

RUMINAL PROTEIN DEGRADATION IN BEEF
CATTLE

By

SCOTT S. SWANEK

Bachelor of Animal Science

Oklahoma State University

Stillwater, OK

1995

Master of Animal Science

Oklahoma State University

Stillwater, OK

1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
Doctor of Animal Science
May, 2007

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Thesis Approved:

Dr. Clinton R. Krehbiel – Thesis Adviser

Dr. Gerald W. Horn

Dr. Hebbie T. Purvis

Dr. Carla L. Goad

Dr. A. Gordon Emslie - Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Clint Krehbiel, who provided revisions, lead me in the right direction, and helped me make sense of it all. Thanks to my committee members, Dr. Gerald Horn, Dr. Hebbie Purvis, and Dr. Carla Goad, who offered their expertise and support. I would also like to acknowledge the lab technicians, Carolyn Lunsford, Donna Perry, Joan Summers and Tim Bodine, without whom my trial analysis would have taken 5 times as long. I would also like to thank my wife for her support and encouragement in obtaining this milestone in my educational career. SSS

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	
Introduction.....	3
In Situ Ruminant Protein Degradation.....	3
Chemical Definition.....	4
Ruminant Metabolism of Protein.....	5
Roughage:Concentrate Ratio and Degradability	7
Monensin.....	9
Protozoa	12
Source of Ruminally Degradable Protein	12
Summary.....	15
Literature Cited.....	16
III. EFFECT OF UREA LEVEL IN STEAM-FLAKED CORN DIETS ON NUTRIENT DIGESTION, N BALANCE AND RUMINAL KINETICS	
Abstract.....	25
Introduction.....	26
Materials and Methods.....	27
Steers.....	27
Sampling.....	28
Laboratory Analysis.....	29
Calculations and Statistics	30
Results and Discussion	30
Implications.....	34
Literature Cited.....	35
IV. EFFECT OF CONCENTRATE:ROUGHAGE RATIO AND MONENSIN ON RATE AND EXTENT OF IN SITU RUMINAL DEGRADATION OF BY PRODUCT FEEDS	
Abstract.....	42
Introduction.....	43
Materials and Methods.....	44
Cattle and Experimental Treatments.....	44
Diets	45
In Situ Dacron Bags.....	45
Rumen Samples	46
Fecal Samples	46
Laboratory Analysis.....	47

Calculations.....	47
Statistical Analysis.....	48
Results and Discussion	48
Effect of Roughage:Concentrate Ratio	49
Effect of Monensin (33 mg/kg).....	52
Implications.....	53
Literature Cited.....	55

LIST OF TABLES

Table

1. Composition of experimental diets (% of DM)	38
2. Effect of increasing dietary urea on intake, fecal output, and total tract digestibility of nutrients	39
3. Effect of increasing dietary urea on nitrogen balance	40
4. Effect of increasing dietary urea on ruminal liquid dilution rate, VFA concentrations, and ammonia concentration	41
5. Ingredient and nutrient composition of experimental diets	59
6. Effects of roughage:concentrate ratio on intake, total tract digestibility, and ruminal kinetics	60
7. Effects of 70:30 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability.....	61
8. Effects of 40:60 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability.....	62
9. Effects of 10:90 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability.....	63
10. Effects of monensin on intake and total tract digestibility	64
11. Effects of monensin on in situ kinetics of ruminal protein degradability.....	64
12. Comparison of in situ protein kinetics to NRC (1996).	65

NOMENCLATURE

ADG	average daily gain
DOF	days on feed
BW	body weight
CP	crude protein
MP	metabolizable energy
NEm	net energy for maintenance
NEg	net energy for gain
DM	dry matter
DMI	dry matter intake
h	hour
d	day
cm	centimeter
kg	kilogram
°C	degrees centigrade

INTRODUCTION

Knowledge of the rate and extent of degradation of protein sources is important in formulating diets with adequate rumen degradable protein (RDP) to support growth of ruminal microorganisms and sufficient rumen undegradable protein (RUP) for enzymatic digestion by the host animal. Ruminal amino acid degradation and ammonia production in excess of microorganism requirements is a waste of dietary crude protein (CP).

The protein fraction of feedstuffs fed to ruminant animals consists of RUP and RDP. Ruminal microorganisms use the RDP fraction to synthesize microbial CP (MCP). Microbial CP and RUP are the major contributors to metabolizable protein (MP), with endogenous CP being a minor contributor. Metabolizable protein is the true protein that is digested post ruminally and absorbed as amino acids (AA) in the small intestine.

Cattle consume CP to supply N to the rumen for microbial growth and AA for maintenance and production of the host. Flow of MCP and RUP to the small intestine is affected by feed intake, as well as the amount and source of dietary protein and energy. Dietary AA escaping ruminal degradation should complement the AA provided by MCP. Feeding protein high in RUP may increase the amount of dietary feed AA reaching the small intestine, but may decrease ruminally synthesized MCP (Clark et al., 1992). The growth of ruminal bacteria is largely dependent on the amount and type of fermentable carbohydrates and the amount of ammonia present in the rumen (Bryant and Robinson, 1962). High-concentrate finishing diets supply a source of readily fermentable organic matter; however, the most efficient N source to supply the rumen with adequate RDP to maximize digestibility has yet to be determined.

The experiments presented in this dissertation were conducted to: 1) determine the effects of increasing RDP levels in isonitrogenous and isocaloric high-concentrate finishing diets on DMI, nutrient digestion, nitrogen balance, and ruminal kinetics, and 2) evaluate roughage:concentrate ratios with or without an ionophore on the rate and extent of in situ ruminal degradation of protein sources commonly fed to ruminant animals. Chapter II provides a review of the literature related to diet type and ionophore effects on ruminal digestion of protein.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Understanding rate and extent of ruminal degradation of various protein sources is important when formulating diets in order to supply enough RDP to optimize rumen microbial growth. Rumen microbial growth is largely dependent on the amount of fermentable carbohydrate and the amount of ammonia present in the rumen (Bryant and Robinson, 1962). Most of the experiments which have evaluated RDP and RUP values of protein feedstuffs have been conducted with roughage diets, and less data is available with grain-based diets. In general, corn-based finishing diets supply the rumen with plenty of readily fermentable carbohydrates. However, the source of N needed to optimize yield and efficiency of MCP along with RUP to provide adequate MP to the host animal has yet to be determined.

In Situ Ruminal Protein Degradation

The most widely used model to describe ruminal protein degradability is the Level II model of the Nutrient Requirements of Beef Cattle (National Research Council, 1996). Ruminal disappearance of protein is a result of ruminal protein degradation and rate of passage from the rumen. Crude protein of feedstuffs consists of multiple fractions that vary widely in their rate and extent of ruminal degradation and are commonly referred to as the A (non-protein N), B (true protein), and C (bound true protein) fractions (Pichard and Van Soest, 1977). These fractions are determined using in situ techniques which have been described in detail (Vanzant et al., 1998).

Fraction A is the percent of CP that is instantly soluble in the rumen at time zero. It includes non-protein N, rapidly soluble protein, and true protein particles that are smaller in size than porosity of the Dacron bags. Fraction C is the fraction considered to be completely undegradable in the rumen and passes completely to the small intestine (NRC, 1996), and is defined by a specified end-point (Nocek and English, 1986). The remaining is the B fraction and is considered to be potentially degradable true protein. Fraction B is the only fraction that is affected by rate of passage, and the extent of degradation of fraction B is determined by the fractional rate of degradation combined with the relative rate of passage. Fraction C plus the undegradable portion of fraction B is considered to be RUP (NRC, 1996).

Chemical Definition

Fraction A can be determined chemically as the percent of total CP that is soluble in borate-phosphate buffer and is not precipitable in trichloroacetic acid (TCA; Roe et al., 1990). Fraction C contains protein that is bound to cell wall and is resistant to microbial and mammalian enzymatic digestion (Krishnamoorthy et al., 1982, 1983), providing no useable ruminal N or post-ruminal AA. It includes lignin bound protein, tannin-protein complexes, and protein that has undergone Maillard reaction (Sniffen et al., 1992). Fraction C can be determined as the percentage of N found as acid detergent insoluble nitrogen (ADIN). The remaining potentially degradable true protein is further subdivided into fractions B1, B2, and B3, based on their different rates of ruminal degradation (Sniffen et al., 1992). Like the A fraction, B1 is also soluble in borate-phosphate buffer; however, B1 is the fraction that is precipitable by the protein denaturant TCA

(Krishnamoorthy et al., 1983). Fraction B1 consists of NPN, amino acids and peptides that are rapidly degradable and converted to ammonia in the rumen (Van Soest et al., 1981). Fraction A and B1 comprise the soluble nitrogen fraction of feedstuffs as defined in the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen et al., 1992), and are determined by incubation in borate-phosphate buffer. Fraction B3 is soluble in acid detergent but insoluble in neutral detergent and can be calculated as the difference of the proportion of N as NDIN and ADIN. Fraction B3 is associated with the cell wall and therefore is slowly degraded in the rumen (Van Soest et al., 1981). The CNCPS describes a high percentage of B3 escaping ruminal degradation (Sniffen et al., 1992). Fraction B2 is the difference of all other fractions ($B2=100-(A+B1+B3+C)$). It is the fraction of N that is insoluble in borate-phosphate buffer less the N that is insoluble in neutral detergent. Fraction B2 represents the potentially degradable true protein portion and is the only fraction that is dependent on relative rates of passage and digestion.

Ruminal Metabolism of Protein

Ruminal protein degradation from feed ingredients is an important factor influencing AA supply to the duodenum. Ruminal proteolysis determines the amount of ammonia, AA, peptides, and branched-chain VFA available for microbial growth and proliferation. The rate and extent of ruminal protein degradation not only affects microbial protein synthesis, but also the amount and type of ruminally undegraded feed protein reaching the duodenum for digestion and absorption. Stern and Hoover's (1979) review of the literature showed that approximately 30 g of N were synthesized per kg of organic matter (OM) truly digested, with values ranging from 10 to 50 g of N per kg OM

truly digested. The amount of protein that is degraded in the rumen depends on microbial proteolytic activity, protein structure and accessibility of the feedstuff to microbes, ruminal retention time, protein solubility, and ruminal pH (NRC, 1985). Rumen bacterial growth is stimulated by peptides and AA acting as multiplying factors, which ultimately affects the rate and extent of protein degradation in the rumen (Argyle and Baldwin, 1989).

