Winter 2009

Evaluation of in vitro methods to determine the digestibility of amino acids in rumen undegraded corn silage

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EVALUATION OF IN VITRO METHODS TO DETERMINE THE DIGESTIBILITY OF AMINO ACIDS IN RUMEN UNDEGRADED CORN SILAGE

BY

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Bachelor of Science, University of Minnesota, 2007

THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Master of Science

in

Animal and Nutritional Sciences

December, 2009

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ACKNOWLEDGEMENTS

I want to thank the Department of Biological Sciences and the University of New Hampshire Graduate School for financially supporting the project, my course work, and my stipend during the school year and for granting me the Summer TA Fellowship during both of my summer terms. The help I have received from the faculty within the Department of Biological Sciences, the staff, and my fellow graduate students is greatly appreciated.

I want to thank the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada. In particular, I want to thank Dr. Hélène Lapierre for her collaboration and assistance with the project. I also want to thank Mario Leonard for his dedicated work during the ruminal in situ incubation and the mobile bag technique preformed at the Dairy and Swine Research and Development Centre.

I want to acknowledge the William H. Miner Institute for allowing us the use of their lab to conduct crude protein analysis. I especially want to thank Stephen Kramer for his help and technical service throughout our stay. I also want to thank Kurt Cotanch for setting up our visit to the Miner Institute.

I want to thank the staff of the University of New Hampshire Fairchild Dairy including Jon Whitehouse, John Weeks, and Roger Comeau for taking care of the cows used in the experiment.

I want to acknowledge Sapienza Analytica, LLC for allowing me to conduct the in vitro experiment and for assisting with the project. In particular, I want to thank the employees of Sapienza Analytica, LLC including Tara Downs, Bobbie Marten, and Dana

Cox for chopping and collecting the whole plant corn samples, for assisting in the completion of in vitro analysis, and for care of the animals used in the experiment.

I especially want to thank Scott Talbot for all of his assistance on the project. Scott and I started our thesis projects at the same time and have been working in a similar field of study. Scott's assistance was instrumental in completing the project and I am grateful for his help.

I want to acknowledge the time that Nancy Whitehouse spent working with me. Nancy was very helpful in the design and statistical analysis of the project. Nancy is involved in about every experiment at both University of New Hampshire dairy farms, and her help is surely appreciated.

I want to thank my committee members including Dr. Sarah Boucher, Dr. Peter Erickson, Dr. Paul Kononoff, and Dr. Donald Sapienza. Their guidance throughout the project not only improved the quality of the thesis but also helped me become a better student.

I want to thank my advisor, Dr. Charles Schwab. I will forever be grateful to Dr. Schwab for giving me the opportunity to study at the University of New Hampshire. Dr. Schwab, and his wonderful wife, Sandy, are the kindest people I know and it has been a pleasure to have them as a part of my life.

Lastly, I want to thank my parents, Roger and Deborrah Fredin. Both of my parents have been extremely generous, forgiving, and supportive during my time in academics.

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ABSTRACT

EVALUATION OF IN VITRO METHODS TO DETERMINE THE DIGESTIBILITY OF AMINO ACIDS IN RUMEN UNDEGRADED CORN SILAGE

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University of New Hampshire, December, 2009

Intestinal and total tract digestibility of crude protein (CP) and amino acids in rumen undegraded protein (RUP-AA) in 5 corn silage hybrids were measured using the mobile bag technique (MBT). Two in vitro methods, the modified three-step procedure (TSP) and an in vitro procedure (IVP) developed by Sapienza Analytica, LLC (Slater, IA), were further evaluated to determine digestibility of RUP-AA in corn silage. Intestinal digestibility of RUP-AA in corn silage varied from 24 to 59%, indicating differences in intestinal digestibility among AA. The TSP and IVP tended to under predict RUP-AA digestibility compared with the MBT. Consequently relationships between the in vitro methods and the MBT for RUP-AA digestibility were poor. The TSP and IVP tended to be better predictors of total tract digestibility compared with the MBT. These results suggest the TSP and IVP are accurate in vitro methods to estimate total tract digestibility of CP and AA.

CHAPTER I

REVIEW OF THE LITERATURE

Introduction

In ruminant nutrition, dietary crude protein (CP) is required both by rumen microbes and by the host animal. Rumen degradable protein (RDP) is needed to meet the N requirements of the rumen microbes for microbial protein synthesis. Ruminally synthesized microbial protein flows to the small intestine where it is available for digestion and the component amino acids (AA) may be absorbed. Ruminally synthesized microbial CP, ruminally undegraded feed CP (RUP), and to a limited degree endogenous CP contribute to the metabolizable protein (MP) pool that is absorbed by the small intestine of ruminant animals (NRC, 2001). Ruminally undegraded feed CP refers to the fraction of dietary protein that is not degraded in the rumen and arrives at the small intestine intact. Endogenous CP represents protein within saliva and sloughed epithelial cells from the gastro-intestinal tract. The absorbed AA provided by ruminally synthesized microbial CP, RUP, and endogenous CP are essential as the building blocks for the synthesis of tissue and milk proteins, as well as other metabolic functions in the body.

The goal of ruminant nutrition is to optimize the efficiency of utilization of dietary N for growth, milk production, and milk protein production (Schwab, 1995). This goal may be accomplished by first providing adequate amounts of RDP for optimal ruminal efficiency with a minimum amount of dietary CP. Feeds should be chosen to

provide the types and amounts of RDP that will meet, but not exceed, the N needs of ruminal microorganisms for maximal synthesis of microbial CP while reducing excretion of wasted N (NRC, 2001). Secondly, types and amounts of digestible RUP should be selected to optimize the profile and amounts of absorbed AA required by the animal. The AA composition of the mixed ruminal microbial population, and of endogenous protein, is assumed to be constant, but the AA composition of RUP may vary among feeds (NRC, 2001). Consequently, the AA composition of the total protein passing to the small intestine may be influenced by the amount and the AA composition of RUP. However, the intestinal digestibility of RUP and individual AA within RUP (RUP-AA)) is highly variable depending on feed type and processing (O'Mara et al., 1997; NRC, 2001). As the contribution of RUP-AA to MP increases, knowing the intestinal digestibility of RUP-AA becomes increasingly important to better meet the AA requirements of dairy cows (Calsamiglia and Stern, 1995).

A ration formulated for a lactating cow typically contains 40-60% forage. Corn silage (CS) is a primary forage in dairy cow rations in the Northeast (Boucher et al., 2007) and continues to be a major forage and energy source in United States (Johnson et al., 1999). Therefore, determining digestibility of RUP and RUP-AA of CS is important. The amount of CP and the proportion of RDP and RUP in forages may vary depending on type, stage of maturity, processing, and storage (Johnson et al., 1999; NRC, 2001). In addition, intestinal digestibility of RUP-AA in forages such as CS may vary.

Current ration evaluation systems do not account for variations in post-ruminal digestibility of RUP-AA. More information is needed regarding digestibility of RUP-AA to improve these models. Advancements in ration evaluation systems will improve

protein utilization by dairy animals and thereby decrease animal N excretion. Data in the literature regarding digestibility of RUP-AA are limited, due to the difficulty in obtaining these estimates in ruminant animals (Boucher et al., 2009b), as ruminants need to be surgically fitted with multiple cannulas to allow for intestinal digestibility estimates. Alternative in vivo and in vitro techniques to measure digestibility of RUP-AA are needed to reduce the need for invasive surgeries.

Milk Protein Synthesis

Amino acids are required by the animal for synthesis of proteins such as body tissue, growth of the fetus, enzymes, peptide hormones, and milk protein. Therefore, it is of importance to understand protein synthesis and in the case of lactating dairy cows, the synthesis of milk proteins in the mammary gland including transcription, translation, and post-translational modification of proteins.

Protein synthesis begins with transcription of messenger RNA (mRNA) from DNA in the nucleus. The enzyme RNA polymerase by binding to the template strand, also called the sense DNA strand, synthesizes mRNA. RNA polymerase will read the DNA base pairs in the 3' prime to 5' prime direction and create the mRNA in the 5' prime to 3' prime direction. Each of the four base pairs, cytosine, guanine, thymine, and adenine, which make up DNA corresponds to a base pair for the creation of the RNA strand. For example, cytosine (C) codes for guanine (G) and vise versa, and uracil (U), replacing thymine, codes for adenine (A) and vise versa.

Translation begins as the new mRNA leaves the nucleus and enters the cytosol. Ribosomes located on the rough endoplasmic reticulum and in the cytosol are responsible for translating the mRNA to AA for polypeptide synthesis. Amino acids are coded by

groupings of three base pairs. There are 20 AA required for protein synthesis, but only four nucleotide base pairs. To compensate, a triplet code, or three nucleotide base pairs called codons translate to each AA. There are a total of 64 codons. Ribosome begins reading the base units of the mRNA until the start codon is read. For example, AUG codes for Met (see appendix B for glossary of amino acids). Translation requires 61 types of transfer RNA (tRNA), one for each codon (except stop codons). Transfer RNA are small RNA molecules that turns back and coil on itself to form a cloverleaf shape, which is then twisted into an angular L shape. One end of the L includes three nucleotides called the anticodon, and the other end has a binding site specific for one of the 20 AA. Each tRNA picks up an AA from the pool of free AA in the cytosol. As the ribosome reads a codon, it must find an activated tRNA with the corresponding anticodon. The ribosome binds and holds the tRNA as a synthetase enzyme forms a peptide bond between the AA and the growing peptide chain. The formation of a peptide bond requires energy and depends on the cleavage of ATP carried by the tRNA. The entire process is then repeated until a stop codon is read and the polypeptide is completed.

Proteins destined for secretion, such as milk proteins, are directed to the Golgi apparatus for packaging into secretory vesicles and secretion via exocytosis. After synthesis in the rough endoplasmic reticulum and cytosol, modifications to secretory proteins may also occur in the Golgi apparatus. These post-translational modifications can change the function of the protein.

Protein synthesis will be met to the extent of the availability of the first limiting amino acid. Therefore, once the first limiting AA is no longer available, synthesis may

cease. This is of particular concern for Met, which is the starting AA for the synthesis of all peptides. Thus, rations for lactating dairy cows should be balanced for AA requirements, in particular the first and second limiting AA to maximize synthesis of protein.

Corn Silage Production

Production of high quality forages used in ruminant animal diets is a principal factor in the success of formulating low cost, energy efficient rations. Several keys to producing and feeding high quality corn silage include hybrid selection, maturity at harvest, adequate packing, and optimal fermentation of the forage.

One goal is to select a hybrid to maximize the amount of energy available as forage to the ruminant animal (Lauer, 2003). This can be accomplished by selecting a hybrid which either maximizes milk produced per ton of harvested corn forage indicating high quality, or milk produced per acre of harvested corn forage indicating high yield. Different corn forage traits include the brown midrib (bmr) variety in which the stover portion of the plant is lower in lignin content and higher in fiber digestibility and a leafy variety in which leaf numbers are increased above the ear (Lauer, 2003). Other varieties include genetically modified hybrids such as Bt or Roundup Ready® corn. University of Wisconsin feeding trails found that bmr corn silage was higher in quality but lower in yield when compared with other varieties and that the leafy corn silage variety was greater in yield but lower in quality when compared with other varieties (Lauer, 2003). Based on quality and yield, the genetically modified varieties typically fell in between the bmr and leafy hybrids (Lauer, 2003).

Corn silage should be harvested when the whole plant is 35 ± 2-3% DM (Kung, 2009). Corn silage harvested at lower DM (< 28-30%) results in the production of excessive fermentation acids, lowered palatability due to this higher acid, and ultimately decreased DM. Corn silage harvested with elevated DM (> 40%) limits fermentation and may be difficult to pack resulting in poor aerobic stability (Kung, 2009). Dry matter of corn forage can be predicted by milk line of the kernel (Ashley, 2001). Once kernels become dented, a milk line appears across the kernel separating the hard starch from the soft dough. This milk line advances down toward the cob as maturity increases. When the hard starch line approaches the cob, a black layer will form. Ideally most corn silage will be harvested from 1/3 milk line to black layer maturity, which represents 32 to 38% moisture (Ashley, 2001). Once the milk line appears whole plant corn DM will increase an average of 0.5 to 0.75% per day (Lauer, 2003). Although milk line is a quick method to predict corn forage moisture, it is not accurate due to difference between hybrids and yearly weather conditions (Roth and Heinrichs, 2001). Therefore, moisture content of corn forage should be determined using a Koster moisture tester, microwave, or oven.

Freshly harvested corn forage should be filled into the silo as quickly as possible and covered with plastic to minimize heating and aerobic respiration. Delivery rate should not exceed the packing rate to ensure a minimal packing density of at least 14-lb/cubic foot (Ross, 2003). Ideal packing density can be achieved by packing 1 to 4 min per ton of forage and layering chopped forage from 6 to 12 inches in the bunker (Ross, 2003). Once the corn forage is properly covered, oxygen levels begin to dissipate and anaerobic fermentation begins. Bacteria present on the corn forage use available plant sugars to produce primarily lactic acid. Lactic acid causes a drop in the pH and

fermentation will continue until enough lactic acid is produced to drop the pH to approximately 4.2, at which point all bacterial action stops. This process will usually take three weeks from the time the silo was filled. If pH does not drop to 4.2, or if overly wet corn forage is harvested, butyric acid is produced and the silage may spoil.

Proper production and management of CS such as choosing types of hybrids best suited for the region and farm system, harvesting at optimal DM, and correctly packing harvested corn forage will increase the nutritive value of CS fed to ruminant animals.

The Nutritive Value of Corn Silage

As a source of nutrition for ruminant animals, the primary contribution of whole plant CS is as a source of fiber and energy (Weiss and Wyatt, 2002). The composition of CS contains stover, the primary source of neutral detergent fiber (NDF) in CS, and grain, the primary source of starch in CS. Minimum recommendations of NDF for dairy cattle are about 25% when NDF from forage comprises 19% of the diet (NRC, 2001). While starch is not believed to be a required nutrient for dairy cows, starch is the principal nonstructural carbohydrate in most feedstuffs (NRC, 2001) and a source of both glucose and propionate used for milk production. Values reported by NRC (2001) and state extension services for NDF and starch content in normal CS range from 30-45 and 25-35% respectively, which makes CS an ideal feed for use in dairy cow and heifer rations to help meet both fiber and energy requirements. The contribution of CS as a dietary protein source is limited due to low concentrations of CP. Reported values of CP content in CS range from (mean ± SD) 9.7 ± 2.2% of DM for immature CS to 8.5 ± 3.9% of DM for mature CS (NRC, 2001). Corn silage is also a poor source of Lys and Met, two frequently limiting AA for lactating cows (Schwab et al., 1992; NRC, 2001).

Quality of CS is affected by stage of maturity at harvest (Johnson et al., 1999). Decreases in digestibility of DM and NFC in CS have been reported with elevated DM (Russell, 1986), thought to be a result of high ADF and lignin concentrations (Russell et al., 1987). Increases in maturity may also have an effect on the rate of ruminal degradation of CP (Johnson et al., 2003). In one experiment, medium maturity CS had significantly greater CP disappearance than more mature CS at 8, 24, and 48 h of ruminal incubation, and in a second experiment early maturity CS had greater CP disappearance compared with advanced maturities at 8, 16, 24, 48, and 96 h of ruminal incubation (Johnson et al., 2003).

Management practices such as mechanically processing whole plant corn, silage additives, and hybrid selection can influence digestibility and quality of CS. Processing whole plant corn can be achieved with the use of onboard processors or rollers to decrease the theoretical length of cut of the forage, reduce cob and stalk size, and to fragment whole corn kernels. Processing often increases starch digestibility in mature CS (Bal et al., 2000; Weiss and Wyatt, 2000), but results have been inconsistent (Johnson et al., 2003). Johnson et al. (2003) reported an increase in ruminal degradation of CP measured at 24 and 48 h incubation for mechanically processed CS when compared with unprocessed CS. However, the increase in CP degradation was not evident for ruminal incubation times less than 24 h. Whole plant corn can be inoculated when chopping to favorably change the fermentation pattern during ensiling. Common additives include *Lactobacillus plantarum*, *L. buchneri*, *Pediococcus acidilactici*, *P. pentocaceus*, and *Enterococus faecium*. Additives are helpful for the prevention of clostridial fermentation,

the enhancement of aerobic stability, and increasing the quality of fermentation (Muck, 2008).

Recently reported data suggests that increased ensiling time of corn silage increases ruminal degradation of NDF and starch (Hallada et al., 2008). Measured by ruminal and intestinal batch culture in vitro methods, ruminal digestibility of NDF at 30 h increased 1.2% per month of ensiling, and total tract starch digestibility increased 1.6% per month of ensiling. Both measurements remained constant after 6 months of ensiling. The increase in nutrient digestibility may be due to the extended time in a low pH environment. It has also been speculated that increased ensiling time may increase protein solubility and increase the rate of microbial degradation of NDF and starch in the rumen.

In Vivo Digestibility Techniques

Ruminally degradable protein, RUP, and RUP-AA digestibility estimates are typically determined by in vivo measurements. In vivo measurements of RDP are obtained using the in situ procedure, while digestibility of RUP and RUP-AA is determined by the mobile nylon bag technique, and nonruminant bioassays.

In Situ Procedure

The in situ procedure has been the most commonly used method for estimating ruminal CP degradation due in part to its low-cost and ease of use (Stern et al., 2007). Feeds weighed into polyester bags are inserted through a ruminal cannula for a predetermined incubation period, typically 0, 2, 4, 8, 16, 48, and 72 hours to measure disappearance of feed CP from the bag. The in situ procedure can then be used to identify the A, B, and C protein fractions, and the rate of degradation (Kd) of fraction B

(NRC, 2001). Fraction A is the protein fraction that disappears from the bag at time 0 and is assumed to be instantly degraded in the rumen. Fraction B includes potentially degradable protein and is affected by the rate of passage. Fraction C is considered to be completely undegradable in the rumen and passes to the small intestine.

Several factors can affect values obtained with the in situ procedure including: particle size of the sample, porosity of the bag, ratio of sample weight to bag surface area, composition of the ration fed to the animal, and attachment of particle associated bacteria (PAB; Stern et al., 1997). Recommendations for a standardized in situ protocol are provided in table 5-6 (pg. 62) of the NRC (2001) in order to decrease potential error when reporting estimates of ruminal protein degradation.

Detachment of microorganisms from rumen undegraded residue. Once the polyester bags are removed from the rumen, it is necessary to rinse the bags to remove microbes from the rumen undegraded residue (RUR; Mehrez and Ørskov, 1977). Ideally, all microbes are removed by rinsing, so that digestibility of CP (Mass et al., 1999) and passage of AA to the small intestine is not overestimated (Whitehouse et al., 1994). However, PAB are more difficult to remove from undigested feed and a method to correct for microbial contamination is necessary (NRC, 2001). Both physical (washing and homogenization using a stomacher) and chemical treatments (neutral detergent solution and methylcellulose), as well as a combination of both types of treatments have been employed to increase the detachment of PAB from RUR (Whitehouse et al., 1994; Martínez et al., 2009).

Mass et al. (1999) proposed refluxing RUR in neutral detergent solution (NDS) without sodium sulfite to remove PAB. Solka floc, a highly digestible fiber byproduct of

the wood pulping industry was ruminally incubated to determine the PAB contamination and capability of NDS to detach PAB from RUR (Mass et al., 1999). The intact solka floc and ruminally undegraded solka floc refluxed in NDS both contained 0.1% N, while the ruminally undegraded solka floc not corrected for microbial contamination contained 0.47% N. These results support that RUR is contaminated with PAB and that refluxing RUR in NDS may remove all PAB.

Whitehouse et al. (1994) evaluated four techniques to detach PAB and other microorganisms from ruminal digesta. The four techniques evaluated were: 1) saline and 0.1% Tween 80 solution shaken with marbles for 1 h in a 4°C water bath; 2) saline, 0.1% Tween 80, and 1.0% methanol solution shaken with marbles for 1 h in a 4°C water bath; 3) saline, 0.1% Tween 80, 1.0% methanol, and 1.0% tertiary butanol shaken with marbles for 1 h in a 4°C water bath; and 4) 0.1% methylcellulose solution for 10 min at 37°C in a shaking water bath. Based on direct counting methods, the four techniques removed 65, 72, 83, or 80% of PAB. The last two techniques appeared to be superior in detaching PAB (Whitehouse et al., 1994).

