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Development of an in Vivo Model to Determine the Biological Value of Microbial Protein

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DEVELOPMENT OF AN IN VIVO MODEL TO DETERMINE THE BIOLOGICAL VALUE OF MICROBIAL PROTEIN

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TAF

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INTRODUCTION

The ruminant's ability to convert low quality and non-protein nitrogen (NPN) to high quality protein is as important as their ability to utilize cellulosic feeds. This unique characteristic will continue to be exploited in the future with increasing emphasis being placed on noncompetitive feedstuffs. There has been considerable progress in developing alternative energy sources in beef cattle diets. There has not been the same success in replacing dietary high quality preformed proteins with urea or other NPN sources in most production situations.

The complexity of the digestive system in ruminants is well recognized by all who work with diet formulation for these species. Digestion by ruminants is a two step process, first by microbes in the reticulorumen, and secondly, true gastric digestion by the host animal. This results in our need to consider two separate but interdependent ecosystems when formulating diets for ruminants.

Since the introduction of NPN as a source of nitrogen for microbial protein synthesis many questions have arisen concerning animal protein requirements. NPN utilization requires readily fermentable carbohydrates, limiting the production situations in which NPN can replace high quality protein. Young rapidly growing cattle or cattle on low quality feeds continue to require supplemental high quality protein like soybean meal to maximize performance. Conceivably, additional protein is required to meet needs for production and possibly to overcome amino acid imbalances.

The need to develop a technique allowing us to directly measure the net protein and amino acid requirements of beef cattle and how ruminal fermentation will modify dietary nitrogen components is apparent. The development of an in vivo model will provide the tool needed to measure the true value of typical protein supplements and screen possible alternative nitrogen sources.

To establish such a model one must first resolve certain aspects of methodology. The objectives of this project were to develop a protein-free diet that is consumed at production intake levels, to validate the use of indigestible acid detergent fiber as an internal phase indicator in this diet and to investigate the potential of urinary allantoin as a means of estimating ruminal microbial protein synthesis.

REVIEW OF LITERATURE

The protein requirements for beef cattle are expressed on a crude protein (CP) basis. This is simply an estimate of the quantity of nitrogen in its various forms needed to account for rumen microbial crude protein (MCP) synthesis, and protein bypass that is necessary to maximize production. Protein source and quality and endogenous nitrogen losses are obscure in this estimate.

Each of these variables must be accounted for and optimally, different nitrogen sources should be used to meet specific needs. High quality protein should be used directly by the host animal. This would allow us to include dietary protein only when MCP synthesis does not meet the animals' amino acid requirements at a given level of production. This assessment is difficult to achieve when the net protein requirements for ruminants has yet to be established (Owens and Bergen, 1983). Quantitating the protein supply is of little value unless its biological value in that organism has been established.

Owens and Bergen (1983) reported that in nitrogen balance and lactation trials NPN supplemented diets often result in poorer performance than when diets are supplemented with preformed protein. Increasing proteolysis in the rumen will increase feed nitrogen loss across the rumen wall as NH_3-N when dietary CP exceeds 12%. Reid (1953) reported that urea was efficiently utilized in concentrate diets when fed at levels equivalent to 12% CP. Utilization decreased decidedly as dietary CP (provided by linseed meal) increased above 18% of the diet.

Burroughs et al. (1973) fed growing steers a basal diet containing 6.2% CP (diet 1). Adding urea to increase CP by 4.6 percentage units (diet 2) improved rate of gain .42 kg/d. Steers fed (diet 2) would be expected to gain more than (diet 1) because CP requirements were greater than 6.2% of diet dry matter. When CP of diet 2 was raised by 2.8 percentage units with urea (diet 3) no change in rate of gain was observed. Feeding an isonitrogenous positive control diet (diet 4, 13.6% CP) supplemented with preformed protein resulted in substantial increases in rate of gain over diets 2 and 3. This suggests that the steers required more amino acids post-ruminally than could be supplied by MCP and that this need was better met by feeding preformed proteins.

Whether MCP contains all the essential amino acids (EAA's) needed by ruminants remains uncertain. Loosli et al. (1949) fed sheep a purified diet containing urea as the sole source of nitrogen. The microbial biomass obtained from this diet was fed to rats and they determined that it did contain all the EAA's needed by nonruminants. The biological value (BV) of the microbial protein was .52. Adding casein to the diet improved the BV to .82. One thing is apparent, the total amino acid needs of high producing ruminants are not met by MCP synthesized from urea alone (Huber and Kung, 1981).

