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EFFECT OF PROTEIN LEVEL AND BYPASS ON MILK PRODUCTION OF COWS FED TOTAL MIXED RATIONS

A Thesis

Presented for the

Master of Science

Degree

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Paula Joan Gaynor

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ABSTRACT

Feed costs represent an estimated fifty to sixty percent of total operating costs of most dairy operations. Cost of protein supplements has traditionally constituted a large percentage of total feed costs, which economically justifies research conducted with the purpose of evaluating the adequacy of low cost protein supplements in meeting dietary protein requirements for milk production.

Three experiments involving a total of 67 Holstein cows in the University of Tennessee Dairy Herd were conducted to determine the adequacy of pelleted protein supplement treated with inorganic water soluble salts (Trial 1), or supplemental dried brewer's grains (Trials 2a and 2b), in supporting milk production when fed with corn silage as a total mixed ration.

Trial 1 was conducted in cooperation with the Central Soya Feed Company of Decatur, Indiana, and involved 27 cows, 24 to 125 days post-partum, arranged in a switch-back design with three treatments and three experimental periods. Treatments consisted of varying concentrate composition of the total mixed ration as follows: 1) 18 % protein, untreated; 2) 15 % protein, treated with inorganic water soluble salts; and 3) 15 % protein, another (distinct) treatment of protein source with inorganic water soluble salts. Cows were fed ad libitum total mixed ration, plus 2.3 kg mixed alfalfa-grass hay per cow per day. Total mixed ration, corn silage, hay, milk, and blood samples were collected and analyzed.

Rations two and three contained substantially higher zinc and iron contents than ration one. Total mixed ration protein levels were 15.0 % (1), 12.6 % (2), and 13.0 % (3). Total dry matter intake, body weight

change, and intake as a percent of body weight were not significantly different ($p>.10$) for the three rations. Milk, fat, and 4 % fat corrected milk, total solids, milk protein, solids-not-fat, and somatic cell counts were not different for the three rations. Plasma and milk zinc were higher for rations two and three ($p<.05$). The 15 % treated protein concentrate used in rations two and three supported milk production equally as well as the 18 % protein concentrate used in ration one.

In Trials 2a and 2b, the milk production and milk composition response to added dietary protein in the form of dried brewer's grains was evaluated. Ration one consisted of the standard milking herd ration composed of 2 parts corn silage and 1 part pelleted 16 % protein supplement blended and fed ad libitum, plus 5.6 kg mixed alfalfa-grass hay per cow per day. Ration two was the same as ration one, plus 2.3 kg per cow per day of dried brewer's grains added to the mixed rations. All cows received a mineral mixture of 1 % lime, 0.7 % MgO, dicalcium phosphate and trace mineral salt in the total mixed ration. Twenty cows within 2 weeks postpartum were assigned to either ration one or to ration two for a minimum of 11 weeks (Trial 2a) or 15 weeks (Trial 2b). All cows were offered 5.6 kg alfalfa-grass hay per day throughout Trials 2a and 2b.

Ration samples were available for Trial 2b only. For this reason, dry matter and protein contents of rations in Trial 2a were assumed to be the same as those found for samples collected in Trial 2b. Milk production and feed intakes and refusals were recorded daily.

Total dry matter intake, total protein intake and milk produced were significantly greater for the control ration, while fat corrected milk produced was not significantly different between the rations (Trial

2a). Contrary to Trial 2a, total dry matter intake, milk and fat corrected milk were greater ($p<.05$) for ration two in Trial 2b, while total crude protein intake was greater for ration one. Unaccounted for differences in ration composition, especially crude fiber content, or environmental differences may be responsible for the inconsistent results obtained between the two trials. The tendency for greater fat corrected milk production on ration two is consistent with the expectation that brewer's dried grains provide an increased supply of amino acids postruminally for absorption, thus enhancing the nutritional value of the total mixed ration consumed by the cow.

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CHAPTER I

INTRODUCTION

Feed costs represent an estimated 50 to 60 percent of total operating costs for most dairy operations. As protein is the most expensive nutrient in the diet of dairy cattle, more efficient utilization of this nutrient by cows would allow the dairyman a possibility of decreasing total feed costs, while maintaining production levels.

The fermentative nature of the ruminant digestive process allows for efficient utilization of high fiber and non-protein nitrogen containing rations. Some low quality plant proteins may be upgraded in quality by rumen microorganisms (Satter and Roffler, 1975). Microbial protein provides nearly all of the protein needed in animals with low protein requirements (Satter et al., 1977). However, at peak lactation and with high feed intakes, the maximum amount of degradable protein which can be utilized by rumen microbes may be less than 60 % (Satter and Roffler, 1975).

Although microbial protein can supply the protein requirements of lactating cows in most situations, some dietary protein sources may already be of greater biological value to the animal than microbial protein, thus protection of these proteins from microbial degradation proves more efficient overall. One method of meeting the protein requirement of high producing dairy cows is to protect high quality protein from degradation by rumen microbes, while maintaining overall digestibility.

Treatment of protein sources with heat, formaldehyde, or protective coatings are viable methods of reducing rumen degradability of ingested protein. Some by-product feeds, such as brewer's dried grains (e.g., processed barley), have excellent inherent rumen bypass qualities.

The objective of Trial 1 was to compare milk production and milk composition response of cows fed total mixed rations containing corn silage and an 18 % protein concentrate with total mixed rations containing 15 % protein concentrates that had been treated (two distinct treatments) with inorganic water soluble salts to increase rumen bypass of protein.

The objective of two other trials conducted was to compare milk production and composition of early lactation cows fed a basal ration of corn silage and 16 % protein concentrate with that of cows fed the basal ration plus added brewer's dried grains.

CHAPTER II

REVIEW OF LITERATURE

1. PROTEIN UTILIZATION IN DAIRY CATTLE

Introduction

Ruminants rely upon a fermentative type digestive process which enables them to utilize dietary non-protein nitrogen (NPN) efficiently, and to upgrade low quality dietary protein to higher quality microbial protein (Satter and Roffler, 1975; Leng and Nolan, 1984). Rumen microorganisms have a tremendous influence on digestive processes in the rumen and on the profile of digesta leaving the rumen (Satter and Roffler, 1975). High quality (in terms of amino acid content) protein may be reduced in quality by rumen microorganism degradation under some dietary conditions, especially when high protein rations are fed (Satter and Roffler, 1975). Protein presented to the small intestine for digestion and absorption consists of microbial protein synthesized in the rumen, and dietary true protein unaltered by rumen microbes (Satter et al., 1977). In animals with low protein requirements, microbial synthesis provides nearly all of the protein needed (Satter et al., 1977). One method of meeting the protein requirement of high producing dairy cows is to increase the relative amount of ruminally undegradable protein reaching the small intestine.

Bypassing ruminal degradation of high quality protein while maintaining the digestibility of protein in the small intestine would benefit production responses if protein requirements of the animal were not being met. Procedures for increasing protein bypass should decrease

dietary protein degradation in the rumen without adversely affecting other aspects of rumen metabolism, and should increase the total supply of non-ammonia nitrogen to the small intestine (Crawford and Hoover, 1984). Theoretically, a combination of urea and a slowly degraded protein source could provide both the rumen microorganisms and the host animal with nitrogen and amino acids of optimum availability while enhancing the utilization of both NPN and natural protein.

Pathways of Nitrogen Metabolism

Pathways of nitrogen utilization in ruminants are qualitatively similar to those of monogastric animals (Hogan, 1975). Nitrogen, both in protein and molecular form, enters the rumen from the diet, from saliva, and to a small extent across the rumen wall (Hogan, 1975; Huber and Kung, 1981). Ingested true protein may be degraded by ruminal microorganisms to ammonia (60 %) or may escape degradation and pass to the lower gut to be digested or excreted in the feces (40 %) (Satter and Roffler, 1975). Rumen microbial enzymes metabolize NPN, and part of protein nitrogen, producing the nitrogenous end products ammonia and amino acids (Hogan, 1975). Ammonia and amino acids are then utilized by rumen microbes to synthesize proteins which, together with NPN components of the microbial cells and undigested protein, pass from the abomasum to undergo enzymatic digestion in the small intestine (Hogan, 1975). The amount of ammonia that can be utilized depends primarily upon the number and prolificacy of bacteria, and therefore upon the energy available (amount of fermentable feed consumed). Excess ammonia is absorbed from the reticulorumen, or passed to the lower gut, where it is absorbed and eventually converted to urea by the liver. Therefore,

the majority of excess dietary ammonia is excreted in urine. Most endogenously produced urea is also execreted in the urine. Thus, three possible sources of amino acids entering the small intestine for digestion and absorption are microbial, dietary, and endogenous protein (Kung et al., 1983).

High producing dairy cows utilize tremendous quantities of dietary protein, energy, and other nutrients for milk synthesis and fetal development each year (Hogan, 1975; Clark, 1975). The most critical period for nutrient supply to the high producing dairy cow is from parturition to peak milk production, which usually occurs between 4 and 10 weeks postpartum (Clark and Davis, 1980). Factors determining peak and persistency of production in dairy cows include genetic merit, environmental stresses, and nutritional adequacy in meeting production demands (Foldager and Huber, 1979; Kronfeld, 1982). Quantities of glucose, amino acids, and acetate extracted from blood by mammary tissue are critical determinants of quantity of milk produced (Clark, 1975). Lactose, synthesized in the mammary gland from glucose and galactose, is considered the primary osmoregulator of total milk volume and quantity of milk protein produced (Kung et al., 1983; Kronfeld, 1982). Therefore, optimal levels of dietary constituents to promote milk synthesis, such as energy and protein, are of utmost concern to dairymen.