Dietary protein supplements can be selected and blended to alter the AA profile of proteins presented to the duodenum for digestion by the host animal (Keery et al., 1993; Erasmus et al., 1993; Christensen et al., 1993). Milton et al. (1997c), Trenkle (1995), and Healy et al. (1995) showed an increase in ADG with the addition of soybean meal (SBM) to urea-based supplements in dry-rolled and steam-flaked corn finishing diets. Soybean meal is a natural protein source that has a high rate of ruminal degradation, providing AA and peptides to the rumen for enhanced microbial growth. Milton et al. (1997b) demonstrated that SBM supplemented steers on dry-rolled corn finishing diets had higher total tract starch digestion, microbial N flow to the duodenum, and efficiency of microbial protein synthesis than steers fed urea supplemented diets. This was supported by in vitro work by Russell et al. (1983) and continuous culture experiments by Griswold et al. (1996) that showed adding AA and peptides to mixed ruminal bacteria increased microbial growth when ammonia was the sole N source.

Milton et al. (1997c) demonstrated that ruminal escape protein sources (blood meal and corn gluten meal) in dry-rolled corn finishing diets had little value for improving ADG and/or feed efficiency compared with feeding SBM. These researchers concluded that MP needs were met through microbial protein production and the

ruminally undegradable fraction of corn protein, and that the increase in supplemental RUP was not efficacious for improving performance. In the same experiment (Milton et al., 1997c), supplementing finishing diets with SBM increased gain efficiency by 4.3% compared with feeding urea. Milton et al. (1997c) suggested that steers fed dry-rolled corn-based finishing diets supplemented with SBM had enhanced MP supply compared with feeding urea, resulting from increased microbial protein synthesis and increased RUP. Milton et al. (1997c) concluded that supplementing diets with a degradable, true protein source not only increased MP, but also improved digestion, energetic efficiency and overall performance throughout the finishing phase.

Fewer experiments have evaluated dietary factors affecting ruminal protein degradation of various protein sources. Legleiter et al. (2005) showed that the level of dietary blood meal and SBM supplementation did not affect their RUP values calculated from in situ degradation data. In contrast, Scholljerges et al. (2005) showed that restricting dietary intake increased the amount of dietary protein that was degraded in the rumen compared with feeding ad libitum. More data are needed evaluating dietary factors, such as roughage:concentrate ratio and ionophores, on ruminal protein degradation in situ.

Roughage:Concentrate Ratio and Protein Degradability

Roughage:concentrate ratio has the potential to affect rate and extent of ruminal protein degradability. Zinn et al. (1994) showed a roughage level (10 vs. 20%) and monensin interaction on ruminal molar proportions of propionate; a 10% roughage diet had a 9.4% greater molar proportion of propionate with monensin supplementation,

whereas the 20% roughage diet had 5.5% lower molar proportion of propionate with monensin supplementation. Zinn et al. (1994) also showed a 20% greater ruminal degradability of feed N with the 20% roughage diet. These data suggest that roughage level influences the efficacy of monensin, and that feed N digestion in the rumen increases as roughage level increases.

Rodriguez-Prado et al. (2004) reported minimal effects of concentrate level on microbial AA profile in 33% and 61% concentrate diets from continuous culture fermenters. In contrast, Hussein et al. (1995) reported that steers fed a 70% concentrate diet had higher concentrations of N (6.0%), OM (2.5%), and 5 to 15% greater concentrations of total, essential, nonessential, and individual AA in mixed ruminal bacteria populations than steers fed a 30% concentrate diet. Therefore, it appears that changes in microbial populations and microbial nutrient composition occur when ruminants are switched from high roughage to high concentrate, which may alter protein degradability.

Rotger et al. (2005) showed that a 30% roughage diet resulted in a greater fluid dilution rate and decreased ruminal ammonia concentrations compared with a 12% roughage diet, but no differences in ruminal pH or total VFA concentrations were observed. These authors also observed that ruminal protein degradation was dietary roughage level and supplement dependent, increasing in peas and lupin seeds, decreasing with sunflower meal, and not changing in SBM when dietary roughage was increased. Similarly, Cronje (1992) determined that 48 h in situ DM degradation increased as the roughage:concentrate ratio increased from 25:75 to 75:25, but the percent change varied among the different feedstuffs common to South Africa. Conversely, Miller et al. (1991)

demonstrated that after 16 h of ruminal incubation DM and N disappearance increased with an 80% concentrate ration (13% CP) compared with low-quality prairie hay (4.5% CP). More data are needed to characterize the effects of roughage:concentrate ratio on ruminal protein degradation of a variety of protein sources.

Monensin

Monensin is primarily used to increase weight gain and feed efficiency of feedlot cattle (Boling et al., 1977) by increasing propionate production and decreasing methane (Goodrich et al., 1984). Monensin belongs to the class of compounds called oxilyc polyether ionophore antibiotics, or commonly referred to simply as 'ionophores'. The basic mode of action of monensin is to modify the movement of ions across biological membranes (Shelling, 1984). The monensin ionophore first binds to the cell membrane in an anionic form, and is then capable of binding to a metal cation such as sodium. The electroneutral ion/ionophore complexes then allow the metal cations entry into the cell. The polar conditions inside the cell causes the cation to dissociate and allows the carboxyl group of the ionophore to bind a proton (hydrogen), and the new electroneutral complex proceeds in the opposite direction out of the cell (Chow et al., 1994). The net effect is an accumulation of intracellular Na^+ , a loss of intracellular K^+ , and a loss of transport activity. Accumulation of intracellular protons decreases cytoplasmic pH (Smith and Rosengurt, 1978), forcing the cell to use its ATP to regenerate the decrease in pH by pumping out excess protons and eventually depleting intracellular ATP. Cells that depend on substrate level phosphorylation for ATP, such as gram positive bacteria, cannot meet their energy demand and eventually lyse.

Monensin is highly hydrophobic and has the capacity to bind to either protons or metal cations, like sodium or potassium. Monensin inhibits the growth of bacteria with gram positive cell wall characteristics with little or no effect on gram negative bacteria, giving gram negative bacteria a selective survival advantage (Chen and Wolin, 1979). The multilayered characteristics of the cell envelope of gram negative bacteria consist of two distinct membranes. The outer membrane serves as a penetration barrier and allows gram negative bacteria to be less sensitive to antibiotics such as monensin (Bergen and Bates, 1984). Gram positive bacteria lack this outer membrane making them more sensitive to monensin. Monensin is lipid soluble and easily enters the lipid membrane of gram positive bacteria that produce acetate, butyrate, H₂ and formate. Monensin mediates primarily a Na⁺-H⁺ exchange, by wrapping itself around the Na⁺ and carrying it into the cell and H⁺ out in a 1:1 exchange (Riddell, 2002). Dawson and Boling (1987) also determined that when the external ruminal potassium concentrations were high, monensin sensitive bacteria were more resistant. Monensin has been demonstrated to be a metabolic inhibitor of hydrogen and formate producing bacteria, but stimulated fumarate reductase, succinate and propionate producing bacteria (Chen and Wolin, 1979).

Schelling (1984) described seven possible system modes of action to help define an animal response to monensin: 1) shift in VFA production; 2) changes in feed intake; 3) changes in gas production; 4) modification of DM digestibility; 5) altering protein utilization; 6) changes in rumen fill and rate of passage; 7) other indirect effects. The remainder of this review will concentrate on monensin's effects on protein utilization.

Numerous studies have demonstrated monensin's ability to reduce ruminal protein degradation and increase the amount of AA N reaching the abomasum in ruminants fed high-forage (Haimoud et al., 1996; Ruiz et al., 2001) and high-concentrate diets (Schelling et al., 1977; Poos et al., 1979; Chalupa et al., 1980; Lana et al., 1997). Monensin has also been shown to decrease ruminal proteolysis and increase feed protein flow to the abomasum. Surber and Bowman (1998) reported that monensin increased feed N flow to the abomasum by 36% but did not alter in situ dry matter degradability of corn or barley. Monensin decreases the rate of free AA degradation in ruminal fluid and lowers ruminal ammonia concentrations (Schelling et al., 1977). Zinn and Borques (1993) showed that 33 mg/kg of monensin in finishing diets increased feed N flow to the duodenum 11.3%, but decreased microbial N flow by 7.4%, with no difference in overall nonammonia N flow to the duodenum. Chen and Russell (1988 and 1989) demonstrated that monensin suppressed the growth of bacteria with high AA deamination activity (approximately 2 to 10% of the total count of rumen bacteria). Obligated AA fermenting bacteria (*Clostridium sticklandii*, *Peptostreptococcus anaerabius* and *Clostridium aminophilum*), with the sole function of deaminating AA, have very high specific activities for ammonia production (Russell et al., 1988; Pastor et al., 1993), using peptides and AA, instead of carbohydrates as an energy source. These gram positive bacteria are sensitive to monensin and their inhibition would explain the lower rumen ammonia levels and the ruminal protein sparing effect observed with monensin supplementation. Yang and Russell (1993) demonstrated that monensin (350 mg/d) supplementation decreased ruminal ammonia concentrations by 30% and a 10-fold decrease in monensin sensitive, AA fermenting bacteria.

Monensin does not affect urease activity (Wallace et al., 1981). This is supported by Goodrich et al. (1984), who reported that monensin improved feed efficiency by 7.8% in diets containing true protein and 1.9% in diets containing urea supplementation. Similarly, Rogerio et al. (1997) demonstrated that monensin had a greater effect on feed efficiency and N utilization on SBM than urea supplemented high-concentrate diets.

Protozoa

Protozoa also contribute to ruminal protein degradation. Although not near as numerous in numbers as bacteria in the rumen, protozoa's large mass makes it a significant portion of rumen microflora. Like gram positive bacteria, ruminal protozoa lack an outer membrane making them more sensitive to monensin (Chow et al., 1994). Ivan et al. (1992) demonstrated that ruminal fluid protozoal numbers for rams supplemented with monensin were lower than rams receiving no monensin.

Source of Ruminally Degradable Protein in Finishing Cattle Diets

Rumen degradable N and carbohydrates are fermented to provide carbon skeletons and energy (ATP) for MCP synthesis. Since MCP provides such a high quality N source to the duodenum for digestion, and Clark et al.'s (1992) review of the literature reports that 59% of non ammonia nitrogen (NAN) reaching the duodenum of dairy cows was supplied by microbial N, it is important to maximize MCP synthesis in the rumen. Ruminal MCP synthesis requires specific nutrients like branched chain fatty acids, sulfur, and trace nutrients (Stern et al., 1994). Hume and Bird (1970) reported that microbial

growth was maximized at a 10:1 N:S ratio, and Rumsey (1978) described a decrease in digestion and feed efficiency when diets were inadequate in dietary sulfur.

