ACNOWLEDGEMENTS

Sincere appreciation is expressed to all the people who contributed to the completion of this thesis. I am especially grateful to Dr. Randy Shaver for his invaluable personal and technical support, guidance, advice, and words of encouragement. The many opportunities and responsibilities he provided me were instrumental in both my professional and personal development. I would also like to thank to all my committee members, Dr. Dave Combs and Dr. Victor Cabrera for their input and advice.

Appreciation is also expressed to my fellow graduate students. The diversity of scientific interests and cultures I have experienced has been invaluable growing experience. The authors would like to specially thank Matías Aguerre for his advice, friendship and for his review of this manuscript prior to submission. Gratitude is also expressed to Peter Crump and Jose Pires for their support in our statistical experimental design.

Finally, I would like to thank to my parents, Juan Alfonso and Martha Teresa, for the prayers and encouragement they have provided and also to my wife Michaela for her support and encouragement during these years.

TABLE OF CONTENTS

LIST OF TABLES

LITERATURE REVIEW

LIST OF TABLES (CONTINUED)

CHAPTER I

LIST OF TABLES (CONTINUED)

CHAPTER III

LIST OF FIGURES

LITERATURE REVIEW

LIST OF FIGURES (CONTINUED)

CHAPTER II

CHAPTER III

EVALUATION OF SLOW-RELEASE UREA (Optigen®) USE IN COMMERCIAL DAIRY HERD DIETS

By

JUAN FRANCISCO INOSTROZA MULET

A thesis submitted in partial fulfillment of the

Requirements for the degree of

MASTER OF SCIENCE

(Dairy Science)

At the

UNIVERSITY OF WISCONSIN-MADISON

2009

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Over the last three decades, the emphasis of ration formulation has shifted from only milk volume and fat to also include milk protein percentage and yield. In recent years, mathematical approaches have allowed for improved models of nutrient requirements and utilization. These models will be used more frequently in the future to support decisions not only regarding the nutrition of dairy cattle, but for other aspects including farm economics and environmental impact (Chalupa, 2007).

Crude protein (CP) is one of the more costly components of a dairy cow ration. While nutritionists are typically concerned with the effect of varying dietary protein concentrations and sources on ration cost, there is a growing appreciation of the cost of inefficient protein utilization. Protein supplied in excess of cow requirements, is converted to urea by the liver and recycled to the rumen or excreted in urine, milk or manure (Broderick, 2003, Burgos et al., 2007). Concern over potential pollution of groundwater by nitrogenous and ammonia emissions from manure has led to limits on how much manure nitrogen may be applied to cropland. Efficient use of protein results in less nitrogen excreted in manure per pound of milk produced (Weiss et al., 2007).

Non-protein nitrogen (NPN) is usually a less expensive nitrogen-dense form of rumen degradable protein (RDP) than oil-seed meals. Urea is the principal form of NPN presently used in ruminants rations (Huber, 1975) and it has shown some advantages when included in dairy cattle rations, specially feed costs reduction since it has shown price advantage that justified its inclusion in dairy concentrates mixtures; however, excess urea in the diet can be toxic to ruminants. The inability of the liver to convert all absorbed ammonia to urea is responsible for the presence of ammonia in peripheral blood which may result in toxicity (Chalupa, 1968). Theoretically, larger amounts of urea could be used for microbial protein production if release rates were matched to usage by the ruminal ecosystem (Siciliano-Jones, 2005). In corn growing areas there has been a marked increase in corn silage feeding to dairy cattle, thus, increasing the supplementation of protein relative to a legume-silage based ration. NRC (2001) suggested that a practical limit for urea in concentrates is between 1.5 and 2%. Higher levels of urea than suggested have resulted in depressed feed intakes and lowered milk yields. Furthermore, detoxification of ammonia due to excess of urea in dairy cattle diets is energy dependent, thereby reducing energy available for productive and reproductive purposes (Butler, 1998). The author stated that, ruminally degradable protein or ruminally undegradable protein in excess of requirement could contribute to reduced fertility in lactating cows. Protein in excess of lactation requirements has been shown to have negative effects on reproduction (NRC, 2001). Dietary protein nutrition or utilization and the associated effects on ovarian or uterine physiology have been monitored with urea nitrogen in plasma or milk; concentrations above 19 mg/dl have been associated with altered uterine pH and reduced fertility in dairy cows.

Mechanisms for reduced fertility include exacerbation of negative energy balance and reduced plasma progesterone concentrations when cows were fed rations that were high in ruminally degradable intake protein. Alternatively, changes in uterine secretions that are associated with high protein intake and elevated plasma urea nitrogen might be detrimental to embryos. The conversion of an ammonia molecule to urea (urea cycle) by liver costs 3 ATP and in other tissues (kidney, muscle and brain), glutamic acid reacts with ammonia to form glutamine, which costs 1 ATP. Trials focused on synchronizing the ruminal production of ammonia with ruminal energy digestion have been done on the development of controlled release urea compounds for more than 30 years. Galo (2003) suggested that slow-release NPN compounds, including isobutylidene diurea, acetylurea, biuret, starea, linseed-oil-coated urea and formaldehyde treated urea have not been as advantageous as urea because a substantial part of the NPN in them may leave the rumen without being converted to ammonia, reducing its incorporation into microbial protein. These data clearly indicate that a new approach to supplying controlled release NPN was needed.

In the first section of this review, the use of urea as a supplement and the use of controlled-release urea in dairy cattle rations are reviewed. In the second section, dietary effects of protein and energy interactions on microbial protein synthesis (MPS), milk production and milk urea nitrogen (MUN) is reviewed. In the third section statistical designs for commercial dairy field trials and the use of a crossover design is described. In the last section of this review, the importance of economic analyses, especially Income Over Feed Costs (IOFC), for supplements and additives is discussed.

USE OF UREA IN DAIRY CATTLE RATIONS

Non-protein nitrogen (NPN) in feedstuffs

Dietary crude protein (CP) for feedstuffs is defined as the nitrogen (N) content multiplied by 6.25. The definition is based on the assumption that the average N content of feedstuffs is 16 g per 100 g of protein (NRC, 2001). Crude protein includes protein and non-protein nitrogen (NPN) and many feedstuffs for livestock contain NPN. Forages generally are higher in NPN when compared with concentrates. Corn silage can contain as high as 50 % of its total nitrogen as NPN and preservation of alfalfa silage results in 45% NPN (Broderick et al., 1999). Alfalfa hay may content between 10 to 20 % of the nitrogen as NPN (NRC, 2001). Nitrogen fractions, the amino acids lysine and methionine, and RUP digestibility of common feedstuffs used in Midwest are presented in **Table 1**.

One of the main objectives of ruminant protein nutrition is to provide adequate rumen degradable protein (RDP) for optimal rumen microbial protein synthesis (NRC, 2001). There are many protein sources with a high content of RDP that are commonly fed to dairy cows. The most common is solvent soybean meal (SBM), which is fed worldwide for supply and cost effectiveness reasons. Other oil seed meals are fed to dairy cows as protein supplements, including cottonseed meal (CSM), canola meal (CNM), sunflower meal (SFM), and linseed meal (LSM).

According to NRC (NRC, 2001), SBM, CSM, CNM, SFM, and LSM contain 57%, 58%, 58%, 84%, and 47% RDP (% of CP), respectively. Conversely, the animal-marine protein supplements blood meal and fish meal contain only 34% and 23% RDP (NRC, 2001), respectively.

In recent years, the price of SBM has been highly volatile thereby causing strong effort to find new alternatives for controlling feed costs. Urea is an alternative, less expensive source of NPN for use in ruminant diets as a source of RDP with 100% RDP (% of CP equivalents [CPE]). Urea is produced commercially from two raw materials, ammonia and carbon dioxide. Large quantities of carbon dioxide are produced during the manufacture of ammonia from coal or from hydrocarbons such as natural gas and petroleum-derived raw materials. This allows direct synthesis of urea from these raw materials (Glibert et al., 2006). Urea is a simple compound that contains 46.7% of nitrogen. Ruminants have the unique ability to metabolize dietary NPN and RDP for synthesis of protein by bacteria within the rumen. Essentially all endogenous urea production occurs in the liver (Huntington and Archibeque, 2000), however, other tissues have the enzyme activity required to urea production (Emmanuel, 1980). The liver removes and detoxifies any excess of ammonia that is absorbed, or diffuses, across all sections of the digestive tract of ruminants by converting the ammonia to urea. Once released into the blood, endogenous urea is excreted in urine and milk or reenters the digestive tract by diffusion into saliva or directly across the gut wall (Huntington and Archibeque, 2000). The authors stated that, urea production, excretion, and recycling to the gut are linked to diet composition, intake, and productive priorities of the animal.

The urea cycle (**Figure 1**) consists of five reactions, two mitochondrial and three cytosolic. The cycle converts two amino groups, one from NH_4^+ and one from aspartic acid, and a carbon atom from HCO_3^-, to the relatively nontoxic excretion product, urea, at the cost of four "high-energy" phosphate bonds, three ATP hydrolyzed to two ADP and one AMP (**Table 2**). Urea-genesis in the liver is closely linked to degradability of dietary N and subsequent absorption of ammonia (Huntington and Archibeque, 2000).

Ammonia is an essential compound factor in the utilization of NPN by ruminants. According to NRC (1976), the following steps are involved in the utilization of urea by ruminants:

(1) Urea → *Microbial urease* → $NH_3 + CO_2$

(2) Carbohydrates → *Microbial Enzymes* → Volatile Fatty Acids + Keto Acids

(3) NH3 + Keto Acids → *Microbial Enzymes* → Amino Acids

(4) Amino Acids → *Microbial Enzymes* → Microbial Protein

(5) Microbial Protein → *Animal Enzymes in the Abomasum and Small Intestine* → Free Amino Acids

(6) Free Amino Acids are absorbed from the small intestine and used by the host animal.

As indicated by the scheme, NPN must first be converted to ammonia and microbial enzymes mediate the reaction.

In the case of urea, the hydrolytic enzyme is urease. Urea is rapidly hydrolyzed under most ruminal conditions. One concern of high levels of urea feeding is excess of ammonia production, which may lead to ammonia toxicity (NRC, 2001). Ruminants depend on the liver to detoxify an excess of ammonia absorbed from the gut. Huntington and Archibeque (2000) stated that the capacity of the liver sometimes is exceeded, and that response is usually associated with excess supply of dietary urea or similar rapidly degradable N source. Non-protein nitrogen compounds are broken down to ammonia, the central component, during the fermentation process in the rumen. As presented in previous scheme, microorganisms in the rumen combine this ammonia with products of carbohydrate metabolism to form amino acids in microbial protein.

Microbial protein passes from the rumen into the abomasum and small intestine for digestion and absorption to meet the daily metabolizable protein requirements of the animal **(Figure 2).**

Urea utilization and milk production.

Ruminants can make efficient use of diets that are low in protein content or of poor quality, because ruminal microbes synthesize good quality protein plus capture recycling urea N that would otherwise be excreted in the urine (Broderick, 2006). Increased amounts of supplemental proteins in the rations above cow requirements increases feed costs and contribute to environmental N pollution.

Nowadays, there is a growing awareness worldwide of the necessity to protect the environment by preventing the contamination of soil and water with excessive amounts of phosphorus (P) and N mainly (Tamminga, 1996). The NRC (2001) stated the NPN sources like urea can be an effective supplier of RDP along with the RDP coming from true protein in supplements for rumen microbial protein production. However, some researchers have found that greater synthesis of rumen microbial protein can be achieved if diets contain only RDP coming from true protein. For example, Brito et al. (2007) replaced supplemental urea N with true protein from SBM, CSM, or CNM and observed that omasal flow of individual AA, essential AA, nonessential AA, and total AA all were lower for cows fed urea. Lower flows of microbial NAN and AA explained depressed yields of milk and milk components on the urea diet than the true protein diet.

Several studies (Boucher et al., 2007, Broderick, 2003, Broderick et al., 2009, Burgos et al., 2007, Olmos Colmenero and Broderick, 2006, Wu and Satter, 2000) showed the effects of supplemental RDP from true protein, true protein plus urea, or urea on milk yield, milk components (% and kg), MUN, and BUN by dairy cows (**Table 3**). Replacement of RDP from true protein sources like SBM with RDP from urea in diets formulated mainly with corn and alfalfa silage resulted in linear decline in milk, fat, and protein yields. Also, linear increases in concentrations of MUN and BUN with increasing concentrations of dietary urea were observed.

Broderick et al. (2009) indicated that replacing RDP from true protein sources with that from NPN sources like urea, may reduce yields of milk and milk components by depressing microbial protein production in the rumen or NPN sources were less effective than true protein for supplying RDP. Boucher et al. (2007) concluded from their study that the optimum ruminal ammonia concentration to support maximum synthesis was between 11 and 13 mg/dl when a corn silage-based diets are fed to lactating cows. Theses results were achieved when concentrations of RDP were 10.0 and 10.8% of the diet DM and dietary urea concentrations were 0.3 and 0.6% (DM basis). On the other hand, Olmos and Broderick (2006) indicated that, according to their study, diets containing 16.5% of CP supported maximal milk, fat and protein yields with reduced MUN and BUN concentrations and reduced N excretion to the environment compared with diets with higher CP content. Regardless the energy content of the diets, feeding 16.7% CP was adequate for supporting high milk, fat, and protein yields and reducing MUN and the excretion of N into the environment.

Since high costs associated with high rates of protein supplementation and environmental concerns have become issues, dietary CP guidelines are being reevaluated. Wu and Satter (2000) found that early lactation diets (8 weeks) for high producing cows (~11,000 kg/308 d) should contain a minimum of 17.5% CP of which RUP is 35 to 37%. Dietary CP in later lactation should be reduced as milk production declines. This reduction should not occur before mid-lactation, and then not be reduced below approximately 16%.

Factors influencing urea utilization are shown in **Table 4**. Broderick (2007) suggested that only a portion of the dietary RDP can be replaced by NPN, because of limited ability of ruminal microbes to utilize ammonia. Ammonia is used best on diets that are high in NFC. Reducing grain particle size increases ruminal starch digestion and increases microbial protein production (Huntington, 1997).

NEW TECHNOLOGIES FOR UREA USE BY RUMINANTS

Use of Controlled-Release Rumen Urea as a Source of NPN in Dairy Cattle Diets.

Over the past 30 years, a number of technologies have been developed to synchronize ruminal NPN release with carbohydrate degradation in the rumen in an attempt to maximize ruminal microbial yield (Tikofsky, 2007).

New products containing urea with biodegradable coatings have been recently marketed for use in ruminant diets. These products differ from earlier attempts in that manufacturing procedures include the addition of a coating designed to slow the rate ammonia release from urea. This strategy to use urea in dairy diets relates to potential problems with the use of unprotected urea such as poor feed intake, depressed milk fat, and even death by toxicity (Huber and Kung, 1981). In addition, Owens (1980) suggested the rapid ruminal hydrolysis of urea as a factor of limiting the utilization of urea as a NPN source.

Akay et al., (2004) suggested that it is important that the rate of ammonia production in the rumen be coordinated with the rate of carbohydrate fermentation since bacterial growth is dependent on ammonia produced in the rumen and energy availability (Newbold and Rust, 1992).

Urea derivates, such as isobutylene diurea (Teller and Godeau, 1986) and biuret (Löest et al., 2001), or urea combined with different substances, such us linseed oil coated urea (Forero et al., 1980) and formaldehyde-treated urea (Prokop, 1976), have been utilized. In addition, approaches to match energy availability with ammonia release in the rumen have been investigated for synchronizing energy availability with ammonia release in the rumen (Forero et al., 1980). Combinations of urea and starch (Deyoe et al., 1968), urea and cellulose (Conrad and Hibbs, 1968), starea a mixture of gelatinized starch and urea (Bartley and Deyoe, 1975, Helmer et al., 1970, Jones et al., 1975, Males et al., 1979), and urea and fatty acids (Wanasundara and Shahidi, 1999) have been also investigated. However, these protected products have not been widely adopted, because it appears that a substantial portion of the NPN may leave the rumen without being converted to ammonia, thus reducing its incorporation into bacterial protein (Akay et al., 2004). In addition, the ammonia formation from these compounds in the rumen, though slower than urea, was still too fast to optimize microbial protein production by rumen bacteria (Owens and Zinn, 1988).

Recently, new sources of controlled release urea have been designed, Optigen® 1200 (Galo et al., 2003), Optigen® (Harrison and Karnezos, 2005) and Nitrosure (Emanuele and Putnam, 2006), for incorporation into dairy and beef cattle rations. Golombesky (2006) and Owens (1980) suggested that a slow-release of urea ruminally could minimize the detrimental effects of unprotected urea and thus enhance the acceptance of urea supplements by the feed industry and the utilization of urea by ruminants.

Golombesky (2006) conducted a trial and concluded that the addition of a slow-release urea to dairy rations, reduced intake without affecting milk production resulting in improved feed efficiency. Slow-release urea (Ruma-Pro) is calcium chloride urea. Eight multiparous and 4 primiparous Brown Swiss cows (117 ± 46 d in milk) were blocked by parity and utilized in a multiple Latin square design. Basal diets were formulated for 16.6% crude protein and 1.55 Mcal/kg of net energy for lactation and contained 35% of dietary dry matter as corn silage, 15% alfalfa hay, 34% of a concentrate mix containing varying proportions of ground shelled corn and soybean meal, and 16% of a constant concentrate premix. Data obtained in an experiment conducted by Forero (1980) indicated that slow-release urea (NIPAK Corporation) improved palatability of urea-containing supplements and effectively slowed ammonia release from urea Eighty-five lactating Hereford cows, were individually fed five different supplements in a 92-day trial.

Supplements contained: 15 or 40% SBM (negative and positive control, respectively), 1.22 kg/head/day; 40% protein (62.5% of crude protein equivalent from SRU), 1.22 kg/head/day; 40% protein (62.5% of crude protein from urea), 1.22 kg/head/day; and 20% protein (62.5% of crude protein equivalent from urea), 2.44 kg/head/day.

These data clearly indicate that a new approach to supply controlled release rumen protected urea, as a NPN was needed. Optigen® is an NPN supplement with a biodegradable coating, a mixture of Vegetable Oil, Beta Carotene, BHT and Citric Acid to coat individual non-protein nitrogen prills, that has controlled release properties using. This product is a highly concentrated nitrogen source with 256% CPE (DM basis; 41% N x 6.25) designed to enhance rumen function by supplying nitrogen to rumen bacteria at a rate that optimizes the conversion into bacterial protein (Tikofsky, 2007). The coating is designed to be inert in the gastrointestinal tract of ruminants, yet release water-soluble urea through pores in the coating.

The passage rate of digesta out of the rumen will vary depending on the level of feed intake. Optigen® provides 7.0% immediately available (soluble) N and 81.5% potentially degradable N with a fractional degradation rate of 23.7%/h as determined In vitro by García-González (2007). *In situ* disappearance of Optigen® was complete within 8 to 16 hours of ruminal incubation, depending on the manufacturing process **(Figure 3).**

Siciliano-Jones and Downer (2005) suggested that the urea in Optigen® diffuses through the coating over a period of approximately 16 hours in the rumen, but the rate of diffusion is a function of the coating integrity and thus may vary. Analyses conducted by FARME Institute, Inc. (Homer, NY; Siciliano-Jones and Downer, 2005) demonstrated the slow-release characteristics of Optigen® under *in situ* conditions **(Figure 4).** Optigen® also increases the N density of the protein supplements in the diet, thereby creating more space for the inclusion of carbohydrates in the ration. The *in situ* nitrogen disappearance rate of Optigen® has been found to be similar to other RDP such as SBM, CSM, LSM or SFL and slower than unprotected urea **(Figure 5).**

Emanuele et al. (2001b) proposed the following objectives for the development of controlled-release urea products: provide an improved NPN source for enhanced rumen health and microbial populations, increase milk production by dairy cattle, increase efficiency of feedstuff utilization, maintain between 6 to 18 mg NH3 – N/100 ml of rumen fluid on a continuous daily basis during a feeding regimen, and decrease manure excretion. Emanuele et al. (2001a) further proposed that controlled-release urea can have several advantages when incorporated into feeding programs for lactating dairy cattle:

1. The amount of supplemental true protein in the ration can be reduced (i.e., SBM);
2. Increase the nutrient density of supplements in the diet, thereby creating more space in the ration for high quality forage (i.e., corn silage).
3. Utilization of forages with low crude protein content is promoted (e.g., tropical grasses);

4. Greater flexibility in diet formulation (Tikofsky, 2007);
5. The volume of cattle manure is lessened, and the nitrogen content of the accumulated manure output is minimized. (Efficient use of proteins results in less nitrogen excreted in manure for every pound of milk produced (Weiss et al., 2007);
6. The ruminal ammonia profile is similar to SBM, and thereby satisfactory for efficient microbial growth;
7. Population and efficiency of rumen microorganisms is increased (**Table 5**); and
8. A higher level of NPN can be fed without detrimental ammonia toxicity.

Siciliano-Jones and Downer (2005) observed in their study that the risk of ammonia toxicity from Optigen® was low and attributed that safety to the controlled-release of nitrogen in the rumen. A limited scale toxicity test and initial field observations showed that the product could be safely fed to lactating dairy cows at up to 454 grams per head per day as shown in **(Table 6).**

Rumen Degradable Protein (RDP) and Rumen-Undegradable Protein (RUP).

The objective of feeding dairy cattle nutritionally balanced diets is to provide a rumen environment that maximizes microbial production and growth. When designing rations for ruminants, the needs of both the animal and the rumen microorganisms must be considered. Dietary CP in ruminant diets serves as a source of metabolizable protein providing both ruminal-degraded protein (RDP) for microbial synthesis and ruminal undegraded protein (RUP). Specifically, the goals of ruminant protein nutrition are to provide adequate amounts of RDP for optimal rumen efficiency and to obtain the desired animal productivity with a minimum amount of dietary RUP, as RUP is usually more expensive than RDP. NRC (1976) suggested that, the efficiency of use of CP is optimized with the inclusion of complementary feed proteins and NPN supplements in the ration. Those supplements should provide amounts and types of RDP that will meet the N needs of ruminal microorganisms for maximal synthesis of microbial crude protein (MCP) and the necessary amounts of digestible RUP that will optimize the profile and amounts of absorbed amino acids (NRC, 2001). Ruminal degradation of dietary feed CP is an important factor influencing ruminal fermentation and AA supply to dairy cattle.

Ruminally degraded feed protein provides a mixture of peptides, free amino acids and ammonia for microbial growth and synthesis of microbial protein. Ruminally synthesized microbial protein supplies most of the AA that will pass to the small intestine (**Table 7**). Rumen undegraded protein (by pass protein) is the second source of absorbable AA for ruminants.

According to NRC (2001) RDP values for feedstuffs (% of CP) are computed using the equation RDP = A + (B * (kd / [kd + kp])). The RUP values for feedstuffs (% of CP) are computed using the equation RUP = (B * (kp / [kd + kp])) + C or RUP = 100 – RDP. These data fit a model with three pools where:

A = Fraction A, % of CP. NPN or protein assumed to be instantly degraded in the rumen

B = Fraction B, % of CP. Protein that is potentially degraded in the rumen.

C = Fraction C, % of CP. Protein that is undegraded in the rumen.

Kd = Rate of degradation of fraction B, % / h.

Kp = Rate of passage from the rumen, % / h.

