EFFECTS OF DIETARY SOYBEAN MEAL AND FISH MEAL ON PROTEIN DIGESTA FLOW IN HOLSTEIN COWS DURING EARLY AND MIDLACTATION

by

Ercole Zerbini

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APPROVED:

Carl E. Polan

William E. Vinson

Joseph H. Herbein

Charles C. Stallings

Kenneth E. Webb, Jr.

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Blacksburg, VA

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INTRODUCTION

A new approach to ruminant nitrogen usage has been recently proposed by NRC (78). A key factor in this method is the total requirement of absorbed protein and, therefore, the estimate of true protein flow to the small intestine. This protein flow is composed of three fractions: microbial protein, undegraded dietary protein and endogenous protein (digestive secretions, epithelial desquamation, etc.). Microbial protein synthesis depends on a number of factors. Energy and nitrogen substrates for microbial growth in the rumen are most important. This protein fraction usually accounts for more than 50% of total protein flow to the duodenum. Dietary protein reaching the abomasum are described by NRC (78) to be composed of undegraded protein that resists microbial degradation in the rumen, and by-pass protein that are flushed out of the rumen without much mixing with ruminal content and therefore evades microbial degradation. NRC (78) and other approaches previously proposed (2,45,128) recognize that microbial protein reaching the small intestine does not meet the animal requirements in situations of rapid growth or relatively high milk production. Therefore, some dietary protein must escape ruminal degradation to supplement microbial protein in the intestine and meet the animal's requirement. The

determination of protein degradability in the rumen is therefore a key factor enabling formulations of dairy rations with greater degree of accuracy than previously done with the crude protein method. In vitro and in situ procedures have been utilized extensively, but both estimates have to be checked against results obtained in vivo. Few data are available on dietary protein degradation and total protein flow to the small intestine in lactating cows at high levels of intake. More research is needed to obtain values of protein degradability applicable to different diets, feeding regimes, and physiological state of the lactating dairy cow.

LITERATURE REVIEW

General Considerations

Degradable protein can be estimated *in vivo* indirectly by difference from total protein flow to the duodenum and the determined microbial protein flow.

Total protein or amino acid flow is determined with animals equipped with reentrant cannulae in abomasum or duodenum, allowing direct measurements of digesta flow. Recently, the use of simple T-type cannulae and of digesta markers have simplified the experimental procedure and allowed more *in vivo* research. The microbial protein fraction is estimated by reference to a "microbial marker," usually a component of the microbial cell or cell wall, or by incorporation of radioisotopes into the microbial cell in the rumen and their measurements in the duodenal digesta.

Factors known to influence microbial yield in the rumen include adequate supply of fermentable carbohydrates and degradable protein. The data obtained with *in vivo* experiments allow the construction of equations to regress dietary parameters on microbial yield. Therefore, prediction of microbial protein and undegraded dietary protein flow to the small intestine will allow the implementation of NRC (78) and other new approaches proposed

recently (2,45,128). Some of the factors influencing microbial yield and flow to the small intestine and degradability of dietary protein in the rumen will be discussed as follows with reference to in vivo experiments.

Microbial Yield and Flow to The Small Intestine

- Energy supply and source
- Nitrogen supply and source
- Effect of rumen digesta turnover rate.
- Expression of efficiency of microbial protein synthesis

Some Aspects of Dietary Protein and Amino Acid Metabolism in The Rumen

- Mechanisms of protein degradability
- In vivo measurements of protein degradability
- Factors influencing protein degradability
- Extent of dietary protein degradation in the rumen.

Amino Acids Flow to The Small Intestine

Effects of Physiological State on Digestion and Nutrient Partitioning

Microbial Yield and Flow to the Small Intestine

1. Energy supply and source

Microbial cell growth is dependent upon the amount of energy released during fermentation. Part of this energy is used for maintenance (Em) and part for growth. Theoretical values for Em range between .75 and 3.5 mmol ATP/g microbial dry matter/h (45). Such great variation is partly due to different digesta turnover rates in the rumen (1) as well as to different microbial species (2).

The theoretical yield of microbial dry matter per unit of energy is about 10-26 g microbial dry matter/mole ATP (78). Assuming that 4.4 moles of ATP are obtained from 162 g of glucose and the microbial cell contains 8.5% nitrogen (N) and 15% ash, the microbial N yield or efficiency of microbial protein synthesis (EMPS) is 20 to 63 g N/kg organic matter apparently digested in the rumen (DOMR) (1).

Contrary to *in vitro* experiments, where starch substrates yielded greater microbial dry matter than cellulose substrates (114), *in vivo* experiments with cattle showed less than average microbial yield with all-concentrate diets (1). ARC (1) reports that microbial yield increases with increasing energy concentration in the diet up to a certain point and then decreases at higher energy concentrations. Lower yields for concentrate diets may be due to: inadequate supply of degradable intake protein (DIP); decrease ATP supply due to increased production of

lactic acid; lower rumen pH; increased protozoa and protozoal ingestion of bacteria; increased microbial turnover in the rumen due to reduced salivation, rumination and rumen liquid turnover rate (19,67). Mathers and Miller (62) showed that organic matter disappearance in the rumen increased linearly with increased barley in the diet, but there was no significant change in microbial non-ammonia nitrogen (NAN) flow to the small intestine thus EMPS decreased from 29.6 to 22.7 g NAN/kg DOMR. Furthermore, EMPS seemed to be unrelated to alterations in rumen fluid VFA and rumen fluid dilution rate.

Chamberlain and Thomas (19) reported values of EMPS ranging from 15.0 to 36.5 g microbial N/kg DOMR. The greatest value was obtained with a hay:concentrate ratio of 5:2. Diets containing only concentrates were associated with a low proportion (.5) of microbial N in the duodenal N. They suggest that with this diet protozoal protein made an appreciable contribution to microbial protein outflow from the rumen. When dairy cows were fed diets containing barley or corn as an energy source, microbial nitrogen and organic matter were less with corn compared to barley diets. EMPS ranged from 21 to 28 g microbial N/kg DOMR and was lower for the corn diet (86). THe effect of physical alteration of energy source is well demonstrated by Meggison et al. (66) who found that EMPS increased from 17.4 to 24.6 g microbial N entering the small intestine/kg organic matter truly

digested in the rumen (TDOMR) by substituting rolled barley with flaked barley in a 7:3 concentrate-hay diet fed to mature Jersey cows. Interactions between forage:concentrate ratio and amounts of intake is illustrated by results reported by Merchen et al.(68). Higher intakes caused an increase in NAN flow to the small intestine due to increase in flow of both bacterial and non-bacterial N. However, wethers fed lower proportions of alfalfa at higher intakes had greater bacterial N reaching the duodenum than when fed greater proportions of alfalfa at higher or lower intakes. EMPS increased with higher intakes irrespective of forage concentration in the diet.

Madsen and Hvelpuld (58) reported results from experiments in which 33 diets were fed to lactating cows. The best estimates of total amino acids flow to the duodenum from analysis of the feed was obtained by dividing diets in two categories: a) barley straw plus great amount of concentrates, and b) silages supplemented with small amounts of concentrates. Diets containing larger amounts of roughages had higher EMPS. They attributed these results to a more steady fermentation in the rumen with roughage diets, but other factors are probably involved.

2. Nitrogen supply and source

Nitrogen requirement varies according to bacteria species (78). Fiber digesters require ammonia (NH_3), and branched chain amino acids for growth; starch digesters require also

NH_3 and some need peptides and amino acids for growth. Minimum concentration of NH_3 in rumen fluid for maximum microbial yield has been indicated to be 5 mg NH_3-N/dl (107) but values of 20 to 22 mg NH_3N/dl have been reported in the literature (70). Russell et al. (103) suggested that the requirement for ammonia is related directly to substrate availability, rate of fermentation and microbial yield.

The efficiency of conversion of degraded dietary protein into microbial protein is not one but seems to be ranging between .5 and .7 (1). If this is true, a loss of 20-30% of degradable intake protein (DIP) should occur in the stomachs. When DIP is supplied in adequate amounts but not excess, reports show that NAN flow to the small intestine approximately equals N intake. This indicates that inefficiency in capturing DIP in the rumen is offset by endogenous N entry preduodenally. Endogenous N originates from sloughed cells of mucosa, abomasal secretions and urea. Bruckental et al. (13) showed that in dairy cows more than 50% of urea derived from N metabolism in the body is passed into the alimentary tract. Cheng and Wallace (20) proposed that urea passes through the rumen wall by diffusion and is hydrolyzed on the inner face of the wall by ureolytic bacteria. Rumen NH_3 concentration seems to regulate the process. Recycling of urea through the rumen wall increased drastically when readily fermentable carbohydrates in the diet were associated with low rumen NH_3 concentration (47).

NRC (78) proposes an efficiency of DIP capture of .9 and consider recycling to be .12 nitrogen intake. This results in an efficiency of 1.02 which could vary according to protein degradability. Verite et al. (128) use 1.0, ARC (2) uses .8, and Kaufmann (45) do not consider it. Forty to 100% of microbial nitrogen has been reported derived from ruminal NH_3-N (46, 81, 109). The direct utilization of amino acids has also been reported, but energetic advantage of their use compared to NH_3 utilization seems to be small (41). Maeng et al. (59) reported that in vitro maximum microbial growth was obtained with 75% of N from urea and 25% from a mixture of amino acids. However, in vivo, the addition of degradable protein to purified diets containing urea increased microbial protein synthesis (104). It is agreed (1) that this type of response could be due to the rate of supply of NH_3 and branched chain fatty acids and/or to the rate of degradation of protein instead of a direct incorporation of amino acids. Ben-Ghedalia (9) obtained an increase in microbial protein synthesis with corn gluten meal, but not with either fish meal or casein supplements in the diet. Zerbini and Polan (130) showed higher proportions of microbial protein in the abomasum of ruminating calves fed a diet supplemented with soybean meal compared to diets containing corn gluten meal or fish meal. Low microbial yields obtained with grass silage (62) were increased by addition of soybean meal to the diet. In addition, Beever

et al. (8) showed that fish meal supplementation increased EMPS from 22.5 to 44 g microbial N/kg DOMR in growing calves fed a corn silage diet. In contrast, Merchen et al. (69) did not find any significant differences in bacterial N flow to the small intestine of steers fed brewers dried grains on a combination of brewers dried grains and urea or soybean meal. Similar results were reported by Petersen et al (92). Steers fed supplements containing urea, soybean meal or a combination of urea, soybean meal and blood meal did not show any treatment difference for organic matter digestibility, NAN, feed N, and bacterial N flow to the duodenum, as well as EMPS. In addition, no increase in bacterial protein flow to the duodenum was reported by substituting urea with peanut meal or fish meal as supplements in a barley based diet or by adding urea or peanut meal to a red clover and barley diet (67, 122). Rooke et al. (101) also reported no significant differences in the amount of microbial N entering the small intestine of Jersey cattle fed a hay-barley diet supplemented with two levels of either untreated or formaldehyde-treated soybean meal. EMPS was also similar for all treatments and averaged 34 g microbial N/kg DOMR.

In spite of some discrepancies among data, it is recognized (1) that in most circumstances, an adequate portion of degradable protein is usually supplied to meet microbial requirements for amino acids, peptides and

branched chain fatty acids. The use of protein instead of NPN as a source of supplementary nitrogen for microbial usage seems to have little advantage except as a more steady supply of nitrogen. However, it is possible that in formulating diets according to NRC (1978) (79) using protein resistant to ruminal degradation such as fish meal, corn gluten meal or dry brewers grains, degradable protein is insufficient and therefore lower microbial flow to the small intestine might be observed.

3. Effect of rumen digesta turnover rate

Solid and liquid outflow from the rumen modify the pool size of microbial mass passing to the small intestine. Van Soest (126) reported that increased forage intake increased microbial flow to the lower gut, probably by increasing saliva flow, pH and thus rumen fluid turnover rate. However, Zinn and Owens (134) reported no increase in EMPS with dry matter intakes greater than 1.8% body weight in steers fed a high concentrate diet. They suggest that lack of linearity in the response to feed intake was due to a deficit of rumen available feed N rather than an effect of ruminal dilution rate. By applying Michelis-Menten relationship, Van Soest (126) developed a regression equation which relates EMPS to rumen fluid dilution rate:

$$1/Y = .14 + .015\ (1/X) \qquad r^2 = .76$$

where: Y = g microbial N/100 g TDOMR

X = fractional rumen liquid dilution rate for

animals at maintenance.

The relatively small relationship between liquid dilution rate and microbial yield in vivo may be attributed to the association of microbes with feed particles. Therefore, dilution of liquid might be greater and unrelated to microbial growth rate (40). To take into account the association of microbes with both solid and liquid phase, Oldham (83) proposed that an appropriate constant Km for microbial outflow from the rumen should be expressed as follows:

$$Km = PsKs + PeKe$$

where: Ps and Pe = proportion of microbial population associated with solid and liquid phase.

Ks and Kl = solid and liquid outflow rates.

Km could then be used to predict microbial growth efficiency as illustrated in the following equation:

$$1/Y_{ATP} = 1/(Y_{ATP}^{MAX} + Me/Km)$$

where:

Y_{ATP} and Y_{ATP}^{MAX} = actual and theoretical maximum yields of microbial dry matter per mole of ATP available

Me = maintenance energy needs of the microbes.

4. Expression of efficiency of microbial protein synthesis

In vivo experiments provide data on flow of microbial matter to the abomasum or duodenum. Results

are usually expressed as microbial yield or nitrogen per kg of organic matter digested in the rumen. However, this may not measure true efficiency (78), but rather microbial wash-out and microbial matter recycled in the rumen. The probable implications of this are described by Oldham (83).

To predict microbial flow and efficiency, it is necessary to estimate extent of organic matter digestion in the rumen. A number of researchers (72,127) have related organic matter digestion in the rumen to overall rate of digestion and rate of passage.

$$D = K_d/(K_d + K_p)$$

where: D = organic matter digestion

K_d = rate of disappearance of organic matter

K_p = rate of digesta passage.

Johnson and Bergen (44) reports that for cattle the true proportion of total organic matter digested in the rumen was .76 ± .1. The large variation was attributed to differences in microbial yield and to measurement techniques (digesta and microbial markers). ARC (1), on the other hand, proposes a value of .65 to represent the proportion of digestible organic matter apparently digested in the rumen. This value was obtained from a range of diets for sheep and cattle. Only experiments which reported rumen NH_3 values greater than 6 mg/dl were used to assure nitrogen availability was not limiting microbial growth. Microbial

nitrogen yields per kg DOMR were summarized for different diets and classes of livestock; however, no clear difference could be associated with class of ruminant or type of diet and the average value of 32 g microbial N/kg DOMR has been proposed for all diets, whether given to sheep or cattle (1). In addition, a quadratic equation was derived from cattle data relating microbial yield (gN/kg DOMR) to metabolizable energy (ME) in the diet (MJ/kg) and the proportion of roughage (R) in the diet.

a. Microbial yield (g N/kg DOMR) = $22.6ME-1.17ME^2-76.6$.

b. Microbial yield (g N/kg DOMR) = $19.6R-4.0R^2+23.1$.

Then it is possible to calculate the amount of degraded nitrogen required by rumen microorganisms (RDN) by taking into consideration the following parameters:

a. Metabolizable energy intake (ME).

b. Factor (F) for conversion of ME to DOM assuming that 18% of apparently digested energy is lost in methane and urine, and that 1 kg DOMR = 19.0 mg digestible energy. Therefore,

$$F = 1/(0.82 \times 19)$$

c. Proportion of DOM digested in rumen (DOMR) = .65

d. Microbial nitrogen yield = 32 g N/kg DOMR.

e. Efficiency of conversion of degraded N to microbial N = 1.0

$$RDN\ (g/d) = ME \times 1/(0.82 \times 19) \times .65 \times 32 = 1.33\ ME.$$

NRC (78) does not propose an average value for

microbial yield/kg DOMR but follows a similar method in deriving equations predicting it. NRC (78) proposes an empirical model in predicting microbial nitrogen flow to the duodenum with parameters easily measured in the field such as dry matter or organic matter intake. The equation for cattle relates microbial N prediction to TDN or NE_l intake.

For diets with >40% roughage:

Microbial N (g/d) = -31.9 + 26.13 TDN (kg).

