EFFECTS OF BETAINE AND CHOLINE SUPPLEMENTATION ON MOHAIR AND MILK PRODUCTION BY GOATS

By

TESHOME SHENKORU

Bachelor of Science
Addis Ababa University
Addis Ababa, Ethiopia
1982

Master of Science
Alemaya University of Agriculture
Dire Dawa, Ethiopia
1987

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Graduate College of the
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Thesis Approved:

J N Owens by DRE
Thesis Adviser
Donald R Gill
Gerald W. Horn
Robert Teeter
James E Breazile
Dean of the Graduate College

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Format of Dissertation

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CHAPTER I

Introduction

Choline has several functions in the body. First, it is a structural component of cell membranes, in the form of phosphtidylcholine and sphingomyelin. Second, they serve as a structural component of phospholipids that are involved in fat digestion, transportation and metabolism. Third, both choline and betaine are methyl donors', contribute carbon for the synthesis of methionine, carnitine, creatine and nucleic acid. Fourth, they serve as precursors for biosynthesis of the neurotransmitter acetylcholine. Supplies of both choline and betaine can become limiting in several species including both the human (Zeisel, 1992) and the Angora goat. In our preliminary experiments with Angora and Alpine goats, infused betaine increased plasma methionine concentrations; this suggests that supply of methyl groups may limit sulfur amino acid recycling or metabolism. Growth of both skin and mohair imposes heavy demands on circulating cysteine. Black and Ries (1979) predicted that 80% of the total pool of combined cysteine and methionine is used for fiber growth; thereby nutritive supplementation with methyl donors may enhance fiber growth. The possibility of using dietary betaine or choline to replace some of the supplemental methionine is attractive because both betaine and choline are lower in cost per unit of methyl group than methionine. Due to the current low market price for mohair, this approach to decrease the cost of mohair production may

prove helpful to goat producers. Choline also is involved in fat and polyamines metabolism. Erdman et al. (1984) indicated that adding non-ruminally protected choline to the diet of lactating cows increased production of 4% fat corrected milk and the fat percentage of milk produced. Post-ruminal infusion of choline in dairy cows increased milk fat percentage (Sharma and Erdman 1988a). Although Erdman and Sharma (1991) reported that ruminally protected choline chloride increased milk production, later experiments using similar supplements of ruminally protected choline detected no beneficial effects (Piva et al. 1993). For dairy goats, a response may or may not be present because goat's milk contains less fat and milk fat of a different fatty acid composition than cow's milk.

Goat milk fat normally contains 35% medium chain fatty acids (MCFA C6 - C14) compared only 17% for milk fat from cows. The three most prevalent MCFA total 15% in milk fat from goats versus only 5% for cows. These medium chain fatty acids have drawn considerable interest from the medical profession because of their unique benefits in certain metabolic disorders of humans (Babayan, 1981). The medium chain length fatty acids have been used to treat mal-absorption syndromes, intestinal disorders, coronary diseases, gallstones, cystic fibrosis, and for supplying energy to pre-mature infants. Medium chain length fatty acids have the unique metabolic ability of providing energy while lowering, inhibiting, and dissolving cholesterol deposits (Kalser, 1971; Tantibhebhyangkul and Hashim, 1975, 1978). Another important features of goat milk is that 40% of all patients sensitive to protein of cow's milk can tolerate goat milk protein (Zemen, 1982), possibly because lactalbumin is immunospecific (differ between species).

Betaine and choline, even at relatively high levels of supplementation, are safe and cause no problems with animal health (Sharma and Erdman, 1988b). It is unlikely that supplemented betaine or choline accumulates or is deposited in animal products because both are very quickly metabolized. However, both choline and betaine also are extensively degraded by ruminal bacteria, so that minimal amounts of dietary choline or betaine that reach the abomasum of ruminants. Consequently, both compounds must be protected from ruminal fermentation if one expects for dietary supplements to increase the supply that reaches the small intestine and is available to absorb.

The objective of our research was to determine the effects of supplying ruminally protected choline or betaine products on performance, mohair growth and characteristics by Angora goats and on milk production and composition by Alpine goats.