Burroughs et al. (1975) introduced a new dietary constraint: the urea fermentation potential (UFP), to be utilized when urea is included in the diet. They estimated MCP synthesis as 10.4% of the total digestible nutrients (TDN). This was based on three

relationships; 1) that 52% of a diet's TDN undergoes digestion within the rumen, 2) that 25% of the digested TDN is transformed into MCP when adequate nitrogen is present, and 3) that 80% of MCP is alpha amino acids. The UFP value is assigned to feedstuffs to ensure that sufficient amounts of NH_3-N are provided to the rumen microflora to allow for maximal fermentation and MCP synthesis and to avoid excess dietary NPN.

Burroughs et al. (1975) proposed the metabolizable protein feeding standard (MPS) as a more complete perspective of protein requirements. This system represents a balance between animal requirements for metabolizable amino acids (MAA's) and its fulfillment by diets composed of a variety of feedstuffs. The UFP is utilized when urea is part of the diet. Although the MPS is an amino acid (AA) standard, a single value metabolizable protein (MP) standard is approximated, and protein quality is overlooked. The MPS also makes the biased assumption that MCP was the sole contributor to metabolic fecal nitrogen (MFN).

Roffler and Satter (1975a) also approached NPN utilization from the standpoint of maximizing MCP synthesis in the reticulorumen. They suggested providing enough urea in the diet to maximize MCP production and to supplement this with added bypass protein to meet greater protein demands for production. Satter and Slyter (1974) demonstrated in continuous culture fermentors, that rumen bacteria can scavenge adequate amounts of NH_3-N from diluted solutions. Concentration of 5 mg NH_3-N$\cdot dl^{-1}$ rumen fluid was sufficient to support maximal rates of

microbial growth. Roffler and Satter (1975a) further reported that ruminal NH_3-N reached 5 $mg \cdot dl^{-1}$ sooner with low energy diets.

Efficiency of NH_3-N utilization is not constant, but varies with ruminal NH_3-N concentrations which in turn depend upon diet composition. The point that NPN supplements are more effectively utilized when added to low protein, high energy diets is well documented (Chalupa, 1973; Conrad and Hibbs, 1968; Reid, 1953).

Protein evaluation of ruminant feeds based on its solubility can not define utilization characteristics adequately. Van Soest (1975) points out that ruminant use of dietary CP is subject to the same factors that affect digestion of energy, namely rate of digestion in the rumen and rate of passage out of the rumen. Decreased protein digestion increases the escape of true protein from the rumen, which can result in more efficient use of dietary protein. In contrast, increasing ruminal protein digestion can exuberate feed nitrogen loss from the rumen (Orskov et al., 1972).

Fox et al. (1981) proposed the Cornell System for meeting protein requirements of beef cattle. They attempted to provide an accurate description of the net protein needs for maintenance and the desired level of production. The rumen submodel proposed by Van Soest et al. (1981) was used to determine the protein contribution from MCP. The net protein for gain was determined by first calculating energy allowable gain as explained by Fox and Black (1977) and then calculating the net protein requirements needed to meet this gain. No consideration of the animals AA requirements are made by this model.

Klopfenstein et al. (1981) proposed the Nebraska Growth System which is an empirical method for determining the protein requirements for growing beef cattle fed corn silage based diets. Biological value of bypass proteins were determined by measuring the average daily gain response when test proteins were added to the urea supplemented diet. Soybean meal was arbitrarily given a protein efficiency (PE) value of of 1.0 and used as a reference for other test proteins. Proteins with a higher bypass value than SBM have a PE greater than 1.0, those with lower bypass potential have values less than one.

This system only ranks protein sources. The Nebraska system does not differentiate between ruminal and animal responses to proteins and does not define animal AA requirements. This latter consideration is a common weakness of each of the systems discussed.

Expanded knowledge of ruminal nitrogen metabolism is needed. It will permit greater control and manipulation of the processes of ruminal fermentation and post ruminal digestion. Further work must be done to establish the amino acid requirement of the ruminant and its interdependent reticulorumen ecosystem.

Purified Diets

Many rumen bacteria, especially cellulolytic species, require ammonia and can survive in media containing urea or ammonium salts as the sole source of nitrogen (Bryant and Robinson, 1962; Hungate, 1966). Because of this a ruminant can be fed a diet containing urea and little or no preformed proteins. Most of the amino-N available at the duodenum under these conditions would be of microbial origin.

Protein-free diets have been used successfully with ruminants (Maeng and Baldwin, 1976; Richardson et al., 1976). Positive weight gain and nitrogen retention were achieved (Loosli et al., 1949; Oltjen et al., 1962a and b) and milk production maintained (Virtanen, 1966) when diets were properly balanced for all nutrients except for protein.