The vast majority of glucose found in ruminant blood is synthesized by the liver from propionate and gluconeogenic amino acids (Clark, 1975). The need for amino acids for both protein synthesis and gluconeogenesis becomes critical in early lactation when other tissues

(liver and kidney) become competitive with the mammary gland for carbon skeletons of amino acids for glucose synthesis (Clark, 1975).

Factors influencing uptake of amino acids by mammary tissue include mammary blood flow, arterial concentration of amino acids, and rates of mammary extraction of amino acids from blood (Mepham, 1982; Kronfeld, 1982). The ratio of cationic, anionic, and neutral amino acids in blood may also be of importance in determining mammary uptake rates of amino acids (Baumrucker, 1984).

The value of escape protein increases as the protein requirement of the animal increases, in terms of efficiency of protein utilization. Supplying nutrients postruminally eliminates energy losses associated with fermentation and lowered protein quality resulting from rumen microbial degradation and resynthesis (Clark and Davis, 1980). Infusion of casein (milk protein) postruminally should supply an ideal profile of amino acids to the mammary gland to enhance milk protein production. Increases in milk yield and milk protein content have been observed when casein was infused postruminally (Clark et al., 1973; Derrig et al., 1974; Vik-Mo et al., 1974). Quality, as well as quantity, of amino acids reaching the small intestine of high producing cows in early lactation might limit milk yield (Kung and Huber, 1983).

2. INTERMEDIARY PROTEIN METABOLISM

Introduction

Many factors are involved in protein metabolism by the ruminant, including the type of protein (natural vs. NPN), rumen degradability of that protein, energy to nitrogen ratio, particle size, retention time in the rumen, sufficiency of the diet for microbial protein synthesis, and

physical and chemical alterations of the protein source (Huber and Kung, 1981). Dietary NPN containing compounds may consist of free amino acids, peptides, nucleic acids, free ammonia, ammonium salts, urea, biuret, nitrate, or other nitrogen containing compounds (Huber and Kung, 1981).

Ration protein levels between 13 and 16 percent are generally recommended to maximize milk production, depending upon level of production (Clark and Davis, 1980; Satter and Roffler, 1975; Moe, 1981; NRC, 1978; Ha and Kennelly,1984). Higher levels (17 % and greater) of ration protein generally result in greater milk production in early lactation, but result in decreased efficiency of protein utilization (Tyrrell et al., 1970). Chalupa (1982) summarized several reports concerning optimum dietary protein level to encourage maximal milk production and concluded that the optimum level of dietary crude protein may be variable, depending upon the protein source. Further, he warned that comparisons of protein sources for milk production may be confounded by changes in body weight, and fiber and fat content of protein sources.

Protein Degradation In the Rumen

Most feeds entering the rumen are initially composed of large, relatively insoluble, complex polymers such as cellulose, hemicellulose, starch, pectins, and proteins (Russell and Hespell, 1981). Physical breakdown of feed particles is achieved primarily through chewing during eating and rumination, rumen movements, and microbial fermentation (Murphy and Nicoletti, 1984). Microbial action does not contribute significantly to reduction in particle size (Murphy and Nicoletti,

1984). Extracellular enzymes of rumen bacteria act on the surface of smaller feed particles, and thus particle size is important to fermentation rate (Hungate, 1966).

Retention time of feed particles in the reticulo-rumen is influenced by level of feed intake, physical characteristics of the feed particle, and associative effects of other ration ingredients (Satter et al., 1977). Ration digestibility is negatively correlated with consumption per unit of time (Tyrrell and Moe, 1975). Depression of about 4 % in ration digestibility is observed for each increase in intake equivalent to maintenance intake (Tyrrell and Moe, 1975). Generally, higher levels of intake are associated with lower levels of metabolizable energy efficiency (Moe, 1981). Metabolizable energy [digestible energy - (energy in urine + energy in fermentation gases)] values are substantially lower for lactating cows than for non-lactating cows fed the same ration (Moe, 1981). Digestibility of cellulose and hemicellulose is depressed 2 to 3 times more than the digestibility of soluble carbohydrates at high intake. Further, most of the systematic bias in estimating digestibility of ration constituents (energy, protein, fiber) can be removed by adjusting for the effect of level of intake on digestive efficiency (Tyrrell and Moe, 1975). National Research Council (NRC) (1978) recommendations for ration protein percent do not readily accommodate the striking changes of milk production relative to feed intake in early lactation (Satter and Roffler, 1975). The amount of feed consumed affects the percentage of protein that should be in the ration to meet production requirements (Satter and Roffler, 1975).

Consumed protein can be divided into soluble vs. insoluble, and degradable vs. non-degradable portions (Chalupa, 1984). Proteins soluble in the rumen liquid phase are generally more rapidly and completely degradable by ruminal microorganisms (Chalupa, 1984). Tertiary structural arrangement of proteins can be an important determinant of their rumen degradability: proteins which have no terminal amino or carboxyl group, and those with excessive crosslinking appear to be less accessible to proteolytic enzymes (Chalupa, 1984).

Protein solubility is dependent upon the distribution of hydrophobic and hydrophilic amino acids on the periphery of the protein molecule (Russell and Hespell, 1981). Hydrophobic residues of protein molecules are usually contained in the interior of the tertiary protein structure, with hydrophilic structures exposed, thus increasing protein solubility (Russell and Hespell, 1981). Soluble nitrogen content of feed is that extracted by a neutral buffer with low ionic strength (Wohlt and Clark, 1978). Insoluble nitrogen is often calculated by subtracting soluble nitrogen from total nitrogen (Huber and Kung, 1981).

Ehle (1984), working with alfalfa hay, theorized that more dense fiber particles of smaller size settle to the lower portion of the rumen digesta mat. Similar stratification of dietary protein molecules during the digestive process presumably occurs. Generally, protein solubility is influenced by feed particle size and density, heat or chemical treatment, and strength or integrity of the plant cell wall which affords protection to the proteins (Satter et al., 1977).

Protein and Energy Interrelationships

The relationship between protein and energy requirements of dairy cows is very complex (Clark and Davis, 1980; Oldham, 1984). Energy yielding and protein yielding nutrients may be used by the rumen microbes or the host animal (Oldham, 1984). Variability in nitrogen to energy ratio effects efficiency of both nutrients' utilization, primarily by altering the population of rumen microbes (Oldham, 1984).

Nitrogen shortage in the rumen causes a decreased population of slow growing cellulolytic bacteria, resulting in decreased cellulose and fiber digestion (Tamminga, 1979; Waldo, 1968). Type and availability of carbohydrate influence nitrogen utilization efficiency by rumen microbes (Oldham, 1984). Journet et el. (1976) and Netemeyer (1982) state that partition of energy into milk decreases with increasing percentage of concentrate and decreasing percentage of milk fat. When dietary protein intake exceeds requirement, protein is deaminated and used as energy, thereby reducing efficiency of protein utilization (Huber and Kung, 1981). Retention time of feeds in the rumen is an important consideration for efficiency of nutrient utilization, since fermentation of substrates must occur to provide energy for microbes to use available nitrogen (Bull and Cleale, 1981).

3. METHODS OF REDUCING PROTEIN SOLUBILITY

Introduction

Even though the profile of amino acids presented postruminally depends extensively on solubility of dietary protein (Huber and Kung, 1981), other factors influence protein metabolism in the rumen. Quantities of various amino acids reaching the small intestine can be

regulated somewhat by protection of protein sources from microbial digestion (Huber and Kung, 1981). Protected proteins are most beneficial in early lactation (up to 12 weeks postpartum) when dietary intake of amino acids and energy lags behind nutrient requirements (Huber and Kung, 1981; Stern et al., 1985; Mielke and Schingoethe, 1981). Grinding, pelleting, rolling, cracking, heat treatment, chemical treatment, and inherent composition of feedstuffs contribute to availability of dietary protein through altering rate of passage, degree of rumen degradation, and microbial protein synthesis (Huber and Kung, 1981; Stern et al., 1985). Solvent or pressure extraction of oilseeds (soybeans, cottonseeds) can reduce rumen degradability of protein in oilseed meals (Stern et al., 1985).

Heat Treated Feeds

Heat causes carbonyl groups of sugars to combine with free amino groups in the Maillard reaction (Bjarnason and Carpenter, 1970). Some heating of forages and concentrates occurs during storage and processing. Brewer's grains are subjected to a certain amount of heat during the drying process which probably contributes to their ruminal bypass qualities. Extent of heat damage in forages, due to improper moisture content at harvest, improper handling, or inadequate storage structures can be quantitated by acid detergent insoluble nitrogen analysis (Goering et al., 1973). Ahrar and Schingoethe (1979) reported that cows fed concentrate mixes containing 50 % of crude protein from heat treated SBM (17 % moisture, 120°C, 25-30 sec) produced slightly more milk during the first 8 weeks of the lactation period, but produced similar amounts of milk compared to control cows during later lactation.