Martínez et al. (2009) evaluated three different techniques to identify the best method to detach PAB from forages incubated in Rusitec fermenters. The three techniques included 1) incubation of residue in saline and 0.1% methylcellulose for 15 min at 37°C in a shaking water bath; 2) residue mixed with saline solution and placed in stomacher standard bags and homogenized with a Stomacher for 5 min; and 3) frozen digesta thawed at 4°C for 24 h followed by repeating technique 2. The observed detachment of PAB, determined using ^{15}N as a microbial marker, was 65, 72, or 69%, respectively.

Refluxing RUR in NDS may remove all PAB from RUR (Mass et al., 1999), however, if the sample has been ruminally incubated for only a short period of time (i.e., 2 to 8 h) digestible sugars and starches and soluble proteins may still be present in the residue. As these nutrients are removed during the NDS wash when analyzing for neutral detergent fiber, they may be washed out during NDS refluxing, depending on the duration and intensity of the technique (Kononoff et al., 2007). This loss of undigested nutrients may cause an overestimation of digestibility. Although the stomacher increases detachment of PAB, the transfer of RUR from polyester bags to stomacher bags may increase loss of material. Incubating RUR in saline and methylcellulose does not remove as much PAB using a stomacher or refluxing in NDS. However, it may be the best method currently available.

Mobile bag technique

First used for estimating digestibility of protein in pigs (Sauer et al., 1983), and further developed for use in ruminants (Kirkpatrick and Kennelly, 1984; Rooke, 1985; Rae and Smithard, 1985), the mobile bag technique is the most commonly used method to determine protein digestibility in the small intestine of ruminants (NRC, 2001; Schwab et al., 2003). Although requiring the need for ruminally and duodenally cannulated animals, the mobile bag technique is relatively easy and provides a more physiological approach to measuring protein digestibility than the use of chemical analysis (NRC, 2001). Feeds are first ruminally incubated, which is generally considered necessary as estimates of protein and AA digestibility (Varvikko and Vanhatalo, 1991; O'Mara et al., 1997; Piepenbrink and Schingoethe, 1998) of intact feedstuffs may differ from protein and AA digestibility estimates of ruminally undegraded feed residues. For example,

Varvikko and Vanhatalo, (1991) measured intestinal digestibility of CP and AA in intact grass silage and grass silage ruminally incubated for 16 h in four cannulated heifers. Crude protein and AA digestibility of the intact grass silage was 78 and 80% respectively, while the CP and RUP-AA digestibility of ruminally undegraded grass silage was 57 and 65% respectively.

Following ruminal incubation, samples are incubated in a pepsin-HCl solution, generally for 1 h, to mimic the conditions of the abomasum. The unopened bags are then introduced into the duodenum via a duodenal cannula and then collected either from the terminal ileum if a cannula is present, or more typically from the feces. After collection from the feces, the bags are rinsed thoroughly to remove endogenous protein and decrease microbial contamination. After correction for microbial contamination, indigestible residue is analyzed for N and AA content and digestibility of N and AA is calculated as the disappearance from the bag.

In the NRC (2001) feed library, intestinal RUP digestibility of CS is 70%. The intestinal digestibility estimates reported by Frydrch (1992), Hvelplund et al. (1992), Kopency et al. (1994), Van Straalen et al. (1997), and values reported by Jarrige (1989) in Table 13.3 of *Ruminant Nutrition: Recommended Allowances and Feed Tables* were summarized to arrive at this value; however, the data used to generate this value are highly variable. Frydrych (1992) and Kopency et al. (1994) measured intestinal RUP digestibility of CS using the mobile bag technique. Corn silage samples were ruminally incubated in situ for 16 h, and RUP digestibility was measured with collection of bags (pore size = 45 μm) in the feces. Digestibility of RUP was observed to be 48% of total RUP in both studies. Hvelplund et al. (1992) also measured intestinal RUP digestibility

of CS using the mobile bag technique. Samples were ruminally incubated in situ for several time points between 2 and 96 h and digestibility of RUP was measured with collection of bags (pore size = 9 μm) in the feces. The mean RUP digestibility for all time points was observed to be 39% of total RUP. Von Keyserlingk et al., (1996) determined RUP digestibility of twelve CS samples using the mobile bag technique in one cow. Samples were ruminally incubated for 12 h in nylon bags (pore size = 52 μm), and bags were collected from the feces. Bags were not incubated in pepsin/HCl prior to intestinal insertions. Intestinal digestibility of RUP was 11% of total RUP. Van Straalen et al. (1997) measured intestinal RUP digestibility of CS using the mobile bag technique in four Holstein cows, three lactating cows fed a 40% forage and 60% concentrate ration and one non-lactating cow fed 95% forage and 5% concentrate diet. Samples were ruminally incubated in situ for 12 h in nylon bags (pore size = 41 μm) and RUP digestibility was determined with collection of bags from the feces. The reported RUP digestibility of CS was observed to be 64% of total RUP.

More recently, Trinácty et al. (2003) evaluated RUP digestibility of CS using three ruminally and duodenally cannulated crossbred cows fed a ration consisting of 89% forage and 11% concentrate. Samples were ruminally incubated for 16 h prior to the mobile bag technique. The observed RUP digestibility of CS was 81%. Danesh Mesgaran and Stern (2005) determined RUP digestibility of CS treated with either 16 or 24 g of urea per kg of DM in four Holstein steers fed a 70% forage, 30% concentrate diet. Samples were ruminally incubated in situ for 12 h in artificial silk cloth (pore size = 48 μm). Bags were then inserted into the small intestine at a rate of one bag every 30 min and collected from the feces. Post ruminal disappearance of RUP for CS containing

either 16 or 24 g of urea per kg of dry matter was 31 and 52%, respectively. Kononoff et al. (2007) measured RUP digestibility of CS in steers fed two different diets consisting of either 0% wet corn gluten feed or 38% wet corn gluten feed using the mobile bag technique with collection of bags from the feces. Corn silage was ruminally incubated in situ for 22 h in Dacron bags (pore size = 50 μm). Intestinal RUP digestibility of CS was observed to be 20% in animals consuming both diets.

The average RUP digestibility from these eight summarized experiments that directly measured intestinal digestibility of RUP of CS (mean ± SD) was 44 ± 22%. This average indicates that the RUP digestibility value of 70% reported in NRC (2001) may be too high. Inaccurate estimates of RUP digestibility of CS by NRC (2001) may lead to over predicting the contribution of RUP to MP and over predicting MP available for metabolic functions including synthesis of tissue and milk protein. Therefore, more accurate estimates of digestibility of RUP in CS may lead to improved predictions of the contribution of RUP to MP.

Van Straalen et al. (1997) also determined intestinal digestibility of RUP-AA and the intestinal digestibility of non-protein-N (NPN) in CS. Non-protein-N was calculated as total N minus AA-N. Total essential AA (EAA) digestibility, total non-essential AA (NEAA) digestibility, and NPN digestibility was 74, 69, and 50%, respectively. Intestinal digestibility of Arg in CS was 100%, whereas the intestinal digestibility of Cys in CS was 53%. The intestinal digestibility of His in CS was extremely low at 5% of available His. Non-protein-N was lower in digestibility than both EAA and NEAA. Van Straalen (1997) suggested that NPN present in the RUR may be linked to components indigestible in the intestine such as lignin, Maillard reaction products, and tannins.

Differences in methodology for the mobile bag technique are quite evident (Beckers et al., 1996) and currently, no standardized protocol exists when using the mobile bag technique (Boucher et al. 2009a). Intestinal digestibility estimates derived from the mobile bag technique can be affected by several factors including: placement of cannulas, site of bag recovery, particle size of the sample, bag porosity (Stern et al., 1997; NRC, 2001), anti-nutritional factors present in the feedstuff (Yin et al., 2002), and length of incubation (Haugen et al., 2006). Incubation time of forages in the rumen can influence intestinal digestibility RUP (Beckers et al., 1996) and digestibility of RUP may be overestimated when length of ruminal incubation periods are too short (Von Keyserlingk et al., 1996). Several estimations of RUP digestibility of forages in the literature are based on ruminal incubations of 16 h or less, which may not reflect the true residence time of forage particles in the rumen (Varvikko and Vanhatalo, 1991, Haugen et al., 2005). The true residence time of forage particles in the rumen for in situ incubation periods has been estimated as 75% of the true mean retention time (TMRT) of forages (Haugen et al., 2006). For example, Haugen et al. (2006) estimated 75% of TMRT for grass pasture samples to vary from 23 to 30 h and Kononoff et al. (2007) estimated 75% of TMRT for CS, alfalfa hay, alfalfa haylage, and brome hay to be 22, 22, 23, and 25 h, respectively. If intestinal digestibility of feeds can be affected by the length of ruminal incubation, then the intestinal digestibility of RUP in forages determined from common in situ incubation times such as 12 and 16 h may not accurately reflect the true intestinal digestibility of RUP in forages.

Precision-fed Rooster Assay

Cecectomized roosters have been used in a precision-fed rooster assay as an animal model to estimate intestinal digestibility of AA in the RUP fraction of feeds for ruminants (Titgemeyer et al., 1990). The ceca are surgically removed so that most of the fermentative capacity of the gastrointestinal tract is removed. To obtain the RUP fraction, feeds are ruminally incubated in situ prior to determining intestinal digestion, and the RUR are crop-intubated to cecectomized roosters. Titgemeyer et al. (1990) estimated digestibility of AA in duodenal digesta using the precision-fed rooster assay and intestinally cannulated steers. Freeze-dried digesta collected from steers was crop-intubated to five cecectomized roosters to determine digestibility of AA. Amino acid digestibility estimates obtained with the precision-fed rooster assay were highly correlated (r^2 = 0.94) with estimates obtained in the cannulated steers. Griffin et al. (1993) determined intestinal digestibility RUP and RUP-AA in raw soybeans, soybean meal (SBM), and extruded SBM using the precision-fed rooster assay. Samples were ruminally incubated for 16 h in calves prior to rooster intubation. Intestinal digestibility of RUP and RUP-AA of the different soybean sources determined by the precision-fed rooster assay corresponded closely with measured N retention of calves fed diets containing the samples (Griffin et al., 1993). Aldrich et al. (1997) also measured the intestinal digestibility of RUP-AA of soybean feeds using the precision-fed rooster assay. Whole and roasted soybeans and extruded SBM were ruminally incubated in steers for 16 h prior to rooster intubation. Intestinal digestibility of RUP-AA in samples measured using the precision-fed rooster assay compared favorably with trypsin inhibitor activity in the whole, roasted, and extruded SBM supporting the precision-fed rooster assay as an in

vivo model to determine intestinal digestibility of RUP-AA of soybean feeds in ruminant animals.

A preliminary trial conducted by Dr. Carl Parsons at the University of Illinois (Urbana-Champaign) in collaboration with the University of New Hampshire, determined that the precision-fed rooster assay is not a suitable in vivo model to estimate intestinal digestibility of RUP of highly fibrous feeds. The bulky nature of the fibrous feeds limits the total amount of residue that can be safely intubated into the cecectomized roosters and limits the total amount of excreta that is generated from each bird for AA analysis (Parsons, personal communication). The RUR was reported to be highly indigestible (data not published).

In Vitro Techniques for Estimating Digestibility of RUP and RUP-AA

Obtaining intestinal digestibility estimates in ruminant animals is expensive, labor-intensive, and requires the use of surgically cannulated animals (Calsamiglia and Stern, 1995). An in vitro procedure to estimate RUP-AA digestibility would allow for more routine, rapid analysis of feeds (Boucher et al., 2009b). In vitro methods used to estimate intestinal digestibility of RUP and RUP-AA include the three-step procedure (TSP) (Calsamiglia and Stern, 1995), a modified version of the TSP (Gargallo et al., 2006), and an unpublished, commercial, in vitro procedure (Sapienza Analytica LLC; Slater, IA; SALLC).

Three-step procedure

The TSP of Calsamiglia and Stern (1995) is the most commonly used in vitro procedure to determine CP and RUP digestibility of protein supplements. The three steps of the procedure include: 1) ruminal incubation, 2) pepsin-HCl incubation, and 3)

pancreatin incubation. Intact feeds are ruminally incubated for 16 h, as this represents the average retention time of feeds in the rumen (Calsamiglia and Stern, 1995). Rumen residue is incubated for 1 h in a 1 N HCl solution containing 1 g/L of pepsin. After incubation, pH is neutralized with 1 N NaOH, a pH 7.8 phosphate buffer containing 3 g/L of pancreatin is added to the solution, and these samples are incubated at 38°C for 24 h. The protein denaturant, trichloroacetic acid (TCA) solution, is added to precipitate undigested protein (Stern et al., 1997). However, TCA is a highly corrosive and toxic acid for humans and the environment (Gargallo et al., 2006). Use of TCA also prohibits AA quantification of the digested sample via cation-exchange HPLC, which is the most common method for AA analysis (Boucher et al., 2009b) and therefore the TSP is not used to estimate digestibility of RUP-AA.

Kopency et al. (1994) measured intestinal RUP digestibility of CS using an in vitro pancreatin assay similar to the TSP of Calsamiglia and Stern (1995). Corn silage samples were ruminally incubated in situ for 16 h, then incubated in a pepsin-HCl solution for 2 h, and in a pancreatin phosphate buffer solution for 26 h. In this study, RUP digestibility of CS was 50%. Kopency et al. (1994) compared in vivo results of 55 various concentrates and forges obtained by the mobile bag techniques to the in vitro pancreatin assay. The correlation coefficient was equal to 0.93.

Danesh Mesgaran and Stern (2005) evaluated the TSP using several plant varieties including CS. Corn silage samples were ruminally incubated in situ for 12 h. After an in situ incubation, all samples were then incubated in a pepsin-HCl solution for 1 h followed by pancreatin phosphate buffer incubation for 24 h. In vitro digestibility of RUR for corn silage containing either 16 or 24 g of urea per kg of dry matter were 35 and

38%, respectively. The relationship between CP disappearance obtained by the mobile bag technique and the TSP was determined for several feeds and a low correlation for total tract ($r^2 = 0.50$) and post ruminal CP digestibility ($r^2 = 0.26$) was reported.

Modified three-step procedure

The modified TSP, developed by Gargallo et al. (2006), allows for the determination of digestibility estimates of RUP-AA. Rumen undegraded residue are incubated in polyester bags in a Daisy^{II} batch incubator with the same digestive enzymes and chemicals used in the TSP of Calsamglia and Stern (1995), except for the exclusion of TCA. The indigestible residue is analyzed for AA content, and intestinal RUP-AA digestibility can be calculated as percentage disappearance of AA from the bags. Gargallo et al. (2006) measured the CP digestibility of several protein supplements with the original and modified TSP. The average estimate of intestinal CP digestion using the modified TSP (71%) was similar to the TSP (69%). A high correlation was also observed ($r^2 = 0.84$) between intestinal digestibility of RUP of protein supplements determined with the original and modified TSP; however, the authors did not validate in vitro RUP digestibility measurements with in vivo data.

Boucher et al. (2009) evaluated several in vitro methods including the original and modified TSP to estimate intestinal digestibility of AA in RUP for supplemental protein feeds such as distillers dried grain with solubles (DDGS), soybean meal (SBM), and fish meal (FM). The average estimate of intestinal RUP digestion obtained from the TSP for DDGS, SBM, and FM was 68, 70, and 71%, respectively. The average estimate of intestinal RUP digestion obtained from the modified TSP for DDGS, SBM, and FM was 83, 86, and 85%, respectively. Of the methods evaluated, the modified TSP was

highly correlated (R^2 = 0.93) with RUP-AA digestibility determined via the precision-fed cecectomized rooster assay.

SALLC in vitro assay technique

A commercially available in vitro procedure was developed by SALLC in 2008 to measure NDF, starch, and protein digestibility both ruminally and post ruminally. Procedures for the ruminal in vitro incubations are outlined by Bossen et al. (2008). Briefly, samples are incubated in rumen fluid, rumen buffer, macro-mineral solution, and micro-mineral solution in an air-jacketed anaerobic incubator at 39°C and 20% CO_2. Bossen et al. (2008) determined ruminal degradation of NDF of several feeds using the in situ procedure, an in vitro method developed by Goering and Van Soest (1970), or an vitro method modified by lowering the pH of the rumen fluid. The modified in vitro method (pH = 6.0) tended to be a better predictor of NDF degradation kinetics than the original in vitro method (pH = 6.8) when compared with the in situ procedure, however the lower pH of the modified in vitro method increased lag time of NDF degradation. Nutrient degradation appeared to be dependent on pH of the system.

Although the in vitro system of Bossen et al. (2008) did not measure CP and AA degradation, other in vitro systems have been evaluated for accuracy in predicting ruminal CP degradation (Broderick 1978; Broderick, 1987). Broderick (1978) measured ruminal degradation of casein using an in vitro system. Ten mL of strained ruminal fluid and McDougall's buffer were mixed in a 1:1 ratio with 10 mg of casein in 50 mL tubes under CO_2 flow and heated to 39°C. Results from the in vitro system were used to predict ruminal degradation and passage rates of casein by measuring the production of ammonia and free AA in the tubes. A similar approach by Broderick (1987) was used to

measure the ruminal degradation and passage rates of casein, meat and bone meal, solvent treated and expellers SBM, and alfalfa hay. Although these results were not compared with in vivo measurements, ruminal degradation rates of the various feeds were in agreement with data obtained using similar in vitro systems.

Ration evaluation model predictions of RUP Digestibility

Ration evaluation models to determine nutrient requirements of dairy cattle currently available include the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992), NRC (2001), and the French Institut National de la Recherche Agronomique (INRA) system (Vérité and Peyraud, 1989). These systems are dependent on ruminal degradation rates of feed CP to accurately predict flows of RUP to the small intestine, and are dependant on RUP digestibility coefficients to predict absorbed MP. The NRC (2001) and CNCPS models are further described below.

Cornell Net Carbohydrate and Protein System

The CNCPS model divides dietary protein into three fractions: non-protein nitrogen (NPN) termed fraction A, true protein potentially degradable in the rumen described as fraction B, and unavailable protein termed fraction C (Sniffen et al. 1992). These fractions are determined by chemical analysis to calculate rate and extent of protein digestion. Fraction A is determined as the protein soluble in borate-phosphate buffer and not precipitated with TCA. Fraction C is determined as the protein that is insoluble in acid detergent solution and is described as acid detergent insoluble CP (ADICP). Fraction C represents protein bound to lignin, protein associated with tannin, and protein affected by mallard reactions that are resistant to rumen microbial degradation and mammalian enzymes. Fraction B is further fractionated into three

different categories, B_1, B_2, and B_3, to estimate ruminal degradation and passage kinetics. Fraction B_1 is determined as the protein soluble in borate-phosphate buffer and is precipitated with TCA. This fraction is believed to be soluble protein that is rapidly and primarily degraded in the rumen. Fraction B_3 is the protein soluble in acid detergent solution but insoluble in neutral detergent solution and is the difference between ADICP and neutral detergent insoluble CP (NDICP). Fraction B_3 may represent protein slowly degraded in the rumen, but largely represents bypass protein. Fraction B_2 is the remaining fraction and is determined by the difference between total CP and the sum of fractions A, B_1, B_3, and C. Flow of total RUP to the small intestine is determined by relative rates of degradation and passage of each feed. Rumen undegraded feeds are not assigned intestinal digestibility coefficients, but instead the model assigns digestibility coefficients to the chemical fractions of feeds. The model assigns intestinal digestibility constants of 100, 100, 100, 80, and 0% for the A, B_1, B_2, B_3, and C fractions. Therefore, the model assumes that RUP from all feeds is digested the same.

NRC (2001)

The latest NRC (2001) model applies an alternative method to estimate RUP digestibility. Instead of relying on protein fractionation through chemicals analysis, the model uses animal data derived from the in situ procedure, described earlier, to determine rates of degradation and passage of each feed. Dependent on degradation and passage rates, the model then calculates RUP supplied by each feed. The assigned RUP digestibility coefficients of each feed were determined from reported data obtained from 54 published studies, 48 studies that employed the mobile bag technique, and 6 studies that used the TSP (Calsamiglia and Stern, 1995). If insufficient data were available to

assign a RUP digestibility value to a particular feed, the French INRA (Vérité and Peyraud, 1989) system RUP digestibility values were used. The RUP digestibility coefficients of feeds in NRC (2001) range from 50 to 95% of total RUP.

French INRA System

The French INRA system for protein (referred to as the Protéines vraies reéllement Digestibles dans l'Lntentin grêle; PDI; Vérité and Peyraud, 1989) determines intestinal digestibility of RUP and the contribution of RUP to MP differently than the CNCPS (Sniffen et al., 1992) or NRC (2001) models. The PDI system takes into account an indigestible nitrogen fraction (IDN) unavailable in both the rumen and the small intestine calculated as IDN = Fecal N – 4.62 × FOM – 9.6 × NDOM where FOM refers to fermentable organic matter and NDOM refers to non-digestible organic matter. The PDI system assumes a constant IDN for each feed. Therefore, the true digestibly of each feed in the small intestine (dsi) is calculated as dsi = (UDN-IDN)/UDN, where UDN refers to ruminally undegraded nitrogen.

