 b; Pacheco et al., 2003; Savary-Auzeloux et al., 2003). The use of isotopic tracers, allow the study of substrate kinetics because the rate of uptake of the isotope is influenced by the concentration of the substrate in plasma (Wolfe, 1992). The development of mass spectrometry, and the development of stable and non-radioactive isotopes, allows measurements of flux or turnover rate that could be performed without health hazards. These measurements are based on the principles of whole body protein turnover (Waterlow et al., 1978). The term turnover means the overall rate at which protein is synthesized or broken down in the body; protein turnover is usually estimated by the measure of the flux of N or by the flux of a particular AA. The flux may be thought as the rate of AA flow through the free AA pool into protein and other metabolic pathways, coming from the food (absorption) or from protein breakdown. The exit routes are by incorporation into protein and by oxidation and excretion (energy). In a given steady state, the transfers in and out are equal; the equation can be synthesized as follows:

Equation 1

Flux = **I** absorption + **B** breakdown = **O** oxidation + **S** synthesis (protein)

(Waterlow et al., 1978; Lobley, 1993)

For mathematical analysis of the turnover or flux of metabolites in living systems, it is usually necessary to divide them into a number of distinguishable volumes referred to as pools or compartments (Waterlow et al., 1978). The pool and compartment are distinguishable volumes of a metabolite (i.e. Lys). Turnover rate or flux means the amount (absolute value) of material transfer from one pool to another per unit of time (i.e.

mmol.h^{-1}). When a model is constructed as a basis for the interpretation of measurements, it is necessary to specify the compartments between which the flux is occurring (see Figure 1). The model follows the transfer of the AA (isotope) into protein and this is equated to the AA movement by selection of an appropriate free pool and measurement of the isotopic enrichment. Irreversible loss rate (ILR) describes accurately the behaviour of the label rather than that of the unlabelled compound, but this expression is used to describe the whole body (WB) net flux (Waterlow et al., 1978) of the metabolite in question.

Figure 1

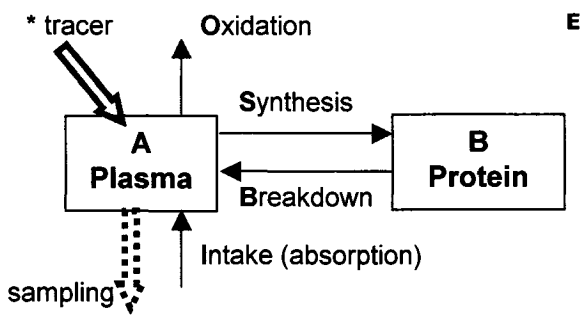

Figure 2

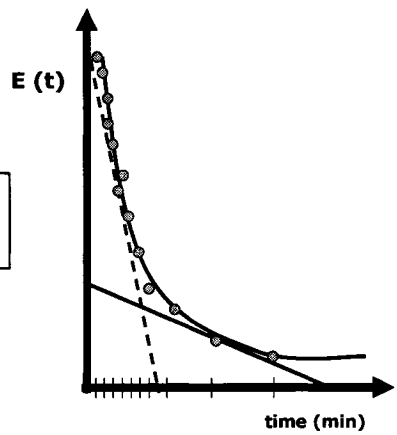

Figure 1: Schematic representation of the two-pool model employed for the single dose study; Injection of bolus (full arrow) and sampling arrow (dashed line).

Figure 2: Schematic representation of isotopic enrichment (E) clearance after a bolus injection in pool A. The decay curve is explained by a steep slope (dash line) and rapid flux (mixing of tracer with blood and extracellular pool) and the less steep slope is explained by an influx from pool B (Shipley and Clark, 1972).

If the studied AA is not limiting in the basal diet, any increased availability should not increase protein synthesis and therefore not change protein degradation (Harris et al., 1994). Therefore, based on the equation (1) the variation in net flux would estimate the variation in absorption.

Several assumptions need to be considered (Shipley and Clark, 1972). For simplicity, it is usually assumed that the system is in steady state, so then the inputs equal the outputs and the pool sizes do not change. These compartments are homogeneous and once in the pool, the isotope mixing is instantaneous. The tracer (labelled compound) behaves exactly as its tracee (unlabelled metabolite). It is assumed that protein acts as a sink for label entering it. Recycling of the label is not considered; this means that the pool is considered to be so large, that within the period of observation the molecules that leave the pool do not re-enter.

The plasma substrate kinetics can be determined equally well by means of a bolus isotope injection or by a constant-tracer infusion (Lobley, 1993; Holtrop et al., 2004). In the case of a single bolus dose, the structure of the system is determined to be that which provides the best fit to the observed decay curves of enrichment against time after injection (Figure 2; equation 2). Enrichments (E) are expressed as tracer-to-tracee ratios, the proportion of labelled over and above unlabelled species (equation 2). Plasma WB net flux or ILR, is the rate of AA flow through the pool (mmol/h or g/d) and can be calculated from the parameters α, β, k_1 and k_2, as described in equation 3 (Shipley and Clark, 1972; Holtrop et al., 2004).

$$Ep(t) = \alpha e^{(-k_1 t)} + \beta e^{(-k_2 t)} \qquad \text{Equation (2)}$$

$$\text{WB flux / ILR} = \text{Dose} / [(\alpha / k_1) + (\beta / k_2)] \qquad \text{Equation (3)}$$

Application of the technique

In ruminants and other mammals whole body and tissue AA fluxes are sensitive to many physiological and nutritional stimuli. Intake has been shown to be a potent regulator of such fluxes. Plasma ILR or net flux, was increased for sheep fed 4 increasing levels of energy (Savary-Auzeloux et al., 2003). This increase was linear for all AA but quadratic for Phe and

Leu and tended to be quadratic for Lys, Ile and Tyr. In lactating dairy cows, whole body AA flux was determined for animals fed fresh forage at 2 levels of intake (Pacheco et al., 2003). Net flux was higher for ad libitum animals compared to those fed restricted, but only significant for Ile, Met and Ala; whereas Glu was the only AA with higher ILR for animals fed restricted. Cronjé et al. (1992) investigated the metabolism of Lys, Leu, and Met in lambs fed roughage diets supplemented with different levels of protein. Lysine, Leu and Met fluxes were increased at higher levels of protein.

Higher metabolizable protein also presented the potential to increase WB Leu net flux (Lapierre et al. 2002) in lactating dairy cows. However, Bequette et al. (1996b) did not increase Leu ILR in protein-supplemented diets. Metabolic events occurring during a parasite infection create a significant demand for additional AA for the animal, particularly for the gastrointestinal tract. Bermingham et al. (2000) investigated the effects on whole body ILR of the additional demands placed by a parasite burden and associated weight loss. Plasma concentrations of essential AA were not different between treatments, and the ILR for Cys and Val were similar between control and infected sheep.

2. 2.2. *In situ* methods to estimate AA availability

The nylon (dracon or polyester) bag technique has been used to measure ruminal degradation and ruminal availability of AA because it is a relatively simple and low-cost method (Stern et al., 1997). The procedure involves suspending bags containing different feedstuffs in the rumen and measuring AA disappearance at various time intervals. It provides an advantage compared to laboratory methods because it involves the digestive process that actually occurs in the rumen of a living animal. However, several factors affect estimates of rumen bag digestion and they need to be controlled and standardized for this technique to yield reliable

results (Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992; Madsen and Hvelplund; 1994; NRC, 2001). The nylon bag technique assumes that all soluble protein is completely and instantaneously degraded in the rumen but Dewhurst et al. (1995) suggested that the technique may not be precise because a high proportion of water-soluble material could leave the bag unfermented. Herold and Klopfenstein (1996) reported that the nylon bag technique overestimates ruminal protein degradation because of food particle losses. These limitations have been addressed by Hvelplund and Weisbjerg (2000) who developed an equation to correct for particle losses, including direct measurement of water soluble N.

The measurement of rumen bag degradation (disappearance) using a single time point of sample incubation does not provide a reliable estimate of ruminal protein degradation (Stern et al., 1997). For this reason, mathematical models have been proposed to combine estimates of ruminal degradation with ruminal outflow rates as a means of accurately estimating ruminal protein degradation (Bach et al. 1998). However, according to Nocek and English (1986) the kind of mathematical model utilized could also affect the estimates of ruminal protein degradability.

The *in situ* mobile bag technique has also been developed to determine intestinal availability of protein (Hvelplund, 1985; Hvelplund and Weisbjerg, 2000). However, in ruminants, this method may require that the feed sample be pre-incubated in the rumen. Once removed from the rumen, these mobile bags are introduced into the duodenum, with (Stern et al., 1997) or without (Vanhatalo et al., 1995) pre-treatment with pepsin-HCl solution; the mobile bags are then recovered from feces or collected at the ileum (Prestløkken and Rise, 2003).

The mobile bag technique is affected by several potential sources of variation: porosity of the bag material, sample-to-surface ratio, animal and

diet effects, retention time, site of bag recovery, and microbial contamination (Stern et al., 1997). According to Hvelplund (1985) and Todorov and Girginov (1991), porosity of the bag material is not an important source of variation; but the free surface area was found to be an important source of variation (Vanhatalo, 1995). Hvelplund et al. (1992) reported negligible effects of ruminal pre-incubation in the evaluation of SBM and cottonseed cake but other authors found important effects of ruminal pre-incubation on the digestion of heat-processed canola seed (Deacon et al., 1988), rapeseed meal and meat and bone meal (Rooke, 1985). Volden and Harstad (1995) concluded that ruminal pre-incubation was not necessary for some feeds (SBM, rapeseed meal, fishmeal) but necessary for others (lupin, barley and oat seeds). Pre-treatment with pepsin HCl is considered important in case of formaldehyde or heat-treated protein. However, Vanhatalo et al. (1995) observed that pre-treatment with pepsin-HCl was not necessary when the mobile bag technique includes pre incubation in the rumen. Recovering the mobile bags from feces instead of the ileum led to a slight increase in the CP and AA digestion of vegetable concentrates (Vanhatalo and Ketoja, 1995) but not for SBM or treated SBM (Prestlokken and Rise, 2003).

When using the *in situ* technique for measuring AA availability, there is the potential for rumen microbial contamination of the residual protein because the amount of undigested protein in the bag is often very small. Some authors suggest that contamination is small enough to be ignored (Hvelplund, 1985; Kohn and Allen, 1992). However, others consider that microbial contamination should be accounted for in cases of low protein feeds and forages (Vanhatalo and Varvikko, 1995) but not for protein supplements (Nocek, 1985; Varvikko, 1986; González et al. 2000). When properly applied, the *in situ* technique is rapid, fairly reproducible and requires minimal apparatus. However, it does require surgically equipping the animal with ruminal and intestinal cannulas, and the use of specialized

care and facilities. Considering the animal welfare concerns regarding the use of cannulated animals and the expense of maintaining them (Broderick et al., 1991), in vitro approaches to measuring AA availability have been investigated.

2. 2.3. *In vitro* methods to estimate AA availability

In vitro procedures for measuring AA availability could be divided in three categories: chemical, enzymatic and microbiological. However, most of them involve a combination of all three. Some microbiological assays were once the primary method of determining the AA content of feedstuffs and were based on the growth of a microorganism that had a specific requirement for the AA studied. Theoretical and practical difficulties with this type of assay have limited their usefulness in modern day investigations (Lewis and Bayley, 1995).

Most chemical procedures for measuring AA availability are specific for Lys (AOAC, 1984). They are based on the premise that the epsilon amino group of Lys must be unbound for Lys to be biologically available. Examples of these chemical methods are the 1-fluoro-2, 4-dinitrobenzenne (FDNB) procedure (Carpenter 1960), the 2,4,6-trinitrobenzene sulfonic acid (TNBS; Kakade and Liener, 1969) and others based on adsorption of dyes (Hurrell and Carpenter, 1975; Goh et al., 1979). Although some studies have shown good correlation with available Lys measured in *vivo* (Faldet et al., 1992 a), there may be chemical reactions other than bound Lys, which lead to reduced intestinal digestion of AA (Stern et al., 1997).

In ruminant nutrition, the final calculation of AA availability in feedstuffs requires an initial estimation of rumen degradation of protein; this process can be simulated *in vitro* with rumen inoculum or protease enzymes. Several factors have been evaluated to optimize the *in vitro* environment

to achieve reliable results from the enzymatic methods (Krishnamoorthy et al., 1983; Mahadevan et al., 1987; Kohn and Allen, 1995). Based on studies with plant or fungal proteases, research has also focused on finding the highest correlations between *in vitro* and *in situ* conditions (Kopečný et al., 1989; Susmel et al., 1993; Tománková and Kopečný, 1995) but to date, there are controversial findings regarding the best choice of protease enzymes to mimic ruminal protein degradation.

Enzymes have long been tested to estimate intestinal digestion. The primary difficulty with this approach has been to identify an enzyme or mixture of enzymes that truly mimics, over a wide variety of feedstuffs, the action of the mixed enzymes *in vivo* (Lewis and Bayley, 1995). Most enzymatic techniques follow a similar protocol; feed samples are incubated at optimal temperature and pH with an enzyme or combination of enzymes. The techniques usually differ in the enzyme combination utilized. The lack of accuracy reported is likely a result of limited spectrum of specificity for any single enzyme (Stern et al., 1997). Although the use of a combination of enzymes improved correlations with *in vivo* methods, *in vitro* measurements may not always accurately predict *in vivo* results (Moughan et al., 1989; van der Poel et al. 1991).

One of the important factors affecting estimates of intestinal digestion of proteins appears to be the sequence used in the procedure: filtration, precipitation, centrifugation and dialysis (Gauthier et al., 1982). The removal of end products is important in avoiding the inhibition of proteases, the stabilisation of pH and to simulate the physiological environment of the gut. Van Straalen et al. (1993) used the pepsin-pancreatin digestion procedure of Antoniewicz et al. (1992) and reported a strong correlation (r=0.9) with the *in situ* mobile bag technique in dairy cows. Calsamiglia and Stern (1995) developed a three-step procedure to estimate intestinal digestion of proteins in ruminants. It is a combination of

the *in vitro* and *in situ* methods, because it includes pre-incubation in the rumen, followed by both pepsin and pancreatin digestion. However, the application of the technique showed a large variation among and within some protein supplements (Stern et al., 1997).

2. 3. Soybean meal as a source of protein for dairy cattle

2.3.1. Characteristics of soybean and soybean meal

Soybean is an important agricultural commodity, with steady increase in worldwide annual production. It represents 56% of oilseed production and 69% of protein meal consumption (ASA 2005). It is one of the world's leading crops because of its wide regions of adaptability and unique chemical composition. Soybean has the highest protein content (40%) among oilseeds and has the second highest oil content (20%) after peanuts. The composition of the whole soybean and its structural parts depends on factors such as variety, growing season, geographic location or environmental stress (Karr-Lilienthal et al., 2004, 2005). On a DM basis, protein composes 40 to 50%, carbohydrates compose 30-35%, and oils around 15 to 30% (Liu, 1999). Refined oil contains more than 99% tryglycerides, the main fatty acids being linoleic (53.2%), oleic (23.4%) and linolenic (7.8%).

In animal nutrition, a number of factors have been identified that could affect the soybean's digestibility, including the presence of biologically active components, the necessary heat treatment and the chemical form of the soy protein itself (Liu, 1999). The presence of proteinase inhibitors (Kunitz and Bowman-Birk) can reduce the protein digestibility by decreasing or inhibiting the action of pancreatic enzymes (Liener, 1994). The action of these inhibitors seems to depend on the animal specie (Swiech et al. 2004; Liener, 1994) and be partially or totally inactivated by

heat and gastric juices (Krogdahl and Holm, 1981). According to Green et al. (1973) proteins in heated soy extract have higher digestibility than those in unheated extracts where the inhibitors were removed. Thus, it appears that the effect of heat treatment on improving protein quality is due not only to the inactivation of trypsin and α-chymotrypsin inhibitors but also to the disruption of the protein's internal nature. Most soybean proteins are globular molecules, and resistant to proteolytic attack (Fukushima 1968). The soybean structure and AA composition determine the properties for food use and condition the ways to alter its digestibility by physical or chemical changes (Kinsella et al., 1985; Ikeda et al., 1995).

Processing methods such as heat or mild alkali treatments generally result in changes in secondary and tertiary structure of soy protein with no breakage of covalent bonds (Liu, 1999). Such physical changes are referred to as denaturation. Moist heat denatures soy proteins very rapidly, that is, protein solubility decreases from 90 to 20% in 10 min at 100°C steaming. Moist heat, although necessary to inactivate lipooxygenase and destroy anti-trypsin agents, rapidly insolubilizes soy proteins (Smith and Circle, 1978). The extent of reduced solubility depends on moisture content and temperature intensity and duration of the process. Prolonged heat above 100°C results in a subsequent increase in protein solubility due to dissociation and degradation of the polypeptides (Wolf, 1978). Given conditions of denaturation, the disulfide bridging within the subunits of SBM protein could be exposed for reduction and exchange in distinct stages rather than co-operatively (Wallace, 1994). The protein could further fold reducing exposure and therefore enzyme attack.

Chemical treatment of soybean leads to a variety of chemical reactions that alter the protein structure by denaturization (Mir et al., 1984; Waltz and Loerch, 1986). In the presence of heat and moisture, a non-

enzymatic browning reaction may occur between the sugar aldehyde group and the free amino acid groups of the protein to yield an amino-sugar complex (Maillard reaction; Adrian, 1974). In the initial stages, the "early" Maillard reactions are the condensation of the carbonyl group of a reducing sugar (aldose) with a free amino group of the AA from the protein into N-substituted glycosylamine (Friedman, 1996). These "Amadori products" are unstable and undergo rearrangements, dehydration or fission. In a third phase these three products can further react with AA to form brown nitrogenous polymers "melanoidins" ("advanced" Maillard reactions). The temperature and/or time of heating result in increased color development. The reaction occurs less readily in foods with high a_w (presence of water) and pH determines the ratio of products formed. Pentose sugars (e.g.ribose) react more readily than hexoses (e.g. glucose), which in turn are more reactive than disaccharides (e.g. lactose; Adrian, 1974; Ashoor and Zent, 1984). Lysine due to its ϵ(epsilon) amino group is the most reactive AA, followed by other basic AA like His and Arg, and by the sulphur AA, Met and Cys (Adrian, 1974; Baxter, 1995). The amino-sugar complex is more resistant than normal peptides to enzymatic hydrolysis and the reversibility of this reaction is dependent upon temperature and time of heat exposure (Mauron, 1981; Marquié, 2001).

Physical or chemical processes alter the structure and solubility of the soybean protein (Kinsella et al. 1985) and these changes will alter the rate of ruminal degradation of soy protein. Soluble proteins are, in general, more susceptible to degradation than insoluble proteins (Wallace, 1994); conversely, when cross-links were introduced into proteins by chemical means, the rate of microbial degradation was decreased (Cleale et al., 1987 a,b ; Faldet et al. 1991; Wallace, 1994). Therefore, several physical and chemical modifications offer protection to proteins from enzymatic digestion by rumen microorganisms.

Soybean meal (SBM) is a by-product of the soybeans after the extraction of oil (Liu, 1999). Soybean meal is the most commonly used protein supplement in beef and dairy diets in North America (Statistics Canada, 2003; USDA-NASS, 2004). North American and European Union restrictions on the use of animal protein supplements (Hasha, 2002; FDA, 2004) are likely to promote even more the use of SBM. It is palatable protein supplement and has a good amino acid balance compared to other plant protein sources (NRC, 2001). Soybean meal has one of the highest percentages of EAA (47.5%) as percent of CP; SBM is particularly a good source of lysine, one of the first limiting AA in ruminant diets (Schwab et al. 1995; Schingoethe, 1996).

Commercial treatment of soybean meal products

Solvent extracted dehulled soybean meal (SE) is produced by cracking, heating (60°C up to10% in moisture) and flaking soybeans and reducing the oil content of the conditioned product by the use of hexane or homologous hydrocarbon solvents to 1%. The extracted flakes are cooked to neutralize anti-nutritional factors (100°C up to 20% in moisture) and ground into a meal. In the expeller soybean meal (EP) process, the soybean are initially cleaned, cracked, and dried. Then transported to tempering devices heated uniformly and fed into expeller presses; a central revolving shift creates pressure within the press causing the mechanical extraction of oil from the ground soybean. The expeller processing involves heating to a maximum of 163°C (Seaman et al. 1993; Liu, 1999).

In the lignosulfonate treatment (LS) of SBM, the product is treated with 8 to 25% (in weight) sulfite liquor from hardwood or softwood processing. Then is heated at 95°C for about an hour producing an early Maillard reaction between the carbonyl group of a reducing sugar (in this case xylose) and the amino groups of the AA composing the proteins. The

temperature, pH, percent moisture and time of reaction is critical so that the reaction does not continue to an irreversible stage that leaves the product ineffective in the small intestine of the ruminant (Winowiski, 1990). The heat and soyhulls process (HS) comprises the mixing the SBM with soyhulls to a final weight ratio of 100: 1-10, adding water to 30-50%. This mixture is further cooked at 95°C up to moisture content of 21-26% and further dried to 12-16% wt in moisture (Heittriter et al., 1998).

Heat treatment of SBM increases ADIN by means of either higher temperature or longer time of heating. Increased ADIN from 1.37 % (of DM) to 33.29 % was reported by McNiven (1994) and Shroeder et al. (1995) in heated SBM (150°C, 90 min) compared to unheated SBM. Demjanec et al. (1995) obtained values of 28 % ADIN by heating SBM at 165°C for 210 min and also increased values of NDF, and ADF. Faldet et al. (1992 a) only affected ADIN in soybeans roasted at 160°C for 90 and 120 min. However, Ljokjel et al. (2000) did not find effects on ADIN for SBM autoclaved at 120 and 130°C. The effect of heat can also reduce Lys concentration (Cross et al., 1992; Shroeder et al., 1995; Demjanec et al., 1995) together with Arg and Cys (Ljøkjel et al. 2000). According to Faldet et al. (1991, 1992 a), available Lys can decrease dramatically in heated SBM (up to 17%). In studies of Shroeder et al. (1995), the amino acids Glu, Gly and Thr were increased comparatively by the effect of heat. Harstad and Prestløkken (2000) found that the treatment with xylose increased the NDF content from 9% to 22%. The Lys content was reduced by 17% while Arg was reduced by 7%. Other authors also found negative effects of lignosulfonate (xylose) treatment on Lys content (Prestløkken and Rise, 2003).

2.3.2. Studies on rumen protected soybean meal

To optimize the amount of absorbable AA for high producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of

rumen undegradable protein (RUP; Schwab, 1995). In studies by Waltz and Stern (1989), protein degradability was decreased from 55.5% in SE to 36.4% in EP. In similar *in situ* studies, RUP values of solvent extracted SBM (SE) were 40.7 compared to 67.1% in expeller SBM (EP; Maiga et al., 1996). Reynal et al. (2003) found mean estimates of RUP of 27% for SE compared to 45% for EP based on omasal samples. The effect of heat, significantly reduced rumen degradation of CP and total AA (Schroeder et al., 1995; Ljøkjel et al., 2000; Ceresnáková et al., 2002). Duodenal flows of N and all individual non-bacterial AA in SBM, were linearly increased by roasting time (Demjanec et al., 1995). In *in vitro* studies, Cleale et al. (1987a) reported that non-enzymatic browning suppressed ammonia release from SBM. The reduction was influenced by many factors including source and quantity of added reducing sugar, moisture, pH, temperature and duration of heat. Can and Yilmaz (2002) studied the effect of heat, heating time and xylose level on RUP compared to untreated SBM. The RUP increased from 34 to 45% and up to 72% for increased heat combined with time or xylose level. All three factors significantly increased the RUP levels of SBM. In all studies, SE presented higher rumen degradation of CP and AA (by 30%) compared to LS (Windschitl and Stern, 1988; Weisbjerg et al., 1996; Prestløkken and Rise, 2003). Cleale et al. (1987 b) increased ruminal escape of SE protein 2.6 times by adding 3 moles of xylose for every 3 mol of Lys at 150°C.

O'Mara et al. (1997) found no significant differences in the AA profile before and after ruminal incubation for 8 and 12 h in formaldehyde treated SBM; however, all the other feedstuffs in the study presented differences in the AA profiles after ruminal incubation compared with the original feedstuffs. The AA profile of the original feed cannot be used to predict the profile of the undegraded residues (Crooker et al., 1987; Erasmus et al., 1994; Cozzi et al., 1995). Lysine and Arg seem to be more degradable as their porportion tends to decrease as a part of total AA after

ruminal incubation. Erasmus et al. (1994) reported a similar finding with 12 different feedstuffs. Methionine, Ile and Leu were reported to be more resistant to ruminal degradation as they increased or tended to increase as a proportion of total AA after ruminal incubation (Tamminga, 1979; Susmel et al., 1989; Erasmus et al., 1994). Maiga et al. (1996) did not find differences between AA profiles of 12-h rumen incubation residues and the original feed for both SE and EP; these authors, found more variation in the NEAA than in the EAA for SE. Harstad and Prestløkken (2000) found that the LS treatment only changed the rumen degradation pattern of Arg, Leu and Thr; contrary to other findings, Lys was numerically less degraded than total AA in lignosulfonate SBM.

The more reactive AA are those with amino side groups like Lys, Arg, His, specifically Lys because of the NH_2 in the epsilon position (Adrian, 1974). This fact makes them more reactive to microbial attack in the rumen. On the contrary, the branched chain AA (BCAA) Leu, Ile and Val are less reactive given the steric hindrance of the bulky side chains (Finley, 1985; Liu, 1999). Some facts even show that after a treatment at 100°C, the SBM protein subunits can maintain a hydrophobic core (Nakamura et al., 1984; Kinsella et al., 1985).

According to Frydrych (1992) and Woods et al. (2003), there is little variation between feeds in intestinal digestion using the mobile bag technique. When determined on 16-h rumen residues, Prestløkken and Rise (2003) found that the *in situ* indigestible AA fraction was lower than 3% in SE and LS. These authors support that there is no practical relevance when differences among AA indigestible fraction are between 0.5 and 1.5%. Faldet et al. (1991) did not find differences in N disappearance from the small intestine for heated soybeans, which averaged 99.7%. However, Demjanec et al. (1995) found linear decreases in intestinal digestion of Lys and quadratic decreases for Phe

and some NEAA, when increasing the heating time from 75 to 210 min. Ceresnáková et al. (2002), also found significant reductions (6 to 16%) in the intestinal digestibilities of the bypass protein, for His, Lys and Arg comparing full fat extruded SBM (FE) to SE. The value for CP intestinal digestion of RUP was higher for FE (99.2%) compared to SE (98.6%).

