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PROTEIN PARTITION AND DIGESTA FLOW IN LACTATING HOLSTEINS FED 2:1 AND 1:2 SOYBEAN MEAL:FISH MEAL

by

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(ABSTRACT)

Attempts to improve upon the crude protein feeding system have strived to characterize feedstuffs with respect to ruminal protein degradability. In vitro an in situ procedures fall short of this goal by not accounting for ruminal turnover. Six lactating cows, fitted with ruminal and duodenal cannulae, were utilized for in vivo determination of protein degradability by employing a double-marker system. Treatments were corn silage-based diets supplemented with 2:1 soybean meal:fish meal nitrogen (SF) or 1:2 soybean meal:fish meal nitrogen (FS) at levels of 15.9 and 16.0 percent protein. Ruminal cobalt ethylenediaminetetraacetic acid (CoEDTA) infusions marked liquid phase (LP) digesta and Yb-soaked hay was used as a solid phase (SP) marker. Duodenal digesta was separated into SP and LP at 3000xg.

Least squares means of LP flows did not differ for total, precipitable protein, or microbial nitrogen, for SF

and FS. Total solid phase flow of dry matter was higher for FS (9.06 kg/d) than for SF (7.97 kg/d), although intakes did not differ. Milk composition and yield did not differ for FS and SF. Average daily gain was 0.12 kg/d for SF and 1.26 kg/d for FS. Whole-tract digestibilties were not different, although rumen digestibility of dry matter was 24.9% for FS and 35.7% for SF, and duodenal recovery of N was 93.8% for SF and 107.5% for FS.

Inclusion of a higher level of fish meal in the diet increased the proportion of feed proteins delivered to the small intestine, increasing weight gain while having no effect on milk production.

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all aspects of dairy farm management. With this focus, I was able to develop an appreciation for dairy cattle which has served me as a source of inspiration in the pursuit of a dairy science education.

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INTRODUCTION

Delivery of amino acids to the small intestine depends on such factors as feed source, intake, energy content of the diet, microbial growth factors, particle size and rate of turnover (37). Before adjustments or refinements in the crude protein system can be accepted, more must be known with regard to a feedstuff's ability to deliver both microbial and undegraded feed proteins to the duodenum. In vitro and in situ procedures have fallen short in their attempts to characterize delivery of amino acids to the duodenum due to the intricacies of the interactions between factors that affect nitrogen flow. Forster et al. (26) proposed that feeding systems should be designed to meet both the amino acid requirements of rumen microorganisms and of the animals' own requirements over and above what is met by rumen microbial protein.

Data from feeding trials suggest a beneficial effect of feeding less degradable protein sources to lactating cows (35, 56). Other researchers have reported no benefits with the possible exception of weight gain (5, 66). In these studies, substitution of undegradable protein sources for more degradable protein sources most likely had the effect of limiting microbial growth in the rumen due to limiting available nitrogen. The beneficial effects of undegradable

protein sources were abolished by the suppression of microbial protein yield.

In this study, ratios of soybean meal:fish meal nitrogen were either 1:2 or 2:1 in order to assess the optimum combination of degradable and undegradable proteins for allowing maximum microbial growth while supplying undegraded feed protein to the duodenum. Analysis of rumen and blood parameters were undertaken in order to better understand the metabolic adjustments occurring in response to treatments. Employment of digesta flow markers enabled calculations of nutrient flow, in order to compare dietary treatments and their relation to factors that affect flow.

LITERATURE REVIEW

NITROGEN UTILIZATION IN DAIRY CATTLE

The optimum feeding system for dairy cattle production is one in which the cost of protein supplementation is kept low, while the complete requirement for essential amino acids is met by a combination of microbial and bypass proteins. Endogenous proteins, consisting primarily of sloughed ruminal epithelium cells, are an additional source of amino acids to the intestine. Contribution by this source is difficult to assess, and is considered to be low (82). Since delivery of essential amino acids to the small intestine depends upon such factors as feed source, intake, energy comtent of the diet, particle size, rumen microbial growth factors and turnover rate, a simple method of estimation of amino acid profile as supplied to the intestine by a feedstuff or ration, is not possible. Although the crude protein system of ration assessment and balancing came into existence before each of these variables was understood, it has doubtless been retained in popular use due largely to the complexity and elusiveness of a more appropriate method of characterizing protein value of a feedstuff (37).

Working within the crude protein system has led to

conflicting recommendations by researchers (62). The NRC requirement for cows in early lactation has been gauged to be inadequate by some (20, 26, 35, 42, 77), adequate by others (51, 87), and unnecessarily high, as deemed necessary to support production of up to 35 kg/d, by Huber and Kung (37). Since protein supplements are typically the most costly feed ingredients in a complete diet, a careful analysis of these differing recommendations is warranted.

Intake has been determined to be a limiting factor in meeting protein requirements in cows in early lactation. Kung and Huber (42) found that intake was not decreased in early lactation cows as crude protein was raised to 17%. Polan et al. (73) had shown a decrease in dry matter intake in cows as crude protein was increased above 12.8%. Intake is also depressed by diets that fall below 11% crude protein, as fermentation by rumen microorganisms is uncoupled, due to a deficiency in available nitrogen for microbial incorporation (5, 64). Cows in early lactation can lose up to one-fourth of their total body protein mass, amounting to upwards of 40 kg, as homeorhetic control mechanisms allow for substantial milk protein production in spite of inadequate intake of dry matter to allow for meeting the protein requirement (6, 8). Once peak production has occurred and the cow begins to produce less milk protein, lost reserves are repleted. Crude protein percent in the diet is positively correlated with the time

required for full repletion (5).

Feedstuffs differ as nitrogen sources, even in the case of isonitrogenous substitutions, as measured by crude protein. Soybean meal has been found to be superior to isonitrogenous additions of urea to a complete feed (77). Heat-treated soybean meal (80) and soybean meal treated with NaOH (57) have been shown to increase nitrogen retention in milking animals and growing calves. Measurements of blood urea- N and rumen fluid NH_3-N indicate that treating soybean meal with NaOH or heat results in decreased proteolysis by rumen microorganisms. Satter et al. (80) concluded that reduced proteolysis of soybean meal resulted in increased delivery of amino acids to the small intestine. Higher urea-N and NH_3-N values are associated with increased N loss, as it was surmised that increased blood levels of urea-N resulted from NH_3 being transformed in the liver to the less toxic urea form. Untreated soybean meal apparently allows rapid NH_3 release by the proteolytic rumen bacteria. The NH_3 diffuses out of the rumen wall into the bloodstream, before being transported to the liver (31, 51, 66). Blood urea is lost to urine in the kidneys, or recycled back to the rumen as saliva. Although some nitrogen is lost in this process, the buffering/recycling mechanism of ruminal nitrogen is rather remarkable, nonetheless.

Another mechanism that allows for the recycling of urea is that whereby blood urea is passed back through the rumen

wall. Orskov (66) cites evidence that this transfer is mediated by a carrier in the event that rumen NH_3 concentration is low. Armstrong and Weekes (3) suggest that this transfer only occurs when energy levels of rumen contents are high. Although both the salivary and rumen wall transport routes help to maximize efficiency of nitrogen utilization, more efficiency is gained when rumen ammonia is not high enough for a substantial increase in blood urea concentration. Certainly, direct uptake of rumen NH_3 by the rumen microorganisms is going to be a more efficient process than is involved in the transport of ammonia to the liver, the conversion of urea, the recycling back to the rumen, and hydrolysis by microorganisms back to NH_3.

RUMEN NON-DEGRADED PROTEINS

The extent to which a protein source is degraded by rumen microorganisms is a characteristic of the feedstuff. Proteins may be hydrolyzed into amino acids which are directly taken up and incorporated into microbial proteins, or they may be deaminated, and the resulting carbon chain fermented by the microorganism as an energy source (66). That proportion of he protein source that resists degradation by the rumen microorganisms and is delivered to the small intestine intact, is nondegraded and is often called bypass protein. Such protein is subject to enzymatic

degradation, as is microbial protein, and both serve as a source of amino acids to be taken up by the epithelial cells of the small intestine (66, 79, 91).

Many factors can influence the extent to which a protein source bypasses the rumen. Huber and Kung (37) stated that not only is extent of bypass a feed characteristic, but it may be affected by passage rate out of the rumen, by altering the microbial population balance or its growth, or by the particle size of the feedstuff. Satter et al. (80) compared rumen digestibilities of whole-extruded, raw soybeans, soybean meal and heat-treated soybean meal, and found large differences in degradabilities. Mathers et al. (50) suggested that in general, animal protein sources bypass to a greater extent than plant proteins.

Estimates of the extent of bypass by a protein have been made by measurements of several different parameters. Rumen NH_3-N values, although in a constant state of flux due to dilution rate, outflow of ammonia through the rumen wall, and rumen environment effects on microbial populations, give an indirect measure of microbial degradation of a protein. Herrington et al. (35) reported lower NH_3-N and blood urea-N when diets were supplemented with protein sources of lower degradability. This was especially pronounced when soybean meal (a degradable protein) was compared to dried brewers' grains (a less degradable protein). Similar results were

reported by Polan et al. (76) and Forster et al. (26). Justification for relating values of urea or NH_3-N to degradability lies in the fact that rate of microbial degradation of proteins in feed is a major factor in the concentration of NH_3-N at any point in time in the rumen. Other factors are involved, however, and may explain the results of Van Horn et al. (90), who found no differences between soybean meal and cottonseed meal with respect to rumen or blood parameters, but found differences in milk yield. Clearly, rumen NH_3-N and blood urea-N can be regarded as no more than indicators of degradability, rather than as reliable measures.

DIRECT MEASURES OF ESTIMATING BYPASS

In vitro solubilities have been used to estimate rumen degradabilities of proteins. Solvents used to dissolve proteins have been the subject of debate for many years (37). Autoclaved rumen fluid stands out as being the closest solvent to the actual rumen, thus narrowing the gap between in vitro procedures and biological reality. Although the actual value of degradability of a feedstuff obtained by these procedures is of questionable value, the method has been kept in use for its ability to rank feedstuffs according to degradability (66, 77, 82).

Procedures involving the use of protease enzymes are a step closer to true rumen degradation of proteins.