Improvements to ration evaluation models to better predict RUP digestibility will allow for greater precision in ration formulation. Currently, no model accounts for differences between digestibilities of RUP-AA within feedstuffs, even though differences have been reported (O'Mara et al., 1997; Boucher et al., 2009a).

Conclusion

Corn silage is a forage commonly fed to lactating dairy cows in the United States. The chemical composition, digestibility and palatability of CS can differ substantially depending upon type of hybrid planted, DM at harvest, processing of the plant, and conditions during fermentation and storage. Few measurements have been reported for

the intestinal digestibility of RUP in CS. The reported RUP digestibility values available in the literature are highly variable. These differences lead to difficulties when comparing the data. Even fewer measurements have been reported for the intestinal digestibility of RUP-AA in CS.

Rapid and affordable methods to determine RUP-AA digestibility are needed to generate accurate data. To date, the most common methods to measure digestibility of RUP-AA are conducted using animal models, which require invasive surgeries and are increasingly expensive to maintain. Therefore, rapid and precise in vitro methods to determine RUP-AA digestibility in both forages and concentrates must be developed and evaluated.

CHAPTER II

EVALUATION OF IN VITRO METHODS TO DETERMINE DIGESTIBILITY OF AMINO ACIDS IN RUMEN UNDEGRADED CORN SILAGE

Abstract

A study was conducted to measure intestinal and total tract digestibility of crude protein (CP) and amino acids (AA) in rumen undegraded protein (RUP-AA) in 5 corn silage (CS) hybrids using the mobile bag technique (MBT). Two in vitro procedures, the modified three-step procedure (TSP) and an in vitro procedure (IVP) developed by Sapienza Analytica LLC (Slater, IA), were further evaluated to determine digestibility of RUP-AA. Samples were incubated in situ in 2 ruminally-cannulated lactating dairy cows for either 16 or 24 h. Profile of AA in rumen undegraded residue (RUR) was consistent between CS samples for both incubation periods. The proportion of essential AA in total AA increased in RUR compared with intact CS. For the MBT, six bags per RUR were inserted through duodenal cannulaes of the same two cows used for the in situ incubation and recovered in the feces. Intestinal digestibility of AA in RUR varied from 24 to 59%, indicating differences in intestinal digestibility among AA. Digestibility values were lowest for Cys and Gly and highest for Arg, Met, and Glu. Intestinal digestibility of total AA ranged from 36 to 62% whereas intestinal digestibility of CP varied from 18 to 43%. This suggests that non-protein-N is less digestible than AA, and probably bound to indigestible components within RUR. The large range in intestinal digestibility of AA was not apparent in the total tract digestibility of AA, which ranged from 78 to 89%.

There was no difference ($P > 0.05$) between intestinal digestibility of AA for RUR ruminally incubated at 16 or 24 h, indicating that 16-h rumen incubation is sufficient to determine digestibility of AA in the RUR of CS. For the TSP, ruminal incubation was followed by a 1-h incubation in pepsin-HCl followed by a 24-h incubation in pancreatin. The TSP tended to under predict RUP-AA digestibility compared with the MBT. For the IVP, CS was incubated in a rumen in vitro inoculum followed by a 2-h pepsin-HCl incubation and 6-h pancreatin/amylase incubation. The TSP and IVP tended to under predict RUP-AA digestibility and consequently poor relationships to MBT digestibility of RUP-AA. The TSP and IVP tended to be better predictors of total tract digestibility compared with the MBT. These results suggest the TSP and IVP are accurate in vitro methods to estimate total tract digestibility of CP and AA.

Introduction

Metabolizable protein (MP) available for absorption is primarily the sum of rumen microbial protein and undegraded dietary protein (NRC, 2001). The efficiency by which tissues use AA for protein synthesis may be improved by more closely matching the essential AA (EAA) profile of MP to the EAA requirements of the animal (NRC, 2001). The AA composition and digestibility of a mixed population of ruminal microbes is assumed to be constant, but the AA composition and digestibility of RUP varies among feeds (NRC, 2001). Consequently, the AA composition of MP may be influenced by the amount of RUP and the AA composition of RUP. This results in a need for more consonant estimates of digestibility of RUP and individual AA within RUP (RUP-AA). Intestinal digestibility of RUP (NRC, 2001) and RUP-AA (O'Mara et al., 1997) is highly variable depending on type of feed and feed processing method. Research to date has focused on intestinal digestibility of RUP-AA in plant and animal protein feeds (O'Mara et al., 1997; Van Straalen et al., 1997; Ceresnáková et al., 2002; Prestlokken and Rise, 2003; Borucki Castro et al., 2007; Boucher et al., 2009a). However, data on digestibility of RUP-AA in forages is limited (Van Straalen et al., 1997; Taghizadeh et al., 2005).

Ruminal incubation may increase digestibility of protein and AA of intact feeds (Hvelplund et al., 1992; Boucher et al., 2009a) and because of the degradation of dietary protein by rumen microbes, the AA profile of rumen undegraded residue (RUR) may differ from the AA composition of the intact feed (Pipenbrink and Schingoethe, 1998). Therefore, feeds are typically ruminally incubated prior to measurements of intestinal digestibility. However, when using single incubation times to estimate RUP digestibility, final values are dependent on the length of time of ruminal incubation (Kononoff et al.,

2007). The most common ruminal incubation time is 16 h, as this represents an average residence time of feeds in the rumen (Calsamiglia and Stern, 1995). However, a 16-h ruminal incubation time may be too short (Varvikko and Vanhatalo, 1991) and may not accurately reflect the true residence time of forages in the rumen (Haugen et al., 2005). Consequently, the effect of residence time of forages on intestinal digestibility measurements must be further studied.

Current ration evaluation systems do not account for variation in post-ruminal digestibility of RUP-AA. More information is needed regarding digestibility of RUP-AA to improve these models, but data available in the literature are limited (Schwab et al., 2003). The most common method to measure intestinal digestibility of nutrients is the mobile bag technique (MBT; NRC, 2001; Schwab et al., 2003). However, the MBT is a labor-intensive, costly, and invasive procedure, which requires cannulation at the site of the duodenum and possibly the ileum (Caslamiglia and Stern, 1995). In vitro methods to estimate protein degradation and digestibility would allow for routine and rapid analysis of feeds (Boucher et al. 2009a). The most common in vitro method to estimate intestinal digestion of proteins in ruminants is the three-step procedure (TSP) developed by Calsamiglia and Stern (1995). Typically this procedure is not used to estimate RUP-AA digestibility because TCA is used to precipitate undigested protein at the end of the TSP procedure and the use of TCA does not allow for AA quantification by ion exchange chromatography. A modified version of the TSP was developed by Gargallo et al. (2006) to eliminate the use of TCA. In the modified version of the TSP, rumen undegraded residue (RUR) are incubated in polyester bags in enzymatic solutions. The

undigested residue is then analyzed for AA content by ion exchange chromatography to calculate disappearance of AA from the bags.

An alternative in vitro procedure (IVP) developed by Bossen et al. (2008) to estimate NDF digestibility is currently available commercially (Sapienza Analytica LLC; Slater, IA; SALLC) to estimate both ruminal degradation of NDF and protein and post ruminal digestion of protein and AA. Instead of ruminally incubating feeds in situ prior to intestinal enzymatic incubations, the IVP incubates feeds in pooled ruminal fluid in an anaerobic and temperature stable environment. A robust in vitro method to estimate RUP and RUP-AA digestibility, along with other nutrient digestibility measurements such as NDF, across a diverse set of feeds including forages would allow for a more cost effective and labor efficient means of analysis.

The objectives of this experiment were 1) to determine the digestibility of RUP-AA in five corn silage (CS) samples ruminally incubated for 16 and 24 h using the MBT, and 2) to validate the modified TSP and IVP to estimate digestibility of AA in CS.

Materials and Methods

Collection of Feed Samples

Five unprocessed CS samples were obtained from SALLC. The first hybrid (CS 1) was harvested in September 2007 at 35% DM and ensiled in a 3 x 18 m concrete stave silo at an estimated packing density of 620 kg/m^3 and ensiled for 360 days. The remaining four whole plant corn hybrids (CS 2 through 5) were chopped in September of 2008 averaging (mean ± SD) 32 ± 2% DM. A representative sample of fresh chopped forage from each of the four hybrids was packed into a mini silo (43 cm length x 30 cm diameter) at an estimated packing density of 477 kg/m^3 and ensiled for 180 days. Wet

silages were removed from the silos and dried in a forced, hot air oven (Sheldon Manufacturing, Portland, OR) at 60°C for 48 h. The silages were ground to pass a 6-mm screen using a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ) in preparation for in situ and in vitro analysis.

Ruminal Incubation at Agriculture and Agri-Food Canada

Two lactating multiparous Holstein cows averaging (mean ± SD) 263 ± 37 DIM housed at the Dairy and Swine Research and Development Center of Agriculture and Agri-Food Canada in Sherbrooke, QC (Ag-Canada) were used in this experiment. Rations fed to the cows consisted of 50% forage and 50% concentrate (Appendix A). Procedures for the experimental protocol were approved by the Institutional Animal Care Committee of the Centre under the Canadian Council on Animal Care through Ag-Canada and the Institutional Animal Care and Use Committee at the University of New Hampshire (UNH). To generate rumen undegraded CS samples for use in the MBT, 8 g of sample (ground 2-mm) were weighed into polyester bags each with a mean pore size of 50 μm and dimensions of 10 × 20 cm (R1020, Ankom Technologies, Macedon, NY) sealed using a thermal impulse heat sealer (International Plastics Inc., Greenville, SC). Prior to incubation, the bags were soaked in warm water for 20 min, placed in a polyester laundry bag (46 x 38 cm), clipped to a stainless steel bar weighing 0.6 kg, and placed in the ventral sac of the rumen. Samples were ruminally incubated in situ for 16 and 24 h. Bags were removed in reverse order of incubation period, so that they were all removed at the same time. Once the bags were removed from the rumen, they were immediately immersed in iced saline solution (0.9% NaCl). Bags were thoroughly washed in a washing machine on a 1 min wash cycle and a 2 min spin cycle 6 times in an automatic

washing machine. Bags were then suspended in a saline solution (0.9% NaCl) containing 0.1% methylcellulose (M-0262, Sigma, St. Louis, MO) for transportation to UNH. To decrease microbial contamination of rumen undegraded residue (RUR), samples were processed according to the procedure of Martinez et al. (2009). Briefly, bags were suspended in a saline solution (0.9% NaCl) containing 0.1% methylcellulose at 38°C for 15 min in a continuous shaking water bath (65 strokes/min; Precision Scientific, Chicago, IL), followed by storage at 4°C for 24 h. Bags were then rinsed with cold tap water until runoff was clear, and lyophilized (Labconco, Kansas City, MO) for 48 h. Residues were composited by sample and incubation period in preparation of the MBT.

Mobile Bag Technique

Eight days after the completion of the in situ procedure, the same two cows housed at Ag-Canada were used for the MBT. Cows were equipped with closed T-shaped duodenal cannulas (Berzins Vet Laboratory Ltd., Edmonton, Alberta, Canada). Undegraded CS samples from the 16 and 24 h ruminal incubation were transferred to polyester bags (5 x 3.5 cm; modified Ankom R510 bag). Three bags from each undegraded CS were introduced into the proximal duodenum of each cow (30 bags per cow) at a rate of 1 bag every 15 min. Upon recovery from the feces, the mobile bags were washed in an automatic washing machine (5 x 1-min wash, 2-min spin) until the rinse water was clear. The bags containing RUR were then frozen. Bags were then lyophilized for 48 h and residues were composited by sample and incubation period for CP and AA analysis.

Ruminal Incubation at the University of New Hampshire

Two lactating multiparous ruminally cannulated Holstein cows averaging (mean ± SD) 43 ± 8 DIM housed at UNH were used in a second experiment to determine the intestinal digestibility of RUP-AA in CS using the modified TSP. Rations fed to the cows consisted of 58% forage and 42% concentrate (Appendix A). To generate RUR of the CS samples for use in the modified TSP, 8 g of sample (ground 2-mm) were weighed into polyester bags (R1020, Ankom Technologies). Bags were tied with plastic fastening ties 2 cm below the top of the bag, soaked in warm water for 15 min, and placed inside of 2 mesh laundry bags (46 × 56 cm; Whitney Design, Inc., St. Louis, MO) for ruminal incubation. Samples were ruminally incubated for 16 and 24 hours. The mesh laundry bags were filled with 10 polyester in situ bags per sample for each incubation period so that 100 bags were in each laundry bag.b Nine metal washers (diameter = 4.3 cm; total weight = 115 g) were tied inside of each mesh laundry bag, and a 60-cm string was tied to one end of the bag. Once the bags were removed from the rumen, they were immediately immersed in cold water. The mesh laundry bags were removed in reverse order of incubation period, such that they could all be removed at the same time. Bags were processed according to the procedure of Boucher et al. (2009a), with modifications. The bags were washed for 5 min 3 times in an automatic washing machine with a final spin. To decrease contamination of particle-associated microbes, bags were processed according to the procedure of Martinez et al. (2009). Bags were then lyophilized for 48 h. Residues were composited by sample and incubation period in preparation of the modified TSP.

Modified Three-step Procedure

Rumen undegraded residue was analyzed using the pepsin and pancreatin digestion steps of the modified TSP procedure described by Gargallo et al. (2006). One gram of RUR collected from the in situ procedure described above was weighed into nylon bags (Ankom R510, pore size 50 μm) in duplicate and heat-sealed. Samples were incubated in Daisy^{II} incubator bottles (Ankom, Fairport, NY) containing 2 L of prewarmed pepsin-HCl (Sigma p7000-100G, Sigma-Aldrich®, St. Louis, MO) solution in constant rotation at 39°C for 1 h. A maximum of 30 nylon bags were placed in each Daisy^{II} incubation bottle. After pepsin-HCl incubation, all liquid was drained and the bags were rinsed with cold tap water until runoff was clear. The washed bags were then placed into the incubation bottles and 2 L of prewarmed pancreatin/KH_2PO_4 (Sigma p7545-100G, Sigma-Aldrich®, St. Louis, MO), solution was added and samples were incubated in constant rotation at 39°C for 24 h. After incubation, all liquid was drained and the bags rinsed with cold tap water until runoff was clear. The washed bags were then dried in a forced hot air oven (Sheldon Manufacturing, OR) at 55°C. Undigested residue was collected from the bags and pooled by sample and incubation period for CP and AA analysis.

Sapienza Analytica, LLC In Vitro Procedure

Five CS samples were incubated using a ruminal IVP developed by SALLC according to methods described by Bossen et al. (2008). Eight g of each sample were weighed into polyester bags each with a mean pore size of 50 μm and dimensions of 10 × 20 cm (R1020, Ankom Technologies, Macedon, NY). Samples were incubated in rumen fluid medium containing rumen buffer, macro-mineral, and micro-mineral solutions. The

final medium used for in vitro incubation was prepared in 4 L wide mouth glass incubator bottles using the following solutions:

Ruminal Fluid: Ruminal fluid and contents were collected from 3 ruminally-cannulated Jersey steers using a utility wet/dry vacuum (Shop-vac; Williamsport, PA) from the ventral sac of the rumen. Rations consisted of 75% forage and 25% concentrate (Appendix A). Ruminal fluid was transferred to a 5 L pre-warmed beaker (39°C) under constant flow of CO_2 within 60 sec of collection.

Rumen Buffer Solution: A total of 4.0 g of NH_4HCO_3 and 35.0 g of $NaHCO_3$ dissolved in 1 L of distilled water.

Macro-mineral Solution: A total of 5.7 g of Na_2HPO_4 – anhydrous, 6.2 g of KH_2PO_4 – anhydrous, and 0.6 g of $MgSO_4 \cdot 7H_2O$ dissolved in 1 L of distilled water.

Micro-mineral Solution. A total of 13.2 g of $CaCl_2 \cdot 2H_2O$, 10.0 g of $MnCl_2 \cdot 4H_2O$, 1.0 g of $CoCl_2 \cdot 6H_2O$, and 8.0 g of $FeCl_3 \cdot 6H_2O$ were dissolved in distilled water and brought to a volume of 100 mL.

The final medium contained 450 mL of pooled rumen fluid, 750 mL of rumen buffer solution, 750 mL of macro-mineral solution, 5 mL of micro-mineral solution, and 6 g of tryptone. The final medium was adjusted to pH of 6.5 using citric acid solution (19.2 g citric acid-anhydrous in 100 mL distilled water). Volume of the final medium within each incubator bottle was adjusted to 2 L with distilled water and allowed to equilibrate to 39°C and 20% CO_2 in an air-jacketed anaerobic incubator (Sheldon Manufacturing, Portland, OR). Once equilibration was achieved, 10 polyester bags (Ankom R1020) containing 8 g of CS were placed into each incubator bottle. Bags were incubated for 16 or 24 h. After incubation, all liquid was drained and the bags were

rinsed with cold tap water (11°C) until runoff was clear. One half of the bags were then placed into 40°C neutral detergent solution (NDS) for 15 min to remove particle-associated bacteria. After NDS wash, bags were again rinsed with cold tap water until runoff was clear. The washed bags were then dried in a forced hot air oven (Sheldon Manufacturing, OR) at 55°C to a constant weight. Undigested residue was collected from the bags and pooled by sample.

The remaining bags were analyzed using in vitro digestion steps to determine the intestinal digestibility of RUP-AA. Samples were transferred without drying from the NDS wash into 5 L incubator bottles containing a pre-warmed solution of 800 mL of rumen buffer solution, 800 mL of macro-mineral solution, 100 ml of sodium azide, 45 ml Triton X, and 255 mL of water and placed into the anaerobic incubator for 2 h. After the incubation, all liquid was drained and the bags were rinsed with cold tap water until runoff was clear. The washed bags were then placed into incubation bottles containing 2 L of pre-warmed pepsin-HCl (MP Biomedicals 102598, MP Biomedicals LLC, Solon, OH) solution (pH $\approx$ 3) and placed into the anaerobic incubator for 2 h. After pepsin-HCl incubation, all liquid was drained and the bags were rinsed with cold tap water until runoff was clear. The bags were placed into incubation bottles containing 2 L of a pre-warmed solution at pH 8 containing pancreatin (EMD Chemicals PX0040-1, EMD Chemicals Inc., Gibbstown, NJ), pancreatic lipase (Spectrum Chemicals L1094, Spectrum Chemicals, Gardena, CA), and diastase (MP Biomedicals 101539, MP Biomedicals LLC), and then placed into the anaerobic incubator for 6 h. After incubation, all liquid was drained and the bags rinsed with cold tap water until runoff was clear. The washed bags were then dried in a forced hot air oven (VWR Scientific, West

Chester, PA) at 55°C to a constant weight. Undigested residue was collected from the bags and pooled by sample.

Chemical Analysis

Intact CS were analyzed by Dairyland Laboratories Inc. (Arcadia, WI) for DM, CP, ADF, NDF, lignin, acid detergent insoluble CP (ADICP), neutral detergent insoluble CP (NDICP), fat, starch, ash, and minerals using wet chemistry. Analysis of CP was determined using a combustion analyzer (AOAC, 2006; method 990.03; Leco FP 528, Leco, St. Joseph, MI). Acid detergent fiber was analyzed by acid detergent extraction (AOAC, 2006; method 973.18). Neutral detergent fiber was analyzed by neutral detergent extraction containing amylase and sodium sulfite according to the methods of Mertens (2002). Lignin was determined using procedures outlined by Goering and Van Soest (1979). Starch was analyzed by a dual enzymatic method (combination of amylo/glucosidase) using a YSI 2700 Select biochemistry analyzer (AOAC, 2006; method 996.11; YSI Incorporated, Yellow Springs, OH). Minerals were analyzed using a PerkinElmer Optima 5300 inductively coupled plasma-optical emission spectroscopy system (PerkinElmer Inc., Waltham, MA). Fat was determined by ether extract (AOAC, 2006; method 920.39). Nonfiber carbohydrate was calculated as 100 – [CP + (NDF – NDICP) + fat + ash].

A portion of the intact corn silages, rumen undegraded residues, MBT residues, and in vitro residues were ground to pass a 0.5-mm screen (Wiley Mill, Thomas Scientific, Swedesboro, NJ) for CP and AA analysis via cation-exchange chromatography (cIEC-HPLC) coupled with postcolumn ninhydrin derivatization and quantitation

(AOAC, 2000; method 982.30; Experimental Station Chemical Laboratories, University of Missouri-Columbia).