Ruminal microbial growth is largely determined by the amount of ammonia N and fermentable OM available for ruminal fermentation (Bryant and Robinson, 1962). High-concentrate corn-based finishing diets provide plenty of fermentable carbohydrates, but are typically deficient in RDP because corn protein is only considered 40% degradable (Sindt et al., 1993) and may not supply adequate RDP to maximize rumen microbial growth. Burroughs et al. (1975) suggested that limiting RDP can restrict ruminal microbial growth and reduce starch digestion. Although urea provides no additional peptides, amino acids, or other growth factors that potentially enhance microbial growth (Zinn and Owens, 1983), performance of cattle fed finishing diets supplemented with urea vs. true protein have been similar in some experiments (Clark et al., 1970; Greathouse et al., 1974; Gleghorn et al., 2004). In contrast, Healy et al. (1995), Trenkle (1995) and Milton et al. (1997) reported that the addition of SBM to urea-based finishing diets improved ADG. Differences in responses among experiments are most likely related in part to source of grain fed and degree of grain processing.

When urea was fed as the sole source of RDP in cattle fed dry-rolled corn-based finishing diets, Milton et al. (1997a) determined by regression analysis of two separate experiments that 0.50 and 0.90% urea diets were optimum for ADG and feed efficiency. Shain et al. (1998) showed a linear response for N intake with increasing urea supplementation levels of 0, 0.88, 1.34, and 1.96% DM, in dry-rolled corn finishing diets. However, they observed no benefit to supplementation above 0.88% urea on cattle

performance, carcass characteristics, or ruminal metabolism, similar to the study by Milton et al. (1997a).

Milton and Brandt (1994) observed that ruminal and total tract digestibility of starch and OM and microbial N flow to the duodenum did not differ when ruminal ammonia N concentrations were above 3.07 mg/100 mL. Similarly, Ortega et al. (1979) observed no change in the rate of ruminal digestion in situ when increasing ruminal ammonia N concentrations from 6.3 to 27.5 mg/100 mL. In contrast, Kang-Meznarich and Broderick (1981) showed a 21% decrease in MCP synthesis when ruminal ammonia N concentrations were decreased from 3.33 to 1.33 mg/100 mL. Ruminal ammonia concentrations appear to be similar for finishing cattle diets supplemented with SBM or urea (Milton et al., 1997b; Zinn, 1995). In the work of Slyter et al. (1979), ruminal OM digestion and VFA production were greatest at a 3.2 mg/100 mL ruminal ammonia concentration, but MCP synthesis was maximized at a much lower ruminal ammonia concentration of 1.6 mg/100 mL. Likewise, Milton et al. (1997a) showed no improvement in MCP synthesis above ruminal ammonia concentrations of 2.2 mg/100 mL, although they did see a performance response to diets that provided ruminal ammonia concentrations above 3.1 mg/100 mL. Shain et al. (1998) reported that increasing level of urea had no effect on ruminal pH, molar proportions of acetate, propionate, butyrate, or acetate/propionate ratio in finishing steer diets. Milton et al. (1997b) also reported no differences among ruminal pH, molar proportions of acetate, propionate, total VFA concentration, or acetate/propionate ratio for SBM vs. urea supplemented in dry-rolled corn finishing diets.

Summary

Understanding rate and extent of ruminal degradation of various protein sources is important for formulating diets to supply enough RDP to optimize rumen microbial growth and MP to support the host. Dietary factors that affect rate and extent of protein degradation of feedstuffs in the rumen have not been well characterized, especially for cattle consuming high-grain diets. More data are needed to determine the effects of RDP levels in high-concentrate finishing diets on digestion and nutrient balance, and to evaluate dietary factors which affect the rate and extent of ruminal degradation of protein sources commonly fed to ruminant animals.

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CHAPTER III

EFFECT OF DIETARY UREA LEVEL IN STEAM-FLAKED CORN DIETS ON NUTRIENT DIGESTION, NITROGEN BALANCE AND RUMINAL KINETICS

S. S. Swanek, C. R. Krehbiel, D. R. Gill, and C. L. Goad

ABSTRACT: Five ruminally cannulated steers (initial BW = 375 ± 34 kg; ADG = 1.16 ± 0.13 kg) were used in a 5 x 5 Latin square design experiment to determine the effects of increasing urea level on intake, nutrient digestion, nitrogen balance and ruminal kinetics. Isocaloric ($NE_m = 2.11$ Mcal/kg; $NE_g = 1.35$ Mcal/kg) and isonitrogenous (2.24% N) steam-flaked corn-based diets with urea concentrations of 0.0, 0.8, 1.2, 1.6, or 2.0% (DM basis) were offered ad libitum to steers. Following nine days of diet adaptation total urine and feces were collected for four days. On day 14, ruminal fluid was collected at 0, 3, 6, 9, 12, 15, 18, 21, and 24 h after pulse dosing with Co-EDTA. Dietary urea concentration did not affect ($P > 0.50$) DM or OM intake. Intake of ADF increased from 0.0 to 0.8% urea, decreased to 1.6% urea, and then increased when 2.0% urea was fed (cubic urea effect, $P < 0.01$). Increasing dietary urea increased (linear urea effect, $P < 0.05$) fecal output of OM and ADF. Although total tract digestibility of OM was not affected ($P = 0.21$) by urea level, total tract ADF digestibility was decreased (linear urea effect, $P < 0.01$). Dietary urea level did not affect ($P > 0.40$) fecal N output, total N balance, or N balance as a percent of N intake. Urinary N output responded quadratically ($P < 0.05$) to increased urea level in the diet. Liquid dilution rate ($P = 0.54$), ruminal fluid pH ($P = 0.13$) and total VFA ($P = 0.16$) were not affected by dietary urea level.

Molar proportion of isobutyrate decreased linearly ($P < 0.01$) as urea level increased.

Our data suggests that from 0.8 to 2.0% urea (DM basis) can be fed to steers consuming steam-flaked corn-based finishing diets with minimal effect on intake, nutrient digestion, or ruminal kinetics.

Key words: rumen degradable protein, nitrogen balance, ruminal metabolism, urea

INTRODUCTION

Urea is a commonly used source of supplemental N in cattle finishing diets because it is inexpensive and easy to incorporate. Compared with sources of true protein, urea is limited to enhancing ruminal OM digestion and does not contribute to unrumen undegradable protein. Although urea provides no peptides, amino acids, or other growth factors that potentially enhance microbial growth (Zinn and Owens, 1983), performance of cattle fed finishing diets supplemented with urea vs. true protein has been similar (Clark et al., 1970; Greathouse et al., 1974; Gleghorn et al., 2004). In contrast, Healy et al. (1995), Trenkle (1995) and Milton et al. (1997c) reported that the addition of SBM to urea-based finishing diets improved ADG. Sindt et al. (1993) concluded that calves expressing compensatory growth require more MP in the finishing diet in order to maximize feed efficiency, whereas calves finished directly after weaning had similar efficiencies whether supplemented with true protein or urea. Milton et al. (1997a,b) determined from regression analysis of two different experiments that 0.5 and 0.9% urea (DM basis) were optimal for ADG and G:F when urea was the sole N supplement in dry-rolled corn-based diets. Similarly, Shain et al. (1998) reported no advantage of supplementing urea above 0.88% in dry-rolled corn-based finishing diets.

Cattle performance data suggest that the proper ratio of rumen degradable:undegradable protein should be fed to maximize performance (Stock et al., 1981; Milton et al., 1997a,b). Providing adequate rumen degradable protein is necessary for maximum microbial CP synthesis, which depends largely on carbohydrate digestion in the rumen (Russell et al., 1983). Therefore, requirements for rumen degradable protein should be greatest with high-grain diets that are based on extensively processed starch (e.g., steam-flaked grains). Gleghorn et al. (2004) reported that ADG by cattle fed steam-flaked corn-based finishing diets responded quadratically to CP concentration, with maximal responses in ADG and other performance variables noted with a 13% CP diet. In addition, carcass adjusted ADG and G:F (both live- and carcass-adjusted) improved as urea supplied more of the supplemental CP. Serum urea N concentrations were affected more consistently by concentration of CP than by source. Therefore, when cattle are finished on diets based on steam-flaked corn, urea seems to be the optimal source of supplemental CP. We hypothesized that urea could replace equivalent amounts of N supplied by SBM up to 2.0% of diet DM for steers fed steam-flaked corn diets. The objective of this experiment was to determine the effect of replacing SBM with increasing urea levels in isonitrogenous and isocaloric steam-flaked corn-based finishing diets on DMI, nutrient digestion, nitrogen balance, and ruminal kinetics.

MATERIALS AND METHODS

Steers. Five ruminally cannulated beef steers (initial BW = 375 ± 34 kg; ADG = 1.16 ± 0.13 kg) were used in a 5 x 5 Latin square design experiment to determine the effects of increasing urea level on nutrient intake, total tract digestion, nitrogen balance

and ruminal kinetics. Diets (Table 1) were formulated to be isocaloric ($NE_m = 2.11$ Mcal/kg; $NE_g = 1.35$ Mcal/kg) and isonitrogenous (2.24% N) steam-flaked corn-based diets with increasing urea levels of 0.0, 0.8, 1.2, 1.6, or 2.0% (DM basis). Steam-flaked corn was purchased from a commercial feedyard and had been flaked to a bulk density of 0.38 kg/L. Steers were fed once daily at 0800. Diets were weighed daily and offered ad libitum to steers on an individual animal basis; all refusals were weighed daily and subtracted from the previous day's feed delivery. Steers were housed individually in concrete slatted floor pens (5×4 m) at the Nutrition Physiology barn at Oklahoma State University. Steers had ad libitum access to fresh water. Experimental procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Sampling. Experimental periods were 14 d in length with 10 d of dietary adaptation and 4 d of sampling. On d 10 of each period, steers were moved from pens and placed in metabolism stanchions to facilitate sample collection. Diet samples were collected daily at feeding during the sampling period, composited by animal within period, dried in a forced air oven (50°C , 48 h), and ground in a Wiley mill to pass a 2-mm screen. On d 11 through 14, total feces and urine were collected and a daily subsample was composited (2.5% of feces and 1% of urine; wet weight basis). A portion of the fecal subsample was dried in a forced-air oven (50°C , 48 h) to determine DM. The remaining subsample was frozen and stored at -20°C for later lyophilization. The subsample of urine was frozen at -20°C for later determination of N concentration. Three hundred milliliters of 6 N HCl was added to each urine collection container to maintain urine pH below 3.