Only the B fraction can be affected by relative rates of passage. The fraction A is considered to be completely degraded in the rumen and all of fraction C is considered to pass from then rumen undegraded. The sum of RDP plus RUP must equal 100%. Requirements for RDP in the generally range 9.5 to 10.5% of the dietary DM for dairy cows depending on diet, animal characteristics and production level NRC (2001). However, Cyriac et al. (2008) suggested that the requirements for microbial RDP can be met with lower concentrations of RDP than those recommended in the current (2001). Mid-lactation dairy cows fed RDP diets with 8.8% (15.9% CP) of DM as RDP maintained DMI, milk yields and milk components compared with cows fed high RDP diets (Cyriac et al., 2008).

Reynal and Broderick (2005) found that decreasing the RDP from 13.2 to 11.7% and CP from 18.8 to 17.7% of DM did not affect milk production or FCM. Gressley and Armentano (2007) observed no difference in milk production when comparing diets with 10.1 or 7.4% . Over feeding protein relative to requirements decreases milk protein secretion efficiency and increases manure N excretion (Kalscheur et al., 2006). Close management of dietary proteins is needed to optimize milk production profitability and minimize environmental risks associated with excessive N excretion in urine and feces (Wu and Satter, 2000). Cyriac et al. (2008) suggested an improvement in milk N secretion efficiencies from 27.7% to 33.5% without a loss in milk production when RDP (% of diet DM) was from 11.3% to 8.8%.

DIETARY EFFECTS OF PROTEIN AND ENERGY INTERACTIONS ON MICROBIAL PROTEIN SYNTHESIS

Nocek and Russell (1988) suggested that since ruminal microorganisms are an important source of AA (50 – 80% of the N reaching the small intestine is likely to be microbial origin) nutritionists should be focus on factors that affect synthesis of microbial protein. Microbial protein could be considered the best source of amino acids for milk protein synthesis (Santos et al., 1998). **Table 7** also shows the amino acid composition of different protein sources in relationship to milk protein and also shows that microbial protein is the closest in AA content to milk protein. When the utilization of each amino acid is considered, microbial protein has the highest score followed by soybean meal.

Schwab (1994) suggested that an ideal ration in terms of digesta flow was 3:1 for lysine (Lys): methionine (Met) as a percentage of the total EAA flowing to the small intestine. If Lys and Met are the first limiting AA for milk production and milk protein synthesis in most dairy cattle diets, then microbial protein has an excellent amino acid balance as shown in **Table 8** (Harrison and Karnezos, 2005).

Bacteria are the principal microorganisms involved in protein degradation and the most abundant in the rumen numbering approximately $10^{10\text{-}11}$ cells per milliliter of rumen contents (Russell and Hespell, 1981). They can be grouped according to the type of substrate fermented and are categorized into eight distinct groups of rumen bacteria. Protozoa are also active and significant participants in ruminal protein degradation. The protozoa population in the rumen is about $10^{5\text{-}6}$ per milliliter of rumen contents and is influenced by feeding practices. Due to their large size (5 – 250 μm long), protozoa comprise a significant portion of the total microbial biomass in the rumen (Williams and Coleman, 1997). Protozoa differ with bacteria in their feeding behavior in that instead of attaching to feeds, they actively ingest bacteria, fungi and small particles of feed. Bacteria are their main source of protein, and higher numbers of protozoa are generally found in the rumen when diets of high digestibility are fed (NRC, 2001). Jouany and Ushida (1999) suggested that protozoa are net exporters of ammonia and with this, defaunation decreases ruminal ammonia concentrations. These microorganisms also contribute to volatile fatty acid production and are involved in sequestering carbohydrates from rapid bacterial attack by engulfing starch grains and other carbohydrates.

Without this function, a significant portion of carbohydrates would be fermented rapidly to lactate, and a lower ruminal pH may result, both aspects are detrimental to overall rumen function (Russell and Hespell, 1981). When mixed rumen bacteria populations or protozoa were incubated with different protein sources, protozoa had lower specific NH_3 production activity than bacteria (Hino and Russell, 1987). There is not too much information available about fungi populations or functions and behavior in the rumen. Jouany and Ushida (1999) and Wallace (1986) suggested that anaerobic fungi have a minimal effects on ruminal protein digestion, because of their low ruminal concentration which is about 10^{3-4} / ml.

Because microorganisms ferment carbohydrates, the association between carbohydrate and protein metabolism is strong (Nocek and Russell, 1988). Optimal microbial protein synthesis results from synchronous utilization of ruminally degraded protein and carbohydrates. Protein degradation often exceeds carbohydrate availability in the rumen, but conversely protein degradation can be too slow to support optimal ruminal digestion of carbohydrates (Herrera-Saldana and Huber, 1989) thereby reducing carbohydrate digestibility.. If there is insufficient ruminally available carbohydrate relative to RDP, N can be lost as NH_3 and microbial protein synthesis reduced (Nocek and Russell, 1988). Therefore, provision of rumen available energy and protein at coordinated rates should allow microbes to obtain ATP and NH3 at the same time needed for cell synthesis, to obtain a better utilization of nutrients in the rumen and increase supply of microbial protein to the small intestine (Oldham, 1984).

Ruminal microbial synthesis and growth depends on the availability of N in the form of peptides, amino acids, NH_3 (Russell et al., 1992) and rumen degradable carbohydrates, the primary energy substrate for ruminal microbes (Firkins et al., 2007). Daily microbial protein synthesis is the product of the efficiency of microbial protein synthesis (MPS) and is defined usually as grams of microbial crude protein (MCP) / 100 grams (or kilogram) of organic matter (OM) digested in the rumen (Hoover and Stokes, 1991).

Most current models recognize the biological need to have rumen degradable carbohydrate in balance with a supply of RDP to meet microbes needs for preformed AA and ammonia (Firkins, 2002). Russell et al. (1992) indicated that nonstructural carbohydrate (NSC) degrading bacteria are the primary users of peptide and AA nitrogen, whereas structural carbohydrate (SC) degrading bacteria only utilize NH_3. Griswold (2003) showed that, when urea was not available and RDP in the diet was increased, NDF, hemicelluloses, and NSC digestibility was improved suggesting that ruminally-available N in the form of peptides and AA can improve the digestion of SC and NSC by the microbial population. Studies reviewed by Oldham (1984) where different concentrations and sources of protein and energy were fed to dairy cows concluded that, form of dietary energy affects protein utilization and that starch often promotes an effect of "protein-sparing".

Herrera-Saldana and Huber (1989) showed that early lactation cows fed a diet synchronized for rapid rumen degradation, where the main sources of starch and protein were barley and cottonseed meal, produced more milk than cows fed a synchronized slowly degraded diet containing milo and brewers dried grains or unsynchronized diets with barley and brewers dried grains or milo and cottonseed meal. Herrera-Saldana (1990) concluded that higher milk yields observed in a previous study (Herrera-Saldana and Huber, 1989) could be explained by the synchronization for rapid fermentation with the more degradable starch and protein stimulating greater microbial protein flow than unsynchronized or less degradable synchronized diets.

Figure 6 illustrates the utilization of protein and carbohydrate by rumen bacteria. When ATP (mainly from CHO fermentation) is available, AA that enter to the microbial cells can be incorporated into microbial protein, but if ATP is not available or is insufficient to support protein synthesis, AA will be fermented as an energy source and ammonia will accumulate (Nocek and Russell, 1988). Supplementing silage-based diets with moderate levels of readily fermented carbohydrates may increase microbial protein synthesis. Increasing the starch content of diets can affect the rumen microbes in a number of ways though, thus making it difficult to predict the effect of starch on rumen microbial protein synthesis (Dewhurst et al., 2000). Karsli and Russell (2001) suggested that the average efficiency of microbial protein synthesis varies primarily according to the type of diet fed **(Table 9).**

A continuous culture study was conducted in by Stokes et al. (1991) to evaluate the effects of dietary RDP and NSC concentrations on microbial metabolism. Continuous culture provides a means to evaluate the effects of nutrients on the metabolism of microbes maintained under controlled conditions of pH, nutrient intake, and passage rates Results were variable, but Hoover and Stokes (1991) concluded that, because NSC has a major influence on total carbohydrate digestion ($r = 0.99$) and RDP affects both carbohydrate digestion and microbial efficiency ($r = 0.71$ and 0.94, respectively), it is appropriate to combine NCS and RDP, as percentages of DM, into a ratio to relate bacterial yield.

Interest in synchrony of release of protein and energy in the rumen derives from the assumption that a lack of synchrony leads to inefficient microbial capture of nitrogen and hence to a reduced efficiency of microbial protein synthesis (Kyoung H Kim, 1999). In conclusion, to reach a high efficiency of microbial protein synthesis it can be useful to synchronize available carbohydrate with RDP. Akay et al., (2004) indicated that microbial metabolism in the rumen is a complex process requiring an understanding of the rate and extent of carbohydrate digestion and ammonia supply for efficient growth in the rumen. Finally, Hoover and Stokes (1991) suggested that, to optimize microbial protein synthesis, an understanding of the interaction of nitrogen and carbohydrates in the rumen is required.

MILK UREA NITROGEN (MUN) AS A MANAGEMENT TOOL TO MONITOR CHANGES IN FEEDING

Overfeeding protein is expensive, may reduce cow performance, and the excess protein is excreted as urinary N that is highly unstable creating an environmental concern. Stoop (2007) suggested that milk urea nitrogen (MUN) has become an important diagnostic tool; i.e. due to the new European legislation the Netherlands will use MUN to monitor dairy herds for N utilization. Broderick and Clayton (1997) suggested that MUN may serve as an index of inefficient N utilization by dairy cows.

Concentrations for MUN in the United States are usually expressed as mg/dl (100 ml = 1 dl or deciliter). Guidelines for interpreting whole herd MUN values (bulk tank milk) for Holstein herds (Ishler, 2008) are presented in **Table 10.**

Nutrition Risk Factors for High MUN Values.

According to Ishler (2008) nutritional and management reasons for MUN levels falling outside recommended ranges that can cause high MUN values (< 10 - > 14 mg/dl) include:

1. Excess dietary CP, RDP, and (or) RUP;
2. Low dietary NFC;
3. Low ruminal NFC degradability;

4. Poor forage quality.
5. Feeding new crop corn silage that may not have the same level of fermentable carbohydrate (less starch or starch is less available) compared to corn silage that has fermented for a period of time.
6. Cows grazing lush pasture can increase their intake of total and degradable protein.
7. Change to a different hay-crop silage that is wetter or higher in protein and/or soluble protein.
8. Feeding corn grain that has a coarse particle size. This may reduce the rate of fermentation in the rumen and may not match with the protein fractions being fed.
9. Shifting from processed corn silage to unprocessed or improperly processed corn silage. This could affect the amount of available fermentable starch.
10. Incorporating more degradable protein sources (e.g. changing from heat-treated soybeans (whole or cracked) to raw soybeans or heat-treated beans that are ground), which results in more rumen ammonia.

Potential benefits from using MUN as a diagnostic tool are improvements in milk yield and composition, body condition score and fertility, lower feed costs and finally less nitrogen excretion to the environment. In addition to assessing dietary CP, RDP and RUP contents, measurement of MUN may provide useful information concerning protein utilization (Nousiainen et al., 2004).

STATISTICAL DESIGNS FOR DAIRY FIELD TRIALS

Dairy cattle research conducted at university experiment stations are important in terms of providing knowledge that can be readily used by the dairy industry (Tempelman, 2009). Research conducted at university stations are characterized for their vigilance, intensive sampling, proximity to laboratories, and compliance to the experimental protocol. However, because of increasing constraints on conducting large-scale animal studies at universities more research is being conducted on commercial dairies. These dairy field trials have implications for experimental design and data analysis.

Feed and pharmaceutical industries rely heavily on commercial field trials for evaluating feed ingredients, feed additives, and other commercial products (St-Pierre and Jones, 1999). In addition, commercial field trials are used to determine the magnitude of response to a nutritional treatment and to provide its economic impact. Field studies on commercial dairy farms, as an experimental unit, have an advantage because large numbers of cows under different environmental conditions can be studied. An advantage of dairy field studies includes the potential to evaluate treatment responses for milk yield and composition across multiple locations and diverse management systems. Field trials provide researchers and extension specialists the opportunity to work as a team with nutritionists, veterinarians, and dairy producers. Also, field trials are often less expensive than research at universities.

A clear disadvantage of field trials is the loss of control of experimental conditions due to environmental and management changes during the trial. Important challenges with on field trials include proper blocking and the randomization of dairy farms to treatments (reference).

There are several critical points to consider when conducting research on commercial dairy farms. First, control of randomization of cows to treatments may be compromised as depending upon the concerns of the farm manager, particularly when it is rather uncertain whether some treatments may have a detrimental effect on various aspects of dairy cattle performance. Second, and concomitant with increasing average herd sizes, a greater number of farms house and manage animals together in pens, thereby complicating issues for true experimental replication (St-Pierre, 2007). Coppock et al. (1970) assembled some of the techniques and procedures useful in field trials as follows:

1. The experiment should be large enough in number of cows, pens, or farms, to be able to draw objective inferences and conclusions. Gill (1969) showed that large numbers of experimental units are necessary to detect small differences. Coppock (1970) reported loss of 20 to 50% of the original trial participants primarily due to study length and the perceived effect of treatment by the farmer.
2. The experimental design should be kept as simple as possible; i.e. two treatments usually the maximum number in most field trials. A control group is essential in all trials. It is useful to set up the statistical design at the beginning of the study (Coppock et al., 1970).

3. Design a simple protocol to record the data, which can be summarized daily, weekly or monthly to monitor the progress of the experiment.
4. Visit the dairymen and nutritionists as often as necessary to keep them convinced of your continued interest, concern, and desire to stay abreast of developments related to the experiment (Coppock et al., 1970). The authors also suggested that it is necessary to select treatments for field-testing focused on increased performance over current control.
5. Keep DHI supervisors informed by sending them a brief description of general procedures of your experiment if the information that you will use comes from DHI records, especially when milk yield, and milk fat, protein, and MUN are the focus of the field trial.
6. Enlist the good will of the herd veterinarians by sending them a description of the experiment and procedures.
7. If possible, avoid experiments that might harm the productivity of cows unless compensation is specified at the beginning of the experiment. For that reason, it is important to create a compromise of compensation to dairy farmers in case a significant loss is incurred.

The following guidelines are important for selecting trial sites:

1. A large cow population on DHI test if many restrictions will be placed upon the participating herds (Coppock et al., 1970).
2. Proximity of the farms if frequent travel to the farms is anticipated.

3. Best experimental performance is usually on owner-operator farms that have minimal labor and management problems. During the initial interview with the prospective participants, gauge their attitude regarding the research. During the initial site visit, it is also important to speak to all those who will be involved with the trial including the herd manager milkers, feeders, etc. Convince everyone associated with the herd the importance of the trial (Coppock et al., 1970).
4. If the objective of the trial involves milk response to a nutrition treatment then it is crucial to involve the nutritionists. Upon the completion of the experiment, data should be summarized as quickly as possible, and the results and conclusions should be sent to all participants whose cooperation made the experiment possible.

Several preliminary planning activities are required before an experiment begins, and these activities are crucial to the success of the experiment. The statistician has a central role in these activities, particularly in relation to the choice of design to be adopted for the experiment that satisfies the available resource constraints (Godolphin, 2004). Tempelman (2009) reported that there are several basic principles that are essential for proper experimental design whether they be applied to university research stations or commercial dairies. The experimental design depends on the objectives of the study and the experiment should be planned in detail. When cows are grouped together in pens on large dairies, then the same randomization principles apply except that pens or farm rather than individual cows, represent the experimental unit. It is a critical factor that dairy farms need to be randomly assigned to treatments in a completely randomized design (CRD) or COD.

St-Pierre and Jones (1999) observed that improper accounting or randomization of uncontrolled changes, like the environment, over time is one of the most common errors. It is also important to focus on a proper assignment of animals or farms to treatment, to replicate the treatment across multiple experimental units. In addition, the authors suggested that, it is crucial to put attention to on-farm oversight mainly to follow the protocols given, because this is one of the common errors. It is also important to apply the correct experimental design to avoid bias and to have the sufficient statistical power for detecting differences that will have biological and economic importance to the dairy industry.

The two-period, two-sequence design has been extensively used and widely studied in the literature. Matthews (1988) reported that one of the earliest uses of COD, and one that is still in use today, is in animal feeding trials. In this design, each of the treatments appears in each sequence and in each period, and with that, the treatment effects are not confounded with the effects of sequence of delivery and periods **(Table 11).** An important area where COD can be used is in field trials with commercial dairy farms. Tempelman (2009) suggested the following example. If there are two different treatments, A and B, to compare within each pen across two time periods, then one-half of the pens would be randomly assigned to the A→B sequence, receiving A in the first period and B in the second period, whereas the other half of the pens would be assigned to the B →A sequence. When specific pens are grouped together in a dairy farm, then the same randomization principle may apply, except that now dairy farm represents the experimental unit rather than pens of cows or individual cows.

An important factor for on-farm studies, in which pen is the experimental unit, involves an appropriate choice for the numbers of pens and cows per pen. It is important here to review what influences classical statistical power. In a simple way to explain the concept of statistical power, it is the probability that one would correctly conclude that two or more treatments have different mean responses. Most researchers understand that the larger the true mean difference between treatment groups, the greater the statistical power will be. Depending on the context, power will be sufficient if this probability is between 70 to 99%. A power analysis is the most common way of determining sample size (Festing and Altman, 2002). The same concept can be applied for on-farm studies in which the experimental unit is the dairy herd or dairy herds considering that several experimental units per treatment are required to establish experimental error such that at least two farms must be randomly assigned to each treatment. Experiments are often designed to measure continuous variables such as the effect of an additive in the diet on milk production and composition. Fixed scenario elements for the power procedure paired t-test to obtain mean differences in our trial are presented in **Table 12**.

Statistical models can be complex, but it is critical to detect if differences exist between the mean of a variable for the treatment groups. A simple formula can be used to compute sample size when power, significance level, the size of the difference between means, and variance of the population means are specified.

To compute sample size for continuous variables, it is necessary to obtain an estimate of the population standard deviation of the variable and the magnitude of the difference that the researcher wishes to detect which is also called the effect. To calculate the sample size for a specific dairy field trial, it is useful to perform some simulations with the power procedure for a paired t test for mean differences with crossover design using SAS (2005).

MONITORING INCOME OVER FEED COSTS (IOFC)

Since feed costs are often the greatest costs associated with milk production, minimizing these costs can improve profitability. The potential incorporation of ingredients into dairy cattle rations requires careful planning and evaluation. Change in feed and milk prices and milk production response to feed supplements are especially important, because they impact income over feed cost (IOFC) (Cabrera et al., 2009).

The single largest dairy farm expense is feed which represents 40% to 60% percent of the total cost of producing milk. Depending on ratio of milk/feed prices, feed costs can represent 70% of total cost of milk production (Smith, 1976). There are some basics tools that nutritionists and producers can use to help manage price volatility. The IOFC measure is one of these tools, because it monitors how well the feeding program is working on a dairy operation (Adkinson et al., 1993). It can be calculated simply by taking the daily bulk tank milk average per cow times the milk price per kilogram minus the feed cost per cow per day. Income over feed cost is a function of milk price, feed costs, and the response of cows to rations (McLaren et al., 2005, Smith, 1976). Because IOFC establishes the relationship between milk outputs for the dollars required to produce the product, it can be readily compared across dairy farms in different regions to evaluate efficiency and profitability. Smith (1976) suggested that achieving maximum IOFC is dependent upon costs of nutrients from available feedstuffs and value of milk as well as the quantitative evaluation of factors affecting milk production (i.e. the inherent capacity of the cow to produce milk, stage of lactation, quantity of grain feeding, forage quality, etc).

Generally, as milk yield increases, IOFC increases as feed costs to produce a liter of milk decrease. Adkinson (1993) stated that the higher IOFC is the more residual income can be applied to other expenses or returned to the owner as profit. Milk yield and feed cost are the two most important areas of management for increasing IOFC for the individual dairy farmer. The cost benefit (profitability) to substitute NPN for preformed proteins supplements depends on a number of factors, but the following four factors are considered to be the most important in an economic analysis:

1. Price of the NPN supplement and the amount fed;
2. Price of the protein supplement replaced and the amount fed;
3. Price of the energy supplement or forage used to fill formulation space and the amount fed; and,
4. Milk response to the change in the ration and the milk price. Milk price can only be influenced by the producer through improved milk composition, quality incentives, and volume premiums (Adkinson et al., 1993).

CONCLUSIONS

Improvements in lactation performance by dairy cows have been reported in the literature in response to feeding Optigen®. However, more research, on-farm evaluations and economic analyses on Optigen® use are warranted. The research objectives for this Thesis research were to determine the effect of Optigen® as a source of dietary nitrogen in commercial dairy farms on milk yield, milk components yields, milk fat, protein, and MUN content and finally determine the IOFC.

REFERENCES

Adkinson, R. W., W. S. Farmer, and B. F. Jenny. 1993. Feeding Practices and Income Over Feed Cost on Pasture-Oriented Dairy Farms in Louisiana. J. Dairy Sci. 76(11):3547-3554.

Akay, V., J. Tikofsky, C. Holtz, and K. A. Dawson. 2004. Optigen® 1200: controlled release of non-protein nitrogen in the rumen. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Annual Symposium: re-imagining the feed industry. T. P. Lyons and K. A. Jacques, ed. Alltech UK, Lexington, Kentucky, USA.

Bartley, E. E. and C. W. Deyoe. 1975. Starea as a protein replacer for ruminants-review of 10 years of research. Feedstuffs, Minnetonka 47:42-44.

Boucher, S. E., R. S. Ordway, N. L. Whitehouse, F. P. Lundy, P. J. Kononoff, and C. G. Schwab. 2007. Effect of Incremental Urea Supplementation of a Conventional Corn Silage-Based Diet on Ruminal Ammonia Concentration and Synthesis of Microbial Protein. J. Dairy Sci. 90(12):5619-5633.

Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007. Effects of Different Protein Supplements on Omasal Nutrient Flow and Microbial Protein Synthesis in Lactating Dairy Cows. J. Dairy Sci. 90(4):1828-1841.

Broderick, G. 2006. Nutritional Strategies to Reduce Crude Protein in Dairy Diets.

Broderick, G. and A. USDA. 2007. Reduced crude protein rations for high producing cows: Production and environmental effects.

Broderick, G. A. 2003. Effects of Varying Dietary Protein and Energy Levels on the Production of Lactating Dairy Cows. J. Dairy Sci. 86(4):1370-1381.

Broderick, G. A. and M. K. Clayton. 1997. A Statistical Evaluation of Animal and Nutritional Factors Influencing Concentrations of Milk Urea Nitrogen. J. Dairy Sci. 80(11):2964-2971.

Broderick, G. A., R. G. Koegel, M. J. C. Mauries, E. Schneeberger, and T. J. Kraus. 1999. Effect of Feeding Macerated Alfalfa Silage on Nutrient Digestibility and Milk Yield in Lactating Dairy Cows. J. Dairy Sci. 82(11):2472-2485.

Broderick, G. A., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. J. Dairy Sci. 92(6):2719-2728.