Calculations and Statistical Analysis

Mobile bag technique and in vitro digestion of CP and AA in the rumen undegraded residue was calculated as follows:

% digested = [(amount of AA in, g, - amount of AA out, g) / amount of AA in, g] × 100.

Differences between CS samples for ruminal DM disappearance were analyzed using the MIXED procedure of SAS (SAS Inst., 2002, Cary, NC). Data were analyzed as a completely randomized design according the following model:

$$Y_{ifk} = \mu + F_i + R_{ij} + L_k + c(F)_{ijkl} + E_{ijkl}$$

Where Y_{ijkl} is the dependent variable; μ is the overall mean; F_i is the fixed effect of the ith corn silage sample (J = 16, 24); R_{ij} is the fixed effect of the ruminal incubation of the ith feed sample and the jth ruminal incuabation; L is the random effect of the kth location of the experiment (k = 1, 2, 3); $c(F)_{ikjl}$ is the random effect of the lth cow with the ith corn silage sample, the jth ruminal incubation and the kth experiment; and E_{ijkl} is the random residual. Tukey's Studentized range test was used to compare least squares means among samples. Significance was declared at $P < 0.05$ and tendencies are reported at $0.05 < P < 0.10$.

Differences between intestinal digestibility of RUP and RUP-AA by ruminal incubation period were determined using the MIXED procedure of SAS (SAS Inst., 2002) according to the following model:

$$Y_{ij} = \mu + F_i + R_{ij} + E_{ij}$$

Where Y is the dependent variable; μ is the overall mean; R is the fixed effect of the ruminal incubation of the ith feed sample.; and E_{ij} is the random residual. Significant differences were declared at $P < 0.05$ and tendencies are reported at $0.05 < P < 0.10$.

The REG procedure of SAS (SAS Inst., 2001) was used to determine the relationship between RUP-AA digestibility measured using the MBT, the modified TSP, and the IVP.

To determine if a mean or linear bias was present in the regression model for RUP-AA digestibility determined via the modified TSP and IVP, the residuals (observed – predicted) were evaluated against predicted values according to the methods of St-Pierre (2003) using the REG procedure of SAS (SAS Institute, 2002) by subtracting the mean predicted value from the individual predicted value. This value along with the residuals were evaluated.

Results and Discussion

The chemical composition and AA profile of the intact CS samples are presented in Table 1 and 2, respectively. Crude protein content in the samples varied between 4.5 and 7.9% while total AA (TAA) content in the samples varied between 3.1 and 4.7%. The NDF content of CS 3 and 4 at 55.6 and 52.9%, respectively were higher than CS 1, 2, and 5 at 36.6, 45.8, and 37.1%, respectively. The NFC content in CS 1 and 5 at 46.2 and 50.9%, respectively were higher than CS 2, 3, and 4 at 41.9, 31.5, and 35.2%, respectively. Starch content of the CS samples averaged 32 ± 9.2%. Essential AA (EAA; % of total AA) ranged from 42 to 45%, similar to values reported in the NRC (2001). The AA in highest concentration were Gly, Leu, and Ala at 10, 11, and 15%, respectively whereas the AA in lowest concentration were Tyr, Met, and Cys, each at 2%

(See appendix B for glossary of amino acids). Concentrations of Trp in CS were below detectable levels and were not reported.

Ruminal Dry Matter Disappearance

Ruminal DM disappearance of CS between locations of experiments are presented in Table 3. Dry matter disappearance at Ag-Canada averaged 57 ± 6.5% and 60 ± 5.1% for CS samples incubated for 16 and 24 h, respectively. Dry matter disappearance observed at UNH averaged 60 ± 4.7% and 66 ± 3.2% for CS samples incubated for 16 and 24 h, respectively. Dry matter disappearance at SALLC averaged 55 ± 7.6% and 54 ± 7.3% for CS samples incubated for 16 and 24 h. Results for ruminal DM disappearance are comparable with published literature data by Van Straalen et al. (1997) and Boucher et al. (2007). Van Straalen et al. (1997) observed 57% DM disappearance after a 12-h ruminal incubation and Boucher et al. (2007) reported a 56% effective ruminal degradability CS. Differences in ruminal DM disappearance were observed for CS samples, dependent on length of ruminal or in vitro incubation. Ruminal disappearance of DM was highest for CS 1 and 5 at 64 ± 2.2 and 64 ± 3.9% respectively and lowest for CS 3 at 51 ± 6.6%, independent of location of the experiment. These results are expected as CS 1 and 5 contain the highest amount of NFC of the five CS samples whereas CS 3 contains the lowest amount of NFC. Typically, DM disappearance was observed to be higher at UNH compared with the Ag-Canada except for CS 1 and 4 incubated for 16 h. The experiment conducted at SALLC yielded the lowest DM degradation results, expect for CS 1, 2, and 3 incubated at 16-h and for CS 1 incubated for 24-h when compared to the results obtained at Ag-Canada. The absence of a difference in DM degradability between the 16 and 24 h samples for the experiment conducted at SALLC may indicate

that rumen microbial activity may have decreased at or before 16-h, which would have diminished degradation of CP and AA. Ration composition at the locations of the experiments may have led to the differences in DM disappearance (Appendix A). The types of rations fed likely resulted in different ruminal microflora within the animals between the sites of the conducted experiments. The ration offered to cows at Ag-Canada contained 50% concentrate and 50% forage compared with the rations at UNH, which contained 58% forage and 42% concentrate and SALLC which contained 75% forage and 25% concentrate. However, ration composition may not affect RUP digestibility. Kononoff et al. (2007) fed two different rations of similar NDF and CP levels containing either 0 or 38% wet corn gluten feed. The differing composition of the rations did not affect RUP digestibility of several feeds including CS (Kononoff et al., 2007).

The CP and AA concentrations and AA profiles of the RUR generated at A-Canada are listed in Table 4. Concentrations of TAA decreased from an average of 3.9% for intact CS to an average of 2.6 and 3% for RUR incubated for 16 and 24 h. Concentrations of CP decreased in RUR after a 16-h rumen incubation from 5.6 to 4.8%, but increased for some RUR samples after a 24-h incubation. The observed decrease in CP and AA was expected, as a substantial portion of the protein in CS is soluble protein, which is rapidly degraded by rumen microbes. Soluble protein (% of CP) of the intact CS was 61 ± 4.2% (data not shown). Van Straalen et al. (1997) reported a greater decrease in CP concentration in CS after a 12 h ruminal incubation from 7.9 to 2.0% and Frydrych et al. (1992) observed a decrease in CP concentration in CS from 9.4 to 7.3% when ruminally incubated for 16 h. The AA in highest concentration within RUR were

Glu, Asp, and Leu at 12.9, 10.0, and 9.8% respectively. The AA in lowest concentration were His and Met, and Cys at 2.2, 2.2, and 1.9% respectively. The concentrations of Arg, Ile, Lys, and EAA increased slightly in RUR compared to intact CS whereas concentrations of Asp, Glu, Pro, and non-essential AA (NEAA) decreased slightly for both incubation periods. Little, if any difference exists between the AA profiles of undegraded CS incubated at 16 or 24 h.

Differences between AA profiles of intact feeds and RUR have been reported for other feeds such as soybean meal (O'Mara et al., 1997; Ceresnáková et al., 2002; Boucher et al., 2009a) and distiller dried grains plus solubles (O'Mara et al., 1997; Kleinschmit et al., 2007). These differences may be attributable to differences in ruminal degradation kinetics of AA within feeds (Prestlokken and Rise, 2003; Borucki-Castro et al., 2007) or particle associated bacteria contamination of the RUR (Boucher et al., 2009a).

Mobile Bag Technique

Digestibility of RUP and AA in RUP (RUP-AA) are presented in Table 5. Intestinal digestibility of RUP varied from 19 to 43% for RUR incubated for 16 h and 18 to 33% for RUR incubated for 24 h. These data are in agreement with previous estimates of digestibility of RUP in CS reported in the literature (Frydrych, 1992; Kopency et al., 1994; Danesh Mesgaran and Stern, 2005; Kononoff et al., 2007), however they are lower than tabular values reported by NRC (2001), estimated at 70%. Frydrych (1992) and Kopency et al. (1994) measured intestinal digestibility of RUP of CS using the MBT. Corn silage was ruminally incubated for 16-h and bags were collected from the feces. Both studies reported that RUP digestibility was 48%. Danesh Mesgaran and Stern

(2005) determined RUP digestibility of CS using the MBT after a 12-h ruminal incubation and bags were recovered from the feces. Intestinal digestibility of RUP was 31 and 52% for rumen undegraded CS treated with either 16 or 24 g/kg DM of urea, respectively. Kononoff et al. (2007) measured RUP digestibility of CS in steers fed two different diets consisting of either 0% wet corn gluten feed or 38% wet corn gluten feed using the MBT with collection of bags form the feces. Corn silage was ruminally incubated in situ for 22 h and intestinal digestibility of RUP was observed to be 20% in animals consuming both diets. The lower RUP digestibility values reported in both the current and previous studies may indicate an overestimation of RUP digestibility in CS by NRC (2001). Typically, RUR is incubated in pepsin-HCl for 1 to 3 h prior to duodenal insertion to mimic conditions of the abomasum. However, It has been demonstrated that intestinal digestibility of RUP and RUP-AA is unaffected by the exclusion of a pepsin-HCl incubation (Vanhatalo et al. 1995), and a pepsin-HCl incubation was not included in the present study.

Digestibility of TAA ranged from 43 to 58% in RUR incubated for 16 h and 35 to 62% in RUR incubated for 24 h. Differences between intestinal digestibility of RUP and RUP-AA have been reported for CS (Van Straalen et al., 1997). Van Straalen et al. (1997) observed an intestinal digestibility of 50% for RUP and 72% for RUP-AA in CS. These differences in digestibility may be due to non-protein-N within RUP linked to components indigestible in the intestine such as lignin, tannins, and Maillard reaction products (Van Straalen et al., 1997).

Digestibility of EAA in RUR ranged from 36 to 61%, similar to digestibility of NEAA which ranged from 35 to 61%. However, digestibility of individual AA varied

considerably in undegraded CS, confirming that AA are not all digested the same. The highest observed average intestinal digestibility was for Arg, Glu, and Met at 58, 57, and 56% respectively, whereas the lowest average intestinal digestibility was for Pro, Gly, Cys at 44, 40, and 24% respectively.

Intestinal digestibility of RUP-AA in CS reported in the literature (Van Straalen et al., 1997; Taghizadeh et al., 2005) are higher than determined here. Van Straalen et al. (1997) reported high intestinal digestibilities of 100, 77, and 76% for Arg, Met, Glu, respectively whereas Cys, Gly, and Pro had lower intestinal digestibilities of 53, 63, and 65%. Taghizadeh et al. (2005) reported an intestinal digestibility of 81, 80, and 75% for Lys, Met, and Ala and lower intestinal digestibility values for Ser, Cys, and Asp at 50, 49, and 38%. The higher values may be due to a shorter (12-h) rumen incubation period for Van Straalen et al. (1997) and Taghizadeh et al. (2005). In addition, neither study reported methods for correction of particle-associated bacteria.

Average digestibility of RUP and RUP-AA ruminally incubated at 16 h or 24 h are presented in Table 6. There is evidence that duration of rumen incubation may affect RUP digestibility in fibrous feeds. Beckers et al. (1996) noted a decrease in intestinal digestibility of RUP in wheat bran from 61 to 59% as rumen incubation time increased from 16 to 24 h. Kononoff et al. (2007) reported an increase in intestinal digestibility of RUP for soy hulls from 82 to 84% and for corn bran from 83 to 87% when rumen incubation time was increased from 20 to 30 h. There were no significant differences for digestibility of RUP and RUP-AA between 16 and 24 h rumen incubation in the present study. These results suggest that a 16-h rumen incubation time may be sufficient to obtain digestibility of RUP-AA for CS. A 16-h rumen incubation time will allow for

more rapid analysis of AA digestibility and also allows for better comparison with literature data commonly where feeds are commonly incubated for 16 h.

Total tract digestibility of CP varied from 58 to 81%, whereas total tract digestibility of TAA ranged from 79 to 91% (Table 7). Estimates of total tract digestibility for CP in CS are in agreement with reported values of 73% by Taghizadeh et al. (2005), but are lower than the values of 83 and 84% reported by Kononoff et al. (2007). Although the difference between total tract digestibility of CP and AA is not as great as the differences observed for intestinal digestibility, the small difference indicates that indigestible non-protein-N components in RUR may be present. The large range in intestinal digestibility of AA was not apparent in the total tract digestibility of AA, which ranged from 70 to 94% for all CS samples. However, observable differences still exist for total tract digestibility of RUP-AA. The AA with the highest average total tract digestibility were Pro, Ala, and Glu at 88, 89, and 89% respectively. The lowest average total tract digestibility was found for Cys, Arg, and Tyr at 78, 80, and 80% respectively. Most AA such as Glu and Met, which were highly digestible in the intestine were also high in total tract digestibility. However, this is not constant for all AA. While Arg was found to have a high intestinal digestibility, the total tract digestibility was low. Conversely, Pro had a low intestinal digestibility and a relatively high total tract digestibility. The lower ruminal degradability was compensated by higher intestinal digestibility, which resulted in a relatively constant total tract digestibility of Pro.

Modified Three Step Procedure

The CP and AA concentrations and AA profiles of the RUR generated at UNH are presented in Table 8. Crude protein and TAA concentrations of RUR all decreased

compared to concentrations present in intact feeds. Concentration of CP and TAA was greater in RUR incubated for 24 h compared with 16 h RUR in samples 2, 3, and 4 whereas CP and TAA concentrations in CS 5 remained the same. The CP and TAA concentrations for all RUR samples determined at UNH were lower than those found at Ag-Canada, indicating a greater ruminal CP and AA degradation by the cows at UNH. This is possibly due to differences in rumen microbial populations of the cows at Ag-Canada and UNH due to the different rations fed. The ration fed at Ag-Canada contained 50% forage and 50% concentrate whereas the ration fed at UNH contained 58% forage and 42% concentrate (Appendix A). However, the AA profile of the RUR generated at UNH and Ag-Canada were similar.

In vitro digestibility of RUP and RUP-AA are listed in Table 9. Digestibility of RUP ranged from 1 to 19%, which are lower than intestinal digestibility values determined by the MBT. Digestibility of TAA in RUR varied from 23 to 46%. These values are also lower than values found using the MBT. Several negative digestibility values were calculated for Cys, Gly, and Pro, which were the lowest AA digestibilities obtained by the MBT. The negative values may be caused by improper or incomplete washing of the Ankom bags after the final step of the modified TSP resulting in contamination of the undegraded CS with residual enzyme from the pancreatin digestion step. Since the concentration of CP and TAA are very low in RUR, small errors in methodology, such as measurements of bag DM and sample DM may lead to errors in the reported data. Contamination of the undegraded residue with enzyme, in addition to the poor digestibility values likely caused the negative digestibility values for Cys, Gly, and Pro. On average, in vitro intestinal digestibility was highest for Lys, Met, and Asp at 58,

51, and 47%. A comparison of the MBT and the modified TSP for RUP, TAA, EAA, and Lys are presented in Figure 1. The modified TSP appears to under predict digestibility of RUP, TAA, and EAA compared to the MBT. In particular, RUP in CS 2 incubated for 16-h was 40% more digestible using the MBT when compared with the modified TSP. Most AA digestibility values were underestimated when using the modified TSP whereas Lys digestibility appears to be consistent between both the MBT and the modified TSP. The lower intestinal digestibility values obtained with the modified TSP may be due to the lower amounts, and possibly the lower quality, of AA available for digestion in the RUR used in the modified TSP. The RUR generated for use in the modified TSP was degraded to a greater extent by the rumen microbes in the cows at UNH compared with rumen microbes from cows used in the other two experiments.

Average in vitro digestibility of RUP and RUP-AA ruminally incubated for 16 h or 24 h are presented in Table 10. In agreement with the RUP-AA digestibility data obtained from the MBT (Table 6) there was no difference in digestibility between CS samples incubated for 16 or 24 h.

Total tract digestibility determined by the modified TSP are presented in Table 11. The total tract digestibility of CP and AA for all CS samples is consistent with values obtained by the MBT. Digestibility of CP ranged from 69 to 84% whereas TAA digestibility varied from 80 to 91%. The total tract digestibility values for TAA are highly agreeable with MBT estimates for TAA. Amino acids highest in total tract digestibility were Leu, Ala, and Glu, each at 90%. The AA lowest in digestibility were Gly, Cys, and Arg at 76, 79, and 80%, respectively. Total tract digestibility of AA was

similar between the MBT and modified TSP as Ala and Glu were highly digestible in both procedures and Cys and Arg were poorly digested in both procedures.

Coefficients of determination of intestinal and total tract digestibility estimates obtained from the MBT and modified TSP are presented in Table 12. Except for Arg (R^2 = 0.81) and Val (R^2 = 0.79), the correlations for RUP and RUP-AA estimates between the two procedures are low. The low estimates may be due to differences in extent of ruminal degradation between the cows at Ag-Canada and UNH as ruminal degradation for most CS samples were greater in cows housed at UNH. Danesh Mesgaran and Stern (2005) observed a low correlation (r^2 = 0.26) for post ruminal CP digestibility between the original TSP and the MBT for two CS samples. However, RUR for the MBT was also generated in a different location using different cows than RUR for the TSP. Ruminal protein disappearance of CS used for the MBT was 38 and 53%, for sample 1 and 2 whereas the ruminal protein disappearance of CS used for the TSP was 40 and 66% for sample 1 and 2. Although ruminal protein disappearance by location was not significant in the experiment by Danesh Mesgaran and Stern (2005), there was a difference of 2 and 13% for CS 1 and 2, respectively. The resulting RUP digestibility using the MBT was 31 and 52% for CS 1 and 2 whereas the RUP digestibility obtained from the TSP was 35 and 38% for CS 1 and 2. The results of Danesh Mesgaran and Stern (2005) in combination with the present study suggest that differences in extent of ruminal protein degradation of CS will result in differences in digestibility of RUP in CS because of differing microbes population in the rumen.

Coefficients of determination for total tract digestibility estimates obtained from the MBT and modified TSP were stronger than the relationships for RUP-AA (Figure 2

and 3). Total tract CP digestibility results obtained by the modified TSP were highly correlated with results obtained by the MBT ($R^2 = 0.91$). Total tract digestibility of TAA were also highly correlated between the two procedures ($R^2 = 0.80$). The lowest correlations for total tract digestibility of AA between the MBT and modified TSP were for Lys ($R^2 = 0.62$), Cys ($R^2 = 0.57$), and Met ($R^2 = 0.27$). This is a concerning limitation of the modified TSP due to the emphasis placed on Lys and Met, as these two AA are often the first and second limiting AA in lactating dairy cow rations (Schwab et al., 1992). Failing to accurately predict the digestibility of Lys and Met in feeds may indicate a need for a more accurate in vitro model. However, total tract digestibility of several of the AA are highly correlated with results obtained by the MBT. Differences in rations between the two experimental sites may have led to differences in RUP-AA digestibility. As ruminal degradability of AA increased, the intestinal digestibility decreased. Therefore, differences in ruminal degradability of AA between the sites would be expected to lead to differing intestinal digestibility of AA. Due to the low number of CS samples tested, a greater number of samples among differing forages should be analyzed to further determine the accuracy of the modified TSP.

The modified TSP overestimated total tract digestibility of CP and AA compared with the MBT (Figure 2 and 3). These results are in contrast to RUP and RUP-AA digestibility data obtained by the modified TSP and may be due to differences in ruminal degradation of protein and AA between the two sites of experiments.

To determine if a mean or linear bias was present in the regression model, the residuals (observed – predicted) were plotted against centered predicted total tract digestibility values (Figure 4) based on the methods of St.-Pierre (2003). The mean bias

and slope bias were nonsignificant for total tract digestibility of CP, TAA, EAA, and Lys, indicating that the modified TSP is a valid in vitro method to determine total tract digestibility of CP and AA in CS.

Sapienza Analytica, LLC In Vitro Procedure

Crude protein, TAA, and profile of AA after the rumen fluid incubation step of the IVP are listed in Table 13. The CP and TAA in RUR generated in the IVP are lower than CP and TAA concentrations of RUR generated at Ag-Canada, except for the TAA concentration of CS 4 incubated for 16-h which was the same.