Values found for intestinal digestion measured *in vitro* are in general, similar or lower than those measured *in situ*. Woods et al. (2003) found *in situ* intestinal digestion of SE to be 96.6% which was higher than *in vitro* values of 81.7%. Maiga et al. (1996), found *in vitro* values of intestinal digestibility of RUP for EP and SE of 88 and 85% respectively, similar to those found by Stern et al. (1994). For lignosulfonate SBM (LS), Calsamiglia and Stern (1995) found values of 87% for *in vitro* undegraded intake protein similar to values of 89 % obtained *in situ* for SE. Stern et al. (1997) found lower values for *in vitro* intestinal digestibility of LS and SE, being 77.9 and 77.6% respectively.

2. 4. Lactation responses and environmental impact of increased amino acid supply in dairy cattle

2 .4.1. Utilization of amino acids for milk protein synthesis

Nitrogen is absorbed from the digestive tract mainly as free AA and ammonia. Ammonia absorption represents approximately half of the absorbed N, with all this absorption transformed into urea across the liver (Lapierre and Lobley, 2001). According to Lapierre et al. (2005) the transfer of hepatic ureagenesis that returns to the gut ranges from 20 to 80%. Although this process is very important for efficiency and contribution via microbial synthesis, anabolism relies on both the amount and profile of absorbed AA.

The absorption of AA was reported not to be directly related to the crude protein and metabolizable protein of the diet (Reynolds et al., 1992; Blouin et al., 2002; Tagari et al., 2004). This may be explained partially because of the role of the gut in AA supply (Lapierre et al., 2006). One possible explanation is directed towards endogenous contributions. Although most attention has been directed to the contribution of RUP and microbial protein to the duodenum, endogenous secretions can represent as much as 20% of duodenal flow (Berthiaume et al., 2001; Ouellet et al. 2002; Ouellet et al., 2005). Another point is the effect of gut metabolism on available AA. During the absorptive process, losses of AA will occur mainly through oxidation of AA by the gut tissues and failure to reabsorb endogenous protein secretions (Lobley and Lapierre, 2003). Measurements of small intestine disappearance (MDV) and portal flow (PDV) have shown large variations between AA in gut-associated losses in dairy cows (Berthiaume et al., 2001; Lapierre et al., 2002).

Nitrogen metabolites are absorbed from the lumen of the gut into the portal vasculature. Hepatic removal involves the prevention of hyper-aminoacidaemia, energy metabolism and synthesis of plasma export proteins. Although the liver removes on average 45% of all AA in portal circulation (Lapierre et al., 2005), there is considerable variation in how the individual AA are managed and released on post-hepatic supply. The group composed by the BCAA and Lys, experience little removal by the liver. The hepatic oxidation of these AA is limited and the enzymes responsible for their catabolism are widely distributed across other tissues (Goodwin et al., 1987; Mabjeesh et al., 2000; Lapierre et al., 2003). A second category of EAA is defined by substantial removal across the liver of the dairy cow, and these are His, Met and Phe (Lobley and Lapierre, 2003). For this group removal seems to be in proportion of the amount

absorbed, even in circumstances when intake is no sufficient to cover requirements (Bach et al., 2000; Lapierre et al., 2000).

The final step is the uptake of AA into milk proteins synthesis. According to Lapierre et al. (2005), splanchnic fluxes of His, Met and Phe appear to be totally captured and secreted quantitatively in milk by the lactating dairy cow. Based on studies by Berthiaume et al. (2006), Raggio et al. (2004) and Tagari et al. (2004) His, Met, Phe and Trp are stoichiometricaly transferred from blood to milk, as supported by Mepham (1982). For other group of AA, the post-liver supply was greater than the mammary gland uptake and milk output. The ratio of mammary to splanchnic flux for the BCAA and Lys, averaged 0.61 with a wide variation associated with dietary intakes and extra-splanchnic oxidation (Lapierre et al., 2005).

2.4.2. Lactation response to amino acid supplementation

The efficiency with which metabolizable protein (MP) is used for the synthesis of milk protein in production systems based on totally mixed rations (TMR) is known to be low (Walker et al., 2004; Jenkins and McGuire, 2005). When the supply of MP is increased, but is not balanced to meet amino acid requirements, increased oxidation and gluconeogenesis from amino acids in the liver, may result in a net increase in the yield of milk and milk components rather than an increase in milk protein concentration (MacRae et al., 2000). Griinari et al. (1997) reported that casein infused alone into the abomasum, increased milk protein yield by 10% whereas a combination of casein and the insulin/euglyceamic clamp increased milk protein yield in 28%. In cows fed a TMR, supplements of rumen protected EAA (Met and Lys) increased milk protein concentration (Chapoutot et al., 1992; Chilliard and Doreau, 1997). Bequette et al. (1998) suggested that His, Lys, Met, Phe and Thr are the most critical EAA for milk protein synthesis. By supplying a

complex well-balanced mixture of AA or casein in the mammary blood, these authors consistently increased milk protein concentration (Bequette et al., 1996a). However, in several experiments involving single AA infusion, plasma concentrations of infused AA increased, but no differences were obtained in protein yield, even though the limiting EAA Lys and Met were fed (Cant et al., 2002). Energy-protein synchrony, hormonal balance and stage of lactation could have a much larger impact on milk protein yield than does the AA supply (Hanigan et al., 1998; Mackle et al., 2000; Cant et al., 2002).

The concentrations of CP in milk and in the diet had no consistent relationship, unless the cows are in a state of severe protein under-nutrition (Emery, 1978; Beever et al., 2001; Jenkins and McGuire, 2005). Genetic variants exist for the major protein fractions derived from the mammary gland and are associated with variations in the amounts of the different casein types expressed and ratio of casein to whey ratio (Ng-Kwai-Hang, 1998). However, nutrition appears to have little effect on the relative rates at which the major proteins are synthesized by mammary secretor cells (MacRae et al., 2000).

The mammary gland is implicated as a key cause for the capture of dietary protein into milk. Studies undergoing a hyperinsulinemic-euglycemic clamp show that both the mammary blood flow and AA extraction can adjust, leading to enhanced milk protein production (Jenkins and McGuire, 2005). The mammary gland has the capacity to alter the uptake of substrates from arterial supply in response to changes in arterial AA concentrations, mammary blood flow, and metabolic activity to improve milk protein. Lapierre et al. (2005) demonstrated that the ratio of milk AA output to portal AA absorption decreased as protein supply increased. Doepel et al. (2004) proposed diminishing returns in milk protein synthesis against AA supply as we approach requirements.

To summarize the available data from the literature, Ipharraguerre and Clark (2005) performed a meta-analysis on the response to supplemental RUP sources in experimental diets. Feeding the supplemental RUP sources increased the passage of non-ammonia non-microbial N (NANMN) to the intestine. Overall supplemented RUP increased NANMN by 26% whereas soy RUP increased NANMN by 25 ± 15%. However there was a 7% depression in microbial N passage to the small intestine. Ruminal outflow of EAA was quantitatively improved by 9% with RUP supplementation but only significantly for blood meal. The response in ruminal outflow of Lys for treated soy proteins was 5 ± 10%. Increasing CP from 14 to 18% with SBM increased milk (2.7 $kg.d^{-1}$) and milk production was maximized (32.8 $kg.d^{-1}$) at 21.4% CP. The effect on performance was also evaluated for different RUP sources compared to iso-nitrogenous control diets. The RUP mix improved milk production but only significantly for treated soy products (expeller SBM), increasing milk yield by 3%. However, in this study, the overall effect of replacing SBM with RUP supplements caused a reduction in milk CP% of around 2%. Bateman (2005) constructed a meta-analysis from the literature (1988 and 2000) and observed that an increase in RUP intake was associated with increased yield of milk and 4% fat-corrected milk. This author supports the important effect of DMI in the relationship between the RUP content of the diet and milk production.

However, Reynal and Broderick (2003) found no difference in milk protein yield, feeding solvent extracted (SE) SBM or expeller (EP) SBM to early lactating dairy cows. Partial replacement of SE for EP did not increase true protein in milk (Ipharraguerre et al., 2005) or milk protein yield (Olmos Colmenero and Broderick, 2006b). The use of lignosulfonate treated SBM (LS) however, increased milk yield and true protein response compared to EP and SE in the studies of Ipharraguerre et al. (2005). Mansfield and

Stern (1994) on the contrary found detrimental effects on milk CP content and yield.

2.4.3. Response in N excretion and environmental impact of AA supplementation

Compared to other species, ruminants make efficient use of diets that are low in protein content or quality because microbes synthesize high quality protein recycling urea-N that would otherwise be excreted in the urine (Van Soest, 1994). However, although dairy cows produce more protein per day than any other animal specie, they are not particularly efficient at converting dietary N into milk N using current management schemes (Bequette et al. 2003). Dairy cows can excrete about 2 to 3 times more N in manure than in milk (Van Horn et al., 1996). In pasture systems, the reduced efficiency of protein utilisation for grazed and conserved grasses and legumes (Beever and Reynolds, 1994) has been overcome by the use of protein supplements. But feeding cows with more protein than needed is wasteful, resulting in elevated feed costs, reduced profits and environmental pollution (Peyraud et al. 1995; Kalscheur et al., 1999; Broderick, 2003). Additionally, high intakes of rumen degradable protein are often associated with reductions in milk quality and increased reproductive problems (Butler, 1997; McCormick et al., 1999).

Dairy farms are regarded as potentially major sources of N pollution (Castillo et al. 2000; Broderick, 2005). In the US and Canada, about one million tons of manure N per year are produced by dairy cows and heifers (Kellogg et al., 2000; Statistics Canada 2001), and only 30% is recycled and applied to cropland. Dairy farms are thought to be significant contributors of nutrients to hypoxia zones in oceans, lakes and ground waters (Carpenter et al., 1998; Burkart and James, 1999; Harter et al., 2002). It is estimated that about 25% of dairy manure N is lost as ammonia under current U.S. practices (Pinder et al., 2003). Although

most estimates of ammonia volatilization are based on total N excretion, urinary N is a much more important source of pollution than fecal N (Lockyer and Whitehead, 1990; Pakrou and Dillon, 1995).

It has been established that the major source of increase in N excretion by dairy cows is related to N consumption (Castillo et al., 2000; Kebreab et al., 2001). The bi-linear relationship between N intake and fecal N excretion obtained based on five experiments by Kebreab et al. (2002) was similar to that obtained by Castillo et al. (2000) based on 580 N balance measurements. Urinary N was exponentially correlated with N intake. The model predicted 80% loss of N in urine for levels of N consumption above 500 g $N.d^{-1}$. Diminishing returns in milk CP are obtained feeding CP values higher than 15%; after 420 g N per day microbial synthesis reaches an optimum and its contribution to the AA-N pool remains constant (Kebreab et al., 2002). Equations obtained from NRC (2001) and Ipharraguerre and Clark (2005a) predict responses in milk production of 0.94 $kg.d^{-1}$ by increasing from 15 to 16 %CP, and values of 0.42 $kg.d^{-1}$ when CP increased from 19 to 20%. In both studies, maximum milk production was achieved at 23% CP. However, in practical conditions the maximum was found at 16.7% CP with detrimental effects in cases of protein over-feeding (Broderick, 2003; Olmos Colmenero and Broderick, 2003 a; Wattiaux and Karg, 2004).

Given the fact that consumption is the primary factor affecting N excreted in dairy cows, nutritional strategies were studied in order to achieve higher values of N efficiency for milk production. The correct tracking of diet composition and the use of a proper model for diet formulation is a key element to avoid over feeding N (Broderick, 2005). Although higher values of milk protein yield could be achieved with increasing amounts of RDP (whereas RUP remains constant), efficiency of N use declines linearly while MUN increases linearly (Kalscheur et al., 2006). In a factorial experiment, 2 sources of supplementary protein were tested SE and a

blend of SE plus animal-marine supplements and ruminally protected Met, given at 3 levels of dietary CP, 14.8, 16.8 and 18.7% (Ipharraguerre and Clark, 2005b). Despite interactions, true protein in milk increased but gross efficiency of N use for milk decreased by increasing N intake, independently of the source of protein fed. The AA pattern fed may contribute to a higher efficiency on N use, and is strongly influenced by the source of RUP fed to the animal (Brito and Broderick, 2004; Broderick, 2005). Research has focused on ruminally protected AA (Felix et al., 1980; Armentano et al., 1997; Kröber et al., 2000). Supplementation with ruminally protected Met allowed a reduction of dietary CP% from 17.5% to 14.7% to obtain similar milk protein yields (Kröber et al., 2000).

Another proposed strategy is feeding to synchronize fermentable energy with RDP in the rumen. The energy supply must match protein supply (ammonia, peptides) to favor microbial uptake and consequently reduce rumen ammonia concentrations, which could further be converted to urea, and contribute to an increase in urinary N excretion (Owens et al., 1986, 1997; Ekinci and Broderick, 1997). Based on this principle, some studies found that replacing alfalfa silage with concentrate (Valadares et al. 2000) or with corn silage (Brito and Broderick, 2003) improved N usage for milk production.

3. RATIONALE AND OBJECTIVES OF THE RESEARCH

a) Rationale for the Research

A review of the literature suggests that information is required regarding the duodenal flow and intestinal digestibility of individual EAA derived from feed RUP; such knowledge is required in order to improve current models of AA nutrition in dairy cattle. Several studies have investigated the effects of lignosulfonate or expeller treatment of soybean meal on metabolizable protein supply; however, there is a lack of information about the intestinal availability of the individual essential amino acids in the different SBM products.

Information is also required regarding rumen degradation kinetics of protein and amino acids in rumen protected SBM products. This information will be helpful to match these characteristics with those of carbohydrates. More research is needed to fully understand the interaction of different protein sources with different carbohydrate sources on the metabolism and animal performance.

The actual use of expensive and labour-intensive experiments (*in situ*, *in vivo* methods) with surgically prepared animals sets the challenge to find new approaches. The whole body Lys net flux seems to be an interesting approach in the measurement of AA availability given the pressing need to develop non-invasive but reliable methods for assessing the availability of AA.

Linear increments in milk production with protein supplementation are usually related to exponential increases in nitrogen excretion. It is therefore a challenge for nutritionists to establish the minimal amount of protein and AA required by the high- producing dairy cow to achieve

optimal, but not necessarily maximal, milk production. Future diet formulation models will include the prediction of the amount excreted in the manure and therefore information on nitrogen outputs to the environment will be required to construct and improve these models.

An additional reason for the research is the need to investigate the relative merits of protein vs. energy supplementation as a means of controlling manure N from dairy cattle, thereby protecting the environment.

b) Research Objectives

The research was undertaken with the following objectives:

1. To determine the effects of physical and chemical treatments of soybean meal on amino acid composition, protein and amino acid degradability in the rumen, and on the disappearance of protein and amino acids entering the small intestine.

2. To quantify, based on an *in situ* procedure, the intestinal availability of amino acids from rumen protected SBM products.

3. To determine whether the assessment of AA availability, based on an *in vitro* procedure, could replace the *in situ* method.

4. To investigate whether the determination of AA availability, using a non-surgical procedure that involves measurement of ^{15}N lysine flux, could be used as a non-invasive alternative to the use of cannulated animals for assessing AA availability based on flow of duodenal digesta.

5. To evaluate the responsiveness in Lys availability obtained with the "lysine flux method" when compared to the novel "plasma response" method of Rulquin and Kowalczyk (2003).

6. To determine whether a dietary supply of RUP from rumen protected SBM would increase milk yield and efficiency of N usage for milk production, or alter milk composition.

7. To determine whether the provision of a source of rapidly fermentable energy in the form of pectin, would have an effect on milk production similar to that of supplying RUP.

8. To determine whether reducing dietary protein by 25% below requirements, would reduce manure N excretion to the environment and increase efficiency of N usage for milk production, without affecting milk yield or composition.

4. EXPERIMENT 1

Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products

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4. 1. ABSTRACT

Four lactating dairy cows equipped with ruminal and duodenal cannulas were used to determine the impact of different methods of treating soybean meal (SBM) on ruminal degradability and intestinal digestibility of CP and amino acids (AA). Solvent extracted SBM (SE), expeller SBM (EP), lignosulfonate SBM (LS) and heat and soy hulls SBM (HS) were incubated in the rumen in nylon bags for 48, 24, 16, 8, 4, 2 and 0 h according to NRC (2001). Additional samples of each SBM product were also incubated for 16 h in the rumen; the residues from these bags were transferred to mobile bags, soaked in pepsin HCl, and then used for determination of intestinal digestibility *in situ* or *in vitro.* Treatment of SBM (EP, LS, HS) protected the CP and AA from ruminal degradation, increasing rumen undegradable protein (RUP) from 42% for SE to 68% for EP. Kinetic analysis of CP and AA degradation in the rumen revealed that, compared to LS and HS, EP exhibited slower rates of degradation but shorter lag phase and a higher proportion of soluble protein. For all SBM products, the pattern of ruminal degradation, at 16 h of incubation, was characterized by extensive degradation of Lys and His, while Met and

the branched chain AA were degraded to the least extent. Estimates of intestinal digestibility of AA and CP were lower when measured *in vitro* than *in situ*; the magnitude of the difference between the two methods was greater (25%) with treated SBM products than with SE (10%). The availability of essential and non essential AA was consistently greater (30%) with treated SBM than with SE. Among the treated SBM products, four EAA (Ile, Leu, Phe and Val) showed differences in availability, with values consistently lower for HS than for LS. This study shows that based on *in situ* measures, heat and chemical treatment of SBM enhanced AA availability, and that compared to HS, expeller and lignosulfonate SBM had a higher potential to enhance AA supply to the small intestine of high producing dairy cows.

Abbreviation key: **SBM**= soybean meal, **SE**= solvent extracted soybean meal, **EP**= expeller soybean meal, **LS**= lignosulfonate soybean meal, **HS**= heat and soyhulls soybean meal, **Glx**= Glu plus Gln, **EAA**= essential amino acids, **NEAA** = non-essential amino acids, **BCAA**= branched-chain amino acids.

4. 2. INTRODUCTION

To optimize the amount of absorbable AA for high producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of RUP (Schwab, 1995). Much research has been conducted on rumen degradability of both plant and animal protein supplements (Erasmus et al., 1994; Cozzi et al., 1995; Ceresnáková et al., 2002). However, the North American and European Union regulations on the use of animal protein supplements in ruminant diets (Hasha, 2002; FDA, 2004) are likely to promote even more research into the use of plant protein concentrates. Soybean meal (SBM) is the most commonly used protein supplement for dairy cattle in North America (Statistics Canada, 2003; USDA-NASS,

2004), and amongst oilseed meals, it has the highest content of essential AA (EAA; NRC, 2001). For these reasons, SBM continues to be extensively studied as a source of amino acids for high producing dairy cattle (Ipharraguerre and Clark, 2005).

Soybean meal has been treated in various ways to enhance the quantity of RUP, and several commercial sources of treated SBM are available for use in diets for dairy cattle. The heat-generating expeller process (EP) is a conventional method to extract oil from soybeans; this process results in an increased proportion of RUP from SBM (Liu, 1999) and does not require the use of organic solvents. Treatment of SBM with lignosulfonate (LS) is an alternative method to increase RUP. The process involves a chemical reaction with sulfite liquors, which are byproducts of wood pulp processing. The method takes advantage of the non-enzymatic browning reaction which results in reduced ruminal degradability of the protein (Cleale et al., 1987 a, b; Can and Yilmaz, 2002). Sulfite liquors from paper mills can be a source of environmental pollution under industrial conditions which do not allow for recovery of sulfites (Smook, 1982). Therefore, the sustainability of the use of sulfite liquors in the manufacture of rumen protected SBM products may be in doubt.

The use of heat and soyhulls (HS) is yet another industrial method for protecting SBM protein from ruminal degradation (Heitritter et al., 1998). This method also involves the non-enzymatic browning but it may be more environmentally acceptable than the LS method because it uses a natural ingredient (soyhulls). There is, however, a lack of published information on the HS method and there is a need for scientific studies to evaluate its efficacy. According to Waltz and Stern (1989), treatment of SBM can increase the supply of AA to the duodenum of ruminants by 40 to 70%, and Bateman (2005) and Ipharraguerre and Clark (2005) have concluded that an increase in RUP can increase milk yield. Based on an exhaustive

analysis of data from 35 research publications Bateman et al. (2005) reported that, although DMI is a major source of variation in RUP supply to the small intestine, there are still other unknown factors that influence the apparent RUP content of feeds. Therefore, any increase in the understanding of the relative merits of protein protection methods, would improve the accuracy of predicting RUP and intestinal amino acid supply from SBM.

In addition to furthering knowledge of RUP from treated SBM, there is a need for research on amino acid availability from these products in order to improve current models for protein nutrition in dairy cattle (NRC, 2001; Rulquin et al. 2001 b). These models rely on the assumption that the AA composition or profile of RUP is identical to that of the original feed, and that the effective degradability of individual EAA is similar to that of the protein in the original feed. González et al. (2000) and Ceresnáková et al. (2002) have reported, however, that the AA composition of RUP from SBM can be modified during passage through the rumen. Based on studies in Europe (Rulquin et al. 2001 a, b), models for AA nutrition of dairy cattle could under or over estimate duodenal availability of AA by 13%, depending on the AA under consideration. According to Bateman et al. (2001), however, North American models (including NRC) for dairy cattle nutrition can result in an error as high as 50 % in predicting the flow of EAA to the small intestine.

Published research dealing with kinetics of ruminal degradation and intestinal availability of individual AA from rumen protected SBM products is quite limited (Ipharraguerre and Clark, 2005). Furthermore, the impact of SBM treatment on intestinal digestion of AA does vary. For example, studies conducted with SBM treated with lignosulfonate or formaldehyde have shown no effects on intestinal digestion of AA (O'Mara et al., 1997; Harstad and Prestløkken, 2000; Prestløkken and Rise, 2003). However,

when SBM was treated with heat, there was a reduction in intestinal digestibility of Lys, Arg and His in the protein escaping ruminal degradation (Demjanec et al., 1995; Ceresnáková et al., 2002); the reduction in intestinal digestibility of individual EAA ranged from 5 to 15 %, suggesting that the response of individual AA to heat treatment is also variable. In order to improve existing models for a metabolizable AA system for dairy cattle, additional knowledge is required concerning intestinal availability of individual amino acids in common feeds.

This study was undertaken to evaluate the impact of multiple methods of treating SBM on ruminal degradability and intestinal digestibility of CP and AA and to compare methods *in situ* and *in vitro* to determine intestinal digestibility of AA.

4. 3. MATERIALS AND METHODS

Four multiparous Holstein cows, with an average BW of 649 (± 46.3 kg) and 158 (± 28.7) DIM at the start of the experiment, were utilized for the study. Animal care procedures followed the guidelines of the Canadian Council on Animal Care (CCAC, 1993), and the protocol was approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre in Sherbrooke, Québec (Agriculture and Agri-Food Canada). Animals were housed in tie stalls with water freely available. The TMR (Table 1) was fed ad libitum twice daily, at 0800 and 1600 h, and the animals were milked daily at 0900 and 2000 h.

Four types of SBM supplements were investigated: solvent extracted SBM (SE; ADM Agri-Industries, Windsor, ON), expeller SBM (EP; SoyPLUS® West Central, Ralston, IA), lignosulfonate treated SBM (LS; Surepro® Land O'Lakes Purina Feed LLC, Saint Paul, MN) and heat treated SBM with soy hulls (HS; Aminoplus® Ag Processing Inc., Omaha,

NE). The EP treatment involves feeding cracked whole soybeans into expeller presses with a central revolving shift; this creates pressure within the press causing the mechanical extraction of oil from the soybeans and heating temperatures up to a maximum of 163°C (Liu, 1999). The LS method involves treatment of SBM with 8-25% (wt/wt) sulfite liquor from hardwood or softwood processing; the product is then heated at 95°C for 1 h resulting in "early" Maillard reactions (Friedman, 1996) with xylose. The HS treatment involves mixing SBM with soy hulls (10:1 wt ratio), adding water to achieve 30-50% moisture, and then further cooking at 95°C to obtain a final moisture content of 12-16% (wt/wt).

The cows were equipped with ruminal (Bar Diamond, Parma, ID) and closed T-shaped duodenal cannulae (Berzins Vet Laboratory Ltd., Edmonton, AB) for *in situ* measurements of ruminal degradability and intestinal digestibility. Ruminal incubations *in situ* started after one week of adaptation to the diet. Samples of each SBM product were milled through a 2 mm screen and subsamples (4 g) were then placed in N free polyester bags (9 x 18 cm) with a pore size of 50 ± 15 µm (R1020 Ankom products, Fairport, NY); the ratio of sample size to surface area of each bag was 12.3 mg/ cm^2 (NRC, 2001). Samples of each SBM product were incubated, in duplicate, in the rumen of each cow for 48, 24, 16, 8, 4, 2 and 0 h. Therefore, there were 2 replicates per cow for each time point of incubation. The bags were inserted in reverse order of incubation period, such that they could be all removed at the same time (NRC, 2001). Before incubation, the bags were soaked in water (39°C) for 20 min, attached to a stainless steel weight and placed in the ventral sac of the rumen. Once they were removed from the rumen, the bags were immersed in 20-L buckets containing cold water, then washed in an automatic washing machine (5 x 1 min wash - 2 min spin) until the rinse water was clear. The bags (with residues) were then frozen.

The same four cows were used to ruminally incubate additional samples of each SBM product in order to estimate intestinal disappearance of AA and CP. Four samples of each feed were placed in the rumen of each cow and incubated for 16 h. At the end of the incubation period, the contents of the bags from each cow were weighed, pooled by feed type and transferred to N free polyester bags (3.5 x 5.5 cm, pore size 50 ± 15 µm; R510 Ankom products, Fairport, NY). All bags were placed in 0.1N HCl solution containing pepsin (1 g/ L; Sigma P7000), and incubated for 1 h at 39°C to mimic abomasal digestion. The bags from each cow, for each SBM product, were handled as follows: three were randomly chosen and frozen for subsequent determination of acid-pepsin losses; five were randomly chosen for measurement of *in vitro* intestinal digestibility according to the 3-step procedure described by Calsamiglia and Stern (1995); the remaining five bags were used to estimate intestinal digestibility using the mobile nylon bag technique (Hvelplund and Weisbjerg, 2000). This latter procedure involves introducing the mobile bags, via the duodenal cannula, into the small intestine of the respective cows, at the rate of 1 bag every 30 minutes. Upon recovery from feces, the mobile bags were washed in an automatic washing machine (5 x 1 min wash- 2 min spin) until the rinse water was clear; the bags (with residues) were then frozen.