Commercially-prepared protease products can be of bacterial, fungal or plant origin (47). Mahadevan et al. (47) reported differences between solubilities as measured by various solvents and resistance to protease enzymes. In addition to lack of agreement of the two procedures, a second problem was reported. The researchers found that a readily soluble fraction of fish meal was more readily hydrolyzed by protease than was a soluble fraction of soybean meal. In contrast, the insoluble fraction of soybean meal was more susceptible to protease degradation than was the insoluble fraction of fish meal. Differential degrees of effectiveness of protease on two phases of protein, soluble versus insoluble, points to a problem that confounds both solubility and protease studies.

Artificial rumen studies, as reported by Orskov (66), have helped to clarify some of the discrepancies noted in other in vitro work. Craig and Broderick (19) reported differential release rates for amino acids depending on protein source. Four-hour incubations showed that 90% of the lysine contained in cottonseed meal was released, whereas the lysine contained in meat and bone meal was released only to the extent of 40%. This conclusion of different release rates of amino acids supports the work of MacGregor et al. (44) who suggested that the amino acid profile of a feedstuff is not necessarily the same profile as that fraction of protein in the feedstuff that bypasses.

While ammonia had long been considered to be the main N-source required by rumen microbial populations, Maeng and Baldwin (46) found that they could double the growth rate as measured by biomass production, by substituting purified amino acids for 25% of the urea in the nitrogen source made available to the microbes. These studies showed that microbial growth responds to more than the rate of release of NH_3-N into the rumen. Teather et al. (85) reported that both Selenomonas and Ruminococcus genera responded by 230% in growth rate as measured by bacterial numbers, when crude protein was increased from 9.4% to 12.5% by supplementing corn silage with soybean meal. Although some of this increase was reportedly believed to be due to amino acid availability to the rumen microorganisms, the response may have been due, at least in part, to a requirement by these microorganisms for a richer energy source than corn silage. This is supported by Owens et al. (70).

During the past decade the dacron bag technique has surged as a method of degradability estimation. This procedure has the advantage over any of the in vitro procedures of being affected by the dynamic state of the rumen environment. Rumen fluid may pass directly over the contents enclosed in the porous bag, allowing for removal of end-products that may suppress activity in in vitro work (60, 66). Estimates of degradability were reported often as time required for half of the nitrogen to disappear from the

contents of the dacron bag. Nocek et al. (60) found that soybean meal had a half-life of 20 h, while fish meal reached the half-degraded point at 55 h. Ganev et al. (28) reported 24 h digestibilities of soybean meal and fish meal to be 89% and 60%, respectively. Similar conclusions regarding differences between soybean meal and fish meal were made by Chalupa (11).

Orskov reported many problems with the bag technique, such as a pore size large enough to admit flow of rumen protozoa is large enough to pass relatively large feed particles (66). Mehrez and Orskov (52) reported a large variation due to animal differences, in work done with sheep. They also reported that the method of rinsing bags with cold water until the rinse water is clear, which is perhaps the best method of removing remaining soluble nitrogen and bacteria from the bags to date, is nevertheless a poor procedure for removal of all bacterial matter, due to the adherence of certain species of microorganisms to feed particles. Despite these problems, Grummer and Clark (32) reported a very high correlation between the solubility estimates for soybean meal and in situ degradability.

Recent work by Meyer et al. (55) has shown the bag technique to be better for use with certain feedstuffs, than others. An examination of numbers of proteolytic bacteria contained in unwashed bags showed fish meal to support a markedly low growth of bacteria. Conclusions drawn by the

researchers were that proteolytic species required more than peptides as a sole energy source to flourish, and that an ample supply of carbohydrates to the microenvironment inside the bag, may allow plant proteins to be degraded more rapidly than animal proteins. True differences between plant and animal protein sources may indeed be somewhat less than most bag studies have reported.

In vivo experiments, although costly, are useful in avoiding problems and false assumptions associated with in vitro and in situ methods (10, 60, 80, 82). Use of digesta flow markers, microbial markers and simple cannulae, either abomasal or post-pylorus duodenal, can work to uncover actual rumen degradability of feed protein, as it exists in the real rumen environment. Markers of solid (particulate) and liquid phase flow can be used to quantitate flow of nutrients out of the rumen (1, 2, 21, 23, 74). Microbial markers are used to quantitate flow of nitrogen (protein) out of the rumen that is due to microbial protein (78). Nitrogen flow due to undegraded protein is calculated to be the fraction of total precipitable protein flow, after accounting for microbial protein flow. Endogenous proteins due in small part to saliva and mainly to sloughed ruminal epithelium cells, have been noted by Leng and Nolan (43) to be a significant contribution to abomasal or duodenal flow of proteins. Correction of non-microbial protein flow for these proteins will give the true picture of undegraded feed

protein flow.

AMINO ACID SUPPLY TO THE SMALL INTESTINE

Infusion of casein into the abomasum has been demonstrated by Clark (12) to increase production of milk protein by 10-15% in high-producing cows. These results suggest that a combination of endogenous, feed and microbial proteins are, at least in the case of high-producing cows, inadequate for maximum production of milk protein by the udder tissue. Oldham (61) noted that circulating methionine (MET) and lysine (LYS) in the blood comprised a relatively smaller proportion of the total array of amino acids than is represented in the casein molecule. Orskov et al. (67) in noting differences between cows fed diets supplemented with urea and fish meal, suggested that increased production in cows fed fish meal could be linked to abomasal flows of MET that were 20% higher than in urea diets and LYS which was 40% higher in the fish meal diet over the urea diet. Orskov (66) has noted that microbial protein is typically lower in its proportions of lysine, methionine, valine, histidine, leucine, proline, serine and glutamic acid, than is casein. Santos et al. (76) determined in 1984 that LYS is the most probable amino acid responsible for limiting the production of casein. Tamminga (83), on the other hand, has suggested that no single amino acid may be assumed to be responsible for limiting milk production.

Several studies have reported increased milk production in high-producing cows when fed undegradable protein supplements (26, 35, 42, 48, 56, 57, 63). Other researchers have reported increased N retention only (29, 68, 83), while some studies found no differences (24, 25). A general concensus which can be drawn from these studies is that high-producing cows may benefit from bypass proteins, while cows in later lactational stages will not (83). A study conducted on commercial farms reported by Miller and Galwey (56), showed that fish meal substituted for soybean meal increased production in cows up through 100 d in lactation. Fish meal is reported to have a LYS content 1.5 times that of soybean meal and MET content 2.3 times higher by Maynard et al. (51). Orskov (66) reported an effect of fish meal in mid-lactation cows as being superior to soybean meal for weight gain, but no differences were found in milk production. Zerbini and Polan (95) found similar rates of gain in calves fed soybean meal and fish meal. If amino acids such as LYS and MET do indeed limit milk production in high-producing cows, repletion of reserves later in lactation may also be determined by LYS and MET reaching the duodenum, while growth in calves may not be as directly linked to these two amino acids.

Protein delivery to the small intestine is affected by rumen retention time. Bull et al. (9) reported greater bypass of feed proteins and greater intake when retention

time was less. Colucci et al. (15) similarly stated that digestibility of protein sources in the rumen is decreased by decreased retention time. Tamminga et al. (84) drew the same conclusion. A greater percentage of feed proteins bypass the rumen for a given feedstuff, if retention time in the rumen can be lowered. Bypass of proteins, then, can be seen as a function of all of the dietary interactions that have a role in nutrient flow.

DIGESTA FLOW MARKERS

Flow markers are required to measure partial tract digestibility or degradability in the rumen. Animals must also be surgically fitted with abomasal or duodenal cannulae. The type of cannula used can influence choice of markers, as a simple T-type cannula is difficult to use to obtain a representative sample of flow. Since maintenance of the reentrant duodenal cannula requires painstaking care, marker systems involving two or more separate phase markers and simple cannulae are preferred (21, 23).

Ellis et al. (21) suggests that since the digestive process in ruminant animals is seen as a two compartment system, at least two phases of flow must be marked to approach biological reality in the description of digesta flow. In two-marker systems, a liquid phase marker is added, in known concentration, to the rumen. A particulate or solid phase marker is either attached to the particles in

the feed, or placed directly into the rumen via the rumen fistula. Since particle size is accepted to be a factor in determining flow of a nutrient, particulate phase markers are often used to mark both forage and protein supplement feed particles (34).

Markers used to measure liquid dilution and flow are frequently one of cobalt ethylenediaminetetraacetate (CoEDTA), CrEDTA or polyethylene glycol (PEG). Both CoEDTA and CrEDTA are simple to quantitate using atomic absorption. Both substances are relatively inert, with no physical side effects. Uden et al. (88), states that with the exception of a small fraction of these substances being excreted in the urine, they meet the requirements of a digesta flow marker rather well. PEG is not excreted in the urine at all, but the quantitative analysis for the marker is prohibitively difficult and riddled with potential sources of error (94).

Particulate or solid phase markers used most widely are rare-earths such as La or Yb, and Cr, either in the form of Cr_2O_3 or in the mordant form attached to fiber. Cr_2O_3 is considered to be less than ideal due to its high density, which causes a non-uniform distribution of marker in the rumen (66). Chromium-mordanting of feed fiber is an improvement over Cr_2O_3, but Mader et al. (45) found that the mordanted fibers were indigestible to the animal. Thus, the mordanting process alters the diet in such a way as to

create a suppression of normal fiber digestibility of up to 12%.

Yb has been found to be a reasonable alternative to the two forms of Cr. In early studies, where Yb was applied to feedstuffs by means of a solution sprayed on to the feed prior to its being offered to the animal, a biphasic flow of Yb was noted (45). The fast-flowing pulse of Yb that surged ahead of the bulk of the element was assumed to be unattached Yb in the form of hydroxide. Erdman and Smith (22) found that Yb binds to sites on fibers with varying affinities. Hays were found to be made up of both high-affinity and low-affinity sites, while silages were lower in Yb binding affinity, in general. Pond et al. (74) found a maximum retention of 9.7 mg Yb per g hay. Coleman et al. (14) suggested that the most effective use of Yb as a marker would be made if only high-affinity binding sites were employed, thus minimizing opportunity for marker migration to other fibers. The researchers soaked the hay in Yb solution and then rinsed the hay thoroughly to remove all Yb bound to low-affinity sites. Retention of marker was found to be 3.5 mg Yb per g hay. Teeter et al. (86) recommended a 12 h soak in ytterbium chloride solution, followed by a 6 h rinse as a method of securing Yb on only the highest affinity binding sites.