Microbial N (g/d) = -30.92 + 11.45 NE_l(Mcal/d).

TDN has been chosen because it estimates well digestible organic matter and because of its large data set. Certain interactions such as those between forage, energy value of the diet and intake need to be taken into account also and eventually be incorporated into the model. Relating microbial yield to TDN or to DOMR accounts for a major variation in microbial yields, some variation remains unaccounted for. Factors, such as diet type and processing, rumen escape estimates, potential substrate degradability in rumen, flow of microbes from the rumen and interaction of N recycling and microbial flow to the small

NRC (78) proposes a regression equation which estimates bacterial crude protein (BCP) from TDN, forage intake and concentrate intake. For diets with <40% roughage:

BCP = 6.25 TDN (8.63 + 14.60 FI - 5.18 FI^2 + .59 CI) r^2=.96

where FI = forage intake (% body weight)

CI = concentrate intake (% body weight).

The amount of ruminally available protein (RAP) then necessary to synthesize BCP is: RAP = BCP/.9

where .9 = efficiency of RAP utilization.

Forage:concentrate ratio and concentrate source greatly influence efficiency of microbial protein synthesis. Greater nitrogen flow to the small intestine is observed with diets containing greater proportions of roughage. However, EMPS may increase with higher intakes even when high amounts of concentrate are present in the diet.

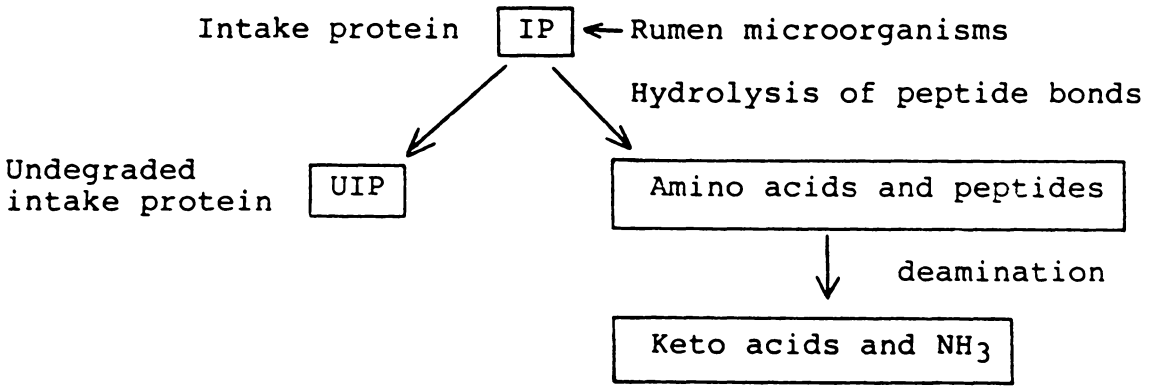
Optimal concentrations of NH_3-N in rumen fluid for maximum microbial growth seems to be greater than 5 mg/dl and the efficency of conversion of degraded protein into microbial protein is less than one. Nitrogen recycling may counterbalance this inefficiency. Diets containing considerable proportions of escape proteins may not supply sufficent degradable protein for maximum microbial protein synthesis.

Liquid and solid dilution rates have to be taken into account to better predict microbial outflow from the rumen. Organic matter digested in the rumen accounts for a considerable part of the variation in microbial yield. ARC (2) proposes constant value of 32 g microbial N/kg organic matter apparently digested in the rumen across diets for sheep and cattle. NRC (78) uses TDN in its prediction equation and takes forage and concentrate intake into account.

Some Aspects of Dietary Protein and Amino Acid Metabolism in The Rumen

Major processes which will be described in the following section are the mechanisms and factors involved in dietary protein degradation by rumen microorganisms. In addition some of the factors involved in NRC (78) and ARC (1) calculations for protein requirements related to protein degradability will be discussed.

1. Mechanisms of protein degradation.



Dietary proteins are broken down in the rumen by microbial proteases to amino acids and peptides. It is suggested (103) that this is the rate limiting step during degradation. This is supported by reports showing very low concentration of free amino acids in rumen digesta (41). Bacteria secrete proteolytic enzymes outside the cell where

protein hydrolysis occurs. Amino acids or peptides can be absorbed directly into the cell (113), further degraded to NH_3 and fatty acids, or leave the rumen with ruminal digesta. The proportion of microbial nitrogen derived from ruminal NH_3-N has been shown to range from .4 to 1 (113). In protozoa, proteolysis takes place inside the cell and, if amino acids are not utilized directly (7-18%), they are excreted back into the surrounding fluid (113,56).

Bacteria and protozoa are both important in ruminal protein degradation. However, more data is available on bacteria than protozoa. Nutritional requirements and interactions play an important role in the degradation process. Although diet *per se* does not seem to affect proteolytic activity, its indirect effect on pH and number and type of microorganisms may influence dietary protein degradation (46).

Bacterial protease appear to have a trypsin-like activity, exposing arginine and lysine to further degradation by other bacterial enzymes (78). This is supported by in vitro and in vivo findings, where disappearance of arginine and lysine in rumen fluid were extremely large (26,117).

2. *In vivo* measurements of protein degradability.

In vivo measurements are extremely important because they are the standard reference used to evaluate in vitro methods (78). These estimates require the use of surgically

prepared animals with cannulae in the omasum, abomasum or proximal duodenum. In addition, there is need of a reliable technique for calculating total digesta passage to the small intestine and one which would allow an estimate of proportional protein flow of microbial origin (117). Cannulae commonly used are re-entrant type or simple T-type. With re-entrant cannulae, digesta flow is determined by total collection or by spot sampling with reference to an indigestible marker. With T-type cannulae and spot sampling, more than one marker is advisable because duodenal digesta samples may not be representative of "true" digesta flowing through. For this reason a dual marker technique has been proposed (30) where a soluble marker (Lm) would be a reference for liquid flow, and a marker bound to feed particles (Sm) which would be a reference for solids flow. Rate of liquid and solid passage are calculated as follows:

Rate of liquid passage = (intake/day of Lm)/Lm concentration in duodenal liquid.

Rate of solid passage = (intake/day of Sm)/Sm concentration in duodenal solids.

Combining data from 1 and 2 will give an estimate of total digesta flow. Recently, Armentano and Russell (5) proposed a method of calculating digesta flow from non-representative samples of digesta in which more than two phase and relative markers can be combined in the calculation of digesta and nutrient flow. However, no matter how sophisticated the

technique is, measurements of digesta flow are subject to error and no marker is ideal. There are problems with complete recovery, suggesting that small quantities might be absorbed by microbes or the host animal. Marker distribution within digesta and association with one or the other phase may not be ideal. Basically, two methods are used for estimating dietary protein degradation in vivo: the regression technique and the "by difference method".

a. Regression technique.

With regression basal diet with the lowest amount of protein to supply adequate nitrogen for microbial synthesis is formulated. The test protein is added to the basal diet in incremental amounts, keeping dry matter intake constant across diets. Microbial protein flow to the small intestine is assumed to be the same for all diets. Duodenal amino acid flow is regressed on amino acid intake, and the increase in flow of amino acids is attributed to the test protein. The undegraded test protein is represented by the slope of the equation. In addition, regressions of duodenal NAN flow on N intake and multiple regression equations including a correction for microbial amino acid flow, gives similar results.

Results on corn gluten meal protein escape in lactating cows by this technique were reported by Stern and Satter (117):

1. $Y = 1183 + .57X \quad r = .81$

where: Y = duodenal amino acid flow (g/day)

X = amino acid intake (g/day)

2. $Y = 74.9 + .55X \quad r = .82$

Y = duodenal amino acid flow (g/day) - bacterial amino acid flow (g/day)

X = amino acid intake (g/day)

3. $Z = 209.3 + .55X + .88Y$

Z = duodenal amino acid flow (g/day)

Y = bacterial amino acid flow (g/day)

X = amino acid intake (g/day)

Proportions of corn gluten meal which escaped ruminal degradation are .57, .55, and .55 with the three different equations, respectively.

The relation between the ratio of duodenal NAN/N intake and nitrogen concentration in the diet was also used to estimate the proportions of undegraded dietary N and microbial N flow to the duodenum (120):

$A = Y - b/X$ where: A = proportion of undegraded NAN

b = microbial N/kg dry matter intake

Y = NAN/N intake

X = N concentration in diet g, N/kg dry matter

Proportions of undegraded protein in duodenal digesta obtained with this technique were reported by Tamminga et al. (120) for two levels of intake in dairy cows.

b. By difference technique.

Undegraded dietary protein is estimated by difference

between total and microbial protein entering the abomasum or small intestine. This method requires an estimate of microbial protein synthesis in the rumen.

UIP = IP - (BCP + endogenous protein)

where: UIP = undegraded intake protein

IP = intake protein

BCP = bacterial crude protein

Estimates of endogenous N are difficult to obtain and in most instances ignored, allowing an overestimate of UIP.

Bacterial crude protein is estimated by using a microbial marker. Some of these are components of the bacterial cell or cell wall, and others are radiolabelled isotopes incorporated into bacterial proteins, phospholipids or nucleic acids (112). As for digesta flow markers, there is no ideal microbial marker and some of the variation in protein escape could be attributed to different microbial markers.

This technique does not allow determinations of protein escape for single feedstuffs, but it estimates undegraded intake protein which includes all diet components (117). However, the method ranks different feedstuffs and also may show some interactions between feedstuffs in the diet which would not be detected by the regression technique.

3. Factors influencing ruminal protein degradability.

An important factor regulating protein degradation in the rumen is microbial access to the protein which can be

influenced by the following:

a. Tertiary structure of protein

Cyclic proteins as well as those containing large numbers of cross-linking bonds (disulfide bonds) or methylene cross-linking (formaldehyde treatment) are relatively resistant to microbial degradation because they are less accessible to microbial proteolytic enzymes (78). Prolamines and glutelins are usually more insoluble than albumins and globulins. However, solubility is not necessarily synonimous of degradability. Mahadevan et al. (60) reported that although soluble, serum albumin and ribonuclease A were resistant to hydrolysis by Bacteroides amylophilus protease. Soluble and insoluble proteins of SBM were hydrolyzed at the same rate; but, soluble proteins from soybean meal, rapeseed meal and casein were hydrolyzed at different rates. They concluded that solubility or insolubility is not an indicator per se of degradability or resistance to degradation by rumen bacterial protease, and that natural resistance of proteins tested was due to the stabilizing effect of disulfide bridges.

b. Animal factors

Ruminal retention time (RRT) dictate time exposure of proteins to microbial attack and therefore influence extent of protein degradation. Tamminga (120) reported escape protein increased from 29 to 45% when intake was increased from 8.2 to 12.9 kg/day in dairy cows. Aim et al. (134)

reported protein degradation decreased 41% when protein supplement intake increased 25%. However, intake effect was different among diets and SBM did not show any appreciable decrease in degradability. Zinn and Owens (133) reported that in steers fed a high concentrate diet, flow of N, NAN, microbial N and feed N to the duodenum increased linearly as level of feed intake increased. Escape protein increased from 44 to 71% of feed protein. It seems that increased feed intake reduces protein degradation, but the effect is reduced when feed intake is greater than 2% body weight (78).

Ruminal escape of SBM and CSM protein decreased from 43 to 24% and from 50 to 43% when the proportion of dietary concentrate was decreased from 80 to 40%, suggesting that roughage level in the diet alters protein escape (29). A report by Elimour and Orskov (29) shows that there were highly significant linear effects of feeding level on fractional outflow rate of Cr-treated FM and SBM supplements from the rumen of sheep and cows. There were no significant differences between protein supplements. Orskov et al. (89) showed that ruminal outflow of protein can vary from .01 to 1.0/h at low or high levels of intake, respectively. The authors suggest that if the degradable fraction of a protein source is degraded rapidly, outflow rate will influence total degradation to a lesser extent than if degraded more slowly. As outflow rate increased, differences in

degradability between fish meal, meat-and-bone meal, soybean meal, cottonseed meal, linseed meal and ground nut meal decreased. They also point out that ranking order of protein supplements for degradation may vary according to outflow rate. Therefore it is not possible from a single value to extrapolate the different rates of outflow. An increased flow of protein to the small intestine of sheep was observed by Prigge et al. (96) with increased rumen fluid dilution rate. Rumen pH is also an important factor influencing protein degradation. Variations in pH can alter protein solubility and microbial proteolytic activity. Optimal pH range is suggested to be between 6 and 7 (120).

Feed processing and storage are also known to influence protein degradability and some of their effects will be reported later.

The regression and by difference techniques are the most used to predict in vivo protein degradation in the rumen. The first allows calculation of degradability values for individual feedstuffs, while, with the latter, degradabilities of complete diets are obtained. Important factors regulating ruminal degradation of dietary proteins are the tertiary structure of protein, feed processing, and ruminal retention time.

4. Extent of dietary protein degradation in the rumen.

In vivo data for dietary proteins is scarce. A large variation in values is probably due to physical nature of

diets, methods of feeding, experimental animals, diets used and intake, variation in feedstuffs, and analytical errors (78). Some peptides and amino acids seem to be more susceptible to degradation than others (26), therefore, it is important to assess the quality of undegraded proteins leaving the reticulorumen (34,36). Some experimental results on protein and amino acid degradation in the rumen are reported below.

Santos et al. (106) reported degradability values of 70, 45, 52 and 46% for soybean meal (SBM), corn gluten meal (CGM), wet brewers grains (WBG) and dry distillers grains (DDG), respectively, when they were included in lactating cow diets. They concluded that diets containing CGM, WBG, or DDG generally supply more total amino acids (AA) to the small intestine than a diet containing SBM. Resistance to microbial degradation in the rumen did not seem to affect AA availability for absorption. Similarly, Loerch et al. (55) reported that diet supplementation with blood meal (BM) or dehydrated alfalfa (DA) resulted in greater N flow to the small intestine of sheep than diet containing SBM. Proportions of ruminal protein escape for SBM, BM and DA were 28.7, 81.7, and 63.3%, respectively.

Zinn et al. (134), in an experiment with Holstein calves fed a 40:60 roughage:concentrate diet containing either casein (CA), SBM, CSM, or CGM, estimated protein degradabilities of 103, 85, 76, and 54%, respectively. CGM

produced the highest NAN flow to the duodenum. In another study percentages of dietary crude protein recovered at the abomasum were greater when steers were fed the whole cottonseed (WCS)/CGM compared to the SBM diet (40).

Values reported above support the concept of varying protein source in the diet and therefore degradability to obtain greater amounts of NAN flow to the small intestine. This would be beneficial to support relatively high milk production yields in lactating cows or rapid growth in young animals.

To decrease the degradability of dietary protein sources of high biological value and increase their proportion in digesta passing through the abomasum, chemical and physical treatments have been developed (119). Some literature values follow. Stern et al. (116) reported that extrusion of whole soybeans at 149 C decreased protein degradation in the rumen from 80 to 60% and increased amino acid flow to the duodenum of lactating Holstein cows, compared to raw soybeans. In a similar experiment with lactating cows, Kung et al. (51) showed that heat-treated SBM was less degradable than SBM (55 vs 66%). Digestion in the small intestine of NAN did not differ between treatments, indicating availability of amino acids for absorption from the two sources was similar. Results summarized by Chalupa (17) show that ruminal protein degradation was decreased by treatment with formaldehyde,

and the amount of NAN entering the small intestine increased significantly.

In summary, there is evidence that by changing protein source in the diet, or by treating protein sources highly degradable in the rumen chemical or physical means, an increase in NAN flow to the duodenum of cattle is obtained. Some production data, relating to these findings are reported below.

Generally, an increase in milk production is obtained by supplementing diets of cows in early lactation with protein sources relatively resistant to ruminal degradation. Oldham et al. (85) showed that replacement of urea-N with fishmeal-N significantly increased yield of milk protein both in early and in mid-lactation. Similarly, Orskov et al. (91) reported higher fat corrected milk for cows fed fishmeal compared to ground nut meal in early lactation. Infusing proteins into the abomasum or duodenum did increase milk production and efficiency of use of absorbed nitrogen. The maximum response was obtained with high producing cows, indicating the greater need for amino acid nitrogen in early lactation.