In the past, protein free diets were low in crude fiber. This is of detrimental consequence since fiber stimulates rumination and saliva production. Crude fiber also provides the "scratch factor" that maintains the rumen epithelium and serves as a support matrix for many bacterial species. Oltjen et al. (1962a) reported that visual observations of animals on purified diets, indicated a drastic reduction in rumination time. They quantitatively determined this by fitting sheep with a pneumograph and recording jaw movements. The periods of chewing separated at regular intervals by short pauses for swallowing and regurgitation, characteristic of rumination were easily distinguishable from irregular chewing recorded during eating. When compared to natural diets, which were composed of corn and alfalfa hay there was actually very little rumination with the purified diets. On the natural diets rumination time was longer (15-20 min), while in the purified diets the periods were short and irregular, averaging 2-5 min.

Oltjen et al. (1962a) included a polyethylene residue to provide the "scratch factor" missing in purified diets. This practice was unsuccessful in increasing rumination time. Virtanen (1966) reported that rumination time in lactating cows was markedly stimulated through the feeding of a .5 Kg of polyethylene pellets per day. In

order to increase saliva production, cows on the purified diet were allowed to chew hard rubber tubing that was placed above their feeding troughs. Virtanen (1966) also reports that the cows chewed the tubing eagerly when urea was in the diet at lower levels. Virtanen theorized that the eagerness to chew the tubing and thus promote the secretion of saliva may have been related to inadequate N intake.

An alternative to artifical replacements for fiber may be fiber sources that supply little or no digestible CP. Corn cobs or wheat straw may be suitable sources for this purpose since the CP is low (corn cobs 3.2%; wheat straw 3.6%; NRC 1984) and digestible CP contributions may be insignificant.

Meachem et al. (1961) reported reduced sheep gains when part of the cellulose fraction of a purified diet was removed and replaced with starch or dextrose. Oltjen et al. (1962b) fed cellulose at varying levels (30%, 40% and 50% of the diet) and varying ratios with highly fermentable carbohydrates (2:1, 1.5:1 and 1:1). They found the ideal diet was 2:1 highly fermentable carbohydrates to cellulose, and that cellulose should be approximately 30% of the purified diet.

Oltjen et al. (1962b) also observed that sheep consumed the purified diet containing urea very slowly. They theorized that it was feasible to assume that by slowly eating the diet the sheep are able to maintain rumen NH_3-N levels within a range consistent with normal rumen function. Virtanen (1966) reported similar observations with cows fed high levels of a purified diet.

Another factor of concern when feeding purified diets is dry matter intake, and the diet's ability to supply the protein levels needed for higher levels of production. Virtanen (1966) reported that a long adaption period to allow the microflora of the rumen to adjust to the new diet and to allow the host animal time to physiologically adapt to the high levels of urea is needed. He further reports that after an adequate period for diet adaptation milk production of cows on experimental diets were comparable to cows fed normal diets (yearly milk production was 4217 kilograms). Oltjen et al. (1962a) reported dry matter intakes (DMI) by lambs fed purified diets to be significantly higher ($P<.05$) than those fed diets composed of corn and alfalfa. They (Oltjen et al., 1962a) also report the ADG of the lambs on the normal diets to be significantly greater ($P<.05$) than the lambs on purified diets. One can conclude that DMI typical of high production situations is attainable when feeding a purified diet. Whether or not the diet would supply the nutrients to support the higher production levels is questionable. Regardless of whether the latter concern could be accomplished or not, the fact that typical DMI could be attained provides the tool needed to screen possible alternative protein and amino acid sources in ruminant diets.

Digesta Markers

Evaluation of digestibility involves the determination of how much of an individual nutrient or feedstuff disappears in the gastro-intestinal tract. The conventional method for studying digestibility is to confine the animals to metabolism crates for total

collection of feces and/or urine. This method, although effective, is extremely labor intensive and does not allow for measurement of partial tract disappearance of diet components.

Another method of determining digestiblity as explained by Hungate (1966), Church (1976) and Faichney (1984) is the indicator method. Indicators are inert materials that are not subject to the fermentative processes of the rumen microflora or enzymatic digestion occurring in the gut, and are not absorbed.

External indicators are added materials such as chromic oxide (Corbet et al., 1963). Natural or internal indicators are those found in plant materials. Lignin (Hate, 1947a), plant chromogens (Reid et al., 1950), and indigestible acid detergent fiber (IADF) (Berger et al., 1979) have been used as internal indicators. Internal indicators have generally met with less enthusiasm due to problems such as lack of sufficient indicator in dry forage or incomplete recovery of material in the feces (Church, 1976).

