# FISHMEAL SUBSTITUTED FOR SOYBEAN MEAL FED AT TWO TDN LEVELS

## FOR LACTATING DAIRY COWS

by

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in

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(ABSTRACT)

Thirty multiparous Holstein and twelve multiparous Jersey cows were used to determine effect of diets (3x2 factorial) containing 0,1,2 kg of fish meal (FM) and energy levels of 70 and 75% TDN on milk production and composition. Basal diets contained 83% corn silage and 10.3% concentrate for the 70% TDN ration and 60% corn silage, 10.3% concentrate and 24.5% high moisture corn for the 75% rations. High moisture corn in the higher TDN diets decreased both acid and neutral detergent fiber, and increased dry matter content. Nitrogen degradability was lower in the higher TDN rations and decreased with increasing fishmeal levels. Dry matter degradability tended to be similar for all diets. Milk and FCM production was similar for all diets. Milk fat percentage was similar for TDN levels but decreased as fishmeal level increased. Milk protein percentage increased with 75% TDN but was not affected by fishmeal. Kilograms of milk protein produced followed a trend similar to milk protein percentage.

Dry matter intake was higher with higher TDN diets, but tended to be lower during the first 4 weeks of the study with increasing levels of fishmeal. Bodyweight fluctuated during the experiment and followed a pattern similar to dry matter intake. Blood plasma urea concentration was similar for all diets.

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God, may his mercy always inspire, protect, and guide me.

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# INTRODUCTION

In recent years much of the research in the area of dairy cow nutrition has been to determine needs of high producing cows in order to maximize production. Results have indicated that higher levels of protein as well as energy are very important during early lactation, because as milk production increases so does protein per unit of energy consumed. More recent work has suggested beneficial effects of feeding less rumen degradable N sources that bypass to the intestine (36). But despite the fact that significant increases in animal performances have been observed, much controversy exists over stage of lactation, type and amount of these protein supplements to be fed. Of the many different sources that have been compared only postruminally casein infusion (11) and use of dietary fishmeal have proven beneficial (35). Furthermore such work has pointed out some controversial data regarding interaction of protein degradability and energy. Animals that have increased milk yield in response to casein infusion have been said to be in negative energy balance. Similar observations have been reported when fish meal has been used, only when cows were restricted in intake or given a protein deficient diet did they show a response.(65).

## REVIEW OF LITERATURE


## IMPORTANCE OF NITROGEN PROTEIN SOURCES


Ruminants digest most of the ingested feed in the rumen prior to gastric and intestinal digestion, and through this process obtain a large portion of energy and protein needed for maintenance and production (50). Since efficiency of protein capture within the rumen depends upon degradability of dietary protein as well as readily available energy (65), different forms of available protein play a role in maximizing such efficiency.


### Non-protein nitrogen (NPN) sources


The amount and proportion of NPN sources useful to high producing dairy cows is questionable. While some studies suggest that its use is limited (21), others claim that urea is compatible with high milk production at 15% crude protein levels (28). NPN can only be utilized as source of nitrogen by the rumen flora (25). But dietary protein in excess of microbial requirements, depending on the nature of the particular protein, may be degraded to ammonia ($NH_3$) and excreted as urea or may escape ruminal degradation and present more true protein for intestinal digestion (27).

When part of the ration protein is replaced by NPN the amount of protein escaping rumen degradation is reduced and quantity of NH3 increases proportionately (47). NPN can be utilized as well as true protein when ruminal ammonia concentration is <5 mg/100 ml and dairy cows may benefit the most when added to high concentrate rations containing 12-13% crude protein (CP) or to all forage rations containing 9-10% CP (48). On the other hand recent studies have suggested that increasing low protein rations from 12% to 14 and 18% using urea and natural protein (SBM) have resulted in milk yields of 30.5, 32.6 and 32.7 kg/day for low , SBM, and urea rations respectively. However in some experiments, both urea and SBM were used to increase CP levels of the ration making it difficult to separate the effect of the two supplements (30). Despite this, inclusion of NPN in dairy cattle ration is profitable so long as production is not diminished and utilization for microbial synthesis is assured (21).

Recently, modified forms of NPN such as Starea, Dehy-100, and biuret have been developed. Their advantage over granular urea is that release of ammonia is slower offering protection against toxicity and improves utilization which allows feeding higher levels of NPN sources (21).

## Natural protein sources.

If maximum efficiency of protein utilization by ruminants is to be achieved there is a need to decrease wasteful fermentation of dietary protein (45). Several SBM treatments have been found to decrease rumen fermentation. Heat or chemicals, such as formaldehyde and tannins, have resulted in reduced protein deamination by rumen microflora. Excessive treatment using any of these methods result in reduced postruminally availability of protein (60).

Kung and Huber (27) conducted an experiment in which heated SBM plus corn silage was compared to SBM plus ammoniated corn silage and SBM plus corn silage. Results indicated that there were no treatment differences in non-ammonia nitrogen (NAN) digestion in the intestine, and partial substitution of N from SBM with N from ammonia added to corn silage did not decrease NAN flow to the small intestine (SI).

Sahlu (46) compared commercial SBM vs heat treated and extruded SBM. Milk production increased with heated SBM( HS) when fed to high producing cows especially from 4 through 7 wk postpartum. No differences were found when same diets were fed to cows producing less than 30 kg of milk.

Stern et al (55) conducted a similar experiment in which SBM was compared to whole SBM and extruded whole SBM (132 and 149 C). Diets containing extruded whole SBM (149 C) decreased protein degradation in the rumen and increased amino acid (AA) flow to the SI.

Treatment of protein sources with formaldehyde has not improved productive performances consistently. Results have been more positive for growth of sheep and steers than for milk production of dairy cows (64). Despite these results, and whatever the method used in protecting proteins there is need for considering the following factors: quantity of protein and AA leaving the rumen (bacterial and dietary protein), digestibility of that particular protein in the rumen, and rumen fermentation rate (25). Also it is important to know whether requirements are met or exceeded by feeding any of these particular protein supplements.

## Post ruminal protein sources

Some feed proteins are able to partially escape degradation in the rumen. But to meet protein requirements of high producing cows it may be important to increase the amount of high quality protein escaping degradation in the rumen (45). Among less degradable protein sources, which have been evaluated in many different trials, are dried brewers grains

(DBG), distillers grains (DG), corn gluten meal (CGM), meat
meal, and blood meal (40). Results have been conflicting and
while some of the studies have increased milk yield others
have not.

Rogers et al (44) compared postruminal infusion of water,
sodium caseinate, SBM, and cotton seed meal. Even when milk
and milk protein yields were increased, FCM production , milk
fat % and yield and milk protein % were not affected by
postruminal infusion of these proteins. Sodium caseinate was
more effective in increasing milk yield( 9%) as compared to
water infusion.

Henderson et al (20) conducted another experiment in which
SBM, cotton seed meal/corn gluten meal (CSM/CGM), and
extruded SBM were used as protein supplements . No signif-
icant differences were found in milk production among the
group of cows receiving SBM and extruded SBM, though the
group fed CSM/CGM produced less milk. Low levels of lysine
(thought to be second limiting AA for milk production) was
the explanation given for the poor performances of this diet.

Polan et al (42) conducted an experiment in which rations
containing 14.5; 16; and 17.5 CP were compared for effect
upon milk production. A basal diet was supplemented with DBG,
wet brewers grains (WBG) and SBM . Milk production was higher

for cows fed the DBG and WBG compared with SBM and for the higher amounts of crude protein. Fish meal (FM) is another protein source resistant to ruminal degradation. It is used extensively in Europe, but very little in the United States for dairy cattle nutrition. Results of feeding trials with FM have consistently produced more increases in milk yields than other ruminal resistant protein sources.

Orskov (36) conducted a series of experiments in which FM replaced barley (Exp 1), FM replaced ground nut meal (GNM, Exp 2) and FM replaced GNM at low and high ME intake (Exp 3). Fat corrected milk yield, milk protein and solids increased when barley was replaced (Exp 1), but no effect was found when replacing GNM (Exp 2). On the other hand when FM replaced GNM in Exp 3; significant increases were found in milk yield for the low ME FM diet at the expenses of live weight loss.

Miller (33) conducted another experiment to measure the effect on milk production when SBM was replaced by FM. FM increased milk yield during the first 4 wk of lactation, but the effect decreased from 5 through 8 wk.