The frozen residues from all bags used in the ruminal and intestinal incubation (*in vitro* or *in situ)* studies were freeze-dried to minimize N losses. At each time point, the respective residues were then pooled by feed type, for each of the four cows (1 replicate), and used for subsequent chemical analyses.

Analytical methods

The DM content of bag residues was determined by freeze-drying; DM content of feed ingredients and TMR was analysed using a forced air oven

maintained at 60°C for 48 h. Dried samples of the TMR and SBM products were ground to pass a 1 mm screen and analyzed for ash and analytical DM with a thermogravimetric analyzer (Model TGA-601, LECO Corporation, St. Joseph, MI). Fat was determined by gravimetric analysis using ISCO SFX™ 3560 supercritical fluid extraction (ISCO Inc., Lincoln, NE) without co-solvent modifiers for extraction of phospholipids. Analyses of NDF and ADF were performed according to the methods of Van Soest et al. (1991) using the Ankom System (ANKOM 200, Fiber Analyzer, Fairport, NY) with heat stable alpha-amylase and without sodium sulfite. Nitrogen was determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI). Nitrogen fractions, defined according to the Cornell Net Carbohydrate and Protein System (CNCPS), were determined on SBM products using the methods of Licitra et al. (1996).

To analyze AA, samples were ground to pass a 0.5 mm screen; SBM products as well as bag residues were acid-hydrolysed with 6 N phenol-HCl for 24 h at 110°C (AOAC, 2000) and AA concentrations of the hydrolysates were determined by the isotope dilution method (Calder et al., 1999). Briefly, 2 ml of the hydrolysate were diluted with 3 ml of ultra pure water and 1 ml of this solution was then combined with 200µl of a mixture of labelled AA (^{13}C and ^{15}N AA isotope standards; CDN Isotopes, Pointe-Claire, QC; Cambridge Isotope Laboratories Inc., Andover, MA), which served as an internal standard. The solution was eluted through a poly-prep chromatography column (Resin 100-200 mesh H, BIO RAD, Hercules, CA) then derivatized with N-(tert-butyldimethylsilyl)-N methyltrifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) 1:1 (394882, 27.0547 Sigma-Aldrich) according to the method of Calder and Smith (1988). Amino acids were quantified by Gas Chromatography Mass Spectrometry (HP 6890 GC System 5973 and Mass Selective Detector, Hewlett Packard, Palo Alto, CA). The amino acids, Met, Cys, Arg

and Pro were analyzed separately by subjecting the samples to performic acid oxidation, followed by HCl hydrolysis (AOAC, 2000); these four AA were analyzed with an AA Analyser Biochrom 20 (Amersham Pharmacia Biotech, NJ, USA). A study of rumen kinetics was not performed for Met, Cys, Arg and Pro because there was insufficient sample to allow for their analyses at all time points of rumen incubation. Tryptophan was not analyzed because it cannot be determined under conditions of acid hydrolysis.

Calculations and statistical analyses

Data from rumen undegraded residues were corrected for particle loss according to Hvelplund and Weisbjerg (2000). Rumen degradability of CP and AA were calculated according to the model of Ørskov and McDonald (1979) and Denham et al. (1989). Rumen degradation (p) was estimated by iterative least-squares non linear procedure (Proc NLIN) of SAS (2001) which yielded the equation parameters, a, b, c and L, each of which is defined below. The Akaike's Information Criterion (AIC; Akaike 1973) was used to assess the relative performance of the model, with and without a lag phase. A model that included the lag phase was accepted, and is described based in the following equation:

Given: $p = a + b\ (1 - e^{-ct})$;

If $t \leq L$: $p = a$,

If $t > L$: $p = a + b\ [1 - e^{-c\ (t-L)}]$

Where,

p = rumen degradation (%)

a = water soluble fraction (%)

b = degradable fraction (%)

c = degradation rate of b fraction (h^{-1})

t = time (h)

L = lag phase (h)

Effective degradability (ED) was calculated according to the following equation, assuming a rumen passage rate (k) of 0.08 h^{-1}:

$ED\ (\%)=a+[bc/(c+k)]\ e^{-kL}$ (Denham et al., 1989)

Availability of AA was calculated as follows, based on the procedure of Berthiaume et al. (2000):

AA availability (% of original feed)=

$$\frac{(100 - \%\ \text{effective degradability}) \times (\%\ \text{intestinal disappearance } \textit{in situ})}{100}$$

Predicted values for a, b, c, L and ED, as well as estimates of intestinal disappearance and availability were analyzed using a randomized complete block design with the MIXED procedure of SAS (2001). The following model was adopted, with feed and cow as fixed and random effects, respectively:

$Y_{ij} = \mu + F_i + c_j + e_{ij}$

where:

Y_{ij} = value of the variable studied on the i^{th} feed, for the j^{th} cow

μ = overall mean

F_i = fixed effect of the i^{th} feed, i = 1 to 4

c_j = random effect of the j^{th} cow, j = 1 to 4

e_{ij} = random error

Pre-planned orthogonal contrasts were designed to test: 1) untreated vs. treated SBM products; 2) the effect of heat vs. the effect of chemical processing of SBM; 3) the difference between two chemical methods of SBM treatment. The contrasts are summarised as follows:

(1) SE vs. EP, LS, HS

(2) EP vs. LS, HS

(3) LS vs. HS

A concordance correlation coefficient was calculated to measure agreement between *in situ* and *in vitro* measures of intestinal disappearance (Lin 1989; 1992; 2000). The criterion for declaring an effect to be statistically significant was predetermined at the 5% level; between 5 and 10% level of probability, values were considered as expressing a tendency.

4. 4. RESULTS AND DISCUSSION

The chemical composition of the SBM studied is presented in Table 2. The CP content of the SBM products showed very little deviation from 50%. The concentrations of NPN and soluble protein were numerically lower in the treated SBM products than in SE. The EP, LS and HS products were all exposed to higher temperatures during the respective processing methods, and heat denaturation of feed proteins is known to reduce their solubility (Liu, 1999). Compared to NRC (2001), NDF, ADF, neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were higher for SE, EP and LS. A possible explanation is the fact that sodium sulfite was not used in the analysis of fibre, a recommendation made by Van Soest et al. (1991). The increases in NDICP and ADICP for the treated SBM products were also the result of exposure to heat (EP) and chemical reactions (LS, HS) during processing (Demjanec et al., 1995; McKinnon et al., 1995). An increase in NDICP reflects an increase in the feed protein fraction that is slowly degraded in the rumen (Mustafa et al., 2000), while an increase in ADICP is an indication of heat damaged protein which leads to reduced protein digestibility (Faldet et al., 1992; Can and Yilmaz, 2002). The concentration of ADICP in the treated SBM products was higher than that in SE; the

maximum value observed here was 8.2% (EP). In studies of the effects of heat treatment on ruminant digestion of CP, no adverse effects on intestinal disappearance were observed for ADICP between 5.1 to 7.0% (%CP) for canola meal (McKinnon et al., 1995) or up to 10.7% ADICP for roasted SBM (Schroeder et al., 1995; Demjanec et al., 1995).

The contents of NDF and ADF were also higher in the treated products than in SE; these increases are likely to be artefacts of the increases in NDICP and ADICP (Van Soest and Mason, 1991). A similar impact of heat treatment on the protein and fibre fractions was found for SBM (Demjanec et al., 1995). Results of the AA analysis of the SBM products (Table 2) reveal that the AA composition of SE was comparable to that reported by Degussa (1996); values for EP and LS, however, were lower than those reported by NRC (2001). Given that there is no published information on the AA composition of HS, the data provided here would be quite useful in diet formulation for dairy cattle. Both EP and LS contained relatively low concentrations of His, Lys and Arg, suggesting binding and crosslinking reactions involving these AA as a result of treatment method (Adrian 1974; Gerrard 2000); this would reduce their release upon acid hydrolysis (Mauron, 1981).

Rumen Kinetics

Particle losses contributed to the disappearance of N from the rumen bags and this source of N disappearance was higher for EP (15.1% of feed N) than for the other SBM products. The estimates of particle loss were 10.4, 10.0 and 6.9% for SE, LS and HS respectively (data not shown).

Parameter estimates of CP and AA rumen degradation are presented in Table 3. The effective degradation of CP was higher ($P < 0.001$) for SE compared to treated SBM products. This observation is similar to that reported by Waltz and Stern (1989) and Maiga et al. (1996). When SE is

compared with treated SBM products, ruminal degradation of CP decreased from 58% to approximately 35%. Ljøkjel et al. (2000) reported a similar reduction (from 63% to 28%) in ruminal degradation of protein by heating SBM to 130°C. When heat (150°C) was combined with xylose treatment of SBM (3% of total SBM, DM basis), ruminal degradation of CP was reduced from 68 to 35% (Can and Yilmaz, 2002). When SBM is heat treated, as is the case with EP, LS and HS, the protein undergoes denaturation, racemization and cross-linking reactions (Rhee and Rhee, 1981; Friedman et al., 1984); this renders the protein less susceptible to microbial enzymes and leads to a reduction in ruminal degradation (Cleale et al., 1987a; Wallace 1994). The results of this study are consistent with previous reports, and confirm the concept that heat and chemical treatment of SBM protects the protein from ruminal degradation.

Among the treated SBM products (EP, LS, HS), there were no differences in the rate of protein degradation nor in effective degradability. The estimate of protein ED reported for EP was similar to that reported by Waltz and Stern (1989) and Maiga et al. (1996), although neither lag phase nor correction for particle losses was considered in these studies. The estimate of CP degradation for LS is also comparable to that reported by Harstad and Prestløkken (2000) although they observed a higher proportion of soluble protein in their SBM products.

Table 3 shows the results of ruminal degradation of EAA. Estimates of ED and rates of ruminal degradation for all EAA were higher ($P < 0.01$) in SE than in the treated SBM products; the SE product also contained larger ($P < 0.05$) quantities of AA in the soluble fraction. Among the treated SBM products, only the branched chain AA (BCAA) and Phe showed significant differences ($P < 0.05$) in effective degradability. When EP was compared to LS and HS, differences were mainly due to a shorter lag phase ($P < 0.10$) for some EAA (Leu, Lys and Phe, and higher solubility (a) for the BCAA

and Phe *(P <0.05)*. One explanation is that the induction of mild non-enzymatic bonding with carbohydrates alters the polarity and net charges of the AA side groups, thereby affecting the solubility of the protein (Liu, 1999). Compared to HS, LS treatment of SBM significantly *(P <0.05)* decreased effective degradability of the BCAA and Phe. The degradation of the HS SBM product was characterized by a longer lag phase (L; *P <0.05)* but higher soluble fraction (a; *P <0.05)* and higher rate of degradation (c; *P <0.05)* for these EAA. It is reasonable to expect differences between LS and HS because during the treatment process the carbohydrate (CHO) added to LS is xylose, while in HS the main CHO added is pectin. Both types of CHO are involved in the "early" Maillard reactions but the resulting linkages are more stable with simple CHO like xylose compared to insoluble CHO like pectin (Adrian, 1974; Rhee and Rhee, 1981). This would result in a higher degree of protection against microbial degradation for some EAA in LS.

The present study provides novel information on effective degradability of EAA in different rumen protected SBM products; such information would be useful to improve current models for predicting duodenal supply of AA in dairy cattle (NRC, 2001; Rulquin et al., 2001). For example, in the NRC (2001) AA submodel, the independent variables utilised to construct the final predicting equations assume that the AA composition of RUP is identical to that of the CP in the original feed; the model also assumes that the rate of ruminal degradation of CP is the same as that of the individual EAA.

Within each SBM product, the effective degradability of the individual EAA's was variable (Table 3). The estimates of ED for BCAA and Phe in EP and HS were higher than values for the other EAA; furthermore, the ED for BCAA and Phe was not constant across SBM products. Other studies have also reported differences in AA composition of the RUP from

SBM compared to the original feed protein (Crooker et al., 1987; González et al. 2000). The present study, with several rumen protected products, confirms that not only are there differences in the effective degradation between CP and EAA but there are differences in ED amongst the individual EAA. Using, for instance, a single ED value for BCAA and Phe, and one based on the CP of original feed CP effective degradation would lead to errors in the calculation of EAA available for absorption.

Throughout the entire study, no corrections were made for bacterial contamination of the nylon bags. The implication of not correcting for bacterial contamination is that the residues in the bag may not reflect the actual AA profile of the RUP. In a review of factors affecting *in situ* ruminal degradation of protein, Nocek (1985) concluded that it was unnecessary to correct for bacterial contamination in protein supplements like SBM. Varvikko (1986) compared the AA composition of RUP in rumen bag residues with and without correction for microbial contamination. The results showed microbial contamination had no significant influence on the AA composition of RUP from protein supplements. It is, therefore, unlikely that, in the present study, the AA profile of the RUP was influenced by bacterial contamination.

Results of AA degradation after 16h of ruminal incubation are presented in Table 4. These data were collected in order to assess the impact of SBM treatment on the pattern of ruminal degradation of AA at a specific time point. Values for the SE product reveal that His and Lys were degraded to the greatest extent; 79% of the His and 77% of the Lys in SE were degraded, while the sulfur AA (Met and Cys) and the BCAA (Ile, Val, and Leu) were least degraded. Among the treated SBM products, His and Lys were also degraded to a greater extent than the other EAA. These findings contrast with those of Maiga et al. (1996) and Prestløkken and Rise (2003) who reported slower release of Lys and His in expeller and lignosulfonate

treated SBM than in solvent extracted SBM. According to Gerrard (2002), Lys, and His are very reactive AA and this makes them more susceptible to degradation. Steric hindrance of the side chain of the BCAA as well as their hydrophobicity reduces the accessibility of microbial enzymes (Finley, 1985; Liu, 1999); this would explain the relatively slow rates of degradation of the BCAA. Studies by Crooker et al. (1987) and Van Straalen et al. (1997) with solvent extracted SBM have also shown slower degradation of Met and Cys compared to other AA. The treatment of SBM did not alter the pattern of degradation at 16 h; those AA with greater extent of degradation in SE reported also higher degradation in treated sources of SBM (EP, LS, and HS).

Intestinal disappearance

Estimates of intestinal disappearance of CP and AA *in situ* (mobile bag technique) and *in vitro* (three-step, enzymatic procedure) are presented in Table 5. The estimates of *in situ* digestion of CP and AA in SE and LS, are similar to those reported by Ljøkjel et al. (2000) and Prestløkken and Rise (2003) for solvent extracted and lignosulfonate treated SBM. However, the values obtained for SE are lower than those reported by O'Mara et al. (1997), but these authors did not perform the pepsin-HCl step during the procedure. Significant differences in digestion of individual EAA were detected among the SBM products. For example, *in situ* digestion of His was lower ($P < 0.05$) with SE than with the other SBM products but its digestion did not differ ($P > 0.10$) among the treated SBM products. Lysine digestion *in situ* was lower ($P < 0.05$) for EP compared to LS and HS, but there was no difference ($P > 0.10$) in Lys digestion between these two chemical methods of SBM treatment. Despite such statistical differences, the values for AA intestinal disappearance *in situ* never differed by more than 3% across or within SBM products. There may, therefore, be no biologically relevant differences (<3%) in the *in situ* measures of intestinal AA disappearance among the treated SBM products.

The estimate of *in vitro* digestion of CP in SE (88%) is similar to that reported by Calsamiglia and Stern (1995) and by Maiga et al. (1996); however, the values for LS and EP are lower than those reported by these authors. Stern et al. (1997) reported values for *in vitro* intestinal CP digestion of LS that are similar to those reported here, but measurements on EP were not made. Differences in intestinal digestion amongst studies may be due to differences in the sources of SBM products. There are no previously published data on intestinal digestion of CP or AA in HS but *in vitro* digestion of CP in HS was not significantly ($P > 0.10$) different from that of the other treated SBM products. *In vitro* digestion of all EAA and most of the NEAA was significantly greater ($P < 0.05$) with SE than with the treated SBM products. There were no significant differences in *in vitro* digestion of EAA between EP and LS or HS, although values for Ile, Lys and Phe tended ($P < 0.10$) to be greater with HS than with LS. Among the treated SBM products, *in vitro* disappearance of EAA ranged from 65% for Leu to 82% for Lys; this variation in *in vitro* digestion of EAA and NEAA was much greater than that observed for *in situ* digestion of the SBM products. To our knowledge, this is the first report of *in vitro* intestinal digestion of AA and therefore more research will be required to confirm this variability of *in vitro* intestinal digestion of AA in rumen protected SBM products.

Estimates of intestinal digestion of CP and AA obtained *in situ* were all greater than those obtained *in vitro* (Table 5); statistical analyses revealed no agreement (*concordance coefficient* < 0.01) between the two procedures. The magnitude of the difference between the two methods was less for SE (10%) than for the treated SBM products (25%). Stern et al. (1997) also found a difference of 22% between values of intestinal digestion *in vitro* and those estimated with the mobile bag technique for SE and LS. Woods et al. (2003) reported higher values for *in situ*

compared to in *vitro* intestinal digestion of CP in SBM. The higher digestion values for the *in situ* method could be explained by the fact that an artificially designed technique to study enzymatic digestion is unlikely to represent exactly the environment or the function of the intestine (Stern et al., 1997). Furthermore, particle losses from the mobile bags during intestinal transit, or loss of material during machine washing of the bags could be additional factors explaining the higher values for the *in situ* method.

Given the fact that the mobile bags were recovered from feces, another possible explanation for discrepancy between *in situ* and *in vitro* AA disappearance is that hindgut bacteria could have contributed to proteolysis in the *in situ* procedure. However, using the mobile bag technique, Prestløkken and Rise (2003) could find no influence of the site of recovery (ileum or feces) on intestinal disappearance of AA from untreated or lignosulfonate treated SBM. Given the lack of agreement between the results *in vitro* and *in situ*, and the discrepancy with published data for *in vitro* intestinal digestion for EP, it seems that the determination of *in vitro* intestinal disappearance of AA cannot be used as a reliable replacement for *in situ* measurements.

Availability of amino acids

This is the first study with dairy cattle to comprehensively evaluate the kinetics of rumen degradation and intestinal availability of AA in SBM products subjected to multiple methods of rumen protection. Such information is needed for the development of diet formulation models to optimize amino acid nutrition of dairy cattle. Calculations of AA availability were based on the *in situ* procedure, and these results are presented in Table 6. The estimates of AA availability of EAA and NEAA were on average 30 % higher ($P < 0.001$) for treated SBM products than for SE; values for individual EAA in SE ranged from 31% for Met to 40 % for Thr.

In contrast, EAA availability for the treated SBM products ranged from 50% to 71%, depending on the particular EAA and the type of SBM treatment. When EP was compared to LS and HS, there was no significant difference in EAA availability. Only three NEAA (e.g. Ala, Arg and Cys) were significantly lower ($P < 0.053$) for EP compared to LS. When compared to LS, the HS SBM product had lower availability values ($P < 0.05$) for the BCAA and Phe. This difference is explained mainly by the significantly higher rumen degradability of BCAA and Phe for HS (Table 3). The present study shows that the main factor contributing to differences in AA availability amongst the SBM products was the difference in rumen degradability; differences in intestinal digestion were minimal.

There is a scarcity of *in vivo* data on intestinal supply of AA from rumen protected SBM products; even less has been published on the availability of EAA from such supplements (Ipharraguerre and Clark, 2005). In a study with wethers fed heated SBM contributing 20% of the dietary CP, Demjanec et al. (1995), observed a linear effect of time of roasting on duodenal flow of non-bacterial AA; there was a significant increase in intestinal disappearance for most EAA but not for Lys and Met. Based on rumen and mobile bag techniques, Schroeder et al. (1995) have also shown that heating SBM increased the amount of available AA in the small intestine of dairy cows. Ipharraguerre et al. (2005) quantified the flow to the duodenum of AA in the non-ammonia non-microbial fraction when expeller SBM or xylose treated SBM partially replaced solvent extracted SBM in the diet of dairy cattle. These authors found that expeller and xylose treatment of SBM tended to increase the duodenal flow of total AA, and that xylose treatment increased the flow of some EAA. However, these authors made no attempt to measure intestinal availability of EAA in the RUP fraction of the SBM products.

4. 5. CONCLUSIONS Experiment 1

Solvent extracted SBM exhibited greater effective degradability of CP and AA when compared to treated SBM products, expeller SBM, lignosulfonate treated SBM, and SBM treated with heat and soyhulls. This was due to a greater fraction of soluble protein and a faster rate of rumen degradation of protein in solvent extracted SBM. Treatment of SBM protected the CP and EAA from ruminal degradation, thereby increasing RUP from 42% to 68%. Estimates of effective degradability of BCAA and Phe differed significantly among the treated SBM products but for the remaining EAA, rumen degradability was similar for all products. Irrespective of the SBM product, the pattern of EAA degradation, after 16 h of ruminal incubation, was characterized by a rapid rate of degradation of Lys and His, and a slower degradation of Met and BCAA compared to that of the remaining EAA. *In situ* intestinal disappearance of AA ranged from 97 to 100% and all values were greater than those obtained *in vitro*. As the magnitude of the difference between the two methods was greater (25%) with treated SBM products than with SE (10%), further development of the *in vitro* method should be considered before it can be used to replace the *in situ* procedure. There were no differences in EAA availability between expeller (EP) SBM and SBM treated with lignosulfonate (LS) or soyhulls (HS). However, when the latter two chemical methods were compared, EAA availability was lower with HS than with LS. The differences were due mainly to lower values of available BCAA and Phe. The results of this *in situ* study suggest that expeller and lignosulfonate treatment of SBM could be used as an effective means of enhancing the supply of EAA to the small intestine of dairy cattle. The use of soyhulls to protect SBM was not as efficacious as the other treatment methods for some EAA.

4. 6. TABLES EXPERIMENT 1

Table 1: Ingredients and chemical composition of the basal diet fed during the *in situ* experiment.

Ingredients, % DM	
Red Clover - timothy silage	33.0
Corn silage	32.0
High moisture shelled corn	21.0
Solvent extracted soybean meal	11.0
Vitamin – mineral supplement[1]	3.0
Chemical Composition, % DM	
DM	40.0
CP	16.5
Fat	3.1
NDF	38.6
ADF	25.7
Ash	8.2

[1] 10.8% Ca; 5.5% P; 3.5% Mg; 13.2% Na; 1.4% K; 2.1% S; 370,000 IU/kg of Vitamin A; 65,000 IU/kg of Vitamin D_3; 1,800 IU/kg of Vitamin E.

Table 2: Chemical composition and AA content of soybean meal subjected to different methods of treatment .