Of the internal indicators lignin has been used most frequently. Lignin was initially considered indigestible but Porter and Singleton (1971) showed that substantial losses of lignin may occur in the ruminant gastro-intestinal tract (GIT), probably most of it in the rumen. Mertens and Ely (1979) point out that although the concept of an indigestible fiber fraction is not accepted by all researchers, several in vitro and in vivo experiments indicate that digestion reaches a maximum that does not equal 100%. This implies that there is an indigestible fiber fraction in ruminant feedstuffs. If this

fraction was determined analytically it would provide the internal marker needed for determining partial and total tract dry matter disappearance.

Weidemier et al. (1983) fed cows diets containing high levels of wheat straw, in an experiment to determine the effect of protein level on dry matter digestibility. In vivo digestibility was determined four ways, 1) total fecal collection, 2) chromic oxide, (external indicator), 3) acid detergent lignin, (internal indicator), 4) indigestible acid detergent fiber (internal indicator). Both chromic oxide and indigestible acid detergent fiber (IADF) were found to be acceptable indicators for predicting DMD with r^2 values of .95 and .98, respectively.

Pritchard and Males (1985) also fed cows diets with high levels of wheat straw and report IADF was a more suitable indicator than $LaCl_2$ when estimating partial and total tract DMD. To correct for the possible disappearance of the indicator in the digestive tract of the animals, Weidemier and Males (1983) ran 48 hour single stage in vitro digestions on the diets fed to cows. The ADF content of the digested residue was determined and this remaining fraction was considered indigestible. Stock et al., (1986) reported a similar procedure when determining the indigestible ADF fraction of diets containing high levels of corn cobs, except the single stage in vitro digestion was extended to 96 hours before the ADF present in the residue was determined.

Weidemier et al. (1983), Pritchard and Males (1985) and Stock et al., (1986) all report success using IADF as an internal indicator in diets containing a high percentage of dietary fiber. This indicates that the indigestible fiber fraction in some diets with fairly high fiber content is suitable as an internal indicator for predicting rumen outflow and partial and total tract dry matter digestibility.

Allantoin

Purine nucleotide catabolism in most non-primates, leads to the eventual formation of the end product allantoin. In ruminants, allantoin appears to be derived largely from nucleic acids of rumen microbial origin. Nucleic acids from feed sources are reported to be rapidly degraded within the rumen (McAllen and Smith, 1972) and do not contribute significantly to the total nucleic acid load reaching the lower gut. Microbial nucleic acid content is related to total microbial protein (Bergen et al., 1980). If a constant relationship exists between absorbed nucleic acids and allantoin production, excreted allantoin might be a suitable indicator of rumen microbial protein outflow (Antoniewicz et al., 1979, 1980, 1981). Topps and Elliot (1965) found a highly significant correlation existed between the concentration of ruminal nucleic acids and the amount of urinary allantoin nitrogen excreted ($r=0.537$; $P<0.01$). These results suggest microbial outflow could be estimated by total urine collection, and analytical determination of allantoin concentration in the urine. This experiment was designed to determine the sensitivity of allantoin

excretion to changes in the level of nutrient supply to rumen microorganisms.

In earlier work Antoniewicz et al. (1979) reported that regardless of the type of diet and body weight of sheep, urinary allantoin output was closely related to digestible organic matter intake (r^2=.8836; P<0.01). They (Antoniewicz et al., 1981) also reported urinary allantoin excretion increased linearly with dry matter intake and was significantly correlated (P<0.01) with dry matter and digestible dry matter intake. They concluded that allantoin in the urine of sheep originates primarily from absorbed microbial nucleic acids. Changes in urinary allantoin reflect differences in quantity of microbial nucleic acid absorbed which are caused by differences in rumen microbial protein outflow.

Owens and Bergen (1983) point out that diet has no major influence on the quality of microbial protein. Except for small endogenous contributions, ruminal outflow protein should be of microbial origin if a purified diet was being fed. Therefore, if nitrogen retention was determined and regressed against microbial protein availability at the duodenum, one could calculate the biological value (BV) of the outflow protein in intact animals based on allantoin excretion and N retention. In this estimation, one must assume that the microbial protein and nucleic acids have similar digestion and absorption rates. If absorption is not similar, allantoin estimation of protein quantity could cause over or underestimation of the BV of the protein.