A number of studies (72, 102, 110) have reported increased milk production with heated SBM fed to high producing cows in early lactation. However, Tamminga (119), in his review reports that response of milk production to formaldehyde treatment of dietary protein is small.

Furthermore, Dijk et al. (27) reported no production increase in cows in early lactation fed extruded soybeans, compared with raw ground soybeans. Henderson et al. (40) reported lower milk production in cows in early lactation receiving a whole cottonseed/corn gluten supplement compared to that of cows fed SBM or extruded SBM. However, no differences were observed between SBM and extruded SBM. Lack of production response and reduced milk protein was observed by Crawford and Hoover (27) from formaldehyde treatments of SBM fed to dairy cattle.

In view of the reports above, it is important to look at the amino acid degradation in the rumen and its possible effect on quality of escape protein. Some discrepancies in animal performance may be due to a process of amino acid selectivity by the microbial population in the rumen. For instance, when the quality of protein in the original fish meal and sunflower meal and their digested residues (from polyester bag incubated in the rumen of sheep) was compared in a rat growth trial, partial degradation within the rumen did not affect protein quality of fish meal, but decreased the protein efficiency ratio of sunflower meal residue (63). Stern et al. (115) reported that in lactating cows 57% of CGM in the diet escaped ruminal degradation. Total AA degradation in the rumen of CGM was 43%. Individual AA degradation was greater for the essentials (EAA) and basic AA and ranged from 22 and 62%. Only 38% of lysine in CGM

escaped degradation. This could represent a disadvantage because CGM is already low in lysine.

Santos et al. (106) found similar flows of lysine to the duodenum of dairy cows fed diets containing SBM, CGM, WBG and DDG. However, intakes of lysine for the SBM diet was about 200% greater than the other diets. Therefore, they suggest that lysine is extensively degraded in the rumen, as well as histidine and phenylalanine in which flows to the duodenum were less than intake. On the other hand, flow of methionine and threonine exceeded intakes of these amino acids for all diets. This is in agreement with McMeniman and Armstrong (65) who reported that in bean (Vicia faba) supplemented diets, significant proportions of all of ingested amino acids except methionine were apparently degraded in the rumen of cattle. In sheep, losses of AA in the rumen when a high-protein diet was consumed were particularly large for glutamic acid, aspartic acid, proline, arginine and leucine. They were least for cystine and threonine (22).

Incubation of casein, soybean meal, peanut meal, meat meal, blood protein and cottonseed meal with rumen fluid showed that individual AA were not released in proportions equal to amounts present in the protein. Alanine, arginine, histidine, lysine and phenylalanine were degraded to a greater extent than total degradation which ranged from 59.2% for casein to 14.2% for blood protein (26).

The amino acid profile of undegraded silage protein which remained in polyester bags after incubation in the rumen of cattle, differed from that of the protein in the original silage (100). After two hours of incubation, arginine, glycine, lysine, phenylalanine and leucine increased, while valine, aspartic acid, glutamic acid, proline and alanine decreased. After correction for bacterial contamination, the silage residues contained markedly greater quantities of isoleucine and leucine, smaller amounts of lysine and basically no methionine. When fraction I leaf protein degradation was measured in vivo and in vitro branched chain AA (valine, leucine and isoleucine) were released in greater proportions than the other AA (82). Degradation of AA in pure cultures of rumen bacteria showed valine and methionine to be most resistant (108). Differences in results obtained in vitro, in situ and in vivo may be due to distribution, amino acid sequence and composition of various classes of protein or in differences in activities of enzymes involved in amino acid degradation (16). NRC (78) ranks AA according to their susceptibility to microbial degradation as follows.

Lysine

Arginine > Phenylalanine > Valine

Threonine Leucine Methionine

Isoleucine

The results summarized suggest that the availability of some

AA in the residue left after proteolysis may differ from that of the AA in the protein supplements from which they were derived. It appears that differences between nitrogen compounds ingested and those arriving at the duodenum may be substantial, particularly when a considerable amount of dietary protein is supplied by forages. NRC (78) suggests that acid detergent insoluble-N be subtracted from intake to give a better estimate of digestible undegraded protein.

Amino Acid Supply to The Small Intestine

Duodenal digesta nitrogen is the sum of undegraded dietary nitrogen compounds, microbial nitrogen and endogenous nitrogen. Oldham and Tamminga (87) indicated that duodenal nitrogen is represented about 35% by essential amino acids, 30% nonessential amino acids, 6% ammonia-N, 4% amides, and 14% unknown.

Amino acid composition of duodenal digesta is influenced greatly by the microbial component. Therefore it is not surprising to find reports (2, 67) which show a small coefficient of variation between duodenal digesta amino acid composition in animals fed different diets containing either NPN or natural protein. Substantial variation from the relatively constant amino acid profile of rumen microrganisms is only seen when a protein resistant to ruminal degradation and with a different amino acid profile than that of microbial protein is supplemented to the diet.

Table 1, adapted from Buttery and Foulds (14), shows the mean amino acid proportions in duoenal digesta, calf whole body and cow's milk. Milk appears to have a higher essential amino acid concentration than duodenal digesta. Since milk production accounts for a considerable proportion of amino acids requirement, a system which considers total amino acid requirements would not provide sufficient amounts of essential amino acids. This criticism was made by ARC (1) on the previous ARC (2) system, but it would apply also

TABLE 1. Essential amino acid proportions in duodenal digesta, calf carcass and milk protein.[1]

Amino acid[2]	Duodenal digesta	Calf whole body	Cow's milk
Leucine	.16	.19	.19
Isoleucine	.10	.08	.11
Valine	.12	.11	.13
Methionine	.05	.05	.05
Phenylalanine	.11	.10	.10
Threonine	.12	.11	.09
Lysine	.15	.18	.15
Tryptophan	.03	.02	.03
Histidine	.04	.07	.05
Total sulfur amino acids	.08	.08	.07
Total aromatic amino acids	.19	.17	.19
Essential/total amino acids	.48	.38	.53

[1]Adapted from NRC (5).

[2]Grams individual amino acid/g total essential amino acids.

to NRC (78) which considers total true protein requirement. Reports (14, 18, 123) show that lysine and methionine are limiting for milk production, particularly on silage based diets. Often milk production shows a positive response to dietary protein supplements or to abomasal infusion of protein (21). Based on these observations ARC (1) suggests that with the present system, the requirements for undegraded intake protein for high producing cows might be underestimated. In addition, as suggested by Buttery and Foulds (89), undegraded protein must not be deficient in any of the essential amino acids.

Effects of Physiological State on Digestion and Nutrient Partitioning

At the onset of lactation the demand of the mammary gland for nutrients is quite large and very appropriately Bauman and Currie (7) refers to "the cow as an appendage on the udder rather than the reverse." Milk secretion becomes the driving force to which other processes become subordinate. After calving, milk yield rises rapidly to a maximum between day 35 and 50 of lactation; thereafter milk production decreases at a rate of about 2.5% per week. Voluntary intake, on the other hand, increases more slowly after parturition and the maximum is reached few weeks after milk yield peak. During early lactation, energy intake from corn silage based rations is usually less than the animal's requirement, therefore, a loss of body weight is usually seen at this stage and the potential milk production may not be obtained. Feed intake remains high for an extended period of time after peak intake and replacement of previously mobilized body tissues occur. By midlactation the cow is able to consume the energy she can utilize, not only for maintenance or milk production but also for body fat deposition. Appetite does not decrease substantially until lactation terminates.

At the beginning of lactation, there is an increase in the volume of digesta and in the weight and capacity for water and solids of the rumen and intestines. Hypertrophy

of the gastrointestinal tract might be an adaptation to increased feed intake, or hormonally regulated or a combination of the two (76, 15, 75, 31). Studies with rats have shown that absorption of leucine and of glucose was increased during lactation and reached a peak on the 10th day of lactation (28). They also observed that the ability of the mucosal cells to absorb changed along with the physiological state of the animal. In ewes maximum enlargement of ruminal mucosa occurred during the sixth week of lactation and was maintained while weaning (31). Fell et al. (31) also reported an increase in food intake and a decrease in dry matter and N digestibility from pregnancy to lactation. They attributed these digestibility changes to a shift in site of digestion and to a greater secretion of metabolic fecal nitrogen associated with gastrointestinal tract hypertrophy. Moon and Campbell (75) reported a constant ratio of rumen weight and feed intake. Correlations developed were as follows:

$M = 245 + 172\ I$ where M = weight of rumen mucosa (g)

$P = 4.0 + .63\ I$ I = food intake (kg/day)

P = length of rumen papillae (mm)

Although prolactin seems to intervene on the hypertrophy of the gastrointestinal tract (33), feed intake seems to be the overriding factor. As suggested by Forbes (33), it seems that the principal effect of lactation on digestion and metabolism is on feed intake, which is increased by the

demand for nutrients for milk production.

A more efficient extraction of nutrients could be expected from hypertrophy of the gut and by an increased blood flow to the digestive organs (37), allowing greater efficiency of utilization of nutrients in a physiological state of high needs. However, there is some confusion about absolute amounts of nutrient absorbed and efficiency of absorption. Decreases or no changes in digestibility are reported in the literature with increased feed intake in lactation (31). It appears that with increased size of the digestive organs more material can be digested leaving digestibility coefficients constant.

The role of prolactin in gut hypertrophy is controversial; however, Muller and Dowling (76) indicated that some metabolic factor was involved because hypertrophy of the jejunum occurred even when it was not subjected to increased digesta flow which occurred in the rest of the digestive tract.

In early lactation, in syncrony with increased size of the gut, there is an enlargement of the liver due to hyperplasia associated with hypertrophy of cells and nuclei. By doing so, the liver can impose a particular emphasis upon certain metabolic pathways. Mackie and Campbell (58) showed that the activities of glucose-6 phosphatase (gluconeogenesis) and arginase (urea cycle) increased steadily during lactation reaching a maximum during late

lactation. This is in agreement with the lower glucose and amino acid oxidation in early lactation compared to midlactation reported by Oldham (83).

The increased rate of lipolysis and decreased uptake of nutrient for synthesis in the adipose tissue, supply oxidative substrates to spare further glucose and amino acids. These events are regulated by homeostatic and homeorhetic mechanisms (7). Bauman and Currie (7) observed that prolactin secretion decreased during the lactation period and they proposed that it was playing a role in partitioning nutrients in liver and adipose tissue toward the mammary gland. Similarly Brockman (11) indicated that less insulin release promotes nutrient movements to the mammary gland. On the other hand hyperinsulinemia increased body gain. Growth hormone may direct lactation by reducing glucose utilization and fat synthesis by extramammary tissues stimulated by insulin by inducing insulin resistance in those tissues. The increase in growth hormone observed in some studies (83) after casein was infused abomasally, or formaldehyde treated proteins may be responsible for some of the response seen and partly explain the lack of response obtained with abomasally infused glucose. Glucose infusion may increase insulin and therefore promote body gain and not milk production. Extra protein supplied to the small intestine has a much greater effect in cows are in negative energy balance. Supplementation of amino acids at the

duodenum increases loss of body weight further and increases milk production. It is possible that growth hormone plays an important role in these metabolic events.

OBJECTIVES

The objectives of this experiment were to determine the effect of highly degradable or relatively undegradable protein sources in the diet on quantity and description of nitrogen compounds entering the small intestine in lactating Holstein cows during early and midlactation.

MATERIALS AND METHODS

Diets

Corn silage based diets were formulated to meet the requirements of 590 kg Holstein cows (79) producing 36 kg/day of milk, 3.5% fat. Fish meal (FM) and soybean meal (SBM) were protein supplements which supplied 54 and 56% of total protein in the diet respectively. FM and SBM diets contained 15.4 and 15.5% crude protein and 20.9 and 20.5% ADF, respectively. Diet formulations and specifications are in Table 2.

Animals and feeding

One to 3 weeks after calving, six lactating Holstein cows from the University Dairy Cattle Center were fitted with rumen cannula and T-type cannulae in the duodenum.

Rumen cannulae (100 mm ID) were manufactured at the Research Division Machine Shop and installed in the rumen by surgical personnel of the Maryland-Virginia College of Veterinary Medicine. Flexible T-type cannulae[1] (25.4 mm ID, 33.0 mm OD) were surgically fitted in the proximal duodenum, about 10 cm from the pylorus. During this period cows were fed twice daily a total mixed diet containing corn silage,

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[1]Manufactured by Dr. D. McGilliard. Department of Animal Science, Iowa State University. Ames, Iowa.

TABLE 2. Dietary ingredients and diet composition.

Item	Diet: Fish meal	Diet: Soybean meal
Ingredients[1,2]		
Corn silage	56.5	52.0
Orchardgrass hay	7.2	7.0
Ground corn	23.1	20.3
Soybean meal[3]	-	17.9
Fish meal[4]	12.9	-
Trace mineral salt[5]	.2	.2
Dicalcium phosphate	-	1.5
Limestone	-	.9
Magnesium oxide	.1	-
Composition		
Dry matter	48.0	50.3
Organic matter[1]	94.7	94.6
Crude protein[1]	15.4	15.5
Acid detergent fiber[1]	20.9	20.5

[1]Dry matter basis.

[2]Total mixed diets were supplemented with 3,190 IU vitamin A and 308 IU vitamin D per kg dry matter.

[3]Contains 48% crude protein, dry basis.

[4]Menhaden, 67% crude protein, dry basis.

[5]Composition (g/100g): NaCl (97-98.5); Fe (>.2); Mn (7.2); Zn (>.2); Mg (>.1); Sulfate (>.04); Cu (>.02); Co (>.007); I (>.007).

alfalfa haylage, high moisture corn and mineral mix. Between 3 and 5 weeks in lactation, cows had apparently recovered from surgery and were assigned to experimental treatments.

In early lactation (28 to 40 d in milk) cows were randomly allotted to FM or SBM diets and fed the assigned diet for 17 days. Then cows fed FM were changed to SBM and vice versa for another 17 days. In midlactation (122 to 140 d in milk) cows were rerandomized to FM or SBM diets and feeding sequence was as in early lactation. A summary of treatment assignments is shown in Table 3. Cows were housed in comfort stalls bedded with saw dust and fed equal portions of the total mixed diet every six hours (200, 800, 1400 and 2000 h). They were milked in a milking parlor twice a day (0650 and 1650 h) and exercised afterwards. Plastic buckets were used as muzzles to prevent cows from eating during this time.

Liquid and solid digesta markers

Cobalt ethylenediaminetetraacetic acid (Co-EDTA) was prepared according to Uden et al. (124) and used as a liquid digesta marker. Fifteen g of Co-EDTA (1.8 g Co) were diluted into 800-900 ml of tap water were infused daily and continuously with a peristaltic pump[2] into the rumen.

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[2]Buchler Multistatic Pump. Buchler Instr. Inc. Fort Lee, N.J.

TABLE 3. Summary of diet assignment to cows in early and midlactation[1].

Stage of lactation	Feeding period	Feeding length (d)	Cow no.					
			1482	1592	1690	1691	1696	1700
E[2]	1	17	FM	FM	SBM	SBM	SBM	FM
E	2	17	SBM	SBM	FM	FM	FM	SBM
M[2]	1	17	SBM	SBM	FM	FM	SBM	FM
M	2	17	FM	FM	SBM	SBM	FM	SBM

[1]FM = fish meal diet; SBM = soybean meal diet.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

Infusion was stopped from 0600 to 0700 h and from 1600 to 1700 h to allow cows to be milked and exercised. Co-EDTA was infused 5 days preceding, and throughout the 3-day collection period.

Ytterbium (Yb) was used as solid marker. Chopped orchard grass hay was labelled with Yb by soaking 10 kg of hay in about 100 l of tap water containing 150 g of $YbCl_3 \cdot 6H_2O$ in solution for 12 h. Thereafter, hay was continuously washed with tap water for 6 h to remove loosely bound Yb (121) and dried in a forced air oven at 65 C for 48 h. Labelled hay contained between 4.4 and 5.7 g/kg of Yb, and replaced 1.0 kg of unlabelled orchard grass hay in the diet during the period of Co-EDTA infusion.

Measurements and sampling

Body weight was recorded at 0700 h three times during each experimental period in both stages of lactation. Individual cow intake was recorded daily by weighing orts at 2000 h. Samples of total mixed diet, corn silage, orchard grass hay, ground corn, soybean meal, fish meal, and orts were taken on day 12, 13 and 14 of each experimental period, sealed in a ziploc plastic bag and frozen at -20 C for subsequent analysis.