Digestibility of RUP and RUP-AA is presented in Table 14. Intestinal digestibility of RUP varied from 20 to 50% whereas digestibility of RUP-TAA ranged from 33 to 55%. Digestibility of RUP-TAA, RUP-EAA, and RUP-Lys are lower than digestibility results from the MBT, but greater than the measured digestibility values from the modified TSP (Figure 5). Although results for RUP-AA digestibility from the IVP are under estimated compared with in vivo results, a linear increase ($P < 0.01$) was observed for intestinal digestibility of RUP-TAA as the portion of TAA increased in RUR (Figure 6). This may explain some of the differences in RUP-AA digestibility between the three procedures. Due to differences in the rate and extent of ruminal protein degradation between the procedures, proportion of TAA in RUR was not consistent in CS between experiments. Therefore, differences in RUP-AA digestibility should be expected between the experiments.

Average in vitro digestibility of RUP and RUP-AA incubated for 16 or 24 h are presented in Table 15. A significant difference ($P < 0.05$) was observed for intestinal digestibility of His, Leu, Ala, Cys, Glu, and Pro and a trend between CS incubated for

either 16 or 24 h. A trend ($0.05 < P < 0.10$) was also observed for intestinal digestibility of Arg, Ile, Lys, Thr, Val, Gly, and Tyr. Corn silage ruminally incubated for 24-h resulted in greater intestinal digestibility for all AA compared to CS incubated for 16-h. However, this effect may be due to incomplete ruminal degradation of CS incubated at 24-h and therefore a greater amount of AA available for intestinal in vitro digestion. No difference was observed between ruminal in vitro DM disappearance of the two incubation periods (Table 3). In particular, the 16-h ruminal in vitro DM disappearance was numerically greater for CS 1, 3, and 4. The lower disappearance of DM for the 24-h ruminal in vitro incubation may be due to an increased lag time caused by lower than expected rumen fluid pH (Bossen et al. 2008). Typically, the pH of pooled rumen fluid from collected steers for the IVP is 6.5 ± 0.3. On collection day, pH of the pooled rumen fluid was 5.9. Furthermore, smaller bag sizes (i.e. Ankom R510) are normally used for the IVP. The present experiment used Ankom R1020 bags to generate sufficient sample size for AA analysis. A decrease in ruminal in vitro DM disappearance may be due to the larger bag size and an increased ratio of sample weight to bag surface area, degradation. Further research may be warranted to identify the size of polyester bag and the ratio of sample weight to bag surface area ideal for the IVP.

Total tract digestibility of AA are similar to values obtained with the modified TSP, but greater than those obtained with the MBT (Table 16). Digestibility of RUP ranged from 75 to 86% whereas digestibility of RUP-TAA varied from 82 to 90%. Total tract digestibility of AA were similar for all CS samples.

A linear was relationship was not observed between intestinal digestibility estimates determined by the MBT and IVP (Table 17). However, a significant linear

relationship was found for total tract digestibility estimates. Coefficients of determination for the IVP and MBT compare favorably to correlations found with the modified TSP and MBT. The modified TSP appears to be a better predictor of total tract digestibility for all AA except for Lys, Glu, and Ser. In particular, the correlation for Lys (R^2 = 0.82) was an improvement over the modified TSP. As with the modified TSP, the IVP over predicts total tract CP and AA digestibility compared to the MBT (Figure 7 and 8). Linear or mean biases were also determined for the IVP (Figure 9). The mean bias and slope bias were nonsignificant for CP, TAA, EAA, and Lys.

Coefficients of determination for digestibility estimates obtained form the modified TSP and the IVP are presented in Table 18. A low correlation was determined for RUP-AA digestibility for both in vitro methods, although the relationship improved when comparing the total tract digestibility of AA indicating that the IVP is a valid in vitro method to determine the total tract digestibility of CP and AA in CS.

Conclusions

Digestibility estimates of RUP-AA in CS ruminally incubated for 16 and 24 h indicate a 16-h ruminal incubation may be sufficient time to generate RUP-AA digestibility estimates. Individual AA in corn silage appear to be ruminally degraded and digested in the intestine at different rates and these differences should be accounted for in ration evaluation systems. Differences between intestinal digestibility of CP and AA in CS suggest that RUP digestibility measurements based on N content, and not AA content, may under estimate the true intestinal digestibility of the undegraded feed. The modified TSP and IVP are adequate predictors of total tract digestibility of CP and AA when compared with the MBT. However, further research may be warranted to evaluate the

relationship between the in vitro procedures and in vivo intestinal digestibility measurements for CS.

Table 1. Chemical composition of intact corn silage samples.

	Sample[1]				
	CS 1	CS 2	CS 3	CS 4	CS 5
DM	41.1	32.6	32,5	40.3	40.7
Item, % of DM					
CP	7.9	4.7	4.5	4.8	6.1
Total AA[2]	4.5	3.4	3.1	3.9	4.7
ADF	24.3	27.6	28.8	25.4	21.7
NDF	36.6	45.8	55.6	52.9	37.1
Lignin	4.0	3.0	2.8	3.0	2.6
ADICP[3]	1.0	0.4	0.7	0.6	0.5
NDICP[4]	2.3	1.0	1.1	1.4	0.7
Fat	3.2	3.2	3.2	3.0	3.1
NFC[5]	46.2	41.9	31.5	35.2	50.9
Starch	32.3	31.5	18.1	36.8	43.0
Ash	6.4	4.7	5.5	4.3	3.2
Ca	1.0	0.4	0.5	0.4	0.3
P	0.2	0.2	0.2	0.2	0.2

[1]CS = Corn silage
[2]AA = amino acids.
[3]ADICP = acid detergent insoluble CP.
[4]NDICP = neutral detergent insoluble CP.
[5]NFC = non-fiber carbohydrates; NFC = 100 – [CP + (NDF-NDICP) + fat + ash].

Table 2. Amino acid profile (% of TAA) of intact corn silage samples.

AA[2]	Sample[1]				
	CS 1	CS 2	CS 3	CS 4	CS 5
	% of TAA				
Arg	3.1	3.1	2.8	3.3	2.9
His	2.1	2.5	2.4	3.1	2.7
Ile	4.8	4.7	4.8	4.4	4.5
Leu	9.8	11.3	11.1	11.1	12.3
Lys	4.3	4.7	5.5	5.3	4.3
Met	1.9	1.9	2.1	2.5	2.2
Phe	4.1	5.0	5.2	5.0	5.2
Thr	5.2	4.7	4.5	4.4	4.3
Val	7.4	6.6	6.9	6.4	6.3
BCAA	21.9	22.5	22.8	21.9	23.1
EAA	42.6	44.4	45.3	45.4	44.6
Ala	13.6	10.0	9.7	9.1	9.2
Asp	7.1	8.4	9.0	8.6	8.1
Cys	1.7	1.9	2.1	2.2	2.0
Glu	14.5	15.0	13.8	14.7	16.1
Gly	6.4	5.6	5.9	5.5	5.2
Pro	7.6	8.4	7.6	8.0	8.5
Ser	4.1	4.1	4.2	3.9	3.8
Tyr	2.4	2.2	2.4	2.5	2.5
NEAA	57.4	55.6	54.7	54.6	55.4

[1]CS = Corn silage, AA = amino acid.

[2]BCAA = branch chain AA, EAA = essential AA, NEAA = nonessential AA; TAA = Total AA.

Table 3. Ruminal degradation of dry matter (DM) after 16 or 24 h rumen incubation (values in % of intact feed).

	Location[1]						
	Agriculture and Agri-Food Canada		University of New Hampshire		Sapienza Analytica, LLC		
	16 h	24 h	16 h	24 h	16 h	24 h	SEM
CS 1	63.4[a]	64.1[a]	63.2[a]	68.1[b]	64.4[a]	61.7[a]	0.51
CS 2	53.4[b]	57.2[c]	61.0[d]	66.2[e]	49.1[a]	50.9[ab]	0.51
CS 3	47.7[a]	54.0[b]	53.5[b]	62.0[c]	45.6[a]	44.5[a]	0.52
CS 4	58.7[b]	60.0[b]	58.7[b]	64.4[c]	53.6[a]	53.1[a]	0.51
CS 5	62.2[a]	66.6[b]	66.0[b]	70.3[c]	60.3[a]	61.2[a]	0.51

[a-d]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]Site of the conducted experiment.

Table 4. Crude protein, total AA, and AA profile (% of TAA) of corn silage samples after a ruminal in situ incubation completed at the Dairy and Swine Research and Development Center in Sherbrooke, Québec.

	Sample[1]									
	16 h					24 h				
	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5
CP	5.9	4.4	4.4	4.5	4.7	6.1	4.9	5.2	5.0	5.5
TAA[2]	3.2	2.4	2.5	2.3	3.0	2.9	2.8	3.0	3.0	3.3
	% of TAA[3]									
Arg	4.7	4.9	4.8	5.0	4.6	4.8	4.9	4.6	4.9	4.5
His	2.3	2.2	2.2	2.3	2.5	2.2	2.3	2.1	2.1	2.2
Ile	5.0	5.3	5.2	5.4	5.3	5.1	5.3	5.6	5.6	4.8
Leu	9.7	9.7	10.0	10.0	10.6	9.6	9.4	9.5	9.4	9.9
Lys	5.4	5.8	6.5	5.9	5.3	5.9	6.8	7.0	7.0	6.1
Met	2.0	2.2	2.2	2.3	2.1	2.2	2.3	2.5	2.1	2.5
Phe	5.7	5.8	5.7	5.4	6.0	5.5	5.7	5.6	5.6	5.7
Thr	5.4	5.3	5.2	5.0	5.0	5.5	5.3	5.3	5.2	5.4
Val	8.1	6.6	7.0	6.8	6.7	8.1	6.8	7.0	7.0	6.4
BCAA	22.8	21.7	22.2	22.2	22.7	22.8	21.5	22.1	22.0	21.0
EAA	48.3	47.8	48.7	48.0	48.2	48.9	48.7	49.1	49.0	47.5
Ala	7.4	7.5	7.8	7.7	7.4	7.4	7.5	7.4	7.3	7.6
Asp	9.7	9.7	10.0	10.0	9.2	9.9	10.6	10.5	10.5	10.2
Cys	2.3	1.8	1.7	1.8	1.8	2.2	1.5	1.8	1.7	1.9
Glu	12.4	13.3	13.0	12.7	13.5	12.5	12.8	12.6	12.6	13.1
Gly	6.4	6.2	6.1	6.3	6.0	6.3	6.0	6.0	6.3	6.1
Pro	6.0	5.8	5.2	5.9	6.4	5.5	5.3	4.9	5.2	5.7
Ser	4.4	4.9	4.3	4.5	4.3	4.4	4.5	4.6	4.2	4.8
Tyr	3.0	3.1	3.0	3.2	3.2	2.9	3.0	3.2	3.1	3.2
NEAA	51.7	52.2	51.3	52.0	51.8	51.1	51.3	50.9	51.0	52.5

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.
[2]TAA = total amino acids.
[3]BCAA = branch chain amino acids, EAA = essential amino acids, NEAA = nonessential amino acids.

Table 5. Intestinal digestibility (%) of AA and CP in ruminally undegraded corn silage determined by the mobile bag technique.

	Sample										
	16 h					24 h					
AA[2]	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	53.3	56.7	57.3	55.6	63.2	41.1	63.8	62.6	60.5	66.1	3.93
His	46.6	42.9	43.6	41.4	59.0	36.1	52.9	51.4	54.0	59.4	4.66
Ile	44.0	44.5	45.2	43.0	55.3	31.6	52.9	57.5	59.7	55.7	5.73
Leu	45.3	48.1	55.1	46.7	64.9	33.7	54.8	56.8	55.7	66.4	3.99
Lys	47.5	41.4	49.9	39.9	55.3	40.1	58.1	61.1	63.2	60.1	5.88
Met	53.3	42.9	62.4	41.4	68.1	52.1	52.9	58.4	54.0	76.3	3.64
Phe	45.1	48.7	49.4	43.0	60.6	29.8	56.1	57.5	54.0	63.1	5.27
Thr	41.6	44.5	45.2	37.8	52.1	36.1	52.9	54.7	50.9	61.0	3.58
Val	45.5	36.5	47.1	41.4	54.7	34.7	47.7	51.4	54.0	57.3	4.66
BCAA	45.1	43.7	50.2	44.2	59.6	33.6	52.1	55.3	56.2	61.2	4.52
EAA	46.2	45.3	50.5	43.8	59.2	35.9	54.7	57.0	56.6	62.4	4.45
Ala	44.8	44.0	47.8	42.5	54.4	37.7	52.9	53.7	51.8	60.5	3.37
Asp	48.5	43.7	51.0	42.3	55.8	39.7	56.3	57.9	57.0	61.5	4.60
Cys	33.3	4.8	29.5	2.3	42.6	20.2	5.8	22.3	26.3	52.6	7.32
Glu	52.1	52.4	56.1	51.1	64.7	43.7	58.5	62.2	61.6	67.6	3.57
Gly	36.1	32.0	39.6	30.2	43.7	26.7	41.1	48.6	48.8	50.1	5.07
Pro	37.8	41.4	45.2	39.9	57.5	23.4	46.2	44.5	50.9	57.9	4.69
Ser	42.5	48.1	43.6	41.4	52.1	36.1	52.9	55.2	54.0	68.4	4.47
Tyr	37.8	45.6	46.3	44.2	57.5	28.2	52.9	56.8	59.1	62.1	4.65
NEAA	44.2	43.5	48.2	41.4	56.1	35.2	51.5	54.4	54.6	61.5	4.13
TAA	45.2	44.4	49.3	42.5	57.6	35.6	53.1	55.7	55.6	61.9	4.29
CP	24.3	42.5	18.9	26.1	19.2	17.7	27.1	27.7	33.2	30.7	5.78

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.
[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 6. Average intestinal digestibility of AA and CP in corn silage samples ruminally incubated for 16 or 24 h determined by the mobile bag technique.

	Digestibility %			
AA[1]	16 h	24 h	SEM	*P*-value[2]
Arg	57.2	58.8	2.48	NS
His	46.7	50.8	2.95	NS
Ile	46.4	51.5	3.63	NS
Leu	52.0	53.5	2.52	NS
Lys	46.8	56.2	3.72	NS
Met	53.6	58.7	2.30	NS
Phe	49.4	52.1	3.33	NS
Thr	44.2	51.1	2.27	*
Val	45.0	49.0	2.94	NS
BCAA	48.6	51.7	2.86	NS
EAA	49.0	53.3	2.82	NS
Ala	50.8	47.2	3.37	NS
Asp	48.3	54.5	2.91	NS
Cys	22.5	25.4	4.63	NS
Glu	55.3	58.7	2.26	NS
Gly	36.3	43.1	3.21	NS
Pro	44.4	44.6	2.97	NS
Ser	45.5	53.3	2.83	NS
Tyr	46.3	51.8	2.94	NS
NEAA	46.7	51.4	2.62	NS
TAA	47.8	52.9	2.71	NS
CP	26.2	27.3	3.66	NS

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

[2]Differences between digestibility of CP & AA in rumen undegraded corn silage marked NS and * were non-significant and $0.05 < P < 0.10$, respectively.

Table 7. Total tract digestibility (%) of AA & CP of corn silage samples determined by the in situ procedure and mobile bag technique.

	Sample[1]										
	16 h					24 h					
AA[2]	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	81.8	78.2	69.5	83.4	86.1	79.1	80.5	72.5	81.8	87.8	1.27
His	85.0	83.6	79.1	89.2	90.9	84.9	85.3	81.1	90.1	92.0	0.41
Ile	84.8	79.7	75.6	82.6	87.3	83.0	81.8	78.0	84.1	88.9	0.84
Leu	86.0	85.5	83.2	88.1	92.8	85.1	87.0	83.5	88.2	93.6	0.46
Lys	83.1	76.7	75.6	83.3	86.7	81.1	79.1	78.0	84.7	86.6	0.93
Met	87.3	78.2	83.7	86.8	92.8	87.2	80.5	78.0	87.9	93.6	1.58
Phe	80.1	80.9	77.2	84.5	89.0	78.0	82.9	79.4	83.8	90.3	0.95
Thr	84.6	79.7	73.7	82.6	86.7	84.5	81.8	76.3	81.8	88.3	0.73
Val	84.7	79.2	78.1	84.5	88.4	83.5	81.4	78.0	84.2	89.8	0.68
BCAA	85.3	82.4	80.1	85.9	90.5	84.1	84.3	80.6	86.2	91.7	0.57
EAA	84.3	81.0	78.0	85.2	89.4	83.1	82.9	79.2	85.3	90.6	0.62
Ala	92.3	86.4	82.6	88.0	91.2	92.2	87.8	84.3	87.9	92.2	0.42
Asp	82.0	79.0	77.5	83.3	87.9	80.7	81.2	78.0	83.5	88.5	0.61
Cys	75.9	70.9	75.6	80.1	87.9	75.7	73.9	70.6	81.8	89.4	1.55
Glu	89.5	86.4	82.9	89.5	93.0	88.8	87.8	84.6	89.7	93.8	0.47
Gly	83.7	75.8	74.2	80.1	84.3	83.6	78.3	76.7	81.8	86.2	0.54
Pro	87.3	87.1	84.5	89.0	92.4	87.2	88.4	84.0	89.9	93.3	0.39
Ser	84.1	79.9	75.6	83.0	87.2	84.0	82.0	78.0	84.4	90.6	0.65
Tyr	79.7	75.1	72.1	82.3	86.8	79.6	77.7	74.8	83.8	88.4	0.57
NEAA	87.1	82.9	79.9	86.1	90.2	86.7	84.6	81.0	86.7	91.4	0.40
TAA	85.9	82.0	79.1	85.7	89.9	85.2	83.9	80.2	86.1	91.0	0.49
CP	79.5	65.3	58.2	68.9	77.9	80.8	70.9	63.1	73.9	78.8	1.12

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.
[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 8. Crude protein, total AA, and AA profile (% of total AA) of corn silage samples after a ruminal in situ incubation completed at the University of New Hampshire.

	Sample[1]									
	16 h					24 h				
	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5
CP	4.5	3.2	3.4	3.5	4.0	4.3	3.7	3.9	3.7	4.0
TAA[2]	2.4	1.9	1.8	2.1	2.4	2.4	2.2	2.3	2.3	2.4
	% of TAA[3]									
Arg	4.5	4.5	4.8	4.6	4.9	4.5	4.9	4.7	4.9	4.7
His	2.7	2.8	2.4	2.6	2.7	2.7	2.5	2.3	2.4	2.3
Ile	5.4	5.1	5.4	5.1	4.9	4.9	5.4	5.6	5.3	5.1
Leu	9.5	9.6	9.5	9.7	9.8	9.4	9.3	8.9	9.2	9.3
Lys	6.3	6.7	7.1	6.7	6.3	6.7	7.8	7.9	7.8	7.0
Met	2.3	2.2	1.8	2.1	2.2	1.8	2.0	1.9	1.9	2.3
Phe	5.4	5.6	5.4	5.6	5.4	5.4	5.4	5.1	5.3	5.1
Thre	5.4	5.1	4.8	5.1	5.4	5.4	5.4	5.1	5.3	5.6
Val	7.2	6.7	7.1	6.7	6.7	7.1	6.9	7.0	6.8	6.5
BCAA	22.1	21.3	22.0	21.5	21.4	21.4	21.6	21.5	21.4	20.9
EAA	48.6	48.3	48.2	48.2	48.2	47.8	49.5	48.6	49.0	47.9
Ala	7.2	7.3	7.7	7.2	7.6	7.6	7.4	7.5	7.3	7.4
Asp	9.9	10.1	10.7	10.3	9.8	10.3	10.8	11.2	10.7	10.7
Cys	2.3	2.2	1.8	2.1	2.2	2.2	1.5	1.9	1.5	1.9
Glu	12.6	12.4	12.5	12.3	12.5	12.5	12.3	12.1	12.1	12.6
Gly	6.8	6.7	6.5	6.7	6.3	6.7	6.4	6.5	6.8	6.5
Pro	5.9	6.2	5.4	5.6	6.3	6.3	5.4	5.1	5.3	5.6
Ser	4.1	3.9	4.2	4.1	4.5	4.0	3.9	4.2	4.4	4.7
Tyr	2.7	2.8	3.0	3.1	2.7	2.7	2.9	2.8	2.9	2.8
NEAA	51.4	51.7	51.8	51.3	51.8	52.2	50.5	51.4	51.0	52.1

[1]CS = Corn silage, samples were ruminally incubated in situ for either 16 or 24 h.
[2]TAA = total amino acids.
[3]BCAA = branch chain amino acids, EAA = essential amino acids, NEAA = nonessential amino acids

Table 9. In vitro intestinal digestibility (%) of AA and CP in corn silage after ruminal incubation determined by the modified three-step procedure.