	Treatment[1]			
Item, % of DM	SE	EP	LS	HS
CP	51.8	47.8	49.2	51.1
NPN, %CP	20.2	16.5	17.1	17.8
Soluble CP, % CP	29.0	18.6	13.9	15.6
Neutral detergent insoluble CP, %CP	4.0	29.5	38.6	29.3
Acid detergent insoluble CP, %CP	1.8	8.2	7.6	3.6
NDF	11.5	29.5	33.0	31.0
ADF	6.3	13.8	12.3	10.1
Fat [2]	0.9	4.2	1.0	0.8
Ash	7.3	7.1	7.8	7.2
Essential AA, % DM				
His	1.26	1.13	1.23	1.29
Ile	2.08	2.10	2.15	2.23
Leu	3.81	3.67	3.69	3.87
Lys	2.99	2.58	2.67	2.89
Met	0.69	0.58	0.66	0.66
Phe	2.44	2.36	2.40	2.49
Thr	1.98	1.90	1.93	2.00
Val	2.12	2.16	2.20	2.28
Non- essential AA, % DM				
Ala	2.15	2.08	2.07	2.17
Arg	3.64	3.24	3.51	3.64
Cys	0.69	0.56	0.64	0.63
Glx [3]	7.74	7.25	7.18	7.94
Gly	2.05	2.08	2.08	2.14
Pro	2.05	1.85	1.95	2.07
Ser	2.57	2.45	2.45	2.56
Tyr	1.81	1.78	1.79	1.80

[1] SE = solvent extracted; EP=expeller; LS=lignosulfonate; HS=heat and soy hulls
[2] excludes phospholipids
[3] Glx = Glu plus Gln

Table 3: Effects of different methods of treatment of soybean meal on the kinetics of ruminal degradation[1] of CP and essential AA

	Treatment[2]				SEM	Contrasts		
Item[3,4]	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS, HS	LS vs HS
ED (%)[5]								
CP	58.3	32.2	38.4	35.4	4.53	<0.001	0.320	0.586
His	66.8	31.7	37.6	36.8	4.59	<0.001	0.206	0.873
Ile	61.9	37.7	34.8	47.9	3.82	<0.001	0.156	0.003
Leu	63.2	37.8	35.7	44.9	4.18	<0.001	0.360	0.025
Lys	61.9	34.0	34.5	36.4	3.91	<0.001	0.656	0.653
Phe	63.7	37.7	35.6	45.4	4.28	<0.001	0.338	0.022
Thr	57.1	32.4	36.4	38.5	4.25	0.003	0.316	0.715
Val	61.0	37.9	31.7	45.5	3.07	<0.001	0.829	0.008
L (h)								
CP	2.0	2.2	9.2	6.5	2.99	0.224	0.115	0.516
His	4.1	2.1	8.1	6.8	3.28	0.645	0.135	0.754
Ile	6.1	7.6	8.0	16.3	4.82	0.135	0.119	0.035
Leu	6.4	7.7	8.7	14.8	4.36	0.064	0.053	0.027
Lys	3.1	2.3	6.6	6.9	2.56	0.412	0.098	0.924
Phe	6.4	7.8	8.8	14.9	4.33	0.057	0.052	0.024
Thr	1.5	2.6	8.7	8.5	3.57	0.245	0.168	0.965
Val	5.9	7.5	6.5	15.3	4.21	0.207	0.243	0.033
a (%)								
CP	9.9	8.4	7.7	6.4	1.46	0.098	0.354	0.487
His	10.4	5.9	6.3	6.8	1.54	0.036	0.707	0.771
Ile	9.2	9.2	5.1	8.7	0.99	0.074	0.013	0.004
Leu	10.1	9.7	5.1	8.4	1.41	0.058	0.018	0.031
Lys	10.7	7.8	5.0	6.4	1.61	0.020	0.156	0.431
Phe	10.2	9.5	5.3	8.6	1.39	0.064	0.041	0.038
Thr	7.1	8.1	5.5	6.7	2.22	0.900	0.399	0.675
Val	9.0	9.2	3.9	8.3	0.90	0.079	0.009	0.005
b (%)								
CP	87.0	88.5	88.4	90.4	1.52	0.203	0.607	0.337
His	85.6	91.0	90.2	90.2	1.54	0.020	0.614	0.999
Ile	88.5	86.5	91.9	88.9	1.14	0.633	0.017	0.092
Leu	87.4	85.9	90.7	89.3	1.48	0.469	0.030	0.480
Lys	85.8	89.1	92.0	90.7	1.64	0.011	0.149	0.436
Phe	86.9	86.0	91.7	88.7	1.58	0.232	0.017	0.116
Thr	89.3	88.6	91.5	90.3	2.38	0.724	0.345	0.693
Val	85.5	85.6	93.1	88.9	2.08	0.157	0.049	0.174
c (h^{-1})								
CP	0.105	0.030	0.044	0.038	0.0012	<0.001	0.363	0.706
His	0.188	0.032	0.044	0.041	0.0331	0.004	0.771	0.946
Ile	0.111	0.041	0.039	0.062	0.0076	<0.001	0.131	0.013
Leu	0.117	0.041	0.042	0.055	0.0075	<0.001	0.116	0.049
Lys	0.122	0.034	0.039	0.041	0.0065	<0.001	0.193	0.648
Phe	0.120	0.040	0.040	0.056	0.0079	<0.001	0.154	0.039
Thr	0.102	0.031	0.042	0.044	0.0068	<0.001	0.138	0.773
Val	0.125	0.042	0.034	0.057	0.0085	<0.001	0.680	0.083

[1] Rumen degradation estimated using the *in sacco* technique

[2] SE = solvent extracted; EP = expeller; LS = lignosulfonate; HS = heat and soy hulls

[3] Model parameters of rumen degradability (p) according to Orskov and McDonald (1979) and Denham et al. (1989):
If lag time L=0 : $p = a + b\,(1- e^{-c\,t})$,
if lag time L>0 : if $t \leq L$: $p = a$ and if $t>L$: $p = a + b\,[1- e^{-c\,(t-L)}]$;

[4] Methionine rumen degradation kinetics was not determined

[5] ED = effective degradability, $a +[bc/(c+k)]\, e^{-kL}$ Denham et al. (1989); estimated with a rate of passage (k) = 0.08 h^{-1}

Table 4: Effects of different methods of treatment of soybean meal on the extent of CP and AA disappearance at 16 h rumen incubation[2]

	Treatments[1]				SEM	Contrasts		
	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS, HS	LS vs HS
CP, %	70.2	37.8	34.9	30.0	1.57	<0.001	<0.001	<0.001
Essential AA,%								
His	78.5	35.2	32.9	31.7	5.56	<0.001	0.244	0.662
Ile	74.4	31.7	29.4	26.3	6.56	<0.001	0.288	0.452
Leu	74.8	32.5	29.2	26.6	5.85	<0.001	0.180	0.481
Lys	77.3	36.2	30.9	30.3	5.65	<0.001	0.049	0.838
Met	68.7	32.2	28.7	28.1	1.67	<0.001	0.092	0.809
Phe	76.0	32.0	27.8	26.5	5.68	<0.001	0.110	0.689
Thr	75.1	33.8	29.3	28.0	5.55	<0.001	0.088	0.703
Val	74.2	32.5	28.2	26.6	6.65	<0.001	0.168	0.693
Non- essential AA,%								
Ala	74.6	32.5	28.8	27.4	5.58	<0.001	0.130	0.666
Arg	74.7	38.5	35.9	29.9	1.37	<0.001	0.008	0.013
Cys	67.7	39.2	33.8	28.6	1.84	<0.001	0.005	0.066
Glx[3]	71.8	38.3	36.8	27.0	1.31	<0.001	0.002	0.004
Gly	75.5	34.4	30.5	28.9	5.36	<0.001	0.088	0.578
Pro	68.1	32.6	29.8	24.9	3.09	<0.001	0.187	0.275
Ser	75.8	34.3	28.4	25.8	5.42	<0.001	0.029	0.440
Tyr	74.1	31.4	27.6	24.7	5.77	<0.001	0.106	0.407

[1] SE = solvent extracted; EP = expeller; LS = lignosulfonate; HS = heat and soy hulls

[2] % Rumen degradation of the treatments at 16 h incubation (*in situ* technique)

[3] Glx = Glu plus Gln

Table 5: Effects of different methods of treatment of soybean meal on intestinal disappearance of CP and AA *in situ* and *in vitro*[2]

	Treatments[1]				SEM	Contrasts			Treatments[1]				SEM	Contrasts		
	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS,HS	LS vs HS	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS, HS	LS vs HS
	In situ[3] disappearance								*in vitro*[4] disappearance							
CP, %	98.5	98.4	99.1	98.9	0.12	0.010	<0.001	0.078	87.5	76.7	74.3	79.4	3.21	0.003	0.968	0.147
Essential AA,%																
His	97.6	98.1	98.9	98.5	0.36	0.025	0.143	0.434	87.6	72.6	74.8	84.0	3.60	0.022	0.151	0.088
Ile	99.3	99.3	99.6	99.4	0.09	0.050	0.007	0.016	89.5	72.8	73.6	83.6	2.96	0 .002	0.094	0.020
Leu	99.2	99.3	99.6	99.4	0.09	0.007	0.021	0.013	86.7	67.3	67.5	86.7	2.80	<0 .001	0.144	0.028
Lys	98.5	98.7	99.3	99.0	0.17	0.008	0.022	0.217	97.8	84.4	82.2	87.8	2.50	<0 .001	0.817	0.092
Met	100.0	100.0	100.0	100.0	-	-	-	-	85.7	76.6	71.2	76.5	3.87	0.036	0.568	0.354
Phe	99.0	99.3	99.6	99.2	0.11	0.015	0.510	0.027	87.7	69.9	69.3	87.7	2.23	<0 .001	0.158	0.013
Thr	98.8	99.1	99.5	99.2	0.14	0.017	0.154	0.170	90.1	73.9	73.7	82.7	3.10	0 .003	0.241	0.059
Val	99.0	99.0	99.5	99.1	0.13	0.077	0.017	0.054	92.3	74.5	76.0	80.4	3.17	0 .001	0.315	0.303
Non- essential AA,%																
Ala	99.0	99.1	99.5	99.2	0.15	0.031	0.036	0.125	89.8	71.7	72.6	80.3	2.48	<0 .001	0.112	0.036
Arg	99.7	99.8	99.8	99.8	0.137	0.287	0.793	0.955	80.1	78.1	60.5	67.2	8.31	0.102	0.053	0.141
Cys	98.5	100.0	99.5	100.0	0.37	0.009	0.533	0.290	90.5	83.9	75.5	81.6	4.18	0.065	0.323	0.326
Glx[5]	99.1	99.4	99.6	99.5	0.15	0.015	0.356	0.489	88.0	74.1	71.5	76.8	3.91	0.013	0.995	0.364
Gly	97.3	97.8	98.7	98.4	0.40	0.015	0.054	0.464	90.7	73.8	75.1	84.9	2.59	0 .001	0.062	0.017
Pro	100.0	100.0	100.0	100.0	-	-	-	-	82.5	67.0	63.4	75.9	3.91	0.012	0.582	0.043
Ser	98.0	98.4	99.1	98.7	0.32	0.030	0.172	0.433	90.2	67.6	68.7	74.6	2.87	<0 .001	0.226	0.131
Tyr	98.2	98.0	99.0	98.4	0.18	0.070	0.004	0.041	94.8	80.0	73.4	85.3	5.01	0 .007	0.887	0.064

[1] SE = solvent extracted; EP = expeller; LS = lignosulfonate; HS = heat and soy hulls

[2] performed on rumen degradation residues after 16 h incubation (*in situ* technique)

[3] % of duodenal degradation of 16 h rumen residues *in situ* (mobile bags)

[4] % of *in vitro* digestion of 16 h rumen residue (Calsamiglia and Stern, 1995)

[5] Glx = Glu plus Gln

Table 6: Effects of different methods of treatment of soybean meal on essential and non essential AA availability[1] (% of original feed)

	Treatments[2]				SEM	Contrasts		
	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS, HS	LS vs HS
Essential AA,%								
His	32.7	69.3	62.0	62.8	5.74	0.001	0.243	0.901
Ile	38.0	62.1	65.1	51.9	3.80	<0.001	0.163	0.003
Leu	36.7	62.0	64.1	54.8	4.15	<0.001	0.373	0.024
Lys	37.7	65.6	65.2	63.9	4.68	<0.001	0.789	0.801
Met	31.3	67.8	71.3	71.9	1.67	<0.001	0.092	0.809
Phe	36.1	62.0	64.3	54.2	4.25	<0.001	0.339	0.020
Thr	40.4	67.2	65.3	55.5	4.45	0.011	0.186	0.166
Val	38.0	61.7	68.1	50.2	3.56	<0.001	0.373	0.002
Non- essential AA,%								
Ala	35.9	62.0	63.5	49.7	4.62	<0.001	0.052	0.008
Arg	25.2	61.3	63.9	69.9	1.35	<0.001	0.008	0.012
Cys	31.9	60.8	65.8	71.4	1.90	<0.001	0.006	0.055
Glx[3]	33.2	67.6	59.8	61.2	5.34	<0.001	0.146	0.807
Gly	37.9	66.3	62.8	64.4	5.12	0.001	0.571	0.792
Pro	31.8	67.4	70.2	75.0	3.09	0.002	0.187	0.275
Ser	37.3	66.9	64.7	55.5	4.15	0.001	0.129	0.124
Tyr	37.4	62.2	64.1	60.1	6.66	0.003	0.980	0.575

[1] (100 - % effective ruminal degradation) x % intestinal disappearance *in situ* ÷ 100

[2] SE = solvent extracted; EP = expeller; LS = lignosulfonate; HS = heat and soy hulls

[3] Glx = Glu plus Gln

4. 7. APPENDIX TABLES EXPERIMENT 1

Appendix Table1: Effects of different methods of treatment of soybean meal on the kinetics of ruminal degradation[1] of non essential AA

	Treatment[2]				SEM	Contrasts		
Item[3]	SE	EP	LS	HS	(n=4)	SE vs others	EP vs LS, HS	LS vs HS
ED(%)[4]								
Ala	60.9	37.6	36.2	38.8	4.82	0.003	0.973	0.689
Glx[5]	66.8	32.2	39.0	38.4	4.16	<0.001	0.100	0.897
Gly	61.4	32.8	36.7	31.6	4.22	<0.001	0.777	0.387
Ser	62.3	32.5	35.7	44.4	3.21	<0.001	0.035	0.052
Tyr	62.3	37.0	35.5	38.7	5.60	0.001	0.987	0.581
L (h)								
Ala	6.2	7.3	8.8	12.1	4.68	0.317	0.317	0.390
Glx[5]	4.1	1.6	10.1	8.0	3.38	0.456	0.044	0.588
Gly	3.1	2.7	8.5	4.6	3.54	0.589	0.314	0.407
Ser	2.8	2.3	8.7	11.5	3.04	0.204	0.056	0.514
Tyr	6.4	7.5	8.9	12.3	4.80	0.238	0.232	0.274
a (%)								
Ala	10.9	10.3	5.8	6.9	1.98	0.156	0.085	0.641
Glx[5]	11.6	7.0	6.6	7.9	1.68	0.046	0.900	0.557
Gly	10.7	8.7	6.7	4.6	1.97	0.093	0.166	0.424
Ser	10.9	8.0	5.0	7.9	1.34	0.013	0.205	0.079
Tyr	10.2	9.8	5.8	6.2	1.93	0.131	0.050	0.851
b (%)								
Ala	85.7	86.7	91.2	90.0	2.01	0.118	0.085	0.621
Glx[5]	84.9	89.8	90.4	89.1	1.81	0.046	0.978	0.589
Gly	86.1	87.0	90.3	92.5	2.26	0.117	0.073	0.414
Ser	85.1	88.7	92.0	89.1	1.54	0.006	0.168	0.092
Tyr	86.8	87.2	91.2	90.8	1.93	0.131	0.050	0.851
c (h^{-1})								
Ala	0.12	0.04	0.04	0.05	0.008	<0.001	0.460	0.479
Glx[5]	0.18	0.03	0.04	0.04	0.029	0.003	0.670	0.946
Gly	0.12	0.03	0.04	0.03	0.007	<0.001	0.376	0.453
Ser	0.12	0.03	0.04	0.06	0.006	<0.001	0.004	0.034
Tyr	0.11	0.04	0.04	0.05	0.009	<0.001	0.464	0.407

[1] Rumen degradation estimated using the *in sacco* technique

[2] SE = solvent extracted; EP = expeller; LS = lignosulfonate; HS = heat and soy hulls

[3] Model parameters of rumen degradability (p) according to Orskov and McDonald (1979) and Denham et al. (1989):
if lag time L=0 : $p = a + b\,(1- e^{-c\,t})$,
if lag time L≠0 : if $t \leq L$: $p = a$ and if $t>L$: $p = a + b\,[1- e^{-c\,(t-L)}]$;

[4] ED = effective degradability, $a +[bc/(c+k)]\, e^{-k\,L}$ Denham et al. (1989); estimated with a rate of passage (k)= 8% h^{-1}

[5] Glx = Glu plus Gln

5. Connecting statement

In Experiment 1, using the in situ method, results showed that the amino acids availability was 30% higher in all the treated soybean meal when compared to solvent extracted soybean meal. The results also showed that treatment with soyhulls was not a suitable method for rumen protection of soybean meal because some of the essential amino acids were more degraded in the rumen with the heat and soyhulls treatment compared to expeller or lignosulfonate. Therefore subsequent investigations were conducted with expeller and lignosulfonate treated soybean meal products.

Because of the importance of lysine as the first limiting amino acids in dairy cattle diets and because of its susceptibility to heat damage following processing to increase rumen undegradable protein, emphasis was placed on investigating the availability of lysine under normal conditions of feeding where treated and untreated soybean meal were included as dietary supplements.

In order to account for physiological aspects of digestion and with the aim to develop new and less invasive techniques, another experiment was conducted to test three different methodologies in order to measure lysine availability.

6. EXPERIMENT 2

Towards Non-invasive Methods to Determine the Effect of Treatment of Soybean Meal on Lysine Availability in Dairy Cows

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6. 1. ABSTRACT

In an attempt to develop a non-invasive method of determining the effect of processing on lysine (Lys) availability in rumen protected soybean meal (SBM) products, the technique of whole body net flux of Lys (Lys flux) was compared with the measurement of intestinally digestible Lys (duodenal flow * intestinal digestibility), and with the plasma Lys response curve method of Rulquin and Kowalczyk (2003). Four multiparous Holstein cows (173 DIM) with ruminal and duodenal cannulas were used in a 4 x 4 Latin square with 14-d periods. They were fed either solvent extracted SBM (SE), expeller SBM (EP) or lignosulfonate treated SBM (LS) as 23% of the diet. The fourth treatment (SE70) consisted in a 70 $g.d^{-1}$ omasal infusion of Lys to cows fed the SE diet. On the last day of each period, 6 blood samples were collected to determine plasma Lys and immediately after, a pulse dose of [2-^{15} N] Lys was given in the jugular vein and jugular plasma samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 16, 19, 25 and 31 min after the injection to determine ^{15}N Lys enrichment. Chromic oxide was included as a digesta marker in order to determine duodenal flow of amino acids (AA). Results of the Lys flux method

revealed no differences in Lys availability among the SBM products. However, the SE70 treatment increased Lys flux by exactly 70 $g.d^{-1}$ above the SE treatment. No differences were observed on duodenal Lys flow, post-ruminal digestion and digestible Lys between the SBM diets (SE, EP, LS). The use of the plasma response curve method confirmed that there were no differences in Lys availability between the different SBM products. Plasma Lys flux was an accurate technique to estimate the increment of Lys availability, as it responded quantitatively to Lys infusion. Further studies should be performed to test the sensitivity of the lysine flux method and to validate underlying assumptions.

Abbreviation key: SBM= soybean meal, **SE**= solvent extracted soybean meal, **EP**= expeller soybean meal, **LS**= lignosulfonate soybean meal, **SE70**= SE+Lys omasal infusion; **EAA**= essential amino acids, **NEAA** = non essential amino acids, **WB**= whole body

6. 2. INTRODUCTION

Dairy cattle fitted with ruminal and intestinal cannulas are often used to estimate the flow and disappearance of nutrients from different segments of the digestive tract (Titgemeyer, 1997), and for determining the supply of essential amino acids (EAA) from feedstuffs (Windschitl and Stern, 1988; Ludden and Cecava, 1995; Mabjeesh et al., 1996). Although helpful, cannulation methods are expensive and labour-intensive, and are subject to errors associated with use of digesta and microbial markers (Stern et al. 1997). Furthermore, when research protocols require much surgical and other manipulation of animals (invasive procedures), questions arise concerning animal welfare and the applicability of such results to intact animals (Swanson, 1994).

Alternative procedures, such as the nylon bag *in situ* technique, also require the use of cannulated animals and do not account for the physiological aspects of digestion, such as mastication and digesta passage. In an attempt to avoid surgically prepared animals and the attendant cumbersome manipulations, researchers may utilize *in vitro* digestion procedures. However, results of amino acid (AA) availability based on *in vitro* procedures are not always consistent with those obtained *in situ* (van de Poel et al., 1991; Stern et al. 1997; Borucki Castro et al., 2006).

Rulquin and Kowalczyk (2003) proposed an approach to determining EAA availability, which relies on the dose response in plasma to duodenal infusion of the test AA. These authors concluded that the technique yielded reliable estimates for availability of methionine but that further tests should be performed before the method could be used for assessing lysine availability.

Isotope dilution techniques have been used to estimate whole body utilization of EAA (Cronjé et al., 1992; Bequette et al., 1996; Pacheco et al., 2003), and changes in the plasma flux of lysine and leucine have been shown to reflect the dietary supply and needs of these EAA in lambs (Cronjé et al., 1992). For these reasons, this metabolic approach has the potential to provide knowledge of the availability of EAA in protein supplements. The flux (Q) of an AA represents the sum of the rate at which the AA leaves the measured compartment (plasma or intracellular pool) for protein synthesis (S) and the rate at which the AA is oxidized (O). Under steady state conditions, this flux equals the sum of the rate at which the AA enters the pool due to absorption/ intake (I) and protein breakdown (B) (Waterlow et al., 1978; Lobley, 1993). The mathematical relationship is as follows: Q = S+O = I+B. Once the EAA under study is not limiting in the basal diet, an increment in absorption of this EAA should not alter

protein synthesis nor protein retention (Harris et al, 1994); protein breakdown, therefore, should remain unchanged. Under such a scenario, any change in flux of the test EAA would estimate a change in absorption and hence, the bioavailability of the EAA in question. Thus, measurements of whole body Lys net flux in plasma (lysine flux) could be a useful non-invasive method and a good metabolic procedure for determining Lys availability in rumen protected protein supplements. The pressing need to improve models for amino acid nutrition of dairy cattle (NRC, 2001; Rulquin et al., 2001) requires the development of non-invasive but reliable methods for assessing the availability of EAA.

The objectives of this study were to: 1) determine whether measurements of lysine flux could be used to determine Lys availability in rumen protected SBM products; 2) to evaluate the responsiveness in Lys availability obtained with the "flux method" when compared to the plasma response method of Rulquin and Kowalczyk (2003) or a method that combines duodenal flow and intestinal digestion of Lys.

6. 3. MATERIALS AND METHODS

Animals and Treatments

Four multiparous Holstein cows, averaging 649 ± 46.3 kg BW and 173 ± 28.7 DIM at the beginning of the study, were used for the experiment. The study was designed as a 4 x 4 Latin square, balanced for residual effects (Cochran and Cox, 1957), with 14-d experimental periods. All cows were equipped with ruminal (Bar Diamond, Parma, ID) and closed T-shaped duodenal cannulas (Berzins Vet Laboratory Ltd., Edmonton, AB). Animal care procedures followed the guidelines of the Canadian Council on Animal Care (CCAC, 1993) and the protocol was approved by the

Institutional Animal Care Committee of the Dairy and Swine Research Centre at Sherbrooke, QC (Agriculture and Agri-Food Ca).

The cows were housed in tie stalls and fed a TMR (Table 1) at 95% of ad libitum intake, as measured prior to the initiation of the experiment. Water was freely available to all cows and they were milked twice each day at 0900 and 2000 h. To achieve steady state conditions, the diets were offered in 12 equal meals, at two-hour intervals, using automated feeders (Ankom, Fairport, NY). The diets consisted of 1) solvent extracted SBM (SE; ADM Agri-Industries, Windsor, ON); 2) expeller processed SBM (EP; SoyPLUS® West Central, Ralston, IA); and 3) lignosulfonate treated SBM (LS; Surepro® Land O'Lakes Purina Feed LLC, Saint Paul, MN). The fourth treatment (SE70) represented an infusion of Lys into the omasum of cows fed the SE diet. Lysine was infused as lysine monohydrochloride (98.5% pure, Ajinomoto Heartland Inc, Eddyville, IA) at the rate of 70 $g.d^{-1}$. This infusion rate was selected to ensure an amount of duodenally available Lys that was 41 $g.d^{-1}$ greater than that predicted by NRC (2001) for the LS diet. The Lys to be infused was diluted in purified water. The solution was then adjusted to pH 3.0 with HCl (final concentration 0.01M) and infused at the rate of 150 $ml.h^{-1}$. The infusion was performed with a peristaltic pump (Masterflex®; Cole Parmer Instrument Co.; Vernon Hills, IL) and the infusion tube was positioned to deliver the solution directly into the omasum (Huhtanen et al., 1997). Cows receiving treatments SE, EP, and LS were infused with saline (0.9%NaCl) via the omasum at an equivalent rate (150 $ml.h^{-1}$).

Each SBM supplement comprised 23 % (DM basis) of the TMR and accounted for approximately 55% of the total dietary protein. The treatments were designed to provide incremental amounts (SE<EP<LS) of duodenal Lys based on the nutrient requirements of dairy cattle (NRC, 2001). To avoid the possibility of restricting rumen microbial protein

synthesis, urea was added at the level of 1% (DM basis) to the EP diet and at 1.4% to the LS diet; this ensured a minimum RDP balance of +100g (NRC, 2001). To assess nutrient flow through the gastrointestinal tract, chromic oxide (5 $kg.ton^{-1}$, fresh basis) was used as a digesta marker; it was mixed with ground corn, and the mixture was pelleted and incorporated in the TMR.

Sampling

The cows were allowed to adapt to their respective diets for 10 d. Samples of the individual diets and orts were then collected on each of the four subsequent days. Milk production was recorded from days 5 to 14. Milk samples were collected daily from days 10 to 14 during the morning and afternoon milkings, and pooled daily in relation to milk production.

On day 12 of each experimental period, a total of six blood samples were collected in order to determine the plasma concentration of Lys, prior to the infusion of ^{15}N lysine. Blood samples were collected at 45 min intervals (covering two feeding cycles), beginning at 0900 and ending at 1245. Immediately after this procedure, a pulse dose of labelled [2-^{15}N] Lys (0.5g/ 5ml saline) was injected into the jugular vein of each cow. Blood samples were collected from the ipsolateral jugular vein at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 16, 19, 25 and 31 min after the bolus injection. To facilitate blood collection, indwelling catheters were inserted in the jugular vein of each cow, one day prior to blood sampling. All blood samples were maintained on ice and then centrifuged at 1,500 *g* for 12 min. To determine plasma Lys concentration, an internal standard consisting of a mixture (200μg) of labelled AA (Raggio et al., 2004) was added to plasma (1 g) harvested from blood collected before the ^{15}N Lys infusion. All processed samples of plasma were frozen at -80°C for subsequent analysis of Lys concentration and isotopic enrichment (IE).

Samples of ruminal fluid (1 L), duodenal digesta (250 ml) and feces (grab samples, 200 g) were collected at 0900, 1100, 1300 and 1500 h on day 13, and at 0800, 1000, 1200 and 1400 h on day 14; all samples were pooled by period. Digesta pH was determined immediately with a pH meter (Accumet 1001, Fischer).

Plasma lysine response curve

At the end of the 4th period of the experiment, the same four cows were used to determine Lys availability based on the plasma response procedure reported by Rulquin and Kowalczyck (2003). This study was designed as two consecutive 4 x 4 Latin Squares each consisting of 4-d experimental periods. The cows were offered the SE diet, and restricted on feed to 95% of ad libitum intake. The diet was fed using automated feeders and was provided in 12 equal meals. In the first Latin Square, the cows were infused, via the omasum, with graded amounts of Lys at the rates of 0, 28, 56 and 84 g.d^{-1}; in the second Latin Square, Lys was infused at the rates of 0, 14, 42 and 70 g.d^{-1}. The preparation and infusion of Lys were performed as described earlier. On day 4 of each experimental period, six blood samples were collected from the jugular vein at 45 min intervals, starting at 0900 and finishing at 1245 (covering 2 feeding cycles). Plasma samples were prepared for lysine analysis, as previously described.

Samples of diets were taken once each week, and milk samples were collected on days 3 and 4 of each experimental period. The relationship between the amount of Lys infused and the concentration of plasma Lys yielded a "plasma response curve", which was used to estimate "available Lys" for the different SBM products. The treatment representing omasal infusion of Lys (SE70, Table 1) served as a "quality control" to assess the recovery of omasally infused lysine.

Analytical methods

Samples of individual feed ingredients, the TMR and digesta were freeze-dried and ground to pass a 1 mm screen. Ash and analytical DM were determined with a thermogravimetric analyzer (Model TGA-601, LECO Corporation, St. Joseph, MI). Nitrogen was determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI). Fat was determined by gravimetric analysis using ISCO SFX™ 3560 supercritical fluid extraction (ISCO Inc., Lincoln, NE), without co-solvent modifiers for extraction of phospholipids. Analyses of NDF and ADF were performed according to the methods of Van Soest et al. (1991) using the Ankom System (ANKOM 200, Fiber Analyzer, Fairport, NY), with heat stable alpha-amylase and without sodium sulfite.