Burgos, S. A., J. G. Fadel, and E. J. DePeters. 2007. Prediction of Ammonia Emission from Dairy Cattle Manure Based on Milk Urea Nitrogen: Relation of Milk Urea Nitrogen to Urine Urea Nitrogen Excretion. J. Dairy Sci. 90(12):5499-5508.

Butler, W. R. 1998. Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle. J. Dairy Sci. 81(9):2533-2539.

Cabrera, V.E., Shaver R.D., and Wattiaux M.A.. 2009. Optimizing Income Over Feed Supplement Costs. Pages 116 - 120 in 4-State Dairy Nutrition & Management Conference, Dubuque, Iowa, USA.

Chalupa, W. 1968. Problems in Feeding Urea to Ruminants. J. Anim Sci. 27(1):207-219.

Chalupa, W. 2007. Precision Feeding of Nitrigen to Lactating Dairy Cows: A Role for Optigen II. Pages 221-226 in Nutritional Biotechnology in the Feed and Food Industries. Vol. 23. T. P. Lyons, K. A. Jaques, and J. M. Hower, ed.

Chandler, P. 1989. Achievement of optimum amino acid balance possible. Feedstuffs 61(26):24.

Conrad, H. R. and J. W. Hibbs. 1968. Nitrogen Utilization by the Ruminant: Nitrogen Utilization by the Ruminant. Appreciation of Its Nutritive Value. J. Dairy Sci. 51(2):276-285.

Coppock, C. E., R. P. Natzke, R. W. Everett, and W. G. Merrill. 1970. Methodology and Procedures in Field Trials in Dairy Science. J. Dairy Sci. 53(7):983-985.

Cyriac, J., A. G. Rius, M. L. McGilliard, R. E. Pearson, B. J. Bequette, and M. D. Hanigan. 2008. Lactation Performance of Mid-Lactation Dairy Cows Fed Ruminally Degradable Protein at Concentrations Lower Than National Research Council Recommendations. J. Dairy Sci. 91(12):4704-4713.

Dewhurst, R. J., D. R. Davies, and R. J. Merry. 2000. Microbial protein supply from the rumen. Animal feed science and technology 85(1-2):1-21.

Deyoe, C., E. Bartley, H. Pfost, F. Boren, H. Perry, F. Anstaett, L. Helmer, D. Stiles, A. Sung, and R. Meyer. 1968. An improved urea product for ruminants. Journal of Animal Science 27:1163.

Emanuele, S., D. Merrill, R. Petcavich, R. Stock, and X. Yang, inventors. 2001a. Feedstock for ruminants with controlled-release non-protein nitrogen. Google Patents, assignee.

Emanuele, S., D. Merrill, R. Petcavich, R. Stock, and X. Yang. 2001b. Feedstock for ruminants with controlled-release non-protein nitrogen. Google Patents.

Emanuele, S. and D. Putnam. 2006. Encapsulating Nutrients to Improve Reproduction and Nitrogen Utilization in Ruminants.

Emmanuel, B. 1980. Urea cycle enzymes in tissues (liver, rumen epithelium, heart, kidney, lung and spleen) of sheep (ovis aries). Comp. Biochem. Physiol. 65B 693.

Festing, M. and D. Altman. 2002. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR Journal 43(4):244-258.

Firkins, J. 2002. Optimizing rumen fermentation. Page 39 in Tri-State Nutrition Conference, Fort Wayne, Indiana.

Firkins, J. L., Z. Yu, and M. Morrison. 2007. Ruminal Nitrogen Metabolism: Perspectives for Integration of Microbiology and Nutrition for Dairy. J. Dairy Sci. 90(13_suppl):E1-16.

Forero, O., F. N. Owens, and K. S. Lusby. 1980. Evaluation of Slow-Release Urea for Winter Supplementation of Lactating Range Cows. J. Anim Sci. 50(3):532-538.

Galo, E., S. M. Emanuele, C. J. Sniffen, J. H. White, and J. R. Knapp. 2003. Effects of a Polymer-Coated Urea Product on Nitrogen Metabolism in Lactating Holstein Dairy Cattle. J. Dairy Sci. 86(6):2154-2162.

García-González, R., J. M. Tricárico, G. A. Harrison, M. D. Meyer, K. M. McLead, D. L. Harmon, and K. A. Dawson. 2007. Optigen® is a Sustained Release of Non-Protein Nitrogen in the Rumen. Dep. of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA.

Gill, J. L. 1969. Sample Size for Experiments on Milk Yield. J. Dairy Sci. 52(7):984-989.

Glibert, P., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating worldwide use of urea global change contributing to coastal eutrophication. Biogeochemistry 77(3):441-463.

Godolphin, J. D. 2004. Simple Pilot Procedures for the Avoidance of Disconnected Experimental Designs. Applied Statistics 53(1):133-147.

Golombeski, G. L., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2006. Slow-Release Urea and Highly Fermentable Sugars in Diets Fed to Lactating Dairy Cows. J. Dairy Sci. 89(11):4395-4403.

Gressley, T. F. and L. E. Armentano. 2007. Effects of Low Rumen-Degradable Protein or Abomasal Fructan Infusion on Diet Digestibility and Urinary Nitrogen Excretion in Lactating Dairy Cows. J. Dairy Sci. 90(3):1340-1353.

Griswold, K. E., G. A. Apgar, J. Bouton, and J. L. Firkins. 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. J. Anim Sci. 81(1):329-336.

Harrison, G. A. and T. P. Karnezos. 2005. Can we improve the efficiency of nitrogen utilization in the lactating dairy cows? Pages 145 - 154 in Recent Advances in Animal Nutrition. Vol. 15. T. P. Lyons and K. A. Jacques, ed. Alltech Inc., Australia.

Harrison, G. A., J. M. Tricárico, M. D. Meyer, and K. A. Dawson. 2007. Effects of urea or Optigen® on fermentation, digestion, and N flow in rumen-simulating fermentors. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K.A., ed. Alltech Inc., Lexington, Kentucky, USA.

Helmer, L. G., E. E. Bartley, and C. W. Deyoe. 1970. Feed Processing. VI. Comparison of Starea, Urea, and Soybean Meal as Protein Sources for Lactating Dairy Cows. J. Dairy Sci. 53(7):883-887.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi, and J. T. Huber. 1990. Influence of Synchronizing Protein and Starch Degradation in the Rumen on Nutrient Utilization and Microbial Protein Synthesis. J. Dairy Sci. 73(1):142-148.

Herrera-Saldana, R. and J. T. Huber. 1989. Influence of Varying Protein and Starch Degradabilities on Performance of Lactating Cows. J. Dairy Sci. 72(6):1477-1483.

Hino, T. and J. B. Russell. 1987. Relative Contributions of Ruminal Bacteria and Protozoa to the Degradation of Protein in Vitro. J. Anim Sci. 64(1):261-270.

Hoover, W. H. and S. R. Stokes. 1991. Balancing Carbohydrates and Proteins for Optimum Rumen Microbial Yield. J. Dairy Sci. 74(10):3630-3644.

Huber, J. T. 1975. Protein and Non-Protein Nitrogen Utilization in Practical Dairy Rations. J. Anim Sci. 41(3):954-961.

Huber, J. T. and L. Kung, Jr. 1981. Protein and Nonprotein Nitrogen Utilization in Dairy Cattle. J. Dairy Sci. 64(6):1170-1195.

Huntington, G. and S. Archibeque. 2000. Practical aspects of urea and ammonia metabolism in ruminants. Journal of Animal Science 77(E-Suppl).

Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk. J. Anim Sci. 75(3):852-867.

Inc., S. I. 2005. SAS user's guide: Statistics. 9.1 ed.

Ishler, V. 2008. Interpretation of milk urea nitrogen values. Page 3. Penn State University, Pennsylvania.

Jackson, M. J., A. L. Beaudet, and W. E. O'Brien. 1986. Mammalian urea cycle enzymes. H. H. M. I. a. D. o. Pediatrics, ed, Baylor College of Medicine, Houston, Texas.

Jones, G. M., C. Stephens, and B. Kensett. 1975. Utilization of Starea, Urea, or Soybean Meal in Complete Rations for Lactating Dairy Cows. J. Dairy Sci. 58(5):689-695.

Jouany, J. and K. Ushida. 1999. The role of protozoa in feed digestion. ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES 12:113-128.

Kalscheur, K. F., R. L. Baldwin, VI, B. P. Glenn, and R. A. Kohn. 2006. Milk Production of Dairy Cows Fed Differing Concentrations of Rumen-Degraded Protein. J. Dairy Sci. 89(1):249-259.

Karsli, M. and J. Russell. 2001. Effects of Some Dietary Factors on Ruminal Microbial Protein Synthesis. TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES 25(5):681-686.

Krebs, H. A. and K. Henseleit. 1932. Untersuchungen uber die harnstoffbildung im tierkorper. Hoppe-Seyler's Physiological Chemistry. 210: 325 - 332.

Kyoung H Kim, J.-J. C., David G Chamberlain,. 1999. Effects of varying the degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in lactating dairy cows consuming a diet of grass silage and a cereal-based concentrate. Journal of the Science of Food and Agriculture 79(11):1441-1447.

Löest, C., E. Titgemeyer, J. Drouillard, B. Lambert, and A. Trater. 2001. Urea and biuret as nonprotein nitrogen sources in cooked molasses blocks for steers fed prairie hays. Animal feed science and technology 94(3-4):115-126.

Males, J. R., R. A. Munsinger, and R. R. Johnson. 1979. In Vitro and in Vivo Ammonia Release from "Slow-Release" Urea Supplements. J. Anim Sci. 48(4):887-892.

Matthews, J. N. S. 1988. Recent Developments in Crossover Designs. International Statistical Review / Revue Internationale de Statistique 56(2):117-127.

McLaren, C. J., K. D. Lissemore, T. F. Duffield, K. E. Leslie, D. F. Kelton, and B. Grexton. 2005. The Association of Herd Milk Production and Management with a Return-Over-Feed Index in Ontario Dairy Herds. J. Dairy Sci. 88(1):419-425.

Newbold, J. R. and S. R. Rust. 1992. Effect of asynchronous nitrogen and energy supply on growth of ruminal bacteria in batch culture. J. Anim Sci. 70(2):538-546.

Nocek, J. E. and J. B. Russell. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. J. Dairy Sci. 71(8):2070-2107.

Nousiainen, J., K. J. Shingfield, and P. Huhtanen. 2004. Evaluation of Milk Urea Nitrogen as a Diagnostic of Protein Feeding. J. Dairy Sci. 87(2):386-398.

NRC. 1976. Urea and Other Nonprotein Nitrogen Compounds in Animal Nutrition. National Academy of Sciences, Washington, DC.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed, Natl. Acad. Sci. Wasington, DC.

Oldham, J. D. 1984. Protein-Energy Interrelationships in Dairy Cows. J. Dairy Sci. 67(5):1090-1114.

Olmos Colmenero, J. J. and G. A. Broderick. 2006. Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows. J. Dairy Sci. 89(5):1704-1712.

Owens, F. and R. Zinn. 1988. Protein metabolism of ruminant animals. The ruminant animal. Digestive physiology and nutrition. Waveland Press, Long Grove, Illinois, USA:227-249.

Owens, F. N., K. S. Lusby, K. Mizwicki, and O. Forero. 1980. Slow Ammonia Release from Urea: Rumen and Metabolism Studies. J. Anim Sci. 50(3):527-531.

Prokop, M. 1976. Evaluation of Slow Ammonia Release Urea (SARU) as a Liquid Supplement for Ruminants. University of Nebraska--Lincoln.

Reynal, S. M. and G. A. Broderick. 2005. Effect of Dietary Level of Rumen-Degraded Protein on Production and Nitrogen Metabolism in Lactating Dairy Cows. J. Dairy Sci. 88(11):4045-4064.

Russell, J. B. and R. B. Hespell. 1981. Microbial Rumen Fermentation. J. Dairy Sci. 64(6):1153-1169.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim Sci. 70(11):3551-3561.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of Rumen-Undegradable Protein on Dairy Cow Performance: A 12-Year Literature Review. J. Dairy Sci. 81(12):3182-3213.

SAS. 2008. User's Guide: Statistics. Version 9.1 ed. SAS Institute, Cary, NY.

Schwab, C. 1994. Optimizing amino acid nutrition for optimum yields of milk and milk protein. SOUTHWEST NUTRITION MANAGEMENT, Tucson:114-129.

Shambaugh, G., 3rd. 1977. Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle. Am J Clin Nutr 30(12):2083-2087.

Siciliano-Jones, J., Downer, J. 2005. Utility and safety of a slow-release nitrogen product: Optigen® 1200. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K. A., ed. Alltech UK, Lexington, KY, USA.

Smith, N. E. 1976. Maximixing Income over Feed Costs: Evaluation of Production Response Relationships. J. Dairy Sci. 59(6):1193-1199.

St-Pierre, N. R. 2007. Design and Analysis of Pen Studies in the Animal Sciences. J. Dairy Sci. 90(13_suppl):E87-99.

St-Pierre, N. R. and L. R. Jones. 1999. Interpretation and Design of Nonregulatory On-Farm Feeding Trials. J. Anim Sci. 77(E-Suppl_2):177-182.

Stanton, T., C. E. Service, and C. S. University. 2006. Urea and NPN for cattle and sheep. Page 3. Colorado State University Extension Service.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. P. Manski. 1991. Impact of Carbohydrate and Protein Levels on Bacterial Metabolism in Continuous Culture. J. Dairy Sci. 74(3):860-870.

Stoop, W. M., H. Bovenhuis, and J. A. M. van Arendonk. 2007. Genetic Parameters for Milk Urea Nitrogen in Relation to Milk Production Traits. J. Dairy Sci. 90(4):1981-1986.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim Sci. 74(12):3112-3124.

Teller, E. and J. Godeau. 1986. Some attempts to improve the nutritive value of urea for dairy cows, 2: Its administration in a slightly soluble form: isobutylidene diurea (IBDH). Archiv fuer Tierernaehrung (German DR).

Tempelman, R. J. 2009. Invited review: Assessing experimental designs for research conducted on commercial dairies. J. Dairy Sci. 92(1):1-15.

Tikofsky, J., Harrison, H.A. 2007. Optigen II: Improving the efficiency of nitrigen utilization in the dairy cow. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K. A., ed. Alltech Inc., Lexington, Kentucky, USA.

Wallace, R. J. and M. C.A. 1986. Influence of the rumen anaerobic fungus Neocallimastix frontalis on the proteolytic activity of a defined mixture of rumen bacteria growing on a solid substrate. Letters in Applied Microbiology 3(2):23-26.

Wanasundara, U. N. and F. Shahidi. 1999. Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. Food Chemistry 65(1):41-49.

Weiss, W. P., N. R. St-Pierre, and L. B. Willet. 2007. Factors Affecting Manure Excretion by Dairy Cows. in Proceedings of the 2007 Tri-State Dairy Nutrition Conference. Ohio State University, Fort Wayne, Indiana, USA, 24-25 April, 2007.

Williams, A. and G. Coleman. 1997. 3 The rumen protozoa. The Rumen microbial ecosystem:73.

Wu, Z. and L. D. Satter. 2000. Milk Production During the Complete Lactation of Dairy Cows Fed Diets Containing Different Amounts of Protein. J. Dairy Sci. 83(5):1042-1051.

Table 1. Nitrogen fractions, amino acids and RUP digestibility of main forages and concentrates used in dairy cow rations in the Midwest (Adapted from Table 15-2a of NRC 2001).

	CP	N Fractions, % CP			Kd	RUP	Met	Lys
Forage	**%**	**A**	**B**	**C**	**% h of B**	**Digest %**	**% CP**	**% CP**
Corn silage, 32-38% DM	8.8	51.3	30.2	18.5	4.4	70	1.53	2.51
Grass silage, < 50% NDF	16.8	60.1	31.8	8.1	8.1	65	1.21	3.28
Legume silage. 40-46% NDF	21.9	57.3	35.3	7.4	12.2	65	1.37	4.41
Legume hay, 40-46% NDF	20.8	44.3	46.9	8.8	17.9	70	1.56	5.09
Concentrates								
Soybean meal, 44%	49.9	22.5	76.8	0.7	9.4	93	1.45	6.28
Soybean meal, 48%	53.8	15	84.4	0.6	7.5	93	1.44	6.29
Soybean meal, expeller	46.3	8.7	91.3	0.0	2.4	93	1.45	6.27
Corn gluten meal, dried	65.0	3.9	90.9	5.2	2.3	92	2.37	1.69
Corn, DDG's	29.7	28.5	63.3	8.2	3.6	80	1.82	2.24
Cottonseed meal	44.9	25.6	55.5	18.9	6.8	92	1.59	4.13
Canola meal	37.8	23.2	70.4	6.4	10.4	75	1.87	5.62
Sunflower meal	28.4	42.0	52.8	5.2	29.2	90	2.29	3.56
Linseed meal	54.2	18.1	48.2	33.7	7.2	60	1.40	5.18

Table 2. Reactions, overall energy requirement and overall equation of the urea cycle adapted from NRC (1976).

Step	Reactant	Product	Catalyzed by	Location
	2 ATP + HCO_3- + NH_4+	Carbamoyl phosphate + 2 ADP + P_i	Carbamoyl phosphate synthetase	Mitochondria
	Carbamoyl phosphate + Ornithine	Citruline + P_i	Ornithine transcarbamylase	Mitochondria
	Citruline + Aspartate + ATP	Argininosuccinate + AMP + PP_i	Argininosuccinate synthetase	Cytosol
	Argininosuccinate	Arginine + Fumarate	Argininosuccinate lyase	Cytosol
	Arginine + H_2O	Ornithine + Urea	Arginase	Cytosol

Overall energy requirement

$NH_3 + CO_2$ + Aspartate + 3 ATP + 2 H_2O → Urea + Fumarate + 2 ADP + 4 P_i + AMP

Overall equation of urea cycle

2 $NH_3 + CO_2$ + 3 ATP + H_2O → Urea + 2 ADP + 4 P_i + AMP + 2 H

Table 3. Effects of dietary CP, urea and CP plus urea on milk production, milk components (% and yield), MUN and BUN by dairy cows.

Reference	Dietary CP % DM	Urea % DM	MUN mg/dl	BUN mg/dl	Milk kg/d	Fat %	Fat kg/d	Protein %	Protein kg/d
Broderick et al. (2009)	16.1	0.0	6.77[a]	8.87[c]	39.3[a]	3.05	1.20	3.22	1.27[a]
	16.1	0.41	7.45[b]	9.89[c]	38.6[a]	3.17	1.19	3.18	1.22[b]
	16.0	0.84	8.13[b]	11.39[b]	38.5[a]	2.92	1.10	3.18	1.21[bc]
	16.1	1.31	9.09[a]	12.78[a]	36.0[b]	3.05	1.11	3.18	1.17[c]
Burgos et al. (2007)[2]	15.0	0.0	7.9[a]	8.2[a]	29.9	4.06	1.27	2.87	0.86
	17.0	0.7	11.9[b]	12.9[b]	31.3	4.20	1.37	2.88	0.90
	19.0	1.5	17.2[c]	18.6[c]	29.7	4.10	1.26	2.91	0.86
	21.0	2.2	24.5[d]	25.8[d]	30.0	4.01	1.24	2.89	0.87
Boucher et al. (2007)[3]	14.9	0.0	11.0[a]	...	33.9	3.11	1.05	2.84[a]	0.96
	15.7	0.3	11.0[a]	...	30.7	3.06	0.95	2.76[b]	0.86
	16.5	0.6	12.5[b]	...	34.6	3.17	1.01	2.79[ab]	0.96
	17.3	0.9	13.2[b]	...	33.0	3.14	1.04	2.77[b]	0.91

[1]Least square means within the same column with different superscripts differ ($P < 0.05$).

[2]Means in the same column without common superscripts differ ($P < 0.05$).

[3]Least square means within the same column without a common superscript differ ($P < 0.05$).

Table 3. Continued...

Reference	Dietary CP % DM	Urea % DM	MUN mg/dl	BUN mg/dl	Milk kg/d	Fat %	Fat kg/d	Protein %	Protein kg/d
Olmos and Broderick (2006)[4]	13.5	...	7.7[d]	10.7[e]	36.3[a]	3.14[b]	1.14	3.09	1.10
	15.0	...	8.5[d]	13.4[d]	37.2[ab]	3.27[ab]	1.20	3.15	1.15
	16.5	...	11.2[c]	17.1[c]	38.3[a]	3.27[ab]	1.24	3.09	1.18
	17.9	...	13.0[b]	21.2[b]	36.6[b]	3.47[a]	1.23	3.18	1.13
	19.4	...	15.6[a]	24.0[a]	37.0[ab]	3.44[a]	1.24	3.16	1.15
Broderick (2003)[5]	15.1	...	9.3[c]	...	33.0[b]	3.51	1.15[b]	2.99[b]	0.99[b]
	16.7	...	12.4[b]	...	34.1[a]	3.66	1.23[a]	3.03[a]	1.02[a]
	18.4	...	15.9[a]	...	34.1[a]	3.60	1.20[a]	3.02[a]	1.02[a]
Wu and Satter (2000)[6]	15.4[p]	…	...	...	36.9[b]	3.97	1.461[b]	2.92[a]	1.086[b]
	17.4[q]	...	...	...	39.5[a]	4.05	1.601[a]	2.84[b]	1.128
	19.3[r]	...	...	...	40.8[a]	3.94	1.626[a]	2.86	1.179[a]
	15.4 – 16.0[s]	...	...	...	30.1[b]	4.06[a]	1.182	3.36[a]	0.972
	16.0 – 17.4[t]	...	...	...	32.9	3.96	1.274	3.19[b]	1.028
	17.4 – 17.9[u]	...	...	...	33.8[a]	3.98	1.297	3.12[b]	1.024
	17.9 – 19.3[v]				33.5[a]	3.72[b]	1.203	3.23	1.050

[4]Means in the same column without common superscripts differ ($P < 0.05$)

[5]Least square means within the same column without a common superscript differ ($P < 0.05$).

[6]Values without superscript do not differ (P > 0.15) from other values within a row and within a lactation period.

[p,q,r]Values corresponds to dietary CP for lactation week 1 to 16 from Wu and Satter (2000)trial.

[s,t,u,v]Values corresponds to dietary CP for lactation week 17 to 44 from Wu and Satter (2000) trial.

Table 4. Factors influencing urea utilization adapted from Stanton et al. (2006).

	OBSERVATIONS
Source of readily available carbohydrates	The single most important factor influencing the amount of urea a ruminant animal can use is the digestible energy or total digestible nutrients (TDN) content of the ration. Rations high in digestible energy (high grain) result in good urea utilization; those that are low in digestible energy (high forage) result in a lowered utilization of urea. The addition to a high forage ration of any feed that will increase TDN will improve urea utilization.
Frequency of feeding urea	A constant or continuous intake of urea will improve its utilization over abrupt or periodic intake.
Level of urea fed	Low levels of urea are utilized more efficiently and with less problems than high levels.
Adequate supply of phosphorus, sulfur and trace minerals	Substitution of urea for natural protein sharply changes the quality and quantity of minerals available for ruminal bacteria and cattle. Although needed only in small quantities, these elements are necessary building blocks for microbial protein synthesis.
Solubility of proteins	Natural proteins such as soybean meal and cottonseed meal have different solubility or rates of hydrolysis in the rumen. The more soluble the protein, the more rapidly it is hydrolyzed to ammonia in the rumen. For this reason, some natural proteins may be more competitive with urea.