Another trial (Mielke and Schingoethe, 1981) showed a similar lack of increased milk production in late lactation of cows fed heat treated (160°C) SBM. Heat treatment (17 % moisture, 120°C, 25-30 sec) caused reduced solubility of soybean and sunflower meals, but did not decrease digestibility of protein sources in the intestines of rats (Schingoethe and Ahrar, 1979). Feeding diets containing extruded whole soybeans (132°C, 149°C) increased availability of total essential amino acids in the small intestine compared to diets containing soybean meal and whole soybeans (Stern et al., 1985). Netemeyer et al. (1982) reported statistically higher milk yields (23.7 kg/cow/d vs. 23.0 kg/cow/d) of cows fed extensively heated SBM (79-115°C, 30 min) compared to regular SBM.

Formaldehyde Treatment

Treating protein with formaldehyde retards rumen degradation, allowing greater digestion and absorption of protein in the small intestine (Huber and Kung, 1981; Crooker et al., 1983). Methylol groups form on the terminal amino group of protein chains, and on the amino group of lysine (Barry, 1976 in Huber and Kung, 1981). Methylol groups condense with primary amide groups of asparagine, glutamine, and arginine to form intra- and intermolecular methylene cross-linkages between protein chains (Ferguson, 1975 in Huber and Kung, 1981). Reversibility of these reactions in the acidic environment of the abomasum dictates the overall effectiveness of formaldehyde treatment (Huber and Kung, 1981). Crooker et al. (1983) and Huber and Kung (1981) cite numerous reports demonstrating consistently increased growth and weight gains of young ruminants fed formaldehyde treated proteins.

Variable response in milk production of dairy cows fed formaldehyde treated protein has been observed, with reasons for lack of a significant positive response attributed to overprotection or underprotection of dietary protein, sufficient supply of protein to the duodenum prior to formaldehyde treatment, use of animals with low protein requirements, decreased microbial protein synthesis in the rumen, or insufficient length of experimental period (Clark et al., 1980 in Crooker et al. 1983, Tamminga, 1979).

Crawford and Hoover (1984) and Crooker et al. (1983) reported no significant increase in milk production of cows fed formaldehyde treated SBM (0.3 g formaldehyde/100 g SBM, both cases) compared to cows fed regular SBM.

Methionine Hydroxy Analogs

The amino acids deemed essential (or indispensible) for milk protein synthesis in the lactating cow include tryptophan, phenylalanine, arginine, histidine, valine, isoleucine, leucine, methionine, threonine, lysine, and cystine (Mepham, 1982). Limiting amino acids in milk protein synthesis are thought to be phenylalanine, methionine, and/or lysine (Derrig et al., 1974; Clark et al., 1977; Spires et al., 1975).

Delivering semi-purified combinations of rumen-protected amino acids for post ruminal digestion and absorption can be achieved through feeding amino acid analogs or derivatives. Derivatives currently being evaluated for their ability to resist microbial degradation are methionine hydroxy analog (MHA), N-(hydroxymethyl)-DL-methionine calcium, and mono-plus di-N-(hydroxymethyl)-L-lysine calcium (Papas et

al., 1984; Emmanuel and Kennelly, 1984; Huber et al., 1984; Wallenius and Whitchurch, 1975).

Feeding MHA to lactating dairy cows has improved fat yields (Huber et al., 1984). Methionine may stimulate microbial growth or metabolic processes in animals, thus altering milk yield and composition (Emmanuel and Kennelly, 1984). Methionine has been observed to stimulate lipid synthesis by ruminal microorganisms (Patton et al., 1968), most likely by providing methyl groups for phosphatidyl-choline synthesis (Emmmanuel and Kennelly, 1984).

Because protein content of milk does not change in methionine treated animals, Papas et al. (1984) postulated that MHA may stimulate milk and milk fat production by enhancing synthesis of the apoprotein fraction of lipoprotein and by providing methyl groups for phospholipid synthesis in the liver, thus increasing availability of lipoprotein needed for milk fat synthesis in the mammary gland (Moore and Christie, 1981).

No significant positive response in milk production was seen by (Papas et al., 1984; Huber et al., 1984; Wallenius and Whitchurch, 1975) when approximately 25 g MHA was fed per cow per day. However, Huber et al. (1984) reported increases in blood serum triglycerides and lipoproteins for cows fed MHA compared to control cows, and suggested that increases of milk fat could have resulted from greater intake of preformed fat by the udder.

Brewer's Dried Grains

Analysis of brewer's dried grains (BDG) on a dry basis is given as 26 % crude protein, 1.50 Mcal/kg Net Energy lactation, and 16 % crude

fiber (NRC, 1978). BDG have been used in a wide variety of rations for different species, and are usually fed as a source of protein, amino acids, and energy. BDG consist of the dried extracted residue of barley malt alone or in mixture with other cereal grains or grain products resulting from the manufacture of wort or beer and may contain pulverized spent hops in an amount not to exceed 3 % (Rounds, 1975). The protein in BDG is slowly degradable in the rumen, and in vivo rumen bypass of BDG protein has been estimated at 61 % (Rounds, 1975). Merchen et al. (1979) reported that feeding BDG and BDG-urea diets tended to result in more valine, leucine, phenylalanine, and methionine entry into the intestinal tract than feeding urea or SBM-urea rations. Improved performance (average daily gains and feed efficiency) of steers fed DBG is attributed to elimination of rumen keratosis and liver abscesses (Murdock et al., 1981). Porter and Conrad (1975) reported similar milk, fat, and fat corrected milk (FCM) production of cows fed 20 % of total dry matter as BDG, wet brewer's grains, dried distillers grains and solubles, and a combination of wheat bran and soybean meal as sources of supplemental protein in the ration of dairy cows.

4. TRACE MINERAL METABOLISM

Introduction

No work has been reported thus far describing treatment of proteinaceous feed sources with inorganic water soluble salts to decrease rumen degradability of protein. Decreased solubility of proteins may result from binding to terminal amino acids by added inorganic water soluble salts.

Copper carbonate and the water soluble forms, copper sulfate, nitrate, and chloride are absorbed to a greater extent than copper oxide or metallic copper (NRC, 1980). Inorganic salts of zinc, such as the oxide, carbonate, acetate, chloride, sulfate, and metallic zinc are readily available sources (NRC, 1980).

Feeding elevated levels of copper, zinc, or iron may influence flavor of milk produced, or may depress feed or water intake, and thus eventually result in decreased milk production.

Copper

Dietary copper levels of 8 to 10 ppm appear to be adequate in ruminant rations (NRC, 1980; NRC, 1978). Copper is absorbed primarily in the duodenum and jejunum, and is excreted in feces, perspiration, and milk (NRC, 1980). The maximum tolerable ration level for cattle is listed as 100 ppm by (NRC, 1980) and as 80 ppm by (NRC, 1978). Cattle accumulate large amounts of excessive copper in the liver before obvious symptoms of toxicity occur. Toxicity is due to the sudden release of large amounts of copper from the liver to the blood causing a hemolytic crisis (NRC, 1980). Cows milk has a copper concentration of .13 ppm with a range between .10 and .16 ppm (Archibald, 1958). Variations in milk copper content are due almost exclusively to stage of lactation, with highest levels found in colostrum (Archibald, 1958). Dietary levels of copper have little impact on milk levels of copper (Archibald, 1958). However, susceptibility of milk to the development of oxidized flavor may be increased by excessive intake of copper (NRC, 1978).

Zinc

The recommended level of dietary zinc is 40 ppm for lactating dairy cattle, with the toxicity threshold estimated to range from 500 to 1500 ppm (NRC, 1978). Zinc is absorbed from the intestinal tract in relation to need and the primary route of excretion is in the feces (NRC, 1980). Miller et al. (1965) fed cows 372 to 1279 ppm zinc as the oxide for 6 weeks and observed no adverse health effects or changes in milk quality or composition. More response was seen in plasma zinc levels than in milk levels with increased dietary zinc (Miller et al., 1965). Zinc deficiency and toxicity are not important practical problems (Miller, 1981). Zinc is known to interfere with copper and iron absorption (NRC, 1980).

Iron

Fifty ppm of dietary iron is recommended, with a maximum tolerable ration level of 1000 ppm (NRC, 1978). Iron absorption takes place almost exclusively in the small intestine, while excretion occurs primarily in feces (NRC, 1980). Iron is distributed widely in feeds and the environment, and iron deficiency is likely to become important only in calves fed only milk (or replacers) (Miller, 1981). Soluble compounds containing iron, such as ferrous sulfate and ferric citrate contain more available iron than compounds such as ferric oxide or iron phytate (Miller, 1981). Symptoms of iron toxicity include lowered feed intake, lowered weight gain and lowered milk production (Miller, 1981). Iron interferes with copper utilization in calves (Humphries et al., 1982).

CHAPTER III

MATERIALS AND METHODS

1. DESCRIPTION OF RATIONS

A cooperative study between the University of Tennessee and the Central Soya Feed Company of Decatur, Indiana was conducted from Fall of 1983 through Spring of 1984 to determine the milk production response to treating proteinaceous feed sources with inorganic water soluble salts prior to pelleting (Trial 1). Two additional feeding trials with dried brewer's grains were conducted; one from fall 1982 through spring of 1983 (Trial 2a), and the other from fall 1983 through spring of 1984 (Trial 2b).