Chapter II

Review of Literature

Chemical structure and properties of choline and betaine

Choline (trimethyl, 2-hydroxy ethyl ammonium hydroxide) is a quaternary ammonium compound found in most biological materials (Figure 1a). Betaine is an oxidized form of choline (Figure 1b). Free choline makes up only a small proportion of the total amount of choline found in biological materials. The most common chemically bound forms are phosphorylcholine, phosphatidylcholine (lecithin) and acetylcholine. Pure choline is a colorless, viscous, strongly alkaline liquid that is notably hygroscopic. Choline is soluble in water, formaldehyde, and alcohol and has no definite melting point. Choline chloride and choline bitartrate are listed in the code of Federal Regulations as nutrients and / or dietary supplements that are generally recognized as safe (GRAS: Fed. Am. Soc. Exper. Bio. 1975). Choline is classified as vitamin, although from a classical standpoint this is not true, because choline does not function as a cofactor in any enzymatic reactions. Instead choline acts more like an amino acid or an essential fatty acid than a vitamin, but based on its biological activity, it does not fit either of these classifications

Betaine is non-toxic and is found widely distributed in a large number of species of plants and animal (Budavari, 1989). The best known betaine accumulators are plants belonging to the sugar beet (*Chenopodiaceae*) family, most organisms, and almost all marine and fresh water invertebrates (Konosu and Yamaguchi, 1982). Beet molasses contains about 6.2% of its dry matter as betaine.

Functions of Choline and Betaine

Choline functions in the body as a structural component of cell membranes when chemically combined to form phosphatidylcholine and sphingomyelin. It also serves as a component of phospholipids that are involved with fat digestion and metabolism. Phospholipids aid in emulsify lipid in the gastrointestinal tract and are necessary for complete absorption of lipid. The second major location of phospholipids is in the blood stream where triglyceride transport relies on lipoproteins that, in turn, are composed partially of phospholipid. The primary transport sites include transport from the site of intestinal absorption (as chylomicrons) to sites of further metabolism (primarily liver) or storage (lipid depots). Secondly, lipoproteins transport lipid from various sites of synthesis to depot sites; with birds, lipogenesis occurs primarily in the liver, for swine synthesis occurs in both the liver and adipose tissue; for ruminants, most lipogenesis occurs in adipose tissue itself. Consequently, the need for lipid transport is much greater for poultry and swine than for ruminants. Another effect of choline on lipid metabolism suggested by Mookerjea (1971) is that choline may influence apolipoprotein synthesis through its modulating effect on uridine diphosphocholine-N (UDP-N) acetylglucosaminyl transferase activity, which is stimulated by phosphatidylcholine.

This transferase enzyme is important for the membrane synthesis required for packing and release of both glycoprotein and lipoproteins. A second possible mechanism is via-cytidine diphosphocholine (CDP-choline), where CDP-choline acts as a cofactor in the transfer of phosphorylcholine into lecithin (Kuksis and Mookerjea, 1978). A third mechanism involves betaine, which indirectly contributes labile methyl groups for the synthesis of carnitine via S-adenosyl-methionine. Carnitine is required for transporting long chain fatty acids across the inner mitochondrial membranes for oxidation (Stryer, 1988). Inadequate carnitine, caused by a deficiency in methyl groups, will impair fatty acid oxidation.

Choline and betaine are sources of labile methyl groups for formation of methionine from homocystine, and of carnitine and creatine from guanidoacetic acid. According to Puchala et al. (1997) and Baker and Czarnecki (1985) betaine, but not choline, enhanced the conversion of high doses of homocystine to methionine. McDowell (1989) indicated that betaine failed to prevent fatty livers and hemorrhagic kidneys in poultry. According to Combs (1992) the activity of phosphatidyl- ethanolamine- N – methyl transferase for the biosynthesis of choline is negligible in young male chicks and is limited in young females chicks. It was also demonstrated (Molitoris and Baker, 1976) that neither methionine nor betaine could replace choline in semi-purified diet of broiler chicks. Hove and Copeland (1954) indicated that rabbit also lack this enzyme. This suggests that choline and betaine may differ in their effects on animal performance.

Choline is a precursor for biosynthesis of the neurotransmitter, acetylcholine. Released at the termination of the parasympathetic nerves, acetylcholine release serves to transmit of nerve impulses from parasympathetic to postsynaptic fibers of both the

sympathetic and parasympathetic nervous systems. This phenomenon results in stimulating or slowing of certain body functions.

Betaine also is involved in osmoregulation, control of cell volume i.e., the ability of a cell to maintain its structure and function through regulating the movement of water in and out of the cell. In the bodies of most animals, water is the major component (Dick, 1979) and water is vital for survival. Animals maintain the intracellular concentration of water crucial for homeostasis by osmoregulation. Most cells adapt to external changes in osmotic pressure or temperature stress by altering the intracellular concentration of low molecular weight organic solute like betaine (Wunz and Wright, 1993) as well as inorganic ions. Alterations in the intracellular load of inorganic ions are limited to alter osmolality because their concentrations can affect protein structure and enzyme function (Burg, 1994). Interest in the medical use of betaine has been stimulated because of its importance as an alternative methylating agent for homocysteine, a metabolic intermediate that plays a major role in chemical manifestation of vascular disease and the genetic defects known as homocystinurias (Clark et al., 1991).

