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

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Summary

A semi-purified diet (SPD) was fed to 12 wether lambs and one fistulated wether and evaluated for acceptability and ability to support growth. Ruminal pH and NH_3 were monitored throughout the diet adaptation period. Treatments included three levels of dry matter intake; low intake (LI) 750 $g \cdot hd^{-1} \cdot d^{-1}$, medium intake (MI) 1125 $g \cdot hd^{-1} \cdot d^{-1}$ and high intake (HI) 1500 $g \cdot hd^{-1} \cdot d^{-1}$. Digestibility of dry matter (DM, 70.6%), acid detergent fiber (ADF, 62.8%), nitrogen (N, 72.8%) and percentage digestible nitrogen retained (33.2%) were not different across intake levels. Indigestible ADF of the semi-purified diet appears to be a suitable solid phase marker for estimating DM digestibility if DM intake is accounted for. Regression analysis indicated indigestible ADF recovered in feces was related to indigestible ADF intake and DM intake as described by the equation Indigestible ADF recovered = 80.3824 + .852 (indigestible ADF intake) + .0426 DM intake; (r^2 = .7057; P<.01).

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

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 improve performance. Amino acid requirements could then be determined indirectly as the sum of microbial protein and additional protein considered optimum. An in vivo model for determining the biological value of microbial crude protein would not only allow us to measure maximum performance attainable from microbial protein but would also provide an effective way of screening or evaluating various dietary nitrogen supplements for high production rates.

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. Using this approach would make it possible to evaluate MCP contributions to amino acid requirements. The objective of the experiment reported here was to develop a diet and identify dietary markers for subsequent amino acid requirement studies.

Materials and Methods

A semi-purified diet (table 1) similar to that used by Maeng and Baldwin (1976) and Oltjen et al. (1962a,b) was prepared except that the ratios of readily fermentable carbohydrates to cellulolytic carbohydrates were changed from 2:1 to 1:1 and feeding frequency was changed from once to twice daily (0600 and 1800 hours).

Twelve crossbred wether lambs ($\bar{x}$ = 30.1 kg) and two fistulated wether lambs ($\bar{x}$ = 87.3 kg) were used in the experiment 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 2) at maintenance (1250 $g \cdot hd^{-1} \cdot d^{-1}$ for the fistulated wethers, 750 $g \cdot hd^{-1} \cdot d^{-1}$ for 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.

Rumen samples were collected on alternate days of the adaptation period from both fistulated wethers. Ruminal content pH was measured and appearance, odor, pH and percentage solids of rumen liquor were evaluated and compared between animals. When all lambs were consuming only the SPD, rumen NH_3 was also measured in these samples by distillation over MgO (AOAC, 1980). Adaptation to the SPD took a total of 14 days.

Lambs were allotted to three intake groups, low (LI), medium (MI) and high (HI), using ear tag numbers and a table of 10,000 random digits (Steele and Torrie, 1980). The LI (maintenance) group was fed 750 $g \cdot hd^{-1} \cdot d^{-1}$. The MI group (1 1/2 x maintenance) was increased to 1125 $g \cdot hd^{-1} \cdot d^{-1}$ and the HI group (2 x maintenance) was increased to 1500 $g \cdot hd^{-1} \cdot d^{-1}$. The method of step-up was to increase feed offered by 100 g units. If this was consumed for two consecutive days, feed intake was increased again. This continued until all lambs had reached target intakes. Feed refusals were removed daily.

After 14 days adaptation at target intake levels, lambs were weighed and put into metabolism stalls. Lambs were allowed 3 days adaptation to their new environment. Feces and urine were collected for two consecutive 3-day collection periods (6 days total). Total feed consumption was monitored and feed refusals were weighed back and subsampled. After 10 days in the stalls, lambs were removed, reweighed and jugular blood samples were taken before morning feeding. Groups were then reassigned intake 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 intake 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 collection periods, 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 1-mm 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 were stored at 2° C during collection periods and then at -18° C until analyzed.

N 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).

Nitrogen retention values reported reflect either the percentage of digestible N retained or N retention in grams.

Single stage, in vitro 48-hour fermentable DM disappearance (Tilley and Terry, 1963) was determined for the semi-purified diet. Indigestible ADF of the semi-purified diet was considered the ADF residue present following a 72-hour in vitro fermentation. Indigestible ADF was checked for its validity for future use as an internal phase marker (Weidemier and Males, 1983). Indigestible ADF found in the feces was used to predict DM digestibility as explained by Church (1976). Predicted DM digestibility was regressed against apparent DM digestibility to determine indigestible ADF value for predicting apparent DM digestibility.

The experiment was statistically analyzed as a 3 x 3 latin square design (Steele and Torrie, 1980). Analysis included using 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.

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, although HI lambs less readily consumed their entire portion. Weight gain improved with increasing DM intake while lambs were in the metabolism crates (table 3).

DM intake, DM digestibility, N intake and N retention (g) are shown in table 3. There were no differences related to period. Therefore, combined mean values will be discussed. DM intake was different between LI and HI ($P<.01$), as were grams of N intake ($P<.05$) and grams of N retained ($P<.05$; table 3). DM digestibility, ADF digestibility and percentage digestible and retained N were not affected by intake level.

Serum urea N (SUN) level was not affected by intake level. Values obtained ($\bar{x}$ = 15.2 $mg \cdot dl^{-1}$) 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.