	Sample[1]										
	16 h					24 h					
AA[2]	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	31.7	39.4	25.8	46.5	46.9	21.8	33.3	42.9	43.9	42.8	5.29
His	35.0	61.2	25.8	61.5	51.3	34.8	42.8	42.9	43.9	42.8	7.35
Ile	43.1	46.2	34.0	51.9	46.9	28.9	39.3	52.4	49.0	48.0	6.09
Leu	39.6	48.7	38.1	54.4	51.3	34.8	44.8	49.9	50.8	52.4	3.46
Lys	51.2	59.6	50.5	63.0	58.3	47.9	58.3	60.8	64.9	61.9	2.64
Met	41.5	51.5	34.0	75.9	61.1	26.7	52.3	52.4	53.2	61.9	7.96
Phe	35.0	41.8	12.0	38.7	35.1	26.7	30.7	30.8	40.5	30.7	5.95
Thr	35.0	46.2	25.8	51.9	43.2	26.7	39.3	48.1	49.0	44.4	6.22
Val	26.9	35.4	25.8	40.8	41.6	14.5	31.9	42.9	39.8	38.8	5.41
BCAA	36.3	43.9	33.1	49.6	47.3	26.7	39.3	48.3	46.8	47.1	4.68
EAA	37.7	47.0	31.6	51.9	47.7	29.7	41.5	47.8	49.0	47.3	4.80
Ala	33.0	40.4	23.9	45.0	42.7	25.2	36.4	40.5	43.9	40.5	4.74
Asp	42.4	46.2	39.5	51.9	46.9	36.2	48.0	52.4	53.2	50.3	3.41
Cys	2.5	3.1	-32.0	27.8	22.1	2.2	-27.1	4.8	-24.8	4.7	16.82
Glu	40.8	47.1	34.0	51.9	51.3	30.2	42.8	48.8	51.3	47.1	4.76
Gly	2.5	11.2	-26.0	11.1	2.7	-17.3	-10.0	-1.9	13.1	4.7	9.33
Pro	-5.0	29.5	-10.0	21.2	30.5	-4.7	4.7	13.5	14.9	12.7	9.35
Ser	24.2	16.9	15.2	39.8	41.6	13.1	16.6	36.6	37.6	42.8	3.78
Tyr	35.0	41.8	20.8	51.9	35.1	18.5	36.4	36.6	37.6	36.5	6.53
NEAA	26.4	34.7	16.9	40.3	37.9	17.3	27.8	35.1	37.6	35.4	5.44
TAA	31.9	40.6	24.0	46.2	42.6	23.2	34.6	41.3	43.2	41.1	4.47
CP	7.8	1.4	11.0	12.6	13.6	4.7	18.9	14.0	14.2	8.2	5.14

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.

[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 10. Average intestinal digestibility of AA and CP in corn silage samples ruminally incubated for 16 or 24 h determined by the modified three-step procedure.

	Digestibility %			
AA[1]	16 h	24 h	SEM	*P*-value[2]
Arg	38.1	36.9	3.34	NS
His	47.0	41.4	4.65	NS
Ile	44.4	43.5	3.85	NS
Leu	46.4	46.5	2.19	NS
Lys	56.5	58.8	1.67	NS
Met	52.8	49.3	5.04	NS
Phe	32.5	31.7	3.77	NS
Thr	40.4	41.5	3.93	NS
Val	34.1	33.6	3.42	NS
BCAA	42.0	41.6	2.96	NS
EAA	43.2	43.1	3.04	NS
Ala	37.0	37.3	3.00	NS
Asp	45.4	48.0	2.15	NS
Cys	4.7	-8.0	10.64	NS
Glu	45.0	44.0	3.01	NS
Gly	0.3	-2.3	5.90	NS
Pro	13.25	8.2	5.91	NS
Ser	27.5	29.3	3.78	NS
Tyr	36.9	33.1	4.13	NS
NEAA	31.3	30.6	3.44	NS
TAA	37.1	36.7	3.25	NS
CP	9.3	12.0	2.83	NS

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

[2]Differences between digestibility of CP & AA in rumen undegraded corn silage marked NS were non-significant.

Table 11. In vitro total tract digestibility (%) of AA & CP of corn silage samples determined by the in situ procedure and modified three-step procedure.

	Sample[1]										
	16 h					24 h					
AA[2]	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	80.9	81.2	65.8	83.6	83.4	80.9	77.5	72.7	82.9	86.5	2.02
His	84.2	90.6	80.4	92.8	91.0	86.2	87.9	84.4	90.7	92.7	1.43
Ile	87.6	87.5	80.4	87.7	89.2	87.6	85.0	84.4	87.2	91.2	1.24
Leu	88.8	90.6	85.7	91.1	92.8	89.4	90.2	88.6	91.4	94.7	0.66
Lys	86.2	87.5	82.9	89.6	88.7	86.2	85.0	84.1	89.2	90.8	0.87
Met	86.7	87.5	84.8	95.6	92.8	88.3	89.3	87.9	92.4	94.2	1.2
Phe	83.3	85.9	75.7	84.7	87.5	83.5	83.9	80.6	86.7	89.8	1.28
Thr	87.1	87.5	78.9	87.7	86.8	87.3	85.0	83.2	87.2	89.2	1.32
Val	86.3	85.7	79.5	86.3	88.5	86.0	84.7	83.6	86.6	90.6	1.05
BCAA	87.7	88.5	82.7	89.0	90.9	87.8	87.5	86.2	89.2	92.9	0.90
EAA	86.3	87.6	80.5	88.7	89.5	86.6	85.9	84.2	88.5	91.6	1.04
Ala	93.2	90.6	83.7	90.5	91.2	92.9	89.9	87.0	90.7	92.9	0.82
Asp	84.6	86.1	80.7	87.3	88.0	84.5	85.7	83.2	87.9	90.3	0.68
Cys	74.7	75.0	69.6	85.2	84.1	77.8	78.5	75.8	82.9	87.0	1.56
Glu	90.1	90.6	84.0	91.1	93.0	89.8	89.9	87.3	91.6	93.9	0.77
Gly	80.3	77.1	62.4	76.4	78.2	79.3	73.2	67.9	77.8	82.2	1.90
Pro	84.5	88.9	79.3	87.8	90.6	85.4	86.9	83.5	88.2	91.5	1.11
Ser	85.4	82.7	77.2	85.9	87.3	85.4	82.7	81.8	85.3	89.7	1.10
Tyr	85.8	83.9	73.9	86.9	87.0	84.5	81.6	79.2	84.8	89.4	1.67
NEAA	87.3	86.9	78.9	87.6	89.2	87.2	85.9	82.8	87.8	91.0	0.95
TAA	86.9	87.2	79.6	88.1	89.4	87.0	85.9	83.4	88.2	91.3	0.99
CP	81.0	74.3	68.8	73.6	79.3	83.7	78.6	71.5	76.3	82.2	0.35

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.
[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 12. Coefficient of determination (R^2 values) of RUP-AA and total tract digestibility of AA estimates obtained using the mobile bag technique and the modified three-step procedure for samples of corn silage.

	RUP-AA		Total tract	
AA[1]	R^2	P > F†	R^2	P > F†
Arg	0.45	0.03	0.87	< 0.01
His	0.00	0.85	0.74	< 0.01
Ile	0.43	0.04	0.81	< 0.01
Leu	0.37	0.06	0.84	< 0.01
Lys	0.23	0.17	0.66	< 0.01
Met	0.00	0.97	0.36	0.07
Phe	0.01	0.78	0.67	< 0.01
Thr	0.13	0.30	0.72	< 0.01
Val	0.37	0.06	0.77	< 0.01
BCAA	0.43	0.04	0.83	< 0.01
EAA	0.26	0.14	0.80	< 0.01
Ala	0.19	0.21	0.86	< 0.01
Asp	0.30	0.10	0.77	< 0.01
Cys	0.00	0.89	0.62	< 0.01
Glu	0.35	0.07	0.87	< 0.01
Gly	0.03	0.62	0.71	< 0.01
Pro	0.22	0.17	0.77	< 0.01
Ser	0.34	0.08	0.92	< 0.01
Tyr	0.14	0.29	0.74	< 0.01
NEAA	0.00	0.17	0.83	< 0.01
TAA	0.23	0.16	0.82	< 0.01
CP	0.07	0.46	0.92	< 0.01

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

†Probability of a significant linear relationship.

Table 13. Crude protein, total AA, and AA profile (% of total AA) of corn silage samples after a rumen in vitro incubation completed at Sapienza Analytica, LLC (Slater IA).

	Sample[1]									
	16 h					24 h				
	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5
CP	4.8	2.7	3.3	3.7	3.8	4.9	3.4	3.7	3.5	3.7
TAA[2]	2.6	1.8	1.6	2.3	2.6	2.6	2.2	1.9	2.3	2.4
	% of TAA[3]									
Arg	4.6	4.3	4.6	4.8	4.6	4.5	4.6	4.6	4.7	4.6
His	2.5	2.5	2.7	2.4	2.5	2.5	2.5	2.3	2.8	2.7
Ile	5.0	4.9	4.6	4.8	4.6	4.9	4.6	4.6	4.7	4.6
Leu	10.4	10.4	10.6	10.5	10.9	10.3	10.2	9.8	10.2	10.5
Lys	5.0	4.9	5.3	5.2	4.6	4.9	5.6	5.2	5.6	5.0
Met	2.1	2.5	2.0	2.4	2.5	2.1	2.5	2.3	2.3	2.3
Phe	5.8	5.5	6.0	5.7	5.9	5.7	5.6	5.8	5.6	5.5
Thr	5.0	4.9	4.6	4.8	4.6	5.3	5.1	5.2	4.7	4.6
Val	7.5	6.8	6.6	6.7	6.3	7.4	6.6	5.8	7.0	6.4
BCAA	22.8	22.1	21.9	21.9	21.8	22.5	21.3	20.1	21.9	21.4
EAA	47.7	46.6	47.0	47.1	46.4	47.5	47.2	45.4	47.4	45.9
Ala	7.5	7.4	8.0	7.6	7.5	7.4	7.6	8.1	7.9	7.7
Asp	8.7	8.6	8.6	8.6	8.0	9.0	8.6	9.2	8.8	8.2
Cys	2.1	2.40	2.0	2.4	2.5	2.5	2.5	2.3	2.3	2.7
Glu	12.9	14.1	13.9	13.3	14.6	12.7	13.7	13.8	13.5	14.1
Gly	7.1	6.1	6.0	6.2	5.9	6.1	6.1	6.3	6.5	6.4
Pro	7.1	7.4	7.3	7.6	8.0	7.0	7.1	6.9	7.0	7.7
Ser	4.2	4.3	4.0	4.3	4.2	4.1	4.1	4.6	3.7	4.1
Tyr	2.9	3.1	3.3	2.9	2.9	2.9	3.1	3.5	2.8	3.2
NEAA	52.3	53.4	53.0	52.9	53.6	52.5	52.8	54.6	52.6	54.1

[1]CS = Corn silage, samples were ruminally incubated in situ for either 16 or 24 h.
[2]TAA = total amino acids.
[3]BCAA = branch chain amino acids, EAA = essential amino acids, NEAA = nonessential amino acids.

Table 14. In vitro intestinal digestibility (%) of AA & CP of corn silage samples determined by the Sapienza Analytica, LLC in vitro procedure.

	Sample										
	16 h					24 h					
AA	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	31.6	32.7	30.9	35.6	41.3	40.1	47.9	40.6	52.9	36.8	4.24
His	21.6	29.4	27.4	26.4	38.5	37.2	43.7	52.5	52.9	39.8	5.05
Ile	37.3	41.1	30.9	44.8	49.7	45.1	58.3	40.6	52.9	45.9	3.76
Leu	43.5	50.1	39.5	45.6	50.3	47.3	62.5	55.3	61.4	52.9	3.23
Lys	37.3	29.4	27.4	41.4	41.3	37.2	57.4	47.2	52.9	42.6	6.00
Met	43.5	52.9	67.7	63.2	53.9	62.3	81.2	52.5	81.1	63.9	8.27
Phe	39.5	37.2	35.5	38.6	47.3	46.2	57.4	43.0	45.0	39.8	4.88
Thr	29.4	41.1	30.9	35.6	41.3	42.1	53.1	47.2	52.9	36.8	4.40
Val	42.5	31.5	32.3	34.2	38.5	47.7	49.5	33.5	49.7	42.0	3.77
BCAA	41.8	42.4	35.5	42.0	46.8	46.9	57.6	45.7	55.9	48.2	2.91
EAA	37.8	39.3	34.6	40.5	45.1	44.8	56.6	45.9	54.7	44.6	3.48
Ala	37.3	45.1	35.5	42.5	48.7	47.7	56.2	52.5	61.2	46.9	4.03
Asp	37.3	39.5	33.0	38.6	41.7	44.4	55.9	52.5	60.3	39.8	4.90
Cys	5.9	5.8	3.2	26.4	38.5	21.6	62.5	28.8	43.4	54.9	8.73
Glu	39.3	46.8	40.1	47.4	52.5	48.4	58.3	56.5	64.2	56.3	2.71
Gly	28.0	24.7	14.0	29.2	34.1	33.6	37.5	30.9	39.4	29.1	4.20
Pro	28.0	29.4	29.6	36.7	46.6	39.1	46.4	44.6	56.0	41.6	4.84
Ser	24.7	32.7	19.3	38.6	35.4	34.1	41.4	40.6	41.1	29.8	4.94
Tyr	32.8	43.5	41.9	38.6	47.3	46.2	53.1	52.5	37.2	48.4	3.22
NEAA	32.8	37.2	31.0	39.5	45.2	41.9	52.2	48.0	55.0	44.6	3.61
TAA	35.2	38.2	32.7	39.9	45.2	43.3	54.3	47.0	54.8	44.6	3.48
CP	25.6	19.5	34.2	35.3	33.1	33.2	48.7	52.3	37.6	39.0	5.50

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.

[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 15. Average intestinal digestibility of AA and CP in corn silage samples ruminally incubated for 16 or 24 h determined by the Sapienza Analytica, LLC in vitro procedure.

	Digestibility %			
AA[1]	16 h	24 h	SEM	*P*-value[2]
Arg	34.4	43.7	2.68	*
His	28.6	45.2	3.19	**
Ile	40.7	48.6	2.38	*
Leu	45.8	55.9	2.04	**
Lys	35.3	47.5	3.80	*
Met	56.2	68.2	5.23	NS
Phe	39.6	46.3	3.09	NS
Thr	35.6	46.4	2.78	*
Val	35.8	44.5	2.38	*
BCAA	41.7	50.9	1.84	**
EAA	39.5	49.3	2.20	**
Ala	41.8	52.9	2.55	**
Asp	38.0	50.6	3.10	**
Cys	16.0	42.2	5.52	**
Glu	45.2	56.7	1.71	***
Gly	26.0	34.1	2.66	*
Pro	34.1	45.5	3.06	**
Ser	30.2	37.4	3.13	NS
Tyr	40.8	47.5	2.04	*
NEAA	37.1	48.3	2.28	**
TAA	38.2	48.8	2.20	**
CP	29.5	42.2	3.48	*

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

[2]Differences between digestibility of CP & AA in rumen undegraded corn silage marked NS *, **, and *** were non-significant, $0.05 < P < 0.10$, $0.01 P < 0.05$, and $P < 0.01$ respectively.

Table 16. In vitro total tract digestibility (%) of AA & CP of corn silage samples determined by the Sapienza Analytica, LLC in vitro procedure.

	Sample[1]										
	16 h					24 h					
AA[2]	CS 1	CS 2	CS 3	CS 4	CS 5	CS 1	CS 2	CS 3	CS 4	CS 5	SEM
Arg	80.3	77.8	69.2	76.4	81.3	81.7	78.4	69.1	82.5	81.8	1.27
His	82.2	83.4	78.9	85.3	88.4	84.9	83.8	85.9	88.6	88.7	1.36
Ile	87.2	85.2	82.4	84.8	89.6	88.1	88.5	82.4	86.9	89.9	0.68
Leu	88.3	88.9	84.6	86.8	91.2	88.4	90.4	87.7	90.6	92.6	0.71
Lys	85.8	82.3	81.5	85.1	87.2	84.9	85.6	84.6	86.7	87.6	0.89
Met	88.0	85.2	91.8	91.0	89.6	91.5	92.8	83.5	95.3	93.2	3.03
Phe	83.0	83.4	80.3	82.0	87.9	84.0	86.5	80.2	83.7	88.3	0.63
Thr	86.9	85.2	81.1	82.3	87.2	87.6	85.6	81.0	86.9	87.6	0.97
Val	88.6	83.1	82.8	82.4	87.6	89.0	85.6	82.7	85.4	89.2	0.66
BCAA	88.2	86.5	83.6	85.1	89.9	88.6	88.6	85.0	88.3	91.2	0.52
EAA	86.4	84.7	82.0	84.2	88.5	87.1	86.9	83.0	87.5	89.5	0.54
Ala	93.3	90.3	85.9	87.7	91.5	94.0	90.5	87.7	91.1	91.8	0.66
Asp	85.1	85.2	83.0	84.3	88.4	85.3	87.2	84.8	89.2	88.7	0.94
Cys	77.1	70.4	75.4	79.7	84.6	75.7	85.6	75.3	84.3	88.7	3.25
Glu	89.5	88.0	84.0	87.8	91.3	90.5	89.2	86.4	91.3	93.0	0.51
Gly	84.6	80.3	76.8	79.7	84.9	84.9	80.8	76.8	81.1	83.9	0.45
Pro	87.0	85.2	82.1	84.6	90.0	88.3	87.2	84.3	89.9	90.2	0.93
Ser	84.9	83.0	79.5	82.6	85.7	86.0	83.4	79.4	85.0	86.1	0.48
Tyr	84.0	81.0	78.9	82.0	87.4	86.4	81.5	78.8	81.3	87.7	0.58
NEAA	88.0	85.6	82.2	85.0	89.3	88.9	87.1	83.7	88.5	90.0	0.56
TAA	87.3	85.2	82.1	84.6	89.0	88.1	87.0	83.4	88.0	89.8	0.54
CP	84.9	78.5	75.3	78.5	84.6	85.0	83.3	79.9	80.4	86.3	1.00

[1]CS = Corn silage, samples were ruminally incubated in situ for 16 or 24 h.
[2]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

Table 17. Coefficient of determination (R^2 values) of RUP-AA and total tract digestibility of AA estimates obtained using the mobile bag technique and the Sapienza Analytica, LLC in vitro procedure for samples of corn silage.

	RUP-AA		Total tract	
AA[1]	R^2	P > F†	R^2	P > F†
Arg	0.07	0.45	0.70	< 0.01
His	0.28	0.12	0.68	< 0.01
Ile	0.16	0.26	0.79	< 0.01
Leu	0.12	0.32	0.64	< 0.01
Lys	0.44	0.04	0.84	< 0.01
Met	0.00	0.99	0.32	0.09
Phe	0.03	0.65	0.63	< 0.01
Thr	0.15	0.27	0.70	< 0.01
Val	0.02	0.67	0.54	0.02
BCAA	0.10	0.37	0.70	< 0.01
EAA	0.16	0.25	0.76	< 0.01
Ala	0.16	0.25	0.80	< 0.01
Asp	0.18	0.22	0.59	< 0.01
Cys	0.03	0.65	0.61	< 0.01
Glu	0.35	0.07	0.84	< 0.01
Gly	0.05	0.52	0.79	< 0.01
Pro	0.19	0.20	0.69	< 0.01
Ser	0.04	0.55	0.80	< 0.01
Tyr	0.13	0.30	0.64	< 0.01
NEAA	0.26	0.14	0.84	< 0.01
TAA	0.22	0.18	0.80	< 0.01
CP	0.04	0.58	0.84	< 0.01

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

†Probability of a significant liner relationship.

Table 18. Coefficient of determination (R^2 values) of RUP-AA and total tract digestibility estimates obtained using the modified three-step procedure and the Sapienza Analytica in vitro procedure for samples of corn silage.