Choline and Betaine Metabolism

Choline is present in the diet mainly in the form of lecithin, with less than 10% as either the free base or sphingomyelin. Choline is released from lecithin and sphingomyelin by phospholipase A_2 of gastro-intestine tract, although some 50 to 60 % of ingested lecithin enters the thoracic duct intact (Chan, 1984). In sheep fed forage diets, free choline is rapidly degraded to trimethylamine with only 10 - 15 % of the dietary choline escaping the rumen degradation. Because choline complexes with phospholipids, many pathways and mechanisms of absorption have been proposed. Most choline

containing phospholipids are thought to be absorbed as lysophosphatidylcholine (lyso-pc). Dietary choline is absorbed from the lumen of the duodenum, jejunum, and ileum via mediated transport by an energy and sodium dependant carrier mechanism (Zesiel, 1992). Only one-third of ingested choline appears to be absorbed intact in ruminants. The remaining, two-third is metabolized by microbes to trimethylamine, and is excreted in the urine (De la Huerga and Popper, 1952). Once absorbed, choline can be acetylated, phosphorylated and oxidized as shown in Figure 2. Lyso-pc absorbed by the intestinal mucosa appears as phosphatidylcholine (pc) in the chyme. At least two pathways are known for this transformation. In the first, lyso-pc is acetylated to form pc by an acyltransferase enzyme (Houtsmuller, 1979). A second path way involves a dismutase enzyme, which combines two lyso-pc molecules to form glycerol, phosphorylcholine and pc. This enzyme may be part of a regulating mechanism, which prevents accumulation of excessive levels of lyso-pc in the lumen of enterocytes. Sphingomyelin absorbed from the intestinal lumen is degraded to phosphate and sphinosine by intestinal enterocytes, followed by absorption by the portal system. Absorbed lecithin enters circulation as lymph via the thoracic duct as chylomicron. A small portion of phospholipids in blood is in the form of very low-density lipoprotein (VLDL). When phospholipids are released into plasma, lipoprotein lipase, present on cell membrane surface lining the blood vessels, acts on both chylomicron and VLDL, via liver lipase to form a choline containing intermediate, low density lipoprotein (LDL). The LDL fraction and the associated phospholipids decrease in size, and the remaining phospholipds, protein and cholesterol form high-density lipoprotein (HDL). Lecithin plus cholesterol acyltransferase convert

free cholesterol to a cholesterol ester and lyso-pc, which can be absorbed directly by brain cells and many other cell types (Houtsmuller, 1979).

Choline and Betaine sources

Synthetic sources

Choline chloride and choline bitartrate are the primary forms of synthetic choline available commercially. Choline chloride is synthesized from natural gases via methanol and ammonia, which are reacted to produce trimethylamine (Griffin and Nyc, 1971). This trimethylamine subsequently is reacted with ethylene oxide to produce choline. For feed supplementation purposes, the chloride salt is produced by reacting choline, and alkaline base, with hydrochloric acid. There also are feed grade sources of betaine; these include betaine hydrochloride, feed grade anhydrous betaine, and purified betaine liquid isolated from beet molasses.

De novo biosynthesis of choline and betaine. De novo biosynthesis of choline occurs either an insufficient substrate supply or lack of ability to synthesize choline at the rate sufficient for animal needs may result in a dietary requirement for maximum productivity. Choline is synthesized in the liver, brain and mammary gland. Choline is synthesized de novo from ethanolamine through addition of methyl groups to form phosphatidylcholine; synthesis is catalyzed by phosphatidylethanoamine-N-methyltransferase (PeMT) via sequential methylation of phosphatidylethanolamine using S-adenosylmethionine as a methyl donor (Zeisel, 1981, and Chap et al., 1988; Rigeway and Vance, 1987). Most PeMT activity is found in the liver (Bjornstad and Bremer, 1981), but substantial activity also is present in the brain (Blusztajin et al., 1979, and

Crews et al., 1981) and mammary gland tissue (Yank et al. 1988) and detectable activity is found in most other tissues (Davis, 1986, and Robinson et al. 1987). Betaine is synthesized from choline by choline oxidase. This choline oxidation takes place in the cell's mitochondria, which means that choline must be transported into the mitochondrion, oxidized to betaine, and finally released back into the cytosol where it can function as a methyl donor (Mann et al., 1938).

***Dietary supply*:** Almost all choline in food is in the form of phosphatidylcholine or sphingomyelin; phosphatidylcholine is the major form in typical in feedstuffs (Neill et al., 1979; Sharma and Erdman, 1989). Mean choline content of selected animal feedstuffs are shown in Table 1. In general, cereal grains and by-products are low in choline, whereas oilseeds by-products are rich sources of choline because of their relatively high phospholipids content.