To analyze AA concentrations, samples of feed ingredients, TMR, and digesta were ground to pass a 0.5 mm screen. Samples were acid-hydrolysed with 6- N phenol-HCl for 24 h at 110°C (AOAC, 2000) and the AA concentrations were determined by the isotope dilution method of Calder et al. (1999). Briefly, 2 g of the hydrolysate were diluted with 3 g of ultra pure water; 1 g of this solution was then combined with a mixture (200 µg) of labelled AA (^{13}C and ^{15}N AA isotope standards, CDN Isotopes, Pointe-Claire, QC; Cambridge Isotope Laboratories Inc., Andover, MA), which served as an internal standard (Raggio et al., 2004). Plasma samples were deproteinized with sulfosalicylic acid (38%) and centrifuged at 27,768 *g* for 10 min. Samples of the supernatant and the hydrolysate were eluted through a poly-prep chromatography column (Resin 100-200 mesh H, BIO RAD, Hercules, CA) and derivatized with N-(tert-butyldimethylsilyl)-N methyltrifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) (394882, 27.0547 Sigma-Aldrich) in a 1:1 ratio, according to the method of Calder and Smith (1988). Measurement of isotopic enrichment of [2- ^{15}N] Lys and of AA concentrations were performed using gas chromatography-mass spectrometry (HP 6890 GC

System 5973 and Mass Selective Detector, Hewlett Packard, Palo Alto, CA).

Milk fat was determined by the Roese-Gottlieb method (AOAC, 2000) and total N was determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI). Milk urea N (MUN) was measured with a Technicon Analyzer (Technicon Instruments Corporation, Tarrytown, NY).

Calculations of ^{15}N flux

The measurement of lysine flux is based on the assumption that, under steady state conditions, and for adult animals fed to requirements, protein synthesis remains constant and protein breakdown remains unchanged (Harris et al., 1994). Therefore, based on Equation 1 (Waterlow et al., 1978), variations in WB Lys net flux would reflect differences in absorbed Lys, hence differences in Lys availability among diets.

WB Lys net flux = S (synthesis) + O (oxidation)

= B (breakdown) + I (absorption) Equation (1)

Calclualtions of net flux of lysine are based on the compartmental model of (Shipley and Clark, 1972) and the plasma ^{15}N Lys enrichment data (Appendix Fig. 1). Isotopic enrichment over time (IEt) is obtained from the ratio of tracer (labelled [2-^{15} N] Lys) to tracee (unlabelled Lys). The plasma ^{15}N Lys enrichment data were subjected to non-linear regression analysis (SAS, 2001) and both single and double exponential models were tested. The data best fitted (reduction of error sums of squares) the double exponential model (Equation 2, below) of Shipley and Clark, (1972) and revealed the existence of two pools. It is assumed that as a result of the steady state, the size of the pools remains constant, and that the sum of inflows equals the sum of outflows from the pool. It is also assumed that tracer and tracee molecules are handled similarly.

$$IE(t) = \alpha e^{(-k_1 t)} + \beta e^{(-k_2 t)} \qquad \text{Equation (2)}$$

When the expression in Equation 2 is plotted on a semi-log scale, it yields two straight lines, where α and β correspond to IE at t = 0, and k_1 and k_2 correspond to the slopes for each line (Shipley and Clark,1972). Whole body Lys net flux was finally calculated using Equation 3 (see below) based on parameters (α, β, k_1 and k_2) of the enrichment curve (Shipley and Clark, 1972; Holtrop et al., 2004).

$$\text{Lys whole body net flux} = \text{Dose} / [(\alpha / k_1) + (\beta / k_2)] \qquad \text{Equation (3)}$$

Statistical Analyses

To establish the "plasma Lys response curve", the data were first analysed using the Proc Mixed procedure of SAS (2001), with cow (random), Latin square, period and dose, as main effects. Both the linear and quadratic effects of infusion dose were tested and the linear model was accepted. Plasma concentrations of Lys (coordinate y) were used to predict values of duodenally available Lys (coordinate x) using the inverse regression method described by Hansen et al. (1999). The dose-response curve was corrected for period and cow effects.

All data from the experiment were analysed using the Proc Mixed procedure of SAS (2001), with cow (random), period and treatment as main effects. Least squares means were obtained and treatment differences were assessed using the following pre-planned contrasts: 1) SE vs. EP, LS, 2) EP vs. LS, and 3) SE vs. SE70. The criterion for declaring an effect to be statistically significant was predetermined at $P \leq 0.10$. Probability values between 10 and 15% level were considered as indicating a tendency.

6. 4. RESULTS AND DISCUSSION

Diet composition and lactation performance

The ingredient and nutrient composition of the experimental diets is presented in Table 1. In general, diets were similar in the content of fibre and protein. As a result of the higher fat content of EP compared to the SE and LS products (Borucki Castro et al., 2006), its inclusion in the diet increased the fat content. Nevertheless, the fat content of the EP diet was much lower than the limit (7% of DM) recommended for dairy cattle (Schauff and Clark, 1992; NRC 2001). The SBM products were allowed to make the dominant contribution to the dietary supply of protein and Lys (53 to 60 % of dietary CP; 70 – 80 % of the dietary Lys) in order to increase the chances of discerning differences due to SBM treatment. Compared to the others, the LS diet seemed to result in lower concentrations of AA, particularly Lys. The lower Lys content of the LS diet could be a consequence of irreversible crosslinkage reactions, which reduces the Lys concentration in feeds (Mauron, 1981; Gerrard, 2002; Borucki Castro et al., 2006). Table 1 reveals that, based on the AA model of NRC (2001), replacing SE with EP or LS would increase the duodenal supply of Lys and the amount of digestible Lys by 21 and 30 $g.d^{-1}$, respectively.

Feed intake and milk production were recorded in order to assess normalcy of the experimental conditions. Although the cows were restricted on feed (90% of ad libitum intake) DMI by one cow was highly variable. Given that wide fluctuations in feed consumption would compromise the "steady state" conditions required for lysine kinetics and the generation of the plasma Lys response curve, results from this cow were excluded from the experiment. Statistical analyses on data from the remaining cows revealed no effects ($P > 0.15$) of dietary treatment on feed intake, milk production or milk composition. Average values for DMI and

milk production were 19.9 (± 0.25) $kg.d^{-1}$ and 26.6 (± 3.29) $kg.d^{-1}$, respectively. Corresponding values for milk protein and milk fat were 3.8 (± 0.24) % and 3.4 (± 0.40) %, respectively. Since the cows were fed to meet their nutritional requirements, the similarity in milk production responses is expected.

Ruminal fermentation

There were no differences ($P > 0.15$) in ruminal pH among treatments (Table 2); the average value for all treatments was 6.05. The concentration of ruminal NH_3 –N and the molar proportion of acetate tended to be greater in cows fed rumen protected SBM products than in those fed the SE diet ($P = 0.105$ and $P = 0.108$, respectively). The inclusion of urea also resulted in greater ($P < 0.01$) concentrations of MUN in cows fed EP and LS compared to SE. Values for MUN (mg/dL) were 18.3, 21.3, and 23.3 (± 0.97) for diets SE, EP and LS, respectively. Ruminal propionate was not affected by treatment ($P > 0.15$). Ipharraguerre et al. (2005) observed higher proportions of acetate and lower proportions of propionate in ruminal fluid of dairy cows fed solvent extracted SBM compared to expeller or lignosulfonate treated SBM.

The concentrations of iso-acids (isobutyrate and isovalerate) and valerate were greater ($P <0.10$) with SE than with the rumen protected SBM products, EP and LS. Similar observations were made in other studies in dairy cows (Mansfield and Stern, 1994; Ipharraguerre et al., 2005). Iso-acids are the end products of the oxidative deamination and decarboxylation of branched chain AA (BCAA; Allison, 1978) and there have been reports of a relationship between the concentrations of iso-acids and NH_3 - N in rumen fluid of cows fed untreated and treated SBM (Mansfield and Stern, 1994; Ipharraguerre et al., 2005). Ludden and Cecava (1995) and Reynal et al. (2003) observed no changes in ruminal concentrations of iso-acids or NH_3-N when raw soybean was replaced with

heat-treated soybean sources. The present study revealed lower levels of iso-acids, but higher concentrations NH_3 – N, in the rumen of cows fed treated SBM products compared to SE; this would not be consistent with the expected trends for ruminal ammonia and iso-acids. The finding that concentrations of ruminal NH_3 N tended to be higher with rumen protected SBM products than with SE could be explained by the fact that urea was added to the EP and LS diets in order to prevent a shortage of RDP, which would limit microbial protein synthesis.

Duodenal flow and digestibility of lysine and other EAA

Table 3 shows results of duodenal flow and digestion of OM, N, Lys and selected EAA. As expected, there were no differences (P >0.15) in OM intake amongst diets, because the cows were restricted on feed. Digestion of OM was also similar (P > 0.15) among diets. Even under conditions of ad libitum feeding, OM digestion by lactating dairy cows was shown to be similar between solvent extracted and expeller or lignosulfonate treated SBM (Mansfield and Stern, 1994; Reynal and Broderick, 2003; Ipharraguerre et al., 2005). Nitrogen intake was greater (P <0.10) in cows fed treated SBM (EP, LS) than those fed SE; this was due to the fact that urea was included in the EP and LS diets, for reasons explained earlier.

Duodenal flows of total N and NAN were unaffected (P > 0.15) by dietary treatment. Post ruminal digestion of protein was also unaffected (P > 0.15); the average value across diets was 75%. Ipharraguerre et al. (2005) reported that, in lactating dairy cows fed ad libitum, the flow of NAN and non microbial NAN was greater with lignosulfonate SBM than with solvent extracted or expeller SBM; however, the differences were explained mainly by a higher intake of diets containing the treated SBM. In studies with dairy cows aimed at comparing solvent extracted SBM with heated SBM (Mabjeesh et al., 1996), expeller SBM (Ludden and Cecava, 1995)

or lignosulfonate SBM (Cleale et al., 1987; Mansfield and Stern, 1994), no differences were observed in post ruminal digestion of protein.

The differences in intake of Lys ($P < 0.15$) and other EAA ($P < 0.15$) between EP and LS (Table 3) may be explained by the lower content of lysine in LS SBM compared to the other SBM products (Borucki Castro et al., 2006). However, this did not affect ($P > 0.15$) the duodenal flow of Lys or other EAA. Omasal infusion of Lys (SE70) tended ($P = 0.108$) to increase the passage of Lys to the duodenum, but only 56 % (39 out of 70 $g.d^{-1}$) of the amount of lysine infused was recovered at the duodenum. Measurements of duodenal flow are highly variable (Titgemeyer, 1997) and this could explain the low duodenal recovery of Lys. Another possible explanation for incomplete duodenal recovery of Lys is that a portion of the infused Lys may have back-flowed to the rumen (Mathison et al., 1995; Bandyk et al., 2001). It is also possible that some of the infused Lys was absorbed at some site between the omasum and the duodenum (Matthews and Webb, 1995; Rémond et al., 2000). Despite these methodological limitations, estimates (Tables 3 and 4) of post-ruminal digestibility of Lys and of digestible Lys (duodenal flow x post-ruminal digestibility) were greater for SE70 than for SE ($P < 0.10$). This was due to an increase in duodenal flow, and a decrease in fecal excretion of Lys (45 vs. 38 ± 2.3 $g.d^{-1}$ for SE and SE70, respectively).

Based on the NRC model (2001), differences in duodenal flow of Lys should have been 21 $g.d^{-1}$ and 29 $g.d^{-1}$ greater, respectively, for EP and LS compared to SE. The present study revealed differences of –10 and 0 $g.d^{-1}$ in duodenal flow of Lys for EP and LS, respectively, compared to SE. The model equations of NRC (2001) are based on empirical data obtained with cows fed, ad libitum, in one or two meals. Feed restriction is known to decrease RUP by reducing rate of passage (Tafaj et al., 2001; NRC, 2001), so differences in feeding conditions may have caused the

difference between values observed in this study and those predicted by NRC (2001). The fact that the duodenal supply of Lys with the SE diet was similar to that of treated products and also higher than predicted by NRC, suggest that there was a higher than expected production of microbial Lys as a result of frequent feeding. Frequent meals have been shown to decrease diurnal fluctuations in ruminal ammonia and lactic acid, thereby promoting efficiency of nutrient utilization in the rumen (Robinson, 1989) and an increase duodenal supply of NAN and microbial N (Cecava et al., 1990; Le Liboux and Peyraud, 1999).

Some previous studies with steers and lactating dairy cows fed ad libitum, twice daily, have failed to show differences in duodenal flow of Lys between solvent extracted and expeller SBM (Ludden and Cecava, 1995; Reynal et al. (2003) or between solvent extracted and lignosulfonate SBM (Windschitl and Stern, 1988). However, on a study with wethers, Demjanec et al. (1995) reported a linear increase in duodenal flow of non-bacterial Lys when the animals were fed roasted SBM rather than what solvent extracted SBM. Ipharraguerre et al. (2005), providing lactating dairy cows with a diet in which either expeller or lignosulfonate treated SBM partially replaced solvent extracted SBM, resulted in an increase in duodenal flow of non- ammonia non microbial Lys. Based on a meta-analysis of published studies with lactating dairy cows, Ipharraguerre and Clark (2005) reported a mean increase of only 3.9% in the intestinal supply of Lys when diets were supplemented with rumen-protected products. Given such marginal responses, rumen protected SBM products would have to make a very large contribution to the duodenal flow of RUP in order to have a substantial impact on the intestinal supply of Lys. In the present study, the contribution to duodenal RUP made by the SBM products was clearly not enough to have an impact on the flow of intestinal lysine.

Estimates of post-ruminal digestibility of Lys (Table 3) as well as the daily amount of digestible Lys (Table 4) were not different (P >0.15) between SE and the rumen protected SBM products (EP, LS). Intestinal digestibility of Lys was lower than that assumed by the NRC (2001) model; it assumes values of 86.5% for RUP Lys, and 80% for endogenous and microbial sources of Lys. Other authors have reported values of 83% and 84% for intestinal digestibility of Lys in diets containing solvent extracted SBM (Windshitl and Stern, 1988; Mabjeesh et al., 1996), and a value of 81% for diets containing lignosulfonate SBM (Windshitl and Stern, 1988).

Estimates of digestible Lys (Table 4) did not differ between untreated (SE) and untreated (EP, LS) SBM products, indicating that SBM treatment did not alter Lys availability. The lower intestinal digestion of Lys for treated SBM and a presumed decrease in the passage of feed RUP reduced the expected differences in duodenal supply of Lys among SBM products.

Plasma lysine response

The reason for constructing the plasma Lys response curve was to quantify the amount of Lys available post-ruminally. This approach was originally developed by Rulquin and Kowalczyk (2003), and has been found to provide reliable estimates of methionine availability in dairy cattle. When used, however, for estimating Lys availability, this method proved to be imprecise (Rulquin and Kowalczyk, 2003). The authors attributed such imprecision to the fact that the maximum supply of Lys from the test feed was only 33% of the duodenal flow of Lys. This explanation may not be adequate because in the study of Rulquin and Kowalczyk (2003), the contribution of methionine in the test diet represented only 20% of the duodenal flow of methionine. In the present study, the supply of Lys from the tested SBM products represented up to 77% of the duodenal supply of Lys.

There was a linear relationship (P <0.001) between omasal infusion of Lys and its concentration in plasma (Fig. 1). As indicated by the high R^2 value (0.77), the relationship is quite reliable. Presented in Table 4 are estimates of plasma Lys for diets containing the different SBM products, as well as the diet, SE70. The increment in duodenal Lys for each experimental diet is also shown in Table 4. The diet, SE 70, was used as a "quality control" treatment, and as indicated earlier, the duodenal recovery of infused Lys was about 60 %. Nevertheless, omasal infusion of Lys did cause a major increase (P < 0.05) in plasma Lys (SE vs SE70) and this was reflected in a large increment in duodenal Lys. Substitution of SE (Table 4) with the treated SBM products (EP, LS) did not alter plasma Lys concentrations (P > 0.15). The results also reveal that, based on confidence intervals (Hansen et al., 1999), there were no significant differences in duodenal increments of Lys among SE, EP and LS. Hence, based on the plasma response method there were no differences in Lys availability (Table 4) among the SBM products.

The usefulness the AA availability method of Rulquin and Kowalczyk (2003) depends largely on the responsiveness of AA in plasma to changes in AA intake and absorption. However, previous research has shown inconsistent responses in plasma AA to changes in AA or protein supply. For example, in studies with dairy cows, Blouin et al. (2002) reported that although splanchnic flux of EAA increased with RUP supplementation, arterial concentrations of EAA in plasma remained unaltered. King et al. (1990) found that plasma concentrations of AA in dairy cows did not reflect differences in dietary or duodenal AA supply; however, the dietary source of RUP did alter extraction of AA by the mammary gland. In a study with early lactating dairy cows, King et al. (1991) reported a linear increase in plasma Lys (abdominal subcutaneous vein) to abomasal infusions (0 – 180 $g.d^{-1}$) of Lys. Working with mid- lactation dairy cows, Guinard and Rulquin (1994) observed increases in arterial concentrations of Lys

following duodenal infusions of Lys (0 to 63 $g.d^{-1}$). However, these authors obtained a plateau in plasma Lys at a Lys an infusion level of approximately 50 $g.d^{-1}$.

Variability in responses in plasma AA seems to be related to the stage of lactation and the level of milk production of dairy cows (Rulquin and Kowalczyk, 2003). Moreover, according to Bergen (1979), the plasma AA profile cannot be used as independent parameter of nutritional status because the size of the free AA pool is much smaller than the fluxes of AA in and out the tissue pools. Nevertheless, measurements of plasma AA are useful if quantitative aspects of lower gut protein flow and digestion are simultaneously studied (Bergen 1979); these conditions existed in the present study. In their review of amino acid metabolism in dairy cattle, Lapierre et al. (2005) concluded that there is very little hepatic extraction of absorbed Lys or BCAA. Therefore, peripheral concentrations of these EAA could reflect changes in their absorption. Given that this study was designed to optimize conditions to achieve a response in plasma lysine to changes in Lys absorption, the plasma lysine response method may have reliably reflected no differences in Lys availability among the SBM products.

Whole body lysine flux

Whole body lysine net flux (Lys flux) was estimated from the decay curve of plasma ^{15}N Lys enrichment (Appendix Table 1 and Appendix Fig. 1). Results presented in Table 4 show no significant differences (P >0.15) in Lys flux among SBM products; estimates of Lys availability were similar among the diets (SE, EP and LS). The lack of treatment effects on Lys availability with the Lys flux method is consistent with the results obtained with the other two methods (Table 4). Consequently, irrespective of the experimental approach used, the processing SBM to reduce increase RUP supply did not alter the availability of Lys in lactating cows fed a corn

silage-based diet. No differences in Lys availability, irrespective of the experimental approach adopted, suggests that no real difference existed in Lys availability among diets containing the different sources of SBM. It must be recognized, however, that a limited number of animals was used in this study, so there may be a need for further research to confirm these findings.

The Lys flux approach to estimating Lys availability relies on the assumption that whole body protein degradation remained unchanged, and that the Lys flux is responsive to changes in intestinal supply and absorption of Lys. Previous research with sheep (Savary-Auzeloux et al. 2003) has shown that Lys net flux (irreversible loss rate) responded linearly to increases in feed intake. In studies with growing lambs, Cronjé et al. (1992) also reported an increase in Lys flux when the animals were fed a high protein rather than a low protein diet. This suggests that Lys flux in ruminants is, in fact, responsive to changes in intestinal amino acid supply. In the present study, the omasal infusion (70 $g.d^{-1}$) of Lys (SE70) increased ($P < 0.10$) the Lys flux by exactly 70 $g.d^{-1}$ when compared to the control diet (SE). This finding, obtained with lactating dairy cattle, is further evidence that Lys flux in ruminants is responsive to duodenal AA supply. The assumption that protein degradation remained unchanged was not verified, and one approach to verify this assumption would have been to measure urinary excretion of 3-methyl histidine. However, this methodology requires total collection of urine for at least 5 days (Plaizier et al., 2000) and may be a cumbersome methodology to perform in female cows.

This research represents the first report of the use of the Lys flux method to determine lysine availability in dairy cattle. There are, however, some limitations in the methodology, as presented. For example, there were a limited number of animals in the study, and the assumption that whole

body protein degradation remains constant, needs to be validated. Nevertheless, this method represents a relatively non-invasive approach to determining amino acid availability in that does not require the use of cannulated animals. Furthermore, the lysine flux approach links intestinal supply of essential amino acids to their metabolic usage. This is a useful direction for future research aimed at improving models of amino acid nutrition in dairy cattle.

6. 5. CONCLUSIONS Experiment 2

Based on a corn-silage diet, the replacement of solvent extracted SBM with different rumen protected SBM products (expeller SBM and lignosulfonate treated SBM) resulted in no difference in the duodenal flow or post ruminal digestion of OM, N and Lys in dairy cattle. Three experimental approaches were evaluated to quantify the availability of lysine in the different diets supplemented with the SBM products. Whether assessed based on duodenal flow of Lys followed by intestinal digestibility, on plasma flux of ^{15}N Lys, or on plasma Lys response to omasal Lys infusions, the study revealed no differences in Lys availability among the diets. The lysine flux approach not only links the intestinal supply of essential amino acids to their metabolic usage, but it avoids the use of cannulated animals, thereby being more acceptable in the context of animal welfare. This is the first report of the use of ^{15}N lysine flux to determine lysine availability in dairy cattle. Further studies should be performed to test the sensitivity of the lysine flux method, and to validate underlying assumptions regarding protein breakdown.

6. 6. TABLES EXPERIMENT 2

Table 1: Ingredients and nutrient composition of the experimental diets

	Treatment[1]			
	SE	EP	LS	SE70
Ingredient composition of TMR, % of DM				
Corn silage	56.0	55.0	54.6	56.0
Corn grain, dry and ground	9.0	9.0	9.0	9.0
Solvent extracted soybean meal	23.0	0.0	0.0	23.0
Expeller soybean meal	0.0	23.0	0.0	0.0
Lignosulfonate soybean meal	0.0	0.0	23.0	0.0
Beet pulp, dry	8.0	8.0	8.0	8.0
Urea	0.0	1.0	1.4	0.0
$NaHCO_3$	0.2	0.2	0.2	0.2
Commercial premix [2]	3.8	3.8	3.8	3.8
Nutrient composition of TMR, % of DM				
DM	49.7	50.6	49.0	49.7
NDF	34.2	35.1	37.3	34.2
ADF	19.8	19.4	20.4	19.8
Fat	2.86	3.35	2.94	2.86
Ash	6.8	7.2	7.1	6.8
CP	19.8	22.0	22.9	19.8
Essential AA, % of DM				
His	0.43	0.46	0.41	0.43
Ile	0.70	0.79	0.72	0.70
Leu	1.54	1.64	1.49	1.54
Lys	0.83	0.85	0.74	0.83
Phe	0.84	0.90	0.81	0.84
Thr	0.69	0.73	0.66	0.69
Val	0.79	0.89	0.83	0.79
Non-essential AA, % of DM				
Ala	1.03	1.07	1.00	1.03
Glx [3]	2.47	2.68	2.34	2.47
Gly	0.75	0.82	0.73	0.75
Ser	0.89	0.93	0.82	0.89
Tyr	0.64	0.69	0.62	0.64
Supply estimated from NRC (2001)				
Metabolizable protein, g d^{-1}	2,476	2,897	3,199	2,476
RDP ,g d^{-1}	3,065	2,296	2,108	3,065
RUP ,g d^{-1}	1,513	1,944	2,287	1,513
Duodenal flow of lysine, g d^{-1}	187	208	217	187
Digestible lysine, g d^{-1}	155	176	184	155

[1] SE= solvent extracted SBM; EP=expeller SBM; LS= lignosulfonate SBM; SE70= SE + omasal infusion of 70 $g.d^{-1}$ Lys

[2] Base Laitière (Shur Gain; Brossard, QC): 8% CP;2.1% fat; 10.5% ADF; 0.84% Na; 2.44% Ca; 0.60% P; 0.36% Mg; 0.33% K; 0.20% S; 71.2 ppm Cu; 257 ppm Mn; 344 ppm Z ; 1.3% Cl; 1.3 ppm Se; 269 ppm Fe; 29,027 IU/kg Vitamin A; 6,450 IU/kg Vitamin D_3 ; 97 IU/kg Vitamin E.