Problems encountered with the use of Yb are many. Quantitating Yb is difficult with atomic absorption, but

much easier and more accurate with costlier neutron activation analysis (74). Combs et al. (16) reports that 70% of Yb is solubilized from binding sites at pH = 2.2, suggesting that normal abomasal pH will release Yb from the fiber to which it has been attached. Since binding affinity to hay is much greater than for silages or concentrates, however, the impact of this solubilization may be minimized by the selective reattachment of Yb to the hay particles as pH increases post-abomasally. Another problem encountered in the use of Yb as reported by Mader et al. (45) is that the immersion or soaking technique of attachment of Yb to hay particles is responsible for the loss of a soluble fraction of the hay, which when lost, lowers the digestibility of the hay. This loss in digestibility is small when compared to the loss in digestibility due to the mordanting of the fiber. A final problem encountered with the use of Yb for marking hay is that smaller particles of hay tend to have more binding sites per unit surface area, resulting in a more densely distributed Yb in smaller particles than in larger particles (22). This problem may be overcome by assuring a homogenous mixture of particle sizes of Yb-marked hay or in the ideal event of assuring a more standard particle size for the immersion process.

Faichney (23) reports that a double marker system can be used to correct for phase migration by markers and for non-representativeness of samples as collected by the

simple, T-type cannula. Armentano and Russell (2) formulated an algorithm for calculation of flows of each phase represented by the number of markers used. Assumptions that must be made are: 1) all markers are infused continuously, 2) phase migration or marker "hopping" does not invalidate calculations, and 3) digesta can be physically separated such that a major portion of marker in question can be attributed to a specific phase. Combs et al. (17) has shown that centrifugation of rumen contents at 1500Xg resulted in greater than 90% of Yb being found in the pellet, while greater than 60% of CoEDTA was associated with the supernatant fraction. Although these separations of marker are incomplete, the system of Armentano and Russell (2) corrects for the nature of the two phases. Flow rates for each phase of digesta that is marked can be calculated.

Use of flow markers has demonstrated variable flow rates for liquid and solid phases. Mertens (54), in looking at ruminal fiber degradation, found that liquid flowed faster than digestible fiber which in turn, flowed faster than lignin. A physiological advantage for the evolution of such a system is that the fraction of the feedstuff that is least available to the animal for nutrient uptake, is allowed the greatest amount of rumen retention and hence, longest exposure to microbial breakdown. Grovum and Williams in 1973 (31) found that particulate flow rate in sheep was not equal to the flow rate for liquid out of the

rumen. Since that time, researchers have recognized the need for describing at least two phases of flow (66).

Stern et al. (81) reported that liquid outflow from the rumen was 9.9% h^{-1} as determined by the natural logarithm of CoEDTA concentration ove time of disappearance from the rumen. Protein supplements were sprayed with La and disappearance of the marker was calculated to be 4.9% h^{-1}. No attempt was made to correct for biphasic flow of the marker as was observed by Mader et al. (45) when the spraying technique of marker application was applied. Hartwell and Satter (34) found similar flow rates in cows, with no significant effect of stage of lactation. Markers used were CrEDTA as liquid flow marker, and Ce and La as particulate flow markers. Liquid outflow rate was determined to be 8.1% h^{-1}, while particulate matter flow was 4.4% h^{-1}. They had theorized that four feedings per day would result in a more uniform rumen outflow, although flow data were not different in the same cows fed once daily.

Marker use is fraught with minor problems that interfere with obtaining perfect results (91). Warner and Stacy (93) reported as early as 1968 that time required for complete equilibration of liquid in the rumen is 1.5 h. Cows feeding ad libitum could conceivably never approach anything near equilibrium of rumen contents. Only the most meticulous of rumen sampling may be successful in avoiding problems of non-homogeneity. Woodford et al. (94) pointed

out that the use of liquid phase markers relied upon the assumption that drinking water was quickly equilibrated with rumen fluid and that there was no net transruminal flux of water. While they set out to test only the first assumption, proof of its falseness serves to encourage a closer look at the other assumption. The researchers found in low-producing cows that 18% of drinking water did not equilibrate with rumen fluid but in fact, bypassed the rumen. They further cautioned that high-producing cows, with greater water intake may bypass a larger proportion of drinking water.

CYTOSINE AS A MICROBIAL MARKER

Early studies concerned with quantifying microbial contribution to the delivery of protein to the small intestine employed diaminopimelic acid (DAPA) as a microbial marker (66). DAPA is a compound found only in bacterial cell walls, hence, it cannot estimate contribution by protozoa. Merry and McAllen (53) compared DAPA concentrations of bacteria harvested from solid and liquid fractions of rumen contents and found differences between types of bacteria.

Schelling et al. (78) reviewed microbial marker studies and recommended cytosine as a good alternative. Cytosine was noted to be more constant in its ratio of cytosine:protein than are other choices of markers across

species of microorganisms. Analytic procedures for cytosine are easier than for other purine or pyrimidine bases. These involve hydrolysis or release of cytosine from microbial compounds in digesta and chromatographic separation techniques. For cytosine to be a reliable marker, dietary and endogenous contribution to cytosine flow must be non-existent or insignificant, at the very least. Feed cytosine has been noted to be rapidly broken down in the rumen, such that cytosine has disappeared from feed in at most 4 h.

GENETIC ASPECTS OF NUTRIENT UTILIZATION

Feed efficiency in lactating cows depends on diet and other environmental factors, as well as on genetic ability of the animal to convert nutrients into milk (7). Efficiency has been defined by Freeman (27) as unit of fat-corrected milk per feed utilized. Hooven et al. (36) defined feed efficiency as fat-corrected milk per unit of NE_L consumed. Freeman (27) concluded that selection for milk would cause at least 70% as much improvement in feed utilization, as would direct selection for the trait. He also determined that the heritability of feed efficiency was 0.48. Hooven et al. (36) determined that the correlation between feed efficiency and milk yield was 0.92. Blake and Custodio (7) suggested that research was needed in the milk yield/tissue balance/appetite complex before any pleiotropic pathway between selection for milk and direct utilization of

dietary nutrients for milk could be presumed. A major confounding problem in previous studies has been inability to account for body tissue balance, undoubtedly under genetic control and exerting an effect on milk production. Grainger et al. (30) in 1985, grouped cows according to breeding value in a nutrient balance study. They found cows of different breeding value to have no differences in N utilization, while cows did differ in energy utilization.

OBJECTIVES

1. To characterize the nature and quantity of proteins entering the duodenum when dairy cows are fed 2:1 and 1:2 ratios of highly degradable:relatively undegradable protein sources.
2. To compare milk production, body weight and rumen and blood parameters in dairy cows fed 2:1 and 1:2 ratios of highly degradable:relatively undegradable protein sources.
3. To calculate duodenal flows of solid phase and liquid phase digesta components as indicators of rumen turnover.

EXPERIMENTAL PROCEDURES

ANIMAL FEEDING AND CARE

Six multiparous Holstein cows, fitted with both ruminal and duodenal T-type cannulae were used in this study. Cows became available in two groups of three cows each, approximately 5 wk apart. Cows averaged 62 to 104 d postpartum for the duration of the study. Cows were housed in comfort stalls, fed at 0200, 0800, 1400 and 2000 h, and milked at 0600 and 1600 h. Composition of the ration, fed in four equal feedings, is found in Table 1. Corn silage was supplemented with either 2:1 soybean meal:fish meal (SF) or 1:2 soybean meal:fish meal (FS) to raise crude protein in the diet to 16%. Cracked corn was added to raise energy content of the diet, and chopped orchardgrass hay was included, to serve both as a carrier for Yb marker, and as a source of fiber in the diets. Percent of requirements met according to NRC (58) by both diets is shown in Table 2. Orts were removed and weights recorded daily at 2000 h. Milk production was recorded daily.

Cows in the first group received SF diet for a 21 d period, and then were switched to FS diet, for a second period of 21 d. The second group of three cows received FS first, followed by SF. Body weights were recorded weekly subsequent to A.M. milking.

TABLE 1. Composition and specifications of diets containing 2:1 and 1:2 soybean meal:fish meal nitrogen.

Ingredient/Specification	SF diet[a,b]	FS diet[b,c]
	- - - - - % DM - - - - -	
Corn silage	60.4	63.4
Orchardgrass hay	7.6	7.8
Fish meal	4.6	8.8
Soybean meal	11.7	5.7
Cracked corn	14.7	14.0
Dicalcium phosphate	0.46	-
Trace-mineral salt	0.25	0.26
Limestone	0.36	-
Magnesium oxide	-	0.003
Dry matter, %	49.9	51.5
Crude protein, %	15.9	16.0
ADF, %	23.7	22.0
NE_L[d], Mcal/kg DM	1.74	1.74

[a] 2:1 soy nitrogen:fish nitrogen.

[b] Expressed as kg dry matter.

[c] 1:2 soy nitrogen:fish nitrogen

[d] Estimated net energy for lactation (58).

TABLE 2. Percent of NRC[a] requirements met by diets containing 2:1 and 1:2 soybean meal:fish meal[b].

Dietary parameter	SF diet	FS diet
NE_L	105	105
Crude protein	109	110
Co	147	148
P	146	149
Na	106	106
Mg	103	102
S	101	99
K	157	148

[a]National Research Council (58).

[b]Requirement met for cow producing 30 kg milk daily of 3.5% fat. Weight of cow is assumed to be 591 kg and with a daily dry matter consumption of 19.5 kg.

MARKER ADMINISTRATION

Cobalt ethylenediaminetetraacetic acid (CoEDTA) was prepared according to the method of Uden et al. (89). Mother liquor of the first crystallization was retained after filtering, and refrigerated for 7 to 10 d in order to obtain a second crop of crystals, thereby increasing yield of precipitated CoEDTA. Direct ruminal infusion of 15 g of CoEDTA dissolved in 800-850 ml water per 24 h was begun on day 5 and continued through day 15 of each treatment period by means of a Buchler Multistaltic four-channel peristaltic pump (Buchler Instruments, Inc., Fort Lee, New Jersey). Plastic tubing carried solution from solution reservoirs to rumen fistula caps, with 18 in of tubing left suspended from the cap into the rumen. Infusions were continuous except for brief periods when cows were taken to the milking parlor and when rumen samples were taken.