(Trial 1). All cows were fed a total mixed ration consisting of two parts corn silage to one part pelleted protein concentrate (as fed basis) blended, and fed ad libitum plus 2.3 kg of alfalfa-grass hay/cow/day. Variations in the concentrate composition distinguish the three treatments (referred to as rations throughout). Treatment 1 consisted of a pelleted 18 % protein concentrate with no treatment to aid in protein escape of ruminal degradation. Treatment 2 consisted of a pelleted 15 % protein concentrate in the mixed ration, in which the proteinaceous ingredients had been subjected to treatment with water soluble inorganic salts prior to the pelleting process to increase rumen escape properties. Treatment 3 consisted of a pelleted 15 % protein concentrate prepared in a similar (yet distinct) manner as treatment 2. No grain was fed in the milking parlor.

(Trials 2a and 2b). Treatment 1 consisted of the standard milking herd ration composed of 2 parts corn silage and 1 part pelleted 16 % protein supplement (as fed basis) blended and fed ad libitum, plus 5.6 kg alfalfa-grass hay/cow/day.

Treatment 2 was the same as treatment 1, plus 2.3 kg/cow/day of dried brewer's grains added to the mixed rations. All cows received a mineral mixture with 1 % lime (dry matter basis) and .7 % MgO (dry matter basis). The remaining portion of the mineral mixture was dicalcium phosphate and trace mineral salt. No grain was fed in the milking parlor.

2. ANIMALS AND EXPERIMENTAL DESIGNS

(Trial 1). Thirty lactating Holstein cows (12 primaparous, 18 multiparous) were selected from the University herd at Knoxville. A switch-back design with three treatments and three experimental periods as described by Lucas (1956) was employed. Animals were randomly assigned to one of six treatment sequences as illustrated in Table 1. The first and second experimental periods were 5 weeks in length, with the final period lasting only 4 weeks due to lack of protein concentrate availability. The cows were from 24 to 125 days postpartum. Three cows did not complete the experiment (see Table 1 footnote).

(Trials 2a and 2b). Twenty high producing Holstein cows (1 first lactation, 13 second lactation, 6 third lactation) from the University herd at Knoxville were assigned to one of the two experimental rations for a minimum of 11 consecutive weeks (Trial 2a). In the second trial involving brewer's dried grains, twenty cows (8 first lactation, 4 second lactation, 5 third lactation, 3 fourth lactation) were assigned

Table 1. Age and Lactation Number of Cows in Treatment Sequences (Trial 1).

Treatment sequence					
1-2-1	1-3-1	2-1-2	2-3-2	3-1-3	3-2-3
[a]					
23 (1)	34 (1)	33 (1)	24 (1)	30 (1)	32 (1)
36 (2)	28 (1)	36 (2)	24 (1)	26 (1)	24 (1)
49 (3)	36 (2)	36 (2)	46 (2)	36 (1)	36 (1)
48 (3)	58 (3)	56 (3)[c]	60 (4)	53 (3)	36 (2)
[b]		[d]			
24 (1)	48 (3)	25 (1)	49 (2)	49 (3)	67 (4)

[a] age in months, lactation number in parenthesis (12 first lactation, 7 second, 6 third, 2 fourth)

[b] dropped from trial due to displaced abomasum

[c] dropped from trial due to injured teat

[d] dropped from trial due to injuries sustained from falling in lot

to one of the experimental rations described previously for a minimum of 15 consecutive weeks (Trial 2b). All cows started the trials within two weeks after parturition.

3. HOUSING, FEEDING, AND MILKING

Cows were housed in tie stall barns equipped with individual feed mangers and individual water bowls. Animals were fed three times daily (0700, 1100, 1600 hours), with refusals and amount of feed offered weighed and recorded at each feeding. Cows were allowed to exercise at the twice daily milkings (0200 and 1400 hours) exercise at the twice daily milkings and were observed for signs of estrus at that time.

4. SILAGE AND HAY

Corn silage for Trials 1 and 2b came from two separate silos. Hay used was from various sources, and was sampled biweekly for analyses.

Representative samples of hay, corn silage, and total mixed rations were obtained weekly for dry matter determination. Ration samples were composited within each experimental period (Trial 1), or every three weeks (Trial 2b) for analysis of crude protein, crude fiber, acid-detergent fiber, acid-insoluble lignin, cellulose, ether extract and ash.

5. COLLECTION OF DATA

(Trial 1, Trials 2a and 2b). All cows were weighed for three consecutive days prior to the experiments, and at weekly intervals thereafter. Milk weights were determined by means of in-line volumetric weigh jars. Percent butterfat and somatic cell counts were obtained

once per month when the entire herd was tested by the Dairy Herd Improvement Association (DHIA).

(Trial 1). Approximately 50 ml milk samples were collected at the 1400 hour milking during the second and the final week of each experimental period using an in-line proportional milk sampler. Milk samples were preserved via addition of 2 ml of 10 percent formalin to each sample, and were analyzed for fat percentage and somatic cell count within three days following collection by the DHIA laboratory at the University of Tennessee, Knoxville. Milk samples were then frozen for subsequent analysis of crude protein, total solids, copper, zinc and iron content.

Blood samples (10-15 cc per cow) were drawn with heparinized (Na heparin, 1500 IU/ml) 20 cc syringes. Samples were taken from the coccygeal vein or artery or the jugular vein in the second and final week of each period. Heparinized 15 cc centrifuge tubes were used to contain blood prior to centrifugation.

6. ANALYTICAL METHODS

(Trial 1). Milk samples. Frozen milk samples were allowed to thaw at room temperature overnight preceeding analysis. Samples were placed in a warm water bath, and homogenized with a polytron to assure sample uniformity and aid in keeping fat solubilized. Total solids were determined by placing 3 ml of each sample in aluminum weighing dishes, which were then placed in a 50° C forced air oven for 24 hours. Crude protein content of milk samples (3-5 ml) was determined by the Kjeldahl method and applying the formula : % crude protein = % nitrogen * 6.38 (AOAC, 1975). Mineral analysis of milk samples (10 ml) involved wet

digestion using 60 ml nitric acid followed by 7 ml of perchloric acid. Samples were then diluted 1:5 with distilled deionized water. Due to extremely low concentrations of copper relative to zinc and iron concentrations, the method of standard addition was utilized by adding .2 ppm copper copper to each sample prior to analysis. Milk copper, zinc, and iron contents were determined using an Instrumentation Laboratory aa/ae 551 Spectrophotometer. Four percent fat corrected milk (FCM) was calculated using the formula:

FCM = [.4 * Milk) + 15 * (Milk * (Fat % / 100)]

where Milk is the kg of milk produced per cow per day, and Fat is the average fat percent of that milk. Milk protein (g) was calculated by multiplying the percent protein in milk by the grams of milk produced. Non-fat solids percent of milk was determined by subtracting percent fat from total milk solids.

(Trial 1). Blood Samples. Blood samples were centrifuged within 2 hr of sampling in a size 2, model K International centrifuge. Samples were centrifuged for twenty minutes at a rotational speed of 4000 times the force of gravity. Plasma (5-8 ml) was decanted off and frozen until subsequent mineral analysis. After allowing overnight thawing, duplicate 2 ml plasma samples were diluted with 2 ml of 10 percent trichloroacetate (TCA) to precipitate proteins (to facilitate sample passage through spectrophotometer), giving a final dilution factor of 1:2. Mineral content of plasma was determined using the spectrophotometer described for milk mineral analysis above.

(Trial 1, Trials 2a and 2b). Feed Samples. Samples of experimental rations and hays were dried in a forced air oven at 50° C for 7 days and weighed hot to determine total dry matter percentage. Composited

samples were ground in a Wiley mill through a 1 mm screen preceeding further laboratory analysis. Crude protein, crude fiber, ether extract, and ash were determined by methods described by AOAC (1975). Acid-detergent fiber, acid-insoluble lignin, and cellulose content of feeds were determined as described by Goering and Van Soest (1970). Feed samples (2 g) were wet digested for mineral analyses using the same procedure described for milk samples. The dilution factor was also 1:5, with no standard addition of copper.

7. STATISTICAL ANALYSIS

Trial 1

Explanation of Switchback Design. Lucas (1956) described treatment sequences for switch-back designs that would permit comparisons of three or more treatments simultaneously using a small number of cows in each sequence. Three treatments and three experimental periods were used in Trial 1. Treatments were allotted randomly to numbers used in the treatment sequence patterns. Cows were then randomly allotted to each sequence pattern as presented in Table 1.

Justification For Use of Switchback Design. During the period of lactation, milk yield of a cow increases steadily during the first 6 to 8 weeks postpartum, then peaks, and thereafter exhibits a steady decline. The rate of decline, or persistency of lactation, varies markedly between cows. Switchback designs cope with this source of error (Cochran and Cox, 1964).

The extremely good efficiency of the switch-back design stems directly from the ability to remove from the error term individual cow differences in persistency of lactation.

Mathematically, this high sensitivity is attested by the relatively low coefficients of variation obtained (Lucas, 1956).