Table 5. Effect of urea or Optigen® on fermentation and N flow in rumen simulating fermentors (Harrison et al., 2007).

	Urea[2]	Optigen[2®]	SE	P[1]	% change
Number of Experiments	17	17			
Numbers of cultures	59	59			
pH[3]	6.37	6.36	0.017	0.71	- 0.1
Total VFA	73.01	76.36	1.8	0.07	4.6
A:P ratio	3.21	3.40	0.05	0.0020	4.6
Ammonia[3], mg/dl	6.24	7.34	0.17	< 0.0001	17.6
True DM Digestibility, %	62.0	62.8	0.45	0.08	1.3
NDF Digestibility, %	45.2	45.5	0.95	0.64	0.6
Bacterial N, g/day	0.338	0.355	0.009	0.02	5.0
g Bact N / kg DMTD	24.2	24.6	0.28	0.15	1.7
Feed N converted to bact N, %	70.7	72.4	1.6	0.05	2.4

[1] Probability of no difference between urea and Optigen® cultures.

[2] Urea and Optigen® added to cultures at equal NPN levels within each experiment.

[3] Culture fluid pH and ammonia measured prior to morning feeding.

Table 6. Preliminary field observations on Optigen® (Siciliano-Jones, 2005)

Dose per cow (g)	Duration of feedings (days)	Number of cows	Location	Toxicity
250	30	440	Western New York	None
300	30	120	Western New York	None
450	30	120	Western New York	None
230	60	130	Western Pennsylvania	None
230	60	180	Northern New York	None
340	30	120	Western New York	None
180	60	65	Eastern New York	None
230	60	75	Northern New York	None
0.23	60	200	Pennsylvania	None
0.34	60	200	Pennsylvania	None

Table 7. Extended chemical scores of protein sources in relationship to milk protein[1].

Protein source	His	Phe	Leu	Thr	Met	Arg	Val	He	Trp	Lys
Blood Meal	100	100	93	86	45	33	70	10	76	91
Fish Meal	77	69	58	68	100	59	59	47	71	80
Feather Meal	11	59	66	59	23	32	38	32	29	13
Meat Meal	67	65	46	59	49	76	51	36	39	58
Meat and Bone Meal	64	64	46	59	49	76	48	36	32	55
Corn Gluten Meal	67	100	100	60	100	36	48	40	30	18
Alfalfa meal, dehydrated	69	100	55	80	60	50	66	51	100	46
Brewers grain	56	100	83	65	78	53	65	74	87	34
Distillers grain w/solubles	74	84	72	63	81	42	53	38	45	24
Soybean meal	89	100	56	74	56	89	60	55	75	70
Microbes	90	97	54	100	97	79	66	61	99	100

[1]Santos *et al.* (1998) adapted from Chandler (1989) and calculated as follow: (percentage of AA in feed protein/ percentage of AA in milk protein) x 100. A score of 100 is the maximum allowed for each value.

Table 8. Ranking of protein sources in relationship to milk protein[1].

Protein source	Met	Lys	Met + Lys
Microbes	97	100	197
Fish meal	100	80	180
Blood meal	45	91	136
Soybean meal	56	70	126
Corn gluten meal	100	18	118
Brewers grain	78	34	112
Meat meal	49	58	107
Alfalfa meal, dehydrated	60	46	106
Distillers grains w/solubles	81	24	105
Meat and bone meal	49	55	104
Feather meal	23	13	36

[1]Santos *et al.* (1998) adapted from Chandler (1989) and calculated as follows: (percentage of AA in feed protein/ percentage of AA in milk protein) x 100. A score of 100 is the maximum allowed for each amino acid value.

Table 9. Average of microbial crude protein (MCP) variation and ranges according to the type of diets (Karsli and Russell, 2001).

Average g MCP/100 g OM	Range	Type of Diet	Nr Studies
13.0	7.5 – 24.3	Forage based	34
17.6	9.1 – 27.9	Forage – Concentrate Mix	34
13.2	7.0 – 23.7	Concentrate based	14

Table 10. Guideline for interpreting whole herd MUN values from bulk tank milk (Ishler, 2008)

Milk Urea Nitrogen (MUN)	Comment*	Suggestions
< 8 mg/dl	Low	Consider MUN as too low if production is less than 70 lbs. and the herd rations are not formulated for low protein (i.e. 16%). For TMR-fed herds, send out an analysis to confirm protein level. For component-fed herds and TMR-fed herds, use DHIA to evaluate individual cows and groups of cows. Evaluate protein and carbohydrate sources.
	Okay	If production is greater than 70 lbs, and the ration is formulated for low protein and well balanced for protein and carbohydrates, then the MUN may be okay.
8 – 10 mg/dl	Slightly low	If the ration is not formulated for low protein and milk production is less than 70 lbs, then there may be some feed management problems and/or ration program issues to address.
	Okay	If production is greater than 70 lbs, and the ration is formulated for low protein and well balanced for protein and carbohydrates, then the MUN may be okay.
12-14 mg/dl	Slightly high	If the ration is formulated for low protein and there are no feed management issues, then closely evaluate the protein fractions (especially soluble protein) and the level and sources of nonstructural carbohydrates.
	Okay	If the ration is formulated for high levels of protein (>17.0%) and there is only one cereal grain source being fed, then the MUN level may be okay. However, there may be opportunities to lower the protein level to reduce N excretion.
>14 mg/dl	High	For TMR-fed herds, send out for analysis to confirm protein level. For component-fed herds and TMR-fed herds, use DHIA to evaluate individual cows and groups of cows. Evaluate protein and carbohydrate sources. Evaluate feed management practices, e.g. sorting.
	Not recommended	If the ration is formulated for high levels of protein (>17.0%), high levels of degradable protein and/or inadequate starch or sugar sources, then the animal is not efficiently using N and excessive levels of N are being excreted.

*Comments and suggestion are based on field observations and do not address every possible explanation for the MUN level being observed.

Table 11. The 2 X 2 crossover design (COD).

SEQUENCE	PERIOD		
	I		II
1	A	→→	B
2	B	→→	A

Table 12. The power procedure - Fixed scenario elements to compute number of pairs of dairy farms utilizing SAS (2008).

FIXED SCENARIO ELEMENTS	
Distribution[1]	Normal
Method[1]	Exact
Number of Sides[1]	1
Alpha[1]	0.05
Standard Deviation for Milk Yield	2.9
Standard Deviation for Protein %	0.11
Standard Deviation for Fat %	0.34
Nominal Power[1]	0.8
Correlation for Milk Yield	0.5
Correlation for Fat and Protein %	0.2

[1] Values for milk yield, fat % and protein %.

Figure 1. Schematic diagram of the urea cycle adapted from Krebs and Henseleit (1932), Shambaugh (1977), and Jackson et al., (1986).

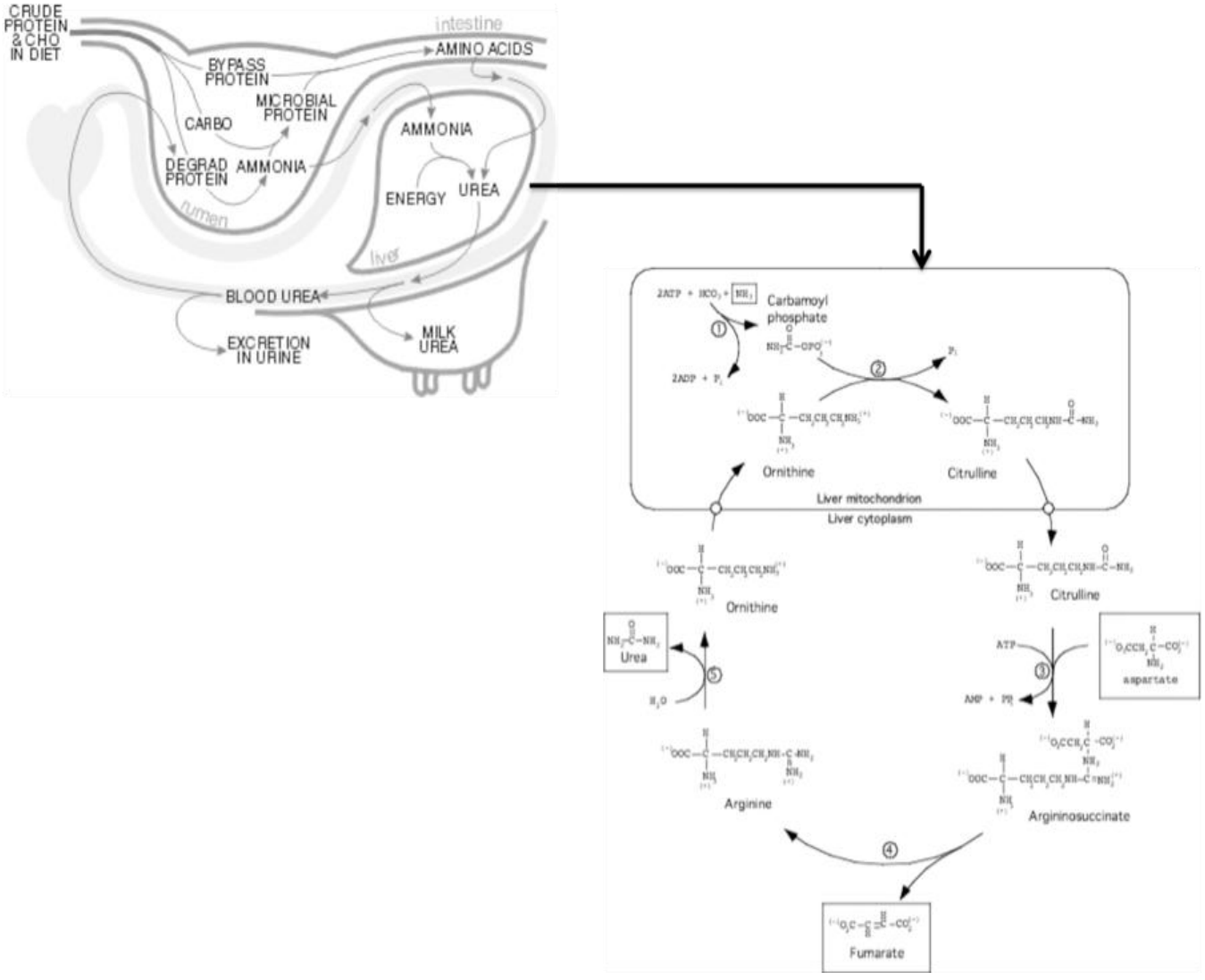


Figure 2. Diagram showing the microbial protein absorption into the small intestine.

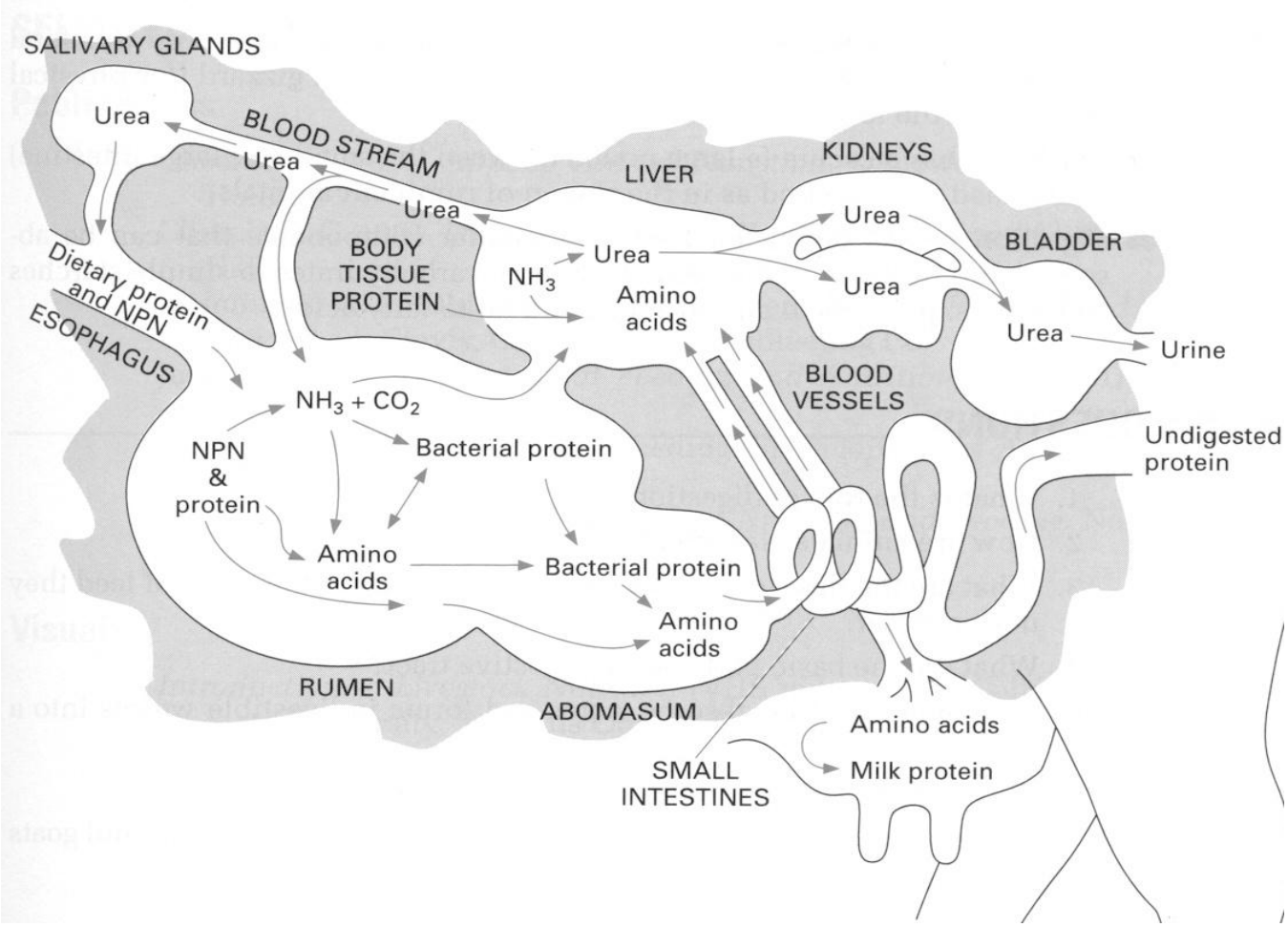


Figure 3. In situ release of nitrogen from several potential nitrogenous substrates in the rumen. Substrates included soybean meal (SBM), distillers grains, a by-pass amino acid source (Amino Plus), urea and six different batches of Optigen® (Siciliano-Jones, 2005).

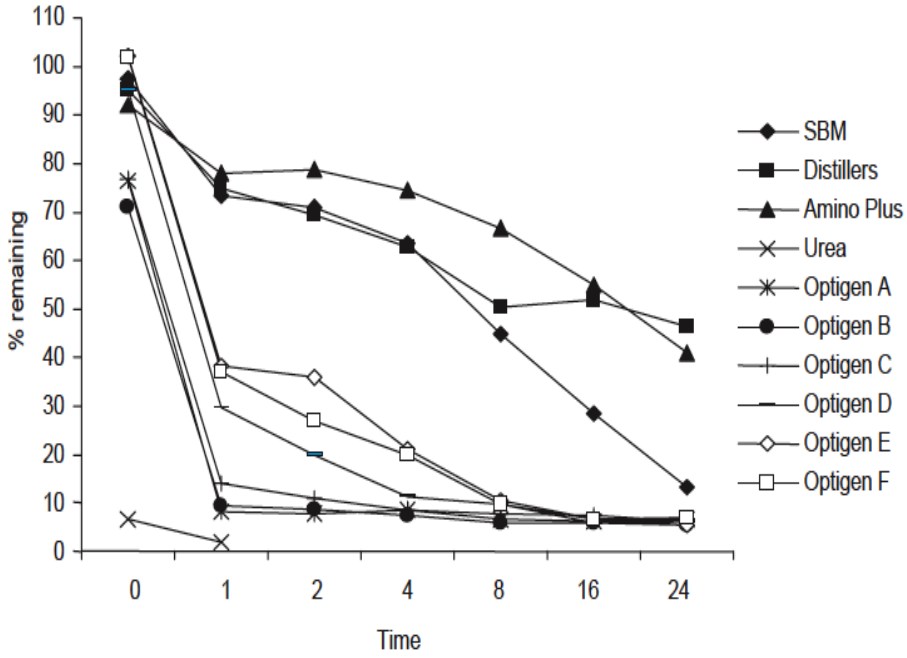


Figure 4. Comparative in situ crude protein digestion of Optigen® 1200, soybean meal and urea (Akay et al., 2004).

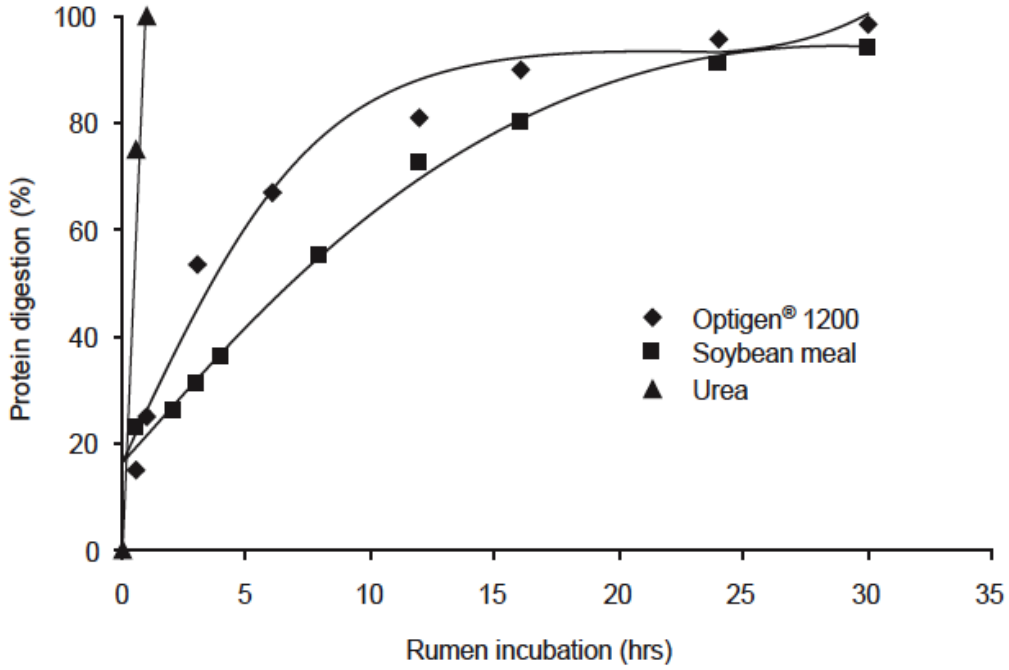


Figure 5. Calculated crude protein degradability. Adapted from García-González et al., (2007) and NRC (2001).

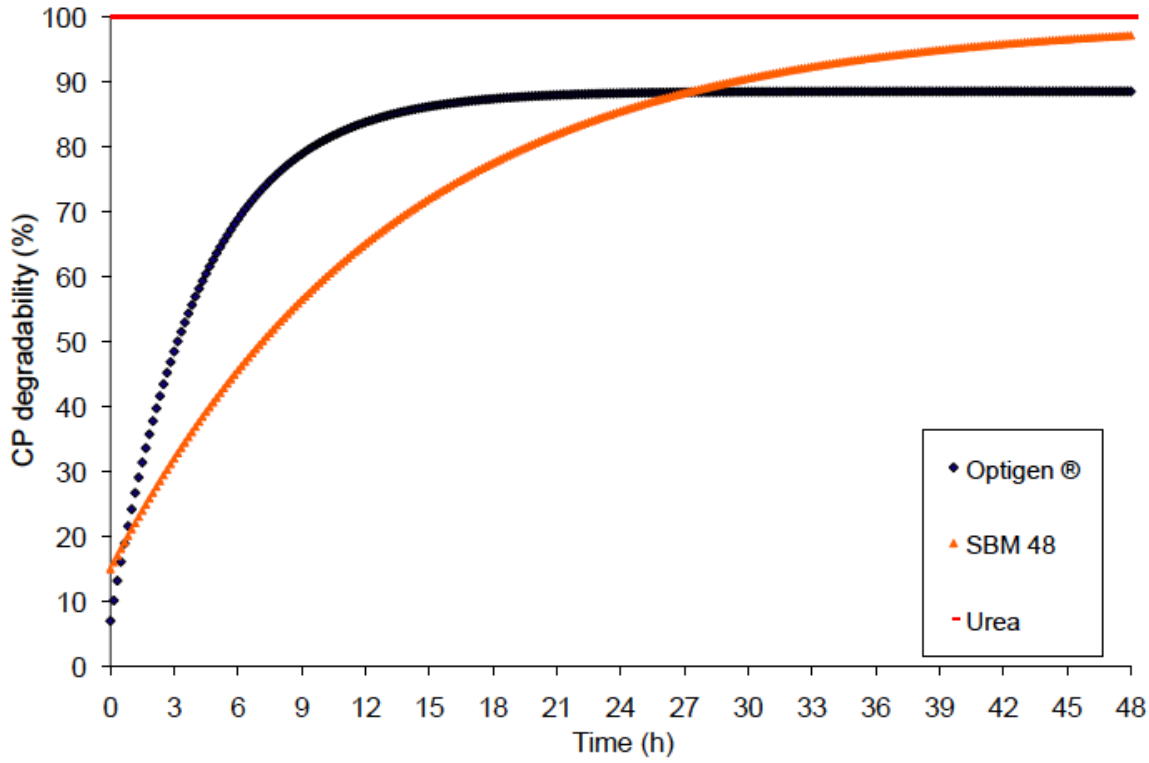
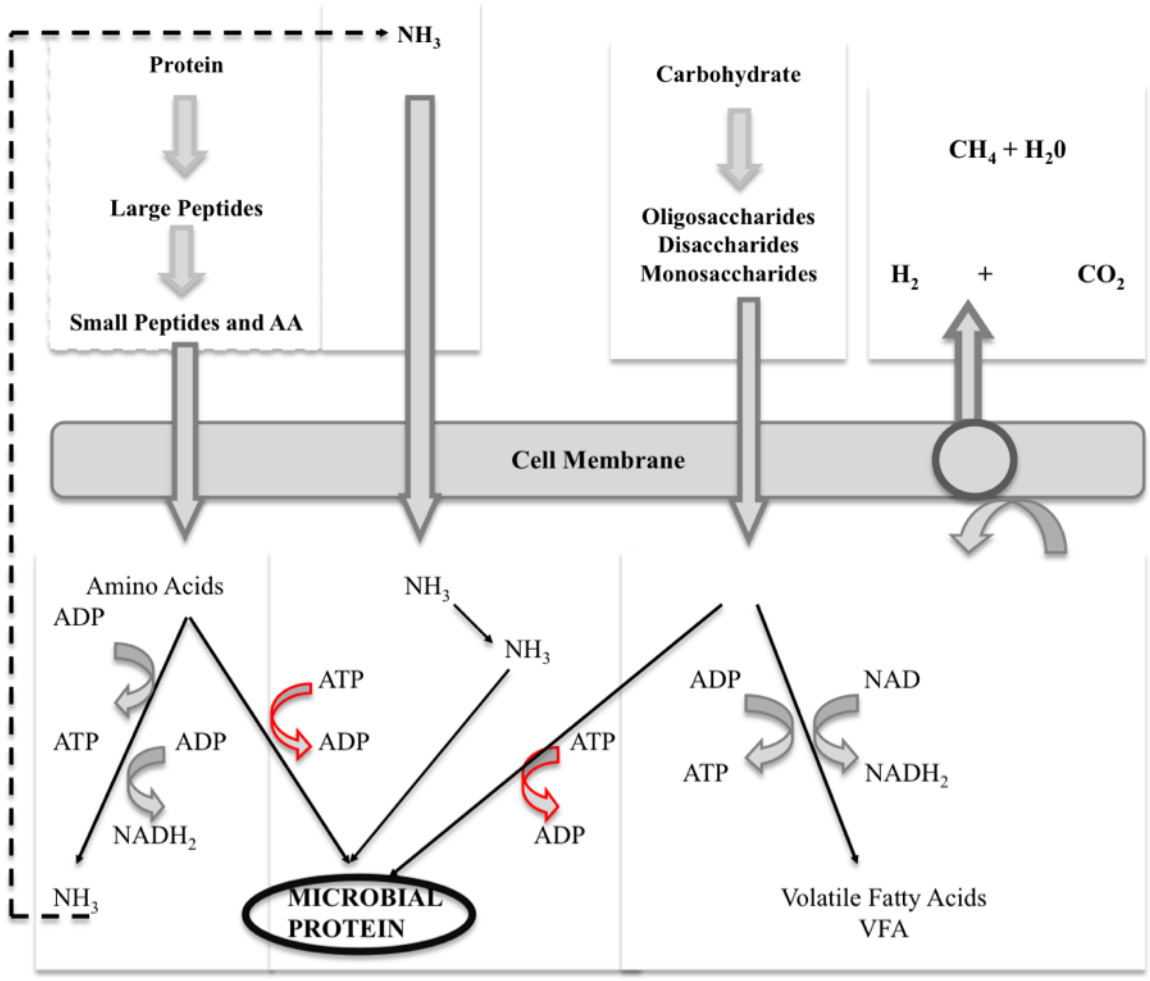


Figure 6. Diagram of utilization of protein and carbohydrate by rumen bacteria adapted from Nocek and Russell (1988).