Six samples of rumen fluid, rumen content, duodenal content, and feces were collected during a period of 3 days (day 12, 13 and 14). Two samples were taken each day 12 h apart. Sampling sequences were 0300 and 1500 h, 0700 and 1900 h and

1100 and 2300 h randomly assigned to day 12, 13 or 14. This sampling procedure was chosen to minimize diurnal variation and feeding time effects. Rumen fluid samples were collected through the rumen cannula and strained through two layers of cheese cloth into a plastic container and pH was measured immediately. A five and 10 ml sample of rumen fluid was pipetted into plastic culture tubes containing 1 ml of 25% (W/V) meta-phosphoric acid or three drops of 98% sulfuric acid and stored at -20 C for subsequent analysis of VFA and NH_3-N, respectively. An additional 50 ml were also stored for cobalt analysis. Approximately 5 l of rumen content from different locations of the reticulorumen were collected into a plastic vessel, thoroughly mixed and a 100 ml subsample was obtained and stored at -20 C. Duodenal digesta was collected by removal of the cannula plug (rubber stopper no. 6 inserted about 20 mm into the cannula and fastened to it with surgical tape) without a diversion gate. From about 800 ml of duodenal content (or the amount collected in 15 min), which was continuously stirred to prevent any settlement of heavy particles, two 100 ml sub-samples were obtained and pH was measured. Samples were stored at -20 C. Grab fecal samples (about 200 g) were collected into aluminum pans, and dried in a forced air oven at 65 C to constant weight.

At 2000 h during day 14, Co-EDTA infusion was stopped and Yb-labelled orchard grass hay was discontinued in the

diet. Samples of rumen fluid, rumen content, duodenal content and feces were then taken every 4 h for the first 36 h and every 6 h for the following 36 h. These samples were stored as described above and were subsequently used to obtain marker dilution curves and to calculate rate of digesta passage. At 1800 h during day 17, 2 l of rumen fluid was strained through two layers of cheese cloth and collected into plastic vessels containing 50 ml of 37% formaldehyde solution to stop fermentation. The bacterial fraction was separated from the fluid by centrifugation according to Meyer et al. (73). Rumen fluid was strained through eight layers of cheese cloth and centrifuged at 200xg for 10 min to remove feed particles. Supernatant was then centrifuged at 20,000xg for 20 min to precipitate bacteria and protozoa. After discarding the supernatant the bacterial residue was washed twice by mixing and centrifuging at 20,000xg for 20 min in saline (.9% NaCl). The bacterial residue was suspended in approximately 100 ml of distilled water and stored frozen at -20 C for subsequent analysis.

Milk production was recorded each milking during each experimental period and milk samples were taken on day 9 and 13 for milk composition analysis. Percent fat, protein and lactose were determined by the Virginia Federation of DHIA laboratory. Blood samples were also collected on day 9 and 13 at 1700 h via tail venipuncture. Two 10 ml samples were

drawn into two plastic test tubes containing 100 µl of .06% (W/V) heparin solution as anticoagulant, placed in ice and brought to the laboratory where they were centrifuged at 200xg for 15 min at 5 C. Plasma obtained was stored at -20 C for subsequent analysis of plasma urea nitrogen.

Feed and digesta samples preparation (day 12, 13 and 14)

Total mixed diets (TMD), corn silage (CS), orts (OS) and fecal samples (FS) were dried at 65 C to constant weight, ground[3] through a 1 mm mesh screen and composited to obtain a sample for each experimental period for TMD and CS and a sample for each cow and experimental period for OS and FS.

Duodenal samples were thawed and composited. Compositing was carried out by stirring individual samples and taking a 25 ml aliquot from each of them to obtain a 150 ml composite. After mixing samples were separated into liquid (LP) and solid phases (SP) by centrifugation at 3000xg for 10 min. Solid phase and bacterial samples (BS) were freeze-dried and ground[4] through a 1 mm mesh screen.

Feed and digesta analysis (day 12, 13 and 14)

1. For dry matter determination, 2 g samples (1 g for bacteria) were dried to constant weight in a forced air oven at 105 C. Samples analyzed were TMD, CS, OS, BS,

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[3]Thomas-Wiley Laboratory Mill Model 4. Arthur H. Thomas Company. Philadelphia, PA. USA.
[4]Cyclone Sample Mill, VD Corp. Boulder, CO.

SP, LP and FS.

2. Organic matter was calculated as the difference between the sample initial dry weight and the residual after ashing at 600 C for 3 h. Samples analyzed were TMD, CS, OS, BS, SP, LP and FS.

3. Total nitrogen analyses were performed using the macro-Kjeldahl method (6). For duodenal liquids approximately a 10 g sample was used. Samples analyzed were TMD, CS, OS, BS, SP, LP and FS. Precipitable-N was measured in SP samples by treatment with 5% trichloroacetic acid (TCA). About 200 mg of sample were weighed in duplicate into 50 ml screw-cap centrifuge tubes. After addition of 10 ml of 5% TCA tightly capped tubes were placed in a 90 C water bath for 10 min. Thereafter they were shaken for 10 min, and content filtered through Whatman No 42 filter paper and washed with hot water. Filtrand was then analyzed for total N. NPN was calculated by difference of total-N in SP and precipitable-N. NPN was measured in LP samples by treatment with 10% TCA. Five g of LP were pipetted into 50 ml screw-cap centrifuge tubes in duplicate. Five g of 10% TCA were added to each tube which were treated as described above for SP samples. However, in this case the filtrate was collected and 5 g of it were pipetted into Kjeldahl flasks for N analysis. Precipitable-N was obtained by difference from total-N in LP and NPN. Amino acid

analysis on TMD, SP and LP samples were done by Health and Nutrition Research Division, Research Laboratories, Eastman Chemical Division, Rochester, N.Y.

4. ADF was analyzed according to Goering and Van Soest (97) on TMT, CS, OS, FS and SP.

5. Ytterbium was extracted from solid samples by a modification of the method of Hart and Polan (38). Sodium-EDTA was used to make up the extraction solution instead of ammonium-EDTA to minimize ionization of Yb during its determination by atomic absorption, thus reducing reading variation. Ytterbium in the extracted solution was measured by atomic absorption[5] with a nitrous oxide-acetylene flame and wavelength set at 398.6 nm. Care was taken to make sure the flame was clear from sodium between samples. Five standard (1, 2, 3, 4, 5 ppm Yb) solutions were prepared with the extraction solution.

Five ml liquid samples were freeze-dried, reconstituted with extraction solution and aspirated into the atomic absorption spectrophotometer as above. This procedure was followed to standardize conditions and to avoid ionization problems with direct Yb measurements on duodenal liquids. To adjust for flame and instrument variation, a sample bracketing was

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[5]Perkin-Elmer model 370 Atomic Absorption Spectrophotometer. Norwalk, Connecticut, U.S.A.

employed. For this, a standard of lower concentration than two unknowns was determined, then two samples analyzed followed by a standard of higher concentration. Readings were from 3 second sample integrations. Concentrations were determined by linear interpolation between the bracketed standards. Samples analyzed were TMD, OS, SP, LP and FS.

6. For SP, LP and FS cobalt was extracted with Yb in the same sample and with the same procedure described above. However, reconstituted LP samples were diluted (1:10) with extraction solution to ensure values in the linear range of the standard curve (1 to 5 ppm). Cobalt in extracted samples was measured by atomic absorption with an air-acetylene flame and wavelength set at 241 nm.
7. Cytosine assay by high pressure liquid chromatography[6] (HPLC) was performed on solid samples (BS and SP) according to Koenig (49) as modified in our laboratory by Armentano and described in Zerbini and Polan (131). For liquid samples, 10 g of LP were weighed into a 15 ml screw top extraction tube, dried in a forced air oven at 90 C for no more than 12 h and hydrolyzed and analyzed as the solid samples. Microbial N as a percentage of the total N in SP and LP was calculated as follows:

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[6]Varian Model 5000 Liquid Chromatograph. Palo Alto, CA, U.S.A.

$$DMN = 100 \times (MN/MC \times DC/DN)$$

where:

DMN = duodenal microbial nitrogen (%),

MN = microbial nitrogen (mg/g DM),

MC = microbial cytosine (µmol/g DM),

DC = duodenal cytosine (µmol/g DM), and

DN = duodenal nitrogen (mg/g DM),

Digesta sample preparation and analyses (Day 15, 16 and 17).

One set of duodenal samples were freeze-dried, ground and analyzed for Yb as previously described for SP. A second set of duodenal samples and rumen samples were centrifuged at 3000xg for 10 min and the supernatant analyzed directly for Co by atomic absorption specrophotometry as described earlier. Unmarked duodenal and rumen fluid were used to prepare standard solutions (1 to 5 ppm).

Parameters on the instruments were as previously described. Rumen fluid samples obtained 4, 8 and 12 h after Co-EDTA infusion had stopped were diluted 2:1 with distilled water to keep them in the range of the standard curve (1 to 5 ppm Co). Marker dilution curves were used to calculate rate of solid and fluid digesta passage.

Analysis of rumen and blood parameters.

Rumen fluid samples previously stored for VFA analysis were thawed and 5 ml of .25% (v/v) isocaproic acid was added as an internal standard to each rumen fluid sample and thoroughly mixed. Samples were then centrifuged at 100xg for 15 min and supernatant was filtered through a 45 µm millipore filter. Filtrates were composited to obtain one sample for each cow during each experimental period. From individual acids a standard was prepared to contain 52.3, 30.0, 5.6, 10.4, 5.3, and 5.0. µmol/ml of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids, respectively. To establish equal conditions for standards and sample, 5 ml of standard solution were added to 5 ml of internal standard and 1 ml of 25% metaphosphoric acid. Concentrations of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids were analyzed with a gas chromatograph[7]. VFA were separated on a glass column packed with 10% SD-1200, 1% H_3PO_4 liquid phase on 80/100 chromosorb W AW packing[8], with a working temperature of 125 C.

Rumen fluid samples collected for NH_3-N analysis were thawed and centrifuged at 100xg for 15 min. Rumen

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[7]Varian, Vista 6000. Palo Alto, CA, U.S.A.
[8]Supelco, Inc. Bellefonte, PA, U.S.A.

NH_3-N concentrations were determined by a modification of the colorimetric procedure of Muramatsu (77). Optical density was measured[9] at 660 nm. Plasma samples were thawed and 1 ml pipetted into a culture tube containing 9 ml of one part 10% sodium tungstate and 9 parts .083 N sulfuric acid. Content was filtered (Whatman No 42) and the filtrate collected was used to determine urea-N according to the colorimetric procedure of Coulombe and Favreau (25). Optical density was measured[9] at 540 nm.

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[9]Bausch & Lomb, Spectronic 1001 Split Beam Spectrophotometer. Rochester, NY, U.S.A.

Negative Control Experiment.

After completion of the experiment a short trial was designed to obtain information on nitrogen flow to the small intestine when SBM and/or FM were removed from the diets used. Diet (CS) ingredients were corn silage, ground corn, orchard grass hay and minerals. Diet formulation and specifications are in Table 4. All six cows were assigned to diet CS. Feeding, marker administration, measurements, sampling and analytical procedures were as described in the previous experiment. This trial was completed on day 14 and no additional samples were taken for marker dilution curves.

TABLE 4. Dietary ingredients and diet composition (CS)[1].

Item	% dry matter
Ingredient[2]	
Corn silage	63.8
Orchardgrass hay	8.4
Ground corn	25.7
Trace mineral salt	.4
Dicalcium phosphate	2.3
Limestone	.4
Composition	
Dry matter	48.9
Organic matter[1]	95.4
Crude protein[1]	8.4
Acid detergent fiber[1]	22.0

[1]Negative control experiment's diet.

[2]Total mixed diets were supplemented with 3,190 IU vitamin A and 308 IU vitamin D per kg dry matter.

Statistical Analysis

Milk, blood, and digesta measurements were subjected to analysis of variance for a crossover design. The model used was as follows:

$$Y_{ijklm} = \mu + S_i + P_{(i)j} + D_k + C_l + SD_{ik} + e_{(ijklm)}$$

where:

μ = effect common to all observations

S_i = effect common to all observations in the ith stage

$P_{(i)j}$ = effect common to all observations in the jth period of the ith stage

D_k = effect common to all observations receiving the kth diet

C_l = effect common to all observations out of the lth cow

SD_{ik} = effect common to all observations in the ith stage and kth diet

e_{ijklm} = effect peculiar to the mth observation on the lth cow receiving the kth diet during the jth period of the ith stage.

i = 1-2

j = 1-2

k = 1-2

l = 1-6

m = 1-n N = 24(n)

The mean square for $P_{(i)j}$ was used to test stage of lactation.

RESULTS AND DISCUSSION

Intake, Milk Production and Body Weight.

These parameters are shown in Table 5. Dry matter intake did not differ among diets or stage of lactation. However, perhaps due to a significant ($P<.05$) increase in body weight in midlactation, feed intake as percent of body weight decreased as lactation progressed. All cows lost weight in early lactation (FM: -.37 kg/day; SBM: -2.93 kg/day). In midlactation cows fed FM diet gained .70 kg/day, while cows fed SBM were still losing weight (-1.63 kg/day). Due to the large standard error (1.43) associated with these measurements, these differences were not significant.

Milk yield and 4% fat corrected milk (FCM) were not different among diets (Table 5), but were higher ($P<.05$) in early than in midlactation. Fish meal in the diet depressed milk fat percent and yield compared to SBM, while protein and lactose percent and yield were lower in midlactation. Milk efficiency (kg FCM/kg feed) was not different among diets or stages of lactation. Similarly, from data of Oldham et al. (84) calculated values of milk efficiency for SBM and FM diets for dairy cows was 1.5, while Crawford and Hoover (27) reported values of 1.37 and 1.21 for diets containing coarse and fine soybean meal respectively. In a

TABLE 5. Dry matter intake, milk yield and composition, and body weight of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Item	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Dry matter intake					
Kg/day	16.4	16.8	16.6	16.6[b]	.6
Percent body weight	3.0	3.1	3.2	2.9[b]	.1
Milk yield, kg/day	27.2	26.8	26.0	25.1[b]	.6
4% FCM, kg/day[4]	23.0	24.2	24.7	22.5[b]	.6
Milk composition, %					
Fat	2.99	3.37[b]	3.02	3.34	.08
Protein	2.94	2.87	2.77	3.05[a]	.09
Lactose	4.88	4.98	4.92	4.93	.07
Milk components yield, kg/day					
Fat	.81	.90[b]	.87	.83	.03
Protein	.79	.76	.79	.76[a]	.02
Lactose	1.32	1.34	1.43	1.24[b]	.03
Milk efficiency, kg FCM/kg feed	1.4	1.5	1.5	1.4	.04
Body weight, kg	551	545	522	573[b]	6
Body weight change, kg/day	+.16	-2.31	-1.67	-.46	1.03

[1] Least square means of 12 observations.

[2] E = early stage of lactation (33-67 d); M = midlactation (131-165).

[3] Standard error common to all means.

[4] 4% fat-corrected milk = (.4 x kg milk) + 15 x kg fat).

[ab] Means for diet or stage of lactation differ; a: $P < .10$; b: $P < .05$.

more recent experiment, data reported by Oldham et al. (85) indicated that milk efficiency for a fish meal diet was 1.5 and 1.2 in early and midlactation, respectively. In addition, they reported that replacement of urea with fish meal increased yield of milk protein both in early and midlactation and depressed milk fat in midlactation. Milk protein increase was not observed in this study, but milk fat depression was quite apparent. Fish oils containing significant amounts of polyunsaturated fatty acids with a chain length of more than 20 carbons may cause milk fat depression when fed to dairy cows (85, 118). In addition, fish meal may influence digestion of carbohydrate in the rumen and therefore influence milk fat. Concentrations of ruminal acetate (Table 6) were lower for diet FM compared to SBM, and since acetate is known to be a precursor of milk fat it may in part explain the differences observed. It was surprising, in view of the positive effect on milk yield reported with fish meal supplementation (85), to observe no differences between FM and SBM. Beneficial effects of escape protein have been obtained especially when cows were in negative energy balance or in conditions of restricted intake. On the other hand, other studies with casein infusion in the abomasum or trials in which protein of low degradability were fed (21, 23) reported little effect on milk production. Orskov et al. (91) reported that when metabolizable energy (ME) intake exceeded 160 MJ/day,

changes in protein degradability produced no response, but at ME intakes below 135 MJ/day, undegradable protein caused an increase in FCM yield, protein content and weight loss. Intakes of ME in this study were greater than 190 MJ/d and similar among diets and stages of lactation. However, the positive body weight change observed with dietary FM may indicate that, extra proteins supplied to the small intestine, may have been directed toward body weight gain.