The treatment pattern results in particularly sensitive comparisons, because it permits the elimination from error of (a) the period effects due to changes in environment, (b) the between-cow variation in production level, and (c) most of the between-cow variation in slope of the lactation curve (Lucas, 1956). In other words, each cow's response is adjusted for a separate regression line and therefore serves as its' own control.

One implicit assumption when using the switch-back design is that all cows used are in the linearly declining portion of their lactation curve. The magnitude of the linear decline does not contribute to the error of the comparison of treatments (Cochran and Cox, 1964). Treatment effects are deliberately confounded with the quadratic component available from partitioning the two degrees of freedom for period. Deviations from linearity on an individual cow basis are thus assumed to be due solely to treatment effects.

From an applied viewpoint, the sensitivity of switch-back or double-reversal trials is beneficial when designing dairy nutrition research as it allows minimization of material, equipment, facility, and labor requirements necessary for evaluating different treatments.

Justification For Analysis Used. Dr. Lucas' paper (1956) was published before the advent of high speed computers and the easy to use software readily available to today's researcher. The statistical methods outlined by Lucas (1956) for evaluating treatment effects begins with the calculation of "difference values." Computational formulas are given for partitioning the degrees of freedom and sums of squares for

main effects, error, and total. Time required to make calculations and pure mathematical errors are vastly reduced when computers are used to analyze data.

Dr. W. L. Sanders, Agricultural Experiment Station Statistician at the University of Tennessee, Knoxville, derived a method for analyzing data from switch-back design experiments (personal communication). Recognizing the deliberate confounding of treatment effects with the quadratic component of experimental period, Sanders wrote a mathematical model which, when fitted to data from a switch-back design experiment via the General Linear Models procedure of the Statistical Analysis System (SAS) computer programs, 1982 version, duplicates exactly the F-statistic for testing the hypothesis of no treatment effects which could have been obtained by Lucas' direct method.

Application to Data. Experimental periods were five, five, and four weeks in length, respectively. The first week of each period was considered an adjustment period and data collected during this time were not used. Response variables (feed intake, milk produced, etc.) taken daily or weekly were averaged within each period to allow the statistical analysis to follow more closely that described by Lucas. Mean values were analyzed via the General Linear Models (GLM) procedure of SAS (1982). The following model, written by Dr. W. L. Sanders (personal communication), was fitted:

$$Y_{ijk} = u + Cow_i + P_j + P*Cow_{ij} + PD_j + Trt_k + E_{ijk}$$

$$i=1, \ldots 27, \quad j=1,2,3, \quad k=1,2,3$$

where: Y_{ijk} = observed response of the ith cow in the jth period on the kth treatment

u = overall mean

Cow_i = effect of the ith individual cow

P_j = (continuous) effect of the jth period

$P*Cow_{ij}$ = effect of between cow variation in slope of lactation curves (adjusts for different persistency of each cow over time)

PD_j = (class variable) effect of the jth period (estimates the environmental period effects throughout duration of the study)

Trt_k = effect of the kth treatment

E_{ijk} = random error associated with the ijkth observation (estimated from pooling of higher order interactions involving PD, Trt, and Cow, which is consistent with with the error term components described by Lucas (1956))

Orthogonal contrasts were used to test for significant treatment differences in response variables.

Trials 2a and 2b

Data from both trials involving added brewer's dried grains were analyzed with the model:

$$Y_{ijk} = u + Trt_i + Cow_{(i)j} + Week_k + Week_k^2 + Week_k^3 + E_{ijk}$$

$i=1,2,\ j=1, \ldots 10,\ k=1, \ldots 11$ (Trial 2a)
$k=1, \ldots 15$ (Trial 2b)

where: Y_{ijk} = observed response of the jth cow on the ith treatment in the kth week

u = overall mean

Trt_i = effect of the ith treatment

$Cow_{(i)j}$ = variation of the jth individual cow within the ith treatment

$Week_k$ = linear component of response over time

$Week_k^2$ = quadratic component of response over time

$Week_k^2$ = cubic component of response over time

E_{ijk} = random error associated with the ijkth observation

Orthogonal contrasts were used to test for significant treatment differences in response variables.

CHAPTER IV

RESULTS AND DISCUSSION

1. TRIAL 1

Ration Analysis

Overall mean composition of the three experimental rations, corn silage and hay is presented in Table 2. Ration composition was similar for all parameters measured except percent crude protein and mineral content. Percent crude protein for ration one was higher (15.04 %) than that of rations two and three (12.62 and 13.00 %, respectively). Copper content of ration three was slightly higher (44.30 ppm) than that of rations one and two (29.20 and 29.70 ppm, respectively). All three rations contained more than the recommended 8 to 10 ppm copper recommended by NRC (1978). Rations two and three contained several times the recommended (NRC, 1978) 40 ppm zinc content, with concentrations well into the 500 to 1500 ppm estimated toxicity range given by NRC (1978) (See Table 2). The three rations also contained many times the recommended (NRC, 1978) 50 ppm of iron, but were within the maximum tolerable level of 1000 ppm (NRC, 1980). Analysis of rations within each of the three experimental periods is given in appendix tables A1, A2 and A3.

Intake Parameters

Total mixed ration intake was highest for cows consuming ration one, while hay dry matter intake was lowest ($p<.10$) for cows on ration one (Table 3). Total dry matter intake was not different ($p>.10$) for all rations, but was slightly lower for ration two, due to low ration

Table 2. Mean Chemical Composition of Total Mixed Rations, Corn Silage, and Hay Fed in Trial 1 (Overall mean).

FEED:[a]	R1	R2	R3	CS	Hay
Dry Matter (%)	52.81	51.83	53.47	35.93	89.76
Crude Protein (%)[b]	15.04	12.62	13.00	8.19	15.26
Crude Fiber (%)[b]	18.26	18.42	16.88	30.89	31.44
Ash (%)[b]	9.09	9.19	9.49	5.63	7.60
Ether Extract (%)[b]	1.99	2.00	1.93	2.12	1.24
Acid Detergent Fiber (%)[b]	19.57	20.22	19.74	32.07	43.36
Lignin (%)[b]	2.70	2.91	2.65	3.56	8.69
Cellulose (%)[b]	16.87	17.31	17.09	28.51	34.67
Copper (ppm)	29.20	29.70	44.30	5.70	45.70
Zinc (ppm)	129.30	1387.50	1225.70	31.00	34.00
Iron (ppm)	305.00	255.33	289.67	143.50	901.50

[a] R1=Ration 1; R2=Ration 2; R3=Ration 3; CS=Corn Silage (See text for description of rations).

[b] Values are on dry matter basis

Table 3. Orthogonal Comparisons of Intake Parameter Means (Trial 1).

TRT:[a]	1 vs 2 and 3		2 vs 3		S. E.
Ration DMI (kg/d)[b]	14.23	14.05	13.92	14.17	0.20
Hay DMI (kg/d)[b]	1.82	1.87 *	1.85	1.88	0.02
Total DMI (kg/d)[b]	16.05	15.90	15.76	16.04	0.20
Ration CPI (kg/d)[c]	2.17 ***	1.85	1.79	1.90	0.03
Hay CPI (kg/d)[c]	0.32	0.33	0.32	0.33	0.00
Total CPI (kg/d)[c]	2.49 ***	2.18	2.17	2.22	0.03
Body Wt. Chg. (kg)	-3.83	1.06	-0.67	2.79	3.57
DMI%BW[d]	2.56	2.55	2.52	2.58	0.03

* (P<.10)

[a] See text for description of rations.

[b] DMI = Dry matter intake

[c] CPI = Crude protein intake

[d] (DMI%BW) = (Total DMI/BW) * 100, intake as a percent of body weight

dry matter intakes. Body weight change was similar ($p>.10$) over time for all cows, regardless of which ration was consumed. Dry matter intake as a percent of body weight was not significantly different ($p>.10$) between the rations.

Milk Yield and Composition

Milk yield (kg/d), fat percent, and fat corrected milk produced were similar, indicating that rations two and three, with reduced protein levels and elevated copper, zinc, and iron content, supported milk production as well as ration one (Table 4). Reduced rumen solubility of the protein source, without decreased digestibility overall, similar to the effect of heat treatment of SBM described by Schingoethe and Ahrar (1979) may have occurred for rations two and three in this study. The lack of a significantly different ($p<.10$) response in milk production in this trial agrees with the results of Crawford and Hoover (1984) and Crooker et al. (1983), who reported no differences in milk production response of cows fed regular or formaldehyde treated SBM (to increase amount of bypass protein). Since intakes and body weight change were similar for all rations, protein requirements were probably partially filled by metabolism of rumen protected proteins. Percent total solids, solids not fat, and somatic cell counts were not different ($p>.10$) for the three rations. Percent milk protein was highest ($p<.10$) for ration three. However, total grams of protein secreted in the milk was similar ($p>.10$) for all rations.

Milk and Plasma Mineral Content

Plasma copper was highest for cows consuming ration three, which contained the highest copper content of the three rations (Table 2).

Table 4. Orthogonal Comparisons of Milk Production Parameter Means (Trial 1).