On day 14 at 0800, 250 mL of Co-EDTA was pulse-dosed intraruminally as a fluid dilution marker. The procedures for preparing Co-EDTA were the same as those described by Uden et al. (1980). Ruminal samples were collected before dosing (0 h) and at 3, 6, 9, 12, 15, 18, 21 and 24 h after dosing to determine pH, Co, ammonia and VFA concentrations. Ruminal fluid samples were collected from several places in the rumen, strained through 4 layers of cheese cloth, composited, and pH was measured using a portable pH meter and combination electrode (HI 9024, Hanna Instruments SRL, Italy). A 10 mL aliquot was acidified with 0.5 mL of 6 N HCL and frozen (-20°C) immediately for ammonia analysis. An 8 mL aliquot was acidified with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen (-20°C) immediately for VFA analysis. A third 10 mL aliquot was frozen (-20°C) immediately for Co analysis.

Laboratory analysis. Ground samples of feed and feces were analyzed for DM and OM according to standard procedures (AOAC, 1996). Nitrogen content of feed and lyophilized feces was determined by the combustion method (Leco NS2000, St. Joseph, MI: AOAC, 1996). Nitrogen content of urine samples was determined by the kjeldahl analysis (AOAC, 1996). Acid detergent fiber concentrations of feed and feces were determined by the Van Soest et al. (1991) method. Feed and fecal total starch content were determined by the starch hydrolysis assay procedure (Megazyme, Bray, Co. Wicklow, Ireland: AOAC, 1996) adopted from McCleary et al. (1997). Ruminal fluid samples for Co and ammonia analysis were thawed and centrifuged at $10,000 \times g$ for 12 min. Concentrations of Co in ruminal fluid samples were determined via ICP analysis at a wavelength of 228.6. Ruminal ammonia concentrations were determined by procedures

adapted from Broderick and Kang (1980). Volatile fatty acid analysis of ruminal fluid was conducted using gas chromatography as outlined by Goetsch and Galyean (1983).

Calculations and Statistics: Fluid dilution rate of Co was calculated by regressing the natural log of marker concentration on time after dosing. Data were analyzed as a 5 x 5 Latin square experimental design using the Mixed procedure of SAS (SAS Inst., Inc., 2003). For total tract digestibility, N balance and liquid dilution analyses, the model included period and urea level. Ruminal pH, ammonia N, and VFA repeated measures were modeled using an autoregressive covariance structure. The model consisted of period, urea level, time of collection, and the urea × time interaction. All models included steer as a random effect. When response means analyzed had a significant ($P < 0.10$) *F*-test, urea level was analyzed for linear, quadratic, and cubic trends using orthogonal contrasts adjusted for unequal spacing of urea levels. All tests for significance were reported at the $P < 0.10$ level.

RESULTS AND DISCUSSION

Dry matter intake, fecal output and total tract digestibility of OM, ADF and starch are shown in Table 2. Dry matter ($P = 0.52$) and OM ($P = 0.51$) intake were not affected by increasing urea level in the present experiment. Similarly, Gleghorn et al. (2004) reported that neither N source nor N concentration affected DMI during the finishing period for steers fed steam-flaked corn-based diets with 11.5, 13.0, and 14.0% CP, and Shain et al. (1994) reported that urea level had no effect on DMI when 80% dry-rolled corn diets were supplemented with urea to provide up to 15% dietary CP. In contrast, Milton et al. (1997c) reported that steers consumed 4.6% more feed when urea vs. SBM

was fed in isonitrogenous finishing diets based on dry-rolled corn. Milton et al. (1997c) attributed the response to high intakes (2.5% of BW) observed across the feeding period. In general, it appears that N source in isonitrogenous diets does not affect DMI by cattle fed high-concentrate diets, consistent with the present data.

In the present experiment, fecal output of OM tended ($P = 0.07$) to increase as urea level increased, although total tract OM digestibility was not affected ($P = 0.21$; Table 2). Similarly, in the study of Milton et al. (1997b), total tract digestibility of OM was not affected by supplemental N, although supplementation with SBM tended (5% increase in OM digestibility) to increase total tract OM digestion compared with urea-supplemented isonitrogenous diets. When dietary N was increased with increasing urea at 0.0, 0.5, 1.0, and 1.5%, no difference in total tract digestion of OM was observed (Milton et al., 1997b). Knaus et al. (2001) showed a 5.5% increase in total tract OM digestion in SBM vs. urea supplemented diets. Potential differences in Knaus et al. (2001) vs. our data may be explained by grain source (cracked corn vs. steam flaked corn, respectively) and roughage level (15 vs. 10% roughage, respectively). In addition, their OM digestibility values were considerably lower compared with values in the present experiment. Overall, these data suggest that N source (SBM vs. urea) does not affect total tract digestibility of OM. In the present experiment, intake of ADF increased from 0.0 to 0.8% urea, decreased to 1.6% urea, and then increased when 2.0% urea was fed (cubic urea effect, $P < 0.01$; Table 2). In addition, increasing dietary urea increased fecal output (linear urea effect, $P < 0.01$) and decreased total tract digestibility (cubic and linear urea effect, $P < 0.01$) of ADF.

There was a cubic effect ($P < 0.05$) of urea level on starch intake; however, fecal

output ($P = 0.69$) and total tract digestibility ($P < 0.75$) of starch did not differ among treatments (Table 2). In contrast, Milton et al. (1997a) determined that ruminal starch digestion was increased 37% by 0.50% urea (DM basis), but higher urea levels did not differ from 0.50%. Milton et al. (1997a) did not feed isonitrogenous diets and the addition of urea was the only supplemental N source. Burroughs et al. (1975) suggested that limiting rumen degradable protein can restrict ruminal microbial growth and reduce energy (starch) digestion. Our diets appeared to contain adequate levels of rumen degradable protein to support OM and starch digestion. A second experiment by Milton et al. (1997b) demonstrated that SBM-supplemented 1.84% N diets increased starch digestibility 5% compared with an isonitrogenous urea-supplemented diet. This is contrary to the present data, and may be explained in part by the higher level of N supplementation and differences in digestibility of the ruminal OM in the diet (i.e., steam-flaked corn in our study vs. dry-rolled corn in the Milton et al., 1997b study). Cooper et al. (2002) reported that cattle fed steam-flaked corn finishing diets had higher rumen degradable protein requirements than cattle fed dry-rolled corn, because of increased ruminal starch digestion.

Nitrogen intake tended ($P = 0.10$) to increase cubically as dietary urea level increased (Table 3). This is contradictory to Milton et al. (1997b) who observed no difference in N intake between 1.84% N diets supplemented from SBM or urea, and Knaus et al. (2001) who showed a 6% decrease in N intake of a 1.8% urea diet compared with an isonitrogenous SBM based dry-rolled corn diet. Total fecal N did not differ among treatments, but urinary N output was lowest for 0.0 and 2.0% urea diets and highest for the 0.8% urea diet. Knaus et al. (2001) showed that fecal N was 11% less for

a 1.8% urea diet compared with other isonitrogenous diets, but urinary N output did not differ among treatments. In the present experiment, overall N balance, expressed as a total (g) or as a % of intake, was not different among treatments. There was no difference in N digestibility among treatments, which is consistent with Milton et al. (1997b) and Knaus (2001).

Ruminal pH was not affected by treatment (Table 4), and is similar to Shain et al. (1998) who showed increasing urea levels (0, 0.88, 1.34, and 1.96% diet DM) had no effect on ruminal pH. In contrast, Milton and Brandt (1994) showed a linear decrease in ruminal pH as level of supplemental urea increased. Liquid dilution rate was not affected by treatment (Table 4). Increasing dietary urea did not affect ($P = 0.16$) total VFA concentration, or molar proportions ($P > 0.10$) of propionate, acetate, butyrate, or the acetate:propionate ratio. However, isobutyrate concentrations were decreased (linear urea effect, $P < 0.01$) with increasing urea. Similar to the present data, Shain et al. (1998) reported that increasing level of urea had no effect on molar proportions of acetate, propionate, butyrate, or acetate:propionate ratio in finishing steer diets. In addition, Milton et al. (1997b) reported no differences in molar proportions of acetate, propionate, total VFA concentration, or acetate:propionate ratio among SBM vs. urea supplemented cattle fed dry-rolled corn finishing diets. Zinn (1995) noted that the addition of urea to high-grain diets had little effect on ruminal fermentation patterns, consistent with the present experiment.

Ruminal ammonia concentrations were not affected ($P = 0.71$) by increasing level of urea supplementation in this study, and all of our values were well above the 3.89 mg/100 mL reported by Shain et al. (1998) as the concentration above which no effects

on cattle performance, carcass characteristics, or ruminal metabolism were observed in high-grain finishing diets. Our ruminal ammonia concentrations were also higher than the 5 mg/100 mL reported by Satter and Slyter (1974) needed to maximize microbial efficiency in continuous-culture fermenters. The ammonia concentrations of this study were consistent with those observed by Milton et al. (1997b) for urea vs. SBM supplemented (1.84% N) dry-rolled corn finishing diets.

IMPLICATIONS

Increasing urea levels in steam-flaked corn diets can satisfy the RDP requirement with little effect on feed intake, nutrient digestion, or ruminal kinetics, most likely due to the high rate of ruminal OM fermentation provided by steam-flaked corn. Our data confirms previously reported literature suggesting that urea can replace natural protein in steam-flaked corn finishing diets.

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Table 1. Composition of experimental diets (% of DM)

Item	Urea level, % of diet DM				
	0.0	0.8	1.2	1.6	2.0
Ingredient composition					
Steam-flaked corn	69.5	67.9	67.9	67.5	67.5
Soybean meal	10.9	5.4	3.1	0.8	0.0
Urea	0.0	0.8	1.2	1.6	2.0
Alfalfa hay	5.0	5.0	5.0	5.0	3.0
Cottonseed hulls	5.0	5.0	5.0	5.0	7.0
Cane molasses	5.0	5.0	5.0	5.0	5.0
Fat	0.5	2.1	2.3	2.5	2.5
Supplement ¹	4.1	8.8	10.5	12.2	13.0
Nutrient composition ²					
NE _m , Mcal/kg	2.10	2.11	2.11	2.11	2.11
NE _g , Mcal/kg	1.34	1.35	1.35	1.35	1.36
CP, %	13.9	14.1	14.4	15.1	14.2
RDP, % of CP	54.5	60.40	63.1	65.7	67.7
NDF, %	14.9	15.6	15.2	15.0	14.9
ADF, %	11.05	12.81	10.61	9.31	12.57
Starch, %	48.5	53.7	47.8	50.1	56.1
Ether extract, %	3.9	5.9	6.0	6.1	6.1
Ca, %	0.60	0.60	0.60	0.60	0.60
P, %	0.30	0.30	0.30	0.30	0.30
K, %	0.89	0.8	0.76	0.70	0.67

¹Supplement includes ground corn and wheat middlings. Total diet formulated to provide 0.12 ppm cobalt, 10 ppm copper, 40 ppm manganese, 0.22 ppm selenium, 30 ppm zinc, 33 mg/kg monensin (Elanco Animal Health, Indianapolis, IN), 10.2 mg/kg tylosin (Elanco Animal Health).