Rumen and Blood Parameters.

Ruminal VFA concentrations are presented in Table 6. Total VFA and acetate (mg/dl) were higher for SBM diet compared to FM. No differences were observed between stages of lactation except for propionate that was significantly higher ($P<.05$) in early than in midlactation. Molar proportions of acetate and valerate tended to be higher for SBM, and valerate was greater ($P<.05$) in early than in midlactation. Acetate/propionate ratio was similar among diets but tended to be higher in midlactation. The reverse was true for efficiency of carbohydrate fermentation (E) calculated according to Orskov et al. (88):

$$E = 100\ (.622\ \text{acetate} + 1.092\ \text{propionate} + 1.56\ \text{butyrate})/(1\ \text{acetate} + 1\ \text{propionate} + 2\ \text{butyrate})$$

where acetate, propionate and butyrate are expressed in their molar proportions. If on one hand, E values would suggest that the fermentation process among diets was similar, on the other, acetate and total VFA concentrations

TABLE 6. Ruminal parameters and plasma urea nitrogen (PUN) in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Item	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Total volatile fatty acids, mg/dl	924.6	982.7[a]	983.6	923.7	22.1
Acetate, mg/dl	445.0	486.0[b]	471.1	459.9	8.9
Propionate, mg/dl	237.1	244.7	268.7	213.1[b]	6.9
Isobutyrate, mg/dl	13.1	14.3	14.9	12.5	1.1
Butyrate, mg/dl	166.9	171.0	162.6	175.4	7.9
Isovalerate, mg/dl	37.8	37.8	38.0	37.7	2.7
Valerate, mg/dl	24.7	28.9	28.4	25.0	1.2
Acetate[4]	56.0	57.3[a]	55.5	57.8	.6
Propionate[4]	24.1	23.3	25.7	21.7	.5
Isobutyrate[4]	1.1	1.1	1.2	1.1	.1
Butyrate[4]	14.3	13.7	13.0	14.9	.5
Isovalerate[4]	2.8	2.6	2.6	2.7[b]	.2
Valerate[4]	1.8	2.0[a]	2.0	1.8[b]	.1
Acetate/Propionate	2.4	2.5	2.2	2.7[a]	.1
Efficiency of carbohydrate fermentation[5]	76.8	76.4	77.3	75.9[a]	.2
Ammonia-N, mg/dl	4.0	8.7[b]	7.4	6.3	.9
Rumen pH	6.3	6.0[a]	5.8	6.4[b]	.1
Rumen fluid dilution rate[6], $\%\cdot h^{-1}$	4.0	3.9	4.2	3.7	.2
PUN, mg/dl	12.4	15.1[b]	13.0	14.5[a]	.6

[1]Least square means of 12 observations.

[2]E = early lactation (33-77 d); M = midlactation (131-164 d).

[3]Standard error common to all means.

[4]Moles/100 moles of acid.

[5](.622 acetate + 1.092 propionate + 1.56 butyrate) x 100(1 acetate + 1 propionate + 2 butyrate). From Orskov (99).

[6]Dilution rate of CoEDTA.

[ab]Means for diet stage of lactation differ, a: $P < .1$; b: $< .05$.

would indicate that more available carbohydrates were accessible for fermentation from soybean meal. Significantly lower ($p>.05$) concentrations of NH_3-N were observed for FM compared to SBM diet, suggesting that NH_3 might have been less available for microbial protein synthesis. However, the value of 5 mg/dl NH_3-N obtained here would be adequate for maximum microbial synthesis according to Roffler and Satter (118). Values of NH_3-N obtained with diet FM are lower than those reported by Mathers and Miller (61). Probable differences are due to differences in treatment and source of fish meal used (71). NH_3-N for SBM was slightly lower than values reported by Roffler and Satter (98) for diets containing between 15 and 16% crude protein. Since the only difference in the diet was protein source, NH_3-N observed in this study reflect differences in protein degradability of fish meal and soybean meal in the rumen. No differences were observed between stages of lactation.

Rumen pH was higher ($P<.1$) for FM compared to SBM and in mid compared to early lactation ($P<.05$). Greater VFA production with SBM might have offset the effect of higher NH_3-N concentration, but it is not certain if the effect of dietary treatment is of biological significance (55).

Cows fed FM had lower ($P<.05$) plasma urea-N (PUN) than those fed SBM. PUN originates from ruminal NH_3-N and from protein catabolism in tissues. Preston (94) suggested that

protein wastage may occur when PUN is greater than 10 mg/dl. Good performance in growing cattle was obtained with a minimum PUN of 7-8 mg/dl (95). Zerbini and Polan (130) reported higher serum urea-N values in calves fed SBM compared to FM diets. Similarly, greater PUN in calves fed 60% ruminally degradable nitrogen (RDN), compared to 30 or 45% RDN was observed by Nocek and Polan (80). They attributed the response to differences in rumen NH_3-N concentrations similar to those observed in this study. PUN values reported here for FM are in agreement with those indicated by Oldham et al. (85) in dairy cows.

PUN in early lactation was lower ($p<.10$) in early lactation for both diets. It is possible that N-conserving mechanisms proposed by Bruckental et al. (12) are working here. They showed that only 25% of urea synthesized by cows in the first and second month of lactation was excreted in urine, and was lower than in midlactation. They attributed this indirect observation to a reduced amino acid oxidation at a time when they are most needed for milk production. Possible aspects of urea recycling into the gastrointestinal tract, particularly the rumen, certainly play a role in early lactation when nutrient supply is not adequate to support potential milk output. Rumen fluid dilution rate was not different between diets or stages of lactation.

Digesta Flow and pH at Proximal Duodenum.

Solid (SP) and liquid (LP) phases are defined in this

study as the pellet and supernatant obtained upon centrifugation at 3000xg for 10 min of whole duodenal digesta. Flow of SP at the duodenum did not differ among diets or between stages of lactation (Table 7), but LP flow was greater ($P<.05$) for SBM diet compared to FM diet, resulting in greater ($P<.05$) total flow of digesta for SBM. As suggested by Firkins et al. (32), it is possible that rapid solubilization of SBM in the rumen increased rumen osmolarity which could cause an increased flux of water through the rumen and therefore lead to increased liquid phase flow. No differences among diets or stages of lactation were observed for whole digesta pH, as well as LP dilution rate, measured from the depletion curve of Co-EDTA. Solid phase dilution rate was greater ($P<.1$) for SBM diet. Although not significantly, LP and SP dilution rates were reduced by 23 and 18%, respectively, in mid compared to early lactation. Hartnell and Satter (39) reported that stage of lactation had no effect on ruminal turnover rate; however, they reported a decrease of 9 and 12% for liquid and hay, respectively, from early to midlactation. Probable effects of turnover rates on digestion will be discussed later in reference to organic matter and nitrogen digestibilities.

Intake, Flow and Digestion of Organic Matter (OM)

Organic matter intake (OMI), liquid and solid phase OM, as well as total OM flow to the duodenum were similar among

TABLE 7. Digesta flow and digesta pH at proximal duodenum in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Item	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Digesta flow:					
Solid phase[4], kg/day	46.2	49.5[b]	47.6	48.0	3.3
Liquid phase[5], kg/day	81.6	101.1[b]	96.1	86.6	6.2
Total, kg/day	127.7	150.5[b]	143.6	134.6	7.3
Liquid phase dilution rate[6], $\%\cdot h^{-1}$	7.5	7.1[a]	8.0	6.5	.3
Solid phase dilution rate[7], $\%\cdot h^{-1}$	3.1	4.1[a]	3.9	3.3	.4
Digesta pH	2.8	2.7	2.8	2.7	.04

[1]Least square means of 12 observations.

[2]E = early lactation (33-67 d); M = midlactation (131-165 d).

[3]Standard error common to all means.

[4]Pellet from centrifugation (3000xg for 10 min) of whole digesta.

[5]Supernatant from centrifugation (3000xg for 10 min) of whole digesta.

[6]Dilution rate of CoEDTA in solution.

[7]Dilution rate of Ytterbium associated with orchard grass hay.

[ab]Means for diet or stage of lactation differ, $P < .05$.

diets and stages of lactation (Table 8). However there was a trend for total OM flow to be lower in midlactation. This resulted in significantly greater apparent and true OM digestibilities at the duodenum. True OM digestibility was calculated by correcting total OM flow to the duodenum for microbial OM flow which was greater ($P<.1$) for SBM compared to FM diet. The proportion of apparently digested OM digested in the stomachs (DOMR) was similar for both diets but increased in midlactation. DOMR values reported here for SBM are well in range with those calculated from literature data (116,106, 119). However, they were lower than those reported by Rooke et al. (99) with Jersey cows fed a grass silage-barley-soybean meal diet. All DOMR values were also lower than .65 which was adopted by ARC (2) for calculation of nitrogen requirements and was chosen as an estimate of the energy available to support microbial synthesis. Differences of DOMR values reflect the large variation seen in the literature (2) and points out the need of different factors for different diets. No significant differences were observed for OM flow in the feces and apparent OM digestibility in total tract among diets or stages of lactation.

Physiological state seemed to have influenced the digestion process in spite of unchanged dry matter intake in early and midlactation. Weston (129) reported that pregnancy and lactation have significant effects on

TABLE 8. Intake, flow, and digestion of organic matter in the digestive tract of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Organic matter	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Intake, kg/day	15.5	16.0	15.7	15.9	.5
At proximal duodenum					
Flow:					
Solid phase[4], kg/day	8.3	8.5	9.0	7.8	.5
Liquid phase[5], kg/day	1.4	1.7	1.6	1.5	.1
Total, kg/day	9.7	10.2[a]	10.6	9.3	.5
Microbial, kg/day	1.6	2.1[a]	2.0	1.8	.1
Digestibility:					
Apparent[6], %	37.9	36.5	32.5	41.9[b]	1.9
True[7], %	48.4	49.8	44.9	53.2[a]	1.9
In stomachs[8], %	52.4	52.7	45.1	60.1[a]	3.3
In feces:					
Flow, kg/day	4.2	4.8	4.4	4.6	.3
Apparent digestibility, %	72.7	70.5	72.1	71.1	1.7

[1] Least square means of 12 observations.

[2] E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3] Standard error common to all means.

[4] Pellet from centrifugation (3000xg for 10 min) of whole digesta.

[5] Supernatant from centrifugation (3000xg for 10 min) of whole digesta.

[6] Percent of intake.

[7] Corrected for microbial organic matter

[8] Percent of apparently digested organic matter digested in the stomachs.

[ab] Means for diet or stage of lactation differ, a: $P < .1$; b; $P < .05$.

digestive functions in sheep and these may influence nutrient supply to the tissues. Fell et al. (31) indicated that in the ewe all alimentary organs hypertrophy during lactation, with ruminal mucosa reaching maximum in the sixth week. In dairy cows (33) volume of rumen contents in early lactation increased simultaneously with feed intake and reached a maximum after peak lactation.

Campbell and Fell (15) suggested that hypertrophy of the alimentary canal in the rat is caused by increased feed intake and is an adaptive response which results in maintenance of a constant coefficient of digestibility at increased food intake. More recently Muller and Dowling (76) implicated prolactin in hypertrophy of the gut during lactation. In the present study, no measurements of rumen or intestine weight or digestive capacity were made. However, since dry matter intake was similar in both stages of lactation, an increased digestive capacity of the stomachs could have resulted in greater OM digestibility preduodenally. Lower liquid and solid phase dilution rates in midlactation support this hypothesis. However, total OM digestibility was similar in both stages of lactation, indicating that intestinal digestion counterbalanced differences observed at the duodenum. Total tract digestibility values are the average of digestibilities calculated using Co and Yb. Individual values are reported in Appendix Table 1.

Intake, Flow and Digestion of Acid Detergent Fiber (ADF)

No differences were observed for intake, flow and digestibility of ADF measured at the proximal duodenum among diets and stages of lactation (Table 9). However, the mean of ADF apparent digestibility in midlactation was 24% greater than that of early lactation. ADF flow in feces was not different between diets, but was significantly lower ($P<.05$) in midlactation, resulting in greater ($P<.05$) ADF apparent digestibility for that stage of lactation. Results indicate that more than 70% of ADF digestion occurred in the stomachs and the rest in the small and large intestine.

ADF digestibility values in early and midlactation reflect those of OM, with the exception that with ADF, differences in the duodenum were consistent with those in the feces. ADF digestion in the small intestine is probably minimal but continues in the large intestine.

Intake, Flow, and Digestion of Nitrogen.

Nitrogen intake was similar among diets and stages of lactation. Total N, SP and LP N, and SP and LP precipitable and non-precipitable N flow to the proximal duodenum were not significantly different among diets and stages of lactation, (Table 10).

Microbial preparations contained between 27.5 and 54.8 umol cytosine per g dry matter and averaged 8.4% nitrogen. Cytosine content of FM and SBM diets were 1.6 and 1.8 μmol/g

TABLE 9. Intake, flow, and digestion of acid detergent fiber (ADF) in the digestive tract of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

ADF	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Intake, kg/d	3.3	3.2	3.2	3.3	.1
At proximal duodenum:					
Flow, kg/d	2.0	2.0	2.1	1.9	.1
Apparent digestibility[3], %	39.4	37.5	34.3	42.6	2.3
Digestion in stomachs[4], %	75.0	74.3	71.6	77.6	4.3
In feces:					
Flow, kg/d	1.6	1.6	1.7	1.5[b]	.1
Apparent digestibility, %	52.6	50.5	48.2	54.9[b]	1.8

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Standard error common to all means.

[4]Percent of intake.

[5]Proportion of apparently digested ADF digested in the stomachs.

[b]Means for stage of lactation differ, $P < .05$.

TABLE 10. Intake, flow, and digestion of nitrogen in the digestive tract of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation (continued).

Nitrogen	Diet[1]		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Intake, g/d	408	432	426	414	21
At proximal duodenum					
Solid phase nitrogen flow[4]:					
Total, g/d	287	268	288	267	13
Precipitable[5], g/d	200	182	200	183	11
Non precipitable[6], g/d	87	86	88	85	4
Microbial, g/d	145	187[b]	116	106	12
Residual[7], g/d	142	81[b]	172	161	12
Liquid phase nitrogen flow[8]:					
Total, g/d	90	97	93	94	5
Precipitable[6], g/d	13	13	13	12	2
Non precipitable[9], g/d	77	84	80	82	4
Microbial, g/d	18	28[b]	24	22	2
Residual[7], g/d	72	69	69	72	5
Total microbial, g/d	164	216[b]	196	183	12
Total residual, g/d	214	149[b]	184	178	12
Total, g/d	378	365	380	361	14
Digestibility:					
Apparent[10], %	6.8	15.7[b]	9.9	12.6	1.6
True[11], %	47.2	65.8[b]	56.7	56.2	2.2
Efficiency of microbial protein synthesis					
Apparent[12]	29.0	40.0[b]	41.0	28.0[a]	3.2
True[13], %	22.2	27.8[b]	28.4	21.6[a]	1.5
Apparent digestibility in intestines[14], %	69.1	65.0[b]	67.7	66.4	1.1
In feces					
Flow, g/d	116	127	122	121	6
Apparent digestibility, %	71.0	70.6	71.2	70.6	1.0

TABLE 10 (continuation).

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Standard error common to all means.

[4]Nitrogen in pellet from centrifugation (3000xg for 10 min) of whole digesta.

[5]Nitrogen in precipitate obtained with 5% trichloroacetic acid.

[6]Calculated by difference from total and precipitable nitrogen.

[7]Calculated by difference from total and microbial nitrogen.

[8]Nitrogen in supernatant from centrifugation (3000xg for 10 min) of whole digesta.