TRT:[a]	1 vs 2 and 3		2 vs 3		S. E.
Milk (kg/d)	26.35	26.32	26.46	26.17	0.19
Fat (%)	3.16	3.24	3.21	3.26	0.08
FCM (kg/d)	23.03	23.32	23.33	23.30	0.31
Total solids (%)	10.77	10.68	10.74	10.62	0.17
Milk protein (%)	3.34	3.34	3.29	3.38*	0.05
Milk protein (g/d)[b]	881.30	874.95	867.99	881.91	13.80
SNF (%)	7.60	7.56	7.70	7.42	0.14
SCC (no./ml)[c]	1031.63	661.61	582.36	740.85	545.93

* ($P<.10$)

[a] See text for description of rations

[b] Milk protein (g/cow/d)=Milk(g/d) * % crude protein

[c] (SCC) somatic cell count; (multiply by 1000 for actual count)

Copper concentration of milk was not different ($p>.10$) for the three rations, and was within the normal .10 to .16 ppm concentration range given by Archibald (1958) in all cases. Plasma copper content was lowest for ration two, which might have been due to depressed uptake of copper resulting from the high zinc and iron contents of the ration.

Humphries et al. (1982) reported that dietary iron of 800 ppm had a marked inhibitory effect on the utilization of dietary copper (4 ppm) in heifer calves. Humphries et al. (1982) postulated that the mechanism of the inhibitory effect of iron on copper utilization might be through the formation of insoluble copper-iron-sulfur complexes in the rumen. Ration copper levels between 8 and 26 ppm are toxic for sheep (NRC, 1975), and generally result in hemolytic crisis. Bremner et al. (1976) reported that lambs receiving 420 ppm supplemental zinc did not develop copper toxicosis when fed rations containing 29 ppm of copper. The protective effect of zinc in copper toxicosis may be due to decreased liver copper or to the stimulatory effect that zinc has on the formation in the liver of relatively non-toxic forms of copper (Bremner et al., 1976). No evidence of depressed copper absorption due to mineral antagonism occurred for ration three, as the plasma copper content was the highest seen for all three rations. Plasma zinc was higher ($p<.05$) for ration two and three compared to ration one (Table 5). Miller et al. (1965) observed a linear increase in plasma zinc levels from a basal value of 2.1 ppm with no added dietary zinc, up to 7.5 ppm with 2000 ppm of added zinc oxide added to the ration. Milk zinc levels were elevated for ration two and three compared to ration one ($p<.001$). Highest zinc content in milk occurred with ration two, which contained the highest level of zinc. Miller et al. (1965) also found increased zinc content

Table 5. Orthogonal Comparisons of Milk and Plasma Mineral Means (Trial 1).

	TRT:[a]	1 vs 2&3		2 vs 3		S. E.
Plasma Cu (ppm)	1.167	1.176		1.148	1.203 *	0.002
Plasma Zn (ppm)	1.151	1.338	**	1.349	1.327	0.006
Plasma Fe (ppm)	0.691	0.696		0.681	0.711	0.002
Milk Cu (ppm)	0.108	0.103	***	0.104	* 0.102	0.000
Milk Zn (ppm)	3.682	4.270		4.367	4.173	0.008
Milk Fe (ppm)	0.365	0.436		0.410	0.461	0.011

* (P<.10) ** (P<.05) *** (P<.001)

[a] See text for description of treatments.

of milk as dietary zinc levels increased. Plasma and milk iron content was similar ($p>.10$) for all three rations.

2. TRIALS 2a AND 2b

Ration Analysis

Analyses of rations collected during Trial 2b are given in Table 6. Corn silage and hay used in Trial 2b was from the same source as in Trial 1 (analyses given in Table 2). Ration and hay samples for Trial 2a were unavailable, and dry matter and crude protein percentages of corresponding rations in Trial 2b were used in calculations of dry matter intake and protein intake for Trial 2a. Caution must be exercised when interpreting dry matter and protein intakes reported for Trial 2a. Analysis of sample composites is given in appendix tables A4 and A5.

Intake Parameters

Ration, hay, and total dry matter intakes were higher for ration 1 in Trial 2a, but were higher for ration 2 in Trial 2b (Tables 7 and 8). Nonascertained differences in dry matter percentages of rations between the two years may partially account for these opposing results.

Ration, hay, and total crude protein intake was similar for both Trials, with ration one resulting in greater total protein intake (Tables 7 and 8).

As no ration samples were available for Trial 2a, the magnitude of the protein intake response in Trial 2a may be different than is shown in Table 7, depending upon actual ration and hay protein contents in that year.

Table 6. Mean Chemical Composition of Total Mixed Rations Fed In Trial 2b.

TOTAL MIXED RATION:[ab]	R1	R2
Dry Matter (%)	54.06	54.73
Crude Protein (%)[c]	15.51	14.32
Crude Fiber (%)[c]	17.71	16.81
Ash (%)[c]	9.05	8.98
Ether Extract (%)[c]	2.28	2.49
Acid Detergent Fiber (%)[c]	20.59	21.32
Lignin (%)[c]	3.74	3.93
Cellulose (%)[c]	16.85	17.39

[a] Corn silage and hay used in Trials 1 and 2b were from same source

[b] R1=Ration 1 (basal ration); R2=Ration 2 (basal ration plus DBG);

[c] Values are on dry matter basis

Table 7. Orthogonal Comparison of Intake and Production Parameter Means (Trial 2a).

TOTAL MIXED RATION:[a]	1	2	S. E.
Intake parameters			
Ration intake (kg/d)[b]	22.82***	20.45	0.27
Hay intake (kg/d)[b]	3.70	3.80	0.05
Ration intake (kg/d)[c]	12.34***	11.91	0.14
Hay intake (kg/d)[c]	3.32	3.41	0.04
Total DMI (kg/d)[d]	15.65***	14.60	0.14
Ration CPI (kg/d)[e]	1.91***	1.62	0.02
Hay CPI (kg/d)[f]	0.51	0.52	0.01
Total CPI (kg/d)[g]	2.42***	2.12	0.02
Production parameters			
Milk (kg/d)	34.78**	33.61	0.28
Fat (%)	2.75	3.07***	0.06
FCM (kg/d)	27.88	28.43	0.35

** (P<.05) *** (P<.001)

[a] Ration 1: basal ration; Ration 2: basal ration plus DBG (See Text)

[b] As fed basis

[c] Dry matter basis (See Table 6; values used were from Trial 2b)

[d] DMI=dry matter intake

[e] Ration crude protein intake = ration dry matter consumed x % protein

[f] Hay crude protein intake = hay dry matter consumed x % protein

[g] Total crude protein intake = Ration CPI + Hay CPI

Table 8. Orthogonal Comparison of Intake and Production Parameter Means (Trial 2b).

TOTAL MIXED RATION:[a]	1	2	S. E.
Intake parameters			
Ration intake (kg/d)[b]	25.07	25.46	0.28
Hay intake (kg/d)[b]	2.95	3.15 **	0.05
Ration intake (kg/d)[c]	13.55	13.93 *	0.15
Hay intake (kg/d)[c]	2.65	2.83 **	0.05
Total DMI (kg/d)[d]	16.20	16.76 **	0.14
Ration CPI (kg/d)[e]	2.10 ***	2.00	0.02
Hay CPI (kg/d)[f]	0.40	0.43 **	0.00
Total CPI (kg/d)[g]	2.51 **	2.43	0.00
Production parameters			
Milk (kg/d)	30.71	33.96 ***	0.21
Fat (%)	3.37	3.37	0.04
FCM (kg/d)	27.67	30.62 ***	0.22

* (P<.10) ** (P<.05) *** (P<.001)

[a] Ration 1: basal ration; Ration 2: basal ration plus DBG (See Text)

[b] As fed basis

[c] Dry matter basis (See Table 6; values used were from Trial 2b)

[d] DMI=dry matter intake

[e] Ration crude protein intake = ration dry matter consumed x % protein

[f] Hay crude protein intake = hay dry matter consumed x % protein

[g] Total crude protein intake = Ration CPI + Hay CPI

Milk Yield and Composition

For Trial 2a, milk yield was higher ($p<.05$) for cows consuming ration one, while fat percent was highest ($p<.001$) for cows on ration two (Table 7). Response of fat corrected milk produced by cows fed the two rations is shown in Figure 1. There was a non-significant trend for greater fat corrected milk production for cows on the ration containing added BDG (ration two).

Opposite results occurred in terms of milk, fat percentage, and fat corrected milk produced by cows on the two rations in Trial 2b when compared to Trial 2a (Table 8). Mean milk production (kg/d) was greater ($p<.001$) for cows on ration one. Fat percentages were not significantly different between the two rations, and therefore a significantly larger ($p<.001$) volume of fat corrected milk was produced by cows on ration two. Mean fat corrected milk production in each week for cows on the two experimental rations during Trial 2b is shown in Figure 2. The trend for greater fat corrected milk production on ration two is consistent with the expectation that brewer's dried grains provide an increased supply of amino acids for absorption post-ruminally, thus enhancing the nutritional value of the total mixed ration consumed by the cow.

One possible explanation for the differences in fat percentage for the two rations in Trial 2a may be that the crude fiber content of ration one may have been substantially lower than the recommended (NRC, 1978) 17 % for optimal rumen acetate production. Inadequate ruminal acetate production, the primary precursor of milk fat synthesis, has long been known to lead to subsequently lowered fat synthesis by the mammary gland.