Oldham (40) compared milk production of cows fed isonitrogenous rations containing urea, SBM, formaldehyde treated SBM, and FM . These N sources were compared at four

levels of inclusion to produce rations containing 103, 123, 143, and 163 g CP/kg dry matter. Milk, FCM and milk protein increased significantly (P<0.05) as ration CP increased. Yields were least with urea averaging 21.5 ; 21.9 ; and .55 kg/d and greatest with FM averaging 27.8; 29.2and .76 kg/d of milk, FCM and protein respectively.

Oldham et al (38) conducted two different experiments to measure the effect of substituting urea with fishmeal on milk contents and yield. Exp 1 ran from 15 through 84 d and Exp.2 from 84 through 175 d of lactation. Replacement of urea with fishmeal significantly increased yield of milk protein in both early and mid lactation (0.515 vs 0.619 kg/d). In mid lactation replacement, fishmeal significantly depressed concentration of milk fat.

In view of these reports it appears that post ruminal resistant protein sources do not always produce increases in milk yields. But FM seems to have a more consistent effect upon milk production, especially in early lactation when high producing cows are in negative energy balance.

## FACTORS AFFECTING PROTEIN DEGRADATION IN THE RUMEN

The extent to which protein is degraded in the rumen depends upon the rate at which it is hydrolyzed and the time it spends

in the rumen (54). Protein hydrolysis is influenced by structure, solubility and processing (10).

True proteins comprise a mixture of albumin , globulin, glutelin and prolamine types of proteins. The albumin and globulin proteins are generally of high quality and are sensitive to processing or chemical alteration as well as rapidly degraded in the rumen. In contrast, glutelin and prolamine proteins are of lower variable quality, resistant to degradation or chemical alteration and more resistant to degradation in the rumen (53). Globulin proteins are highly soluble in salt solutions. Since rumen fluid of cows is essentially a salt solution, globulin proteins are attacked and broken down very rapidly in the rumen . Oats contains 80% of the protein as globulin protein, whereas corn protein is composed of prolamine (54).

Protein solubility (in buffer solutions) is related to postruminally availability of protein (66). But even when nitrogen solubility of a protein and its degradability in the rumen are related they do not equate one to the other (21). A simple nitrogen-solubility measurement describes only the fraction of total nitrogen immediately available for bacterial degradation, and is not descriptive of other nitrogen fractions (65). Also solubility or short term nitrogen

degradability appears invalid for predicting ruminal degradable nitrogen especially in total mixed rations (35).

Storage and processing of feeds greatly affects nitrogen utilization by ruminants (57). Heat and formaldehyde treatments have been the most common methods used for modifying rumen degradability of protein sources(31). Heating has been related to reducing nitrogen degradation in the rumen by the Malliard reaction . This refers to the combining of carbonyl groups of sugars with the free aminogroups of proteins (6). Formaldehyde treatment affects rumen degradation by forming methylol groups on the terminal aminogroups of protein chains and on the aminogroups of lysine (7). Formaldehyde treatment of high quality protein such as casein and whey have been shown to increase milk yield , but similar treatment of plant proteins has not always been beneficial (12).

The time dietary ingredients spend in the rumen is another important factor that affects rate of degradation. Retention time is closely related to particle size and level of intake ( 57). Fine or small particles normally flow out of the rumen more rapidly and without any further size reduction (37). The fractional rate for outflow of protein particles can vary from 0.01 to 0.10 h depending on protein structure and level of intake.(34).Outflow rates of 0.01/ h have been found by feeding ground diets at maintenance levels, these rates have

increased to 0.02/ h  with same level of feeding with long forage diets (15). On the other hand feeding SBM diets that differed in particle size 1.8 mm (coarse) and 1 mm (fine) have had no effect upon out flow rate and degradability (16).


## NITROGEN DEGRADATION IN THE SMALL INTESTINE


Dietary protein is degraded in the range of 60 to 80% in the rumen, leaving 20 to 40% to escape for later digestion (25). Therefore the principal N-containing compounds entering the small intestine (SI) of ruminant  animals are by-pass protein, microbial protein, nucleic acids and residual ammonia (30). The extent of protein digestion in the small intestine has been the subject of many studies and reviews in recent years, but many questions still remain (31).   Apparent digestibility values have been normally used to quantify digestion of total nitrogen, non-ammonia nitrogen (NAN) and total amino acids (TAA). Such values has been calculated by measuring differences between amounts entering and leaving the SI (3).


Digestibility of approximately 0.70 has been calculated as normal which includes endogenous nitrogen sources entering the SI But it is also believed that small ammounts of AA nitrogen are absorbed from the reticulo-rumen in the free form (29).  Differences in absorption of specific AA from the SI

also exist, thus utilization of digestibility values to quantify intestinal digestion has many uncertainties and more information is needed in the area of protein digestion in the lower tract.

## PROTEIN AND ENERGY INTERRELATIONSHIPS

Rumen microbial activity results in two major processes: microbial degradation of dietary compounds (mainly carbohydrates and proteins) and synthesis of organic compounds into microbial mass (58). Therefore protein supply of the host animal (ruminants) depends to a large extent on an adequate energy supply to the rumen microorganism. Maximization of the energy to the host animal also depends on protein supply of the microorganism. Protein and energy metabolism are highly interrelated (2).

In dairy cows two different and important aspects of the interrelationship between protein and energy yielding nutrients can be identified. First, a change of protein input can influence productivity by changing overall plane of nutrition, changes in digestibility and intake. Secondly, changing protein supply to tissues can alter the pattern and efficiency of absorbed nutrient use, such as changes in glucose and fatty acid metabolism (39).

In early lactation when both AA and glucose are in short supply, oxidation of glucose appears to be reduced (9). It also appears that AA oxidation is reduced and at the same time mobilization of adipose tissue is stimulated as shown by an increase entry of fatty acids into the blood (29).

Furthermore, when AA supply to the tissue is low relatively to availability of energy yielding nutrients, the metabolic process is either to dispose of surplus energy by producing heat or store it as fat. If amino acid uptake is increased metabolic need for fat deposition is reduced and as a result net tissue mobilization occurs (38). When protein increases amino acid supply to the intestine usually milk yield increases at the expenses of body weight in deficient rations (36). In contrast, it can also be said that production responses to protein can be in part or all induced by changes in energy intake, rather than optimization of energy partition toward product formation as a consequence of protein (AA balance).

In many experiments with dairy cows, protein supplementation has produced increase in dry matter intake (DMI 0.23 to 0.31) kg DMI CP% (40). Milk yield response to increased intake (change in FCM/change DMI) stimulated by protein supplementation is relatively high and quite variable (mean value of 2.05 - 2.06 kg FCM/DMI) (38). This contrasts with

the level of response to variation in the plane of energy nutrition which usually is 0.4 to 1 kg milk/kg increase in concentrate intake of DM. Additional concentrate can or may replace forage and only part of the increment of nutrient intake is directed towards milk production and the rest is deposited in tissue (23).

This suggest that milk production responses to protein are greater than for energy, and that efficiency may also be higher if the protein source increases amino acid supply to the small intestine.

## ENERGY METABOLISM

Digestion of dietary carbohydrates, protein and lipids in the cow leads to the production of volatile fatty acids in the rumen, cecum and colon, and to glucose, amino acids and longer chain fatty acids in the small intestine (59). Methane and heat also result from this digestion and they can represent losses of energy and protein to the animal.

As much as 9% of energy ingested in feed is converted to methane and lost through eructation, and between 5 to 9 % of the energy can also be lost as heat which result from different metabolic processes within the animal (45). Metabolism of the products of rumen fermentation is quite variable and controversial. Enough information has been ob-

tained to have a fair understanding of such processes. Figure 1 illustrates the different pathways that may take place during degradation and absorption of feedstuff from the gut and passage to the extrahepatic tissues (58).

## Carbohydrates

Degradation of carbohydrates differs between various classes: free sugars (4-5 monomers especially saccharose) are degraded almost instantaneously, non-structural carbohydrates (fructosan and starches) are next, structural carbohydrates (lignin, hemicellulose and cellulose) are last, the latter degraded after a lag phase (56). Carbohydrates bypassing the rumen are subjected to enzymatic digestion in the small intestine and microbial degradation in the cecum and colon (31).

Readily degradable carbohydrate contribution to total energy digested in the small intestine is little, because more than 90% of this fraction will disappear in the reticulo-rumen. Non-structural carbohydrates will contribute most to total energy depending on the type of diet fed (49).