[9]Nitrogen in filtrate obtained from filtration of liquid phase treated with 10% trichloroacetic acid.

[10]Percent of intake.

[11]Corrected for microbial nitrogen.

[12]Grams microbial nitrogen/kg organic matter apparently digested in the stomachs.

[13]Grams microbial nitrogen/kg organic matter truly digested in the stomachs.

[14]Percent of nitrogen at duodenum.

[ab]Means for diet or stage of lactation differ; a: $P < .1$; b: $P < .05$.

dry matter and was assumed completely degraded in the rumen. Microbial N flow either in SP or LP was greater (P<.05) for SBM compared to FM diet, but similar values were observed in early and midlactation. Residual N was calculated as the difference from total and microbial N. It was larger (P<.05) for FM solid phase, but not for FM liquid phase. Total microbial N was higher (P<.05) for SBM, compared to FM diet.

Apparent N digestibility in the stomach was greater (P<.05) for SBM. Nitrogen recovery at the duodenum was 93.2 and 84.3% of N intake for FM and SBM, respectively reflecting more N loss on SBM diet. True N digestibility was calculated by subtracting microbial N from total N flow to the duodenum and dividing by N intake. This value, which was larger (P<.05) for the SBM diet, represents the degradability of dietary protein in the rumen. Apparent N digestibility in the intestines expressed as a percent of duodenal N was higher (P<.05) for FM diet, but values for stages of lactation were similar.

Total tract N digestibility was similar among diets and stages of lactation, although N flow in feces from SBM were slightly higher than that of FM diet. Apparent (AEMPS) and true (TEMPS) efficiencies of microbial protein synthesis in the rumen were greater (P<.05) for SBM diet, and in early lactation (P<.1). AEMPS represents grams of microbial N synthesized per kg of OM apparently digested in the

stomachs, while to calculate TEMPS OM apparently digested in stomachs is corrected for microbial OM. Both values are presented to make comparison with literature data easier since only one or the other is usually reported. From a number of reports (51, 68, 99, 106, 111, 116, 133, 134) for diets supplemented with soybean meal fed to cattle or sheep, AEMPS ranged from 14.5 to 39.0. TEMPS was usually lower except for values of 40.3 and 44.2 reported by Santos et al. (106) and Stern et al. (116), respectively, with lactating cows which were considerably higher than those reported in this study also. Santos et al. (106) attributed these discrepancies in part to DAPA which they used as microbial maker. However, it is unlikely that this is the case because DAPA may underestimate and certainly not overestimate microbial flow compared to RNA or cytosine which are present in protozoa also (54). Probably solid and liquid digesta markers are at least as much responsible for this variation as microbial markers. Literature values for fish meal diets (111, 64, 24) ranged from 19 to 29 g N/kg OM apparently or truly digested in the rumen. These values agree with those reported here for FM diets. Smith et al. (111) reported no AEMPS or TEMPS differences for FM or SBM diets, and in general much of the variation is attributed to energy supply in the diet rather than to protein source. Factors recognized to influence EMPS are fluid and solid turnover rates in the rumen.

Selected correlations of flow and digestive parameters are in Table 11. Rumen fluid turnover rate (calculated from Co-EDTA depletion curve) was correlated with TEMPS and microbial flow to the duodenum for both diets. However, much higher correlation coefficients were obtained with duodenal fluid turnover rate and both TEMPS and microbial flow. Hobson (42) and Orskov (90) reported increased microbial growth with increased dilution rate in vitro and in vivo. Similar results were reported by Rode et al. (97) who reported positive correlations between TEMPS and turnover rate of rumen contents as measured by Ce, Yb and CoEDTA. They suggest that cerium may become associated with the microbial mass and by doing so, would estimate microbial mass turnover rate. In this study dilution rate of Yb at the duodenum was not correlated with TEMPS although more than 50% of the microbial mass was associated with the solid phase. As suggested by Oldham (89), it is more appropriate to consider microbial outflow as the weighed mean of liquid and solid turnover rates. Rode et al. (97) went further to suggest that in dairy cows at high levels of intake microbial protein synthesis is influenced more by solid turnover rate than liquid dilution rate and that the physical nature of the diet had more effect on TEMPS than its chemical composition.

Organic matter intake was positively correlated with microbial N flow for both diets, but especially for SBM.

TABLE 11. Selected correlations of flow and digestive parameters in Holstein cows fed a fish meal (FM) or soybean meal (SBM) supplemented diet.

Parameters[1]	Diet	
	FM	SBM
	R^2	
RFDR and TEMPS	.35	.63[a]
RFDR and DTMN	.51[a]	.54[a]
DLDR and TEMPS	.89[b]	.72[b]
DLDR and DTMN	.91[b]	.55[a]
OMI and DTMN	.53[a]	.73[b]

[1]RFDR = ruminal fluid dilution rate (CoEDTA).
TEMPS = efficiency of microbial protein synthesis.
DTMN = microbial nitrogen flow to proximal duodenum.
DLDR = duodenal liquid phase dilution rate (CoEDTA).
OMI = organic matter intake.

[2]Coefficient of correlation.

[ab]Significant R, a: $P < .1$; b: $P < .05$.

This points out the close association between degradable N and fermentable carbohydrates in the rumen and microbial synthesis.

Differences in AEMPS or TEMPS observed in this study originated from the greater flow of microbial N to the duodenum for the SBM diet rather than to differences in OM digestion in the stomachs. Protein source effect (i.e., rumen degradability) here was significant, but literature reports are conflicting. For example, while Stern et al. (116) reported similar flows of microbial N for SBM or heat-treated SBM diets, Rooke et al. (101) showed significant differences. Armentano et al. (4) and Merchen et al. (68) did not observe significant differences between SBM and dried brewers grains. On the other hand, greater microbial N proportions (% total N) in the duodenum were observed by Ling and Buttery (54) in sheep for SBM compared to FM diets. Similar findings were reported by Zerbini and Polan (130) in ruminating calves. Since energy in the diets was similar, this would indicate a higher availability of NH_3-N (or other nutrients) with the SBM diet. This hypothesis is supported by higher rumen NH_3-N concentrations obtained with this diet.

Although total N flow to the duodenum was slightly higher for FM diet, differences were not significant. However, N recovery (% N intake) was significantly higher for FM (93.2%) compared to SBM diet (84.3%). These values

compare favorably to those reported in the literature for protein sources susceptible to different degree of degradation in the rumen (68, 93, 111, 132, 133). Some of the values reported by the Wisconsin group (97, 106, 116), are considerably higher. Differences in digesta markers and possibly in digesta flow estimates may account for differences.

It is apparent in this study that any advantage obtained by a greater proportion of dietary protein escaping rumen degradation as with FM, was partly offset by less microbial protein flow to the duodenum. The goal for feeding escape protein is to supply greater quantity and possibly quality of amino acids to the small intestine. The results of this study indicate that microbial growth in the rumen was in part limited when FM was fed compared to SBM, probably due to an insufficient supply of ruminally degradable protein. This point addresses the advantages of new systems (2, 78), which go beyond expressing protein requirements as crude protein but instead estimate the requirement for degradable and undegradable protein in the diet. An example is shown in Table 12 which compares protein requirement calculations with the ARC (2), NRC (1985) (78), and NRC (1978) (79) methods. NRC (1978) (79) was used to formulate FM and SBM diets. Although percent crude protein in the diet is similar for the 1978 and 1985 NRCs (78,79), the partition of this dietary protein in

TABLE 12. Daily protein requirements for a 600 kg Holstein cow producing 36 kg of milk at 3.5% fat calculated with different methods.

Requirements	ARC[1]	NRC[2]	NRC[3] Present study[4] FM	NRC[3] Present study[4] SBM
Dry matter intake, kg/day				
Calculated	20.7	20.2	21.8	22.3
(Observed)[5]			(16.4)	(16.8)
Digestible undegraded intake protein, kg/day	775	1280	-	-
Ruminally available protein, g/day	1781	2572	-	-
Intake protein, g/day	2556	3350	-	-
Intake protein in dry matter, %	12.3	16.6	16.1	16.0
Undegraded protein needed in diet, %				
Calculated	30.3	38.2	-	-
(Observed)[5]			(52.8)	(34.2)
Degraded protein needed in diet, %				
Calculated	69.7	61.8	-	-
(Observed)[5]			(47.2)	(65.8)

[1]Agricultural Research Council (U. K.). 1980 (2).

[2]National Research Council (U.S.A.). 1985 (78).

[3]National Research Council (U.S.A.). 1978 (79).

[4]Actual milk productions were 27.2 and 26.8 kg/day at 2.99 and 3.37 % fat for diets supplemented with fish meal (FM) or soybean meal (SBM), respectively.

[5]Observed in the present study.

undegradable and degradable portions and the results obtained in this study show that only SBM diet approached requirements. Diet FM would not have contained enough degradable protein to meet requirements calculated with the new method, even if crude protein requirements were met, but it nearly supplies the degradable protein specified by the ARC (2) system. However, considerable difference exists between ARC and NRC in amount of protein required in the diet to support the same amount of milk production.

The greater N digestibility in the intestine for FM diet observed, supports earlier reports (125, 105) which indicate that for a given feedstuff, intestinal digestibility of nitrogen increases as nitrogen bypass increases.

Intake, Flow and Digestion of Amino Acids.

Total amino acids (TAA) intake (Table 13) was significantly ($P<.05$) greater for SBM diet in spite of similar nitrogen intakes. Reports (90, 130) show that fish meal protein has a greater fraction A (soluble and completely degradable nitrogen compounds) than soybean meal. Greater non protein nitrogen in the FM diet, perhaps due to more NPN in fish meal compared to soybean meal supplement and to a greater proportion of corn silage in FM, could account for differences observed in amino acid intake. Factors associated with analytical procedure of amino acid analysis of diets could also have contributed to differences

TABLE 13. Amino acid intake, flow, and digestion in the stomachs of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements in early and midlactation.

Amino acid	Diet[1]		Stage of lactation[1,2]		
	FM	SBM	E	M	SE[3]
Intake, g/day	1341	1678[b]	1520	1499	52
Flow to duodenum, g/day	1220	1185	1208	1198	63
Microbial[4], g/day	742	962[b]	880	823	51
Residual[5], g/day	480	225[b]	330	374	63
Disappearance in stomach, g/day	133	495[b]	308	321	55
Degradation in stomachs, %	64.6	87.0[b]	77.0	74.5	4.3

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Standard error common to all means.

[4]Microbial maino acid composition from Hoogenraad and Hind (43) and Keyser (48).

[5]Total amino acid flow to duodenum minus microbial amino acid flow.

[b]Means for diet or stage of lactation differ ($P < .05$).

observed. Lower amino acid recovery (g/kg dry matter), obtained with FM diet (Appendix Table 2) supports this hypothesis. Amino acid flow to the duodenum was similar for both diets). Microbial amino acid (MAA) flow was greater for SBM, but residual amino acid flow was higher for FM diet. Microbial dry matter was not analyzed for amino acids. Published values (43, 48) have been used to calculate MAA flow to the proximal duodenum. Such calculations have the potential for inaccuracies but relative differences between diets or stages of lactation should not be affected. For both diets, there was a loss of TAA in the stomachs. However, this loss was significantly greater for SBM (29.5%) than FM diet (9.9%) because of different ruminal degradation of TAA in FM (64.6) and SBM (87.0). No differences were observed for TAA intake, flow, and digestion in the stomachs between stages of lactation. Intake, flow and digestion of essential (EAA) and nonessential (NEAA) amino acids showed similar trends obtained with TAA (Table 14). Duodenal recovery of EAA and NEAA was significantly higher ($P<.05$) for SBM diet and recovery of EAA greater than NEAA. In addition, recovery of EAA and NEAA in the duodenum was greater in early lactation for FM, but the reverse was observed for SBM diet. Rumen degradability of EAA and NEAA was greater for SBM diet with a tendency for EAA to be degraded to a greater extent than NEAA for both diets. The ratio EAA/NEAA in duodenal solid

TABLE 14. Essential (EAA) and nonessential (NEAA) amino acid intake, flow and digestion in the stomachs of Holstein cows fed fish meal (FM) or soybean meal as protein supplements during early or midlactation.

Item	Diet		Stage of lactation[1,2]		SE[3]
	FM	SBM	E	M	
Intake, g/day					
EAA	549	681[b]	613	617	21
NEAA	792	997[b]	906	883	31
Flow to duodenum, g/day					
Solid phase[4]:					
EAA	421	385	412	393	20
NEAA	509	482	506	485	26
Liquid phase :					
EAA	122	135	120	137[b]	7
NEAA	168	184	169	182	10
Recovery at duodenum[6], %					
EAA	99.1	76.4[b]	89.1	86.0	1.6
NEAA	85.3	66.4[b]	76.0	75.7	1.5
Degradability in stomachs, %					
EAA	66.5	92.0[b]	80.6	77.9	5
NEAA	63.3	83.5[b]	74.6	72.2	4

[1] Least square means of 12 observations.

[2] E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3] Standard error common to all means.

[4] Pellet from centrifugation (3000xg) of whole digesta.

[5] Supernatant from centrifugation (3000xg) of whole digesta.

[6] Percent of intake.

[b] Means for diet or stage of lactation differ ($P < .05$).

and liquid phases for FM and SBM diets were .83, .73, .80, and .73, respectively. This represents an increase of about 15% compared to EAA/NEAA ratio in the diet, indicating a considerable quality improvement of the protein fraction from mouth to duodenum. Except for alanine, valine, threonine and methionine, individual amino acid flows to the duodenum were lower than intakes (Table 15). Lysine and tyrosine seemed to increase for FM, but to decrease for SBM diet.

Individual amino acid intakes were greater for SBM compared to FM diet except for methionine and histidine. There were no differences between stages of lactation except for alanine, valine, and cysteine which were lower in midlactation. Duodenal methionine flow was greater ($P<.05$) for FM diet, while the contrary was observed for cysteine. For all other individual amino acids measured no significant differences were observed between diets or stages of lactation (except glycine). Duodenal recovery of individual amino acids (% of intake) was greater ($P<.05$) for FM diet, except for alanine, valine and cysteine. Recoveries greater than 100% were observed for alanine, valine, and methionine for both diets. Threonine, lysine and tyrosine were greater than intake only for FM diet. Generally, recovery was similar in both stages of lactation; however, alanine, valine and histidine recovery were greater in midlactation. Individual amino acid degradability in the stomachs was

TABLE 15. Individual amino acid intake, flow, and digestion in the stomachs of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation (continued).

Amino acid	Intake, g/day					Flow to duodenum, g/day				
	Diet[2]		Stage of lactation[2,3]			Diet[2]		Stage of lactation[2,3]		
	FM	SBM	E	M	SE[4]	FM	SBM	E	M	SE[4]
ALA	80	72[a]	81	71[b]	3	86	84	84	86[a]	5
GLY	90	71[b]	83	78[b]	3	76	67	75	68[a]	4
VAL	5	5[b]	5	4[b]	.2	57	56	56	57	3
THR	57	69[b]	61	65	2	65	67	66	65	4
SER	73	92[b]	84	81	3	65	67	66	65	4
LEV	139	175[b]	156	159	5	107	107	109	105	6
ILE	53	64[b]	57	59[b]	2	52	51[b]	51	52	3
CYS	22	28[b]	27	23[b]	.8	18	25[b]	23	22	1
PRO	104	120[b]	114	110[a]	4	66	65[b]	67	64	4
MET	10	11[b]	10	11[a]	.4	35	29[b]	31	32	2
ASP	143	201[b]	173	171	6	133	130	130	133	7
PHE	67	94[b]	80	82	3	59	59	60	59	3
GLU	236	348[b]	291	294	11	184	179	183	180	10
LYS	79	97[b]	86	91	3	90	78	81	87	4
TYR	43	65[b]	53	55	2	49	50	49	50	3
ARG	105	132[b]	124	112	4	51	45	51	46	5
HIS	33	35	35	34	1	27	27	27	28	2

[1]Percent of intake.

[2]Least square means of 12 observations.

[3]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[4]Standard error common to all means.

[ab]Means for diet or stage of lactation differ, A: $P < .10$; b: $P < .05$.