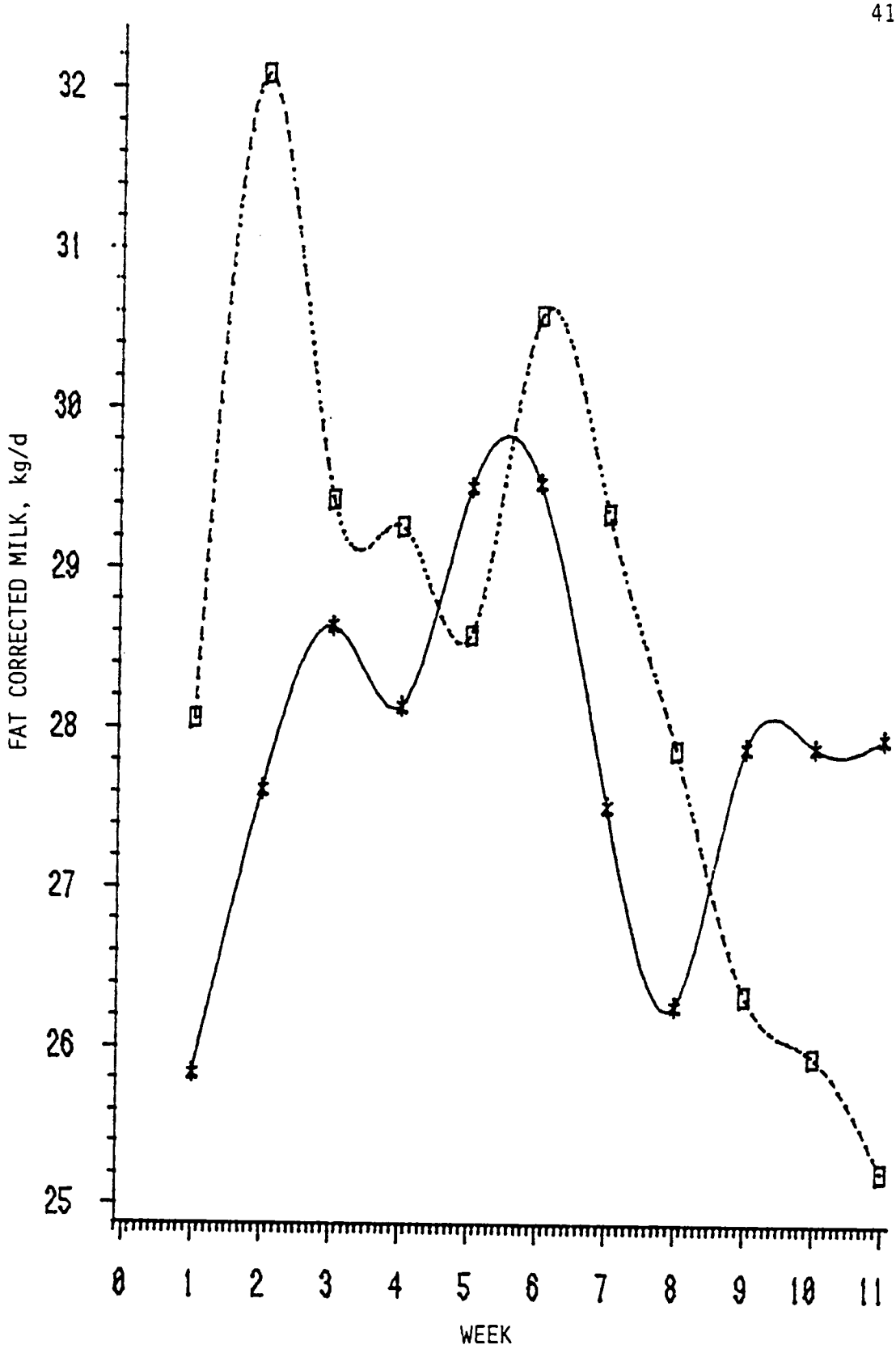


Figure 1. Response of fat corrected milk production of cows fed the control (Ration 1) (*——*) or dried brewers grain ration (Ration 2) (□----□) in Trial 2a.

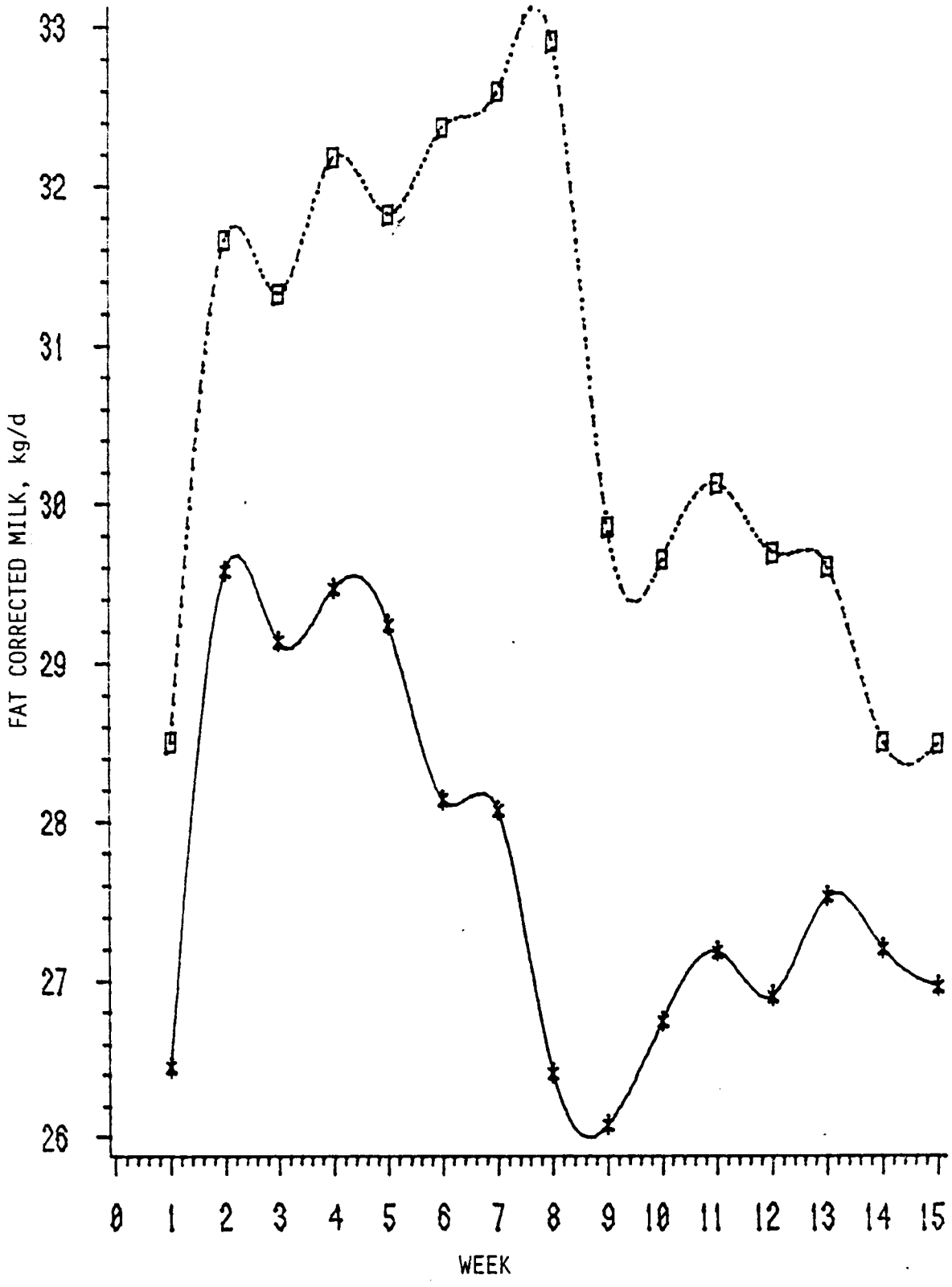


Figure 2. Response of fat corrected milk production of cows fed the control (Ration 1) (*——*) or dried brewers grain ration (Ration 2) (□----□) in Trial 2b.

Chalupa (1982) summarized several reports dealing with milk production response to increasing dietary crude protein by commenting that milk production increases almost linearly as dietary crude protein is increased. Moreover, most of the increase in milk production can be accounted for by increasing feed consumption.

In Trial 2a, assuming ration protein levels listed for trial 2b, higher dry matter intakes were observed with greater ration crude protein, but without a concomitant increase in fat corrected milk production as Chalupa (1982) indicated should occur in this situation. On the other hand, ration two (with lower crude protein than ration one) supported significantly ($p<.001$) greater milk production in Trial 2b. Increased rumen bypass of undegraded protein for the BDG containing ration may have contributed to the performance and efficiency of cows on ration two in Trial 2b.

Merchen et al. (1979) fed rations containing BDG or a combination of BDG and urea to abomasally canulated steers. BDG and BDG-urea supplementation resulted in greater plant nitrogen flow to the abomasum when compared to the urea or SBM-urea. Further they reported than feeding BDG and BDG-urea diets tended to produce more valine, leucine, phenylalanine, and methionine- essential for milk synthesis (Mepham, 1982)- into the intestinal tract than feeding urea or SBM-urea rations. A response similar to this could have occurred in the two trials presented here as well.