CHAPTER II

Effect of Optigen® on Milk Yield and Composition in Commercial Wisconsin Dairy Herds

J. F. Inostroza[1], R. D. Shaver[1], V. E. Cabrera[1], and J. M. Tricárico[2]

University of Wisconsin, Madison 53706

Address correspondence to R.D. Shaver, University of Wisconsin-Madison, Department of Dairy Science, 1675 Observatory Drive, Animal Sciences Building, Madison, WI 52706-1284 (608) 263 3497, and Email: rdshaver@facstaff.wisc.edu

[1]Department of Dairy Science, University of Wisconsin, Madison, WI, USA

[2]Alltech Inc., Brookings, SD, US

ABSTRACT

The objective of this field trial was to determine the effect of Optigen® (blended, controlled-release urea), as a source of dietary nitrogen, on milk yield, composition and component yields in commercial Wisconsin dairy herds. The number of lactating cows within herd averaged 148 cows ranging from 58 to 550 cows across the 16 trial herds. Within herd, cows were fed a single-diet as a total mixed ratio **(TMR)**. Control TMR **(CON)** for each herd was formulated by the herd nutritionist according to production level. The treatment TMR **(OPT)** for each herd contained 114 g/cow/d Optigen® replacing an equivalent amount of supplemental crude protein **(CP)**, primarily from soybean meal, to provide iso-nitrogenous control and treatment TMR. Diet formulation space created by the use of Optigen® was filled with dry matter **(DM)** from either corn grain or corn silage at the discretion of the herd nutritionist in the treatment TMR. Across the 16 trial herds, TMR contained 56±3% forage comprised of 43±9% corn silage and were formulated for 17.1±0.4% CP and 30.5±1.7% neutral detergent fiber **(NDF)** as a DM basis. Herds were randomly assigned to either OPT-CON or CON-OPT treatment sequence in a crossover design with two 30-d feeding periods. Records of weight and composition (fat, protein) and milk urea nitrogen **(MUN)** of bulk tank milk shipments were obtained for each herd over the 60-d trial. The numbers of cows with milk in the bulk tank for each shipment were recorded for each herd over the 60-d trial. Average per cow daily milk yield and component yields were then calculated. Data were analyzed using the mixed model procedure of SAS with period, sequence and treatment as fixed effects and herd as a random effect.

Milk yield was 0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON. Under the conditions of this study, Optigen® (114 g/cow/d) was an effective iso-nitrogenous replacement for soybean meal in lactating dairy cow diets.

Key Words: Milk yield, dairy cows, controlled-release urea.

INTRODUCTION

Attempts to achieve slow release of NPN in ruminants have varied not only in method, but also in degree of success. Urea coated with different substances has been utilized. Past research efforts to match ruminal ammonia and energy availability include combinations of urea and starch (Deyoe et al., 1968), urea and cellulose (Conrad and Hibbs, 1968), and urea and fatty acids (United, 1977). However, most products have either released ammonia too rapidly or bound the nitrogen too tightly thereby limiting ammonia availability (Males et al., 1979). Owens (1980) observed that a coated product released ammonia slowly, enhancing the acceptability of urea containing diets and reducing urea toxicity. A slow release urea product should be useful to reduce toxicity, enhance acceptability of supplements and use of urea, and improve performance in ruminant species. In 2005, Alltech (2009) developed a biodegradable blended controlled-release NPN product (Optigen®) using a mixture of Vegetable Oil, Beta Carotene, BHT and Citric Acid to coat individual non-protein nitrogen prills.

The objective of this field trial was to determine the effect of Optigen® (blended, controlled-release NPN), as a source of dietary nitrogen, on milk yield, protein and fat composition and component yields, and milk urea nitrogen (MUN) in commercial Wisconsin dairy herds.

MATERIALS AND METHODS

Dairy Farms and Cows

The number of lactating Holstein dairy cows within herd averaged 148 cows ranging from 58 to 550 cows across the 16 trial commercial herds. All of the cows were in second lactation or greater. Eight collaborating nutritionists from two different nutrition companies in Wisconsin identified 16 trial herds out of thirty dairy farms that were willing to participate in this commercial dairy field trial. The participating herds were paired by location, production level, percentage of corn silage (DM basis) in the diet, and number of cows. We performed a power analysis for a crossover design using the POWER procedure (Paired t test for mean difference) from SAS (2008) to estimate the number of herds required to detect a statistical significant difference with an alpha of 0.05 and beta of 0.80.

Experimental Design and Treatment Diets

The 16 dairy herds were randomly assigned to treatments in a crossover design with 30-d periods using the herd as experimental unit. Tempelman (2009) stated that, "The experimental unit is the smallest unit to which and individual treatment is imposed.

For group-fed animals, the group of animals in the pen or the paddock is the experimental unit; therefore, groups must be replicated" (Excerpted from *The Journal of Dairy Science* in its Instruction to Authors). In a crossover design, treatment sequences are randomly allocated to each experimental unit or subject. The reduction in variability from taking multiple measurements on a subject allows for more precise treatment comparisons (SAS, 2008). The simplest design is the AB/BA crossover, in which each subject, in our case the dairy farm, receives each of two treatments in a randomized order. Therefore, the dairy farm may be used as an experimental unit in commercial dairy field trials, since it fulfills the basic requirements for randomization and replication. Thus, for each farm, we had a total of 60 observations on milk production and composition; 30 observations for the first period and 30 observations for the second period.

Two treatment sequences were used in the trial, Control-Optigen® (CON-OPT) and Optigen®-Control (OPT-CON). Herds were randomly assigned to either the CON-OPT or OPT-CON treatment sequences in a crossover design with two 30-d feeding periods **(Figure 1)**. Treatment sequences were randomly allocated to herds within each of the eight herd pairs. This randomization scheme resulted in a balanced row (period)-column (sequence) crossover design. Each of the two sequences (CON-OPT and OPT-CON) was presented with equal frequency (8 occurrences) in each period to avoid confounding the period and treatment effects. Thus, the same numbers of farms were allocated to both groups, providing the maximum information per experimental unit, and equivalently the smallest sampling variances.

Each farm was assigned to their experimental diet for a 30-d period. Within farm, cows were fed a single-diet TMR. Control TMR (CON) for each herd was formulated by the herd nutritionist according to production level. The treatment TMR (OPT) for each herd contained 114 g/cow/d Optigen® replacing an equivalent amount of supplemental CP, primarily from soybean meal, to provide iso-nitrogenous control and treatment TMR. The product was provided by Alltech Inc. to the farms. Diet formulation space created by the use of Optigen® was filled with DM from either corn silage, high moisture shelled corn or corn grain at the discretion of the herd nutritionist in the treatment TMR. Across the 16 trial herds, TMR contained 56 ± 3% forage comprised of 43 ± 9% corn silage and were formulated for 17.1 ± 0.4% CP and 30.5 ± 1.7% NDF (DM basis).

TMR Sample Protocol and Analysis

Milk yield and composition for each bulk tank or tanker shipment were recorded along with the number of cows contributing to each shipment from an on-farm log. Data was collected for each farm in each treatment period everyday. Records of milk yield and composition (fat, protein, and MUN) of bulk tank milk shipments were obtained for each herd over the 60 d trial from April 2008 to June 2008. Average per cow daily milk yield and component yields were then calculated.

The largest error in feed analysis is improper sampling methods on the farm (Undersander et al., 2005). The TMR was sampled at the start of the trial and every 30-d thereafter. Samples of the TMR for wet chemistry analysis were sent to Dairy One Labs (Ithaca, NY) for the following nutrients: dry matter (DM), crude protein (CP), soluble protein (SP), neutral detergent fiber (NDF), acid detergent fiber (ADF), fat, starch, sugar, non fiber carbohydrates (NFC), total digestible nutrients (TDN; 2001 NRC summative energy equation), net energy for lactation (NE_L), Ca, P, K, Na, and sulfur. Split samples were sent to Alltech's Lab (Brookings, SD) to run in vitro gas production and to Dairyland Laboratories (Arcadia, WI) for additional analysis of CP and NDF by wet chemistry procedures. The nutrient analysis of feed ingredients used by the nutritionists in their diet formulations was obtained.

Statistical Analysis

Data were analyzed using the mixed model procedure of SAS (2008) with period, sequence and treatment as fixed effects and herd as a random effect. All measurements of this trial were condensed to monthly means across the 30 d to get one value for each farm for each 30 d period. When running long-term lactation studies, it may be useful to use weekly averages with week as a repeated measure to test for week by treatment effects. However, with short-term studies, repeated measures may not be as meaningful as the average over the period and repeated measures also adds complexity to the analysis.

If a treatment was significant in the model, differences between treatment least squares means were determined using SAS (2008) with statistical significance declared at $P < 0.05$ and trends at $P > 0.05$ to $P < 0.10$.

The model used for the lactation performance data was: (complete the model description below)

$$Y_{ijk} = \mu + seq_i + farm_{ij} + per_k + trt_h + e_{ijk}$$

where,

μ = Overall mean effect

seq_i = Effect of the i^{th} sequence (i = 1,2)

$farm_{ij}$ = Random effect of the j^{th} farm on the i^{th} sequence (j = 1 to 16)

per_k = Fixed effect of the k^{th} period (k = 1,2)

trt_h = Fixed effect of the hth treatment (h = 1,2); being a function of i and k)

e_{ijk} = Random residual error

RESULTS AND DISCUSSION

Diet Composition

Ingredient and nutrient composition of experimental diets (mean ± standard deviation) are presented in **Tables 1 and Table 2,** respectively. The forage to concentrate ratio was 55.5% forage: 44.5% concentrate for the control diet and 56.0% forage: 44.0% concentrate for the treatment diet (DM basis). Dry matter content of formulated control diets averaged 53.7 ± 3.9% and for formulated treatment diets the average was 53.2 ± 5.1%. Dietary CP concentrations were similar for both formulated rations, averaging 17.1 ± 0.4%. However, chemical analysis from Dairy One Labs **(Table 3)** showed higher CP concentrations for control and treatment samples with 18.2 ± 0.9% and 18.4 ± 0.7% (DM basis), respectively. A similar trend was observed for the chemical analysis from Dairyland Laboratories **(Table 4)** with 17.9 ± 0.8% CP for the control samples and 18.1 ± 0.6% CP for treatment samples (DM basis). The NRC (2001) recommended CP minimums between 15.2% and 16.7% (DM basis) for mid lactation cows (> 12 weeks relative to calving). Formulated dietary ADF concentrations averaged 19.9% for control and 20.0% for treatment diets, while dietary NDF concentrations averaged 30.5% and 30.4% for control and treatment diets, respectively (DM basis). Formulated NFC composition averaged 39.8 ± 2.2% for the control diet and 40.1 ± 2.4% for treatment diet (DM basis).

Chemical analyses from Dairyland Laboratories showed similar values for NDF (28.6 ± 2.1% for control samples and 29.2 ± 2.0% for treatment samples) and also for NFC averaging 41.5 ± 2.5% for control and 40.4 ± 2.0%for treatment samples (DM basis). Formulated dietary starch content averaged 25.3 ± 2.2% for control and 26.2 ± 2.0%for treatment diets, respectively (DM basis). Lower values were found in the Dairy One Labs analysis where starch content averaged 23.0 ± 2.9% for control and 22.6 ± 4.6% for treatment diets, respectively (DM basis). Finally, diets were formulated to be similar in content of NEL (1.69 ± 0.08and 1.68 ± 0.09 Mcal/kg for control and treatments diets, respectively), while calculated NEL from TMR chemical analysis were slightly lower (1.65 ± 0.04and 1.64 ± 0.04 Mcal/kg for control and treatment diets, respectively (DM basis).

Large differences observed for NDF and NFC values between Dairy One Labs and Dairyland Laboratories were possibly due to the presence or absence of sodium sulfite in their NDF procedure, which causes greater NDF values and consequently lower NFC values. If one laboratory measures NDF using sodium sulfite but while another laboratory does not, the NDF concentrations will differ between laboratories (St-Pierre and Weiss, 2009). Nutrient and chemical composition of experimental diets (mean ± standard deviation) when soybean meal 48% was replaced by 114 grams of Optigen® and the space was filled with corn silage are presented in **Table 5**. The DM content averaged 50.2 ± 5.3% and 53.6 ± 5.6%, for control and treatment diets, respectively.

Dietary CP concentrations averaged 17.6 ± 0.9% for the control diet and 18.2 ± 0.6% percent the treatment ration diet. Soluble CP was higher on treatment diet averaging 52.5 ± 5.6% of CP, and 47.5 ± 6.6% of CP for the control. This can be explained by the partial replacement of soybean meal with Optigen® in the treatment diet. Starch content was also higher in treatment diets on average, and possibly due to an increase of 2.2% of corn silage in the diet (DM basis) to fill the space. The NEL (Mcal/kg) contents were identical for treatment and control diets averaging 1.65 ± 0.02 Mcal/kg DM.

Nutrient and chemical composition of experimental TMR diets (mean ± standard deviation) when soybean meal 48% was replaced by 114 grams of Optigen® and the space was filled with high moisture shelled corn and corn grain ground are presented in **Table 6** and **Table 7** respectively. The quality of forages included in control and treatment diets are presented in **Table 8**.

Milk Yield and Composition

Response variables evaluated were yields of milk, fat percentage and yield, protein percentage and yield, and MUN. Least square means for the effect of Optigen® supplementation on production responses are shown in **Table 9**. Milk yield was 0.5 kg/d greater ($P < 0.01$) when Optigen® was supplemented at approximately 114 g/cow/d. Other researchers (Tikofsky, 2007, Varga and Ishler, 2008) reported a milk yield response when formulating dairy diets with Optigen® . In a field study, Tikofsky and Harrison (2007) reported an increase of 1.6 kg/cow/d on a dairy in Central Kentucky.

Cows were fed a similar ration consisting of mixed hay, corn silage, alfalfa silage, soybean meal, soy hulls, shelled corn and mineral premix. The ration was reformulated to include 125 g/cow/d of Optigen®. Other changes in ration ingredients included reductions in distillers grains and alfalfa haylage inclusion and increases in corn silage and soy hull inclusion. These changes in the formulation resulted in a treatment diet that contained higher dry matter (59.6% vs. 50.5%) starch (24.3% vs. 23.1%) contents, and lower NDF (30.8% vs. 35.6%), and CP (15.9% vs. 17.9%) contents (DM basis).

In addition, Varga and Ishler (2008) observed a numerical increase (P = 0.11) of 1.2 kg/d in milk yield when the Optigen® was fed to dairy cows. The researchers used a completely randomized block design with two treatments and 60 cows per treatment. Cows were blocked by production average, lactation number, and days in milk. The duration of the trial was 90 d. Diets were reformulated with 112 g/d/cow Optigen® by increased levels of corn silage and ground corn in the diet and decreasing levels of canola meal and heat-treated SBM. De Almeida et al. (2009) concluded that there was no detectable treatment effect (P = 0.16) on milk yield, although yield was numerically greater with Optigen® (39.4 versus 36.9 kg/d at d 30 and 38.1 kg/d versus 36.4 at d 60 of treatment). The study evaluated the effects of partial replacement of soybean meal with Optigen® on milk yield and composition. Thirty-four lactating Holsteins were blocked by milk production, lactation number and DIM. Data was analyzed with the mixed procedure of SAS. No detectable treatment effects were found for milk composition, MUN, and SCC either.

In our study, yields of milk fat and protein and milk protein percentage were unaffected ($P > 0.10$) by treatment. We did, however, observe a trend ($P = 0.07$) for a lower milk fat content when herds were fed Optigen®. Varga and Ishler (2008) reported a numerical reduction milk fat percentage ($P > 0.10$ when cows were fed Optigen®. A study conducted by Akay et al. (2004) with a similar slow-release coated urea product showed similar results. Diets that contained the urea product increased ($P < 0.01$) milk yield 3.7 kg/d, decreased ($P < 0.01$) milk fat and protein percentages, and increased ($P < 0.01$) fat and protein yields. The author attributed the response to a combination of improved bacterial protein synthesis and availability of greater concentrations of degradable carbohydrates. A dairy field trial reported by Tikofsky and Harrison (2007) showed a milk yield response of 1.8 kg/d when 100 grams of Optigen® was included in diets to replace unprotected urea. This trial was done in a 120-cow commercial herd in Ohio. Milk yield and DMI were observed over 30 d and the researchers attributed the response to maximizing rumen function through the consistent supply of NPN from Optigen® increasing both microbial yield and efficiency.

Chalupa (2007) reported that Optigen® influenced ruminal fermentation, increasing and improving microbial efficiency and growth. In continuous culture fermentors, Optigen® increased microbial yield by 5% (Chalupa, 2007). The CPM-Dairy (Chalupa and Sniffen, 2006) was used to simulate the impact of improved microbial growth on nitrogen excretion (0.232 g/d for OPT versus 0.220 g/d for CON). Capture of dietary nitrogen in ruminal microbes with dietary Optigen® increased by 5%.

This increased microbial growth led to 6% more nitrogen in milk with a decrease of 7% in urinary nitrogen excretion reducing ammonia emissions. An increase in milk production of 1.8 kg/cow/day was also reported by Chalupa and Sniffen (2006). Maximizing the ruminal production of microbial protein may be one of the theories that explain increased in milk production with the use of slow-release controlled urea. Efficiency of microbial growth is usually expressed as grams of microbial protein per gram of fermentable carbohydrate or fermentable DM (Chalupa, 2007). Tikofsky and Harrison (2007) reported an increase ($P < 0.10$) in bacterial N yields when cultures were fed with Optigen® versus urea. This increase was 29% when 50 grams of Optigen® was fed and 60% greater yields compared with urea when 150 grams of Optigen® were fed to the cultures.

The difference in milk production might be explained by the slow-release characteristics of Optigen®. **Figure 2** shows in situ nitrogen disappearance of Optigen® as compared with that of urea and soybean meal, and it was observed that its nitrogen disappearance pattern in the rumen is similar to that from soybean meal but different when compared with urea (García-González et al., 2007).

As a result, Optigen® is an NPN source that is available to the ruminal ecosystem at a slow rate (Siciliano-Jones, 2005). While the idea of using Optigen® to better synchronize protein and carbohydrate availability in the rumen continues to be examined, it may have a practical benefit in dairy cow rations. Optigen® has a CP equivalent (CPE) of 256% on a dry matter basis (41% N x 6.25).

This allows nutritionists to safely increase CP density of the supplemental CP, thereby creating space for other critical nutrients, such as digestible fiber or non-fiber carbohydrates. Owens (1980) demonstrated that; 1) SRU achieved more uniform release of ammonia-nitrogen into the ruminal fluid than prilled urea, and 2) SRU was unlikely to produce toxic ruminal ammonia levels. Palmer et al. (2007) concluded that Optigen® was estimated to have a lower level of quickly degraded nitrogen compared with SBM (**Figure 3**). In situ nitrogen disappearance from diets containing soybean meal (SBM) or 150 g of Optigen® to replace a portion of SBM and a fitted model Ørskov (Orskov et al., 1980, Ørskov et al., 1980, Ørskov and McDonald, 1979) are presented in **Figure 4**.

García-González et al. (2007) reported that over 75% of the NPN supplied in Optigen® was available in the rumen within the first 8 h of incubation, while ruminal and plasma ammonia and urea-N concentrations were lower than those in steers supplied with urea (**Figure 5A and 5B respectively**). The rate of diffusion is a function of coating integrity and may be varied in a predictable manner (Siciliano-Jones, 2005).

Nitrogen and carbohydrates are the major nutrients that support microbial growth (**Figure 6**), but the quantity and composition of these nutrients is hardly constant (Harrison and Karnezos, 2005). Golombeski (2006) observed that the addition of highly fermentable sugars in combination with a slow-release nitrogen source reduced intake without affecting milk production which improved feed efficiency. From the same study, milk fat percentage was unaffected by the addition of slow-release urea which was in accordance with the study (Galo et al., 2003).

Replacement of soybean meal with slow-release NPN did not alter true protein percentage or yield, demonstrating that it can be an alternative source of N in dairy cow diets without causing inefficient use of N (Golombeski et al., 2006). Data obtained in a experiment conducted by Forero et al. (1980) indicated that a slow-release urea product improved palatability of urea-containing supplements and effectively slowed ammonia release from urea.

By increasing the CPE density of the protein supplements used in our study, diet formulation space was created which allowed the nutritionists to feed more forage or grain. With a reduced RUP in the Optigen® diets compared to control (**refer to Table 2**), and having created this space in the diet for more rumen degradable carbohydrates, bacterial protein synthesis might have improved which could explain the greater milk yield observed in our trial. Some observations suggest that feeding urea hourly to simulate slow release may increase the synthesis of microbial protein due to a better synchronization with carbohydrates (Males et al., 1979, Rush et al., 1976). Because hourly feeding of a supplement is an impractical management procedure, efforts have been intensified to find an ideal controlled release urea product. Henning et al. (1993) suggested that such a product should ensure that the total amount of ruminally available N is sufficient for the total amount of carbohydrate expected to be available in the rumen each day.