Chopped orchardgrass hay was marked with Yb by a modified method of Teeter et al. (86). $YbCl_3 \cdot H_2O$, at the rate of 15 g per kg hay was dissolved in minimum tap water to cover hay. After a 12 h soaking period, marked hay (YH) was rinsed in a continuous stream of tap water for 6 h. YH was then dried in loosely-packed burlap sacks in a forced-air oven at 50 C. Drying time was approximately 48 h. Batches of YH were thoroughly hand-mixed prior to feeding. YH was substituted for chopped orchardgrass hay in dietary

treatments at the rate of 1% of total mass of as-fed dietary components. Diets containing YH were fed beginning at 2000 h on day 5 of each treatment period, and continued through day 15 at 2000 h, when YH was replaced with chopped orchardgrass hay.

SAMPLING PROCEDURES

Samples of diets and feed components were collected on days 13, 14 and 15 of each treatment period. Corn silage and mixed ration samples were frozen at -20 C. Milk samples were taken for both A.M. and P.M. milkings on days 8 and 14. Samples were delivered to the Virginia Federation of DHIA laboratory for direct testing of milk components.

Blood was taken by tail vein/artery puncture approximately 3 h after the afternoon feeding on days 8 and 14 of each treatment period. Blood was drawn into heparinized vacutainers and then placed on ice for transport back to the laboratory. Blood was centrifuged at 3000xg (Beckmann Model J2-21) to separate plasma, which was frozen in plastic tubes at -20 C.

Digesta samples during continuous infusion of CoEDTA and feeding of YH were taken on days 13-15 during each of the four treatment periods. For each treatment period, the 12 h interval of 0300 and 1500 h, 0700 and 1900 h, or 1100 and 2300 h was randomly assigned to each of days 13-15 to serve as sample collection times. In this manner, two

collection periods per day across three sampling days represented 4 h intervals. Fecal grab samples were collected first, with approximately 200 g samples placed in tared aluminum foil pans. These were weighed prior to placement in a 60 C forced-air oven for a drying time of approximately 3 d. After shutting off the CoEDTA pump, rumen samples were taken. Grab samples of many locations in the rumen were placed into a 4 l beaker until approximately 2 l of loosely-packed rumen contents was obtained. After thorough hand-mixing, subsamples were packed into 100 ml plastic cups, and capped. Handfuls of rumen contents were squeezed through four layers of cheesecloth until 100 ml rumen fluid was obtained in a second plastic cup. Aliquots of rumen fluid of 5 ml each were pipetted into two plastic tubes, one containing 2 drops of concentrated sulfuric acid, and the other containing 1 ml of 25% metaphosphoric acid. These were capped, inverted several times, and frozen at -20 C for later analysis of rumen ammonia and volatile fatty acids, respectively. Remaining rumen fluid was measured for pH and was capped and frozen for CoEDTA and Yb analysis. All rumen contents not placed in sample containers were returned to the rumen, and the CoEDTA infusion continued.

Duodenal digesta samples for each cow were taken upon completion of rumen sampling in each sampling period. Cannulae were unstoppered, and the resulting initial gush of duodenal digesta was allowed to fall to the floor.

Subsequent gushes of digesta were collected in a 4 l plastic beaker until 1000 ml of digesta was obtained. After secure replacement of cannula stoppers, digesta samples were stirred on a magnetic stir plate to obtain a homogeneous suspension. Duplicate 100 ml samples were quickly scooped from the stirring samples in tared plastic cups and capped and frozen at -20 C. Measurements of pH were taken in the stirring sample and recorded.

At 2000 h on day 15, Yb intake was discontinued by removal of orts, and YH was replaced by unmarked hay in subsequent feedings. CoEDTA infusion was also stopped at this time. Digesta samples for marker depletion determination were collected at 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 46, 52, 48, 64 and 70 h after markers were removed. Rumen whole contents and fluid samples were collected as before in 100 ml plastic cups, and capped and frozen. Fecal samples were also taken in the manner of the earlier sampling. Single duodenal samples for marker depletion were scooped from 1000 ml whole duodenal digesta collected from each cow per each sampling time. These were capped and frozen for later analysis of Co and Yb content.

On days 19-21 in each treatment period, a single, 2 l sample of rumen fluid was aspirated from one cow of the group each day, and preserved with 50 ml of 37% formaldehyde solution. The microbial fraction was separated from rumen fluid by retaining the supernatant fraction from

centrifugation at 200xg and then collecting the pellet left by 20000xg centrifugation. Resuspension of the pellet in isotonic buffer solution and centrifugation again at 20000xg was carried out twice per each pellet obtained, thereby washing microbial fractions free from as much contamination as possible. The thrice-spun pellets were resuspended in approximately 100 ml distilled water and frozen at -20 C. Microbial samples were then freeze-dried to 40 C and ground in a Cyclone mill.

LABORATORY ANALYSIS

Corn silage and mixed ration samples were freeze-dried, while raised to 20 C. Samples were then ground through a 1 mm screen in a Wiley mill. Soybean meal and cracked corn samples were composited and ground in a Cyclone mill. Fish meal samples were composited. Duplicate samples of feedstuffs and mixed rations were analyzed for nitrogen by the Kjeldahl procedure. Acid detergent fiber was analyzed by the method of Van Soest (4, 92). Absolute dry matter was determined by drying approximately 500 mg of air equilibrated sample at 100 C in a forced air oven to a constant weight.

Feces samples were weighed after drying in 60 C oven for dry matter determination. Samples were ground in a Wiley mill through a 1 mm screen. Samples representing days 13-15, which were taken during the continuous infusion of

CoEDTA and feeding of YH, or prior to marker depletion (PMD), were composited to represent a single sample per cow and treatment period.

Duodenal samples representing PMD collection were thawed and composited within cow and treatment combination by scooping equal 26 ml aliquots from samples stirring over a stir-plate. Composites were spun at 3000xg (Beckmann model J2-21) for 15 min. Supernatant was poured off into 100 ml plastic cups to represent liquid phase (LP) digesta. Pellets were scraped into 100 ml plastic sample cups and centrifugation jars rinsed with distilled water into sample cups to represent solid phase (SP) duodenal digesta. LP composite samples were refrozen at -20 C and SP composites were freeze-dried to 40 C. Duodenal samples collected on days 16-18, which were used to quantify marker depletion (MD) were either freeze-dried or oven-dried at 60 C to constant weight. SP composites and MD samples were ground in a Cyclone mill when dried.

PMD samples of rumen fluid for ammonia and volatile fatty acids were centrifuged at 3000xg. One ml aliquots of the supernatant were combined to composite within cow and treatment combination. Samples for volatile fatty acid analysis were filtered through 45 µm millipore filter. Both composite sets of samples were refrozen at -20 C.

Whole rumen contents were freeze-dried to 40 C for PMD samples. MD samples were dried to a constant weight in 60 C

forced-air oven. Dried samples were broken up with a mortar and pestle and then ground in a Cyclone mill. PMD samples were composited.

For fecal, SP duodenal, LP duodenal, MD duodenal, and rumen contents samples, absolute dry matters were obtained by drying approximately 500 mg of sample in a forced-air oven at 100 C to a constant weight. LP duodenal samples were collected by pipetting aliquots from stirring composite samples on a stir-plate. Kjeldahl analysis was performed on PMD composites of fecal, SP duodenal, microbial samples and LP duodenal samples. Approximately 5 g of LP sample was used in each Kjeldahl tube, and all samples were run in duplicate. SP and LP nitrogen was divided into protein nitrogen and non-protein nitrogen by precipitation of proteins with 10% trichloroacetic acid. Acid-detergent fiber was determined in PMD fecal and SP duodenal composite samples, and run in duplicates according to the method of Van Soest (4, 92).

Cytosine was extracted from microbial, LP duodenal and SP duodenal samples by the method reported in Zerbini and Polan (95), for estimation of microbial contribution to duodenal nitrogen. Cytosine was separated by high performance liquid chromatography on a 25 cm Partisil-10 SCX L column (Whatman Inc., Clifton, New Jersey) at a temperature of 30 C. The mobile phase was .14 M ammonium phosphate, adjusted to pH 3.5 with 85% H_3PO_4. Injection

volume was 100 µl for LP samples, 50 µl for SP samples, and 20 µl for microbial samples. Flow rate was set at 0.6 ml/min and detection at 254 nm. Microbial N as a percentage of total N in SP and LP duodenal digesta was calculated as follows:

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

where:

DMN = duodenal microbial nitrogen, %

MN = microbial nitrogen, mg/g DM

DN = duodenal nitrogen, mg/g DM

MC = microbial cytosine, µmol/g DM

DC = duodenal cytosine, µmol/g DM

Marker determination (Co and Yb) was made by atomic absorption on a Perkin-Elmer model 370 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Connecticut). Baseline values representing continuous infusion of CoEDTA and feeding of YH were measured in PMD composites of fecal, SP duodenal, LP duodenal and rumen fluid samples. Simultaneous extraction of Co and Yb in samples was carried out by a modified method of Hart and Polan (35). Extraction solution was 0.1 M disodium ethylenediaminetetraacetic acid. Samples of 0.2 g were measured into 50 ml screw-top plastic tubes, and 20 ml of extraction solution added. Capped tubes were shaken for 30 min on a wrist-action shaker. Samples were filtered through

Whatman No. 1 filter paper and the filtrate stored in refrigeration until analyzed. MD samples were extracted for fecal and duodenal Yb and Co. Rumen content samples were assayed for Yb, only, and rumen fluid MD samples were assayed for Co, only. Liquid samples were diluted 1:6 with extraction solution, shaken and filtered as were solid samples.

Concentrations of acetic, propionic, butyric, valeric, isobutyric and isovaleric acids were measured in composited PMD samples of rumen fluid on a Varian Vista 6000 gas chromatograph (Varian Instruments, Palo Alto, California). A glass column packed with 10% SP-1200, 1% H_3PO_4 liquid phase on 80/100 chromasorb WAW packing was used. Isocaproic acid was used as an internal standard.