Results with sheep indicate that molar proportions of VFA measured in the rumen fluid are in broad agreement with the molar proportions that are produced. This agrees best with

| FOOD CONSTITUENTS | DIGESTION | | |
|---|---|---|---|
| | IN RUMEN | IN SMALL INTESTINE | IN CAECUM AND COLON |
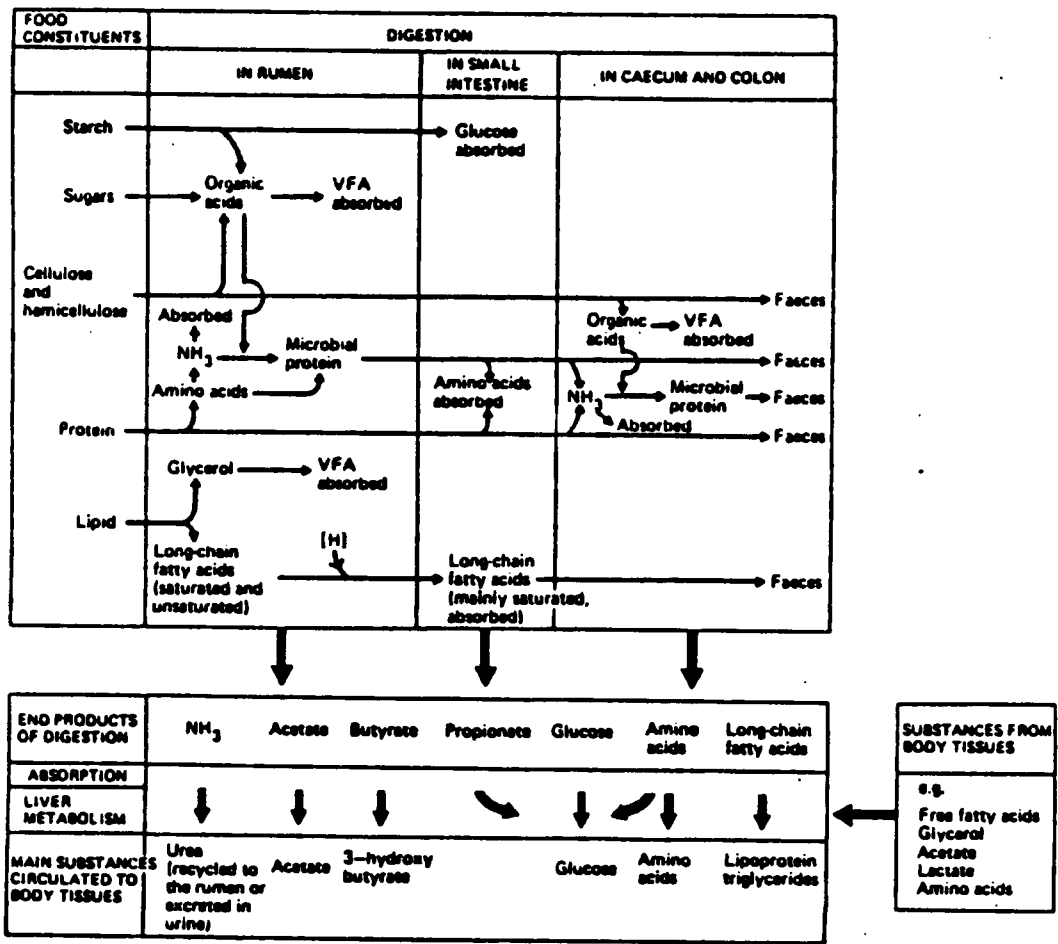
Figure 1. Different metabolic pathways taking place during degradation and absorption from the gut and transfer to hepatic tissues of the products of rumen fermentation

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LITERATURE REVIEW

high forage diets. Results with cattle are more variable than with sheep and some of this variation reflects differences in production rates of molar proportions of VFA and extent of ruminal fermentation of diets (56).

## Volatile Fatty Acids.

Acetate is metabolized very little during absorption, but butyrate is completely converted to 3-hydroxybutyrate.

During early lactation when fatty acids are being mobilized from adipose tissue a greater proportion of acetate is de-rived  from this endogenous source. The breakdown of long chain fatty acids in the liver may also lead to an increase in the endogenous production of 3-hydroxybutyrate (59).

A small proportion of propionate is converted to lactate (17), but it is  recognized that propionate is the major glucose precusor. Even the quantity of amino acids used for gluconeogenesis is dependent on the supply of propionate up-take from the rumen (17).

## Glucose

Lactating cows derive little if any, glucose from the diet so they must depend on gluconeogenesis to meet requirements

for milk production and other glucose demanding processes (61).

Generally the more propionate presented to the liver the more glucose is produced (15). ·However there seems to be a limit to conversion of propionate to glucose as well as differences in the metabolism of glucose and propionate (17). Infusion of glucose and propionate to lactating and non-lactating cows have shown that hepatic glucose production decreased in lactating cows when glucose was administrated but did not when propionate was used. Also there were no differences in glucose production when both substrate were infused to dry cows (38). In another study in which barley and corn were compared for energy, milk production increased with the barley diet relative to corn. The differences were associated with increased propionate with the barley diet as compared with increased glucose with the corn diet (56).

Results of continuous intraabomasal or intradoudenal infusion of glucose indicated that 1 to 1.5 kg can be absorbed daily, although infusion of 400 to 500 g over short periods of time (up to 4 h) indicate an apparent absorption of only 75% (56).

# LITERATURE SUMMARY AND RESEARCH OBJECTIVES

It is evident from the literature reviewed that the source of nitrogen fed is quite important, especially during early lactation. At that point requirements of high producing cows are not being met due to metabolic limitations and feed intake. Thus, in order to maximize production, it may be important to include in the diet of these animals a source of nitrogen that will escape rumen degradation which should supply more amino acids for absorption from the small intestine. However, feeding ruminally resistant N sources has not always shown the expected increase in animals productive performances. Feeding fishmeal N sources has more consistently improved production than other protein sources. Cows in negative energy balance have responded more dramatically to fishmeal and the response declined as cows advanced into lactation toward a positive energy balance.

The present study had two main objectives: 1) Determine response in milk production, milk composition and certain physiological measurements due to substitution of SBM by fishmeal.

2) Determine any possible interaction between energy (TDN levels) and N protein source on milk production and composition of cows at 90-100 days in lactation.

# MATERIALS AND METHODS

## ANIMALS

Thirty multiparous Holstein and twelve multiparous Jersey cows (90 15 days in lactation) were chosen from the University dairy herd. Assigned cows entered the experiment on Tuesday over a period of eight consecutive weeks, (Appendix Table 1). Cows were stratified by anticipated production then randomly assigned equally to treatments. Tie stalls were used to feed and house the Holsteins and free stalls with access to Calan Doors was used to house Jerseys.

## DIETS

Six different diets (3x2 factorial) contained either 70 or 75 % total digestible nutrients (TDN) and soybean meal (SBM) replaced with either 0, 1 or 2 kg of fishmeal per 100 kg of dietary dry matter. These were fed twice a day (6 A.M. and 1 P.M.) as total mixed rations. Forage:concentrate ratio was 82:18 and 60:40 respectively (Table 1).

Table 1.  Diet Composition

| Ingredients[a] | TDN | 70% | | | 75% | | |
|---|---|---|---|---|---|---|---|
| | Fishmeal[b] | 0 | 1 | 2 | 0 | 1 | 2 |
| Corn silage | | 82.7 | 83.5 | 82.4 | 60.0 | 60.0 | 60.0 |
| Soybean meal | | 7.0 | 4.2 | 3.3 | 5.8 | 3.0 | 0.0 |
| High moisture corn | | 0.0 | 0.0 | 0.0 | 23.9 | 24.7 | 25.7 |
| Fish meal (FM) | | 0.0 | 2.0 | 4.0 | 0.0 | 2.0 | 4.0 |
| Concentrate[c] | | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 | 10.3 |

[a]
   % Dry matter basis
[b]
   Approximate intake of fishmeal per cow daily
[c]
  See Table 2 for concentrate composition

Table 2. Concentrate Composition.

| Ingredients | % of D.M. | % of C P | % of TDN |
|-------------|-----------|----------|----------|
| Soybean meal | 53.6 | 50.2 | 100 |
| Urea | 9.1 | 49.8 | - |
| Vit-Min mix * | 33.3 | - | - |

| * Mineral-Vitamin | %; ppm; I.U. |
|-------------------|--------------|
| Calcium | 13.5% |
| Phosphorus | 6.25 |
| Magnesium | 2.25 |
| Sulfur | 1.70 |
| Sodium bicarbonate | 25.0 |
| Sodium Chloride | 12.5 |
| Vitamin A ( IU/kg) | 88,000 |
| Vitamin D ( IU/kg) | 25,000 |

## SAMPLING

Individual feed ingredients as well as TMR were sampled weekly and stored at -20 C for further analysis. Milk samples (A.M. and P.M.) were collected biweekly and quantified for fat and protein content by infrared analysis in a 4 channels automated milk analyzer (Multispect M model). Monthly production records were revised and average milk production were calculated.