TABLE 15 (continuation). Individual amino acid intake, flow, and digestion in the stomachs of Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Amino acid	Recovery at duodenum[1], %					Degradability in stomachs, %				
	Diet[2]		Stage of lactation[2,3]			Diet[2]		Stage of lactation[2,3]		
	FM	SBM	E	M	SE	FM	SBM	E	M	SE
ALA	108.2	115.3[b]	104.2	119.3[b]	3.5	58.1	80.1[b]	75.2	62.8	5.8
GLY	84.0	94.5[b]	90.2	88.4	3.4	54.8	69.4[a]	61.7	62.5	4.9
VAL	1285.7	1251.1	1094.7	1442.0[b]	43.2	-254.1	62.9[b]	-15.6	-175.6	72.8
THR	114.3	95.9[b]	110.9	99.4	3.4	60.6	83.4[b]	72.1	72.0	5.8
SER	89.7	72.1[b]	80.3	81.5	2.5	55.7	74.6[b]	66.8	63.5	3.9
LEV	76.6	60.9[b]	71.1	66.4	1.5	65.7	82.6[b]	73.9	74.4	3.3
ILE	97.7	80.5[b]	89.8	88.5[a]	1.9	79.1	102.0[b]	94.5	86.6	5.5
CYS	90.1	89.7	85.0	94.7	3.9	94.4	96.8	96.9	94.3	6.6
PRO	62.7	54.2[b]	58.6	58.4	1.3	62.0	73.6[b]	68.2	67.4	2.3
MET	342.7	275.6[b]	312.3	306.0	9.9	-9.3	129.0[b]	81.6	38.1	17.9
ASP	92.6	64.6[b]	78.6	78.6	1.3	66.7	90.0[b]	80.6	76.1	3.9
PHE	87.7	62.6[b]	77.8	72.4	1.5	85.3	104.4[b]	96.1	93.5	4.9
GLU	78.1	51.1[b]	66.9	62.3	1.4	59.6	81.7[b]	70.2	71.1	2.7
LYS	116.6	80.8[b]	101.8	95.7	2.8	54.2	90.4[b]	74.1	70.5	4.9
TYR	114.3	76.3[b]	99.7	90.9	2.6	96.8	117.6[b]	110.2	104.1	7.6
ARG	49.0	34.9[b]	43.3	40.6[a]	4.2	84.3	99.7[b]	90.4	93.5	4.8
HIS	83.0	75.0[b]	76.8	81.2[a]	1.6	55.7	72.2[b]	67.3	60.6	3.4

significantly greater for SBM diet except for cysteine and tyrosine. No significant differences were measured between stages of lactation. Of the EAA, valine, methionine, lysine and histidine were least degraded for FM diet, but were degraded considerably for SBM diet. In both diets lowest degradabilities were observed for glycine, valine, serine, proline and histidine. Greatest differences were observed for valine and methionine which went from an apparent negative degradability for FM to a greater than 100% degradability for SBM diet. Amino acid composition of duodenal solid and liquid phase are in Appendix Table 3, 4, 5 and 6.

Results obtained in this study do not show the increased amino acid flow to the duodenum compared to intake which is generally observed when treated or naturally degradation-resistant protein sources are present in the diet (24, 52, 65, 106, 116). However, the lower amino acid intake and essentially the same or slightly higher amino acid flow to the duodenum observed for FM diet, led to a greater duodenal amino acid recovery for that diet. Apparently more dietary amino acids were escaping rumen fermentation and flowing to the small intestine with FM compared to SBM diet. Endogenous contribution of EAA and NEAA to the digesta in the stomachs should be modest, and its influence relatively small since its amino acid profile is similar to that of digesta (36). In addition, for

comparative purposes, it is assumed that endogenous contribution is similar across diets. The results indicate that individual amino acids degradability in the stomachs among protein sources may vary greatly.

Earlier findings (130) have suggested that amino acid profiles of bag residues of fish meal, peanut meal, soybean meal and sufflower meal after 9 h of incubation were similar to that of the original sample. However, more recent reports have shown that the amino acid profile of undegraded silage protein from in situ incubation differed from that of the protein in the original silage (88). Similarly, Craig and Broderick (104) have shown that alanine, arginine, histidine, lysine and phenylalanine were degraded to a greater extent than total degradation. Leucine and isoleucine tended to be released to a lesser extent. In this study degradability values for arginine, tyrosine, phenylalanine, cysteine and isoleucine were greater than total degradability, while methionine and valine were the least degraded. Similar findings were reported by Chalupa (17) who indicated that arginine and threonine were rapidly degraded. Lysine, phenylalamine, leucine and isoleucine showed a lesser degree of degradability; and valine and methionine were relatively resistant to degradation (17). The techniques used here do not allow calculations of endogenous amino acid supply and may contribute to some of the discrepancies seen above.

Chalupa (17) estimated ruminal degradation of essential amino acids of alfalfa protein by using in vitro rate constant and reported that their half-lives were 2 h or less. He further pointed out that to bypass the rumen these amino acids had to be present in the diet greatly in excess of requirements. However, methionine seems to be relatively resistant to ruminal degradation. Methionine, lysine and probably histidine are thought to be limiting for milk production. Therefore their supply to the small intestine is considered particularly important in early lactation. Diets supplemented with fish meal showed lower than total degradability for methionine, lysine and histidine, while, except for histidine, these amino acids were highly degraded for the SBM diet. Greater flows of lysine and methionine to the small intestine in cows fed FM diet did not result in any difference in milk production. The total amino acid flow to the duodenum was similar between diets and this perhaps had a greater effect on animal performance than higher flows of lysine and methionine.

Percent recovery of EAA at the duodenum for diet FM was much greater than that for SBM diet. At the same protein intake, fish meal supplied more amino acids to the small intestine than soybean meal giving it potential as a protein supplement in early lactation. Diets supplemented with fish meal must contain enough degradable protein to allow maximal microbial growth in the rumen. This is at least partially

accounted for in the new NRC method (78).

The contribution of microbial amino acids to the duodenal TAA is also apparent, in particular for valine and methionine which showed substantial increases in the duodenal digesta compared to dietary intake.

Negative Control Experiment.

Results obtained in this experiment were not compared statistically to data obtained in the preceding trial. However, some reference to those results will be made and Tables 16, 17, 18, 19, 20 and 21 show the average for FM and SBM diets also. Dry matter intake, milk yield and composition, and body weight are shown in Table 16. The drastic decrease in dietary protein supply (about 50%) caused a decrease in milk yield and efficiency, but milk composition was maintained. All six cows except one which decreased milk production to about 5 kg/day, lost weight during the 14-day experimental period at an average rate of 1.0 kg/day.

There was an apparent decrease in VFA production except for isovalerate (Table 17). Proportion of acetate and valerate increased, while propionate seemed to decrease. Acetate/propionate ratio was therefore increased but efficiency of carbohydrate fermentation was similar. These results were expected in view of the greater proportion of corn silage in the diet compared to diet supplemented with fish or soybean meal (SBM-FM).

Rumen NH_3-N and plasma urea-N were drastically decreased by feeding the CS diet. This reflected the relatively low supply of ruminally degradable nitrogen and triggered N-sparing mechanisms suggested by very low concentrations of PUN. Recirculation of blood urea to the

TABLE 16. Dry matter intake, milk yield and composition, and body weight of Holstein cows fed a corn silage-ground corn diet (CS) in midlactation (165-204 d).

Item	CS[1]	± sd	SBM-FM[2]
Dry matter intake			
Kg/day	14.0	1.0	16.6
Percent body weight	2.5	.3	3.1
Milk yield, kg/day	18.1	4.2	27.0
4% FCM, kg/day[3]	16.5	3.8	23.6
Milk composition, %			
Fat	3.42	.45	3.18
Protein	2.80	.22	2.91
Lactose	4.76	.38	4.93
Milk components yield, kg/day			
Fat	.61	.15	.86
Protein	.51	.09	.78
Lactose	.87	.24	1.33
Milk efficiency, kg FCM/kg feed	1.2	.02	1.45
Body weight, kg	572	41	548
Body weight change, kg/day	-1.0		-

[1]Means of six observations ± standard deviation.

[2]Average least square means of SBM and FM diets for same parameters. For individual diet values see Table 5.

[3]4% fat-corrected milk = (.4 x kg milk) + (15 x kg fat).

TABLE 17. Ruminal parameters and plasma urea nitrogen (PUN) in Holstein cows fed a corn silage-ground corn diet (CS) in midlactation (165-204 d).

Item	CS[1]	± sd	SBM-FM[2]
Total volatile fatty acids, mg/dl	754.3	150.7	953.7
Acetate, mg/dl	412.6	87.5	465.5
Propionate, mg/dl	150.6	21.3	240.9
Isobutyrate, mg/dl	11.6	2.8	13.7
Butyrate, mg/dl	122.5	30.7	169.0
Isovalerate, mg/dl	40.1	12.5	37.8
Valerate, mg/dl	16.9	3.9	26.8
Acetate[3]	62.5	2.8	56.7
Propionate[3]	18.7	1.8	23.7
Isobutyrate[3]	1.2	.1	1.1
Butyrate[3]	12.6	1.3	14.0
Isovalerate[3]	3.5	.4	2.7
Valerate[3]	1.5	.1	1.9
Acetate/propionate	3.8	.5	2.5
Efficiency of carbohydrate fermentation[4]	74.2	.9	76.6
Ammonia-N, mg/dl	1.4	.7	6.9
Rumen pH	6.5	.2	6.2
PUN, mg/dl	2.7	.8	13.8

[1]Means of six observations ± standard deviation.

[2]Average of SBM and FM diets for same parameters. For individual diet values see Table 6.

[3]Moles/100 moles of acid.

[4](.622 acetate + 1.092 propionate + 1.56 butyrate) x 100/(1 acetate + 1 propionate + 2 butyrate). Orskov (88).

gastrointestinal tract could be important to maintain digestive efficiency when lactating cows are fed low protein diets.

Solid and liquid digesta flow to the small intestine (Table 18) were somewhat higher than flow obtained with FM and SBM diets. However, the organic matter data (Table 19) was less than reported for previous diets. Indications are that duodenal samples collected contained less dry matter compared to those collected for SBM-FM. This agrees with the concept that with increasing proportions of forage in the diet mastication and rumination increased as does liquid outflow from the rumen. Mean apparent and true organic matter digestibility at the duodenum and total organic matter digestibility were lower compared to SBM-FM. A greater proportion of total digested organic matter of the CS diet was digested in the rumen compared to SBM-FM indicating a shift in site of digestion from stomachs to intestines with SBM-FM. Acid detergent fiber intake was lower, and ADF and OM digestibility, decreased considerably with diet CS (Table 20). Nitrogen flow and digestibility are shown in Table 21. Microbial N for diet CS was lower than the average SBM-FM but was essentially the same as reported for FM. This would indicate nitrogen from fish meal contributed very little to microbial protein synthesis when diet FM was fed to cows in early or midlactation. The much greater microbial flow obtained with SBM diet would

TABLE 18. Digesta flow and digesta pH at proximal duodenum in Holstein cows fed a corn silage-ground corn diet (CS) in midlactation (165-204 d).

Item	CS[1] ± sd	SBM-FM[2]
Digesta flow:		
Solid phase[3], kg/day	52.9 7.7	47.9
Liquid phase[4], kg/day	112.1 25.5	91.4
Total, kg/day	165.0	139.2
Digesta pH	2.5 .2	2.8

[1]Means of six observations ± standard deviation.

[2]Average least square means of SBM and FM diets for same parameters. For individual diet values see Table 7.

[3]Pellet from centrifugation (3000xg for 10 min) of whole digesta.

[4]Supernatant from centrifugation (3000xg for 10 min) of whole digesta.

TABLE 19. Intake, flow, and digestion of organic matter in the digestive tract of Holstein cows fed a corn silage based-ground corn diet (CS) in midlactation (165-204 d).

Organic matter	CS[1]	±	sd	SBM-FM[2]
Intake, kg/day	13.4		1.0	15.8
At proximal duodenum				
Flow:				
Solid phase[3], kg/day	7.5		1.0	8.4
Liquid phase[4], kg/day	1.5		.3	1.6
Total, kg/day	9.0		.8	10.0
Microbial, kg/day	1.6		.2	1.9
Digestibility:				
Apparent[5], %	32.6		6.8	37.2
True[6], %	45.1		6.9	49.1
In stomachs[7], %	80.7		7.0	52.6
In feces:				
Flow, kg/day	5.9		1.0	4.5
Apparent digestibility, %	55.9		7.1	71.6

[1]Means of six observations ± standard deviation.

[2]Average least square means of SBM and FM diets for same parameters. For individual diet values see Table 8.

[3]Pellet from centrifugation (3000xg for 10 min) of whole digesta.

[4]Supernatant from centrifugation (3000xg for 10 min) of whole digesta.

[5]Percent of intake.

[6]Corrected for microbial organic matter.

[7]Percent of apparently digested organic matter digested in the stomach.

TABLE 20. Intake, flow, and digestion of acid detergent fiber (ADF) in the digestive tract of Holstein cows fed a corn silage-ground corn diet (CS) in midlactation (165-204 d).

ADF	CS[1]	± sd	SBM-FM[2]
Intake, kg/day	2.8	.3	3.3
At proximal duodenum:			
Flow, kg/day	2.1	.4	2.0
Apparent digestibility[3], %	25.3	11.6	38.5
Digestion in stomachs[4], %	75.5	12.1	74.7
In feces:			
Flow, kg/day	1.8	.3	1.6
Apparent digestibility, %	33.5	5.2	51.6

[1]Means of six observations ± standard deviation.

[2]Average least square means of SBM and FM diets for same parameters. For individual diet values see Table 9.

[3]Percent of intake.

[4]Proportion of apparently digested ADF digested in the stomachs.

TABLE 21. Intake, flow, and digestion of nitrogen in the digestive tract of Holstein cows fed a corn silage-ground corn diet (CS) in midlactation (165-204 d).

Nitrogen	CS[1]	± sd	SBM-FM[2]
Intake, g/day	191	13	420
At proximal duodenum			
Solid phase nitrogen flow[3]:			
Total, g/day	204	20	278
Precipitable, g/day	141	15	191
Non precipitable, g/day	62	8	87
Microbial, g/day	148	21	166
Residual, g/day	56	22	112
Liquid phase nitrogen flow[7]:			
Total, g/day	64	14	94
Precipitable, g/day	9	4	13
Non precipitable, g/day	55	13	81
Microbial, g/day	17	4	23
Residual, g/day	47	13	71
Total microbial, g/day	165	22	190
Total residual, g/day	103	26	182
Total, g/day	268	16	372
Efficiency of microbial protein synthesis			
Apparent, g N/kg DOMR[9]	40.3	13.1	34.5
True, g N/kg TDOMR[10]	28.3	6.1	25.0
N recovery, % of intake	140.9	11.2	88.8
True digestibility[11], %	45.9	13.5	56.5
Apparent digestibility in intestines[12], %	56.7	2.9	67.1
In feces:			
Flow, g/day	116	10	122
Apparent digestibility, %	39.0	6.3	71.1

[1]Means of six observations ± standard deviation.

[2]Average least square means of SBM and FM diets for same parameters. For individual diet values see Table 10.

[3]Nitrogen in pellet from centrifugation (3000xg for 10 min) of whole digesta.

[4]Nitrogen in precipitate obtained with 5% Trichloroacetic acid.

[5]Calculated by difference from total and precipitable nitrogen.

[6]Calculated by difference from total and microbial nitrogen.

[7]Nitrogen in supernatant from centrifugation (3000xg for 10 min) of whole digesta.

[8]Nitrogen in filtrate obtained from filtration of liquid phase treated with 10% trichloroacetic acid.

[9]Organic matter apparently digested in the stomachs.

[10]DOMR corrected for microbial organic matter.

[11]Corrected for microbial nitrogen.

[12]Percent of nitrogen at duodenum.

support this hypothesis. The "by difference technique" was used here to calculate rumen degradability of soybean and fish meal as follows:

From Table 2 we can calculate that soybean meal and fish meal supplied 53.4 and 55.5% of total crude protein in the diet respectively. Possible contributions of endogenous N to residual N are ignored in the following calculations.