[3] Glx = Gln plus Glu

Table 2: Ruminal fermentation parameters of lactating dairy cows fed diets containing different soybean meal products

	Treatment[1]				SEM	Contrast		
Item	SE	EP	LS	SE70	n=3	SE vs EP, LS	EP vs LS	SE vs SE70
pH	5.97	6.09	6.14	6.00	0.114	0.252	0.680	0.843
NH_3 – N, mg/dL	22.8	26.2	25.7	25.3	1.09	0.105	0.742	0.215
Total VFA, m*M*	118.3	115.1	114.7	119.3	6.21	0.571	0.954	0.886
Acetate, %	69.1	71.2	71.4	69.2	1.32	0.108	0.902	0.924
Propionate, %	18.2	17.2	17.1	19.1	1.50	0.466	0.942	0.576
Butyrate, %	9.61	9.11	9.10	8.68	0.295	0.186	0.964	0.073
Isobutyrate, %	0.76	0.58	0.64	0.73	0.048	0.037	0.287	0.534
Valerate, %	0.90	0.80	0.79	0.90	0.023	0.038	0.878	0.947
Isovalerate, %	1.44	1.10	1.03	1.34	0.121	0.079	0.704	0.613
A : P ratio[2]	3.9 : 1	4.1 : 1	4.2 : 1	3.8 : 1	0.33	0.430	0.631	0.610

[1] SE = solvent extracted SBM; EP = expeller SBM; LS = lignosulfonate SBM; SE70 = SE +omasal infusion of 70 $g.d^{-1}$ Lys

[2] A : P = acetate : propionate

Table 3: Intake, flow and post-ruminal digestibility of OM, N, Lysine and essential AA[1] of lactating dairy cows fed diets containing different soybean meal products

	Treatments[2]				SEM	Contrast		
	SE	EP	LS	SE70	n = 3	SE vs EP,LS	EP vs LS	SE vs SE70
OM								
Intake, kg.d^{-1}	17.4	17.4	17.0	17.1	0.19	0.376	0.273	0.359
Passage to duodenum, kg.d^{-1}	10.8	10.8	10.0	9.9	0.78	0.715	0.518	0.468
Apparently digested post-ruminally, %	59.8	58.8	56.8	59.4	3.96	0.704	0.745	0.937
N								
Intake, g.d^{-1}	587	700	676	581[2]	16.7	0.015	0.380	0.811
Passage to duodenum, g.d^{-1}	631	631	637	588	37.6	0.947	0.914	0.490
Passage to duodenum as NAN, % of duodenal N	89.2	87.3	87.3	88.9	1.01	0.220	0.992	0.809
Apparently digested post-ruminally, %	76.1	73.0	74.1	77.7	2.07	0.389	0.732	0.625
Lysine								
Intake, g.d^{-1}	155	153	140	152[3]	3.4	0.134	0.074	0.495
Passage to duodenum, g.d^{-1}	213	203	213	252	11.9	0.784	0.601	0.108
Apparently digested post-ruminally, %	78.8	75.0	77.2	85.0	1.76	0.296	0.447	0.092
Histidine								
Intake, g.d^{-1}	80	84	77	79	1.9	0.909	0.085	0.672
Passage to duodenum, g.d^{-1}	63	68	70	57	4.1	0.314	0.795	0.380
Apparently digested post-ruminally, %	79.6	79.7	79.8	80.3	1.35	0.915	0.961	0.720
Branched chain AA								
Intake, g.d^{-1}	567	608	562	557	14.8	0.384	0.115	0.675
Passage to duodenum, g.d^{-1}	597	617	619	547	36.9	0.676	0.975	0.419
Apparently digested post-ruminally, %	75.9	77.0	74.3	77.9	1.02	0.853	0.170	0.268
Phenylalanine								
Intake, g.d^{-1}	157	164	149	154	3.7	0.912	0.071	0.600
Passage to duodenum, g.d^{-1}	156	162	165	141	8.8	0.524	0.840	0.330
Apparently digested post-ruminally, %	76.8	76.6	76.0	77.1	2.35	0.873	0.886	0.942
Threonine								
Intake, g.d^{-1}	129	134	122	127	2.7	0.744	0.053	0.566
Passage to duodenum, g.d^{-1}	162	161	163	148	8.1	0.999	0.860	0.334
Apparently digested post-ruminally, %	74.6	73.6	73.9	76.2	1.97	0.743	0.909	0.628

[1] Pre-oxidation of samples was not performed so methionine values are not presented.
[2] SE = solvent extracted SBM; EP = expeller SBM; LS = lignosulfonate SBM; SE70 = SE + abomasal infusion of 70 $g.d^{-1}$ Lys
[3] excludes Lys infusion

Table 4. Estimates of available lysine obtained by different methods in lactating dairy cows fed diets containing different soybean meal products

Method	Measurement	Treatment[6]				SEM	Contrast		
		SE	EP	LS	SE70	n=3	SE vs EP,LS	EP vs LS	SE vs SE70
Digesta Marker [1]	Digestible lysine, g d^{-1}	168	152	165	214	3.4	0.570	0.527	0.081
Plasma response	Plasma Lys , µM	89	80	85	141	7.4	0.53	0.69	0.02
	Post-ruminal increments [2], $g.d^{-1}$	13	4	9	68	-	-	-	-
	[C.I.] [3]	[6.6, 19.4]	[-3.7, 11.2]	[1.7, 15.5]	[60.4, 74.8]				
Lys flux [4]	Lysine net flux, $g.d^{-1}$	294	311	281	365	13.7	0.909	0.219	0.035
		Lysine Availability[5] (%)							
Digesta Marker [1]		100	90	98	127		ns	ns	$P < .10$
Plasma response		100	96	98	125		ns	ns	*
Lys flux [4]		100	106	95	124		ns	ns	$P < .05$

[1] [duodenal Lys flow x post-ruminal Lys digestibility]

[2] "x" value obtained based on inverse regression of plasma response curve (Hansen et al., 1999)

[3] 95% confidence interval

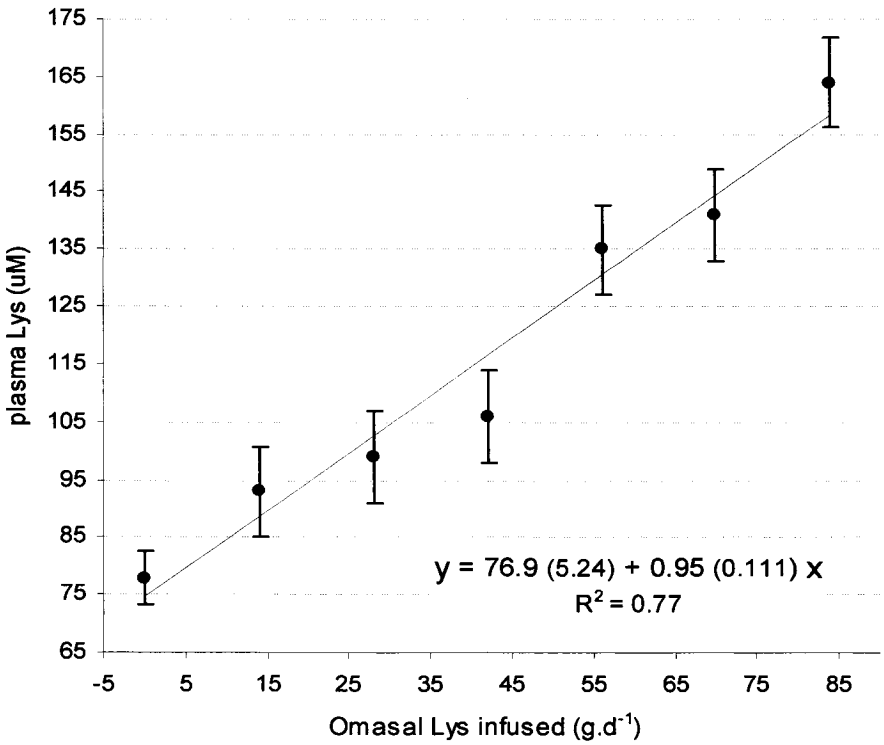
[4] metabolic indicator based on decay curve after bolus dose ^{15}N Lys

[5] relative to the SE diet (100)

[6] SE = solvent extracted SBM; EP = expeller SBM; LS = lignosulfonate SBM; SE70 = SE + omasal infusion of 70 $g.d^{-1}$ Lys

6. 7. FIGURES EXPERIMENT 2

Figure.1: Plasma lysine response to omasal infusion of lysine in lactating dairy cows fed solvent extracted soybean meal



6. 8. APPENDIX TABLES EXPERIMENT 2

Appendix Table 1: Mean values of parameter estimates obtained by fitting a model[1] of Plasma enrichment in dairy cows fed diets containing different soybean meal products

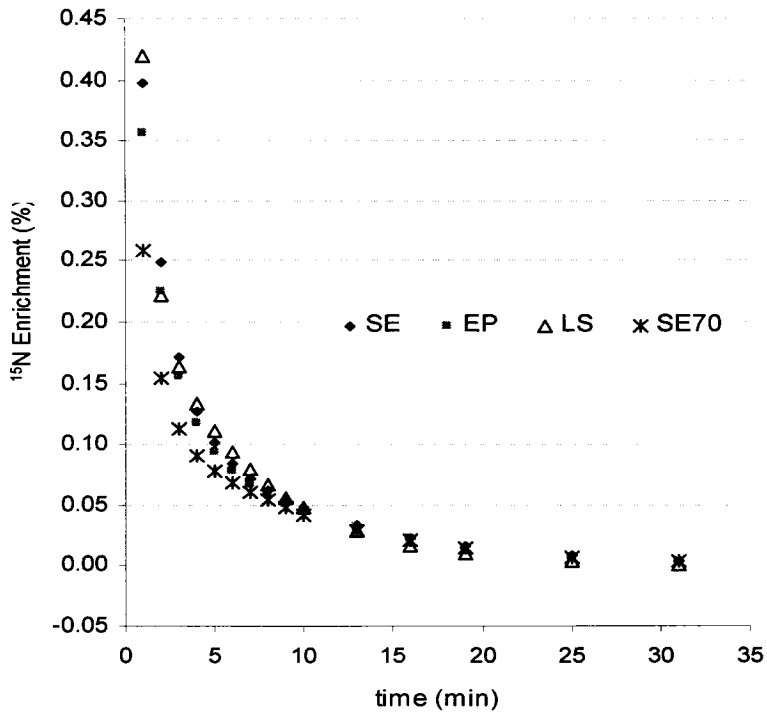
Parameter[1]	Treatment[2]				SEM
	SE	EP	LS	SE70	n=3
α	0.53	0.47	1.08	0.41	0.220
β	0.16	0.15	0.26	0.14	0.030
K_1	0.73	0.74	1.68	1.11	0.202
K_2	0.12	0.12	0.17	0.12	0.013

[1] Equation (2) : Enrichment = $\alpha e^{-k_1.t} + \beta e^{-k_2.t}$

[2] SE = solvent extracted SBM; EP = expeller SBM; LS = lignosulfonate SBM; SE70 = SE +omasal infusion of 70 $g.d^{-1}$Lys

6. 9. APPENDIX FIGURES EXPERIMENT 2

Appendix Figure 1. Plasma ^{15}N Lys enrichment for dairy cows fed different soybean meal products[1]



[1] SE = solvent extracted SBM; EP = expeller SBM; LS = lignosulfonate SBM; SE70 =SE +omasal infusion of 70 $g.d^{-1}$Lys

7. Connecting statement

Growing concerns about the environment present a challenge to nutritionists to provide the minimal amount of protein and amino acids to high- producing dairy cows to achieve optimal, but not necessarily maximal, milk production. In order to achieve efficiency, another goal in diet formulation is to maximize the amount of nitrogen output in milk, while minimizing the amount excreted in the manure.

The manipulation of protein degradability and energy sources in the rumen may increase synchrony and therefore an increment in the capture of ruminal NH_3-N for milk N, which results in lower excretion of N to the environment. A subsequent trial was performed, therefore, to evaluate the total replacement of solvent extracted with expeller soybean meal, and the partial replacement of the energy source (corn starch) with beet pulp (pectins) on the efficiency of nitrogen use for milk production.

To increase the chances to discern differences in nitrogen efficiency and milk production, the diets were fed ad libitum and the animals selected were early lactating dairy cows, to be able to express their maximum potential in milk production.

8. EXPERIMENT 3

The Effect of Soybean Meal and Carbohydrate Source on Lactation Performance and Nitrogen Balance in Dairy Cattle

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8. 1. ABSTRACT

Eight multiparous Holstein cows in early lactation were used to determine the impact of different sources of soybean meal (SBM) and energy source on milk production and efficiency. The experiment was conducted as a 4 x 4 Latin Square design with 21-d periods. Isocaloric alfalfa based diets were fed ad libitum and supplemented with: 1) high moisture shelled corn (HMSC; diet ASCG), 2) HMSC + solvent extracted SBM (SE; diet ASSE), 3) HMSC + expeller SBM (EP; diet ASEP), and 4) ASSE with dried sugar beet pulp unmolassed (BP; diet ASBP). There was no evidence of energy deficit among diets based on BW changes or values of plasma glucose or NEFA. Supplementation with SE or EP increased DMI whereas DMI was decreased with BP. Higher efficiency of N usage for milk production on ASCG was, however, associated with lower levels of milk and protein yields. Plasma His, Leu, Val and Phe tended to increase with RUP supplementation (ASEP). The use of BP increased the concentration of His and Val in plasma. However, diet ASSE tended to produce higher milk protein and fat yields compared to ASEP, and tended to increase milk

yield and milk protein composition compared to ASBP. No significant changes were observed between protein-supplemented diets (ASSE, ASEP or ASBP) on either N digestion, excretion or efficiency for milk production. The inclusion of BP or the replacement of SE with EP in protein supplemented diets decreased lactational performance compared to diets based on AS supplemented with HMSC and SE; the use of RUP (ASEP) or the replacement of starch with pectin (ASBP) had no effect on N efficiency or reduction of N excretion to the environment.

Abbreviation key: **AS**= alfalfa silage, **HMSC**= high-moisture shelled corn; **SBM**= soybean meal, **SE**= solvent extracted soybean meal, **EP**= expeller soybean meal, **BP**= beet pulp, **EAA**= essential amino acids, **NEAA** = non essential amino acids, **ASCG** = AS+ HMSC; **ASSE** =ASCG+SE; **ASEP** =ASCG+EP; **ASBP** =ASSE+BP.

8. 2. INTRODUCTION

Responses to RUP supplements are highly variable and can be explained mainly by variations in the type of control diet, microbial protein synthesis, and the type and amount of RUP in the diet (Ipharraguerre and Clark, 2005). In a recent review of the impact of the source and amount of CP on the intestinal supply of N fractions and performance of dairy cows, these authors concluded that compared to solvent extracted SBM, rumen-protected soy products increased milk output by +2.7%, but no effects were reported on milk composition. The lack of effect of rumen-protected soy products on milk protein was consistent with our own data showing that feeding treated SBM (EP, LS) had no effect on the duodenal supply of EAA (Borucki Castro et al. 2006b). However, in a meta-analysis based on the literature between 1988 and 2000, Bateman (2005) observed that an increase in RUP intake was associated with increased yields of milk and

4% fat-corrected milk. This author also demonstrated the important effect of DMI on the relationship between RUP content of the diets and milk production. In our previous study (Borucki Castro et al., 2006b), dry matter intake was restricted (95% of ad lib), which might have decreased the potential responsiveness to RUP supplementation.

Marginal gains in milk protein yield at higher protein intakes could bring exponential increases in terms of N excretion and pollution to the environment in dairy cows (Kebreab et al., 2001). If this is the case, the cost of RUP supplementation will be difficult to justify. Together with the correct choice of RUP supplement, several authors suggest that the selection of the dietary energy source is an important management tool to increase performance and efficiency of N usage in dairy cattle (Børsting et al., 2003; Broderick, 2005). Hristov and Ropp (2003) found that the inclusion of fermentable fiber (corn, beet pulp and brewer's grains) enhanced the conversion of ruminal ammonia into milk N compared to sources of starch (barley and molasses).

Therefore, the objectives of this experiment were to study the effect of RUP from treated soybean meal and the effect of the energy source (starch vs. pectin) on milk yield, milk composition and N usage in lactating dairy cows.

8. 3. MATERIALS AND METHODS

Animals and Treatments

Eight multiparous Holstein cows averaging 658 ± 28 kg BW and 80 ± 20 DIM at the beginning of the study, were used in a 4 x 4 Latin square design with 21-d experimental periods, balanced for residual effects

(Cochran and Cox, 1957). Cows were housed in tie stalls with free available water and milked twice a day at 0900 and 2000 h. Animal care procedures followed the guidelines of the Canadian Council on Animal Care (1993); the protocol was approved by the Institutional Animal Care Committee of the Dairy and Swine Research Centre in Sherbrooke, QC (Agriculture and Agri-Food Canada).

The cows were fed ad libitum with a diet based on alfalfa silage (AS) and a mineral and vitamin pre-mix (Table1). The TMR was either supplemented with: 1) high moisture shelled corn (HMSC) in diet **ASCG**; 2) HMSC + solvent extracted SBM (SE; ADM Agri-Industries, Windsor, ON) in diet **ASSE**; 3) HMSC + expeller SBM (EP; SoyPLUS® West Central, Ralston, IA) in diet **ASEP**; and 4) diet SE replacing 50% of HMSC with pelleted dried sugar beet pulp unmolassed (Meunerie Sawyerville, Cookshire, QC) in diet **ASBP**.

Sampling

After 16 d of adaptation to the diet, from day 17 to 21, samples of individual feedstuffs, TMR and orts were collected and pooled by period. Orts were taken daily prior to AM feeding. During these 5 days, feces and urine were weighed daily and a representative sample (2%) was taken and frozen immediately; samples were then pooled by period. Urine was collected in stainless steel containers with sulphuric acid added (10ml H_2S0_4 98% / L urine) to maintain pH < 2.0 (Spanghero and Kowalski, 1997) and feces were collected in pre-weighed plastic lined plywood boxes. On day 21, blood samples were collected from the coccygeal vein, 1 hour before and 3 hours after the morning meal, into two vacutainers (1 with heparine and 1 with EDTA). Blood samples were kept on ice and immediately centrifuged at 1,500 *g* for 12 min. To determine plasma AA concentrations, an internal standard consisting of a mixture (200 μg) of labelled AA (^{13}C and ^{15}N AA isotope standards, CDN Isotopes, Pointe-

Claire, QC; Cambridge Isotope Laboratories Inc., Andover, MA; Raggio et al., 2004) was added to 1 g of plasma from the samples. Plasma was then frozen at –80°C until analysed.

From d 17 to 21, milk yield was recorded and samples from AM and PM milkings were collected and pooled on a daily basis according to milk production. Cows were weighed on 3 consecutive days at the same time of the day, at the start of each period and at the end of the last period of the Latin Square.

Analytical methods

Samples of feed, orts and feces were freeze-dried and ground to pass a 1 mm screen. The DM content was analysed using a forced air oven maintained at 60°C for 48 h. Ash and analytical DM was determined with a thermogravimetric analyzer (Model TGA-601, LECO Corporation, St. Joseph, MI). Nitrogen was determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI) except for acidified urine samples, where N was measured by micro-Kjeldhal analysis (FOSS Tecator Kjeltec System, Brampton, ON). Analyses of NDF and ADF were performed on feeds according to the methods of Van Soest et al. (1991) using the Ankom System (ANKOM 200, Fiber Analyzer, Fairport, NY) with heat stable alpha-amylase and without sodium sulfite. Fat analysis was determined for the TMR by gravimetric analysis using ISCO SFX™ 3560 supercritical fluid extraction (ISCO Inc., Lincoln, NE) without co-solvent modifiers for extraction of phospholipids.

To analyze AA concentrations, samples of feed ingredients and TMR were ground to pass a 0.5 mm screen. Samples were acid-hydrolysed with 6-N phenol-HCl for 24 h at 110°C (AOAC, 2000) and the AA concentrations were determined by the isotope dilution method (Calder et al., 1999).

Briefly, 2 g of the hydrolysate were diluted with 3 g of ultra pure water; 1 g of this solution was then combined with a mixture (200 µg) of labelled AA, which served as an internal standard (Raggio et al., 2004). Plasma samples were deproteinized with sulfosalicylic acid (38%) and centrifuged at 27,768 *g* for 10 min. Samples of the supernatant and the hydrolysate were eluted through a poly-prep chromatography column (Resin 100-200 mesh H, BIO RAD, Hercules, CA) and derivatized with N-(tert-butyldimethylsilyl)-N methyltrifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) 1:1 (394882, 27.0547 Sigma-Aldrich) according to the method of Calder and Smith (1988). Measurements of AA concentrations were performed using gas chromatography-mass spectrometry (HP 6890 GC System 5973 and Mass Selective Detector, Hewlett Packard, Palo Alto, CA).

Concentrations of plasma glucose were determined by colorimetric methods (kit 600 PAP #448668 ROCHE; Mississauga, ON) and non-esterified fatty acids -NEFA- (kit NEFA C #994-75409 WAKO Chemicals USA; Richmond; VA) using a Microplate Spectrophotometer System (Spectra Max 250, Molecular Devices, Sunnyvale, CA). Energy was determined by combustion using an Adiabatic Bomb Calorimeter (Parr®Instruments Co. Moline, IL) in TMR, milk and digesta. Milk samples were analysed for total solids using a thermogravimetric analyser TGA 601 (LECO; St. Joseph; MI). Milk fat was determined by the Roese-Gottlieb method (AOAC, 2000). Total N and protein- N in milk were determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI) according to AOAC (2000). Milk urea N was measured with a Technicon Analyzer (Technicon Instruments Corporation, Tarrytown, NY) based on colorimetric methods (Huntington, 1984; Reynolds et al., 1989).

Calculation of Energy and Nitrogen Balances

Values of energy lost in heat and methane production were estimated as 34% and 6% respectively, as proportion of gross energy intake based on the work of Sutter and Beever (2000). Energy retained was estimated as:

ER = GEI – (EF + EU + EM + EH + EMe)

Being, ER = energy retained, GEI = gross energy intake, EF = fecal energy, EU= urinary energy, EM = energy in milk, EH= heat energy and EMe = energy lost as methane.

Calculations for Nitrogen balance were as follows:

NR = NI – (NF + UN + NM)

Being, NR = nitrogen retained, NI = nitrogen intake, NF= fecal N, UN = urinary N and NM = N excreted in milk.

Statistical Analyses

Data was analysed using Proc Mixed of SAS (2000), with cow (random), period and treatment as main effects. The pre-planned contrasts are described as follows: 1) ASCG vs. other treatments (ASSE, ASEP and ASBP), 2) ASSE vs. ASEP, and 3) ASSE vs. ASBP. The criterion for declaring an effect to be statistically significant was predetermined at the 5% level and corrected by Bonferroni at $P \leq 0.02$. Values between 2 and 10% level of probability were considered as expressing a tendency.

8. 4. RESULTS AND DISCUSSION

Composition of diets

Ingredient and nutrient composition of diets are presented in Table 1. The treatments were planned to be isocaloric. The ASCG diet was formulated to measure the efficiency of N usage from the basal diet without protein supplementation. The concentration of CP was lower for this diet (15.6%)

and also presented a lower concentration of AA. The other treatments were supplemented with either SE or EP so their %CP (18%) and AA concentrations were higher. Values of NDF and ADF were not different except for ASEP due to higher NDF in EP; artefact fibre is created in this feed (EP) because of protein cross-linkage given the effect of heat (Demjanec et al. 1995; Borucki Castro et al., 2006a). The alfalfa silage non protein- N and pH averaged respectively 61.4 (%CP) and 4.2, indicating that it was well preserved (Dulphy and Demarquilly, 1981; Appendix Table 1). The nutritive value was as expected for a medium quality alfalfa silage according to NRC (2001).

Nutrient intakes and digestibility

Protein supplementation with SE or EP increased intake ($P < 0.02$) of DM and OM for all diets compared to ASCG (Table 2); previous research have also reported increased DMI when alfalfa based diets were supplemented with protein in high yielding dairy cows (Grings et al., 1991; Dhiman and Satter, 1993). The ASCG diet tended to have lower DM and OM digestibility ($P < 0.10$). Dry matter and OM digestibilities have been reported to increase as dietary CP concentration increases (Van Soest, 1994; Kauffman and St. Pierre, 2001).

Intakes of DM or OM were not different ($P > 0.10$) between ASSE vs. ASEP. No differences were reported when SE was partially replaced by EP in lactating dairy cows fed diets based on alfalfa and corn silages (Reynal and Broderick, 2003; Ipharraguerre et al., 2005; Olmos Colmenero and Broderick, 2006b). However, DMI and OMI were reduced when cornstarch was partially replaced by BP in ASBP ($P < 0.02$). Other studies found the same effects of the inclusion of BP on DMI (Mansfield et al., 1994; Voelker and Allen, 2003a). The aim of replacing part of the HMSC with BP was to increase the synchrony between the release of AA and N-NH_3 present in the rumen with a highly digestible source of CHO,

like those found in BP (Robert et al., 1989). This source (BP) has been shown to reduce ruminal N-NH_3 in vitro (Bach et al., 1999; Hristov and Ropp, 2003) and to prevent negative effects of low ruminal pH (Voelker and Allen, 2003c) given its high NDF content (Table 2). No effect was found however, by the inclusion of BP (ASBP) on DM and OM digestibility ($P > 0.10$).

Energy balance

Lower intakes on ASCG resulted in lower energy intakes ($P < 0.02$) compared to the other diets (Table 2). Energy excreted in feces ($P > 0.10$) was similar to the other treatments, therefore digestible energy was lower ($P < 0.02$) in ASCG. This diet presented lower energy excreted in milk and urine ($P < 0.02$), but no differences were found in energy retention compared to the other diets ($P > 0.10$). Energy- retained estimates indicated that the animals were in negative energy balance on all of the diets. However, based on BW changes or values of plasma glucose or NEFA, there was no evidence of energy deficit among diets. According to Beever et al. (1998) high producing dairy cows may be in negative energy balance although no changes are perceived in BW of the cows up to week 20 of lactation. Plasma NEFA levels are recognized as indicators of body fat mobilization and together with decreased glucose levels in plasma, these parameters have been associated with negative energy balances in early lactation dairy cattle (Rabelo et al., 2005; Clark et al., 2005; van Knegsel et al., 2005). Values of NEFA recorded in this study are similar to those reported by Accorsi et al. (2005) for early lactation cows.

No significant differences were found in energy ingested, excreted or retained between diets ASSE and ASEP (Table 2). Although ASSE tended to have higher energy intakes ($P < 0.10$) no differences were found ($P > 0.10$) in energy excretion. Tendencies ($P < 0.10$) for higher energy excretion in heat and methane for ASSE were associated with higher

intakes of this diet, because these values were estimated based on gross energy intake (Sutter and Beever, 2000). The inclusion of RUP has been reported to improve energy balance in other studies. Santos et al. (1999) reported lower BW changes for lactating dairy cows fed either steam-flaked sorghum or corn when dairy cows were fed a marine-blend source of RUP compared to solvent extracted SBM (44%CP). However, in the study of Santos et al. (1999) there was no evidence of body fat mobilization (NEFA levels) and this difference could have been explained by a 9% higher intake of net energy of lactation in diets with the RUP supplement.

The inclusion of beet pulp caused negative energy balance in other studies. Replacing beet pulp for corn or barley in the diet of dairy cows fed wilted grass and maize silages resulted in increased mobilization of body reserves and a negative energy balance (de Visser et al., 1990); this was caused by a higher output of milk energy and lower intakes with the BP diet. Hindrichsen et al. (2006) found differences in energy losses via feces, urine and heat in dairy cows when a diet based on maize and grass silages was supplemented (1:1) with concentrates either rich in lignified-fibre, non lignified-fibre, pectins, fructans, sugars or starch. In the present study, the inclusion of BP decreased the excretion ($P< 0.02$) of energy in feces, but had no effect ($P > 0.10$) on digestible energy compared to ASSE. No differences were observed in body condition score or in plasma metabolites that would reflect differences in energy balance between ASSE and ASBP.

N balance

There were linear relationships between N intake and N losses in feces, urine and N secretion in milk (Fig. 1). An increment of 1 g in N consumed increased N in feces and in milk by 0.18 g, respectively, whereas urinary N increments were 0.63g per g of N intake. Kebreab et al. (2001) also

found linear increases in feces and milk with higher N consumption. However, in a further study, Kebreab et al., 2002 found bi-linear relationships between N intake and milk and fecal N. In their study, a less steep slope was explaining the responses in fecal or milk N for intakes of N higher than 420 g d^{-1}. In the present study, the animals utilized had higher potential of milk production whereas in the studies of Kebreab et al. (2001, 2002) they managed animals with milk yields of 7000 $kg.year^{-1}$. Although these authors preferred the exponential relationship between N intake and urinary N, in the present study, both the exponential and linear relationships between N intake and urinary N presented the same coefficient of determination $R^2 = 0.74$.