Blood from the tail vein was taken at 4, 6 and 8 wk (3.5 h after the 1 P.M.feeding) into evacuated plastic tubes containing 0.1 ml of heparin. Tubes were then placed in iced containers for transit to the laboratory. There, samples were centrifuged for 20 min at 5000 xg at 0 C to separate plasma (Beckman Model J2-21). After aspiration plasma was stored at -20 C awaiting analysis.

Individual cow feed intake and bodyweight were recorded 4 d a week (Monday-Friday) and every two weeks respectively.

## LABORATORY ANALYSIS

Frozen feed samples were ground with dry ice in a Wiley mill to pass a 5mm screen. After thorough mixing duplicate samples were measured for Kjeldahl nitrogen and nitrogen solubility in a potassium phosphate buffer according to Crooker et al (11).

For acid and neutral detergent fiber determinations sub-samples of frozen ground feeds were dried at 50 C and further ground to pass 1 mm screen. NDF determination was done by a modified Van Soest procedure with 2 g of amylase added after samples have been in the refluxing apparatus for .5 h (62).

Absolute dry matter was determined for all samples by placing 2 g in a forced air oven at 100 C for 24 h and recording weight loss.

Rumen degradability of nitrogen and dry matter were estimated for TMR by the in situ bag method (35).Ten g (as fed basis) of total mixed rations were placed in duplicate in nylon bags (10 x 20 cm, 50-70 ug pore size) to provide a surface exposure of 16-22 mm /mg. Residence time in the rumen was 72, 24, 12, 6, 2, and 0 h. Immediately after removal from fistulated cows, bags were rinsed with warm water and brought to the laboratory. Individual bags were placed in plastic container and washed overnight with running tap water. Bags were then opened and placed in a forced air oven at 50 C for 48 h. Dried contents were ground to pass a 1mm screen.

Degradability  of dry matter and nitrogen were then deter-
mined by using the following method (4).

Total dry matter= A + B + C

Total nitrogen =  A + B + C

A= fraction readily degraded

B= fraction degraded at a measurable rate

C= fraction not degraded

Fraction C was determined for dry matter and nitrogen as
follows.

$$C = (\text{72h DM residual/ original DM})$$

$$C = \frac{\text{72h DM residual} \times \text{\% crude protein of residual}}{\text{Original DM} \times \text{\% crude protein of diet}}$$

Fraction B was estimated by calculating the natural log of
the different time  intervals and constructing the regression
line from which the intercept and slope of the regression
were calculated

$$\ln \left[ \frac{\text{residual N,DM}}{\text{original N,DM}} - C \right]$$

With these values fraction B was estimated by derivation from the equation that measures fraction of feed remaining in the rumen at any time (FFRR).


FFRR= Be + C ln (FFRR - C ) = lnB-kt

   intercept B = 2.3


A= 1 - B - C



Degradability (N, DM) = A + ( B x Kd) / (0.05 + Kd)


where Kd=slope of the line

 0.05 = constant (rate of ruminal turnover   ).


Plasma samples were thawed in water bath at 40 C for 1 min and one ml of plasma was deproteinized with 9 ml of a solution containing one part of 10% sodium tungstate and nine parts of 0.092 N sulphuric acid. The resulting filtrate (Whatman # 42) was used for quantification of plasma urea according to Coulombe and Favreau (14).


STATISTICAL ANALYSIS


Data were analyzed using the general linear model (GLM) of the statistical analysis system package SAS (49).  Milk, fat,

protein, dry matter intake, and body weight were analyzed by an analysis of variance with a split plot design. Main treatment effects (fishmeal and TDN) were tested using the mean square of cow nested within interaction of fishmeal TDN and breed. A regression equation was calculated and used to determine effect of fishmeal upon fat (%) of milk. Fat = 3.866-(0.2913 *FM)- [(-0.03222) (FM)]. Effect of week as well as interaction of week with fishmeal and TDN were tested by the mean square of the error term (see Appendix Table 2 for an example of ANOVA).

Data from the in situ bag technique were analyzed using linear regression to calculated degradability values of the different fractions.

## RESULTS AND DISCUSSION

## MILK PRODUCTION

Mean treatment values for milk production and milk fat and protein content are given in Table 3. Milk and 4% FCM production were not significantly different for any of the six diets fed. However milk yield tended to be greater (on the average 1.5 kg) with higher TDN rations.

Figure 2 shows that there was considerable fluctuation in milk production for both levels of TDN and fishmeal during the experiment. Production declined during the last 4 weeks of the study, but production of cows on the higher TDN rations was nearly maintained. A production decline of approximately 8 to 10% per month is normal (22). FCM production followed no particular pattern due to the differences in fat measurements.

## MILK COMPOSITION.

Milk fat percentage was significantly different (P<.05) due to fishmeal inclusion in the diet (Table 3).
A regression equation calculated and plotted for the three different levels showed that increasing fishmeal linearly decreased fat percentage (Figure 3).

Table 3.  Milk production and composition of cows fed
          different amounts of TDN and fishmeal.

|              | Diets[1] | | | | | |
|--------------|------|------|------|------|------|------|
| Trait        | 1    | 2    | 3    | 4    | 5    | 6    |
| Milk kg/d    | 24.60 | 26.50 | 26.50 | 27.30 | 27.60 | 28.11 |
| FCM kg/d     | 24.70[a] | 25.60[b] | 25.11[b] | 27.40[a] | 26.04[c] | 25.60[d] |
| Fat %        | 3.83[a] | 3.60[a] | 3.58[b] | 3.83[c] | 3.38[a] | 3.18[b] |
| Protein %    | 3.30 | 3.25 | 3.17 | 3.60 | 3.30 | 3.11 |
| Fat kg/d     | 0.87[a] | 0.88[b] | 0.86[a] | 0.97[b] | 0.88[b] | 0.83[b] |
| Protein kg/d | 0.78 | 0.83 | 0.79 | 0.93 | 0.87 | 0.85 |

[1]
  See table 2 for diet description
  Means with different superscripts are significant at $P<.05$
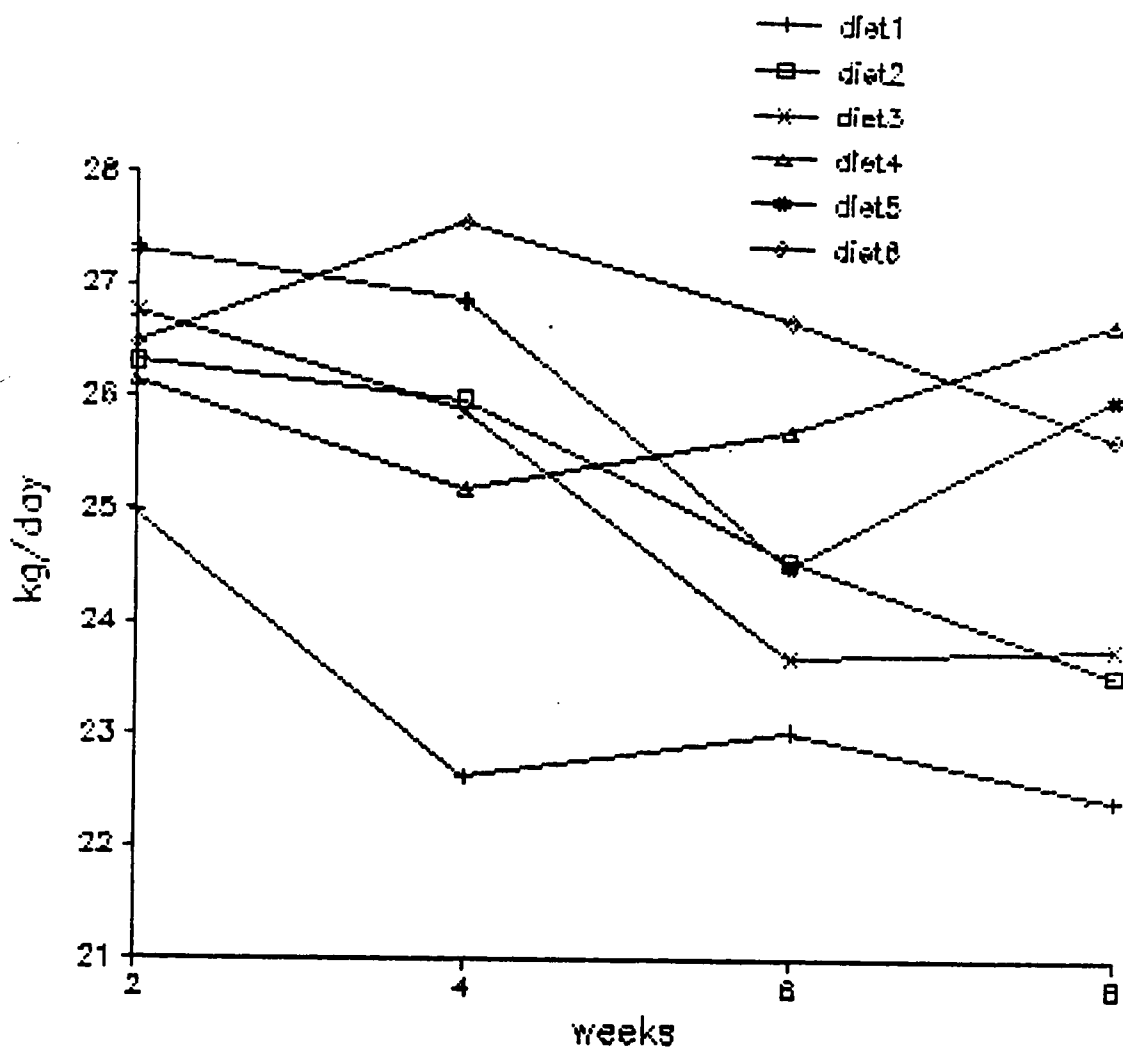
**Figure 2. Effect of fishmeal and TDN levels on milk production.**