The validity of allantoin as an indicator of rumen microbial protein outflow over a wide range of metabolizable energy (ME) intake levels has not been thoroughly tested. Antoniewicz (1981, 1980, 1979) fed ME levels of .70 to 1.69 x maintenance and found a linear relationship between microbial protein production and increasing ME intake ($r=.90$; $P<0.01$). Intake levels as high as 2.00 to 2.5 x maintenance which occur in production situations should be considered. Urinary allantoin excretion may not increase linearly with higher ME ranges. Higher levels of ME intake may result in a decline in the appropriateness of allantoin for estimating rumen microbial outflow. One possible cause for this decline is that uric acid the substrate for allantoin production, may exceed the catabolic pathway ability to metabolize it to allantoin. Another possible avenue for decreasing the linear relationship of allantoin, to ME intake, may be an increase in the salvage of nucleic acids at higher levels of production. Investigation of these possibilities are necessary to validate allantoins ability to estimate MCP production over a broader range of production situations.

EXPERIMENT DISCUSSION

Summary

A semi-purified diet (SPD) was fed to 12 wether lambs ($\bar{x}$ = 34.9 kg) and one fistulated wether and evaluated for acceptability and ability to support growth. Treatments included three levels of dry matter intake (DMI); low intake (LI 723 $g \cdot hd^{-1} \cdot d^{-1}$), medium intake (MI 1085 $g \cdot hd^{-1} \cdot d^{-1}$) and high intake (HI 1446 $g \cdot hd^{-1} \cdot d^{-1}$). Digestibility of dry matter (DM 70.6%), acid detergent fiber (ADF 62.8%), nitrogen (N 72.8%) and percentage digestible nitrogen retained (PDNRT 33.2%) were not different across intake levels. Indigestible ADF (IADF) of the semi-purified diet appeared to be a suitable solid phase marker for estimating DM digestibility (DMD) if DMI is accounted for. Regression analysis indicated IADF recovered (IADFR) in feces was related to IADF intake (IADFI) and DMI as described by the equation IADFR = -80.3824 + .852 (IADFI) + .0426 (DMI); (r^2 = .7057; P<.01). Allantoin excretion in the urine was evaluated as a predictor of microbial protein synthesis. Allantoin excreted in the urine was different across DMI levels (P<.05). Urinary allantoin (A) $mg \cdot 100^{-1} g$ dry matter digested ($\bar{x}$ = 55 mgA$\pm$4.5) was not different across intake levels. However total allantoin excretion did not increase with increasing DMI.

(Key Words: Semi-purified Diet, Lambs, Dry Matter Digestibility, ADF Digestibility, Digestible N Retained, Indigestible ADF, Allantoin.)

Introduction

Because of the inherent contribution of microbial protein to the ruminant's protein requirement, it is probably not sufficient to only measure the animal's amino acid requirements. More importantly, research should concentrate on quantifying microbial protein supply and determining the quantity and quality of additional protein needed to optimize performance. Amino acid requirements could then be determined indirectly as the sum of microbial protein and the additional protein considered optimum. An in vivo model is needed for determining the biological value of microbial crude protein. Ideally this model would also provide an effective way of screening or evaluating various dietary nitrogen supplements for animals in a variety of production conditions.

Ruminants provided with nonprotein nitrogen and carbohydrates can grow, reproduce and lactate (Loosli et al., 1949; Viraten, 1966; Oltjen, 1969) when consuming no amino acids (AA) or dietary protein. A protein free diet approach may make it possible to evaluate MCP contributions to amino acid requirements. The objective of the experiment reported here was to develop a protein-free diet suitable for this purpose and to determine whether dietary IADF would be a suitable marker for subsequent studies.

Materials and Methods

A semi-purified diet (Table 2) similar to that used by Maeng and Baldwin (1976) and Oltjen et al. (1962a,b) was prepared except that the ratio of readily fermentable to cellulolytic carbohydrates was

changed from 2:1 to 1:2 and feeding frequency was changed from once to twice daily (0600 and 1800 hours).

Twelve crossbred wether lambs ($\bar{x}$ = 34.9 kg) and two ruminally fistulated wether lambs ($\bar{x}$ = 87.3 kg) were used in experiments to evaluate acceptability of the semi-purified diet. Lambs had previously been treated for internal and external parasites and received injectable vitamins A, D and E.

Initially all lambs were fed a control diet (Table 1) at maintenance (1250 $g \cdot hd^{-1} \cdot d^{-1}$ for the fistulated wethers, 750 $g \cdot hd^{-1} \cdot d^{-1}$ for the other wether lambs). All crossbred lambs and one fistulated wether were adapted to the semi-purified diet by initially replacing 20% of the control diet with semi-purified diet. If the new diet was accepted for two consecutive days, the semi-purified diet component was increased by 10% of the daily feed offered. This procedure was followed until all lambs were consuming only the semi-purified diet 14d.