	RUP-AA		Total tract	
AA[1]	R^2	P > F†	R^2	P > F†
Arg	0.07	0.47	0.74	< 0.01
His	0.00	0.95	0.56	0.01
Ile	0.03	0.64	0.57	0.01
Leu	0.18	0.22	0.76	0.01
Lys	0.28	0.11	0.43	0.04
Met	0.00	0.88	0.17	0.23
Phe	0.01	0.78	0.62	< 0.01
Thr	0.14	0.28	0.52	0.02
Val	0.09	0.40	0.40	0.05
BCAA	0.04	0.56	0.66	< 0.01
EAA	0.09	0.41	0.64	< 0.01
Ala	0.23	0.16	0.79	< 0.01
Asp	0.26	0.13	0.65	< 0.01
Cys	0.01	0.81	0.57	0.01
Glu	0.27	0.12	0.82	< 0.01
Gly	0.16	0.25	0.74	< 0.01
Pro	0.09	0.39	0.57	0.01
Ser	0.13	0.32	0.70	< 0.01
Tyr	0.02	0.68	0.59	< 0.01
NEAA	0.20	0.68	0.77	< 0.01
TAA	0.15	0.27	0.72	< 0.01
CP	0.59	< 0.01	0.90	< 0.01

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA; TAA = total AA.

†Probability of a significant liner relationship.

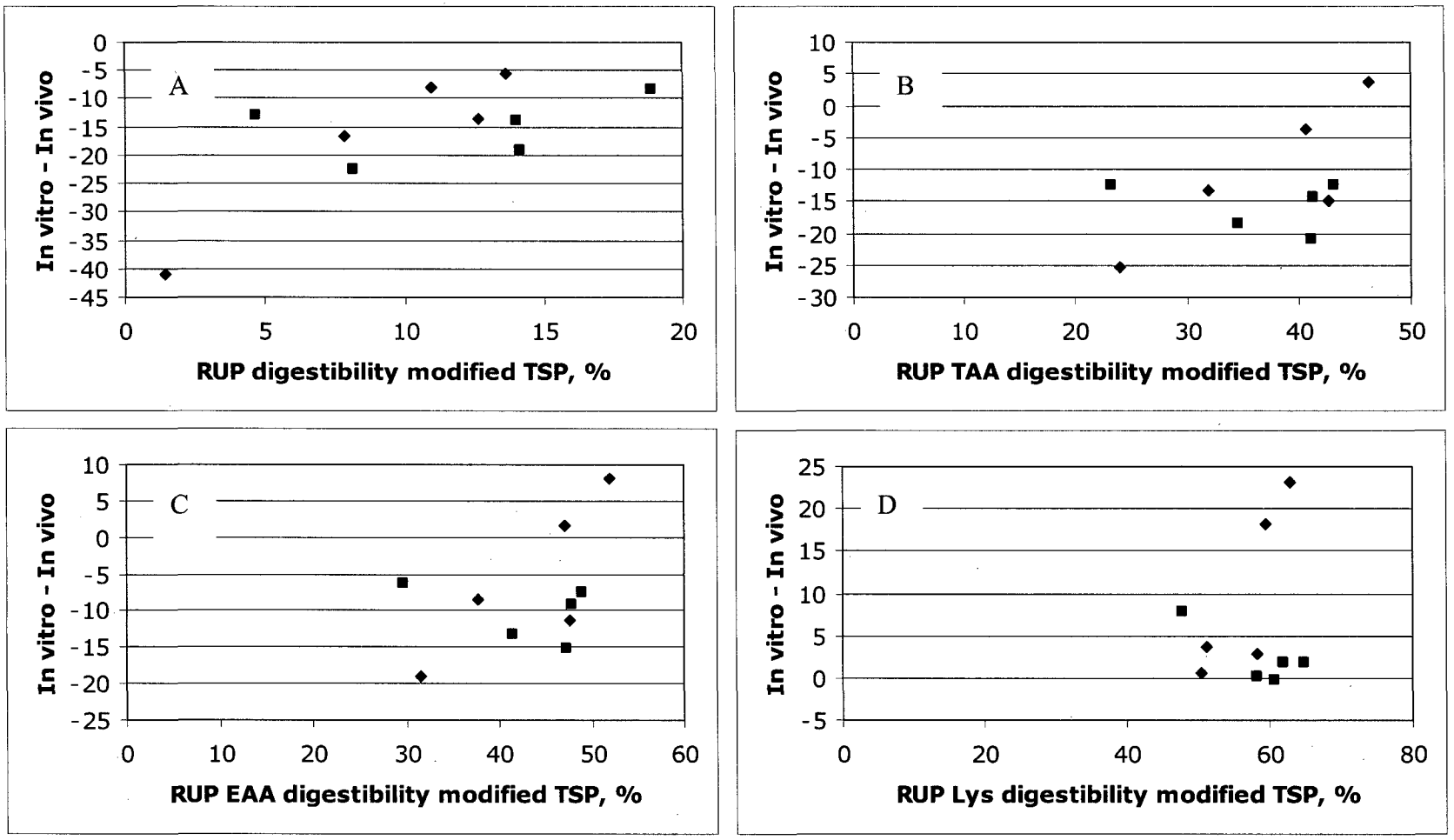

Figure 1. Plots of RUP (A), RUP TAA (B), RUP EAA (C), and RUP Lys (D) digestibility of corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) measured using the modified three-step procedure minus RUP digestibility obtained from the mobile bag technique.

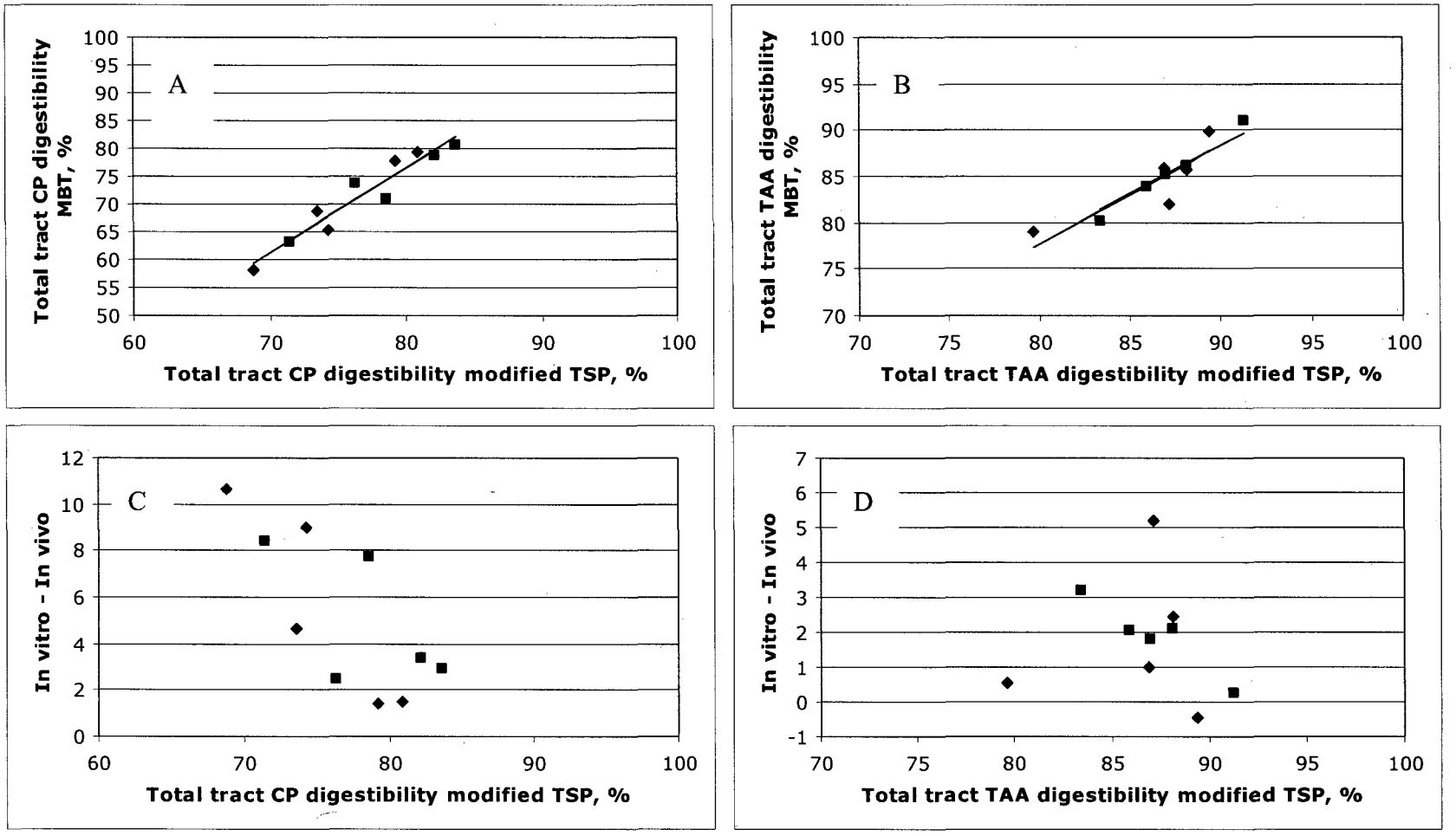

Figure 2. Comparison of total tract digestibility of CP (A; Y = -46.15 + 1.53X; $R^2 = 0.92$; $P < 0.001$; n = 10) and total AA (B; Y = -7.60 + 1.07X; $R^2 = 0.82$; $P < 0.001$; n = 10) in corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the modified three-step procedure and mobile bag technique, and plots of total tract digestibility of CP (C) and total AA (D) measured using the modified three-step procedure minus total tract digestibility using the mobile bag technique.

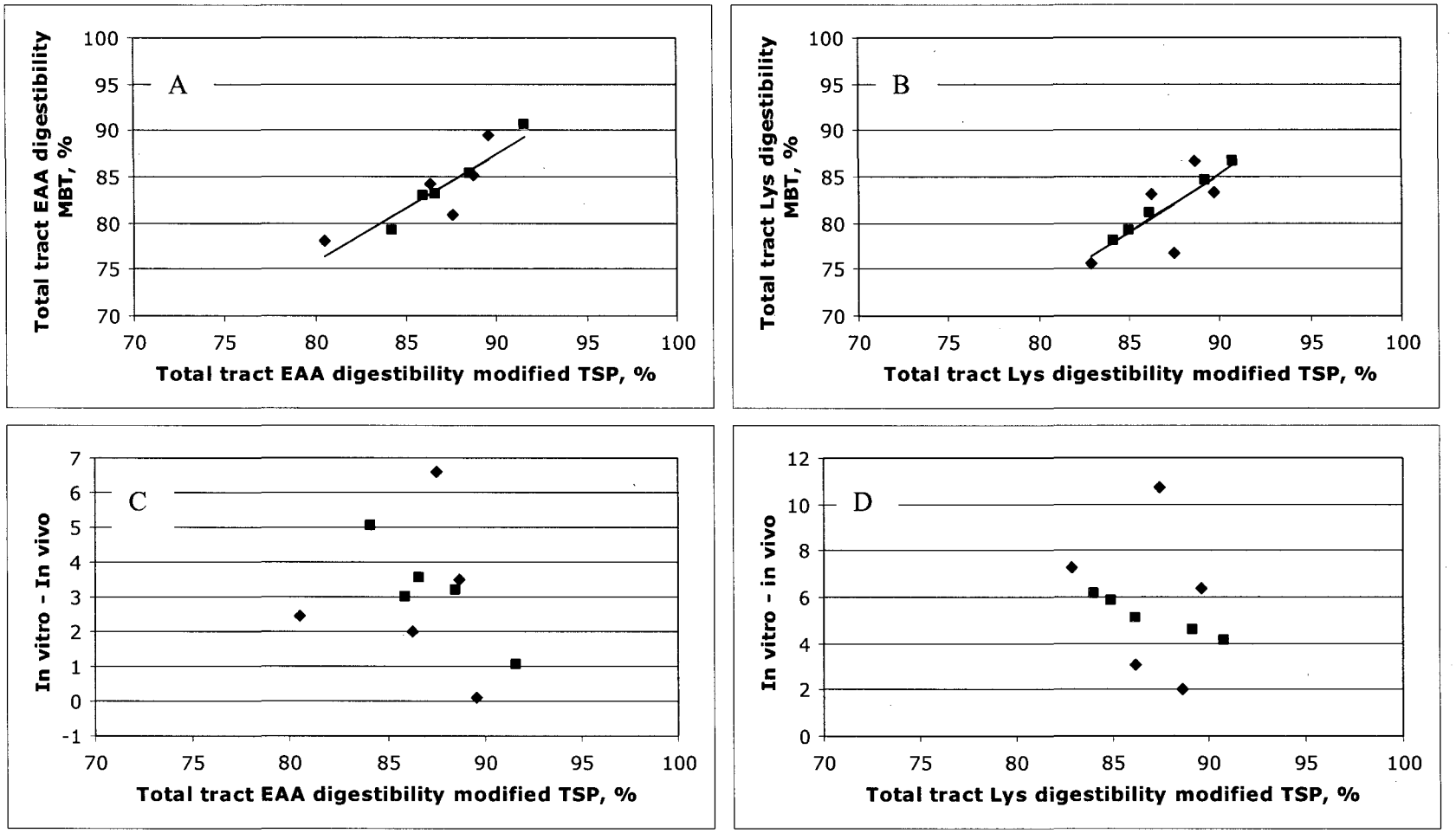

Figure 3. Comparison of total tract digestibility of essential AA (A; Y = -18.16 + 1.17X; R^2 = 0.80; $P < 0.001$; n = 10) and Lys (B; Y = -28.29 + 1.26X; R^2 = 0.66; $P < 0.01$; n = 10) in corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the modified three-step procedure and mobile bag technique, and plots of total tract digestibility of essential AA (C) and Lys (D) measured using the modified three-step procedure minus total tract digestibility using the mobile bag technique.

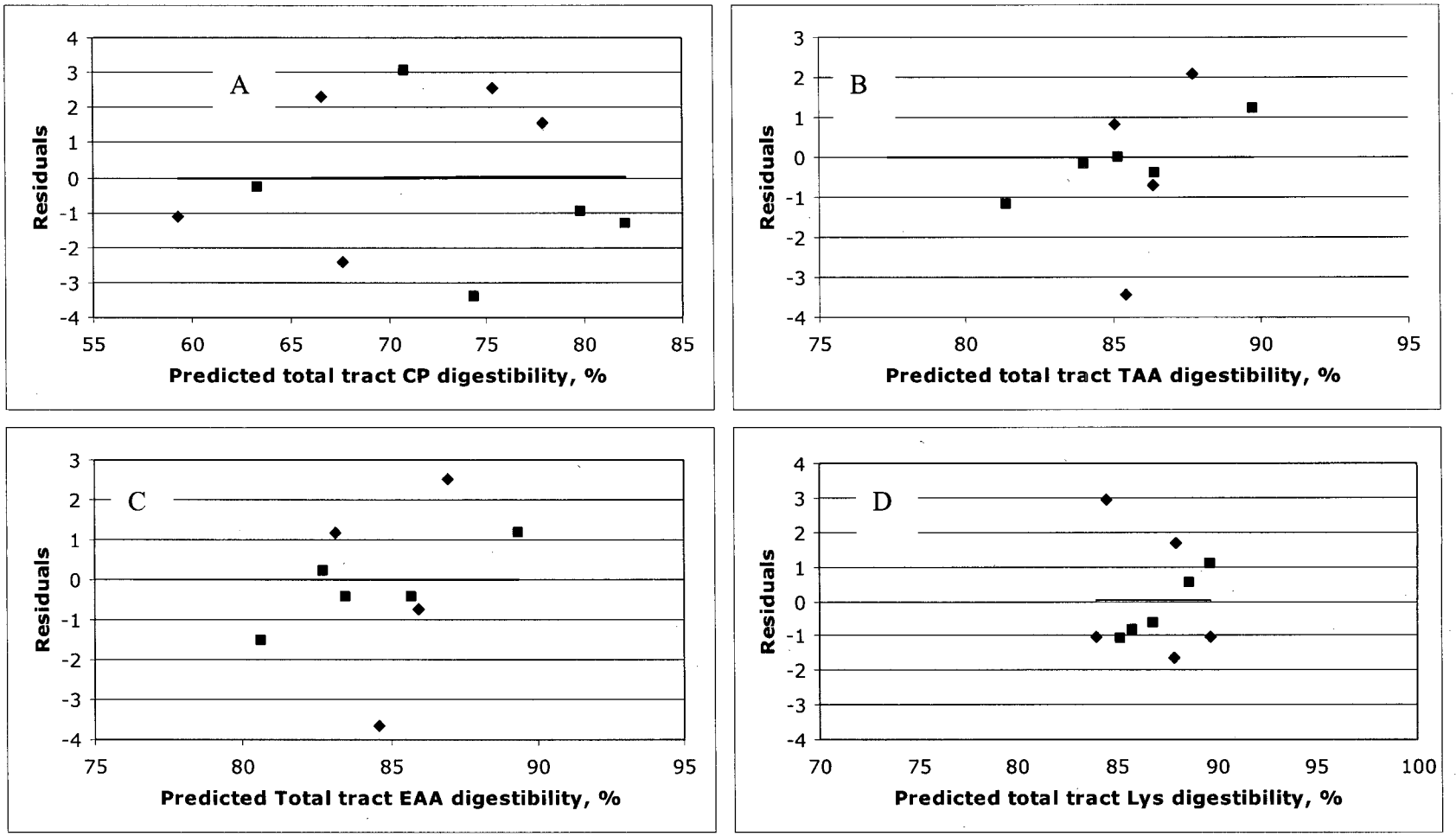

Figure 4. Residual plots of predicted total tract CP (A; $Y = 3.03E^{-22} + 1.70E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), TAA (B; $Y = 1.57E^{-5} + -6.93E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), EAA (C; $Y = 0 + 6.10E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), and Lys (D; $Y = -2.00E^{-5} + -2.06E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10) digestibility in corn silage samples incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the mobile bag technique and the modified three-step procedure.

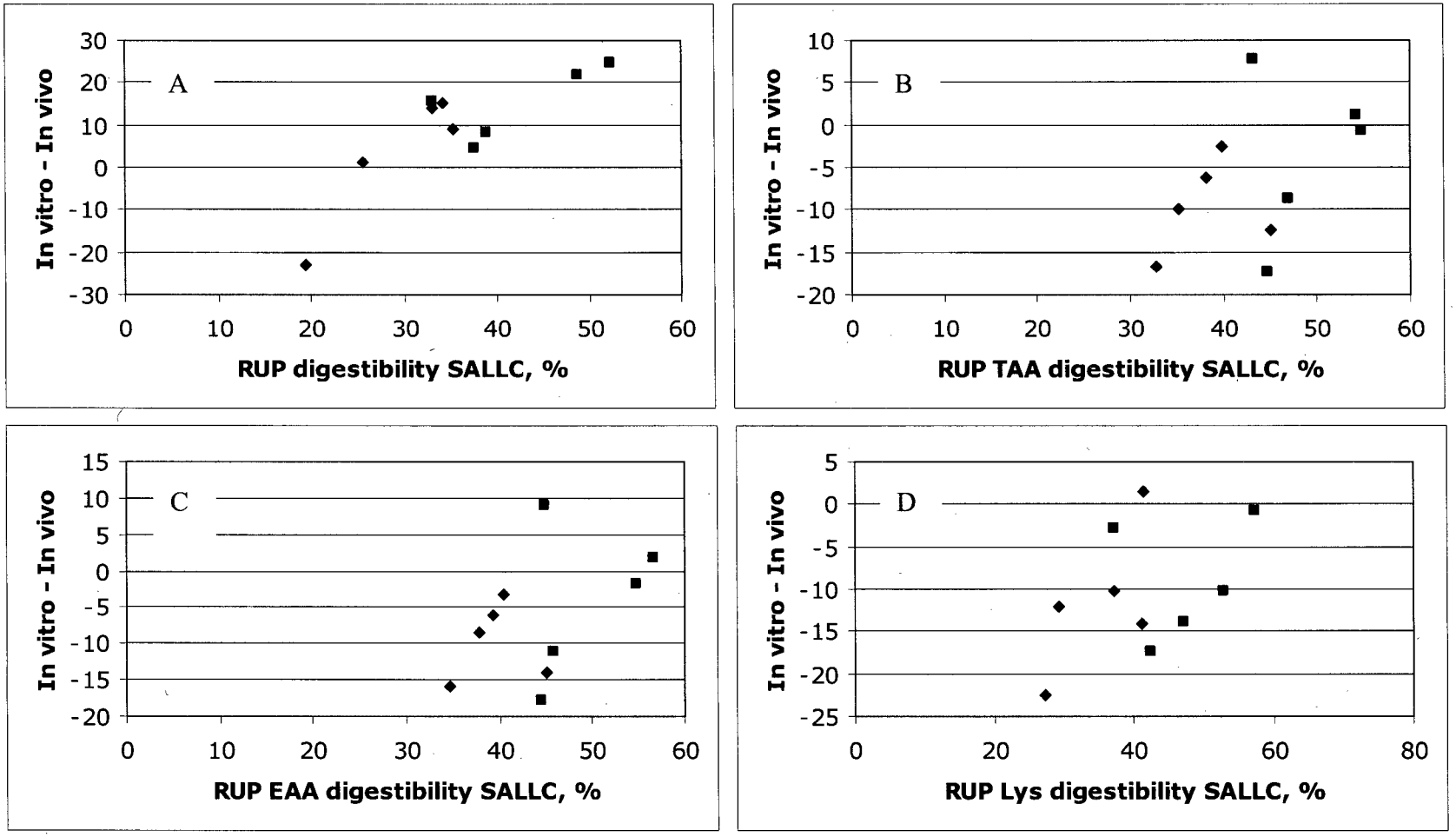

Figure 5. Plots of RUP (A), RUP TAA (B), RUP EAA (C), and RUP Lys (D) digestibility of corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) measured using the Sapienza Analytica in vitro procedure minus RUP digestibility obtained by the mobile bag technique.