The ASCG was the only diet that differed with the other diets in N excretion, digestion and efficiency ($P < 0.02$; Table 3). Cows fed no protein supplement (ASCG) produced less MUN ($P < 0.02$), which corresponded with lower concentrations of PUN ($P < 0.02$; Table 5) and lower urea N excretion (as a % of urinary N) for this diet ($P < 0.02$). Dietary CP is the best single predictor of MUN, which is strongly related to PUN and urinary N (Hoff et al., 1997; Kohn et al., 2002; Nousiainen et al., 2004). In the present study, the ASCG diet had a CP value of 15.6% so an increase in milk N excretion ($P < 0.02$) with protein supplementation (SE or EP) was expected. In studies of Olmos Colmenero and Broderick (2006a), the inclusion of protein supplementation also increased milk N secretion with incremental CP (13.5 to 19.4%) in lactating dairy cows given a 50% forage ration. Higher efficiency of N usage for milk production ($P < 0.02$) on ASCG was however associated with lower levels of milk and protein yields ($P < 0.02$; Table 5). Milk N as proportion of intake N linearly decreased with N intake (Kebreab et al., 2002; Broderick, 2003; Olmos Colmenero and Broderick, 2006a). No differences in N excretion in either feces or urine ($P > 0.10$) were found between diets with different protein degradabilities (ASSE vs. ASEP) or different CHO sources (ASSE vs.

ASBP). Nitrogen excreted in milk tended to be higher for ASSE compared to ASEP and ASBP (P= 0.106, P= 0.101; respectively). For diets ASSE, ASEP and ASBP, CP was 18%, so cows fed these diets secreted more N in milk but also excreted more N in urine, which is considered the sink for "extra" not utilised N (Broderick, 2003; Wattiaux and Karg, 2004). Values of 16.5 %CP were reported to be optimum for milk N secretion and efficiency as a % of N intake (Wu and Satter, 2000; Broderick, 2005).

One of the objectives of this study was to increase N usage for milk protein either by increasing RUP or by improving rumen conditions with the inclusion of BP in the diet. However, no significant changes were obtained between these diets either in N digestion, N excretion or in N efficiency for milk production. Olmos Colmenero and Broderick (2006b) also found no differences in N usage for milk (% N intake) when partially replacing SE (9.6 %DM) with EP (5.9 %DM) in alfalfa-corn silage diets (20:35 % of DM) containing 16.6% CP. Similar to the present study, these authors also found lower values of MUN with EP supplementation (P < 0.02); in both studies, values of urinary N or urea N excreted (% total urinary N) were not affected (P > 0.10). In another study, Kalscheur et al. (1999) linearly increased the efficiency of N usage for milk, by increasing the RUP from 5.5 to 7.3 (% of DM) using a mixture of fish meal, distillers grains and corn gluten meal for early lactating dairy cows. The dietary CP was reduced from 17% to 15% DM. The positive effects of RUP inclusion were accompanied by a reduction in milk fat and protein yields in early lactation cows, but there were no effects on the milk output of mid and late lactation cows. Gross N efficiency (milk N / intake N) was increased in diets containing highly digestible RUP (blood meal and poultry meal) compared to feather meal (Noftsger and St.Pierre, 2003); the greatest increase was recorded with Met-supplemented diets. Baker et al. (1995) found that the AA profile of the supplemented RUP formulated according

to CNCPS provided greater efficiency of true protein N in milk (lower NPN as % of milk N) compared to diets formulated without this consideration.

Increasing or decreasing dietary CP has a clear effect on N excretion to the environment and on N usage for milk (Kröber et al., 2000; Kebreab et al., 2002; Olmos Colmenero and Broderick, 2006a). However, the effects of RUP inclusion on N usage for milk or N excretion may depend on factors like DMI (Bateman, 2005), type of diet (Shinfield et al., 2001, 2003; Wattiaux and Karg, 2004), stage of lactation (Kalscheur et al., 1999), and RUP quality (Baker et al., 1995; Noftsger and St-Pierre, 2003).

Amino acid profiles in plasma

Circulating levels of most of the EAA, except for Met, Phe and Thr, were lower in cows fed the control diet ASCG ($P < 0.02$; Table 4). There were no differences in AA content among diets, but for Glu and Gly, which were higher for ASCG ($P < 0.02$). Increasing the supply of metabolizable protein was also reflected in the plasma AA profile in the study of Raggio et al. (2004). The tendency to increase circulating levels of His, Leu, Val and Phe ($P < 0.10$) associated with RUP supplementation (ASEP vs. ASSE) were in line with our previous findings that the treatment of SBM increased the bypass and availability of BCAA and Phe in EP compared to SE (Borucki Castro el al., 2006a). Other studies found no differences in arterial concentrations of EAA, but found that the splanchnic net flux (Blouin et al., 2002) or the mammary extractions of EAA (King et al., 1990) were increased with more RUP in isonitrogenous diets. Plasma profiles may not always be responsive to different protein supplements or increased RUP. Similar plasma Lys concentrations were found for cows fed SE or EP (23 % DM) in a corn- silage based diet (Borucki Castro et al., 2006b), with no differences between treatments in Lys availability. Faldet et al. (1992) found no differences in plasma AA concentrations of growing heifers fed different heat-treated soybeans. Plasma AA profiles are useful

to reflect nutritional status but only under determined conditions of steady state (Waterlow et al., 1978; Bergen, 1979). The inclusion of BP tended to increase ($P< 0.10$) His and Val concentrations in cows fed ASBP compared to ASSE; these AA concentrations were numerically higher in BP compared to HMSC (Appendix Table 1) and were possibly responsible for this difference.

Production and composition of milk

Protein supplementation increased milk and FCM yield, and milk CP, true protein and lactose production compared to ASCG ($P < 0.02$; Table 5). The milk yield response to increased protein supplementation of alfalfa diets in early lactation is well documented (Dhiman and Satter, 1993; Cardoniga and Satter, 1993; Olmos Colmenero and Broderick, 2006a). True protein yield increased 14 to 18% and fat yield increments were 6 to 8 % in ASSE and ASBP diets compared to ASCG. Milk protein and fat yields tended to be higher for ASSE compared to ASEP ($P\leq 0.10$). In the present study, the substitution of SE for a treated SBM product (EP) and the partial substitution of HMSC with BP (ASBP diet) both had a detrimental effect on lactation performance. The effect of increased %CP combined to the effect of RUP (EP) was studied by Olmos Colmenero and Broderick (2003a,b) in diets based on alfalfa and corn silage (20:35% DM basis). Milk production was not improved by feeding RUP (EP) or by increasing RDP (SE) in diets containing between 16.6 and 19.4 %CP. Other authors reported no responses in milk yield or composition to the inclusion of RUP (McCormick et al., 2001; Reynal and Broderick, 2003). In agreement with the present study, Titgemeyer and Shirley (1997) also found a reduction in milk protein when EP replaced SE, however these authors found that RUP supplementation increased FCM.

Based on the present study, replacing SE (ASSE) with EP (ASEP) would not improve the efficiency of N usage for milk. Differences in EAA

composition amongst soy protein sources were on average 5%; EP had lower concentration of EAA, especially for Lys and His. The lack of improvements in lactation performance and efficiency could possibly be explained by the fact that RUP from EP may have not significantly contributed to the intestinal supply of AA (Ipharraguerre et al., 2005; Borucki Castro et al., 2006b). Negative effects on microbial protein could be a possible explanation (Ipharraguerre and Clark, 2005) but no evidence of this effect is reported in the present study. The higher bypass potential of EP (Borucki Castro et al. 2006a) was reflected in plasma only for those AA with lower rates of degradation, such as the branched chain AA and Phe (Table 4). Other authors reported that the imbalance in the EAA supply might be the cause for the lack of responses to RUP supplementation (Baker et al., 1995; Noftsger and St-Pierre, 2003; Broderick, 2005). According to Bateman (2005) increased DMI is an important factor in the response to RUP. In the present study, the total replacement of SE by EP had no effect on DMI ($P > 0.10$).

Milk and milk protein yields tended to decrease with ASBP compared to ASSE ($P < 0.10$). Responses to the inclusion of beet pulp in diets for dairy cows are not conclusive. Hristov and Ropp (2003) found that although the inclusion of fermentable fiber (corn, beet pulp and brewer's grains) enhanced the conversion of ruminal ammonia into milk N compared to sources of starch (barley and molasses), milk and protein yield were not affected. Mansfield et al. (1994) found no differences in milk yield, but found increased fat concentration and lower milk CP yield and concentration, when beet pulp replaced half of the corn. These diets were based on alfalfa pellets and corn silage with forage to concentrate ratio of 47:53. Other authors found opposite results (O'Mara et al., 1997) or no differences in milk production or composition (Friggens et al., 1995; Delahoy et al., 2003) when corn sources were replaced with non-forage fiber sources.

8. 5. CONCLUSIONS Experiment 3

The supply of beet pulp, partially replacing high-moisture shelled corn to improve the utilization of N coming from RDP (SE), decreased DMI and tended to decrease milk and milk protein production. The use of beet pulp (ASBP) improved the concentration of His and Val in plasma, but had no effect on N efficiency. Replacing untreated SBM (SE) with treated SBM (EP) did not improve lactation performance or increase N usage for milk protein secretion. This fact was probably explained by the lack of improvement on the supply of intestinally digestible EAA for EP compared to SE.

8. 6. TABLES EXPERIMENT 3

Table 1: Ingredients and nutrient composition of the experimental diets

	Treatment[1]			
	ASCG	ASSE	ASEP	ASBP
Ingredient composition of TMR, % of DM				
Alfalfa silage	55.0	55.7	56.8	50.9
Corn, high moisture, shelled	33.3	21.9	20.3	11.7
Soybean meal, solvent extracted	0.0	11.9	0.0	11.7
Soybean meal, expeller	0.0	0.0	12.2	0.0
Sugar beet pulp[2]	0.0	0.0	0.0	15.3
Soybean, hulls	4.8	4.8	4.9	4.7
Calcium soaps of fatty acids[3]	4.8	4.0	4.1	3.9
Vitamin-mineral premix [4]	0.8	0.8	0.8	0.8
Sodium bicarbonate	0.8	0.8	0.8	0.8
Sodium phosphate	0.2	0.2	0.2	0.2
Nutrient composition of TMR				
DM, %	42.7	42.6	41.9	44.4
Gross Energy, Mcal.kg^{-1}	4.68	4.72	4.69	4.68
CP, % of DM	15.6	18.3	18.3	17.9
NDF, % of DM	35.6	33.5	37.2	35.3
ADF, % of DM	24.4	25.2	25.7	26.5
Fat, % of DM	7.41	6.65	6.84	6.43
Ash, % of DM	10.3	10.7	10.6	11.0
Essential amino acids, % of DM				
His	0.29	0.36	0.37	0.35
Ile	0.61	0.72	0.76	0.73
Leu	1.15	1.33	1.35	1.29
Lys	0.69	0.87	0.86	0.86
Phe	0.64	0.78	0.79	0.76
Thr	0.58	0.69	0.70	0.67
Val	0.75	0.86	0.90	0.87
Non essential amino acids, % of DM				
Ala	0.83	0.93	0.94	0.90
Glx [3]	1.46	1.91	1.92	1.82
Gly	0.65	0.77	0.79	0.76
Ser	0.69	0.84	0.84	0.81
Tyr	0.44	0.53	0.54	0.52

[1] ASCG=alfalfa silage diet + high-moisture shelled corn; ASSE=ASCG + solvent extracted soybean meal; ASEP=ASCG + expeller soybean meal; ASBP= ASSE+ sugar beet pulp
[2] unmolassed and dry, in pellets
[3] Megalac®, JEFO Nutrition Inc. (St Hyacinthe, Qc)
[4] Commercial mix, Omni 0 -0-13 (Shur Gain; Brossard, Qc): 18.9%Na; 29% Cl; 0.5% Ca;

0.4% P; 13% Mg; 4% K; 5% S; 6,200 ppm Mn; 330 ppm Fe; 110 ppm Co; 193 ppm I ; 1,500 ppm Cu; 7,000 ppm Z ; 46 ppm Se; 1,030,000 IU/kg Vitamin A; 182,000 IU/kg Vitamin D_3 ; 4,320 IU/kg Vitamin E.

Table 2: Intake and digestibilities of DM, OM and Energy balance and status of lactating dairy cows fed alfalfa silage supplemented with different energy and soybean meal sources

	Treatment[1]				SEM	Contrast[2]		
	ASCG	ASSE	ASEP	ASBP	n=8	ASCG vs others	ASSE vs ASEP	ASSE vs ASBP
				Intake				
DM, kg.d^{-1}	20.2	22.0	21.3	20.7	0.59	0.007	0.152	0.014
OM, kg.d^{-1}	18.1	19.6	19.0	18.5	0.51	0.007	0.140	0.007
Energy, Mcal.d^{-1}	94.7	104.0	100.4	97.0	2.73	0.002	0.087	0.002
				Digestion				
DM, %	68.0	69.2	68.9	69.3	0.61	0.070	0.648	0.892
OM, %	69.0	70.5	70.2	71.3	0.60	0.010	0.674	0.248
Energy, %	66.5	68.3	67.6	68.8	0.60	0.003	0.313	0.391
				Energy partition				
Feces, Mcal.d^{-1}	31.7	32.9	32.5	30.2	1.07	0.735	0.604	0.002
Heat[3], Mcal.d^{-1}	32.2	35.4	34.1	33.0	0.93	0.002	0.081	0.002
Methane[3], Mcal.d^{-1}	5.7	6.2	6.0	5.8	0.16	0.002	0.058	0.002
Urine, Mcal.d^{-1}	2.7	3.4	3.4	3.4	0.16	<0.001	0.662	0.716
Milk, Mcal.d^{-1}	24.0	27.1	26.3	26.3	1.12	<0.001	0.234	0.277
				Energy status				
Energy retained, Mcal.d^{-1}	- 1.6	- 1.1	- 1.9	- 1.8	1.20	0.999	0.385	0.490
BW change, kg.d^{-1}	+ 0.4	+ 4.7	+ 8.5	+ 8.5	4.78	0.231	0.578	0.582
Glucose, mg/ dL	61.4	62.9	63.0	63.5	1.29	0.110	0.881	0.611
NEFA, μEq / L	154	150	149	151	9.0	0.694	0.867	0.992

[1] ASCG=alfalfa silage + high-moisture shelled corn; ASSE=ASCG + solvent extracted soybean meal; ASEP=ASCG + expeller soybean meal; ASBP= ASSE+ sugar beet pulp
[2] Contrast correction by Bonferroni, significant at $P \leq 0.02$
[3] Estimated loss as heat (34%) and methane (6%) in proportion of gross energy ingested (Sutter and Beever, 2000)

Table 3: Nitrogen balance and N parameters in blood of lactating dairy cows fed alfalfa silage supplemented with different energy sources and soybean meal products

	Treatment[1]				SEM	Contrast[2]		
	ASCG	ASSE	ASEP	ASBP	n=8	ASCG vs others	ASSE vs ASEP	ASSE vs ASBP
N Intake, $g.d^{-1}$	524	694	679	671	17.8	<0.001	0.325	0.138
N excreted in feces, $g.d^{-1}$	189	216	215	207	6.8	<0.001	0.815	0.138
N apparently digested in the total Tract, %	63.7	68.2	68.3	69.1	0.87	<0.001	0.632	0.819
N excreted in urine, $g.d^{-1}$	133	261	252	257	9.2	<0.001	0.140	0.509
Urea N excreted in urine, $g.d^{-1}$	99	229	217	225	8.2	<0.001	0.187	0.667
N excreted in milk, $g.d^{-1}$	158	188	182	181	6.3	<0.001	0.106	0.101
N retention, $g.d^{-1}$	45	28	32	27	11.6	0.090	0.734	0.878
N usage for milk N, % intake	30.1	27.2	27.0	26.7	0.79	<0.001	0.537	0.840
PUN[3], mg/ dL	9.2	17.2	16.5	15.9	0.87	<0.001	0.367	0.113
MUN[3], mg/dL	9.7	17.1	15.7	16.7	0.64	<0.001	0.014	0.494

[1] ASCG=alfalfa silage + high-moisture shelled corn; ASSE=ASCG + solvent extracted soybean meal; ASEP=ASCG + expeller soybean meal; ASBP= ASSE+ sugar beet pulp
[2] Contrast correction by Bonferroni, significant at $P \leq 0.02$
[3] PUN = plasma urea nitrogen and MUN = milk urea nitrogen

Table 4: Plasma essential and non- essential amino acids of lactating dairy cows fed alfalfa silage supplemented with different energy sources and soybean meal products

	Treatment[1]				SEM	Contrast[2]		
	ASCG	ASSE	ASEP	ASBP	n=8	ASCG vs others	ASSE vs ASEP	ASSE vs ASBP
Essential AA, µM								
His	18	43	52	53	4.0	<0.001	0.047	0.030
Ile	113	145	159	155	9.1	<0.001	0.147	0.308
Leu	135	163	185	161	10.4	0.004	0.040	0.803
Lys	66	85	81	85	6.0	<0.001	0.529	0.982
Met	20	19	18	18	1.1	0.119	0.445	0.257
Phe	42	45	50	43	2.2	0.139	0.077	0.425
Thr	88	102	90	98	6.4	0.162	0.137	0.587
Trp	59	65	68	66	3.0	<0.001	0.365	0.731
Val	174	249	279	280	13.9	<0.001	0.052	0.043
Non essential AA, Mm								
Ala	262	280	262	269	12.4	0.438	0.153	0.386
Gln	292	290	271	267	13.2	0.276	0.290	0.213
Glu	55	49	48	50	1.9	0.004	0.788	0.630
Gly	376	327	294	302	16.5	<0.001	0.112	0.223
Ser	83	80	77	71	4.3	0.166	0.566	0.100
Tyr	56	59	60	54	3.4	0.645	0.777	0.266

[1] ASCG=alfalfa silage + high-moisture shelled corn; ASSE=ASCG + solvent extracted soybean meal; ASEP=ASCG + expeller soybean meal; ASBP= ASSE+ sugar beet pulp

[2] Contrast correction by Bonferroni, significant at $P \leq 0.02$

Table 5: Milk production and composition from lactating dairy cows fed alfalfa silage supplemented with different energy sources and soybean meal products

Item	Treatment[1]				SEM	Contrast[2]		
	ASCG	ASSE	ASEP	ASBP	n=8	ASCG vs others	ASSE vs ASEP	ASSE vs ASBP
Milk, $kg.d^{-1}$	37.2	41.8	41.0	39.9	2.8	<0.001	0.437	0.062
CP, %	2.73	2.91	2.85	2.91	0.086	<0.001	0.174	0.999
CP, $kg.d^{-1}$	1.01	1.20	1.15	1.15	0.040	<0.001	0.097	0.097
True Protein, %	2.24	2.44	2.38	2.45	0.098	<0.001	0.234	0.827
True Protein, $kg.d^{-1}$	0.82	1.00	0.96	0.96	0.033	<0.001	0.106	0.214
Fat, %	3.39	3.27	3.19	3.30	0.131	0.052	0.356	0.689
Fat, $kg.d^{-1}$	1.24	1.34	1.26	1.31	0.064	0.085	0.073	0.398
4% FCM[3], $kg.d^{-1}$	33.4	36.6	35.3	35.6	1.70	0.004	0.182	0.274
Lactose, %	4.97	5.02	5.06	5.02	0.079	0.348	0.601	0.964
Lactose, $kg.d^{-1}$	1.86	2.09	2.07	2.00	0.117	0.002	0.768	0.173
TS[4], %	11.8	12.0	11.8	12.0	0.22	0.301	0.285	0.669
TS[4], $kg.d^{-1}$	4.38	4.97	4.82	4.77	0.226	<0.001	0.239	0.120

[1] ASCG=alfalfa silage + high-moisture shelled corn; ASSE=ASCG + solvent extracted soybean meal; ASEP=ASCG + expeller soybean meal; ASBP= ASSE+ sugar beet pulp

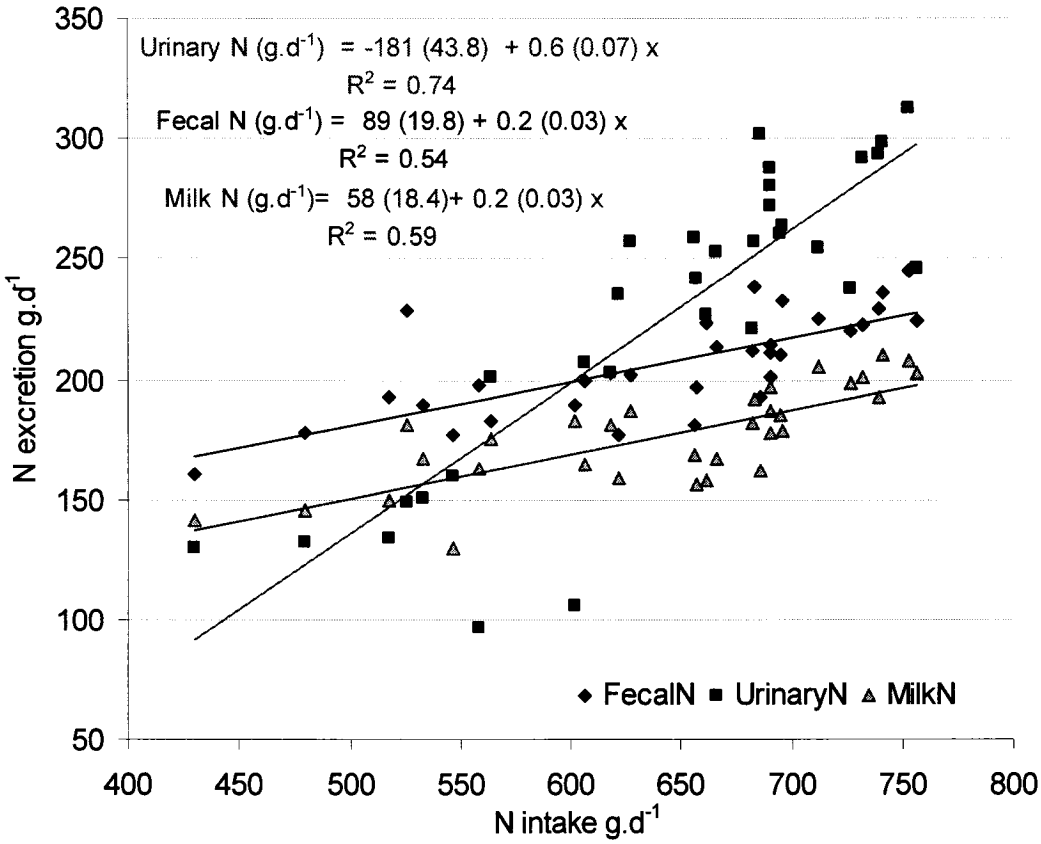
[2] Contrast correction by Bonferroni, significant at $P \leq 0.02$

[3] 4% FCM = 0.4 (kg milk) + 15 (kg fat) Gaine's formula (NRC 2001)

[4] TS = total solids

8. 7. FIGURES EXPERIMENT 3

Figure 1: Nitrogen excretion in feces, urine and milk as a function of N intake ($g.d^{-1}$)



8. 8. APPENDIX TABLES EXPERIMENT 3

Appendix Table 1: Nutrient composition of the TMR ingredients

	Alfalfa silage	Corn grain[1]	Sugar beet pulp[2]	Soybean meal	
				solvent extracted	Expeller
DM, %	32.9	75.2	91.8	92.4	92.6
CP, % of DM	19.0	10.3	10.1	51.8	47.8
Fat, % of DM			0.05	0.9	4.2
NDF, % of DM	45.0	12.0	50.8	11.5	29.5
ADF, % of DM	34.1	3.8	29.8	6.3	13.8
Ash, % of DM	10.9	1.8	8.6	7.3	7.1
pH	4.2	-	-	-	-
N - NH_3, % Ntotal	2.4	-	-	-	-
NPN, %CP	61.4	-	-	20.2	16.5
Soluble CP, % CP	69.0	-	-	29.0	18.6
Neutral detergent insoluble CP, %CP	12.4	-	-	4.0	29.5
Acid detergent insoluble CP, %CP	5.8	-	-	1.8	8.2
Essential AA, % of DM					
His	0.28	0.26	0.31	1.26	1.13
Ile	0.69	0.30	0.31	2.08	2.10
Leu	1.17	1.20	0.59	3.81	3.67
Lys	0.79	0.29	0.60	2.99	2.58
Phe	0.68	0.47	0.35	2.44	2.36
Thr	0.66	0.36	0.45	1.98	1.90
Val	0.85	0.43	0.48	2.12	2.16
Non-essential AA, % of DM					
Ala	0.91	0.77	0.45	2.15	2.08
Glx[3]	1.30	1.56	0.83	7.74	7.25
Gly	0.71	0.38	0.42	2.05	2.08
Ser	0.78	0.51	0.51	2.57	2.45
Tyr	0.40	0.40	0.48	1.81	1.78

[1] Corn grain, high moisture, shelled
[2] unmolassed and dry, in pellets

[3] Glx = Glu plus Gln

9. GENERAL CONCLUSIONS

Effective rumen degradability of protein (CP) and amino acids (AA) from solvent extracted SBM was greater than that of expeller SBM, lignosulfonate treated SBM, or SBM treated with heat and soyhulls. This was due to a greater fraction of soluble protein and a faster rate of rumen degradation of protein in solvent extracted SBM (SE). The treatment of SBM increased RUP from 42% to 68%. Estimates of effective degradability of branched chain AA (BCAA) and phenylalanine differed significantly among the treated SBM products but for the remaining essential AA (EAA), rumen degradability was similar. Irrespective of the SBM product, after 16 h of ruminal incubation, the degradation of lysine and histidine was greatest; methionine and BCAA were degraded to a lesser extent than the remaining EAA. Based on the mobile bag technique, *in situ* intestinal disappearance of AA ranged from 97 to 100% and all values were greater than those obtained *in vitro*. As the magnitude of the difference between the *in situ* and *in vitro* methods was greater (25%) with treated SBM products than with SE (10%), further development of the *in vitro* method should be considered before it can be used to replace the *in situ* procedure. Investigations into the availability of EAA using the in situ method, revealed no differences between expeller SBM and SBM treated with lignosulfonate or soyhulls. However, EAA availability was lower with SBM treated with soyhulls compared to lignosulfonate; this was explained by lower estimates of availability for BCAA and phenylalanine.