²Nutrient values are based on tabular values for individual feed ingredients (NRC, 1996) except for CP, ADF and starch, which are laboratory results.

Table 2. Effect of increasing dietary urea on intake, fecal output, and total tract digestibility of nutrients

Item	Urea level, % of diet DM					SEM	<i>P</i> > <i>F</i>
	0.0	0.8	1.2	1.6	2.0		
Intake							
DM, kg	9.4	9.6	10.1	9.6	9.4	0.5	0.52
OM, kg	8.7	8.8	9.3	8.9	8.7	0.5	0.51
ADF, kg ¹	1.04	1.22	1.07	0.90	1.19	0.06	< 0.001
Starch, kg ^{2,3}	4.56	5.13	4.83	4.83	5.30	0.26	0.03
Fecal output							
OM, kg ³	0.91	0.98	1.07	0.99	1.09	0.08	0.07
ADF, g ⁴	343	370	398	383	470	32.5	0.008
Starch, g	77.6	87.7	97.5	105.0	103.8	17.5	0.69
Digestibility							
OM, %	89.2	88.9	88.5	88.5	87.4	0.86	0.21
ADF, % ^{1,4}	66.4	70.0	62.8	55.5	60.4	3.29	0.01
Starch, %	98.3	98.3	98.0	97.8	98.0	0.39	0.75

¹Cubic urea effect (*P* < 0.01).

²Cubic urea effect (*P* < 0.05).

³Linear urea effect (*P* < 0.05).

⁴Linear urea effect (*P* < 0.01).

Table 3. Effect of increasing dietary urea on nitrogen balance

Item	Urea level, % of diet DM					SE	<i>P</i> > <i>F</i>
	0.0	0.8	1.2	1.6	2.0		
N intake, g ¹	209	215	232	232	214	12.0	0.10
Fecal N output, g	30.6	30.4	31.8	31.4	32.3	2.42	0.81
N digestibility, %	85.9	85.9	86.3	86.1	84.9	1.03	0.44
Urine N output, g ²	80.0	109.3	98.1	100.	86.1	7.8	0.06
N balance, g	98.6	75.4	102.	100.	95.9	13.7	0.44
N balance, % of N intake	46.4	35.4	43.8	41.1	44.0	5.4	0.41

¹Cubic urea effect (*P* < 0.10).

²Quadratic urea effect (*P* < 0.05).

Table 4. Effect of increasing dietary urea on ruminal liquid dilution rate, VFA concentrations, and ammonia concentration

Item	Urea level, % of diet DM					SEM	<i>P</i> > <i>F</i>
	0.0	0.8	1.2	1.6	2.0		
Liquid dilution rate,	4.68	4.39	5.63	4.34	3.49	1.01	0.54
Ruminal pH	5.67	5.79	5.73	5.74	6.00	0.10	0.13
Area < pH 6.0, pH*h	9.29	7.74	8.46	9.04	6.10	1.54	0.45
VFA							
Total, mM	111.8	114.2	110.8	108.1	98.0	5.74	0.16
Acetate (A), %	44.7	47.4	44.7	45.7	45.9	1.62	0.58
Propionate (P), %	42.5	39.8	45.5	42.6	42.0	2.61	0.46
Isobutyrate, % ¹	1.15	0.74	0.43	0.35	0.47	0.18	0.01
Butyrate, %	7.57	6.94	5.89	6.19	6.35	0.61	0.21
Isovalerate, %	1.66	3.19	1.46	2.81	2.49	0.75	0.35
Valerate, %	2.38	2.02	1.99	2.33	2.78	0.33	0.46
A:P	1.09	1.32	1.00	1.21	1.20	0.16	0.49
NH ₃ , mg/100 mL	5.91	6.80	7.01	5.89	6.09	0.86	0.71

¹Linear urea effect (*P* < 0.01).

CHAPTER IV

EFFECTS OF ROUGHAGE:CONCENTRATE RATIO AND MONENSIN ON RATE AND EXTENT OF IN SITU RUMINAL DEGRADATION OF BYPRODUCT FEEDS

S. S. Swanek, C. R. Krehbiel, D. R. Gill, and C. L. Goad

ABSTRACT: Nine mature (681 ± 91 kg) crossbred steers with ruminal cannulas were used in an incomplete 6×6 Latin square design experiment to evaluate three roughage:concentrate ratios (70:30, 40:60, and 10:90) with (33 mg/kg) or without (0 mg/kg) monensin on the rate and extent of in situ ruminal degradation of soybean hulls (SH), wheat middlings (WM), dried distillers grains with solubles (DDG), cottonseed meal (CSM), high protein soybean meal (SBM), and porcine blood meal (BM); and their effects on ruminal kinetics. Monensin had no affect ($P > 0.10$) on DM, OM, NDF or starch intake, output or digestibility. However, monensin decreased ($P < 0.01$) in situ ruminal degradation of N and DM B fraction, and increased ($P < 0.01$) the C fraction of N and DM. Rate of N and DM disappearance expressed as %/h was also decreased ($P < 0.01$) by monensin. Ruminal protein degradation was decreased by 6.7% ($P < 0.01$) and RDDM decreased by 4.1% ($P < 0.01$) for monensin supplementation, suggesting monensin has a protein sparing effect. Total VFA concentration was not affected by monensin. Dry matter and OM intake did not differ between roughage:concentrate ratios. Fecal DM, OM, and NDF output decrease ($P < 0.01$) and digestibility increased ($P < 0.01$) with increasing level of concentrate. Starch output was the lowest ($P = 0.01$) for the 70:30 roughage:concentrate ratio ration and starch digestibility did not differ among

roughage:concentrate ratios. Total VFA concentration increased ($P < 0.01$) as concentrate level in the diet increased. Molar proportions of individual VFA did not differ among roughage:concentrate ratios. There was no roughage:concentrate ratio x byproduct interaction, however as the level of concentrate increased the B fraction and the rate of degradation decreased and the C fraction increased, resulting in decreased DM and N ruminal degradation with increased levels of concentrate. All of the byproducts reacted this way with the exception of blood meal, which remained relatively constant across treatments.

Key words: in situ degradability, monensin, protein, ruminants, roughage:concentrate ratio

INTRODUCTION

The two components of dietary CP are rumen degradable protein (RDP) and rumen undegradable protein (RUP). Rumen degradable protein provides peptides, free amino acids (AA), and ammonia for microbial growth and synthesis. Knowledge of the extent of degradation of different protein sources is important in formulating diets with adequate RDP to support ruminal microorganisms and sufficient RUP for enzymatic digestion by the host animal. Ruminal AA degradation and ammonia production in excess of microorganism requirements is a waste of dietary CP. Obligate AA fermenting bacteria, *Clostridium sticklandii*, *Peptostreptococcus anerabius* and *Clostridium aminophilum*, sole function is deaminating AA and ammonia production (Russell et al., 1988; Pastor et al., 1993), using peptides and AA instead of carbohydrates as an energy source. These gram positive bacteria are sensitive to monensin and their inhibition can in

part explain the rumen protein sparing effect seen with monensin supplementation. Zinn and Borques (1993) showed the 33 mg/kg of monensin finishing diets increased feed N flow to the duodenum 11.3%. Cronje (1992) determined that 48 h in situ DM degradation increased as the roughage:concentrate ratio increased from 25:75 to 75:25, but the percent change varied among the different feedstuffs common to South Africa. The objective of this experiment was to evaluate three roughage:concentrate ratios (70:30, 40:60, and 10:90) with (33 mg/kg) or without (0 mg/kg) monensin on the rate and extent of in situ ruminal degradation of soybean hulls (SH), wheat middlings (WM), dried distillers grains with solubles (DDG), cottonseed meal (CSM; solvent process), dehulled soybean meal (SBM), and porcine blood meal (BM). These byproducts were chosen as feedstuffs of interest from previous literature where we looked at 15 byproduct feeds (Swanek et al., 2001).

MATERIALS AND METHODS

Cattle and Experimental Treatments. Six ruminally cannulated mature beef steers (initial BW 681 ± 91 kg) were allotted randomly to 1 of 6 treatments in a 6 x 6 Latin square design experiment to determine: 1) the effect of dietary roughage level on the rate and extent of in situ ruminal degradation of byproduct feeds; 2) the ruminal protein sparing effect of monensin on byproduct feeds; and 3) the effects of dietary roughage levels and monensin on ruminal digesta kinetics. Treatments included: 1) 30% concentrate, 70% roughage, 33 mg monensin/kg of diet; 2) 30% concentrate, 70% roughage, 0 mg monensin/kg of diet; 3) 60% concentrate, 40% roughage, 33 mg monensin/kg of diet; 4) 60% concentrate, 40% roughage, 0 mg monensin/kg of diet; 5)

90% concentrate, 10% roughage, 33 mg monensin/kg of diet; and 6) 90% concentrate, 10% roughage, 0 mg monensin/kg of diet. Composition of the experimental diets is shown in Table 1.

Steers were housed individually in concrete slatted pens (5 × 4 m) at the Nutrition Physiology barn and had free choice access to fresh water. Steers were allowed 22 d of dietary adaptation. On d 22 steers were placed in metabolism crates and samples were collected from d 23 through d 28. At the beginning of Period 3, three steers were removed from the experiment and replaced with three alternate steers of similar size and BW, due to their inability to comfortably stand up and lie down in the metabolism stanchions. Additional experimental periods were used so that replacement steers could complete the Latin square. Experimental procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Diets. The grain portion of the ration was dry-rolled corn and the roughage portion was alfalfa hay and cotton seed hulls. Diets were weighed daily and offered ad libitum to steers on an individual basis; refusals were weighed back daily and subtracted from the intake of each steer. Diet samples were taken daily at feeding during the sampling period, composited, dried in a forced air oven (50°C, 48 h), and ground in a Wiley mill to pass a 2-mm screen for later determination of DM, OM, N, starch, and NDF.

In Situ Dacron Bags. Two grams of ground (2-mm screen) as-fed feed was placed in Dacron bags (5 × 13 cm) in order to maintain the recommended sample size: surface area ratio between 10 and 20 mg/cm² (Vanzant et al., 1998). On day 23 at 2000, the 48-h Dacron bags were suspended below the particulate mat layer in the rumen.