Figure 3 also shows that there was a trend toward less fat % produced with higher TDN diets, but no statistical difference was found. Weight of fat produced was similar for all diets. Only diet 4 (75% TDN and 0 kg FM) produced .1 kg more of fat than the other 5. The difference was due to more milk or more fat percentage, depending on the comparison.

The drop in fat percentage due to inclusion of fishmeal in diets of lactating cows has also been reported by Oldham (41) who fed fishmeal to substitute for urea-N sources. Cows in mid lactation produced less fat percentage when fed fishmeal diets. Fishmeal may exert its effect on milk fat because the presence of fish oils may adverely affect rumen fermentation or may alter lipid metabolism in liver or mammary tissue. Reduction of fat percentage could have also been in part due to lower amount of fiber in the higher TDN diets. But as mentioned before, no interaction between fishmeal and TDN levels was found.

In general, raising the proportion of concentrate in diets to approximately 60% of ration dry matter increases milk yield and feed intake of cows in early lactation. Milk fat test decreases as grain feeding increases (23).
Milk protein percentage was significantly different (P< .05) for TDN, fishmeal and interaction of fishmeal and TDN (Table
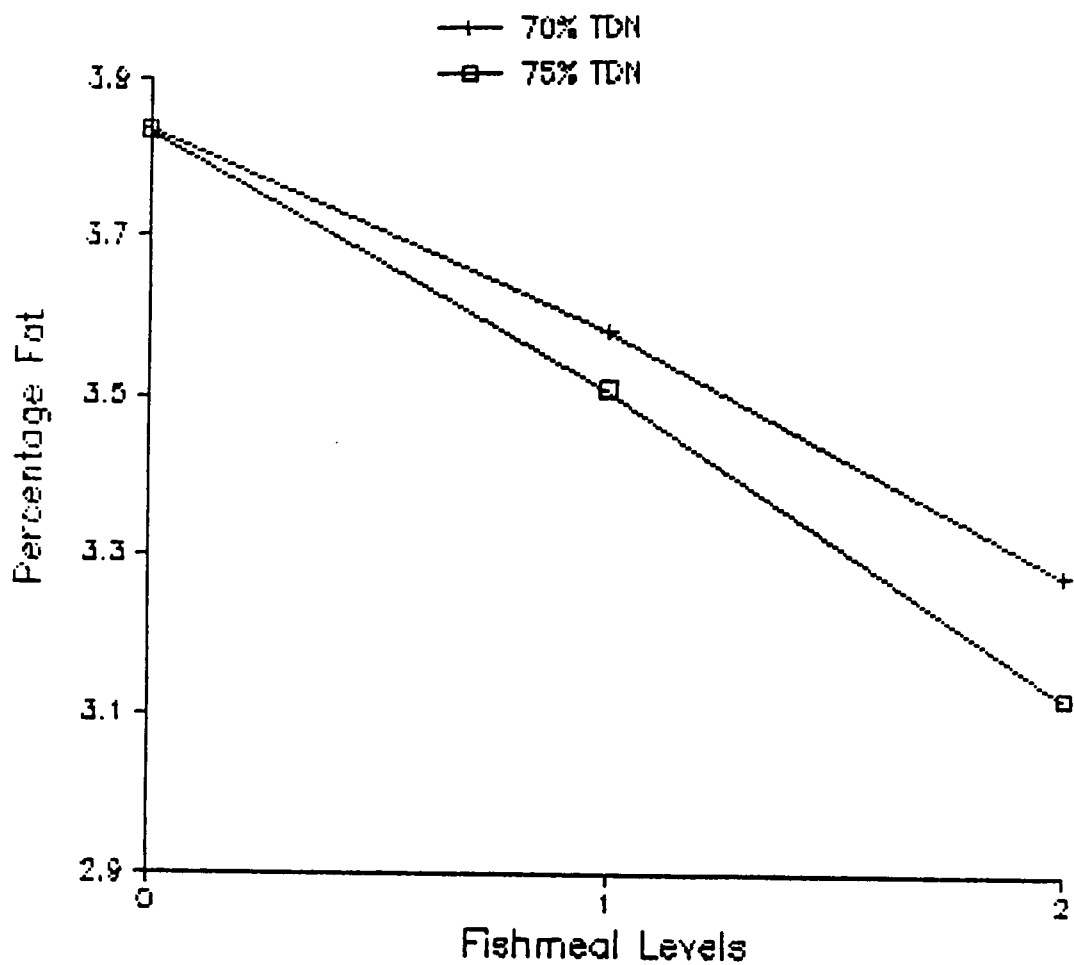
Figure 3. Effect of fishmeal levels on milk fat production.

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RESULTS AND DISCUSSION                                    33

Table 4.  Effect of TDN and fishmeal and interaction of
         TDN x fishmeal levels on milk protein percent-
         age.

| Fishmeal[1] | 70% TDN | 75% TDN |
|---|---|---|
| 0 | 3.4[a] | 3.9[b] |
| 1 | 3.3[a] | 3.5[a] |
| 2 | 3.3[a] | 3.3[a] |

[1]
 kg/100 kg DM
Values are least square means
letters with different superscript are significantly different
at P <.05.

4). However, amount of protein produced was significantly different only for 75% TDN level (Figure 4).

The response of milk protein to increasing levels of energy has also been reported by Broster (8). On the other hand responses of milk protein to N levels and sources have varied. Feeding protected soybean (16% CP) increased protein during 3 to 6 wk of lactation compared to unprotected soybean. The reverse effect was observed during 7 to 10 wk and 11 to 16 wk of lactation (18). Cows in this study were in later lactation and responded somewhat like the cows in later stages of the cited study.

## DRY MATTER INTAKE

Daily dry matter intakes (kg/d) for the total period and for the six different diets are given in Table 5. Effects of TDN and fishmeal on dry matter intake are in Figure 5. Effect of fishmeal levels are in Figure 6. There was significant differences (P<.05) due to TDN effect on dry matter intake but no differences were found for fishmeal levels or interaction of TDN x fishmeal. Diet 4 (75% TDN and zero kg FM) showed the greatest intake, whereas diet 3 (70% and 2 kg FM) showed the least intake (Figure 5).