Rumen ammonia was determined in duplicate samples of acid-preserved, composited rumen fluid samples using a modified procedure of Chancey and Marbach (12). Optical density was read at 650 nm. Plasma urea nitrogen was determined in plasma deproteinized by adding 1 ml of plasma to 9 ml of a 1:9 mixture of 10% sodium tungstate and 0.083 N sulfuric acid, and then filtered through Whatman No. 42 filter paper. Duplicate aliquots of filtrate were assayed for urea nitrogen using the colorimetric method of Coulombe and Favreau (18). Optical density was read at 540 nm. Both colorimetric procedures were performed with the use of a Bausch and Lomb Spectronic 1001 split-beam spectrophotometer

(Bausch and Lomb Inc., Rochester, New York).

Milk Samples were analyzed within 96 h of collection on a Multispec Infrared Analyzer (Berwind Instruments, Ltd., York, England) for percent protein, fat, lactose, and solids-not-fat. Samples were preserved with potassium dichromate prior to analysis.

CALCULATIONS AND STATISTICAL ANALYSIS

Flows of SP and LP duodenal digesta were calculated using the double-marker system of Armentano and Russell (2). Rumen turnovers of SP and LP digesta were calculated from slopes of natural logarithms of duodenal concentrations of markers as percents of PMD levels of Co and Yb over the 70 h of MD sample collection. Digestibilities were calculated using:

$$1 - (MI/MO) \times (DCO/DCI)$$

where:

MI = marker concentration of digesta inflow, ppm

MO = marker concentration of digesta outflow, ppm

DCO = digesta constituent outflow, g/g DM

DCI = digesta constituent inflow, g/g DM

Milk production and composition, rumen, blood, duodenal and fecal parameters, intake and body weight were analyzed by analysis of variance (SAS, procedure GLM) (79) with a crossover design. Mean squares of individual cow within treatment group were used to test the effect of group (see

Appendix, model A). Diet effects and diet by treatment group interaction were tested by the mean square error. Effects of diet on rate of marker depletion expanded this model to contain the effect of hour after removal of marker and a diet by hours removed interaction, as follows:

$$Y_{ijkl} = \mu + G_i + C_{J(i)} + D_k + H_l + GD_{(ik)} + DH(kl) + E_{ijkl}$$

where:

Y_{ijkl} = observation of l^{th} hour depleted in k^{th} diet of j^{th} cow within i^{th} treatment group

μ = parametric mean within population

G_i = effect of i^{th} treatment group

$C_{j(i)}$ = effect of j^{th} cow within i^{th} treatment group

D_k = effect of diet

H_l = effect of lth hour after depletion begun

$GD_{(ik)}$ = interaction effect of treatment group and diet

$DH_{(kl)}$ = interaction effect of hour after depletion begun and diet

E_{ijkl} = random error effect.

To test the effect of breeding value, or genetic level on diets, cows were grouped in pairs according to cow index for product dollars. Means of cows per group are shown in Table 3. Mean squares of cows within genetic level grouping were used to test for differences between genetic levels (see Appendix, model B). The interaction of diet and genetic level was tested by the error mean square to detect

TABLE 3. Means for cow indexes in three genetic level groupings used in the statistical analysis.

	Group		
	1	2	3
Milk, kg	185	28	-576
Fat, %	-.02	+.01	+.05
Fat, kg	5	1	-17
Percentile rank	91	75	5

diet differences across 3 genetic levels. The model was as follows:

$$Y_{ijk} = \mu + GL_i + C_{j(i)} + D_k + GLD_{ik} + E_{ijk}$$

where:

Y_{ijk} = observation of k^{th} diet on j^{th} cow within i^{th} genetic level

μ = population parametric mean

GL_i = effect of i^{th} genetic level

$C_{j(i)}$ = effect of j^{th} cow within i^{th} genetic level

D_k = effect of k^{th} diet

LD_{ik} = interaction of genetic level and diet

E_{ijk} = random error effect

RESULTS AND DISCUSSION

PRODUCTION PARAMETERS

Milk yield, composition and component yields are shown in Table 4. Yield was not different between the SF and FS diets. Orskov (66) suggested that cows in post-peak lactation status would not benefit from replacement of soybean protein with less degradable protein sources with respect to milk yield. However, Miller and Galwey (56) reported increases in milk yields in commercial herds when fish meal was substituted for soybean meal in the rations of cows averaging 70 d postpartum. Majdoub et al. (48) fed diets as high in protein as FS and SF, and found that maximum milk production was achieved in diets containing protein sources of lower degradability.

No significant differences were noted in milk composition when cows received SF or FS. Herrington et al. (35) reported lower fat percent in lactating cows fed dried brewers grains as opposed to the more soluble soybean meal. Perhaps SF and FS diets, being intermediate in terms of compositions of degradable and undegradable proteins did not differ enough to influence differences in fat percent.

Body weight and gain are also shown on Table 4. Cows receiving FS and SF did not differ in average body weight. Average daily gain, calculated from four weekly weights per

TABLE 4. Milk composition, yield, component yield and body weight of cows fed 2:1 (SF) and 1:2 (FS) soybean meal:fish meal 62-104 d postpartum.[a]

	SF	FS	SE
	- - - - - - - % - - - - - - -		
Composition			
Fat	3.09	3.02	0.08
Protein	2.86	2.82	0.08
Lactose	5.13	4.95	0.11
Solids	9.04	8.84	0.19
	- - - - - - - kg - - - - - - -		
Yield			
Milk	27.0	27.2	0.4
Fat-corrected milk	23.3	23.3	0.5
Fat	0.83	0.82	0.03
Protein	0.77	0.77	0.02
Lactose	1.38	1.35	0.03
Body weight	537	534	2
Gain, kg/d	0.12	1.26	0.26*

[a]Values are least squares means, 6 observations per diet.

*$P < .05$.

cow per dietary treatment was 0.12 kg/d for cows receiving SF, and 1.26 kg/d for cows receiving FS. The difference was significant. Majdoub et al. (48) reported N retention was highest in cows in the eighth to tenth week after calving. Orskov (66) noted that inclusion of undegradable protein sources in the diet in post-peak lactating cows caused increased weight gain. In this study, depletion of nitrogen reserves after parturition may have been greater than normal and closer to the proposed limit of 25% total body proteins as reported by Botts et al. (8) and Bauman and Currie (6). Protein losses due to cannulation surgery may have been a factor in addition to the typical loss of reserves during early lactation. Perhaps repletion of protein reserves was increased in the FS diet over SF, and partially explains the difference in gains.

RUMEN AND BLOOD PARAMETERS

Total ruminal production of volatile fatty acids did not differ between diets, as shown in Table 5. Ratio of acetate:propionate did not differ either, nor did molar proportions of acetate, propionate, isobutyrate or isovalerate. Valerate was higher in SF at 1.87% of total volatile fatty acid concentration, compared to 1.53% for FS. This is similar to the findings of Santos et al. (76) which were that valerate concentration increased with increased ruminal protein digestion. SF, being higher in soybean meal

TABLE 5. Rumen volatile fatty acid composition, rumen ammonia-N, plasma urea-N, and pH in cows fed 2:1 (SF) and 1:2 (FS) soybean meal:fish meal 62-104 d postpartum.[a]

	SF	FS	SE
Total VFA, mM	84.1	87.2	2.2
Acetate/propionate	3.05	3.13	0.08
	- - - - molar % - - - - -		
Acetate	59.7	60.6	0.6
Propionate	20.3	19.3	0.3
Butyrate	13.1	15.4	0.6*
Isobutyrate	1.10	1.05	0.06
Valerate	1.87	1.53	0.07*
Isovalerate	3.10	2.95	0.19
	- - - - - mg/dl - - - - - -		
NH_3-N	10.2	10.3	0.3
Plasma urea-N	18.0	13.4	0.8*
Rumen pH	5.90	6.05	0.03*
Duodenal pH	2.80	2.79	0.11

[a]Values are least squares means, 6 observations per diet.

*$P < .05$.

than FS, would be expected to be more fully degraded in the rumen than FS. Butyrate was higher in the rumen of cows fed FS, comprising 15.4% of total volatile fatty acid concentration, compared to 13.1% for SF. This may be explained as a consequence of ruminal degradation of fats contained in fish meal. An alternative explanation arises when rumen pH data is considered. Rumen pH was higher at 6.05, on the average, in cows receiving FS, while cows receiving SF averaged 5.90. Orskov (66) reports that cellulolytic bacteria are slowed in the rumen at pH below 6.2. Perhaps the pH difference between the two diets is due to different activity rates of cellulolytic bacteria. Butyrate could be expected to increase as cellulolysis increases, which may explain the apparently differing rates of production of butyrate. An alternate explanation for observed butyrate differences could be different rates of uptake of butyrate by ruminal epithelium cells, if influenced by pH of the rumen.

Rumen ammonia nitrogen was not different between diets. At 10.2 and 10.3 mg/dl for SF and FS, respectively, ammonia nitrogen concentration was high enough to not be limiting microbial growth, according to Tamminga (83). Both diets contained enough readily-degradable protein to maintain rumen ammonia at levels sufficient for maximum incorporation of nitrogen by microbial populations (32).

Plasma urea nitrogen was higher at 18.0 mg/dl in cows

receiving SF, than when they received FS (13.4 mg/dl). Lack of agreement between rumen ammonia values and plasma urea may be explained by the sampling schedule. Rumen fluid samples were composites of samples collected at 1 h pre-feeding and 1 and 3 h post-feeding. Across these times, rumen ammonia nitrogen averaged the same between diets. Plasma urea nitrogen was determined in blood samples taken 3 h post-feeding only. It is possible that fluctuations in rumen ammonia values due to diurnal or hours post-feeding variation were obscured by the sampling schedule and compositing, while urea nitrogen values reflected diet differences in rates of ammonia release. Higher urea nitrogen in plasma of cows receiving SF implied that more nitrogen left the rumen and was converted from ammonia to urea by the liver than when the cows received FS, at least in the hours preceding the 1700 h sampling of blood. Higher urea nitrogen also implies that more nitrogen was probably excreted in the urine of cows receiving SF, as urea is cleared from the plasma in the kidneys and not efficiently reabsorbed. Nitrogen retention is expected to be lower in cows consuming diets lower in undegradable protein sources (66) and gaining less weight.