Subsequent Dacron bags were added to represent 24, 16, 8, 4, and 2 h time periods. On day 25 at 2000, all Dacron bags were simultaneously removed from the rumen and washed in cold water in a washing machine on the delicate setting (1 min rinse and 2 min spin) 5 times in accordance with Coblenz et al. (1997). Zero hour bags were not incubated in the rumen, but were included in the washing procedure. Dacron bags were dried in a forced-air oven (50°C, 48 h), air equilibrated and weighed to determine DM disappearance. N remaining in the bags was determined by a combustion method (Leco, NS2000, St. Joseph, MI) and assumed that feedstuffs contained 16 g N per 100 g of protein.

Rumen Samples: On day 27 at 0800, 250 mL of Co-EDTA was pulse-dosed intraruminally as a fluid dilution marker, and 1 kg of a blend of Erbium acetate-labeled dry rolled corn and Ytterbium acetate-labeled alfalfa (in the same ratio as the experimental diet) replaced 1 kg of the diet as a particulate matter marker. Ytterbium labeling procedures for alfalfa were the same as outlined by Teeter et al. (1984), whereas Erbium labeling procedures for corn were the same as outlined by Sindt et al. (1993). Ruminal samples were collected before dosing (0 h) and at 3, 6, 9, 12, 15, 18, 21 and 24 h post dosing to determine pH, Co and VFA concentrations. Ruminal fluid samples were collected from several places in the rumen, strained through 4 layers of cheese cloth, composited, and pH was determined using a combination electrode. An 8 mL aliquot was acidified with 2 mL of 25% (wt/vol) metaphosphoric acid and frozen (-20°C) immediately for VFA analysis. An additional 10 mL aliquot was frozen (-20°C) immediately for Co analysis. Ruminal particulate samples will be dried in a forced air oven (50°C, 48 h), and ground through a Wiley Mill to pass a 2-mm screen for Er and Yb

analysis. Cobalt-EDTA preparation was the same as described by Prigge and Varga (1980).

Fecal samples: On day 23 through 26 total feces were collected daily and a subsample composited. A portion of the subsample was dried in a forced-air oven (50°C, 48 h) to determine DM. The remaining subsample was frozen and later lyophilized to determine N, ash, NDF and starch.

Laboratory analysis. Ground samples of feed and feces were analyzed for DM and OM according to standard procedures (AOAC, 1996). Nitrogen content of feed, lyophilized feces, insitu samples was determined by the combustion method (Leco NS2000, St. Joseph, MI: AOAC, 1996). Neutral detergent fiber concentrations of feed and feces were determined by the Van Soest et al. (1991) method. Feed and fecal total starch content were determined by the starch hydrolysis assay procedure (Megazyme, Bray, Co. Wicklow, Ireland: AOAC, 1996) adopted from McCleary et al. (1997). Ruminal fluid samples for Co analysis were thawed and centrifuged at 10,000 X g for 12 min. Cobalt concentration in ruminal fluid was determined by ICP analysis. Ytterbium and Er from ruminal particulate samples was extracted by rocking a 0.2 g sample for 1 h in EDTA solution. Ytterbium and Er concentrations were determined by ICP analysis.

Calculations: Fluid dilution rates of Co and particulate passage rates of Yb and Er were calculated by the regression of the natural log of the marker concentration over time. Extent of ruminal CP degradation was determined by the Mathers and Miller (1981) equation: Extent of CP degradation (percent) = $A + B \cdot [K_d / (K_d + K_p)]$, where A is the instantly soluble fraction of N (percentage of original N weighed into the bag) that disappeared at 0 h by the rinsing procedure. The C fraction is considered to be

completely undegradable and is defined as the N remaining at 48 h (Nocek and English, 1986). The remaining N is the B fraction and is considered to be potentially degradable true protein. Fraction A, plus the rumen degradable portion of the B fraction, is considered to be RDP. Rumen undegradable protein can be assumed to be 100-RDP. A constant rate of passage (K_p) from the rumen of 0.05 h^{-1} was used. The rate (K_d) of N disappearance from the rumen was estimated as the slope of the regression of the natural logarithm of the percentage of the nonsoluble N remaining vs. incubation time in the rumen.

Statistical Analyses. Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., 2003). The model for total tract digestibility and rate of passage included period, concentrate level, monensin, and concentrate level \times monensin. The model for in situ kinetic data included period, concentrate level, monensin, protein source, and all the two and three way interactions. Ruminal pH and VFA data were analyzed as repeated measures. The model consisted of period, concentrate level, monensin, time of collection, and the two and three way interactions. All models included steer as a random effect. Restricted maximum likelihood methods were used to estimate variance components, and the Satterthwaite method was used to approximate degrees of freedom. For each analyzed variable, the covariance structure of the repeated measures was selected based on fit statistics. When an effect was significant ($P < 0.05$), concentrate level Least squares means were separated using least significant difference methods. All tests for significance were reported at the $P < 0.05$ level.

RESULTS AND DISCUSSION

There were no roughage:concentrate ratio \times monensin interactions ($P > 0.10$) for response variables measured in this experiment. Therefore, main effects of roughage:concentrate ratio and monensin are shown and discussed separately.

Effects of roughage:concentrate ratio

Intake of DM (expressed as kg/d or as %BW) did not differ among roughage:concentrate ratio (Table 2). Starch intake increased ($P < 0.01$) and NDF intake decreased ($P < 0.01$) with increasing level of concentrate. Fecal DM, OM, and NDF output decreased ($P < 0.01$) with increasing level of concentrate. Rotger et al. (2005) reported that DM and NDF digestibility were greater in a 30:70 diet compared with a 12:88 roughage:concentrate ratio diet, which is contrary to the present results. As expected, fecal output of starch was lowest ($P = 0.01$) for the 70:30 roughage:concentrate ratio treatment. Apparent total tract digestibility of DM, OM, and NDF increased ($P < 0.01$) as concentrate level increased, whereas apparent total tract starch digestibility did not differ ($P = 0.28$) among roughage:concentrate ratios.

Particulate passage rates determined from labeled corn ($P = 0.94$) and alfalfa ($P = 0.37$) did not differ among roughage:concentrate ratio treatments. Liquid dilution rate was not significantly different ($P = 0.26$) among treatments; however, there was a numerical increase for the 70:30 roughage:concentrate ratio diet. The numeric increase in liquid passage rate with increasing roughage is consistent with Rotger et al. (2005), who reported that calves fed a 30% roughage diet had a greater liquid dilution rate than calves fed a 12% roughage diet. In addition, Choat (2001) showed liquid dilution rate

increased as roughage level increased from 7.5, 15, 25 and 35% of the diet. In the present experiment, ruminal pH decreased ($P < 0.001$) and total VFA concentration increased ($P < 0.01$) as concentrate level increased (Table 2). In contrast, Rotger et al. (2005) reported no effect of roughage level on ruminal pH or total VFA concentrations in 30% vs. 12% roughage diets. Rotger et al. (2005) also noted that feeding the 30% roughage diet tended ($P = 0.098$) to increase the molar proportion of acetate, and decreased isovalerate molar proportion compared with feeding a 12% roughage diet. It is generally accepted that increasing concentrate increases molar proportions of propionate and thus decreases the acetate:propionate ratio. In the present experiment, molar proportions of individual VFA and acetate:propionate did not differ ($P \geq 0.42$) among roughage:concentrate ratios. Reasons for the lack of response are unclear.

In situ kinetics of ruminal DM and CP degradability are shown in Table 3a,b,c. There was a roughage:concentrate ratio \times byproduct interaction ($P < 0.05$) for fractions B and C for in situ DM degradability. In general, the potentially rumen degradable fraction B of byproduct feeds decreased and the rumen undegradable fraction C increased as concentrate level increased with the exception of BM, which remained similar across roughage:concentrate ratios. A similar response was observed for rate of DM degradability and rumen degradable DM, which decreased as concentrate level increased except for BM (roughage:concentrate ratio \times byproduct interaction, $P < 0.05$). Results from the present experiment are similar to Cronje (1992) who determined that 48 h in situ DM degradation increased as the roughage:concentrate ratio increased from 25:75 to 75:25, although the percentage change varied among different feedstuffs common to South Africa. Conversely, Miller et al. (1991) demonstrated that after 16 h of ruminal

incubation DM and N disappearance increased with an 80% concentrate ration (13% CP) compared with low-quality prairie hay (4.5% CP). Discrepancies among experiments are most likely related to forage quality, ruminal ammonia concentrations, and OM fermentation.

There were no roughage:concentrate ratio \times byproduct interactions ($P > 0.10$) for fractions B and C of in situ ruminal CP degradability (Table 3a,b,c). In situ ruminal CP degradability decreased ($P < 0.01$) with increasing concentrate level. Similar to DM, a roughage:concentrate ratio \times byproduct interaction ($P < 0.05$) was observed for rate of ruminal CP degradability, which decreased as dietary concentrate level increased except for BM (Table 6). Rotger et al. (2005) observed that the effects of increasing dietary roughage level on ruminal protein degradation was supplement dependent, increasing with peas and lupin seeds, decreasing with sunflower meal, and resulting in no change for SBM. It is interesting to note that the Nutrient Requirements of Dairy Cattle (NRC, 2001) reports two values for RUP, one value for a 25% roughage diet (DMI = 2% BW), and a second value for a 50% roughage diet (DMI = 4% BW; typical DMI for a lactating dairy cow). As a result of the greater DMI, in all cases the RUP values reported are higher for the 50% roughage diet.

Within roughage:concentrate ratio, in situ kinetics varied among byproduct feeds (Table 3). Rate of ruminal CP degradation was SBM>SH=CSM>DDG>WM>BM ($P < 0.05$). Rankings were similar for all roughage:concentrate ratios except for the 10:90 roughage:concentrate ratio, in which SH>CSM=DDG ($P < 0.05$). Rankings for rumen degradable protein were WM>SBM=SH>DDG>CSM>BM ($P < 0.05$),

WM>SBM>SH=DDG>CSM>BM ($P < 0.05$), and WM>SBM>SH=DDG>CSM>BM ($P < 0.05$) for 70:30, 40:60, and 10:90 roughage:concentrate ratio, respectively.

Vanzant et al. (1998) discussed the potential effects of roughage:concentrate ratio on digestion of substrates in situ. They suggested that dietary factors that influence the rumen microbial population (e.g., starch content) will potentially affect the rate and (or) extent of digestion within synthetic bags. Weakley et al. (1983) reported lower in situ digestibilities of protein-based concentrates when the proportion of dietary roughage decreased. Weakley et al. (1983) suggested that this response may have been related to physical, as well as microbial differences. Slime produced on high-grain diets could block pores. In addition, the abrasive action associated with incubation in a forage mat may help to clear foreign materials from pores and thus increase digestion of substrates (Vanzant et al., 1998). Therefore, in addition to changes in dietary substrate and microbial populations, physical effects associated with the in situ methodology might influence rates and extent of ruminal digestion of protein sources in situ.