Dry matter intake fluctuated throughout the experiment (Figure 5), but no differences were found when data was ana-

RESULTS AND DISCUSSION                                          35
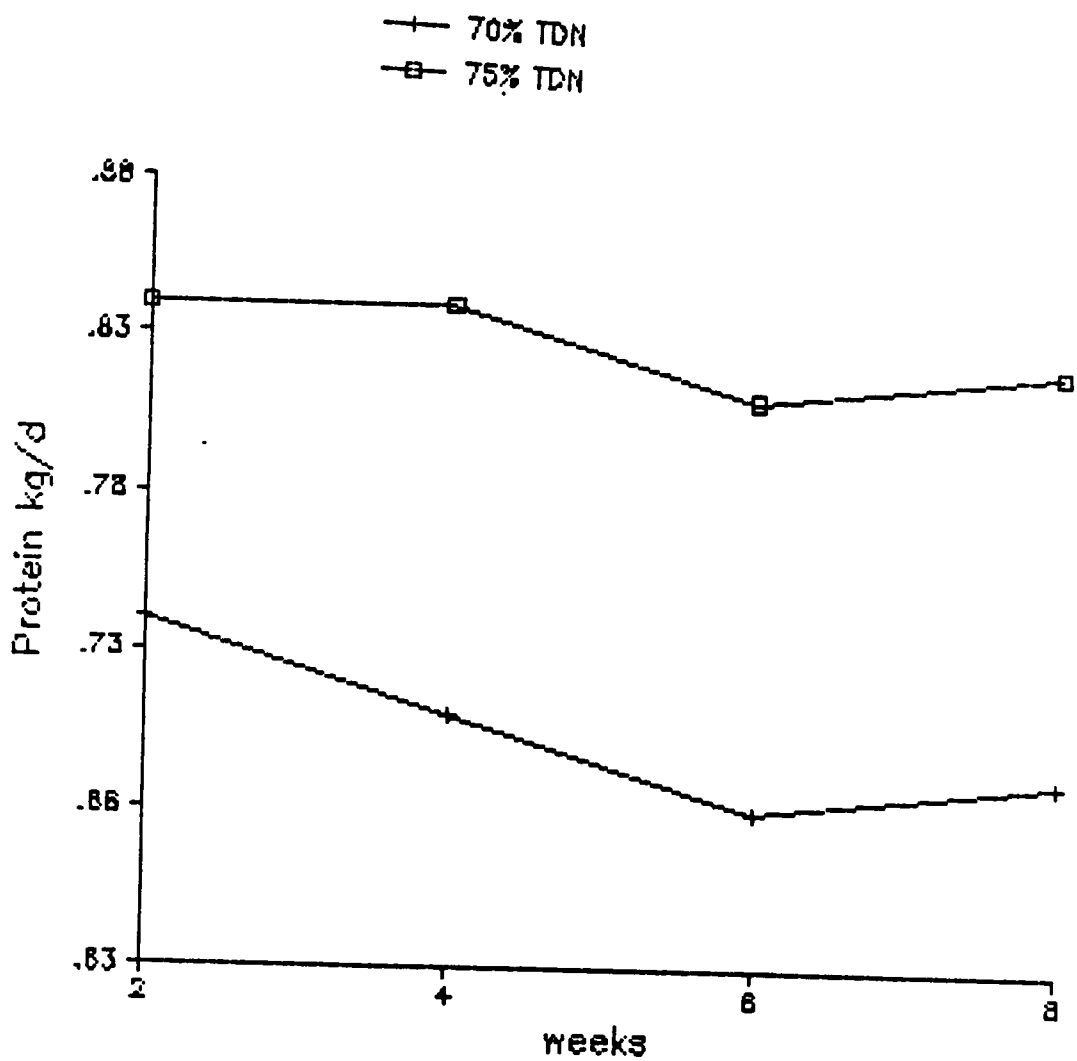
Figure 4. Effect of TDN levels on milk protein pro-
         duction (kg/d).

RESULTS AND DISCUSSION

Table 5. Least square means of weekly dry matter intake for cows fed different amounts of TDN and fishmeals.

| Weeks | Diets[1] | | | | | |
|-------|------|------|------|------|------|------|
|       | 1    | 2    | 3    | 4    | 5    | 6    |
| 1     | 16.0 | 16.2 | 15.2 | 17.6 | 16.4 | 17.4 |
| 2     | 17.2 | 17.3 | 16.2 | 19.0 | 17.6 | 17.4 |
| 3     | 17.6 | 18.3 | 15.5 | 19.2 | 17.3 | 17.6 |
| 4     | 17.9 | 17.8 | 16.3 | 18.6 | 17.6 | 17.2 |
| 5     | 16.6 | 17.9 | 16.4 | 18.5 | 17.3 | 16.9 |
| 6     | 16.3 | 17.4 | 16.3 | 18.5 | 17.7 | 17.7 |
| 7     | 16.4 | 16.4 | 16.1 | 18.1 | 17.3 | 17.6 |
| 8     | 17.3 | 17.2 | 17.6 | 18.5 | 16.8 | 17.6 |
| 9     | 17.3 | 17.7 | 17.9 | 18.5 | 17.8 | 18.0 |

[1] see table 2 for diet description

RESULTS AND DISCUSSION
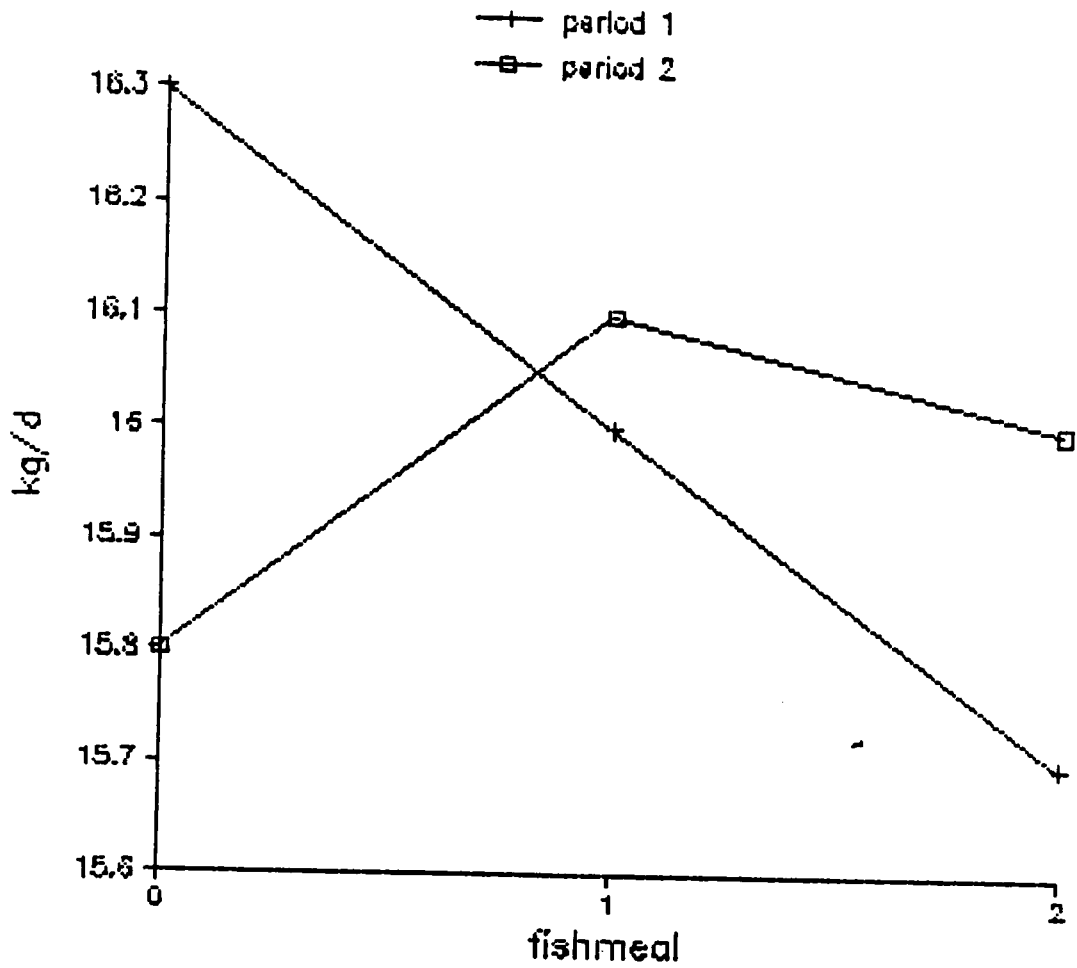
Figure 5.  Effect of TDN and fishmeal levels on dry mat-
ter intake (kg/d).

RESULTS AND DISCUSSION

lyzed as two separate periods, wk 1 to 4 and wk 5 to 9 (Table 6). However, a trend toward a decrease in intake due to fishmeal levels during the first 4 wk of the study existed. Dry matter intake showed an increase during the last 4 wk of the study, however no significant differences were found (Figure 7). A possible explanation for this pattern on dry matter intake is that cows may have gone through a process of adaptation to fishmeal. Oldham reported that cows fed fishmeal diets have significantly (P.<.05) lower intake (41).

## BODY WEIGHT

There were non-significant differences in bodyweight due to the effect of TDN and fishmeal levels, or interaction of TDN x fishmeal. Over all the experiment weight gain occurred for all six diets fed (Figure 6). Weight gain followed a pattern consistent with the intake data (Figure 5).

Table 6.  Least square means of weekly dry matter intake
          kg/d.