Duodenal digesta pH was not different between diets. This suggests that abomasal secretion of acid compensated for different acidity of digesta leaving the rumen.

FLOW OF DRY MATTER

Intakes of dry matter (Table 6) when cows received FS and SF were not different. Diets varying more greatly in relative degradability of protein supplements have shown increased intake in diets high in undegradable protein sources (35). Flows of dry matter at the proximal duodenum were noted to be different, however, with SP dry matter accounting for 9.06 kg/d in cows receiving FS and 7.97 kg/d in cows receiving SF. Liquid phase dry matter flows were not different between the diets, although combined-phase flows were different for total dry matter flow. 10.71 kg/d of dry matter flowed past the duodenum, of cows receiving FS, while flows in cows receiving SF amounted to 9.36 kg/d. Apparent digestibility of dry matter in the rumen, which was calculated from the ratio of duodenal flow of dry matter over intake of dry matter, was calculated to be higher in SF. When flow of bacterial dry matter was subtracted from total duodenal flow to represent true flow of dietary dry matter, the values obtained for rumen digestibility of dry matter were also significantly different. Ruminal disappearance of dry matter corrected for microbial dry matter for SF was 48.4%, compared to 39.5% for FS. This fact explains how it is possible that although intakes were constant, duodenal flow of FS was greater. If less dry matter was degraded in the rumen, then more would appear in duodenal flow.

TABLE 6. Intake, digesta flow, and digestibility of dry matter in the digestive tract of cows fed 2:1 (SF) and 1:2 (FS) soybean meal:fish meal 62-104 d postpartum.[a]

	SF	FS	SE
Intake, kg/d	14.5	14.7	0.3
Proximal duodenum			
Solid flow[b], kg/d	7.97	9.06	0.18*
Liquid flow[c], kg/d	1.59	1.65	0.11
Total flow, kg/d	9.36	10.71	0.20*
Digestibility (rumen)			
Apparent, %	35.7	24.9	0.8*
Corrected[d], %	48.4	39.5	0.3*
Feces			
Flow, kg/d	4.51	4.61	0.17
Apparent digestibility %	68.9	68.3	1.4

[a]Values are least squares means, 6 observations per diet.

[b]Pellet obtained by 3000xg centrifugation of whole digesta.

[c]Supernatant obtained by 3000xg centrifugation.

[d](Total DM flow - microbial DM flow)/DM intake*100.

*$P < .05$.

Armentano et al. (1) has reported ranges of duodenal dry matter flow of 8.0 to 9.7 kg/d. Cows used in their study produced less milk than cows in this study, indicating that dry matter flows obtained are similar, relative to milk produced. Dry matter flow was highest in the diet containing dried brewers grains. FS, being higher in undegradable protein sources than SF, should then, in fact, be more like dried brewers grain diets than SF. Results in this study agree with those of Armentano et al. (1).

Fecal flow of dry matter did not differ between SF and FS and averaged 4.56 kg/d. Apparent dry matter digestibility was shown to be not different between diets, as well. Diets averaged 68.6% in overall digestibility of dry matter. In order for duodenal dry matter flow to be higher for FS than SF, while fecal flow of dry matter is equal, more dry matter must have been digested in the gastrointestinal tract between the proximal duodenum and anus for FS than for SF. Orskov (66) reported that digestibility of endogenous and feed protein was greater in the lower tract than the digestibility of microbial protein.

FLOW OF NITROGEN IN THE DIGESTIVE TRACT

Intakes of nitrogen were not different between diets, as shown in Table 7. LP nitrogen flow was also not different. When proteins were precipitated from LP digesta with addition of 10% (w/vol) trichloroacetic acid, analysis

TABLE 7. Intake, digesta flow and digestibility of nitrogen fractions in the digestive tract of cows fed 2:1 (SF) and 1:2 (FS) soybean meal:fish meal 62-104 d postpartum.[a]

	SF	FS	SE
N intake, g/d	368	375	8
Solid phase protein flow[b], g/d	177	231	9*
Liquid phase protein flow[c], g/d	5.5	3.7	1.3
Solid phase N flow, g/d	239	311	15*
Liquid phase N flow, g/d	91	89	8
Total N flow, g/d	331	400	7*
Total microbial N flow, g/day	155	181	4*
Total endogenous and feed N flow, g/day	176	220	4*
Total microbial N, %	46.6	44.7	0.9
Total endogenous and feed N, %	47.8	59.5	0.8*
Duodenal N recovery, %	93.8	107.5	2.7*
Fecal output N, g/d	106.9	105.3	1.1
Apparent N digestibility (whole tract), %	71.0	71.8	.6

[a]Values are least squares means, 6 observations per diet.

[b]Protein precipitated with 5% trichloroacetic acid.

[c]Protein precipitated with 10% trichloroacetic acid.

*$P < .05$.

of LP protein nitrogen was also found to show no differences between diets. Solid phase nitrogen flow to the duodenum was higher (311 g/d) for FS than for SF (239 g/d). Protein nitrogen, as precipitated by 5% trichloroacetic acid in SP composites also had a higher flow to the duodenum for FS (231 g/d) than for SF (177 g/d). Total nitrogen flow to the duodenum was 400 g/d for FS and 331 g/d for SF. This difference was due almost entirely to differences in SP protein nitrogen flow.

Total microbial nitrogen flow was greater for FS than for SF, as estimated by cytosine content of duodenal digesta. While cytosine content in samples was not different, flows were greater as calculated for solid phase digesta, yielding greater estimates of microbial flow. Grummer and Clark (32) estimated maximal incorporation of ammonia by microbial populations when rumen ammonia-N concentrations were far less than found for both FS and SF diets. Van Soest (91) reported maximum production of microbial dry matter with faster-than-normal turnover of rumen contents. Owens and Isaacson (70) also concluded that bacterial production increases with increased turnover assuming sufficient microbial growth substrates. Leng and Nolan (43) concluded that approximately 75% of total rumen microbial mass is associated with feed particles. A possible explanation for increased flow of microbial nitrogen in FS (181 g/d) vs SF (155 g/d) is that increased

flow of SP dry matter translates into increased availability of growth-promoting substrate to microbial populations. Higher plasma urea nitrogen in cows fed SF indicates that more nitrogen crossed the rumen wall in SF than in FS, perhaps decreasing total microbial uptake of nitrogen.

Armentano et al. (1) reported values of total nitrogen flow to the duodenum that were approximately 25% less than for SF. Total dry matter flow was also less. On the other hand, Stern and Satter (82) fed a diet made up of 50% soybean meal which yielded 364 g/d of microbial nitrogen flow. Robinson and Sniffen (75) reported 266 g/d of microbial nitrogen flow in the duodenum. Results for FS and SF are more intermediate than these values, as intakes of degradable protein are lower than studies reporting higher microbial nitrogen flow, and intakes of FS and SF were greater in terms of nitrogen than those reported by Armentano et al. (1).

Flow of endogenous and feed nitrogen, as calculated by subtracting estimated microbial nitrogen flow from the total, are also shown in Table 7. Flows for FS (220 g/d) were higher than for SF (176 g/d). Since contribution of endogenous nitrogen could be assumed to be constant for these two diets, the difference reflected must represent increased flow of undegraded dietary nitrogen in the FS diet over the SF diet. These findings were supported in dacron bag studies by Chalupa (11), who found soybean meal to be

60% degraded in the rumen, while fish meal was 30% degraded. Meyer et al. (55) reported that numbers of proteolytic organisms found inside the microenvironment of the dacron bag was lower with fish meal than whole rumen contents, indicating that fish meal does not support a representative rate of growth of bacteria. The bag technique may estimate lower degradability values for fish meal than actually occurs in the natural rumen system.

Percent of total nitrogen flow represented by microbial and endogenous and feed sources is also shown in Table 7. Microbial protein nitrogen flow as percent of total was not different between the diets. Duodenal flow of endogenous and feed nitrogen was 59.5% for FS and 47.8% for SF. Differences were significant. Duodenal recovery of N as a percent of N intake was 107.5% for FS and 93.8% for SF. By this indicator, FS diet was more effective at delivery of nitrogen to the duodenum. The loss of some dietary N in SF prior to reaching the duodenum is supported by higher plasma urea-N values when feeding this diet.

Fecal nitrogen flow was not different between diets. Apparent whole-tract digestibility of nitrogen did not differ for SF and FS, and averaged 71.4%. This value agrees very closely with what has been reported by Kung et al. (41).

FLOW OF ACID DETERGENT FIBER IN THE DIGESTIVE TRACT

Intake of acid detergent fiber between FS and SF differed, as shown in Table 8. Although dry matter intakes were not different, acid detergent fiber content in the corn silage fed across treatment periods differed such that intake averaged 3.43 kg/d in SF and 3.23 kg/d in FS, which were significantly different. Duodenal flows of acid detergent fiber, along with rumen digestibility, fecal output and whole-tract digestibility, were not different for SF and FS. Cow variation was very large and cow within period effects were significant as tested by the error mean square for each of these parameters. Hartnell and Satter (34) reported similarly large individual cow differences in a related study.

MARKER DEPLETION

Decline in marker concentration after cessation of administration differed for markers of different phases of duodenal digesta (Figure 1). Ytterbium, representing flow of solid phase or particulate digesta averaged a decline of 4.5% h^{-1} between diets, which were not significantly different. Figure 2 shows marker concentrations expressed as the natural logarithm of percent of markers remaining in hours after depletion. Statistical analysis of this data showed no diet differences, although individual cow differences were large for each marker. Disappearance of Co in duodenal digesta averaged 7.9% h^{-1} for the two diets. R^2

TABLE 8. Intake, digesta flow and digestibility of acid detergent fiber in the digestive tract of cows fed 2:1 (SF) and 1:2 (FS) soybean meal:fish meal 62-104 d postpartum.[2]

	SF	FS	SE
Intake, kg/d	3.43	3.23	0.07*
Duodenal flow, kg/d	2.02	2.49	0.21
Rumen digestibility, %	41.1	23.1	7.3
Fecal output, kg/d	1.62	1.74	0.11
Whole tract digestibility, %	56.7	45.8	3.6

[a]Values are least squares means, 6 observations per diet.