Diets	FM	SBM
Total N intake, g/day	408	432
From fish meal=408x.534=218	From soybean meal = 432x.555=240	
From CS = 408-218=190	From CS = 432-240=192	
Residual N flow to duod =214		149
From CS =190x.541=103	From CS = 192x.541=104	
From fish meal= 214-103=111	From soybean meal = 149-104= 45	

Fish meal degradability = (1-111/218) * 100 = 49.1%

Soybean meal degradability = (1-45/240) * 100 = 81.3%

These values, although slightly higher, compare well with those reported in the literature for fish meal and soybean meal degradabilities calculated with the regression technique or the in situ method. Problems still remain for calculation of endogenous N. True and apparent efficiency of microbial synthesis for diet CS was similar to SBM but

greater than FM diet. Less organic matter digested in the rumen certainly contributed to these differences in microbial efficiency.

Nitrogen recovered in the small intestine with CS diet was about 41% greater than N intake. On the contrary, N loss was observed with SBM-FM diets. True N digestibility in the rumen (or degradability) was similar for diets CS and FM but much lower than SBM diet (see Table 8). Apparent N digestion in the intestines and total N digestibility were lower than values reported for SBM-FM, particularly for the latter. Recovery of nitrogen in the duodenum greater than intake obtained with CS diet is probaby due to a combination of factors including nitrogen recycling in the rumen and endogenous secretions. Relatively low concentrations of NH_3-N in the rumen favors urea flux through the rumen wall, its hydrolysis to NH_3 by rumen wall adherent bacteria and therefore its utilization by rumen microorganisms.

Low nitrogen intake, substantial quantity of microbial N synthesized in the rumen and endogenous N all contributed to the relatively low nitrogen digestibility. If such a great effort is made by the animal to recycle nitrogen losses into the gastrointestinal tract via microbial form, it makes little sense to excrete such a large quantity in the feces. It is possible that a substantial quantity of N in the forage, which comprised more than 70% of the ration was acid detergent insoluble nitrogen (ADIN), and therefore

indigestible. NRC (1) suggests that the ADIN fraction of intake nitrogen be subtracted when formulating diets for ruminants.

SUMMARY AND CONCLUSIONS

Effects of fish meal or soybean meal supplementation in the diet on digesta and digesta components flow to the small intestine during early and midlactation were investigated using six Holstein cows cannulated in the rumen and the proximal duodenum.

Dry matter intake and milk production did not differ among diets, but milk fat was depressed when fish meal diet was fed. Protein source did not affect organic matter digestibility , but organic matter flow to the duodenum decreased in midlactation for both diets. Organic matter intake in early and midlactation was similar, but less organic matter reached the duodenum in midlactation perhaps due to an increased digestive capacity of the stomachs.

Nitrogen intake and flow to the duodenum were similar among diets and stages of lactation. However, microbial nitrogen flow and efficiency of microbial protein synthesis were greater for the soybean meal diet. Since energy content of the diets was similar, ruminally available nitrogen with fish meal diet probably limited microbial growth. This is supported by the lower values of rumen ammonia-N observed with fish meal supplementation. Dietary proteins in the soybean meal diet were more degraded than protein in the fish meal diet. Recovery of dietary nitrogen in the

proximal duodenum was greater for the latter. Although there were no differences in total nitrogen digestibilities, nitrogen digestibility in the intestines was greater for fish meal diet. This indicated greater intestinal digestibility of escape protein compared to microbial nitrogen.

Total amino acid intake was greater with soybean meal in the diet, but essential and nonessential amino acid flow to the duodenum were similar among diets. Greater flow of microbial amino acids was observed with soybean meal, while greater recovery of dietary amino acids was observed with fish meal. Except for alanine, valine, threonine and methionine, individual amino acids flow to the duodenum were lower than intake. Individual amino acid degradability was greater for soybean meal except for cysteine and tyrosine. Of the essential amino acids, valine, methionine, lysine and histidine were less degraded for fish meal, but were degraded considerably for soybean meal diet. Least degraded for both diets were glycine, valine, serine, proline and histidine.

In this study, the advantage of more dietary nitrogen escaping rumen degradation obtained with the fish meal diet was offset by less microbial protein synthesis in the rumen compared to soybean meal. These results indicate that microbial growth was limited by insufficient ruminal supply of degradable nitrogen with fish meal. This supports the

need of systems which estimate the requirements of degradable and undegradable protein in the diet. From the amino acid data it is apparent that similar nitrogen intakes did not translate into similar amino acid intakes, suggesting the need of expressing nitrogen requirements and dietary supply in terms of amino acids and not just protein. Furthermore, observed individual amino acid degradabilities showed a great variability between amino acids and among protein sources. However, because of the considerable impact of microbial amino acids, no differences between diets were observed on duodenal amino acid flow.

If the goal is to supply more amino acids to the small intestine in early lactation by feeding proteins resistant to ruminal degradation, the diet should contain sufficient quantity of degradable protein to allow maximum microbial protein synthesis. The lack of response to fish meal supplementation in early lactation could be attributed to a depressed microbial protein synthesis with this diet.

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APPENDIX

APPENDIX TABLE 1. Flow in feces and apparent digestibility of organic matter acid detergent fiber, and nitrogen in lactating Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation[1].

	Diet								Stage of lactation[2]							
	FM				SBM				E				M			
Item	Co	SE	Yb	SE	Co	SE	Yb	SE	Co	SE	Yb	SE	Co	SE	Yb	SE
Flow to feces:																
Organic matter, kg/day	4.2	.6	4.3	.3	5.0	.6	4.5	.3	4.4	.6	4.4	.3	4.8	.6	4.4	.3
Acid detergent fiber, kg/day	1.5	.04	1.6	.1	1.5	.04	1.6	.1	1.7	.05	1.6	.1	1.4	.04	1.6	.1
Nitrogen, g/day	113	5	119	10	123	5	131	10	123	5	121	10	113	5	129	10
Apparent digestibility:																
Organic matter, %	72.9	3.6	72.5	1.4	68.8	3.6	72.3	1.4	72.1	3.6	72.1	1.4	69.6	3.6	72.6	1.4
Acid detergent fiber, %	53.3	1.9	51.8	2.6	51.2	1.9	49.8	2.6	47.7	1.9	48.7	2.5	56.9	1.9	52.9	2.5
Nitrogen, g/day	71.9	1.0	70.6	1.6	71.4	1.0	69.8	1.6	71.0	1.0	71.5	1.6	72.3	1.0	68.9	1.6

[1] Least square means of 24 observations per diet or stage of lactation.

[2] E = early lactation (33-67 d); M = midlactation (131-165 d).

APPENDIX TABLE 2. Amino acid composition (g/kg dry matter) of fish meal (FM) and soybean meal (SBM) diets fed to Holstein cows during early and midlactation[1].

Amino acids	Diet			
	FM		SBM	
	E[2]	M[2]	E[2]	M[2]
ALA	5.25	4.51	4 51	4 02
GLY	5.70	5 29	4 32	4 03
VAL	22	.30	.30	.22
THR	3.22	3 99	4.10	4 04
SER	4 56	4.36	5.49	5 40
LEV	8.15	9 18	10.52	10 08
ILE	3.14	3.36	3 69	3 80
CYS	1.41	1.26	1 84	1.47
PRO	6 32	6.57	7 42	6.69
MET	.69	55	.52	.72
ASP	8.42	9 47	12.39	11.25
PHE	3.88	4.58	5.73	5.40
GLU	13.47	16.18	21.49	19.57
LYS	4.05	6.10	6.27	5.22
TYR	2.35	3.15	4.03	3.61
ARG	6.38	6.60	8.55	6.97
HIS	2.06	2.00	2.10	2.04
EA[3]	31.88	36.60	41.79	38.50
NEAA[4]	49.54	50.79	61.49	56.04
AL[5]	81.42	87.59	103.28	94.54

[1]Analysis of one composite sample (from eight individual samples) for FM or SBM diets in early or midlactation.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Essential amino acids included: VAL, THR, LEU, ILE, MET, PHE, LYS, ARG, HIS.

[4]Nonessential amino acids included: ALA, GLY, SER, CYS, PRO, ASP, GLU, TYR.

[5]EAA + NEAA.

APPENDIX TABLE 3. Amino acid composition (g/16 g N) of duodenal digesta solid phase (dry matter) in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Amino acids	Diet[1]		Stage of lactation[1,2]		SE
	FM	SBM	E	M	
ALA	3.57	3.58	3.50	3.65[b]	.08
GLY	3.10	2.96	3.06	2.99[b]	.06
VAL	2.43	2.42	2.38	2.46[a]	.05
THR	2.79	2.93	2.88	2.84	.10
SER	2.83	2.95[b]	2.84	2.94	.04
LEU	4.68	4.78	4.75	4.71[a]	.07
ILE	2.23	2.25	2.19	2.29[a]	.04
CYS	.55	.67[b]	.59	.63	.02
PRO	2.87	2.97[a]	2.92	2.92	.04
MET	1.64	1.45[b]	1.50	1.59[a]	.02
ASP	5.63	5.58	5.49	5.72[b]	.07
PHE	2.65	2.69	2.65	2.68	.04
GLU	7.97	7.83	7.85	7.96	.10
LYS	3.76	3.34	3.39	3.71	.07
TYR	2.11	2.15	2.11	2.16	.07
ARG	2.29	1.92	2.15	2.05	.28
HIS	1.23	1.22	1.19	1.27	.02

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67d); M = midlactation (131-165 d).

[ab]Means for diet or stage of lactation differ, a: $P < .10$; b: $P < .05$.

APPENDIX TABLE 4. Amino acid composition (g/kg dry matter) of duodenal digesta solid phase in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during ealry and midlactation.

Amino acid	Diet[1]		Stage of lactation[1,2]		SE
	FM	SBM	E	M	
ALA	7.52	7.56[b]	6.58	7.51[b]	.21
GLY	6.51	5.41[b]	5.78	6.15[b]	.14
VAL	5.11	4.43[b]	4.49	5.05[b]	.14
THR	5.88	5.36[a]	5.42	5.82	.19
SER	5.95	5.40[b]	5.35	6.00[b]	.11
LEU	9.84	8.73[b]	8.93	9.64	.16
ILE	4.69	4.11[b]	4.13	4.68[b]	.11
CYS	1.15	1.22	1.10	1.28[a]	.05
PRO	6.02	5.42[b]	5.48	5.97[b]	.08
MET	3.46	2.66[b]	2.85	3.27[b]	.08
ASP	11.86	10.20[b]	10.37	10.69[b]	.22
PHE	5.57	4.90[b]	4.99	5.49[a]	.09
GLU	16.77	14.32[b]	14.80	16.29[a]	.23
LYS	7.92	6.14[b]	6.45	7.61[b]	.18
TYR	4.43	3.94[b]	3.96	4.41[b]	
ARG	4.77	3.49	4.13	4.13	.56
HIS	2.60	2.24[b]	2.24	2.60[b]	.05
EAA[3]	49.86	42.05[b]	43.63	48.28	.07
NEAA[4]	60.22	52.47[b]	53.40	59.30[b]	.96
TOTAL[5]	110.08	94.53[b]	97.03	107.58[a]	1.53

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Essential amino acids included: VAL, THR, LEU, ILE, MET, PHE, LYS, ARG, HIS.

[4]Nonessential amino acids included: ALA, GLY, SER, CYS, PRO, ASP, GLU, TYR.

[5]EAA + NEAA.

[ab]Means for diet or stage of lactation differ, a: $P < .10$; b: $P < .05$.

APPENDIX TABLE 5. Amino acid composition (g/16 g N) of duodenal digesta liquid phase in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplements during early and midlactation.

Amino acid	Diet[1]		Stage of lactation[1,2]		SE
	FM	SBM	E	M	
ALA	3.95	3.95	3.81	4.09	.18
GLY	3.55	2.97	3.44	3.08	.25
VAL	2.51	2.67	2.43	2.75	.12
THR	2.73	2.92	2.64	3.00	.12
SER	2.47	2.55	2.36	2.67	.13
LEU	4.15	4.51	4.21	4.46	.18
ILE	2.11	2.33[b]	2.07	2.37	.10
CYS	1.78	2.28[b]	2.19	1.88	.09
PRO	2.54	2.59[b]	2.51	2.62	.10
MET	.96	.73[b]	.79	.90	.06
ASP	5.75	6.15[b]	5.59	6.31	.24
PHE	2.11	2.43[b]	2.15	2.39	.09
GLU	7.38	7.94	7.39	7.94	.30
LYS	4.02	3.76[a]	3.61	4.16	.16
TYR	2.00	2.26[a]	2.00	2.27	.09
ARG	1.89	2.37[a]	2.10	2.16	.19
HIS	.97	1.02	.94	1.05	.04

[1]Least square means of 12 observations.

[2]E = early stage of lactation (33-67 d); M = midlactation (131-165 d).

[ab]Means for diet or stage of lactation differ, a: $P < .10$; b: $P < .05$.

APPENDIX TABLE 6. Amino acid composition (g/kg) of duodenal digesta liquid phase in Holstein cows fed fish meal (FM) or soybean meal (SBM) as protein supplemetns duringe early and midlactation.

Amino acid	Diet[1]		Stage of lactation[1,2]		SE
	FM	SBM	E	M	
ALA	.275	.238[b]	.232	.280[b]	.010
GLY	.245	.178[b]	.211	.212[b]	.014
VAL	.175	.161	.149	.188[b]	.007
THE	.190	.177	.162	.205[b]	.008
SER	.172	.154	.144	.183[b]	.008
LEU	.289	.271	.256	.305[b]	.010
ILE	.147	.141	.126	.162[b]	.006
CYS	.123	.137[a]	.133	.127	.005
PRO	.177	.156[b]	.153	.180[b]	.006
MET	.066	.044[b]	.048	.063[a]	.004
ASP	.400	.372	.341	.431[b]	.015
PHE	.146	.147	.131	.163[b]	.006
GLU	.514	.478	.450	.542[b]	.018
LYS	.280	.228[b]	.022	.286[b]	.010
TYR	.139	.137	.122	.155[b]	.155
ARG	.131	.143	.130	.144	.012
HIS	.068	.062	.058	.072[b]	.003
EAA[3]	1.493	1.374	1.281	1.586[b]	.057
NEAA[4]	2.045	1.849[a]	1.785	2.109[b]	.071
TOTAL[5]	3.538	3.223	3.066	3.695[b]	.127

[1]Least square means of 12 observations.

[2]E = Early stage of lactation (33-67 d); M = midlactation (131-165 d).

[3]Essential amino acids included: VAL, THR, LEU, ILE, MET, THE LYS, ARG, HIS.

[4]Nonessential amino acids included: ALA, GLY, SER, CYS, PRO, ASP, GLU, TYR.

[5]EAA + NEAA.

[ab]Means for diet or stage of lactation differ, a: $P < .10$; $P < .05$.

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EFFECT OF DIETARY SOYBEAN MEAL AND FISH MEAL ON PROTEIN DIGESTA FLOW IN HOLSTEIN COWS DURING EARLY AND MIDLACTATION

by

Ercole Zerbini

(ABSTRACT)

Six lactating Holstein cows fitted with rumen cannulae and T-type cannulae in the proximal duodenum were used to measure digesta and nitrogen compounds flow to the small intestine during early and midlactation. Fish meal and soybean meal provided 54 and 56% of the protein in the diets composed of corn grain, corn silage and orchardgrass hay, and which contained 15.4 and 15.5% crude protein and 20.9 and 20.5 acid detergent fiber. Spot samples of digesta were collected from the rumen, duodenum and rectum over a period of 72 hours. Co-EDTA and ytterbium were used as liquid and particulate digesta markers to estimate flow and digestibility of nutrients. Cytosine was used as microbial marker. True organic matter digestibility in the stomachs was 48.4, 49.8, 44.9, and 53.2% for fish meal and soybean meal diets and early and midlactation respectively. Protein degradability in the stomachs were 47.2, 65.8, 56.7, and 56.2% for fish meal and soybean meal diets and early and

midlactation respectively. Amino acids intake was greater for soybean meal diet but total amino acids reaching the the duodenum were similar for both diets. Valine, methionine, lysine and histidine were less degraded for fish meal but were extensively degraded in soybean meal diet. Least degraded for both diets were glycine, valine, serine, proline and histidine. The advantage of greater quantity of protein escaping ruminal degradation with fish meal supplementation was counterbalanced by less microbial synthesis in the rumen partly explaining the similar response obtained with diets especially in early lactation.