                 Period 1                   Period 2
----------------------------------------------------------------
weeks     1     2     3     4     5     6     7     8     9
          a
intake  15.5  16.2  16.2  16.2  15.9  15.9  15.6  16.1  16.4


----------------------------------------------------------------

a
 kg dry matter /day
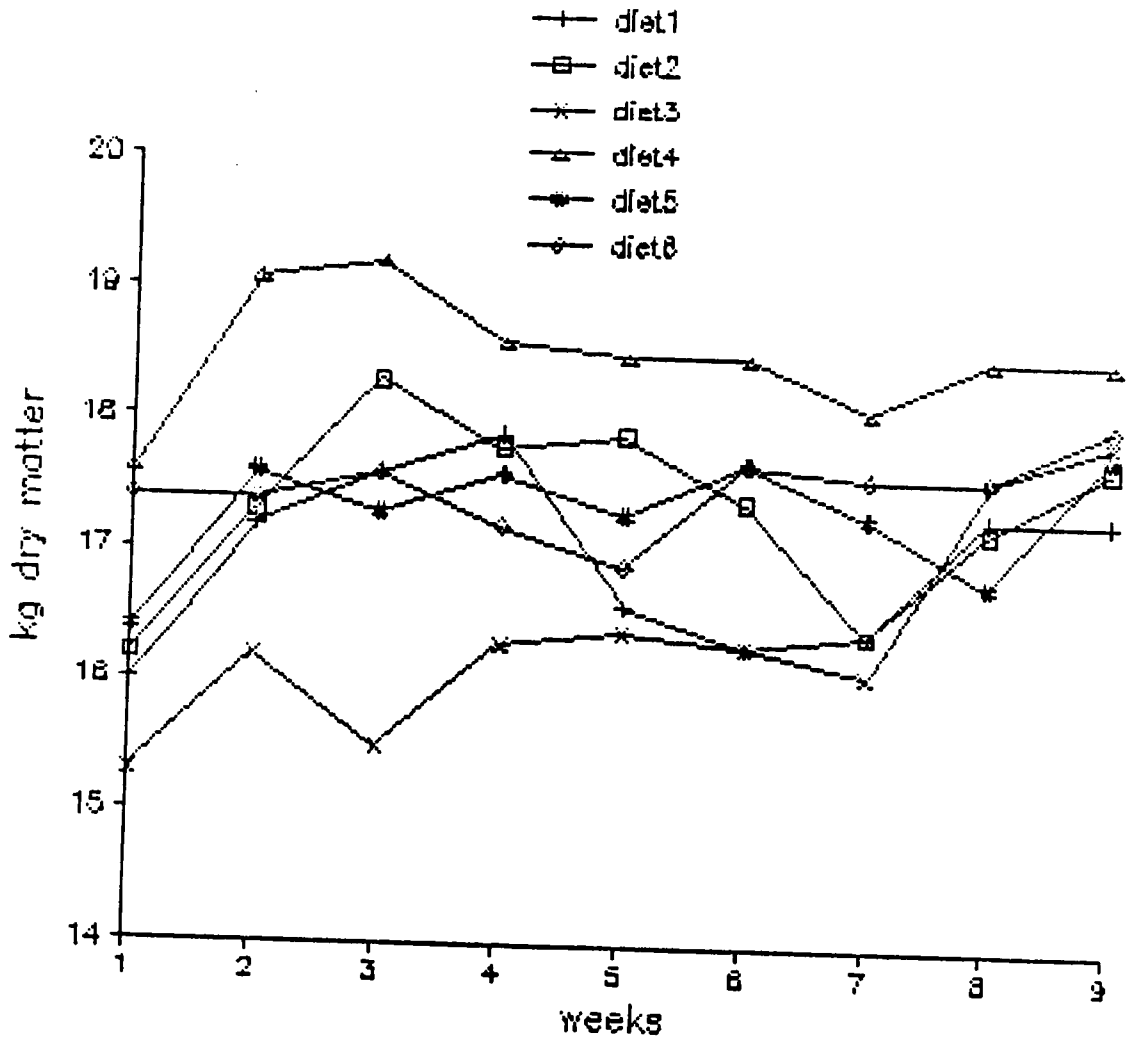
Figure 6.  Effect of fishmeal on dry matter intake.
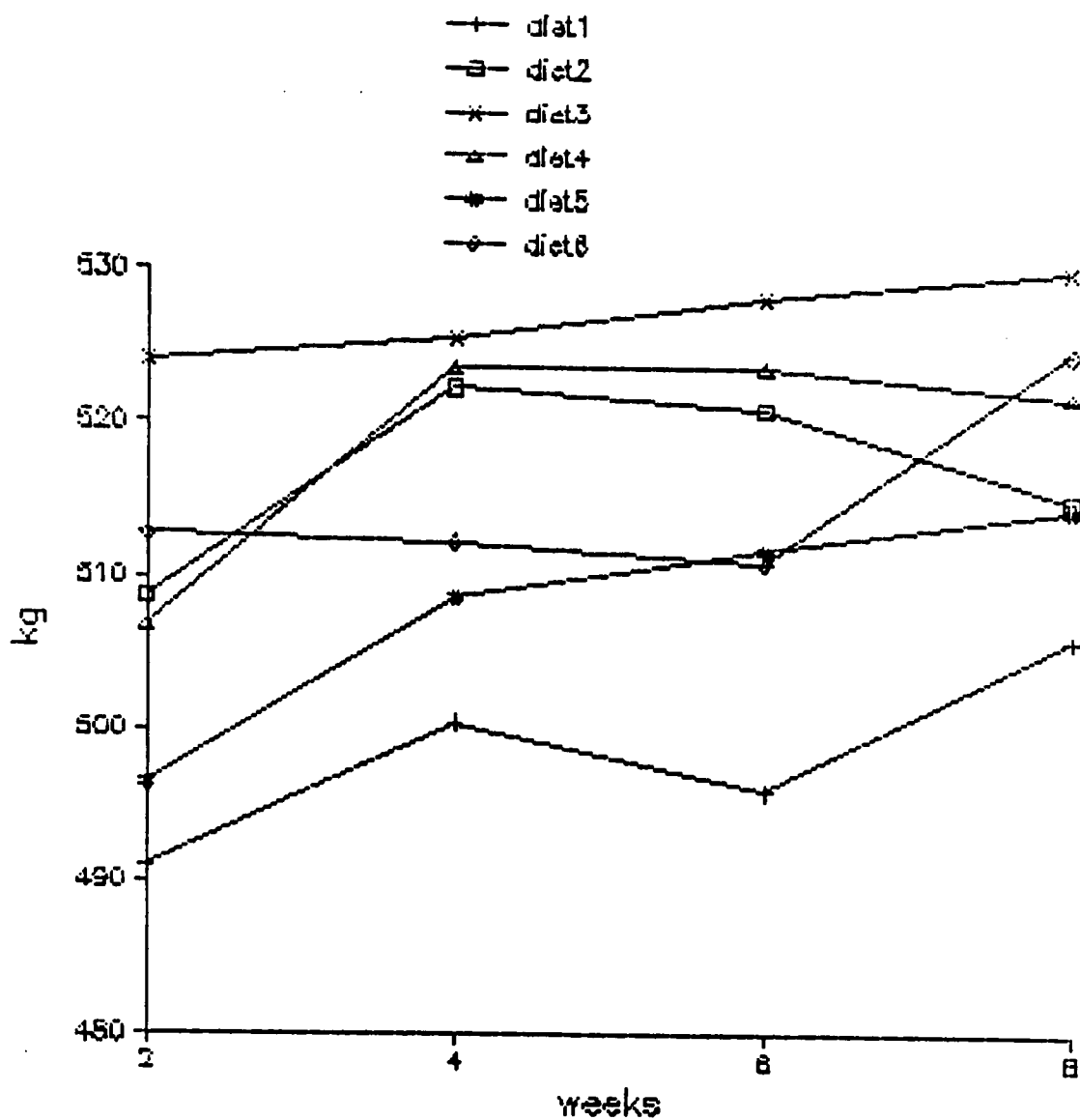
Figure 7. Effect of TDN and fishmeal levels on bodyweight

## PLASMA UREA

Means of plasma urea concentration of cows fed different TDN and fishmeal levels were lower than expected (Table 7) and did not differ among the diets fed.This could suggest that efficiency of nitrogen utilization did not differ.

Another possible reason for these low plasma urea concentrations could be that responses of blood urea to rumen ammonia concentration takes several hours (51). If not enough time is allowed before sampling it could be reflected on the measurements. Nonetheless samples were taken 3.5 h after the afternoon feeding which has been considered adequate time postfeeding

Table 7.  Effects of TDN and fishmeal levels on plasma
          urea concentration.