MUN was affected by treatment ($P < 0.01$) averaging 12.4 and 13.2 mg/dl for control and Optigen® treatment, respectively. These MUN values are in the normal range established by researchers and the industry of between 10 and 14 mg/dl (Ishler, 2008, Wattiaux, 2005), and thus was probably not physiologically significant. Higher MUN when herds were fed Optigen® may have been related to the slightly higher diet CP content observed when it was fed. Varga and Ishler (2008) found the same results and reached the same conclusion regarding MUN in their study, where MUN increased ($P < 0.01$) from 8.6 mg/dl for the control to 9.8 mg/dl for the Optigen® treatment.

The single dietary factor most closely associated with MUN is dietary CP. Energy intake, especially rumen available energy needed to capture rumen available N, is another factor that influences MUN. Wattiaux (2005) suggested that, under common feeding conditions of the Midwest USA, MUN values of approximately 12 mg/dl are associated with a diet of approximately 16.5% of CP, was an optimal situation that does not penalize milk production, but avoids unnecessary losses of urinary nitrogen. Although dairy cows use feed CP with great efficiency, they still secrete 2-3 times more N in manure than they secrete in milk. In feeding trials with cows receiving diets formulated from typical Midwest USA ingredients there were no increases in yields of milk and protein with more than about 16.5% dietary protein (Broderick, 2006). Data from a trial conducted by Broderick (2003) clearly showed that, over-feeding CP decreases the efficiency of capture of protein into milk protein with a corresponding increase in excretion of urea nitrogen in urine, and showed no differences in milk production when diets were increased from 16.7% to 18.4% of CP.

CONLUSIONS

Milk yield was 0.5 kg/d greater ($P < 0.01$; 35.9 vs. 35.4 kg/d) when commercial WI dairy herds were fed 114 g/cow/d Optigen® than when they were not fed Optigen® in diets formulated to be iso-nitrogenous. Milk fat percentage tended ($P < 0.07$) to be lower when the herds were fed Optigen®, but the numerical difference between the treatments was small (3.69% vs. 3.72%). Milk fat yield and milk protein percentage were unaffected by treatment. A 10 g/cow/d increase in milk protein yield when the herds were fed Optigen® approached a trend ($P < 0.13$). Milk-urea nitrogen was greater ($P < 0.01$) when the herds were fed Optigen®, but only by 0.8 mg/dL and both control and treatment average MUN values fell within the normal expected range for high-producing dairy herds. Under the conditions of this study and with Optigen® fed at 114 g/cow/d, it was an effective partial substitute for soybean meal as an RDP source when diets were formulated to be iso-nitrogenous. Results indicate that Optigen® can be an alternative RDP source in diets for lactating dairy cows when the economics of supplementation are favorable. The crossover design with herd as the experimental unit appears to be a feasible approach for evaluating the efficacy of feed additives on commercial dairy farms.

ACKNOWLEDGMENTS

We would like to thank Alltech Inc., for providing partial financial support for this research trial. Appreciation is extended to CP Feeds and Badgerland Nutrition Inc. staff Todd LeNoble and Jeniffer Meyer, and also to Vita Plus Corporation staff Spence Driver, John Weinkes, Rod Sjoerdsma, Randy Greenfield and Irv Haugen, and Mike Yahr of West Bend Elevator Inc. for their assistance in selection of the dairy farms that participated in our trial and their work on ration reformulations. The authors would also like to thank the dairy farmers of Wisconsin who were willing to participate in this field trial: Daug & Brenda Schmitt, Wayne & Corey Leis (Leis Farms), Joe Brantmeier & Sons (Hillrose Dairy), LLC Ton Tienor & Andy Fisher (Riverside Dairy), Randy and Mary Bonde (Bonde Acres Dairy), Andy and Joan Meyer (A & J Meyer), Mark Beyer (Hilltop Dairy Farm), Dick & Jacob Halverson (Halverson Dairy), Larry & Kim Voights (Voight Crest Holsteins), Gordon & Joyce Herr, Scott Bass & Julie (Bass Holsteins), Mike & Kim Plager (Plager Land Holsteins), Dennis & Jane Vellema (Vellema Farms), Randy & Brenda Kamphuis, Mike & Kathy Pamperin (Cool Breeze Dairy) and, Greg & Ellen Goeller (Goeller Family Farms LLC).

REFERENCES

Akay, V., J. Tikofsky, C. Holtz, and K. A. Dawson. 2004. Optigen® 1200: controlled release of non-protein nitrogen in the rumen. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Annual Symposium: re-imagining the feed industry. T. P. Lyons and K. A. Jacques, ed. Alltech UK, Lexington, Kentucky, USA.

Alltech, I. 2009. Optigen ® Lexington, KY.

Broderick, G. 2006. Nutritional Strategies to Reduce Crude Protein in Dairy Diets.

Broderick, G. A. 2003. Effects of Varying Dietary Protein and Energy Levels on the Production of Lactating Dairy Cows. J. Dairy Sci. 86(4):1370-1381.

Chalupa, W. 2007. Precision Feeding of Nitrigen to Lactating Dairy Cows: A Role for Optigen II. Pages 221-226 in Nutritional Biotechnology in the Feed and Food Industries. Vol. 23. T. P. Lyons, K. A. Jaques, and J. M. Hower, ed.

Chalupa, W. and C. Sniffen. 2006. Balancing Rations on the basis of Amino acids: The CPM-Dairy Approach. Pages 96-102 in Southwest Nutrition and Management Phoenix, AZ, USA.

Conrad, H. R. and J. W. Hibbs. 1968. Nitrogen Utilization by the Ruminant: Nitrogen Utilization by the Ruminant. Appreciation of Its Nutritive Value. J. Dairy Sci. 51(2):276-285.

De Almeida, R., P. R. Barreto, V. L. De Souza, D. F. Ferreira, R. F. De Lima, C. Pereira, W. Giardini, and M. N. Pereira. 2009. Partial Replacement of Soybean Meal by Protected Urea Effects on Milk Yield and Composition. Universidade Federal do Paraná, Paraná, Brasil.

Deyoe, C., E. Bartley, H. Pfost, F. Boren, H. Perry, F. Anstaett, L. Helmer, D. Stiles, A. Sung, and R. Meyer. 1968. An improved urea product for ruminants. Journal of Animal Science 27:1163.

Forero, O., F. N. Owens, and K. S. Lusby. 1980. Evaluation of Slow-Release Urea for Winter Supplementation of Lactating Range Cows. J. Anim Sci. 50(3):532-538.

Galo, E., S. M. Emanuele, C. J. Sniffen, J. H. White, and J. R. Knapp. 2003. Effects of a Polymer-Coated Urea Product on Nitrogen Metabolism in Lactating Holstein Dairy Cattle. J. Dairy Sci. 86(6):2154-2162.

García-González, R., J. M. Tricárico, G. A. Harrison, M. D. Meyer, K. M. McLead, D. L. Harmon, and K. A. Dawson. 2007. Optigen® is a Sustained Release of Non-Protein Nitrogen in the Rumen. Dep. of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA.

Golombeski, G. L., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2006. Slow-Release Urea and Highly Fermentable Sugars in Diets Fed to Lactating Dairy Cows. J. Dairy Sci. 89(11):4395-4403.

Harrison, G. A. and T. P. Karnezos. 2005. Can we improve the efficiency of nitrogen utilization in the lactating dairy cows? Pages 145 - 154 in Recent Advances in Animal Nutrition. Vol. 15. T. P. Lyons and K. A. Jacques, ed. Alltech Inc., Australia.

Henning, P. H., D. G. Steyn, and H. H. Meissner. 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. J. Anim Sci. 71(9):2516-2528.

Ishler, V. 2008. Interpretation of milk urea nitrogen values. Page 3. Penn State University, Pennsylvania.

Jonker, J. S., R. A. Kohn, and J. High. 2002. Use of Milk Urea Nitrogen to Improve Dairy Cow Diets. J. Dairy Sci. 85(4):939-946.

Kertz, A. F. 1998. Variability in Delivery of Nutrients to Lactating Dairy Cows. J. Dairy Sci. 81(11):3075-3084.

Males, J. R., R. A. Munsinger, and R. R. Johnson. 1979. In Vitro and in Vivo Ammonia Release from "Slow-Release" Urea Supplements. J. Anim Sci. 48(4):887-892.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed, Natl. Acad. Sci. Wasington, DC.

Ørskov, E., F. Hovell, and F. Mould. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Tropical Animal Production 5:195-213.

Ørskov, E. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, Cambridge 92(part 2):499-503.

Owens, F. N., K. S. Lusby, K. Mizwicki, and O. Forero. 1980. Slow Ammonia Release from Urea: Rumen and Metabolism Studies. J. Anim Sci. 50(3):527-531.

Palmer, M., D. Wilde, and R. Fawcett. 2007. A Comparison of Protein Degradation profile of Soybean Meal and Slow Release Nitrogen Source. Alltech UK Ltd, Lincolnshire, UK.

Ratkowsky, D., M. Evans, and J. Alldredge. 1993. Cross-over experiments: design, analysis, and application. CRC Press.

Rush, I. G., R. R. Johnson, and R. Totusek. 1976. Evaluation of Beef Cattle Range Supplements Containing Urea and Biuret. J. Anim Sci. 42(5):1297-1308.

SAS. 2008. User's Guide: Statistics. Version 9.1 ed. SAS Institute, Cary, NY.

Siciliano-Jones, J., Downer, J. 2005. Utility and safety of a slow-release nitrogen product: Optigen® 1200. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K. A., ed. Alltech UK, Lexington, KY, USA.

Sniffen, J., R. Beverly, C. Mooney, M. Roe, and A. Skidmore. 1993. Nutrient Requirements Versus Supply Strategies to Account for Variability. J Dairy Sci 763160:3178.

St-Pierre, N. R. and L. R. Jones. 1999. Interpretation and Design of Nonregulatory On-Farm Feeding Trials. J. Anim Sci. 77(E-Suppl_2):177-182.

St-Pierre, N. R. and W. P. Weiss. 2009. Application of Analytical and Statistical Tools in the Feed Industry: Controlling Diet Variation. Ohio state University, Columbus, OH.

Stewart, R. L., J. M. Tricárico, D. L. Harmon, W. Chalupa, K. R. McLeod, G. A. Harrison, J. H. Clark, R. García-González, and K. A. Dawson. 2008. Influence of Optigen® on Nitrogen Behavior in Lactating Dairy Cows. University of Kentucky, Lexington, KY, USA.

Tempelman, R. J. 2009. Invited review: Assessing experimental designs for research conducted on commercial dairies. J. Dairy Sci. 92(1):1-15.

Tikofsky, J., Harrison, H.A. 2007. Optigen II: Improving the efficiency of nitrigen utilization in the dairy cow. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K. A., ed. Alltech Inc., Lexington, Kentucky, USA.

Udén, P., P. Robinson, and J. Wiseman. 2005. Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. Animal feed science and technology 118(3-4):181-186.

Undersander, D., R. D. Shaver, J. Linn, P. Hoffman, and P. Peterson. 2005. Sampling Hay, Silage, and Total Mixed Rations for Analysis. University of Wisconsin-Extension, Madison, WI.

United, N. 1977. Protein and Non-Protein Nitrogen for Ruminants. Recent Developments in the Use of New Sources. P. Press, ed. Economic Comision of Europe, London.

Varga, G. A. and V. Ishler. 2008. Effects of Optigen® on Milk Production, N Balance and Diet Cost in High Producing Cows. The Pennsilvania State University, University Park, PA. USA.

Wattiaux, M. 2005. Fine-tuning test-day MUN records for DHI-related variables. Pages 75-83.

Table 1. Ingredients of Control (CON) and Optigen® (OPT) TMR diets[1] formulated by the nutritionists.

	CON	OPT
Item	**% of DM**	
Forage	55.5	56.0
Corn Silage	23.1	24.2
Alfalfa Silage	28.0	27.3
Other Forage[2]	4.4	4.5
Concentrate	44.5	44.0
Corn Grain Ground	9.0	8.5
High Moisture Corn	13.3	14.6
Soybean Meal 48%	3.5	1.7
Other Plant Protein[3]	3.5	3.5
Animal Protein[4]	0.8	0.8
High Fiber By-products[5]	9.4	9.2
Min/Vit/Additive Mix[6]	5.0	5.7

[1] Values are means of the 32 diets used during the trial. OPT diet contained 114 grams (0.25 oz) of Optigen ®.

[2] Most common ingredients used during the trial were: balage, hay, and wheat straw.

[3] Most common ingredients used during the trial were: expeller meal, cottonseed meal, soy plus, distillers grains, wet distillers grains, corn gluten meal, roasted soybeans, and vita soy.

[4] Most common ingredients used during the trial were: pork blood meal, fishmeal, meat, and bone pork.

[5] Most common ingredients used during the trial were: peas and oats, corn gluten feed, soybean hulls, beet pulp ground, citrus pulp, cottonseed fuzzy, and whole cottonseed.

[6] Most common minerals used during the trial were: Dynamate (18%K, 11% Mg, 22% S), Magox 54%, calcium carbonate 38%, monocalcium phosphate 21%, dicalcium phosphate 18.5%, salt, sodium sesquicarbonate, bicarbonate of soda, and trace minerals Se, Zn, Cu, Mn, and Co. Most common vitamins were: Vitamin A 30000, Biotin 1%, and Vitamin E 20000. Additives most used were: yeast, mannan oligosaccharides, Megalac®, Rumensin® and Omnigen-AF. Included 114 grams of Optigen® (0.25 oz).

Table 2. Nutrient composition of Control (CON) and Optigen® (OPT) TMR diets[1] formulated by the nutritionists.

	CON	OPT
Nutrient	**% of DM**	
DM, %	53.7 ± 3.9	53.2 ± 5.1
CP, %	17.1 ± 0.4	17.1 ± 0.4
Soluble CP, % of CP	34.1 ± 3.1	36.6 ± 3.4
RUP, % of CP	35.9 ± 1.9	34.8 ± 2.3
RDP, % of CP	64.1 ± 1.9	65.2 ± 2.3
NEL, Mcal/kg	1.69 ± 0.08	1.68 ± 0.09
NDF, %	30.5 ± 1.3	30.4 ± 2.0
ADF, %	19.9 ± 0.8	20.0 ± 1.0
Forage NDF, %	23.1 ± 1.1	23.4 ± 1.2
NFC, %	39.8 ± 2.2	40.1 ± 2.4
TDN, %	72.9 ± 2.5	72.6 ± 2.7
Starch, %	25.3 ± 2.2	26.2 ± 2.0
Sugar, %	3.5 ± 0.8	3.2 ± 0.8
Fat, %	4.8 ± 0.7	4.9 ±0.8
Ca, %	1.0 ± 0.1	1.0 ±0.1
P, %	0.4 ± 0.02	0.4 ± 0.02
Na, %	0.4 ± 0.1	0.4 ± 0.1
K, %	1.4 ± 0.1	1.4 ± 0.1
Sulfur, %	0.2 ±0.02	0.2 ± 0.02

[1] Values are mean ± standard deviation of 32 diets used during the trial.

Table 3. Chemical composition of Control (CON) and Optigen® (OPT) TMR samples[1] analyzed at Dairy One Labs (Ithaca, NY).

	CON	OPT
Nutrient	**% of DM**	
DM, %	50.5 ± 3.9	50.8 ± 5.0
CP, %	18.2 ± 0.9	18.4 ± 0.7
Soluble CP, % of CP	49.8 ± 7.0	52.5 ± 5.3
NEL, Mcal/kg	1.72 ± 0.04	1.69 ± 0.06
NDF, %	33.6 ± 1.8	35.4 ± 2.8
ADF, %	22.7 ± 2.1	23.6 ± 2.9
NFC, %	37.4 ± 2.1	34.7 ± 2.9
TDN, %	72.6 ± 1.4	71.4 ± 1.9
Starch, %	23.0 ± 2.9	22.6 ± 4.6
Sugar, %	2.9 ± 0.7	3.3 ± 0.8
Fat, %	5.6 ± 0.7	6.0 ± 1.0
Ca, %	1.0 ± 0.1	0.9 ± 0.1
P, %	0.4 ± 0.03	0.4 ± 0.04
Na, %	0.4 ± 0.1	0.3 ± 0.1
K, %	1.5 ± 0.2	1.5 ± 0.3
Sulfur, %	0.2 ± 0.1	0.2 ± 0.1

[1] Values are mean ± standard deviation of 32 TMR samples.

Table 4. Chemical composition of Control (CON) and Optigen® (OPT) TMR samples[1] analyzed at Dairyland Labs (Arcadia, WI).

	CON	OPT
Nutrient	**% of DM**	
CP, %	17.9 ± 0.8	18.1 ± 0.6
NEL, Mcal/kg	1.65 ± 0.04	1.64 ± 0.04
NDF, %[2]	28.6 ± 2.1	29.2 ± 2.0
NFC, %	41.5 ± 2.5	40.4 ± 2.0
TDN, %	71.8 ± 1.4	71.4 ± 1.4
Fat, %	5.2 ± 0.5	5.4 ± 0.6

[1] Values are mean ± standard deviation of 32 TMR samples.

[2] Analyzed utilizing Na_2SO_3 in the procedure.

Table 5. Nutrient and chemical composition of Control (CON) and Optigen® (OPT) TMR diets[1] when space was filled with corn silage.

	CON	OPT
Item	**% of DM**	
Forage	55.2	55.7
Corn Silage	22.6	24.8
Alfalfa Silage	28.3	26.6
Other Forage[2]	4.3	4.3
Concentrate	44.8	44.3
Corn Grain Ground	7.5	7.6
High Moisture Corn	14.9	15.0
Soybean Meal 48%	3.7	2.0
Other Plant Protein[3]	4.4	4.6
Animal Protein[4]	0.9	1.0
High Fiber By-products[5]	8.6	9.0
Min/Vit/Additive Mix[6]	4.6	5.2
Nutrients, % of DM[7]		
DM, %[8]	50.2 ± 5.3	53.6 ± 5.6
CP, %[9]	17.6 ± 0.9	18.2 ± 0.6
Soluble CP, % of CP[8]	47.5 ± 6.6	52.5 ± 5.6
NEL, Mcal/kg[10]	1.65 ± 0.02	1.65 ± 0.02
NDF, %[10]	28.1 ± 1.7	28.5 ± 1.4
NFC, %[10]	42.3 ± 2.0	41.0 ±1.5
TDN, %[8]	72.9 ± 1.7	71.8 ± 1.7
Starch, %[8]	24.1 ± 2.9	24.9 ± 2.7

[1] Values are means of the 16 diets used during the trial. OPT diet contained 114 grams (0.25 oz) of Optigen®.

[2, 3, 4, 5, 6] See content of ingredients on footnotes from Table number 2.

[7] Values are mean ± standard deviation of 16 diets used during the trial.

[8] Values are mean ± standard deviation from Dairy One Lab analysis (Ithaca, NY).

[9] Values are mean ± standard deviation (Avg. between Dairy One Lab and Dairyland Lab).

[10] Values are mean ± standard deviation from Dairylan Labs (Arcadia, WI).

Table 6. Nutrient and chemical composition of Control (CON) and Optigen® (OPT) TMR diets[1] when space was filled with high moisture shelled corn.

	CON	OPT
Item	**% of DM**	
Forage	54.6	55.2
Corn Silage	20.5	20.5
Alfalfa Silage	33.0	33.6
Other Forage[2]	1.1	1.1
Concentrate	45.4	44.8
Corn Grain Ground	7.9	0.7
High Moisture Corn	17.2	24.2
Soybean Meal 48%	1.6	0.3
Other Plant Protein[3]	2.2	1.4
Animal Protein[4]	0.2	0.2
High Fiber By-products[5]	10.0	10.4
Min/Vit/Additive Mix[6]	6.4	7.5
Nutrients, % of DM[7]		
DM, %[8]	51.2 ± 2.5	48.8 ± 1.3
CP, %[9]	18.5 ± 0.6	18.7 ± 0.5
Soluble CP, % of CP[8]	52.3 ± 7.8	58.0 ± 4.4
NEL, Mcal/kg[10]	1.65 ± 0.04	1.64 ± 0.04
NDF, %[10]	28.6 ± 2.1	29.2 ± 2.0
NFC, %[10]	41.5 ± 2.5	40.4 ± 2.0
TDN, %[8]	72.0 ± 1.0	70.3 ± 3.5
Starch, %[8]	22.3 ± 3.4	20.8 ± 1.1

[1] Values are means of the 6 diets used during the trial. OPT diet contained 114 grams (0.25 oz) of Optigen®.

[2, 3, 4, 5, 6] See content of ingredients on footnotes from Table number 2.

[7] Values are mean ± standard deviation of 6 diets used during the trial.

[8] Values are mean ± standard deviation from Dairy One Lab analysis (Ithaca, NY).

[9] Values are mean ± standard deviation (Avg. between Dairy One Lab and Dairyland Lab).

[10] Values are mean ± standard deviation from Dairylan Labs (Arcadia, WI).

Table 7. Nutrient and chemical composition of Control (CON) and Optigen® (OPT) TMR diets[1] when space was filled with corn grain ground.

	OPT	CON
Item	**% of DM**	
Forage	56.4	56.8
Corn Silage	25.5	25.5
Alfalfa Silage	24.4	24.7
Other Forage[2]	6.4	6.7
Concentrate	43.6	43.2
Corn Grain Ground	12.1	14.6
High Moisture Corn	8.3	8.2
Soybean Meal 48%	4.4	4.2
Other Plant Protein[3]	2.9	2.9
Animal Protein[4]	1.0	1.0
High Fiber By-products[5]	10.3	8.9
Min/Vit/Additive Mix[6]	4.6	5.4
Nutrients, % of DM[7]		
DM, %[8]	50.7 ± 1.6	47.3 ± 2.6
CP, %[9]	18.6 ± 0.5	17.9 ± 0.8
Soluble CP, % of CP[8]	52.2 ± 7.0	50.8 ± 3.7
NEL, Mcal/kg[10]	1.63 ± 0.06	1.64 ± 0.05
NDF, %[10]	30.0 ± 2.6	30.0 ± 1.9
NFC, %[10]	39.3 ± 2.4	39.5 ± 2.3
TDN, %[8]	72.2 ± 0.8	72.0 ± 1.4
Starch, %[8]	21.2 ± 2.5	22.7 ± 1.3

[1] Values are means of the 10 diets used during the trial. OPT diet contained 114 grams (0.25 oz) of Optigen®.

[2, 3, 4, 5, 6] See content of ingredients on footnotes from Table number 2.

[7] Values are mean ± standard deviation of 10 diets used during the trial.

[8] Values are mean ± standard deviation from Dairy One Lab analysis (Ithaca, NY).

[9] Values are mean ± standard deviation (Avg. between Dairy One Lab and Dairyland Lab).

[10] Values are mean ± standard deviation from Dairyland Labs (Arcadia, WI).

Table 8. Mean and variation for main forages incorporated into Control and Treatment TMR and comparison with NRC (2001) tabular values.