Crude protein content of corn cobs was determined to be 3.2%. Acid detergent insoluble N equalled 3.07% crude protein, indicating corn cobs probably contribute little or no digestible protein to the SPD.

In vitro DM digestibility at the SPD was found to be 74.8% for period I and 77.2% for periods II and III.

Indigestible ADF of the diet was given consideration for use as an internal phase marker for predicting partial and total tract DM digestibility in future studies. Indigestible ADF values determined for the diet were 10.2% for period I and 7.1% for periods II and III. The change in the indigestible ADF of the semi-purified diet was due to a change in source of corn cobs between periods I and II.

Indigestible ADF intake (IADFI) and DM intake were used in a multiple regression to predict the indigestible ADF recovered (IADFR) in the feces. This equation was defined as IADFR = 80.3824 + .852 (IADFI) + .0426 (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. 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/dl in lambs fed semi-purified diet and serum urea N from these lambs had a mean value of 15.2 mg/dl. These values represent acceptable parameters 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 intake, it was observed that N retention increased with increasing DM intake (table 3). 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 DM and N intake.

In the past semi-purified diets have been low in crude fiber. Corn cobs were included in this diet to provide the "scratch factor" to maintain ruminal epithelium as well as provide the indigestible fiber needed for use as an internal phase marker for quantitating rumen DM outflow (Pritchard and Males, 1985, Weidemier and Males, 1983). Regression analysis of intake versus recovery indicated we can utilize indigestible ADF as a digestion marker in this diet.

Literature Cited

AOAC. 1980. Official Methods of Analysis (13th Ed.). Association of Official Analytical Chemists, Washington, DC.

Church, D. C. 1976. Digestive Physiology and Nutrition of Ruminants. O and B Books, Corvallis, Oregon, pp 99-131.

Fawcett, J. K. and J. E. Scott. 1960. Spectrophotometric determination of urea-N in plasma or serum. J. Clin. Path. 11:419.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis apparatus, reagents, procedures and some applications. Agriculture Handbook. No. 379, ARS, USDA.

Loosli, J. K., H. H. Williams, W. E. Thomas, F. H. Ferris and L. A. Maynard. 1949. Synthesis of amino acids in the rumen. Science 110:144.

Maeng, W. J. and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rates and yields: Effects of amino acid additions to a purified diet with nitrogen from urea. J. Dairy Sci. 59:648.

Oltjen, R. R. 1969. Effects of feeding ruminants non-protein nitrogen as the only nitrogen source. J. Anim. Sci. 28:623.

Oltjen, R. R., R. J. Sirney and A. D. Tillman. 1962a. Purified diet studies with sheep. J. Anim. Sci. 21:277.

Oltjen, R. R., R. J. Sirney and A. D. Tillman. 1962b. Effects of three levels of minerals and three levels of cellulose on the performance of sheep fed purified rations. J. Anim. Sci. 21:302.

Preston, R. L., D. D. Schakenberg and W. H. Pfander. 1965. Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr. 86:281.

Pritchard, R. H. and J. R. Males. 1985. Effect of crude protein and ruminal ammonia N on digestibility and ruminal outflow in beef cattle fed wheat straw. J. Anim. Sci. 60:822.

Reid, J. T. 1953. Urea as a protein replacement for ruminants: A review. J. Dairy Sci. 36:955.

Roffler, R. E. and L. D. Satter. 1975a. Relationship between ruminal ammonia and non-protein nitrogen utilization by ruminants. I. Development of a model for predicting nonprotein nitrogen utilization by cattle. J. Dairy Sci. 58:1880.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC. SAS Institute Inc., p. 956.

Tilley, J. M. A. and R. A. Terry. 1963. A two stage technique for in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104.

Viraten, A. I. 1966. Milk production of cows on protein free feed. Science 153:1603.

Weidemeier, R. D., J. R. Males and C. T. Gaskins. 1983. Effect of dietary crude protein on dry matter digestibility of wheat straw diets in cattle. J. Anim. Sci. 57:1568.

TABLE 1. COMPOSITION OF THE SEMI-PURIFIED DIET[a]

Ingredient	%
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	1.0 ppm
Z_nO	64 ppm
$Na_2SeO_3.5H_2O$	.219 ppm
Vitamin E	1.49 ppm
Vitamin A	17 ppm
Crude protein	12.0
Ca	.365
P	.287
Mg	.06
K	.50
S	.20
I	.80 ppm
Se	.10 ppm
Zn	50 ppm

[a] Calculated percent dry matter basis.
[b] A commercial mixture of di and mono calcium phosphates containing 26.50% Ca and 18.70% P.

TABLE 2. LAMB RECEIVING DIET[a]

Ingredient	%
Corn cobs	39
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 mono calcium phosphates containing 26.30% Ca and 18.70% P.

TABLE 3. EFFECT OF INTAKE LEVEL ON SEMI-PURIFIED DIET UTILIZATION

Intake level	DMI[a]	DMD[b]	NI[a]	NR[a]	ADG[a]
LI	723.33[c]	74.10	13.16[d]	2.30[d]	73
MI	1018.27	68.80	18.45	5.27	176
HI	1224.89[c]	68.83	21.51[d]	7.04[d]	210
SEM	± 101.39	± 1.57	± 2.29	± 2.36	± 19.84

[a] g/hd/d.
[b] Percent dry matter digestibility.
[c] Means differ (P<.01).
[d] Means differ (P<.05).