Effect of monensin (33 mg/kg)

Monensin had no effect on DM, OM, NDF or starch intake, fecal output, or apparent total tract digestibility (Table 4), which is consistent with the findings of Zinn (1994). Particulate passage rates determined from labeled corn ($P = 0.49$) and alfalfa ($P = 0.33$) were not affected by feeding monensin, although liquid dilution rate tended ($P = 0.08$) to be greater when monensin was fed. In contrast, Schelling (1984) reported that monensin may decrease ruminal particulate passage and liquid dilution rate.

The slight numeric increase in total VFA concentrations (97.9 vs. 104.3 mM) with the monensin diets were not significant ($P = 0.14$), and inconsistent with the 20% increase in VFA concentration observed by Lana and Russell (1997) in three all forage diets. Molar proportion of acetate tended ($P = 0.07$) to decrease and molar proportion of propionate numerically ($P = 0.15$) increased with monensin supplementation, resulting in a numerical ($P = 0.17$) decrease in acetate:propionate. Molar proportion of isobutyrate tended ($P = 0.07$) to increase when monensin was fed, whereas molar proportions of butyrate ($P = 0.43$) and valerate ($P = 0.84$) were not affected by feeding monensin.

There were no monensin \times byproduct feed interactions ($P > 0.10$) for in situ ruminal kinetics. Across all sources, monensin decreased ($P < 0.01$) the percentage of potentially rumen degradable DM and CP, and increased ($P < 0.01$) the percentage of rumen undegradable DM and CP (Table 5). Rate of DM and CP degradation (%/h) and percentage of rumen degradable DM and CP were decreased by 15.2, 16.5, 4.3, and 7.1%, respectively, when monensin was fed. Present data are consistent with numerous studies demonstrating monensin's ability to reduce ruminal protein degradation and increase the amount of amino acid nitrogen reaching the abomasum in high-forage (Haimoud et al., 1996; Ruiz et al., 2001) and in high-concentrate (Poos et al., 1979; Lana et al., 1997) diets.

IMPLICATIONS

Increasing level of concentrate resulted in a decrease in ruminal DM and N degradation of byproduct feeds by decreasing the potentially degradable true protein fraction and the rate of ruminal degradation, and increasing the ruminally undegraded

fraction. With the exception of blood meal (which remained relatively constant), all byproducts used in this experiment responded this way. Monensin showed a protein sparing effect regardless of roughage:concentrate ratio. Because dietary factors affect in situ degradation, in situ incubations should be conducted in rumens of animals that are consuming the diet of interest. Thus, to generate data directly applicable to a particular production situation, diets that most closely approximate those production conditions should be used.

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Table 1. Ingredient and nutrient composition of experimental diets

Item	Roughage:Concentrate Ratio		
	70:30	40:60	10:90
Ingredients, % DM			
Alfalfa hay	67.5	40.0	10.0
Cotton seed hulls	2.5	0.0	0.0
Corn, dry rolled	25.4	49.7	79.5
Cane molasses	4.3	4.3	4.3
Soybean meal (48%)	0	4.6	3.8
Urea	0	0	1.0
Supplement ^{a,b}	0.3	1.4	1.4
Nutrient composition			
NE _m , Mcal/kg	1.51	1.79	2.08
NE _g , Mcal/kg	0.89	1.12	1.35
CP, %	14.1	14.1	14.0
Ca, %	1.06	1.06	0.61
P, %	0.26	0.29	0.29

^a Supplement included salt and limestone. Formulated to provide 0.3 ppm zinc, 30 ppm selenium, 40 ppm manganese, 10.5 mg/kg tylosin, 3,500 IU/kg vitamin A.

^b Experimental diets including monensin were formulated to include 33 mg/kg monensin.

Table 2. Effects of roughage:concentrate ratio on intake, total tract digestibility, and ruminal kinetics

Item	Roughage:Concentrate Ratio			SEM	P
	70:30	40:60	10:90		
Nutrient intake					
DM, kg	12.71	11.50	12.79	1.04	0.20
DM, %BW	1.78	1.68	1.80	0.17	0.57
OM, kg	11.44	10.58	12.21	0.95	0.09
Starch, kg	1.71 ^a	3.25 ^b	5.04 ^c	0.22	< 0.01
NDF, kg	5.54 ^a	3.35 ^b	2.07 ^c	0.32	< 0.01
Nutrient output					
DM	3.80 ^a	2.41 ^b	1.45 ^c	0.38	< 0.01
OM, kg	2.90 ^a	1.89 ^b	1.17 ^c	0.30	< 0.01
Starch, g	31.8 ^a	86.8 ^b	88.6 ^b	17.6	0.01
NDF, kg	2.10 ^a	0.97 ^b	0.39 ^c	0.19	< 0.01
Digestibility, %					
DM	73.1 ^a	81.2 ^b	89.2 ^c	1.9	< 0.01
OM	72.8 ^a	80.1 ^b	88.2 ^c	1.9	< 0.01
Starch	96.1	94.7	96.3	1.3	0.28
NDF	63.0 ^a	71.7 ^b	79.1 ^c	2.7	< 0.01
Rate of ruminal passage, %/h					
Corn	2.85	2.40	2.66	0.92	0.94
Alfalfa	1.90	4.47	4.56	1.52	0.37
Liquid dilution rate	6.01	4.98	4.62	0.69	0.26
Ruminal pH	6.30 ^a	5.90 ^b	5.60 ^c	0.10	< 0.001
Total VFA, mM	86.6 ^a	107.7 ^b	108.9 ^b	9.0	< 0.01
Acetate, mol/100 mol	60.8	62.3	60.2	2.1	0.42
Propionate, mol/100 mol	23.7	23.2	24.2	1.8	0.78
Acetate:Propionate	2.65	2.88	2.68	0.20	0.58
Isobutyrate, mol/100 mol	0.80	0.74	0.89	0.25	0.46
Butyrate, mol/100 mol	10.8	10.1	10.7	0.80	0.46
Isovalerate, mol/100 mol	2.18	2.01	2.23	0.15	0.46
Valerate, mol/100 mol	1.42	1.65	1.68	0.24	0.62

^{a,b,c}Means within rows differ (P<0.05).

Table 3a. Effects of 70:30 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability

Item	70:30						SEM
	SH	WM	DDG	CSM	SBM	BM	
DM							
A, %	22.8	22.8	22.8	22.8	22.8	22.8	
B, % ^a	64.1 ^u	64.1 ^u	64.1 ^u	64.1 ^u	64.1 ^u	64.1 ^u	2.3
C, % ^a	13.1 ^u	13.1 ^u	13.1 ^u	13.1 ^u	13.1 ^u	13.1 ^u	2.3
%/h ^{a,b}	2.18 ^u	2.18 ^u	2.18 ^u	2.18 ^u	2.18 ^u	2.18 ^u	0.10
RDDM, % ^{a,c,d}	50.7 ^u	50.7 ^u	50.7 ^u	50.7 ^u	50.7 ^u	50.7 ^u	1.4
CP							
A, %	24.1	24.1	24.1	24.1	24.1	24.1	0.0
B, % ^e	64.0 ^u	64.0 ^u	64.0 ^u	64.0 ^u	64.0 ^u	64.0 ^u	2.2
C, % ^e	11.9 ^u	11.9 ^u	11.9 ^u	11.9 ^u	11.9 ^u	11.9 ^u	2.2
%/h ^{a,b}	2.06 ^u	2.06 ^u	2.06 ^u	2.06 ^u	2.06 ^u	2.06 ^u	0.12
RDP, % ^{c,f}	52.1 ^u	52.1 ^u	52.1 ^u	52.1 ^u	52.1 ^u	52.1 ^u	1.5

^a Interaction of roughage:concentrate and byproduct feed ($P < 0.05$).

^b Estimated as the slope of the regression of the natural logarithm of the percent remaining vs. ruminal incubation time.

^c Determined according to the Mathers and Miller (1981) equation assuming a constant passage rate (k_p) of ruminal undegradable CP of 0.05 h^{-1} .

^d RDDM = rumen degradable DM.

^e Effect of roughage:concentrate ($P < 0.01$).

^f RDP = rumen degradable protein.

^{u,v,w,x,y,z} Means within rows within a roughage:concentrate differ ($P < 0.05$).

Table 3b. Effects of 40:60 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability

Item	40:60						SEM
	SH	WM	DDG	CSM	SBM	BM	
DM							
A, %	22.8	22.8	22.8	22.8	22.8	22.8	
B, % ^a	51.3 ^u	51.3 ^u	51.3 ^u	51.3 ^u	51.3 ^u	51.3 ^u	2.3
C, % ^a	25.9 ^{u,v}	25.9 ^{u,v}	25.9 ^{u,v}	25.9 ^{u,v}	25.9 ^{u,v}	25.9 ^{u,v}	2.3
%/h ^{a,b}	1.58 ^u	1.58 ^u	1.58 ^u	1.58 ^u	1.58 ^u	1.58 ^u	0.10
RDDM, % ^{a,c,d}	42.4 ^u	42.4 ^u	42.4 ^u	42.4 ^u	42.4 ^u	42.4 ^u	1.4
CP							
A, %	24.1	24.1	24.1	24.1	24.1	24.1	0.0
B, % ^e	61.1 ^u	61.1 ^u	61.1 ^u	61.1 ^u	61.1 ^u	61.1 ^u	2.2
C, % ^e	14.8 ^u	14.8 ^u	14.8 ^u	14.8 ^u	14.8 ^u	14.8 ^u	2.2
%/h ^{a,b}	1.89 ^u	1.89 ^u	1.89 ^u	1.89 ^u	1.89 ^u	1.89 ^u	0.12
RDP, % ^{c,f}	49.8 ^u	49.8 ^u	49.8 ^u	49.8 ^u	49.8 ^u	49.8 ^u	1.5

^a Interaction of roughage:concentrate and byproduct feed ($P < 0.05$).

^b Estimated as the slope of the regression of the natural logarithm of the percent remaining vs. ruminal incubation time.

^c Determined according to the Mathers and Miller (1981) equation assuming a constant passage rate (k_p) of ruminal undegradable CP of 0.05 h^{-1} .

^d RDDM = rumen degradable DM.

^e Effect of roughage:concentrate ($P < 0.01$).

^f RDP = rumen degradable protein.

^{u,v,w,x,y,z} Means within rows within a roughage:concentrate differ ($P < 0.05$).