CHAPTER V

SUMMARY AND CONCLUSIONS

A total of 67 Holstein cows in the University of Tennessee, Knoxville dairy herd were used in three trials to evaluate the milk production and milk composition response to varying levels and sources of dietary protein.

In Trial 1, 27 cows from 24 to 125 days postpartum completed 14 weeks in a switch-back design involving three treatments and three experimental periods. Cows were fed a 2:1 corn silage to 16 % crude protein concentrate in a total mixed ration, plus 2.3 kg of alfalfa-grass hay per cow per day. Ration one contained an 18 % protein concentrate, untreated for bypass. Rations two and three contained 15 % protein concentrate mixes which had been subjected to treatments with inorganic water soluble salts to increase rumen bypass properties of the protein.

Feed samples were collected weekly, composited by ration and period, and analyzed for crude protein, crude fiber, acid-detergent fiber, acid-insoluble lignin, cellulose, energy, copper, zinc, and iron concentrations.

Milk weights were recorded at the twice daily milkings. Milk samples were collected in the second and final weeks of each experimental period, and frozen for later analysis of crude protein, total solids, non-fat solids, copper, zinc, and iron content. Milk fat percentages were determined on milk samples collected at the beginning and end of each experimental period and were also obtained monthly from Dairy Herd Improvement Association records.

Blood samples were taken from the coccygeal or jugular vein in the second and last week of each experimental period. Plasma was analyzed for copper, zinc, and iron concentration.

Results indicated that dry matter consumption, actual milk production, milk fat percentage, non-fat solids, fat corrected milk, somatic cell counts, total solids, and body weight change were not significantly different between treatment groups ($p>.10$).

Rations two and three contained much higher zinc contents than ration one. Plasma mineral analysis showed that copper content was highest (1.203 ppm) for ration three; zinc was higher for rations two and three; and, iron was not different for all rations. Milk zinc was highest for rations two and three as well.

The 15 % crude protein supplement used in total mixed rations two and three supported milk production equally as well as the 18 % crude protein concentrate used in ration one. Economic advantages of using the 15 % protein concentrate vs. the 18 % protein concentrate when feeding total mixed rations should be considered, as well as the long term physiological effects associated with the elevated dietary zinc and iron levels found with these two rations.

In Trials 2a and 2b, 20 early lactation cows were assigned to either a basal total mixed ration composed of a 2:1 mixture of corn silage to 16 % concentrate, or to the basal diet plus 5.6 kg of added brewer's dried grains for a minimum of 11 weeks (2a) or 15 weeks (2b). Ration samples (2b only), milk weights, ration and hay dry matter intake, and milk production were recorded.

Total dry matter intake, total protein intake and milk produced were significantly higher for the ration that did not contain BDG

(ration one), while fat corrected milk produced was not significantly different between the rations (Trial 2a). Contrary to Trial 2a, total dry matter intake, milk and fat corrected milk were higher ($p<.05$) for ration two in Trial 2b, while total crude protein intake was highest for ration one. Unaccounted for differences in ration composition, especially crude fiber content, or environmental differences, may be responsible for the inconsistent results obtained between the two trials. The trend for greater fat corrected milk production on ration two is consistent with the expectation that brewer's dried grains provide and increased supply of amino acids postruminally for absorption, thus enhancing the nutritional value ot the total mixed rations consumed by the cow.

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APPENDIX

Table A1. Mean Chemical Composition of Total Mixed Rations, Corn Silage, and Hay Fed in Trial 1, Period 1.

FEED:[a]	R1	R2	R3	CS	Hay
Dry Matter (%)[b]	50.67	48.52	50.60	33.75	89.89
Crude Protein (%)[b]	15.36	11.93	12.54	8.32	14.65
Crude Fiber (%)[b]	16.31	17.30	14.61	29.43	38.07
Ash (%)[b]	9.92	9.58	9.79	5.43	7.91
Ether Extract (%)[b]	2.05	2.04	2.07	2.11	0.83
Acid Detergent Fiber (%)[b]	17.77	19.33	19.69	28.30	44.17
Lignin (%)[b]	2.48	2.74	2.61	3.33	8.20
Cellulose (%)[b]	15.29	16.59	17.08	24.97	35.97
Copper (ppm)	32.50	27.00	30.50	5.00	116.00
Zinc (ppm)	146.00	1301.50	1114.00	26.00	62.00
Iron (ppm)	336.00	245.00	249.00	153.00	1704.50

[a] R1=Ration 1; R2=Ration 2; R3=Ration 3; CS=Corn Silage (See text for description of rations).

[b] Values are on dry matter basis

Table A2. Mean Chemical Composition of Total Mixed Rations, Corn Silage, and Hay Fed in Trial 1, Period 2.

FEED:[a]	R1	R2	R3	CS	Hay
Dry Matter (%)	49.02	50.08	52.60	35.32	89.66
Crude Protein (%)[b]	14.66	12.95	13.83	8.73	17.45
Crude Fiber (%)[b]	19.88	19.52	18.54	30.95	16.02
Ash (%)[b]	8.63	9.09	9.13	6.60	7.80
Ether Extract (%)[b]	1.73	1.79	1.81	1.70	1.42
Acid Detergent Fiber (%)[b]	21.59	22.20	20.86	34.91	41.26
Lignin (%)[b]	2.70	2.81	2.66	3.57	8.71
Cellulose (%)[b]	18.89	19.39	18.20	31.34	32.55
Copper (ppm)	23.00	29.00	51.00	5.00	12.00
Zinc (ppm)	129.00	1343.00	1306.00	39.00	19.00
Iron (ppm)	306.00	234.00	291.00	143.50	901.50

[a] R1=Ration 1; R2=Ration 2; R3=Ration 3; CS=Corn Silage (See text for description of rations).

[b] Values are on dry matter basis

Table A3. Mean Chemical Composition of Total Mixed Rations, Corn Silage, and Hay Fed in Trial 1, Period 3.

FEED:[a]	R1	R2	R3	CS	Hay
Dry Matter (%)	58.73	56.90	57.21	38.71	89.73
Crude Protein (%)[b]	15.11	12.99	12.64	7.53	13.69
Crude Fiber (%)[b]	18.59	18.43	17.50	32.28	40.22
Ash (%)[b]	8.71	8.90	9.56	4.85	7.10
Ether Extract (%)[b]	2.18	2.17	1.91	2.55	1.40
Acid Detergent Fiber (%)[b]	19.34	19.13	18.67	33.00	44.64
Lignin (%)[b]	2.91	3.19	2.67	3.78	9.16
Cellulose (%)[b]	16.43	15.94	16.00	29.22	35.48
Copper (ppm)	32.00	33.00	55.00	7.00	9.00
Zinc (ppm)	113.00	1518.00	1257.00	28.00	21.00
Iron (ppm)	273.00	287.00	329.00	134.00	99.00

[a] R1=Ration 1; R2=Ration 2; R3=Ration 3; CS=Corn Silage (See text for description of rations).

[b] Values are on dry matter basis

Table A4. Mean Chemical Composition of Composites of Total Mixed Rations Collected During the First and Second Three Weeks of Trial 2b.

TOTAL MIXED RATION:[ab]	R1	R2	R1	R2
THREE WEEK PERIOD:	1		2	
Dry Matter (%)	53.01	53.04	52.91	49.30
Crude Protein (%)[c]	14.88	15.26	15.58	13.68
Crude Fiber (%)[c]	19.58	17.73	17.45	17.46
Ash (%)[c]	9.03	9.23	9.02	8.33
Ether Extract (%)[c]	2.35	2.34	2.39	2.79
Acid Detergent Fiber (%)[c]	20.36	20.96	20.68	22.19
Lignin (%)[c]	3.66	3.76	3.89	3.74
Cellulose (%)	16.70	17.20	16.79	18.45

[a] Corn silage and hay used in Trials 1 and 2b were from same source

[b] R1=Ration 1 (basal ration); R2=Ration 2 (basal ration plus DBG);

[c] Values are on dry matter basis

Table A5. Mean Chemical Composition of Composites of Total Mixed Rations Collected During the Third and Fourth Three Weeks of Trial 2b.

TOTAL MIXED RATION:[ab]	R1	R2	R1	R2
THREE WEEK PERIOD:	3		4	
Dry Matter (%)	53.28	56.78	57.03	59.78
Crude Protein (%)[c]	15.23	14.39	16.33	13.95
Crude Fiber (%)[c]	18.55	19.20	15.49	12.83
Ash (%)[c]	8.72	8.56	9.43	9.30
Ether Extract (%)[c]	2.10	2.31	2.27	2.51
Acid Detergent Fiber (%)[c]	22.18	21.65	19.15	20.47
Lignin (%)[c]	3.66	3.90	3.74	4.31
Cellulose (%)[c]	18.52	17.75	15.41	16.16

[a] Corn silage and hay used in Trials 1 and 2b were from same source

[b] R1=Ration 1 (basal ration); R2=Ration 2 (basal ration plus DBG);

[c] Values are on dry matter basis

VITA

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