Three experimental approaches were used to quantify the availability of lysine in corn silage-based diets supplemented with either expeller or lignosulfonate SBM. Whether assessed based on duodenal flow of Lys followed by intestinal digestibility, on flux of ^{15}N lysine, or on plasma Lys response to omasal Lys infusions, there were no differences in lysine

availability among the diets. This is the first report of the use of ^{15}N lysine flux to determine lysine availability in dairy cattle. This approach not only links the intestinal supply of essential amino acids to metabolic usage but it avoids the use of cannulated animals, thereby being more acceptable in the context of animal welfare. Further studies should be performed to test the sensitivity of the lysine flux method and to validate underlying assumptions.

A performance trial aimed at evaluating the milk production response to rumen protected protein showed no improvement in milk yield, milk composition or efficiency of N usage when solvent extracted SBM was replaced with expeller SBM. Given the fact that EAA in RUP was significantly higher in EP than in SE in Experiment 1, but duodenal flow of EAA was similar between EP and SE in Experiment 2, expeller SBM may not have improved the supply of EAA under normal conditions of feeding. No evidence is presented on possible negative effects of these RUP sources on EAA of microbial origin. Providing a source of readily fermentable energy, in the form of beet pulp, did not alter milk production or reduce N excretion in manure. However, the use of beet pulp as a partial (50%) replacement for corn starch did increase the concentration of histidine and valine in plasma.

The results of this research suggest that, based on *in situ* procedures, expeller and lignosulfonate treatment could be used as an effective means of enhancing the supply of rumen protected EAA to the small intestine of dairy cattle compared to solvent extracted SBM. However, under conditions of normal feeding, responses in available EAA, and lactation performance and efficiency are not observed. There are limitations therefore, in the use of *in situ* methods to predict rumen undegradable protein and amino acids. The use of rumen-protected products may result

in adaptive responses in rumen function that need to be further and fully explored.

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11. APPENDIX

11.1. APPENDIX 1:

EXPERIMENT 1 - Experimental error and animal effects

Variable - Essential AA Rumen degradation kinetics	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Effective degradability (%)		
CP	55.59	38.31
His	38.15	29.73
Ile	13.26	38.86
Leu	17.22	44.60
Lys	25.43	24.49
Phe	18.44	46.29
Thr	54.24	0
Val	24.18	4.49
Lag phase (h)		
CP	23.76	1.69
His	24.92	8.29
Ile	16.24	68.71
Leu	7.53	64.76
Lys	13.95	6.53
Phe	7.47	63.95
Thr	38.22	0
Val	17.37	45.26
Soluble fraction "a" (%)		
CP	4.89	2.92
His	5.85	1.39
Ile	1.18	2.24
Leu	2.45	4.32
Lys	4.66	3.62
Phe	2.69	3.82
Thr	11.77	3.35
Val	1.93	0.58
Degradable fraction "b" (%)		
CP	283	0
His	6.14	1.04
Ile	3.89	0
Leu	5.64	1.00
Lys	4.55	4.24
Phe	4.59	3.43
Thr	12.61	4.98
Val	13.01	0
Rate of degradation "c" (h^{-1})		
CP	2.96×10^{-4}	5.1×10^{-5}
His	2.82×10^{-3}	4.96×10^{-4}
Ile	7.7×10^{-5}	1.23×10^{-4}
Leu	5.1×10^{-5}	1.51×10^{-4}
Lys	4.1×10^{-5}	1.09×10^{-4}
Phe	5.8×10^{-5}	1.65×10^{-4}
Thr	1.4×10^{-4}	0
Val	2.2×10^{-4}	0

Variable – 16 h rumen incubation residue (% original feed)	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
CP	5.74	4.06
His	7.20	2.10
Ile	9.24	0.10
Leu	7.24	0.12
Lys	6.76	1.01
Met	10.77	0.39
Phe	7.14	0.30
Thr	7.60	2.23
Val	9.84	0.12
Ala	7.38	0.23
Arg	7.56	0
Cys	12.54	1.01
Glx[3]	5.76	1.11
Gly	6.76	0.32
Pro	37.42	4.25
Ser	8.68	1.30
Tyr	7.68	1.00

Variable – Availability (% original feed)	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
His	28.31	19.60
Ile	13.57	25.95
Leu	16.70	4.70
Lys	15.71	6.66
Met	10.77	0.39
Phe	17.52	5.96
Thr	11.25	3.79
Val	17.25	2.25
Ala	13.67	3.26
Arg	7.29	0
Cys	12.89	1.55
Glx[3]	45.41	22.20
Gly	14.42	4.95
Pro	35.50	2.57
Ser	14.39	4.21
Tyr	14.13	7.18

Variable – Intestinal digestion in situ (% 16 rumen incubation residue)	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
CP	0.03	0.03
His	0.26	0
Ile	0.01	0.02
Leu	0.01	0.02
Lys	0.06	0
Met	0	0
Phe	0.03	0
Thr	0.03	0.01
Val	0.02	0.02
Ala	0.03	0.02
Arg	0.01	0.06
Cys	0.46	0.08
Glx[3]	0.05	0
Gly	0.25	0.09
Pro	0	0
Ser	0.01	0.20
Tyr	0.06	0

Variable – Intestinal digestion in vitro (% 16 rumen incubation residue)	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
CP	15.46	11.32
His	30.51	17.67
Ile	9.84	4.67
Leu	18.21	10.35
Lys	19.71	8.58
Met	96.79	0
Phe	14.24	13.67
Thr	17.53	11.19
Val	16.48	13.94
Ala	18.07	8.09
Arg	202.9	10.46
Cys	83.99	0
Glx[3]	45.81	0
Gly	16.89	3.43
Pro	43.99	0
Ser	22.16	5.21
Tyr	19.62	54.16

Variable - Nonessential AA Rumen degradation kinetics	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Effective degradability (%)		
Ala	66.88	2.97
Glx	29.52	26.77
Gly	52.17	1.20
Ser	21.65	10.59
Tyr	49.04	54.56
Lag phase (h)		
Ala	20.97	56.82
Glx	23.07	12.97
Gly	32.96	4.90
Ser	27.72	0
Tyr	13.86	72.97
Soluble fraction "a" (%)		
Ala	9.72	2.31
Glx	7.85	0.67
Gly	10.09	1.66
Ser	3.32	2.43
Tyr	6.60	5.50
Degradable fraction "b" (%)		
Ala	9.55	2.87
Glx	9.34	0.50
Gly	10.87	5.03
Ser	3.66	4.13
Tyr	6.60	5.50
Rate of degradation "c" (h^{-1})		
Ala	1.4×10^{-4}	9.4×10^{-5}
Glx	2.1×10^{-3}	4.4×10^{-4}
Gly	1.2×10^{-5}	3.7×10^{-4}
Ser	4.6×10^{-5}	7.9×10^{-4}
Tyr	1.3×10^{-5}	1.7×10^{-4}

11. 2.1. APPENDIX 2.1:

EXPERIMENT 2 - Experimental error and animal effects

Variable - Intake, duodenal flow and post ruminal digestion	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
OM		
Intake, kg/d	0.095	0
Passage to duodenum, kg/d	1.62	0.056
Apparently digested post-ruminally, %	43.05	0
N		
Intake, g/d	720.9	50.31
Passage to duodenum, g/d	3874.6	0
Passage to duodenum as NAN,% of duodenal N	0.50	0.16
Apparently digested post-ruminally, %	11.78	0
Lysine		
Intake, g/d	32.51	0
Passage to duodenum, g/d	390.6	0
Apparently digested post-ruminally, %	8.53	0
Histidine		
Intake, g/d	10.34	0
Passage to duodenum, g/d	42.69	3.41
Apparently digested post-ruminally, %	4.98	0
Branched chain AA		
Intake, g/d	0.24	0.82
Passage to duodenum, g/d	0.57	0.74
Apparently digested post-ruminally, %	0.27	0.18
Phenylalanine		
Intake, g/d	37.16	0
Passage to duodenum, g/d	211.9	0
Apparently digested post-ruminally, %	0.99	0.88
Threonine		
Intake, g/d	19.80	0
Passage to duodenum, g/d	179.7	0
Apparently digested post-ruminally, %	10.65	0

Variable - Rumen fermenta-tion parameters	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
pH	0.019	0.018
NH_3 – N, mg/dL	3.25	2.99
Total VFA, m*M*	2.71	4.41
Acetate, %	1.70	3.38
Propionate, %	3.01	3.48
Butyrate, %	0.24	0.71
Isobutyrate, %	0.09	0.32
Valerate, %	0.08	0.25
Isovalerate, %	0.10	0.40
A : P ratio[2]	0.75	2.51

Variable - Lys availability	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Digestible Lys, g/d	0.12	0.69
Plasma Lys, µM	0.03	0.58
WB Lys flux, g/d	0.07	0.78

Variable - ^{15}N Lys decay curve parameters	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
α	0.29	0.46
β	0.11	0.29
k_1	0.11	0.61
k_2	0.08	0.16

Variable - Plasma response curve	Experiment al error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Plasma Lys = f (omasal Lys infusion)	122.3	0

11. 2.2. APPENDIX 2.2:

EXPERIMENT 2 - Calibration curve – Bootstrapped inverse estimates

Diet	Plasma Lys (y value)	Variable estimate: (value x) post ruminal Lys increment	Bootstrapped SE
Solvent extracted SBM	89.241	4.373	0.876
Expeller SBM	80.405	1.704	1.103
Lignosulfonate SBM	85.052	3.108	0.963
Solvent extracted + 70 g/d omasal infusion	141.37	20.124	2.570

Calibration curve

95% confidence intervals – Bootstrap percentile method

Diet	Lower limit	Upper Limit
Solvent extracted SBM	3.035	6.349
Expeller SBM	0.172	3.975
Lignosulfonate SBM	1.743	5.226
Solvent extracted SBM + 70 g/d omasal infusion	18.279	25.997

11. 2.3. APPENDIX 2.3:
EXPERIMENT 2 - Model choice and parameter estimates for individual cow ^{15}N Lys enrichment decay curves

849	1	SE	0.97983	0.99909	double exponential	10.73	0.37	double exponential	24.76	12.08	0.43	0.08
849	2	SE 70	0.97235	0.99864	double exponential	10.77	0.43	double exponential	30.85	11.74	0.58	0.10
849	3	EP	0.97761	0.99877	double exponential	13.39	0.54	double exponential	34.75	10.48	0.50	0.09
849	4	LS	0.98907	0.99920	double exponential	8.16	0.42	double exponential	31.14	10.49	0.37	0.08
853	1	EP	0.96129	0.99725	double exponential	34.37	2.15	double exponential	163.2	22.06	1.28	0.15
853	2	SE	0.98565	0.99675	double exponential	13.77	2.44	double exponential	43.71	17.93	0.54	0.13
853	3	LS	0.97743	0.99709	double exponential	8.48	0.83	double exponential	21.56	10.35	0.45	0.08
853	4	SE 70	0.98648	0.99956	double exponential	3.69	0.09	double exponential	14.68	7.76	0.32	0.07
2002	1	LS	0.99204	0.99349	double exponential	5.00	3.63	double exponential	31.52	1.81	0.21	0.04
2002	2	EP	0.98676	0.99642	double exponential	10.13	2.09	double exponential	31.71	12.51	0.41	0.10
2002	3	SE 70	0.97446	2002	double exponential	7.87	1.99	double exponential	19.91	7.93	0.39	0.08
2002	4	SE	0.97576	0.99652	double exponential	12.53	1.41	double exponential	34.23	13.54	0.62	0.11

11.3. APPENDIX 3:

EXPERIMENT 3 - Experimental error and animal effects

Variable - intakes, digestibilities and energy status	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Intake		
DM, $kg.d^{-1}$	0.84	1.98
OM, $kg.d^{-1}$	0.58	1.48
Energy, $Mcal.d^{-1}$	0.001	0.50
Digestibility		
DM, %	2.12	0.84
OM, %	1.97	0.89
Energy, %	1.57	1.32
Energy partition		
Feces, $Mcal.d^{-1}$	2.30	6.87
Heat, $Mcal.d^{-1}$	1.82	5.04
Methane, $Mcal.d^{-1}$	0.055	0.158
Urine, $Mcal.d^{-1}$	0.114	0.089
Milk, $Mcal.d^{-1}$	1.91	8.13
Energy status		
Energy retained, $Mcal.d^{-1}$	3.54	8.07
BW change, $kg.d^{-1}$	0.59	0.46
Glucose, mg/ dL	6.08	7.30
NEFA, μEq / L	538.0	107.3

Variable - Nitrogen balance	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
N intake, $g.d^{-1}$	822.5	1705.8
N excreted in feces, $g.d^{-1}$	134.1	239.0
N apparently digested in the total tract, %	4.21	1.79
N excreted in urine, $g.d^{-1}$	154.2	527.2
Urea N in urine, $g.d^{-1}$	294.4	245.3
N excreted in milk, $g.d^{-1}$	75.32	241.1
N retention, $g.d^{-1}$	505.9	566.6
N use for milk N, % intake	2.52	2.46
PUN, mg/ dL	2.21	2.16
MUN, mg/dL	1.13	2.14

Variable - Plasma AA	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Essential AA, µM		
His	63.86	66.69
Ile	353.8	313.2
Leu	389.3	479.4
Lys	119.1	164.2
Met	6.56	3.49
Phe	27.16	10.13
Thr	222.9	99.14
Trp	41.64	31.23
Val	847.22	702.64
Non essential AA, µM		
Ala	559.8	668.3
Gln	1198.43	199.9
Glu	21.11	8.46
Gly	1585.3	603.1
Ser	123.6	26.88
Tyr	61.49	29.72

Variable - Lactation Performance	Experimental error $\sigma^2 e$	Random effect of cow $\sigma^2 cow$
Milk, $kg.d^{-1}$	3.33	38.16
CP, %	0.008	0.051
CP, $kg.d^{-1}$	3.1×10^{-3}	9.4×10^{-3}
True Protein, %	0.010	0.065
True Protein, $kg.d^{-1}$	2.3×10^{-3}	6.2×10^{-3}
Fat, %	0.028	0.109
Fat, $kg.d^{-1}$	0.007	0.026
4% FCM, $kg.d^{-1}$	3.28	19.73
Lactose, %	0.027	0.023
Lactose, $kg.d^{-1}$	0.018	0.092
Total solids, %	0.055	0.330
Total solids, $kg.d^{-1}$	0.059	0.349

11. 4. APPENDIX 4:

Formulaire d'approbation des methods experimentales sur les animaux

COMITÉ INSTITUTIONNEL DE PROTECTION DES ANIMAUX
CENTRE DE RECHERCHE ET DE DÉVELOPPEMENT SUR LE BOVIN LAITIER ET LE PORC DE LENNOXVILLE

FORMULAIRE D'APPROBATION DES MÉTHODES EXPÉRIMENTALES SUR LES ANIMAUX

(Révisé décembre 2002)

Numéro du projet: (Ne rien inscrire, le numéro du projet sera attribué par l'Administration)

Évaluation du mérite scientifique Date: 03 03 13

Évaluation du CIPA Date: 03 04 03

Titre du projet: The effect of different physical and chemical treatments applied to soybeans and/or soybean mea on the digestion and metabolism of amino acids from soybean meal in lactating dairy cows.

Mots clés: Recheche, évaluation de méthodes, canulation

Résumé vulgarisé du protocole: Nous nous proposons d'utiliser quatre vaches avec canules au rumen et au duodénum ainsi que 8 vaches non canulées pour étudier l'effet de différents traitements appliqués au soya sur la dégradabilité ruminale l'absorption intestinale et le métabolime dans le corps entier des acides aminés. Tout ceci dans le but de connaître les effets de ces traitements sur la biodisponibilité des acides aminés et de caractériser les besoins de vaches laitières et les apports en acides aminés des rations.

Numéro de projet en référence à un protocole antérieur: (s'il y a lieu)

Chercheur responsable: **Robert Berthiaume**

Collaborateurs(trices) du CRDBLP de Lennoxville:

1. Hélène Lapierre
2.
3.
4.

Autres collaborateurs(trices) impliquées: (Nom et institution)

1. Leroy Phillip, Université McGill
2. Pascal Dubreuil, FMV, Université de Montréal
3.
4.

Noms, qualifications professionnelles et formation des personnes qui manipulent les animaux
(Personnel de recherche, étudiants(es) 1er, 2e et 3e cycle, stagiaires post-doctoraux, etc.)

1. Sylvia Borucki, étudiante au Doctorat

2. Sylvie Provencher, technicienne

3. Personnel de l'étable

4.

- **Si le projet ne nécessite pas l'utilisation d'animaux, passez au point 13**

Phase animale A. Date de début : Mai 2003

B. Date de fin : Octobre 2003

Section A. Porcs

B. Bovins laitiers — B

Financement externe — Oui X / Non

Si oui, par: West Central Soya

Projet réalisé à des fins réglementaires (études cliniques) pour l'obtention d'une approbation par Santé Canada ou d'autres organismes réglementaires — Oui / Non X
(Si oui, répondez aux 2 questions suivantes)

A- Le projet a été planifié selon les lignes directrices réglementaires les plus récentes — Oui / Non

Justifiez:

B- Le nombre d'animaux utilisés ne dépasse pas les nombres exigés par les autorités réglementaires — Oui / Non

Justifiez:

1. **Classification d'utilisation des animaux**

Choisir à quelle classe du CCPA appartient votre projet en vous référant à l'annexe B. (N'inclure qu'une classe) — 01 | 17 | 02

2. **Catégorie de techniques invasives en expérimentation animale**

Choisir à quelle catégorie du CCPA appartient votre projet en vous référant à l'annexe C (Catégorie A, B, C, D ou E) *D dans le cas de la vache qui sera canulée — B

3. **But de l'utilisation des animaux**

Choisir une catégorie de 1 à 5 selon l'annexe D | 1

4. Type d'expérimentation: Aigu, chronique ou laboratoire

Préciser si les interventions sont aigues, au niveau du laboratoire, chroniques ou mixtes selon la définition du CCPA: (Choisir A, B, C ou M) | C

A. Aigu: toute étude au cours de laquelle un animal est euthanasié de façon humanitaire *dans le seul but de récolter certains tissus*.

B. Laboratoire: expérience *in vitro* et/ou échantillons récoltés à l'abattoir chez des animaux d'origine inconnue.

C. Chronique: toute autre étude où l'animal n'est pas euthanasié.

M. Mixte: étude ayant une phase animale pour collecter des données et qui se termine par l'euthanasie de l'animal (avec ou sans prélèvement de tissus).

5. Utilisation des animaux

A. Espèces animales utilisées et nombre exact d'animaux requis ou achetés au total (Définir pour chaque expérience, si nécessaire)

Espèce	Nb
vaches laitières canulées au rumen et au duodénum	4
vaches laitières	8

B. L'expérience nécessite-t-elle une contention (Immobilisation d'un animal par divers moyens pour restreindre ses activités) autre que celle utilisée dans la régie normale des animaux?

Oui [X]
Non [ ]

Si oui, **précisez:** Durée: 15 min. Pose de catheters jugulaires pour fluidothérapie.

6. Remplacement, réduction et raffinement

A. Est-ce que l'utilisation de méthodes alternatives (la culture de tissu, le prélèvement de matériel (biologique ou autres) qui ne nécessitent pas d'animaux vivants peut s'appliquer?

Oui [ ]
Non [X]

Justifiez: Il n'existe pas d'autres méthodes pour mesurer le transit digestif.

B. Sur quelle base statistique vous êtes-vous appuyé pour établir le nombre d'animaux à utiliser pour cette expérience?
Justifiez en fonction du choix des espèces et du nombre d'animaux:

Oui [ ]
Non [ ]

Nous avons réalisé trois projets au cours des dernières années comportant les mêmes types de mesure. En utilisant le CV observé(15% dans ces projets nous avons pu estimer le nombre de replicats nécessaires. Quatre vaches sont nécessaires pour détecter des différences entre les traitements de 15 à 20% avec une puissance de 90% à P<0.05.

C. Est-ce que la/les technique(s) utilisée(s) réduit/réduisent au minimum le niveau de détresse imposée aux animaux? Oui [X] Non [ ]

Justifiez:

Les canules utilisées sont beaucoup moins invasives que les canules réentrantes.

7. Description des points limites inhérents à l'expérimentation

(L'expression *point limite* est définie comme le moment auquel la souffrance et/ou la détresse d'un animal est arrêtée, minimisée ou diminuée en prenant des mesures comme celle d'euthanasier l'animal de façon humanitaire, de mettre fin à une procédure qui le fait souffrir ou en le traitant pour soulager sa souffrance et/ou sa détresse)

A. Types et fréquence des observations: Les animaux sont oscultés 1 fois/jour.

B. Choix des points limites: Température rectale(2 fois/jour):température supérieure à 39,1°C ou inférieure à 38°C. Etat de santé général: anorexie partielle ou totale, signes de coliques, atonie ruminale. Détresse cardio-respiratoire. Bande HEMATEST pour détecter la présence de sang dans les fèces à tous les jours.

C. Choix des interventions au besoin: Selon l'évolution des symptomes le Dr Martineau recommande les traitements appropriés.

D. Qui a la responsabilité de l'observation/surveillance des animaux?

Nom(s): Robert Berthiaume, Hélène Lapierre, Roger Martineau , Sylcia Borucki et Sylvie Provencher

Formation:
R. Martineau, DMV
R. Berthiaume, PhD Sciences animales
H. Lapierre, PhD Sciences animales
S. Borucki, Msc PhD candidate
S. Provencher, technicienne

8. Devenir des animaux utilisés dans le projet

A. Est-ce que les animaux sont sacrifiés dans le cadre du projet? Oui [ ] Non [X] S/O [ ]

Si **oui**, est-ce que la méthode d'euthanasie est conforme aux normes acceptées parle CCPA? (Décrire la méthode) Oui [ ] Non [ ]

Si **non**, décrire et justifiez la méthode qui sera utilisée:

B. Préciser le devenir des animaux vivants à la fin du projet:. Retour dans le troupeau

9. Pointer les types d'interventions qui seront pratiquées chez les animaux:
(Cocher et décrire, si nécessaire, le nombre d'animaux ou de fois, quantité par prélèvement, etc.)

Jeûne							
Gavage							
Injections		s.c.		i.m.		i.v.	
		autre: (préciser)					

Prélèvements	X	sang		lymphe		salive	
	X	liquide ruminal	X	lait	X	urine	
	X	fèces	X	autre: (préciser)		digesta duodénal	

Pose de cathéters	X	type: (préciser)	jugulaires pour hydrothérapie et médication si nécessaire

Autre: (préciser) canules ruminales et duodénales. Trois des 4 vaches utilisées possèdent déjà des canules ruminales et duodénales. Il nous faudra opérer une quatrième vache pour le projet. Cette vache sera gardée par la suite.

Préciser la fréquence, la durée et/ou la quantité pour chaque type d'intervention:
Infusions: mesure du débit du digesta et du temps de résidence dans le rumen.
- Infusion d'un marqueur indigestible (Co-EDTA) dans le rumen.
- des jours 7 à 14 de chaque période expérimentale (4 fois)

Prélèvements de digesta:
- 4 échantillons par jour , 250 ml /échantillon
- jours 12,13,et 14.

Collecte de fèces et d'urine: effectuées pendant 4 periodes de 5 jours.

10. Chirurgies ou procédures nécessitant une anesthésie ou une analgésie:

A. Est-ce que les animaux seront soumis à une forme de chirurgie (incluant les biopsies) ou à toute autre procédure pouvant nécessiter une anesthésie ou une analgésie?

Oui	X
Non	

▶ Si **non**, passez au point 11
Si **oui**, répondez aux questions suivantes:

- Laquelle? Canules ruminales et duodénales (1 vache)
- Quel est le nombre maximum prévu par jour? 1
- Est-ce que l'animal sera sous anesthésie locale? Oui ☐ Non X
- Est-ce que l'animal sera sous anesthésie générale? Oui X Non ☐
- Est-ce que des soins post-opératoires seront donnés? Oui X Non ☐

- Donner le nom, la dose, la fréquence et la voie d'administration de tous les médicaments des catégories suivantes utilisés lors des procédures:

  Tranquillisant : ACEPROMAZINE, 25 Mg I.V.

  Pré-anesthésique : Glycéril guaiacolate plus thyobarbiturique inductio i.v. à effet

  Anesthésique : halothane, maintien de l'anesthésie à effet

  Analgésique : fluvexin ou kétoprofen, 1mg/kg, au besoin

  Antibiotique : Pénicilline, débutant 12 heures avant la chirurgie et pendant une semaine

- Est-ce que la personne qui fera cette procédure l'a déjà faite auparavant? Oui [X] Non [ ]

  Si non, qui sera là lors de la première procédure pour lui enseigner? (nom et formation)

- Si aucune anesthésie ou analgésie n'est utilisée, justifiez:

B. Description détaillée des procédures chirurgicales: (inclure une copie des références, le cas échéant)
La canule duodénale sera implantée sur une vache tel que décrit dans l'article de ***Robinson et Kennelly(1990).***
N..B.: Ce type de chirurgie a été réalisé a plusieurs reprises à Lennoxville. Les vaches suivantes: 853, 831 et 339 ont été opérées en 2001 et se portent bien. Ces vaches ont complétés une lactation et se sont reproduits normalement.

11. Caractéristiques des produits utilisés ne nécessitant pas l'obtention de permis:

NOM	VOIE (im., sc., orale, etc.)	DOSE
Co-EDTA	Rumen	1 g/j

- Y a-t-il des effets secondaires critiques fréquents ou importants suite à l'apport de ce(s) produit(s)? Oui [ ] Non [X]
- Si oui, lesquels?

12. Caractéristiques des produits utilises nécessitant l'obtention de permis: Aucun [ ]

NOM	VOIE (im., sc., orale, etc.)	DOSE

	Agents infectieux (ex: bactérie, virus)	
	Matériel biologique (ex: vaccin)	
	Agent chimique (ex: médicament)	
	Radioisotope (ex: I_{131})	
	Agents à biorisque	
X	Autres	Anesthésiant

- Est-ce que vous avez l'approbation institutionnelle relative à l'utilisation de ces produits?
- **Précisez** pour chaque produit:

Le Dr Dubreuil possède les autorisations requises

- Quels sont les effets secondaires ou toxiques suite à l'apport de ce(s) produit(s)?

Aucun