Rumen samples were collected on alternate days of the adaptation period from both fistulated wethers. Ruminal content pH was measured and percentage solids in rumen liquor was evaluated. When all lambs were consuming only the SPD, rumen NH_3-N was also measured 6 hr after feed to the fistulated wethers on three alternate days. Determination of rumen NH_3-N was done by distillation over MgO (AOAC, 1980).

Following adaption to the semi-purified diet, lambs were randomly allotted to three dry matter intake (DMI) groups: low (LI),

medium (MI) and high (HI). The LI (1.2 x maintenance) group was fed 723 $g \cdot hd^{-1} \cdot d^{-1}$. Feed offered the MI group (1.7 x maintenance) was increased to 1085 $g \cdot hd^{-1} \cdot d^{-1}$ and the HI group (2.2 x maintenance) feed was increased to 1446 $g \cdot hd^{-1} \cdot d^{-1}$. If feed offered was consumed for two consecutive days, the amount offered was increased by 100 g. This continued until all lambs had reached target intakes. Orts were removed daily.

After adaptation at target intake levels (14d), lambs were weighed and put into metabolism stalls. Lambs were allowed 3 days adaptation to their new environment. Feces and urine were then collected for two consecutive 3d collection periods. Feed consumption was monitored and orts were weighed and subsampled. After 10 days in the metabolism stalls, lambs were removed, reweighed and jugular blood samples were taken before morning feeding. Groups were then reassigned DMI levels (LI-MI, MI-HI, HI-LI) and allowed another 14 days adaptation for the next replicate. This procedure was repeated three times, so that each group of lambs was fed at each DMI level.

Blood samples were stored at 2° C for 24 hours and allowed to clot. Serum was separated by centrifuging at 7800 x g for 30 minutes. Serum was decanted and stored at -18° C until analyzed for urea nitrogen (SUN) as described by Fawcett and Scott (1960).

At the end of each collection period, daily feces and orts were pooled for each lamb and subsampled for dry matter (DM), nitrogen (N) and acid detergent fiber (ADF) determination. Feed and orts were dried in a forced draft oven at 100° C for 24 hours. Feces were dried in a

forced draft oven at 56° C for 48 hours. All samples were then ground through a 1mm screen and stored in airtight containers for subsequent analysis.

Urine was collected in vessels containing 100 ml of a 30% HCl solution. Urine output of <1000 ml was diluted to volume (1 liter) with deionized water to avoid salt precipitation. Pooled subsamples (10%) of the urine for each lamb were stored at 2° C during collection periods and then at -18° C until analyzed. Samples (10 ml) of the pooled urine were centrifuged at 1000 x g for 60 min to remove salts. The urine was then stored at 2° C until analyzed for allantoin content as described by Young and Conway (1942).

Nitrogen content of urine, dry feed, feces and orts was determined by the Kjeldahl method (AOAC, 1980). Acid detergent fiber of dried feed, feces and orts was determined as described by Goering and Van Soest (1970). In vitro, single stage 48 hr fermentable DM disappearance (Tilley and Terry, 1963) was determined for the semi-purified diet.

Indigestible ADF (IADF) was evaluated for future use as an internal phase marker. Indigestible ADF of the semi-purified diet was considered to be the ADF residue present following a 72 hr in vitro fermentation (Weidemier et al., 1983). Indigestible ADF (IADF) found in the feces was used to predict dry matter digestibility (DMD) as explained by Church (1976). Predicted DMD was regressed against apparent DMD to determine IADF value for predicting apparent DMD.

The experiment was statistically analyzed as a 3 x 3 latin square design (Steele and Torrie, 1980). Analysis included using the general linear model (GLM) [SAS, 1985] to calculate missing values that occurred in period I, collection II. Independent variables included in the model were intake level, period, group, collection, lamb and appropriate interactions.

A modification in the statistical analysis to generate the correct error term for testing significance of effects was needed. Because of the small size of the latin square, and the missing value that occurred in period I collection II, the proper error term for calculating the appropriate F test was not produced by GLM. A factorial design was implemented in which each group x period cell was considered a treatment. The model sums of squares (SS) from the analysis of variance derived from the factorial was used to calculate this correct error term for the latin square design (Table 3). The model SS of the latin square was subtracted from the model SS of the factorial design to produce the error term. The degrees of freedom associated with the error term were determined similarly. The appropriate error term was then used to test for significant effects due to level of intake, period and group.

Orthogonal comparisons of effects were based on the comparisons LI and HI vs MI and LI vs HI.