	DM, %		CP, %		ADF, % of DM		NDF, % of DM		NEL, Mcal/kg	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Control										
Hay										
Legume	82.8	1.4	18.8	0.6	32.2	2.5	42.8	3.6	1.37	0.02
Silage										
Alfalfa	50.7	5.8	19.9	2.2	31.8	2.3	41.3	2.4	1.39	0.03
Corn	36.3	3.5	7.4	0.6	24.1	2.1	40.2	2.7	1.52	0.04
HMSC	74.6	1.5	9.4	0.4	3.6	0.2	11.7	0.4	1.94	0.03
Optigen®										
Hay										
Legume	83.1	1.1	19.4	0.5	33.4	2.0	43.6	2.9	1.37	0.02
Silage										
Alfalfa	50.5	5.3	19.6	2.2	33.5	4.9	42.8	4.4	1.34	0.06
Corn	36.6	3.7	7.3	0.4	24.2	2.2	40.3	2.9	1.50	0.04
HMSC[1]	76.3	4.2	9.3	0.7	3.4	0.5	11.7	0.6	1.98	0.01
NRC (2001)										
Hay										
Legume[2]	83.9	3.2	20.8	2.3	33.4	2.0	42.9	1.2	1.28	...
Silage										
Alfalfa[3]	42.9	1.0	21.9	1.8	35.2	2.1	43.2	1.5	1.22	...
Corn[4]	35.1	...	8.8	1.2	28.1	3.3	45.0	5.3	1.45	...
HMSC	71.8	...	9.2	0.7	3.6	1.6	10.3	2.7	1.90	...

[1] HMSC: High Moisture Shelled Corn.

[2] Mid maturity (40 – 46% NDF).

[3] Mid maturity (40 – 46% NDF).

[4] Normal (32 – 38% DM).

Table 9. Effect of diet reformulation with controlled-release urea (Optigen®) on milk yield and component yields, and milk composition.

	Control	Optigen®
Variable		
Dairy Farms, n	16	16
Cow average/farm, n	148	148

	Control	Optigen®	SEM	P-Value
Variable[1]				
Milk Yield, kg/d	35.4	35.9	0.2	< 0.01
Fat, %	3.72	3.69	0.02	0.07
Fat Yield, g/d	1317	1322	8	NS
Protein, %	2.98	2.97	0.01	NS
Protein Yield, g/d	1055	1065	6	0.13
MUN, mg/dl	12.4	13.2	0.3	< 0.01

[1] All data are least square means.

Figure 1. Trial protocol.

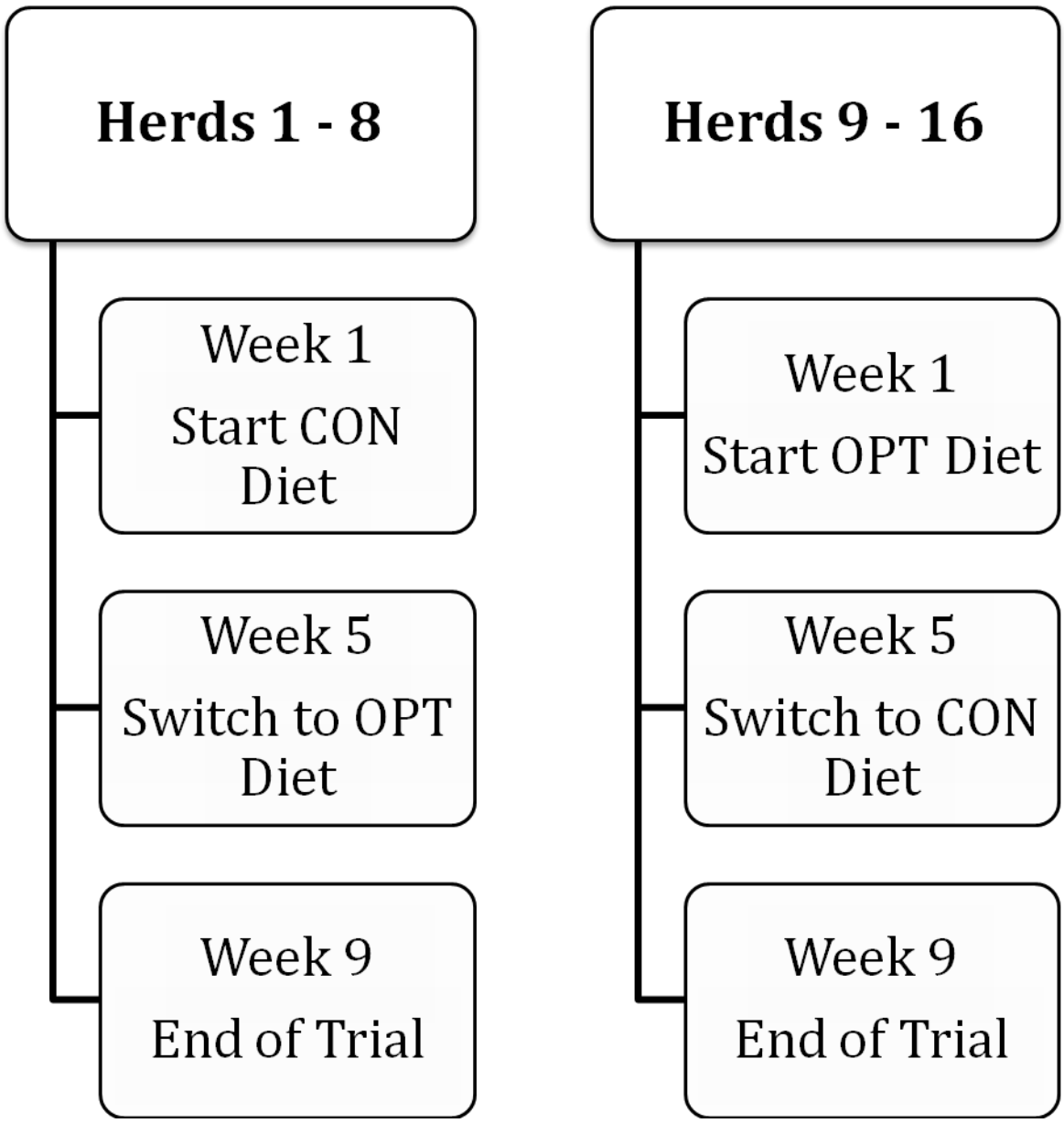


Figure 2. Calculated crude protein degradability using NRC (2001) and García Gonzalez *et al*. (2007).

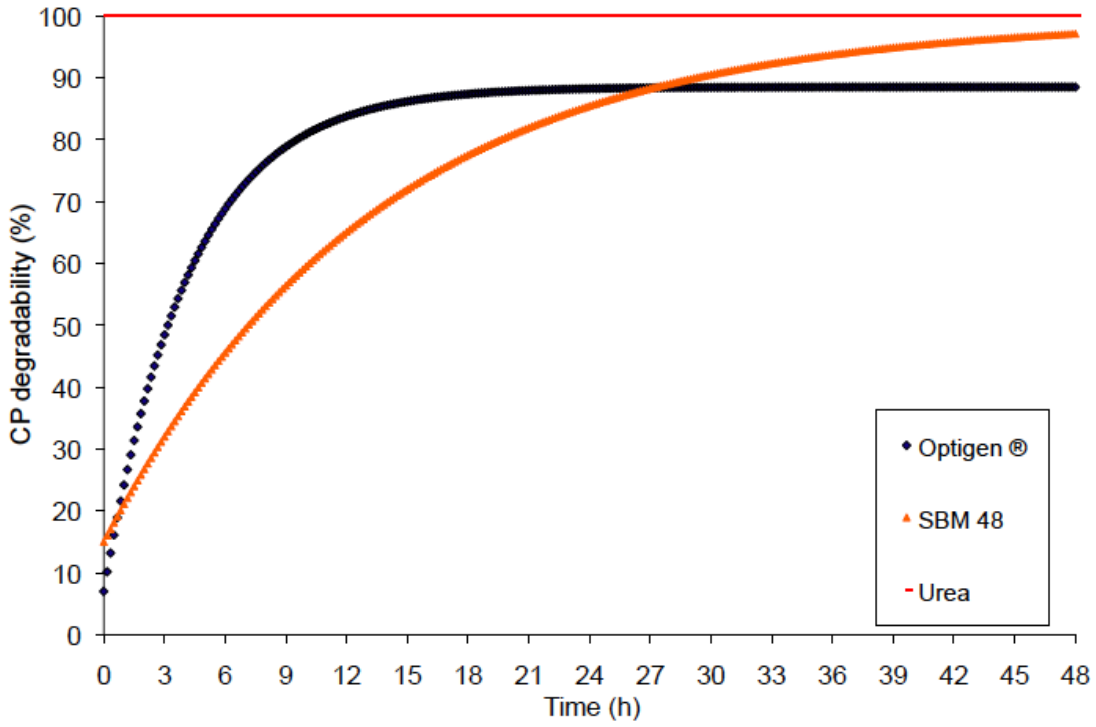


Figure 3. Protein degradation over time for Soybean meal and Optigen® (Palmer et al., 2007).

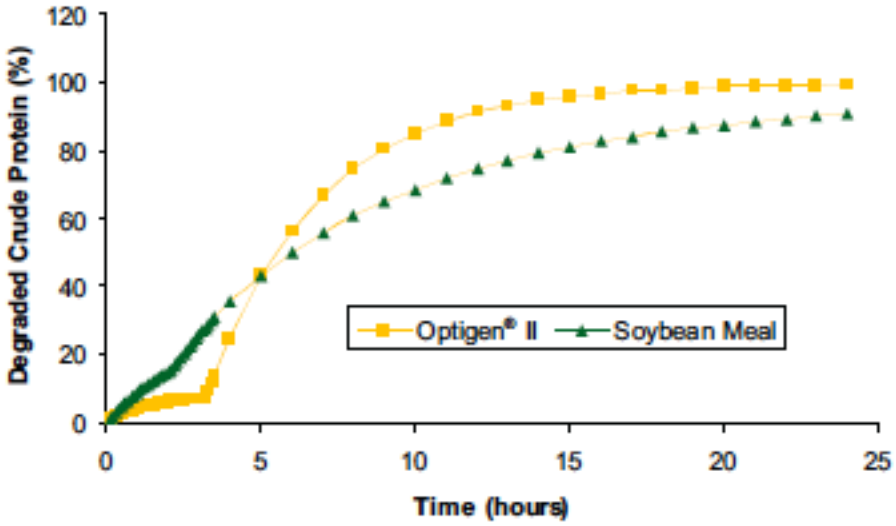


Figure 4. In situ nitrogen dissapearence (B) from diets containing soybean meal (SBM) or 150 g of Optigen® to replace a portion of SBM and fitted to Ørskov model (Ørskov and McDonald, 1979).

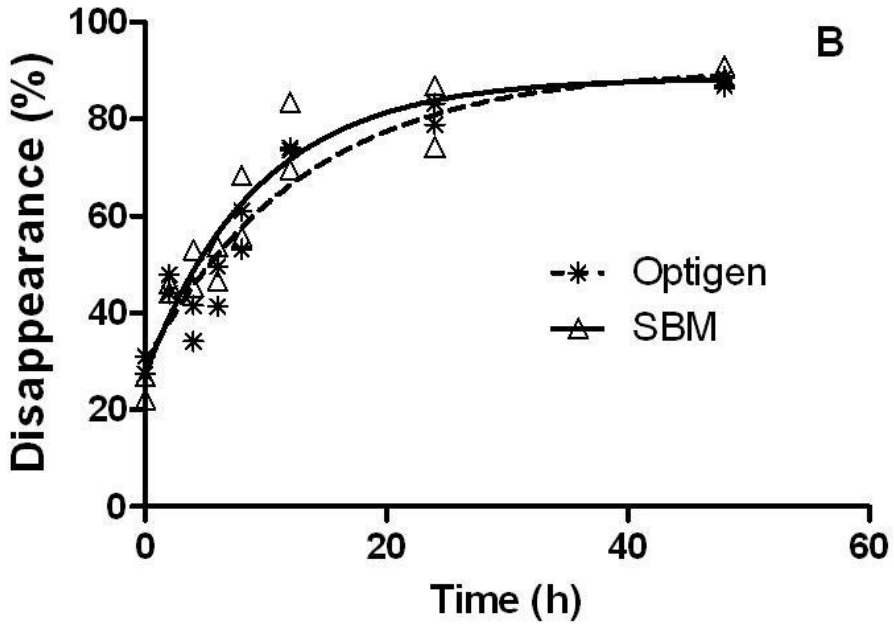
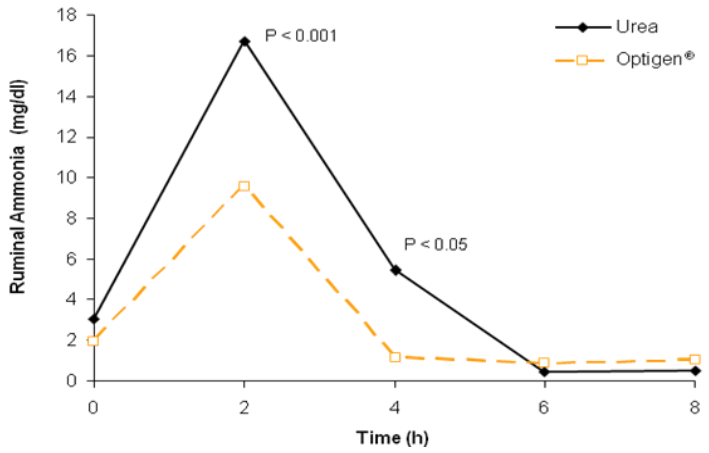


Figure 5. A) Ruminal ammonia concentrations (mg/dL) observed over the time when supplemental Optigen® or Urea were intra-ruminally supplied. B) Blood plasma ammonia concentrations (mg/dL) observed over the time when supplemental Optigen® or Urea were intra-ruminally supplied. Data presented with permission of García-González (2007).

A)



B)

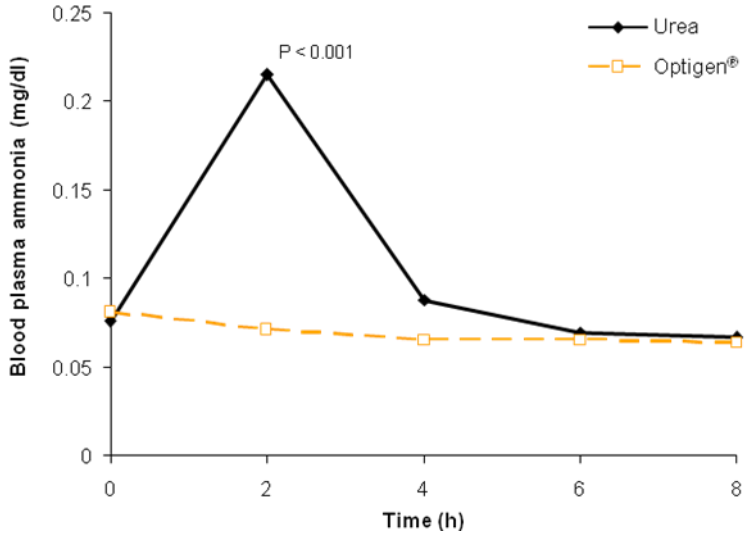
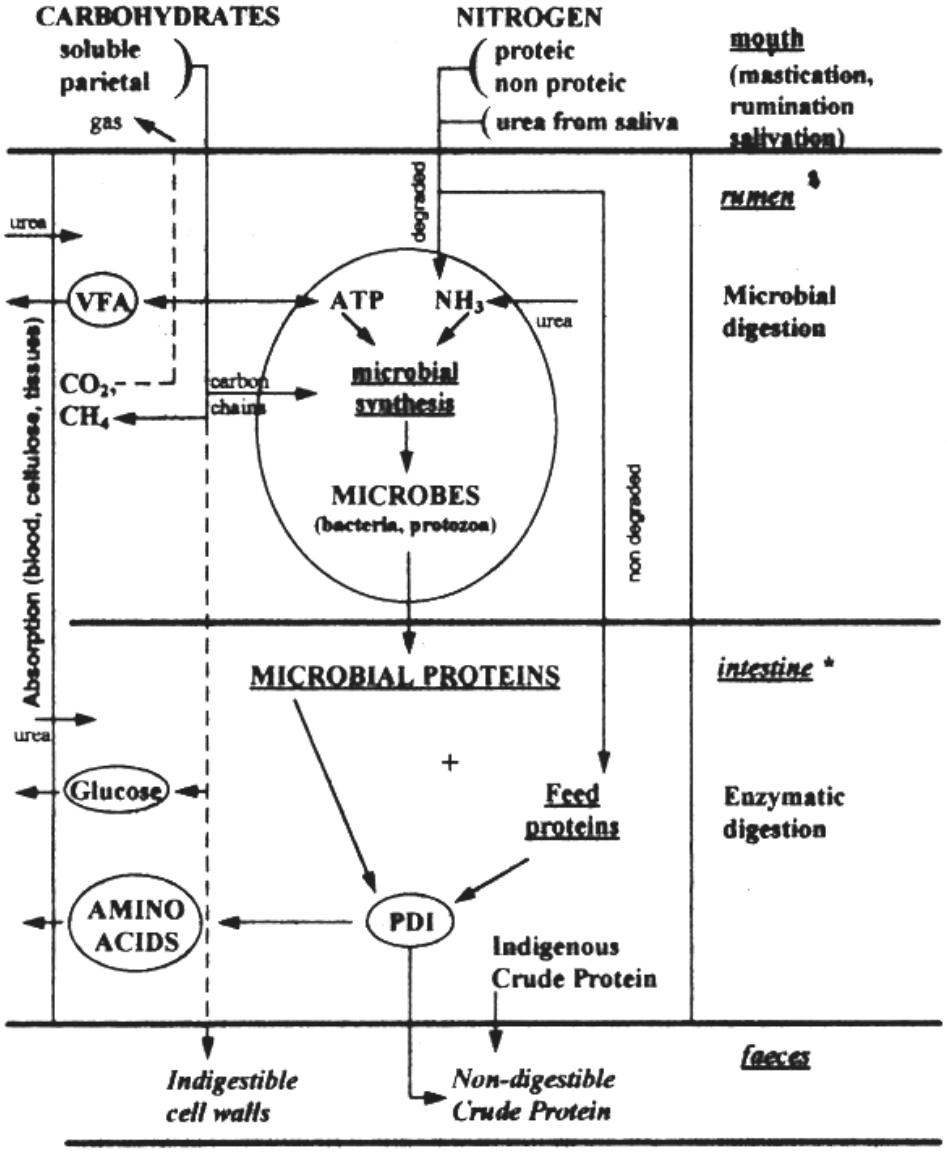


Figure 6. Simplified schematic diagram illustrating the digestive utilization of crude proteins by a ruminant (Adapted from FAO, 2009).



*The large intestine, which has not been shown for better clarity, hosts celluloytic bacteria (but not protozoa). This is where celluloytic fermentation and bacterial synthesis (the source of PIM) takes place, allowed by the urea in the blood (passing through the wall), the source of NH3, and by the few constituents that are still degradable (source of energy). There is no absorption of amino acids at this stage in the digestive tract. A small proportion of these microbial proteins are fermented into ammonia, which will join the overall ammonia pool, the major proportion that remains being excreted in the feces.

CHAPTER III

Evaluation of the economic impact of Optigen® use in commercial dairy herd diets with varying feed and milk prices

J. F. Inostroza[1], R. D. Shaver[1], V. E. Cabrera[1], and J. M. Tricárico[2]

University of Wisconsin, Madison 53706

Address correspondence to R.D. Shaver, University of Wisconsin-Madison, Department of Dairy Science, 1675 Observatory Drive, Room 280 Animal Sciences Building, Madison, WI 52706-1284; (608) 263-3497; rdshaver@wisc.edu.

[1]Department of Dairy Science, University of Wisconsin, Madison, WI, USA

[2]Alltech Inc., Brookings, SD, US

ABSTRACT

The objective of this study was to evaluate the impact of Optigen® (controlled-release urea) use in commercial dairy herd diets on feed cost and income over feed cost. Results from a field trial with 16 Wisconsin dairy herds randomly assigned to treatment sequences of either Optigen® **(**OPT; 114 g/cow/d replacing an equivalent amount of supplemental CP to provide iso-nitrogenous TMR; TMR formulation space created by the use of OPT was filled with either dry corn, corn silage or high moisture corn DM) to control (CON) or CON to OPT in a cross-over design with two 30-d feeding periods was used in the economic analysis.

Milk yield in the field trial was 0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON; data were analyzed using the mixed model procedure of SAS with period, sequence and treatment as fixed effects and herd as a random effect. An economic simulation analysis was performed using the OPT feeding rate and milk yield response from the field trial and monthly soybean meal-48 ($0.373 ± 0.054/kg), dry corn ($0.188 ± 0.020/kg), corn silage ($0.059 ± 0.005/kg), and high-moisture corn ($0.149 ± 0.016/kg) prices (as-fed basis) and milk prices ($0.38 ± 0.03/kg) for the period January through December, 2008. The cost of OPT was set at $1.63/kg. Thirty-two combinations of varying feed and milk prices were simulated. Under the conditions of the simulations performed in this study, OPT reduced feed cost only when corn silage was used to fill formulation space while milk income minus feed cost was increased by OPT for all scenarios.

A decision-tool spreadsheet was developed to allow for further economic simulation analyses with the ability to vary the milk yield response to OPT, the cost of OPT, and the CP and energy supplements evaluated.

Key Words: Controlled-release urea, economics, feed cost, dairy cows

INTRODUCTION

Costs of ingredients and nutrients utilized for the production of milk historically have comprised over 50% of the total costs of milk production. In recent years, ratios of milk/feed prices have declined and at present, feed costs may account for 60%, or more, of total costs of milk production, hence, it is important that correct decisions are made to maximize returns on supplemental feed expenses. Changes in prices and milk production related to feed supplements are specially important, because they impact directly on economic profitability of dairy farms (Cabrera et al., 2009). Although an additive may be beneficial in production research studies, it also needs to be economical. There is a need for tools to evaluate cost benefit of using feed additives on dairy farms. To measure the economic impact of Optigen® we designed a user-friendly Excel application to evaluate Income Over Feed Cost (IOFC), which is a major determinant of profits or losses in a dairy production enterprise. The IOFC is a function of milk price and yield, value of replaced feeds, and additive costs.

One of the more expensive components in a dairy cow ration is crude protein (CP). As a result, urea may be used in dairy rations as a less expensive alternative to rumen degradable protein (RDP) from plant origin, such as soybean meal or cottonseed meal (Akay et al., 2004). Urea can only be used as a source of nitrogen when there is an adequate supply of readily fermentable carbohydrate available to synthesize bacterial protein (Nocek and Russell, 1988).

Urea can be utilized in ruminant diets, because it is hydrolyzed to ammonia in the rumen and can be incorporated by microbes into amino acids and bacterial protein that are subsequently utilized by the host animal (Huntington and Archibeque, 2000). While the objective of using Optigen® to better synchronize nitrogen and carbohydrate availability in the rumen continues to be researched, its use may have a practical benefit in dairy cattle rations. Siciliano-Jones (2005) suggested that a slow-release urea can replace some soybean meal protein to meet RDP requirements. The result of this replacement is a net gain of formulation space available for feeding more forage or grain dry matter.

Since feed costs can represent as much as 60% of total costs of producing milk on a dairy farm, feed costs savings, even a few dollars per ton, add up to significant changes over a year on dairy farms, especially large scale farms (Bethard, 1998). Therefore, a complete farm budget is not needed to determine the profitability of these specific changes in the operation of the farm. The analysis could be accomplished by using a partial budget (PB), which means that only the relevant costs and incomes are included in the analysis. Partial budgets can be used to analyze practical management decisions, such as adopting new technologies or purchasing new equipment, facilities, and machinery. Since PB is best adapted to small changes in the business and indicate that the change will increase, decrease, or not change the net income, it could be used as part of the analysis for modifying feeding management and complement an IOFC analysis.