$P < .05$.

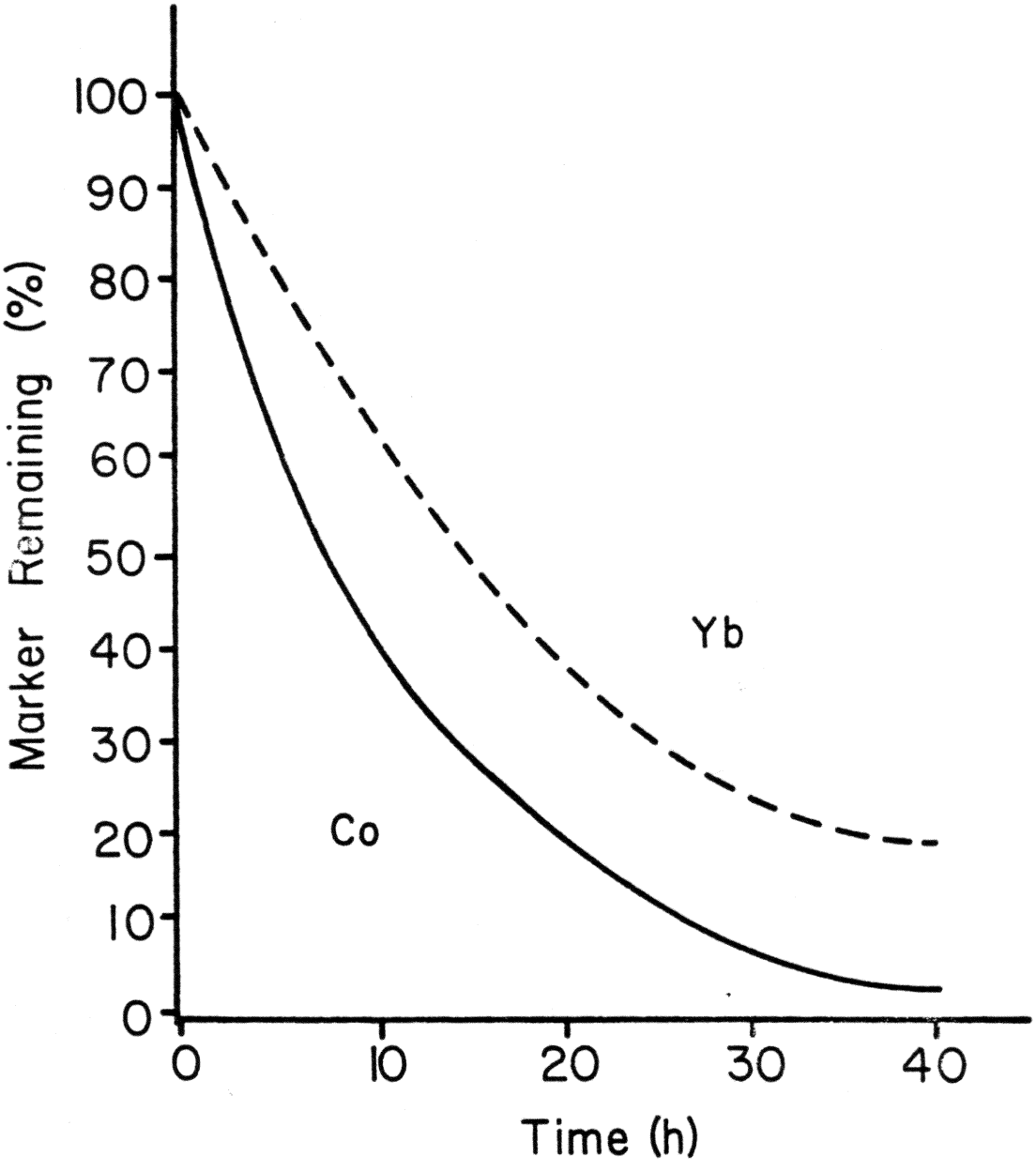

FIGURE 1. Marker concentration of whole duodenal contents as percent of baseline values of Co(——) and Yb (--) established during continuous infusion of markers. Data are least squares means of marker concentrations across all cows and diets.

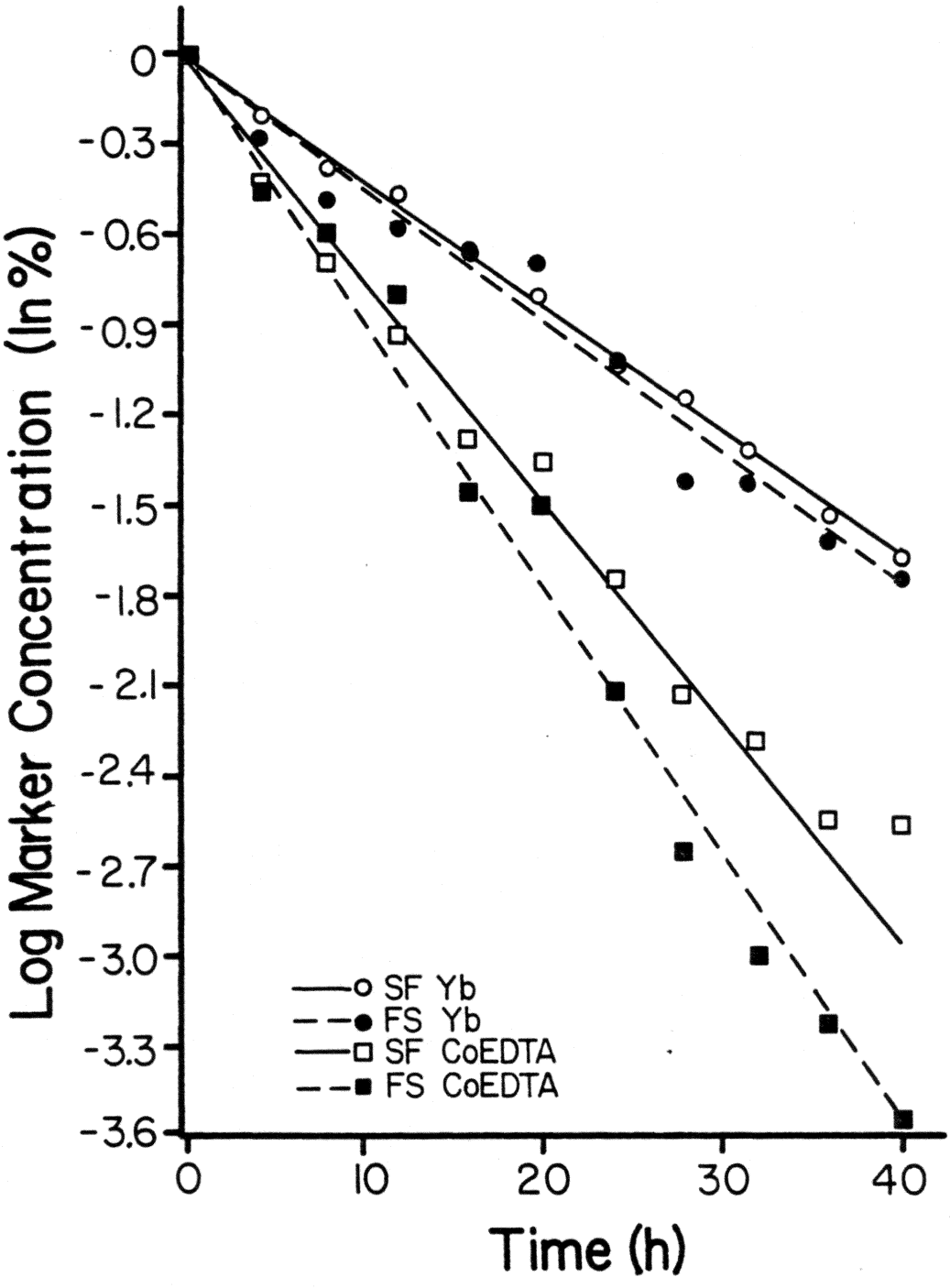

FIGURE 2. Marker concentration in whole duodenal digesta as the natural log of percent of baseline values of Co and Yb established during continuous infusion of markers. Data are least square means.

values for these regressions were .70 for Yb and .72 for Co. Stern et al. (80) used similar techniques to mark solid and liquid phases and obtained 4.9% h^{-1} for solid phase depletion and 9.6% h^{-1} for liquid phase depletion. Robinson and Sniffen (75) reported large animal variation in similar studies, and found solid-phase depletion of marker to be 4.4% h^{-1}, while liquid phase depletion of marker was 13.2% h^{-1}. Hartnell and Satter (34) reported solid phase turnover of 4.4% h^{-1} and liquid phase turnover of 8.1% h^{-1}. These results agree fairly closely with findings for Co and Yb in SF and FS.

Rumen values of Co and Yb depletion, as measured by rumen fluid and rumen while contents, respectively, showed results similar to duodenal measures. Greater total variation was evident for both types of rumen samples, suggesting that duodenal flows were more representative of rumen levels of both markers than were rumen grab samples. Warner and Stacy (93) suggested that equilibrium time of rumen fluid is 1.5 h after an event such as intake of water. Factors such as this contributed to overall sample variation.

Appearance of Co and Yb in the feces is depicted in Figure 3. Marker levels shown are percents of the total marker found in feces during the continuous infusion of CoEDTA and feeding of YH. Cow differences were quite large, although diets did not differ for either Co or Yb depletion.