Table 3c. Effects of 10:90 roughage:concentrate ratio and byproduct feed on in situ kinetics of ruminal protein degradability

Item	10:90						SEM
	SH	WM	DDG	CSM	SBM	BM	
DM							
A, %	22.8	48.2	22.8	48.2	22.8	48.2	
B, % ^a	44.2 ^u	26.6 ^v	44.2 ^u	26.6 ^v	44.2 ^u	26.6 ^v	2.3
C, % ^a	33.0 ^u	25.3 ^v	33.0 ^u	25.3 ^v	33.0 ^u	25.3 ^v	2.3
%/h ^{a,b}	1.21 ^u	0.44 ^v	1.21 ^u	0.44 ^v	1.21 ^u	0.44 ^v	0.10
RDDM, % ^{a,c,d}	37.3 ^u	61.6 ^{v,y}	37.3 ^u	61.6 ^{v,y}	37.3 ^u	61.6 ^{v,y}	1.4
CP							
A, %	24.1	51.0	24.1	51.0	24.1	51.0	0.0
B, % ^e	58.8 ^u	40.2 ^v	58.8 ^u	40.2 ^v	58.8 ^u	40.2 ^v	2.2
C, % ^e	17.1 ^u	8.8 ^v	17.1 ^u	8.8 ^v	17.1 ^u	8.8 ^v	2.2
%/h ^{a,b}	1.82 ^u	0.76 ^{v,w}	1.82 ^u	0.76 ^{v,w}	1.82 ^u	0.76 ^{v,w}	0.12
RDP, % ^{c,f}	47.9 ^u	73.7 ^v	47.9 ^u	73.7 ^v	47.9 ^u	73.7 ^v	1.5

^a Interaction of roughage:concentrate and byproduct feed ($P < 0.05$).

^b Estimated as the slope of the regression of the natural logarithm of the percent remaining vs. ruminal incubation time.

^c Determined according to the Mathers and Miller (1981) equation assuming a constant passage rate (k_p) of ruminal undegradable CP of 0.05 h^{-1} .

^d RDDM = rumen degradable DM.

^e Effect of roughage:concentrate ($P < 0.01$).

^f RDP = rumen degradable protein.

^{u,v,w,x,y,z} Means within rows within a roughage:concentrate differ ($P < 0.05$).

Table 4. Effects of monensin on intake and total tract digestibility

Item	0 mg/kg	33 mg/kg	SEM	<i>P</i>
Nutrient Intake				
DM, kg	12.76	11.90	1.04	0.19
DM, %BW	1.79	1.72	0.17	0.48
OM, kg	11.80	11.01	0.95	0.19
Starch, kg	3.47	3.20	0.26	0.12
NDF, kg	3.67	3.64	0.39	0.92
Nutrient Output				
DM, kg	2.58	2.52	0.49	0.30
OM, kg	2.03	1.94	0.38	0.71
Starch, g	60.9	77.3	22.5	0.31
NDF, kg	1.19	1.12	0.24	0.70
Digestibility, %				
DM	81.5	80.9	2.4	0.74
OM	84.7	84.3	2.0	0.62
Starch	96.3	95.1	1.3	0.17
NDF	70.2	72.3	3.6	0.43
Rate of ruminal passage, %/h				
Corn	2.23	3.04	0.93	0.49
Alfalfa	2.81	4.47	1.40	0.33
Liquid dilution rate	4.56	5.85	0.60	0.08
Ruminal pH	5.95	5.92	0.09	0.72
Total VFA, mM				
Acetate, mol/100 mol	62.4	59.8	2.0	0.07
Propionate, mol/100 mol	22.8	24.6	1.7	0.15
Acetate:Propionate	2.87	2.60	0.16	0.17
Isobutyrate, mol/100 mol	0.72	0.89	0.07	0.07
Butyrate, mol/100 mol	10.3	10.8	0.70	0.43
Isovalerate, mol/100 mol	2.03	2.25	0.13	0.17
Valerate, mol/100 mol	1.61	1.56	0.19	0.84

Table 5. Effects of monensin on in situ kinetics of ruminal protein degradability

Item	0 mg/kg	33 mg/kg	SEM	<i>P</i>
DM				
A, %	30.8	30.8		
B, %	41.4	37.2	1.5	<0.01
C, %	27.8	32.0	1.5	<0.01
%/h ^a	1.14	0.99	0.06	<0.01
RDDM, % ^{b,c}	48.7	46.7	1.0	<0.01
CP				
A, %	24.1	24.1		
B, %	53.1	47.8	1.4	<0.01
C, %	22.8	28.1	1.4	<0.01
%/h ^a	1.62	1.39	0.07	<0.01
RDP, % ^b	46.6	43.5	0.9	<0.01

^a Estimated as the slope of the regression of the natural logarithm of the percent remaining vs. ruminal incubation time.

^b Determined according to the Mathers and Miller (1981) equation assuming a constant passage rate (k_p) of ruminal undegradable CP of 0.05 h⁻¹.

^c RDDM = rumen degradable DM.

^d RDP = rumen degradable protein.

Table 6. Comparison of in situ protein kinetics to NRC (1996)

Item	NRC, 1996	Roughage:Concentrate Ratio		
		70:30	40:60	10:90
Soybean hulls				
CP, % DM	12.2	12.7	12.7	12.7
RDP, % of CP	75.0	52.1	49.8	47.9
RUP, % of CP	25.0	47.9	50.2	52.1
Wheat middlings				
CP, % DM	18.7	17.4	17.4	17.4
RDP, % of CP	79.0	75.5	74.4	73.7
RUP, % of CP	21.0	24.5	25.6	36.3
Soybean meal				
CP, % DM	54.0	49.9	49.9	49.9
RDP, % of CP	66.0	57.3	55.1	51.7
RUP, % of CP	34.0	42.7	44.9	48.3
Cottonseed meal				
CP, % DM	46.1	44.9	44.9	44.9
RDP, % of CP	57.0	40.3	38.7	33.0
RUP, % of CP	43.0	59.7	61.3	67.0
Dried distiller's grains/solubles				
CP, % DM	30.4	29.7	29.7	29.7
RDP, % of CP	48.0	48.5	47.2	45.4
RUP, % of CP	52.0	51.5	52.8	54.6
Porcine blood meal				
CP, % DM	93.8	95.5	95.5	95.5
RDP, % of CP	25.0	6.8	7.4	6.7
RUP, % of CP	75.0	93.2	92.6	93.3

VITA

Scott S. Swanek

Candidate for the Degree of

Doctor of Animal Science

Thesis: PROTEIN DEGRADATION IN RUMINANTS

Major Field: Animal Science

Biographical:

Personal Data: Born in Edmonton, Alberta, Canada, on August 22, 1973, the son of Rod and Mary Swanek. Husband of Christina Swanek and father to Taylor, Madison, Mikail, and Jonathan.

Education: Graduated from Lethbridge Collegiate Institution, Lethbridge, Alberta, Canada in May 1991; received Bachelor of Animal Science from Oklahoma State University, Stillwater, Oklahoma in May 1995. Completed the requirements for the Master of Animal Science Degree at Oklahoma State University, Stillwater, Oklahoma in December 1997. Completed the requirements for the Doctor of Animal Science at Oklahoma State University, Stillwater, Oklahoma in May 2007.

Professional Experience:

In November 2005 I started my own business, Swanek Nutrition Consulting, providing nutritional advice and ration balancing, economic assessments, customized protein blending and bidding, and complete analysis of production records, environment, and cow comfort to dairy producers in Minnesota, Iowa, and Wisconsin. Prior to this I was employed by Standard Nutrition Company for almost two years providing similar services in southeast Minnesota, to what I am currently doing. Other work experiences include, graduate research assistant, Oklahoma State University; Relief A.I. technician, Genex Cooperative Inc.; Dairy specialist, MoorMan's Inc. / ADM Alliance Nutrition; General laborer, Oklahoma State University Purebred Beef Livestock Center; Ultrasound research assistant, Agriculture Canada Research Station.

Name: Scott S. Swanek

Date of Degree: May 2007

Institution: Oklahoma State University

Location: Stillwater, OK

Title of Study: RUMINAL PROTEIN DEGRADATION IN BEEF CATTLE

Pages in Study: 65

Candidate for the Degree of Doctor of Animal Science

Major Field: Animal Nutrition

Scope and Method of Study: In Exp. 1, five ruminally cannulated steers were used in a 5 x 5 Latin square design experiment to determine the effects of increasing urea level on intake, nutrient digestion, nitrogen balance and ruminal kinetics. Isocaloric and isonitrogenous steam-flaked corn diets with urea concentrations of 0.0, 0.8, 1.2, 1.6, or 2.0% (DM basis) were offered ad libitum to steers. Total urine and feces, and ruminal fluid were collected. In Exp. 2, nine ruminally-cannulated steers were used in a 6 x 6 Latin square design experiment to evaluate three roughage:concentrate (70:30, 40:60, and 10:90) ratios with or without monensin on the extent and rate of in situ ruminal degradation of soybean hulls (SH), wheat middlings (WM), dried distillers grains with solubles (DDG), cotton seed meal (CSM), soybean meal (SBM), and porcine blood meal (BM), and on ruminal kinetics.

Findings and Conclusions: In Exp. 1, dietary urea level did not affect ($P>0.10$) DM intake. Urea level did not affect ($P>0.10$) fecal output of OM and starch, or OM, N, or starch digestibility. Urea concentration did not affect ($P>0.10$) total N balance, N balance as a percent of N intake, or liquid dilution rate and pH. Our data suggests that urea levels up to 2% of diet DM can be used in steam-flaked corn finishing diets without altering intake, nutrient digestion, or ruminal kinetics. In Exp. 2, monensin had no effect ($P>0.10$) on DM, OM, NDF or starch intake, output or digestibility, or total VFA concentration. Monensin decreased ($P<0.01$) the N and DM B fraction of in situ ruminal degradation and the rate of N and DM degradation, and increased the C fraction of N and DM. Ruminal protein degradation was decreased by 6.7% ($P<0.01$) for monensin supplementation, suggesting monensin has a protein sparing effect. Dry matter and OM intake, starch digestibility and molar proportions of individual VFA did not differ among roughage:concentrate ratios. Fecal OM and NDF output decreased ($P<0.01$), and digestibility and total VFA concentration increased ($P<0.01$) with increasing level of concentrate. As level of concentrate increased, the B fraction and the rate of degradation decreased and the C fraction increased, resulting in decreased DM and N ruminal degradation ($P<0.01$). All of the byproducts responded similarly except blood meal, which remained relatively constant across treatments. Because dietary factors affect in situ degradation, in situ incubations should be conducted in rumens of animals that are consuming the diet of interest.

ADVISER'S APPROVAL: Dr. Clint Krehbiel