The IOFC is a common measure of performance of a feeding program and is a function of milk price and yield and feed costs (Adkinson et al., 1993). Simpler models to measure the impact of diet changes due to the inclusion of new ingredients or additives could be developed that provide solutions to dairy farmers, nutritionists and consultants sufficiently similar to solutions from more complex models. A decision-tool spreadsheet could allow for further economic simulation analyses with the ability to vary the milk yield response to OPT, the cost of OPT, milk price, and the CP and energy supplement costs evaluated.

The objectives of this study were to evaluate the impact of Optigen® (controlled-release urea) use in commercial dairy herd diets on feed cost and income over feed cost in commercial Wisconsin dairy herds, and to develop a user-friendly Excel spreadsheet decision tool to evaluate IOFC.

MATERIALS AND METHODS

An economic simulation analysis was performed using the OPT feeding rate and milk yield response from our field trial (refer to Chapter II) and monthly soybean meal-48 ($0.373 ± 0.054/kg), dry corn ($0.188 ± 0.020/kg), corn silage ($0.059 ± 0.005/kg), and high-moisture corn ($0.149 ± 0.016/kg) prices (as-fed basis) and milk prices ($0.38 ± 0.03/kg) for the period January through December, 2008 (**Table 1**). The cost of OPT was set at $1.63/kg. Thirty-two combinations of varying feed and milk prices were simulated in a user-friendly Excel spreadsheet (Optigen® Evaluator) designed to help dairy producers, nutritionists, consultant and extension agents evaluate the Income Over Feed Costs of supplementing Optigen®. These combinations varied depending on amount and costs of ingredients used to perform reformulations that involved soybean meal, corn silage, high moisture corn, and corn grain. The price of milk and the price and amount of Optigen® fed were also important factors in these calculations. Instructions for the proper use of the Optigen® Evaluator decision tool are presented in **Table 2**. These instructions were a modification of the Income over Feed Supplement Cost analysis proposed by Cabrera et al. (2009). National Research Council (2001) tables were used as a reference feedstuff nutrient composition (DM % and CP%). Soybean meal (SBM) was chosen as the protein supplement for partial replacement in the diet simulations by Optigen®, because they have been shown to have similar ruminal degradation curves (García-González et al., 2007, Palmer et al., 2007). Other protein supplements that could be partially replaced by Optigen® in dairy cattle diets are presented in **Table 3**.

RESULTS AND DISCUSSION

Least squares means for milk production composition are provided in **Table 4**. Milk yield was 0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON **(Refer to Chapter II)**. Under the conditions of the simulations performed in this study, OPT reduced feed cost only when corn silage was used to fill formulation space while milk income minus feed cost was increased by OPT for all scenarios. A decision tool spreadsheet was developed to allow for further economic simulation analyses with the ability to vary the milk yield response to OPT, the cost of OPT, and the CP and energy supplements evaluated. Results of the economic analysis using the decision tool are presented in **Table 5**.

Milk income minus feed cost was greater in all the diets that contained Optigen®. This was especially evident when the diet formulation space created by use of the product was filled with corn silage, where feed cost was reduced and milk income minus feed cost was $ 0.21 ± 0.051/cow/day greater. Change in nutrient composition of diets during trial period March to June 2008 after reformulation with Optigen® and IOFC when corn silage was utilized to fill formulation space are presented in **Table 6**. When diet formulation space was filled with high-moisture corn, cost benefit to use the product was $ 0.15 ± 0.040/cow/day, and when dry corn grain was used the cost benefit was $ 0.015 ± 0.039/cow/day. The lower cost-benefit when high-moisture corn and dry corn grain were used to fill the formulation space was due to a higher feed cost compared with corn silage, which had a feed cost (OPT-CON) of $ -0.020 ± 0.039.

Optigen® effects on milk yield (+0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON) could be due to changes in populations or functions of ruminal microbial species, their interrelationships with the extra energy supplements, and subsequent effects on microbial efficiency and growth (Akay et al., 2004). Logically, if more dietary nitrogen can be captured by ruminal microorganisms, the efficiency with which dietary nitrogen is captured in milk should increase (Chalupa, 2007).

A study conducted by Varga and Ishler (2008) reported that Optigen® reduced diet costs when partially replacing soybean meal and increasing forage while maintaining the supply of metabolizable protein and milk yield. They also reported that Optigen® numerically increased milk yield by 1.2 kg/d ($P<0.11$). Urea, an NPN compound, can be relatively inexpensive per unit of crude protein equivalents (CPE) compared with true protein supplements, such as soybean meal. Urea can be utilized in ruminant rations because it is hydrolyzed to ammonia in the rumen and can be incorporated by microbes into microbial protein, which can be subsequently digested and absorbed post-ruminally, thereby serving as a source of amino acids for the host animal (Galo et al., 2003). Depending on protein supplement prices, the use of Optigen® may help lower feed costs. In addition, by increasing the CPE density of dietary protein supplements space was created in formulations that afforded nutritionists an opportunity for manipulating dietary carbohydrate fractions.

Comparisons of RDP and RUP prices between different protein sources, urea and Optigen® are presented in **Table 7**. To obtain values of RDP ($/kg), RDP ($/kg), and RUP digestible ($/kg), formulas were adapted from Bethard (1998) to express values in metric system:

(1) % CP x (% RDP ÷ 100) = RDP as a % of DM

(2) (% RDP of DM ÷ 100) x (% DM x 908 kg/ton) = kg RDP/ton as-fed

(3) $/ton ÷ kg RDP/ton as fed = $/kg RDP

(4) Same steps to calculate RUP.

The method described considered RUP digestibility but not palatability or quality. With current (July, 2009) ingredient market prices, Optigen® had a higher price ($2013/ton) compared with SBM ($480/ton) and other protein sources, however was one of the cheapest sources of RDP ($0.76/kg) compared with SBM ($1.92/kg) due to its density (high DM, CP and RDP percentages). Considering nutritional aspects, prices and cow performance, Optigen® can be a partial substitute for SBM or other potential RDP supplying ingredients depending on market prices.

CONLUSIONS

Optigen® increased milk yield 0.5 kg/cow/d ($P < 0.01$) in a field trial on commercial WI dairy farms. Use of Optigen® decreased feed cost only when diet formulation space was filled with corn silage in the simulation analysis. The simulation analysis revealed that Optigen® increased IOFC by up to \$0.21 ± 0.051/cow/d. Depending on feedstuff prices, milk price, additive price and the amount of additive used, Optigen® may lower feed costs when formulation space is filled with forage and can increase IOFC, but the economics depends greatly on the aforementioned factors and inter-relationships. The Optigen® Evaluator tool can be used to evaluate the impact of fluctuating market price scenarios worldwide on the economics of Optigen® use in dairy cattle diets.

ACKNOWLEDGMENTS

We would like to thank Alltech Inc., for providing partial financial support for this research trial. Appreciation is extended to CP Feeds and Badgerland Nutrition Inc. staff Todd LeNoble and Jeniffer Meyer, and also to Vita Plus Corporation staff Spence Driver, John Weinkes, Rod Sjoerdsma, Randy Greenfield and Irv Haugen, and Mike Yahr of West Bend Elevator Inc. for their assistance in selection of the dairy farms that participated in our trial and their work on ration reformulations. The authors would also like to thank the dairy farmers of Wisconsin who were willing to participate in this field trial.

REFERENCES

Adkinson, R. W., W. S. Farmer, and B. F. Jenny. 1993. Feeding Practices and Income Over Feed Cost on Pasture-Oriented Dairy Farms in Louisiana. J. Dairy Sci. 76(11):3547-3554.

Akay, V., J. Tikofsky, C. Holtz, and K. A. Dawson. 2004. Optigen® 1200: controlled release of non-protein nitrogen in the rumen. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 20th Annual Symposium: re-imagining the feed industry. T. P. Lyons and K. A. Jacques, ed. Alltech UK, Lexington, Kentucky, USA.

Bethard, G. 1998. Economic evaluation of dairy feeds. Pages 1-8. College of Agriculture and Home Economics, New Mexico State University, Las Cruces, NM.

Cabrera, V. E., R. D. Shaver, and M. A. Wattiaux. 2009. Optimizing Income Over Feed Supplement Costs. Pages 116 - 120 in 4-State Dairy Nutrition & Management Conference, Dubuque, Iowa, USA.

Chalupa, W. 2007. Precision Feeding of Nitrigen to Lactating Dairy Cows: A Role for Optigen II. Pages 221-226 in Nutritional Biotechnology in the Feed and Food Industries. Vol. 23. T. P. Lyons, K. A. Jaques, and J. M. Hower, ed.

Galo, E., S. M. Emanuele, C. J. Sniffen, J. H. White, and J. R. Knapp. 2003. Effects of a Polymer-Coated Urea Product on Nitrogen Metabolism in Lactating Holstein Dairy Cattle. J. Dairy Sci. 86(6):2154-2162.

García-González, R., J. M. Tricárico, G. A. Harrison, M. D. Meyer, K. M. McLead, D. L. Harmon, and K. A. Dawson. 2007. Optigen® is a Sustained Release of Non-Protein Nitrogen in the Rumen. Dep. of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA.

Gould, B. W. 2009. Understanding Dairy Markets. Agricultural and Applied Economics, UW Madison, Madison, WI.

Huntington, G. and S. Archibeque. 2000. Practical aspects of urea and ammonia metabolism in ruminants. Journal of Animal Science 77(E-Suppl).

Nocek, J. E. and J. B. Russell. 1988. Protein and Energy as an Integrated System. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. J. Dairy Sci. 71(8):2070-2107.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed, Natl. Acad. Sci. Wasington, DC.

Palmer, M., D. Wilde, and R. Fawcett. 2007. A Comparison of Protein Degradation profile of Soybean Meal and Slow Release Nitrogen Source. Alltech UK Ltd, Lincolnshire, UK.

Siciliano-Jones, J., Downer, J. 2005. Utility and safety of a slow-release nitrogen product: Optigen® 1200. in Nutritional biotechnology in the feed and food industries. Proceedings of Alltech's 21st Annual Symposium. T. P. Lyons, Jacques, K. A., ed. Alltech UK, Lexington, KY, USA.

Varga, G. A. and V. Ishler. 2008. Effects of Optigen® on Milk Production, N Balance and Diet Cost in High Producing Cows. The Pennsilvania State University, University Park, PA. USA.

Table 1. Feedstuffs prices period January to December 2008.

	Soybean Meal[1]	Corn Silage[2]	Corn Grain[3]	Corn[2]	Milk[3]
	Solvent, 48%	32–38% DM	Ground Dry	HMSC	Class III
	$/kg As Fed	$/kg As Fed	$/kg As Fed	$/kg As Fed	$/kg
Jan-2008	0.350	0.052	0.156	0.124	0.43
Feb-2008	0.399	0.056	0.178	0.141	0.37
Mar-2008	0.398	0.058	0.185	0.146	0.40
Apr-2008	0.387	0.062	0.202	0.160	0.37
May-2008	0.372	0.063	0.207	0.164	0.40
Jun-2008	0.475	0.065	0.215	0.170	0.45
Jul-2008	0.411	0.063	0.206	0.163	0.40
Aug-2008	0.389	0.063	0.207	0.163	0.38
Sep-2008	0.393	0.061	0.197	0.156	0.36
Oct-2008	0.277	0.055	0.172	0.136	0.38
Nov-2008	0.299	0.054	0.167	0.132	0.34
Dec-2008	0.320	0.053	0.161	0.127	0.34
Average	0.373	0.059	0.188	0.149	0.38
SD	0.054	0.005	0.020	0.016	0.032

[1] Feedstuffs magazine, period January to December 2008.

[2] Values of corn silage and HMSC were calculated on a corn grain ($/bushel) base.

[3] Gould (2009).

Table 2. Instructions to use the IOFC spreadsheet (Optigen® Evaluator). Adapted from Cabrera et al. (2009) Income over feed supplements costs recommendations.

SECTIONS	RECOMMENDATIONS
I – Set protein source (See Figure 1)	In this section you need to input the DM%, CP% and price ($/kg) as fed (AF) of the protein feedstuffs to be used. Initial necessary data of composition of feeds (DM% and CP%) comes from NRC (2001) table number 15-1. Cells marked in yellow are input data that can be overwritten as desired. Main protein sources considered relevant to be included in dairy cows diets for worldwide conditions are presented.
II – Set energy source (See Figure 2)	In this section you need to input the DM%, CP% and price ($/kg) as fed (AF) of the energy feedstuffs to be used. Initial necessary data of composition of feeds (DM% and CP%) comes from NRC (2001) tables number 15-1 and 15-2a. Cells marked in yellow are input data that can be overwritten as desired. Main energy sources considered relevant to be included in dairy cows diets for worldwide conditions are presented.
III – Optigen® Evaluator (See Figure 3)	This section is divided in sub-sections for better understanding.

Table 2. Continued...

SUB-SECTIONS	RECOMMENDATIONS
1 – Input data	Cells marked in yellow are input data that can be overwritten as desired.
1.1 – Optigen®	Set Optigen® amount (kg/cow/d) and price ($/kg).
1.2 – Select a source of protein to be ***replaced***	Use drop box menus to select protein ingredients previously updated in Section I. Potential feedstuffs to be replaced like Soybean Meal, Canola meal, Sunflower Meal, and Cottonseed Meal are included.
1.3 – Select a source of energy to ***add*** to diet	Use drop box menus to select energy ingredients previously updated in Section II. Potential feedstuffs to be added like Corn Silage, High Moisture Corn, and Corn Grain Ground are included.
1.4 – Milk Increase/Decrease assumption	Use right/left arrows to adjust the amount of milk (kg/cow/d) assumed to be increased because the use of Optigen® and reformulation. Maximum amount is adjusted up to 500 cc and minimum of 0 cc.
1.5 – Milk Price	Use right/left arrows to adjust milk price ($/cwt) according to market.
2 – Analysis (Output data – Results)	Output data were to see results, but you are not allowed to change because they may include formulas.
2.1 – Optigen®	Amount (kg DM) and value ($/cow/d) because of adding Optigen® to the diet.
2.2 – Source of protein	Amount (kg DM) and value ($/cow/d) because of source of protein replaced.
2.3 – Source of energy	Amount (kg DM) and value ($/cow/d) because of source of energy replaced.
2.4 – Value of change in milk production	Value associated to milk increase/decrease (1.4) and milk price (1.5).
2.5 – Value of Using Optigen®	Cost benefit because of reformulation.

Table 3. Potential protein sources to be partially substituted by Optigen® in dairy cow rations.

Protein Source	CP[1]	RDP	RUP	RUP digest[1]	Met[1]	Lys[1]
	%	% of CP	% of CP	%	% of CP	% of CP
Soybean Meal, 48%	53.8	65	35	93	1.44	6.29
Canola Meal	37.8	64	36	75	1.87	5.62
Linseed Meal	32.6	47	53	85	1.76	3.69
Cottonseed Meal	44.9	58	42	92	1.59	4.13
Sunflower Meal, w/hulls	28.4	84	16	90	2.29	3.56

[1] Mean values from NRC (2001). Table 15-2a.

Table 4. Effect of diet reformulation with control-release urea (Optigen®) on milk, milk components, and MUN.

	Control	Optigen®		
Variable				
Dairy Farms, n	16	16		
Cow average/farm, n	148	148		
	Control	**Optigen®**	**SEM**	**P-Value**
Variable[1]				
Milk Yield, kg/d	35.4	35.9	0.2	< 0.01
Fat, %	3.72	3.69	0.02	0.07
Fat Yield, g/d	1317	1322	8	NS
Protein, %	2.98	2.97	0.01	NS
Protein Yield, g/d	1055	1065	6	0.13
MUN, mg/dl	12.4	13.2	0.3	< 0.01

[1] All data are least square means.

Table 5. Economic impact of Optigen® use in dairy herd diets.

CP Supplement Replaced by OPT	Ingredient Used to Fill Formulation Space	Feed Cost OPT - CON ($/cow/day)	Milk Income OPT – CON ($/cow/day)	Milk Income - Feed Cost ($/cow/day)
SBM-48	Dry Corn	0.047 (± 0.027)	0.192 (± 0.016)	0.15 (± 0.039)
SBM-48	Corn Silage	-0.020 (± 0.039)	0.192 (± 0.016)	0.21 (± 0.051)
SBM-48	HM Corn	0.042 (± 0.028)	0.192 (± 0.016)	0.15 (± 0.040)

Table 6. Change in nutrients composition of diets during trial period (March to June 2008) after reformulation with Optigen®, and IOFC when corn silage was utilized to fill formulation space.

	CON	OPT
DM, %[1]	50.5 ± 3.9	50.8 ± 5.0
CP, %[2]	17.9 ± 0.8	18.1 ± 0.6
Soluble Protein, % of CP[1]	49.8 ± 7.0	52.5 ± 5.3
RDP, % of CP[3]	64.1 ± 1.9	65.2 ± 2.3
RUP, % of CP[3]	35.9 ± 1.9	34.8 ± 2.3
NEL, Mcal/kg[2]	1.65 ± 0.04	1.64 ± 0.04
Forage, % of DM[3]	55.5 ± 3.1	56.0 ± 3.0
NDF, % of DM[2]	28.6 ± 2.1	29.2 ± 2.0
NFC, % of DM[2]	41.5 ± 2.5	40.4 ± 2.0
TDN, % of DM[2]	71.8 ± 1.4	71.4 ± 1.4
Starch, % of DM[1]	23.0 ± 2.9	22.6 ± 4.6
Milk Production, kg/d[4]	34.5	34.9
Milk Price Class III, $/cwt[5]	0.18	0.18
IOFC, $/cow/d[6]		0.21

[1] Dairy One Analysis (Ithaca, NY).
[2] Dairyland Labs (Arcadia, WI).
[3] Nutritionists ratio formulation.
[4] Assuming 0.5 kg/d/cow greater ($P < 0.01$) for OPT than for CON.
[5] Class III price from period March to June 2008 (Gould, 2009).
[6] Income Over Feed Cost from period March to June 2008.

Table 7. Comparisons on CP, RDP and RUP prices between different proteins sources, Optigen® and urea.

Protein Source	DM[1]	CP[1]	Ton[2]	RDP[3]	RUP[3]	RUP digest[3]
	%	%	$	$/kg	$/kg	$/kg
SBM, 48%	89.5	53.8	480	1.92	2.54	2.73
Canola Meal	90.3	37.8	330	1.67	2.97	2.78
Linseed Meal	90.3	32.6	275	2.20	1.77	2.29
Cottonseed Meal	90.5	44.9	330	1.53	2.12	2.30
Sunflower Meal	92.2	28.4	237	1.19	6.24	6.94
Urea	99.0	281	567	0.21	...	...
Optigen®	99.0	256	2013	0.76	...	...

[1] NRC (2001).

[2] Feedstuffs, July 2009.

[3] Values calculated using Bethard (1998) and NRC (2001) information.

Figure 1. Section I, protein feedstuffs (NRC, 2001) example.

SECTION I
PROTEINS FEEDSTUFFS (NRC,2001) 7th Revised Edition

Name	DM %	CP %	Price ($/k)
BLOODMeal, ring dried	90.2	95.5	
BLOODMeal, batch dried	90.2	95.5	0.550
BREWERS GRAINSDried	90.7	29.2	
BREWERS GRAINSWet	21.8	28.4	0.057
CANOLAMeal, mech. Extracted	90.3	37.8	0.298
CORN, YELLOWDistillers grains with solubles, dried	90.2	29.7	0.150
CORN, YELLOWGluten meal, dried	86.4	65.0	0.575
COTTON SEEDMeal, solvent, 41% CP	90.5	44.9	0.300
FISH BYPRODUCTSAnchovy, meal, mech.	92.0	71.2	
FISH BYPRODUCTSMenhaden, meal, mech.	91.2	68.5	0.890
LINSEED (FLAX)Meal, solvent	90.3	32.6	0.315
MEATMeal, rendered	93.9	57.6	
MEATMeat and bone, rendered	94.0	54.2	0.285
PEANUTMeal, solvent	92.3	51.8	
SAFFLOWERMeal, solvent	93.5	29.0	0.130
SOYBEANMeal, expellers, 45% CP	89.6	46.3	
SOYBEANMeal, solvent, 44% CP	89.1	49.9	0.335
SOYBEANMeal, solvent, 48% CP	89.5	53.8	0.408
SOYBEANSeeds, whole	90.0	39.2	
SOYBEANSeeds, whole roasted	91.0	43.0	0.450
SOYPLUS	90.0	42.5	0.370
SUNFLOWERMeal, solvent	92.2	28.4	0.180

Figure 2. Section II, energy feedstuffs (NRC, 2001) example.

SECTION II
ENERGY FEEDSTUFFS (NRC,2001) 7th Revised Edition

Name	DM %	CP %	Price ($/k)
BAKERY BYPRODUCTByproduct meal	84.7	12.5	0.150
BARLEYGrain, rolled	91.0	12.4	
CORN, YELLOWGrain, cracked, dry	88.1	9.4	
CORN, YELLOWGrain, ground, dry	88.1	9.4	0.166
CORN, YELLOWGrain, steam-flaked	88.1	9.4	
CORN, YELLOWGrain, rolled, high moisture	71.8	9.2	
CORN, YELLOWGrain, ground, high moisture	71.8	9.2	0.110
CORN, YELLOWShell, high moisture	70.0	9.1	0.116
CORN, YELLOWGrain and cob, dry, ground	89.2	8.6	
CORN, YELLOWGrain and cob, high moisture, ground	67.1	8.4	
CORN, YELLOWHominy	88.5	11.9	0.120
CORN, YELLOWSilage, normal 32-38% DM	35.1	8.8	0.061
FATS AND OILSTallow	99.8	0.0	
FATS AND OILSVegetable oil	100.0	0.0	
OATSGrain, rolled	90.0	13.2	0.109
POTATOByproduct meal	5.4	10.5	
RICEBran	90.6	15.5	0.140
SORGHUM, GRAIN TYPEGrain, dry rolled	88.6	11.6	
SORGHUM, GRAIN TYPEGrain, steam-flaked	88.6	11.6	
SUNFLOWEROil seeds, whole	91.8	19.2	
WHEATGrain, rolled	89.4	14.2	
WHEATMiddlings	89.5	18.5	0.130

Figure 3. Section III, Income Over Feed Cost example (Optigen® Evaluator).

SECTION III

Optigen® Evaluator

J.F. Inostroza, V.E. Cabrera, R.D. Shaver, J.M. Tricárico

1	***INPUT DATA***	As Fed kg/cow/d	Price $/kg
1.1	Optigen®	0.114	1.63
1.2	Select a source of protein to be replaced: SOYBEANMeal, solvent, 48% CP		0.408
1.3	Select a source of energy to add to the diet: CORN, YELLOWSilage, normal 32-38% DM		0.061
1.4	Milk Increase/Decrease because use of optigen	kg/cow/d 0.500	
1.5	Milk Price		$/cwt 18.00

2	***ANALYSIS***	Amount kg DM	Value $/cow/d
2.1	Optigen®	0.113	-0.186
2.2	SOYBEANMeal, solvent, 48% CP	-0.620	0.283
2.3	CORN, YELLOWSilage, normal 32-38% DM	0.507	-0.088
2.4	Value of change in milk production		0.198
2.5	**Value of Using Optigen®**		$/cow/d 0.21