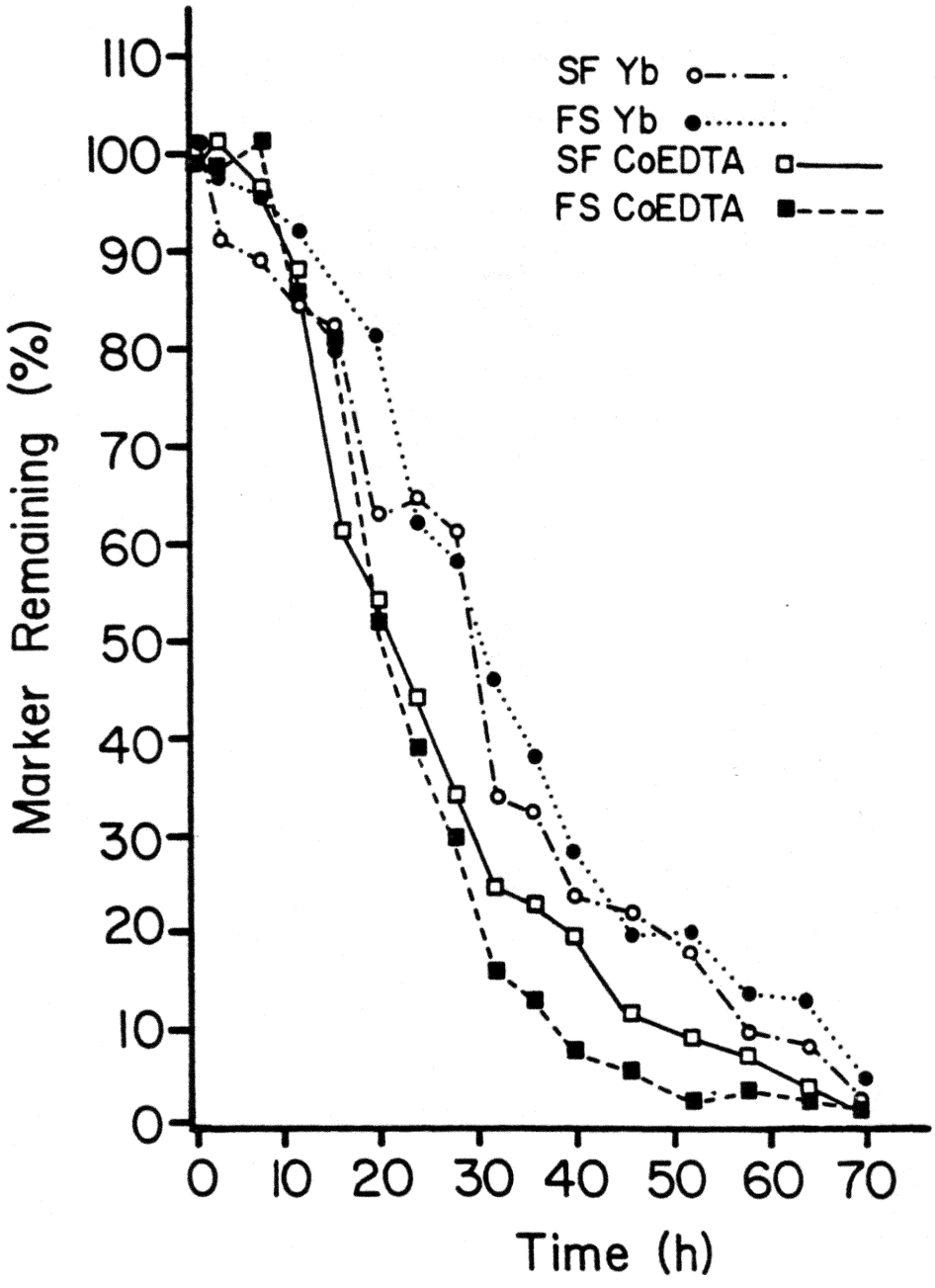

FIGURE 3. Fecal depletions of marker represented as percent of baseline established during continuous infusion. Values for FS-Yb (·), SF-Yb (°), FS-Co (□) and SF-Co (□) are least squares means.

Values remained fairly constant for Yb, up to 12 h after feeding of YH stopped. Co levels fell more quickly after the infusion of CoEDTA was discontinued. Fifty percent of Co was depleted in feces in 20 h, while Yb took 32 h for 50% depletion. Van Soest (91) reported whole-tract retention times in large heifers of 29 h for liquids and 79 h for solids. No such large difference was found in these data. The fact that these animals were lactating cows producing substantial yields of milk makes a comparison to heifer data questionable. Cows producing milk in early lactation have intakes much greater than heifers, thereby increasing flow rate of digesta. Combs et al. (16) reported that up to 70% of Yb bound to hay was solubilized at abomasal pH. Since binding sites per unit mass would be greatest in the smallest particles, marker hopping of Yb may occur in such a manner that the passage of Yb is increased. Pulsatile flow of digesta in the small intestine allows for faster passage of smaller particles (17). Erdman and Smith (22) cautioned that such marker hopping of Yb would yield higher readings of Yb concentrations further along the digestive tract than would be realistic for the feedstuff originally marked.

EFFECTS OF GENETIC LEVEL

Statistical analysis of all parameters studied by model B (see Appendix) revealed that no significant effect of genetic level x diet was evident in these data. Genetic

level, as tested by the mean square for cow within genetic level, was not significant for any of the parameters tested. Cow effects were large, in general, and diet differences shown as significant were found for basically the same parameters that were found significant by using model A. These results agree with those found by Grainger et al. (30), who found no differences in nitrogen utilization when cows were grouped by breeding value. These results support conclusions of Blake and Custodio (7) who stated that genes for milk production have no pleiotropic effect on protein, starch or fiber utilization. Freeman (27) calculated a heritability of .48 for fat-corrected milk produced per net energy of lactation consumed, and Hooven et al. (36) found a correlation of .92 between milk production and feed efficiency. Perhaps energy utilization is more closely related to genotype for milk production than is protein utilization.

GENERAL DISCUSSION

Inclusion of additional fishmeal in the diet allowed for increased quantities of undegraded feed proteins to reach the duodenum. Microbial synthesis of proteins was not limited by available nitrogen, as both diets supported similar levels of rumen ammonia. Increased flow of particulate matter in the FS diet may have allowed for increased growth rates of rumen microbial populations, as

microbial content of digesta was the same for both diets despite higher flow in FS.

Although no differences were noted between diets with respect to milk composition or yield, gain was greater when cows received FS. Maynard et al. (51) showed that fish meal was 1.5 times as rich in lysine as is soybean meal, and is 2.3 times richer in methionine. Although MacGregor et al. (44) warns that the amino acid composition of a feed protein is not the same as the amino acid composition of the protein that bypasses, and since methionine is relatively resistant to proteolytic enzymes (28), perhaps the altered recovery in the duodenum of essential amino acids is responsible for differences in gain. Tamminga (83) concluded that limiting milk production would require deficiency of more than a single amino acid based upon research to date, but little has been concluded about rate of protein reserve repletion. Barney et al. (5) showed a response of percent crude protein on body weight gain in post-peak lactation cows. Orskov (66) reported advantages of undegradable proteins in weight gain when no milk production response was evident.

Quantification of ytterbium was difficult in many respects. Machine drift required zeroing of absorbance with a blank containing deionized water between each sample read. Readings recorded were machine-integrated over continuous readings for a period of 10 sec for each sample. Duplicate readings taken in this manner were accepted if agreement was

within 10%. Large variation was found in Yb values for ration samples. Erdman and Smith (22) reported binding of Yb to smaller particles when concentrations of bound Yb were measured. This is due to the fact that smaller particles, having a greater surface area:mass ratio, also have a greater binding site number:mass ratio. Variation encountered in ration samples may have been attributed to this phenomenon, were not the YH in the storage barrel of uniform quality, nor the YH evenly distributed in the ration by the mixing process. An indicator of this problem was the fact that in some instances, three cows on the same diet were found to have widely varying Yb concentration in rumen content samples, while at the same time, day-to-day differences in Yb concentration in the ration also varied widely. Digestibilities calculated using inflow and outflow values of Yb varied much more than did digestibilities calculated with Co concentrations. Whole-tract digestibilities calculated during two treatment periods were as much as 10% lower when calculated with Yb, than when calculated with corresponding Co values. Flows of solid phase digesta may subsequently have been overestimated slightly, as both types of calculations required the use of Yb values. Successive extractions of the same sample yielded much smaller variation than was found among different samples, giving greater credence to the problem existing in uniform distribution of Yb in the rations,

themselves. Based on this assumption, digestibilities reported in this study are those calculated with Co concentrations, only. Estimates of both markers were required for flow calculations.

Cow differences were very large, especially in relation to characteristics of flow. Evidence of genetic differences for nitrogen utilization and for other, associated parameters tested by model B (see Appendix) may have been uncovered if cow numbers were greater.

CONCLUSIONS

1. Increasing partial substitution of fish meal for soybean meal in diets adequate for crude protein and energy increased flow of feed nitrogen to the duodenum without negatively affecting rumen microbial growth.
2. Increasing partial substitution of fish meal for soybean meal resulted in decreased rumen concentrations of valerate, and increased concentrations of butyrate.
3. Increasing partial substitution of fish meal for soybean meal resulted in no changes in milk yield or composition, but increased rate of gain in cows 62-104 d into lactation.
4. Ytterbium was difficult to detect using atomic absorption, and regulation of Yb intake was difficult in mixed rations containing Yb-marked hay.
5. Individual cow variation is very large in relation to digesta flow characteristics, while grouping cows by cow index for production dollars showed no effects of breeding value on digesta flow characteristics.

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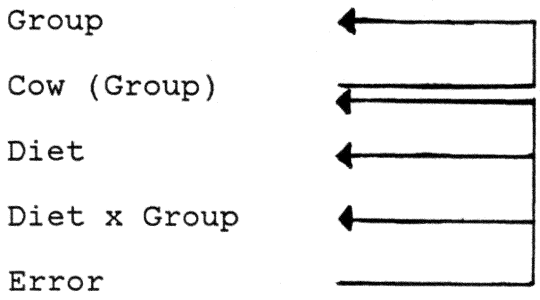
APPENDIX

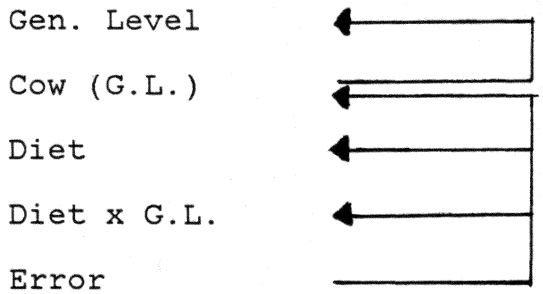
APPENDIX

STATISTICAL MODELS

Model A



Model B



The vita has been removed from the scanned document