Results

Lambs adapted to the diet rather quickly. Weight gain improved ($P<.05$) with increasing DM intake while lambs were in the metabolism

crates (table 4). Crude protein content of corn cobs was determined to be 3.2%. Acid detergent insoluble N of the corn cobs was 3.07% crude protein, indicating corn cobs probably contribute little or no digestible protein to the SPD. In vitro DMD of the SPD was found to be 74.8% for period I and 77.2% for periods II and III.

Dry matter intake, DMD, N intake (NI) and N retention (NR) are shown in table 4. There were no differences related to period, therefore, combined mean values will be discussed throughout. Dry matter intake was different between LI and HI ($P<.01$). Increased DMI resulted in increased ($P<.05$) NI and NR. Percent dry matter digestibility (70.6 $\pm$ 1.57), ADF (62.8 $\pm$ 2.37) and N digestibility (72.8 $\pm$ 1.77) and digestible nitrogen retained (33.2 $\pm$ 5.25) were not affected by intake level.

Allantoin N excretion was affected by DMI level ($P<.05$). Orthogonal contrast of LI and HI vs MI and LI vs HI showed trends ($P<.10$) of increasing allantoin excretion with increasing DMI (Table 5). Daily totals of allantoin N excretion reported for LI and MI are in line with values reported by Antoniewicz et al. (1981) for animals receiving metabolizable energy (ME) at 1.0 to 1.5 X maintenance. Increasing ME to 2.0 x maintenance (HI) did not increase daily allantoin N excretion (Table 5).

Serum urea N (SUN) concentrations were not affected by intake level. Values obtained ($\bar{x}$ = 14.4 $mg \cdot dl^{-1}$ $\pm$ .78) were in line with values reported by Preston et al. (1965) as being typical for lambs on

growing or finishing diets with adequate amounts of dietary crude protein.

Dietary IADF was considered for use as an internal phase marker for predicting partial and total tract DMD in future studies. Indigestible ADF values determined for the diet were 10.1% for period I and 7.6% for periods II and III. The change in the IADF of the semi-purified diet was due to a change in the source of corn cobs.

Indigestible ADF intake (IADFI) and DMI were used in a multiple regression to predict the IADF recovered (IADFR) in the feces. This equation was defined as g IADFR = -80.3824 + .8520 (g IADFI) + .0426 (g DMI); [r^2 = .7057; P<.01]. This indicates a strong relationship between intake and recovery.

Discussion

The semi-purified diet used was acceptable to lambs and supported growth (Table 4). These results are consistent with those of Loosli et al. (1949), Oltjen et al. (1962a,b) and Maeng and Baldwin (1976). The semi-purified diet was formulated to contain 12% crude protein. This value is considered to be the maximum value for efficiently utilizing urea (Reid, 1953). Rumen NH_3-N was 4.44 $mg \cdot dl^{-1}$ in lambs fed the semi-purified diet and serum urea N from these lambs had a mean value of 14.4 $mg \cdot dl^{-1}$. These values represent acceptable ranges in the nutritional physiology of the ruminant (Roffler and Satter 1975a; Preston et al., 1965).

When the semi-purified diet was fed at three levels of DMI, it was observed that N retention increased with increasing DMI (table 4).

Since little degradable N was provided from the corn cobs, we can presume that nearly all of the protein retained was of microbial origin. This indicates that we can control the quantity of microbial protein available to the animal by controlling DMI and consequently NI.

When ME intakes range from .79 to 1.69 x maintenance, urinary allantoin excretion is linearly related to ME intake (Antoniewicz et al., 1979; Antoniewicz et al., 1980 and Antoniewicz et al., 1981). Allantoin N excreted in the urine increased with increases in digested dry matter until ME was fed at levels above 1.7 x maintenance. It appears that at levels slightly above 1.7 x maintenance a threshold is reached at which allantoin N no longer increases linearly with increases in digested dry matter. Levels of ME above 1.69 x maintenance have not been thoroughly tested for their effect on allantoin N production. These results indicate that allantoin N could be utilized as a marker for microbial protein synthesis as long as ME does not exceed 1.7 x maintenance.

Storm et al. (1983) reported the biological value (BV) of microbial protein, in lambs maintained on intragastric infusions of amino acids to be 80. The apparent BV determined for each intake level of the SPD was: LI = .24; MI = .36; HI = .39, respectively (Table 6). The BV of urea is equal to zero. The observed increase in the apparent BV of the SPD feeding levels, indicates that increasing DMI increases absorption of microbial protein by the host animal. The low BV along with the high dry matter digestibility percentage (70.6%) suggest that

the growth of ruminal microbes was probably uncoupled from fermentation.