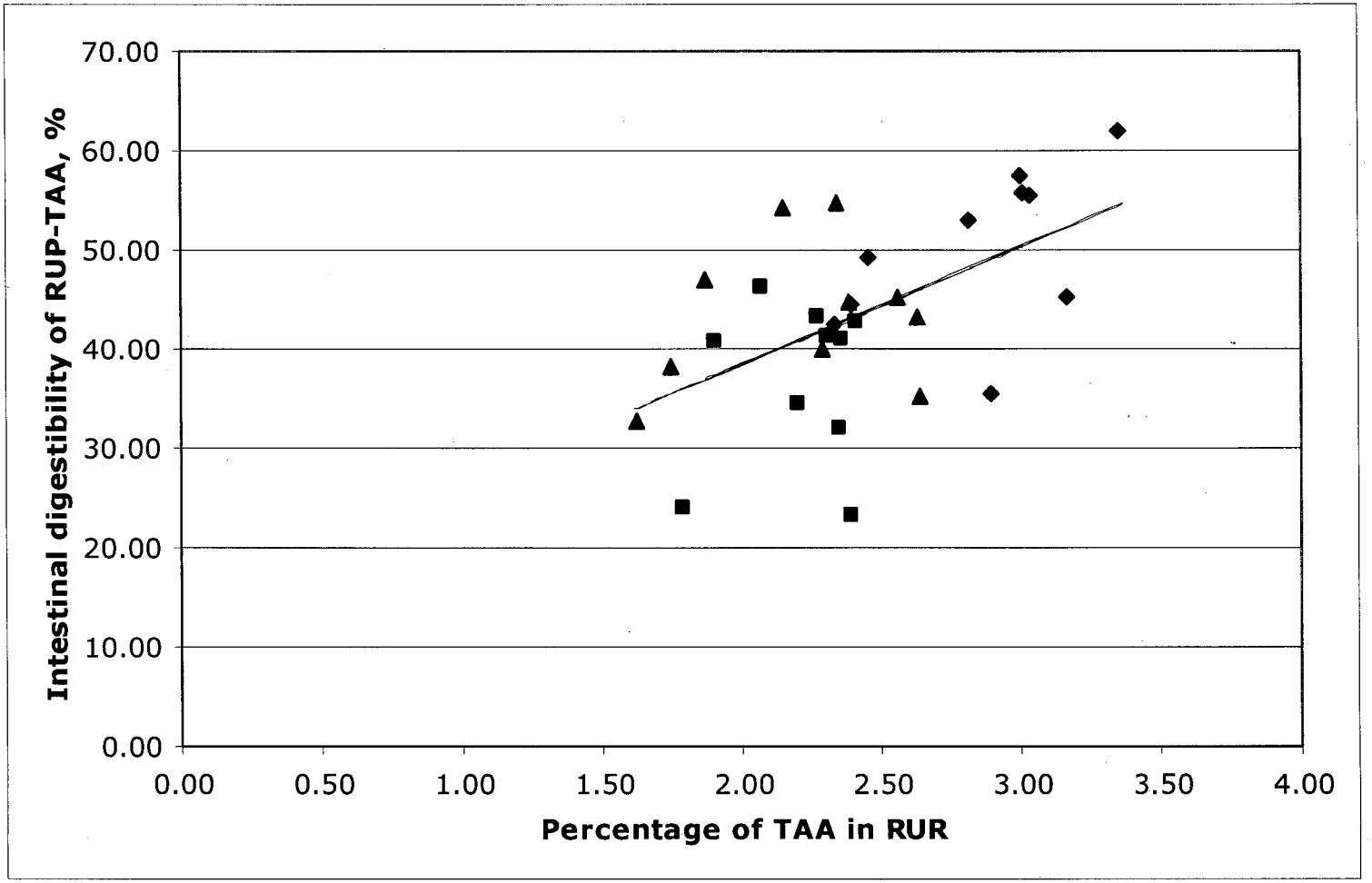

Figure 6. Comparison of the percentage of TAA in RUR obtained by the mobile bag technique (♦; n = 10), the modified three-step procedure (■; n = 10), and the in vitro procedure of Sapienza Analytica (▲; n = 10) to the intestinal digestibility of RUP TAA (Y = 14.79 + 11.84X; $R^2 = 0.27$; $P < 0.01$; n = 30).

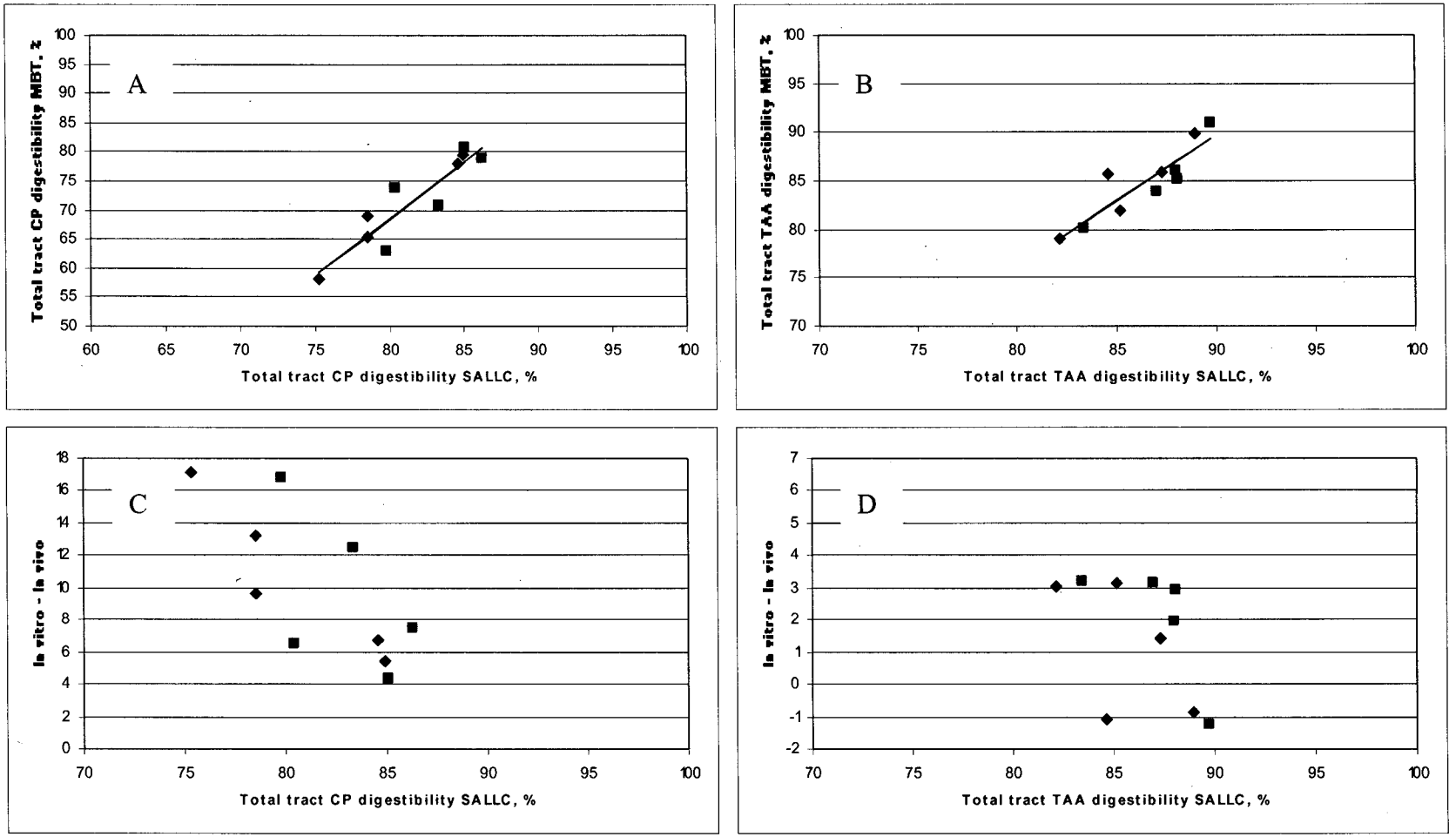

Figure 7. Comparison of total tract digestibility of CP (A; Y = -87.48 + 1.95X; R^2 = 0.84; P < 0.001; n = 10) and total AA (B; Y = -32.77 + 1.36X; R^2 = 0.80; P < 0.001; n = 10) in corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the Sapienza Analytica in vitro procedure and mobile bag technique, and plots of total tract digestibility of CP (C) and total AA (D) measured using the Sapienza Analytica in vitro procedure minus total tract digestibility using the mobile bag technique.

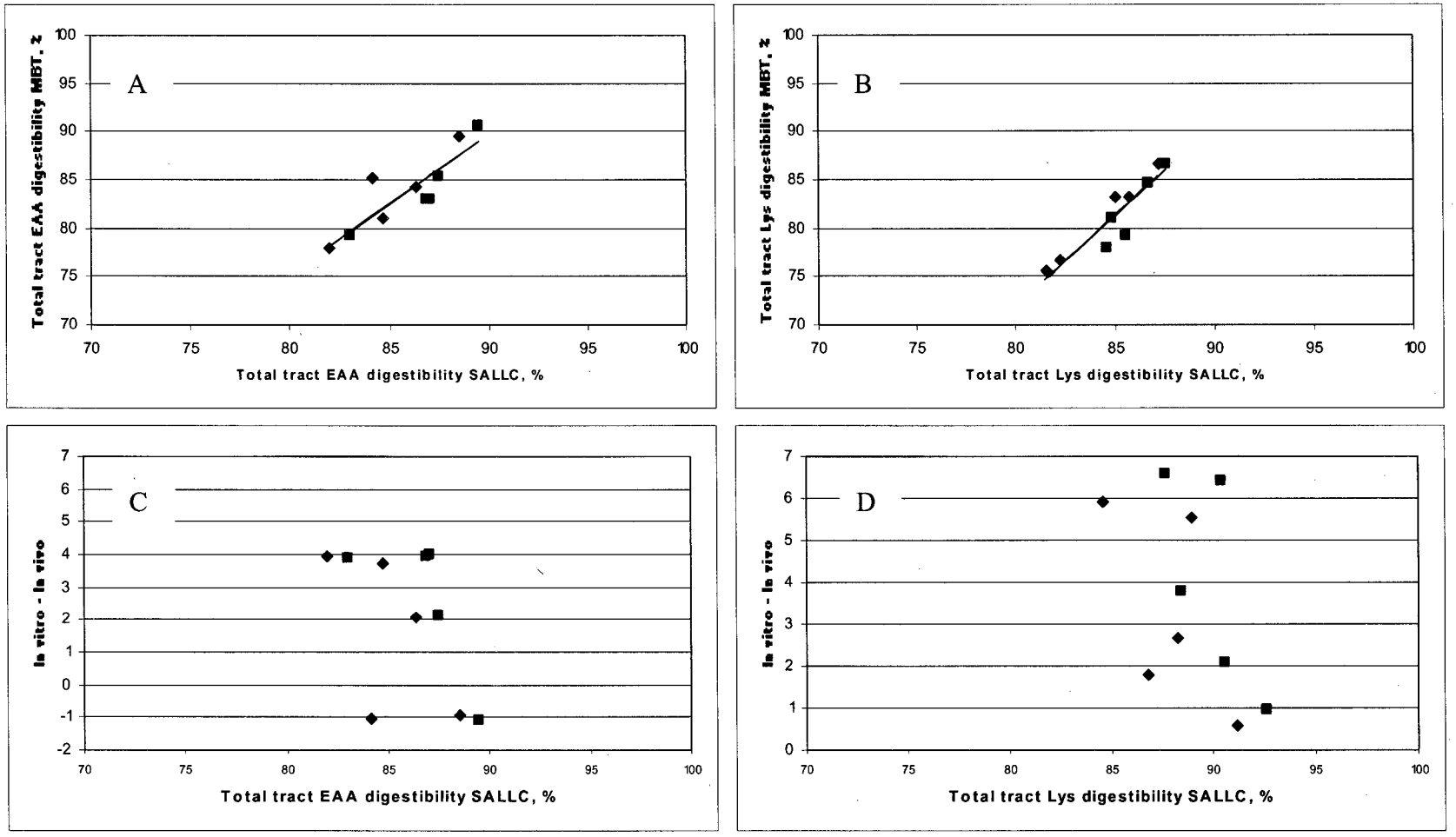

Figure 8. Comparison of total tract digestibility of essential AA (A; Y = -41.17 + 1.45X; R^2 = 0.76; *P* = 0.001; n = 10) and Lys (B; Y = -76.72 + 1.86X; R^2 = 0.84; *P* < 0.001; n = 10) in corn silage incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the Sapienza Analytica in vitro procedure and mobile bag technique, and plots of total tract digestibility of essential AA (C) and Lys (D) measured using the Sapienza Analytica in vitro procedure minus total tract digestibility using the mobile bag technique.

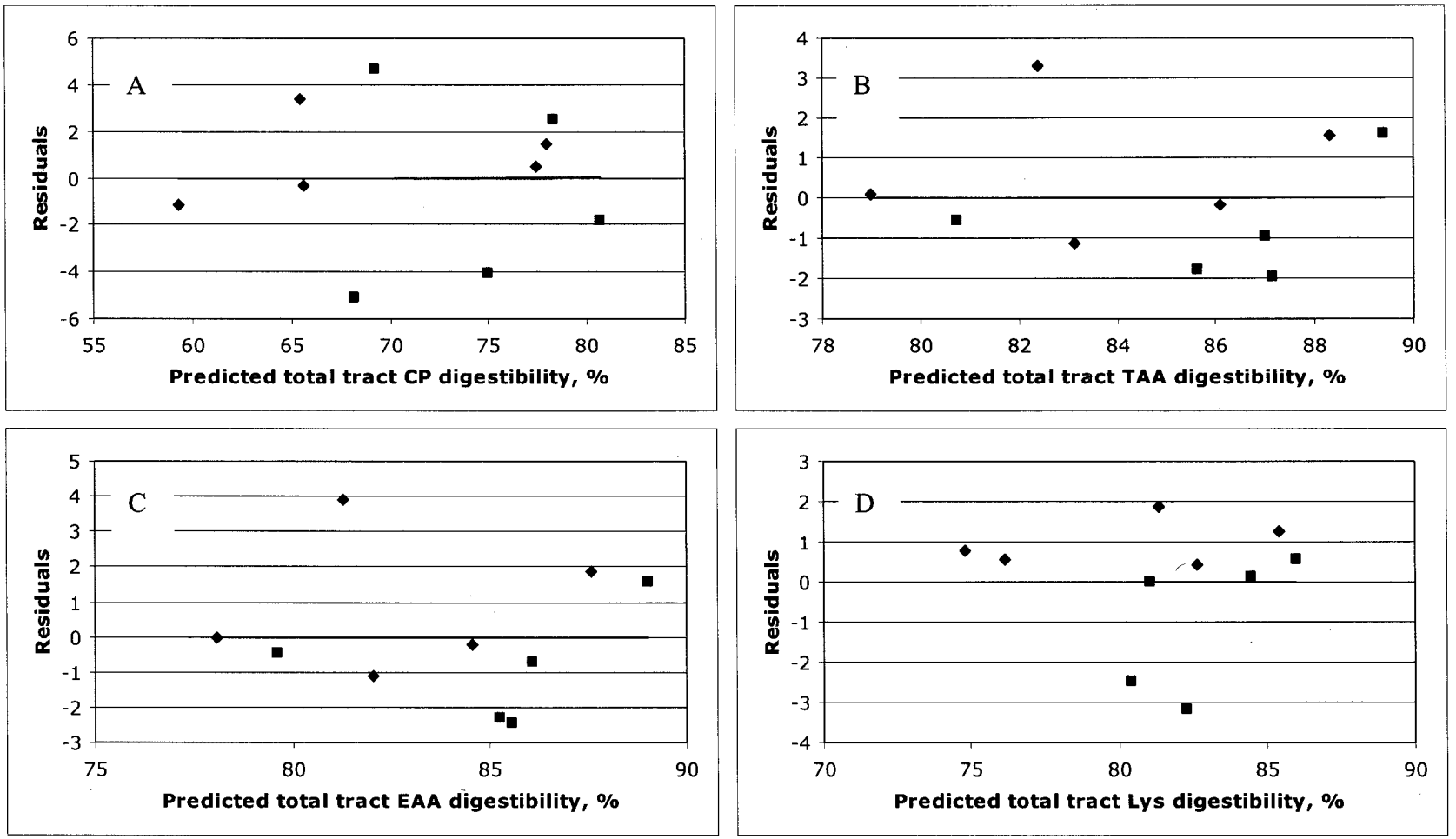

Figure 9. Residual plots of predicted total tract CP (A; $Y = -1.00E^{-5} + 1.49E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), TAA (B; $Y = -1.00E^{-5} + -8.05E^{-7}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), EAA (C; $Y = 1.00E^{-5} + 2.96E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10), and Lys (D; $Y = -1.00E^{-5} + 2.05E^{-6}X$; $R^2 = 0.00$; $P = 1.00$; n = 10) digestibility of corn silage samples incubated for 16 h (♦; n = 5) and 24 h (■; n = 5) determined by the mobile bag technique and the Sapienza Analytica in vitro procedure.

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Appendix A. Ingredient and composition of the experimental diets.

Table 1. Ingredient composition of the total mixed ration fed to lactating cows at Agriculture and Agri-Food Canada (Sherbrooke, QC).

	Percent (DM basis)
Corn silage	23
Hay silage	21
Corn grain	29
Soybean meal	12
Dry hay	6
Beet pulp	4
Soy supplement	2
Mineral mix	2
Megalac	1
Calcium carbonate	0.5

Table 2. Ingredient composition of the total mixed ration fed to lactating cows at the University of New Hampshire (Durham, NH).

Ingredient	Percent (DM basis)
Corn silage	38
Energy mix[1]	23
Soy/urea mix	13
Grass silage	12
Alfalfa hay	7
Vitamin/mineral mix[2]	3
Provaal	2
Megalac	1

Table 3. Ingredient composition of the total mixed ration fed to steers at Sapienza Analytica (Slater, IA).

Ingredient	Percent (DM basis)
Corn silage	79
Energy mix	13
Vitamin/mineral mix	4
Alfalfa hay	4

Appendix B. Glossary of amino acids and corresponding three letter codes.

Amino acid	Three letter code	Amino Acid	Three letter code
Arginine	Arg	Alanine	Ala
Histidine	His	Aspartic acid	Asp
Isoleucine	Ile	Cysteine	Cys
Lysine	Lys	Glutamic acid	Glu
Methionine	Met	Glycine	Gly
Phenylalanine	Phe	Proline	Pro
Threonine	Thr	Serine	Ser
Valine	Val	Tyrosine	Tyr

Appendix C. Institute for Animal Care and Use Committee Approval.

University *of* New Hampshire

Research Integrity Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
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13-Aug-2009

Whitehouse, Nancy
Animal and Nutritional Sciences
Dairy Nutrition Research Center
30 O'Kane Road
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IACUC #: 080702
Project: Evaluation of an In Vitro Method to Estimate Corn Silage Amino Acid Digestibility Post Ruminally in the Dairy Cow
Category: B
Approval Expiration Date: 21-Jul-2010
Modification Approval Date: 12-Aug-2009

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the requested modification to the protocol for this study:

Changes per 6/12/09 memo

with the following comment(s):

Approval at this time is to implement the requested modification at the UNH site; work at the site in Quebec may not commence until the UNH IACUC has received a copy of that site's IACUC approval documentation.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
Whitehouse, Jon
Fredin, Shane