------------------------------------------------------
                 a
Diets               1 .  2    3    4    5    6
                b
concentration   4.7  4.4  4.3  4.7  4.4  4.4

------------------------------------------------------
a
 See Table 2 for diet description

b
 mg/dl

Results of laboratory analysis of the six total mixed rations (TMR) fed are shown in Table 8. Dry matter, acid detergent fiber (ADF), and neutral detergent fiber (NDF) differed as expected between 70 and 75% TDN diets. These values reflect the effect of adding high moisture corn to higher TDN diets in order to raise the energy content. Dry matter degradability tended to be similar for all diets. But degradability of nitrogen was lower for the higher TDN diets and contrary to expected, Fraction A (readily degradable portion) was lower for higher TDN. This could have been due to greater quantity of SBM in lower TDN diets. Also nitrogen degradability varied with inclusion of fishmeal levels, but it only occurred in the higher TDN rations (Table 8).

Table 8.  Chemical composition of total mixed ration fed.

| Diets [a] | Dry Matter | Crude Protein | Acid Det.Fiber | Neutral Det.Fiber | DM Deg | Nitrogen Deg |
|------|------------|---------------|----------------|-------------------|--------|--------------|
| 1 | 39.05 | 14.60 | 22.80 | 37.60 | 65.3 | 76.1 |
| 2 | 39.60 | 15.10 | 23.20 | 35.60 | 64.4 | 73.3 |
| 3 | 40.50 | 15.20 | 22.70 | 34.70 | 63.6 | 72.8 |
| 4 | 45.00 | 15.30 | 19.20 | 31.00 | 66.2 | 70.2 |
| 5 | 44.80 | 15.10 | 19.00 | 29.40 | 66.7 | 67.1 |
| 6 | 44.90 | 14.70 | 18.60 | 27.00 | 64.0 | 65.9 |

[a]
  see Table 2 for diet composition
[b]
  as a % of dry matter
[c]
  dry matter degradability In situ method.

## GENERAL DISCUSSION AND CONCLUSIONS

Most of the experiments concerning feeding of ruminal resistant protein sources to high producing cows have been conducted during early stages of lactation when animals are in negative energy balance and demands for energy and protein are high.

Few experiments have tested the effect of protein sources beyond early lactation and only limited reports of interrelationship between energy and protein can be found.

In this particular study an attempt was made to determine the effect of partially replacing soybean meal with fishmeal on milk production and composition of milking cows fed diets differing in forage to concentrate ratio and TDN.

Results obtained show that there was no effect on milk and 4% FCM production due to inclusion of fishmeal in the diet. Cows fed higher TDN level diet tended to produce more milk though no statistical differences were found.

On the other hand milk composition did show a response to levels of TDN and fishmeal. An inverse relationship between levels of fishmeal and fat percentage was found (P<.05). Although not statistically measurable, a negative inter-

action effect of fishmeal and higher TDN levels on fat percentage also existed, which is in agreement with others (41).

Milk protein production responded to higher TDN levels.However there was response to inclusion of fishmeal which contradicts literature reports (36, 41, 11). Less degradable protein sources has increased milk protein, though most of the reports have been with animals in early stages of lactation. The response to higher TDN intake could be related to an increase of protein escaping rumen degradation mediated by an increase in dry matter intake as appeared the case with this study.

Dry matter intake was higher and nitrogen degradability lower for diets with 75% TDN (P<.05) compared to 70 % TDN. A possible explanation is that a higher flow from the rumen occurred due to particle size reduction. Diets containing 75% had more grain and with corn silage as the only source of forage, movement from the rumen could have been faster.

From these observations it was concluded that substitution of soybean meal with fish meal did not produce an increase in milk yield in cows with modest demands.

Milk composition seems to be affected by energy levels and the interaction with fishmeal tends to reduce both milk fat and protein yield.

Finally responses to both TDN and fishmeal levels were not clear. Perhaps different results could be obtained with animals in early stages of lactation

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# APPENDIX A.  APPENDIX

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Table 9.  Appendix.Table 1.Schedule of cows assignment
          to experiment (Diets 1, 2 and 3).

| Diet | Breed | Cow # | Date started | Days in lactation |
|------|-------|-------|--------------|-------------------|
| 1 | H | 1535 | Nov/ 6/84 | 100 |
| 1 | H | 1518 | Dec/18/84 | 90 |
| 1 | H | 1645 | Jan/29/85 | 90 |
| 1 | H | 1544 | Jan/29/85 | 95 |
| 1 | H | 1430 | Jan/29/85 | 95 |
| 1 | J | 1898 | Dec/11/84 | 80 |
| 1 | J | 1894 | Dec/18/84 | 75 |
| 2 | H | 1528 | Nov/ 6/84 | 95 |
| 2 | H | 1640 | Dec/25/85 | 95 |
| 2 | H | 1625 | Jan/21/85 | 90 |
| 2 | H | 1517 | Jan/29/85 | 93 |
| 2 | H | 1569 | Jan/29/85 | 92 |
| 2 | J | 1900 | Nov/27/84 | 75 |
| 2 | J | 1896 | Dec/11/84 | 75 |
| 3 | H | 1426 | Nov/ 6/84 | 80 |
| 3 | H | 1442 | Nov/27/84 | 91 |
| 3 | H | 1461 | Dec/11/84 | 97 |
| 3 | H | 1499 | Dec/25/84 | 95 |
| 3 | H | 1549 | Jan/29/85 | 90 |
| 3 | J | 1897 | Dec/ 4/84 | 75 |
| 3 | J | 1902 | Dec/11/84 | 75 |

Table 10.  Appendix.Table 2.Schedule of cows assignment
          to experiment (Diets 4, 5 and 6).

| Diet | Breed | Cow # | Date started | Days in lactation |
|------|-------|-------|--------------|-------------------|
| 4 | H | 1412 | Nov/12/84 | 75 |
| 4 | H | 1632 | Dec/18/84 | 96 |
| 4 | H | 1414 | Jan/22/85 | 92 |
| 4 | H | 1467 | Jan/22/85 | 92 |
| 4 | H | 1560 | JAN/29/85 | 96 |
| 4 | J | 1578 | Nov/ 1/84 | 100 |
| 4 | J | 1901 | Dec/11/84 | 75 |
| 5 | H | 1384 | Nov/27/84 | 80 |
| 5 | H | 1513 | Dec/18/84 | 91 |
| 5 | H | 1650 | Jan/22/85 | 96 |
| 5 | H | 1520 | Jan/22/85 | 90 |
| 5 | J | 1651 | Dec/ 4/84 | 80 |
| 5 | J | 1893 | Jan/ 8/85 | 75 |
| 6 | H | 1447 | Nov/13/84 | 90 |
| 6 | H | 1415 | Nov/20/84 | 90 |
| 6 | H | 1657 | Dec/11/84 | 92 |
| 6 | H | 1444 | DDc/25/84 | 95 |
| 6 | H | 1652 | Jan/22/85 | 95 |
| 6 | J | 1895 | Nov/1 /84 | 95 |
| 6 | J | 1539 | Jan/15/85 | 80 |

Table 11.  Appendix Table 3.Anova for milk fat percentage

| Source | D. F. | Type I SS | F value | PR> F |
|--------|-------|-----------|---------|-------|
| breed | 1 | 123.38 | 841.56 | 0.0001 |
| fishmeal | 2 | 6.65 | 22.67 | 0.0001 |
| energy | 1 | 1.52 | 10.35 | 0.0017 |
| fishmeal*energy | 2 | 1.14 | 3.90 | 0.0230 |
| fishmeal*breed | 2 | 2.61 | 8.90 | 0.0003 |
| fm*energy*breed | 2 | 0.91 | 6.22 | 0.0142 |
| cow(fm*energy*breed) | 31 | 19.43 | 4.28 | 0.0001 |
| week | 3 | 0.53 | 1.21 | 0.3082 |
| week*fishmeal | 6 | 0.46 | 0.53 | 0.7856 |
| week*energy | 3 | 0.58 | 1.32 | 0.2709 |
| week*fm*energy | 6 | 0.77 | 0.88 | 0.5115 |

TEST OF HYPOTHESIS USING THE TYPE III MS FOR
COW(FM*ENERGY*BREED)

| Source | D. F. | Type III S S | F value | PR> F |
|--------|-------|--------------|---------|-------|
| Fishmeal | 2 | 9.19 | 7.30 | 0.0025 |
| Energy | 1 | 0.31 | 0.49 | 0.4911 |
| Fishmeal*energy | 2 | 1.20 | 0.95 | 0.3979 |

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