This point is further supported by the values reported for allantoin production per 100 g digested dry matter (Table 6). These values are substantially lower than those reported by Antoniewicz et al. (1981) on lambs receiving natural protein diets with similar ME levels. It is possible that the levels of allantoin production were higher because the natural protein diets provided the subtrate needed by the rumen microflora for maximum microbial protein synthesis.

Stock et al. (1986) reported that adding dried delactose whey to a SPD fed to steers increases bacterial N ($P<.09$) and amino N flow ($P<.06$) reaching to the abomasum. It was thus concluded that when NPN supplied all the supplemental N in low protein high roughage diets, dried delactose whey increased microbial protein synthesis.

This information outlines the need to determine the substrates necessary to provide optimum microbial protein synthesis, and the additional amino acid requirements of the host animal. The diet and model developed in this experiment should provide the tools needed for screening alternative protein sources and their effect on the protein status of the ruminant animal.

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APPENDIX

TABLE 1. LAMB RECEIVING DIET

Ingredient	% DMB[a]
Corn cobs	39.0
Corn grain	42.5
Alfalfa	10.0
Soybean meal	6.0
Molasses	1.0
Dical[b]	1.0
Trace mineralized salt	.5

[a] Percent dry matter basis.

[b] A commercial mixture of di- and monocalcium phosphates containing 26.30% Ca and 18.70% P.

TABLE 2. COMPOSITION OF THE SEMI-PURIFIED DIET

Ingredient	% DMB[a]
Corn starch	31.888
Solka-Floc	28.931
Corn cobs	30.020
Animal fat	2.499
Urea	4.269
DiCal[b]	1.490
K_2SO_4	.584
Trace mineralized salt	.300
MgO	.017
KI, ppm	1.0
ZnO, ppm	64.0
$Na_2SeO_3.5H_2O$, ppm	.219
Vitamin E, ppm	1.49
Vitamin A, ppm	17.0
Crude protein	12.0
Ca	.365
P	.287
Mg	.06
K	.50
S	.20
I, ppm	.80
Se, ppm	.10
Zn, ppm	50.0

[a] Calculated percent dry matter basis.

[b] A commercial mixture of di- and monocalcium phosphates containing 26.50% Ca and 18.70% P.

TABLE 3. EXPLANATION OF STATISTICAL ANALYSIS

Dependent Variable _____:		
Source	DF	Sums of Squares (SS)
Model	6	---
Error	64	
Total	70	

Source	DF	Type III Sums of Square
Level	2	---
Period	2	---
Group	2	---
Error	2	

* Error = Model SS Factorial - Model SS Latin Square

** DF associated with error = DF Model SS Factorial - DF Model SS Latin Square

* Error termed used for proper F test
** DF associated with error was always equal to 2

TABLE 4. EFFECT OF INTAKE LEVEL ON SEMI-PURIFIED DIET UTILIZATION

Intake level	DMI[a]	DMD[b]	NI[a]	NR[a]	ADG[a]
LI	723[c]	74.10	13.16[d]	2.30[d]	73.0[d]
MI	1018	68.80	18.45	5.27	176.0
HI	1224[c]	68.83	21.51[d]	7.04[d]	210.0[d]
SEM	± 101	± 1.57	± 2.29	± 2.36	± 20.0

[a] $g \cdot hd^{-1} \cdot d^{-1}$.
[b] Percent dry matter digestibility.
[c] Means differ ($P<.01$).
[d] Means differ ($P<.05$).

TABLE 5. EFFECT OF LEVEL OF DIGESTIBLE DRY MATTER ON ALLANTOIN N SECRETED IN SHEEP FED A SEMI-PURIFIED DIET

Intake level	Digested dry matter[a]	Allantoin N[b]
LI	533[c]	262
MI	707	474
HI	842[c]	408
SEM	± 15.0	± 32.0

[a] $g \cdot hd^{-1} \cdot d^{-1}$.
[b] mg/d.
[c] Means differ ($P<.01$).

TABLE 6. EFFECT OF LEVEL OF INTAKE ON N UTILIZATION AND ALLANTOIN EXCRETION

Intake level	Biological value[a]	N retained[b]	Allantoin N[b]
LI	24.33	442.9[c]	49.0
MI	36.52	730.6	67.0
HI	39.08	758.8[c]	49.0
SEM	± 4.29	± 59.6	± 4.5

[a] 100 (Nitrogen retained/nitrogen digested).
[b] mg/100 g digested dry matter.
[c] Means differ (P<.05).