The betaine concentration of common feedstuffs is shown in Table 2. Corn, milo, soybean meal, and meat or fish by-product meals contain very little betaine. With such diets, supplementation may be necessary to satisfy an animal's requirement for betaine. Betaine can be provided by the including condensed beet molasses solubles (25-200 g betaine /kg), feed grade betaine hydrochloride (722 g betaine / kg), feed grade anhydrous betaine (> 970 g betaine/kg), or purified betaine liquid (> 470 g betaine / kg) in the diet. Diets containing forage (e.g. alfalfa and /or small grains forage from wheat or barley) or their products may contain sufficient betaine to satisfy an animal's requirement. However, betaine concentrations and bioavailability may vary considerably, depending on the crop's growing conditions. In general the betaine content of plant tissues rises as the soil moisture level decreases or salinity increases (Kidd et al., 1997).

Choline requirement of animals

Dietary requirements for choline by growing poultry, swine, cats and dogs are suggested to be between 1000 and 3000 mg/kg diet. When concentrations in typical feeds (Table 1) are contrasted with these requirements, the need for supplementing non-ruminant diets with choline become obvious. However, one should be careful when calculating animal requirement using reference values. This is because choline content of foods is extremely variable, presumably as a result of biological or analytical variation. Analytical assays for choline and betaine are variable. Thus, reference values should be used only as starting point in terms of calculating choline or betaine adequacy or needs for diet supplementation. According to McDowell (1989) little is known about the bio-availability of choline or betaine from natural feedstuffs. Molitoris and Baker (1976) reported that bio-availability of choline from de-hulled regular soybean meal and whole soybeans ranged from 60 to 75% based on the chick assay method. Pre-ruminant calves placed on a choline deficient diet developed choline deficiency symptoms in 6 - 8 days (Johnson et al. 1951). Choline requirements of mature ruminants are unknown due to degradation of choline in the rumen. According to Sharma and Erdman (1989) ruminal destruction of free choline ranged from 79-99% for various feed types and choline sources. Work with dairy cattle demonstrated that approximately 77% of dietary choline was degraded in the rumen (Atkins et al., 1988). Although the post-ruminal need for choline has not been determined precisely, Erdman and coworkers (1985, 1987) proposed that the post-ruminal choline requirement for lactating cows was 30 g/d based on the fact that supplementing dairy cow diets to this level of choline tended to increase fat yield (Erdman et al., 1984). In sharp contrast with this 30 g value, Zinn et al. (1987),

extrapolating from the requirement for swine, proposed that 1 g post-ruminal choline was needed daily by growing steers. Loss in milk can be large. A cow producing 50 kg of milk each day secretes 9.6 g of choline. Atkins et al. (1988), using mature steers fed a 50% corn silage diet, estimated that the post ruminal supply of choline was 14 to 17 g. He noted that ruminant diets contain choline, but only 1.3 g of the 26 g of choline they added to the diet escaped ruminal digestion. This indicates that most post-ruminal choline is derived from microbes that synthesize phosphatidylcholine. Choline synthesis has been associated with ruminal protozoa (John and Ulyatt, 1979). Owens (unpublished data) calculated choline synthesis and ruminal degradation by combining data from various literature studies. The value he estimated with steers was that 1 g choline flowed out of the rumen daily. The choline yield from protozoa would be much less than 1 g with feedlot diets or defaunated ruminants. Under such conditions, choline should be deficient, so increasing the post-ruminal supply should have a beneficial effect on animal performance. Feedstuffs rich in choline content may increase post-ruminal supply of available choline slightly, but ruminal escape is limited and the contribution of feedstuffs to the total choline requirement of dairy cows would be minimum. For example, if the post-ruminal choline requirement of a dairy cow is 30 g /d, the cow would have to consume 39 kg /d of fish-meal to meet this need, a condition that is neither practical nor economically feasible. Thus, in order to increase the post-ruminal supply of available choline, synthetic sources containing much higher amounts of choline that are protected from rumen degradation must be developed.

Factors affecting choline and betaine requirements

Response to dietary supplementation of choline depends on various dietary factors. These include the 1) basal dietary supply of methionine, betaine, myo-inositol, folacin, and vitamin B_{12}, 2) level and composition of fat, carbohydrate, and protein in the diet, and 3) age, sex, and growth rate of animals. These factors influence on the lipotropic action of choline and thereby the requirement for choline (Mookerjea, 1971). Studies by Welch and Couch (1955) have shown that supplemental vitamin B_{12} and folacin can reduce the requirement for choline in rats and chicks. Biosynthesis of labile methyl from a formate carbon requires folacin, while B_{12} plays a role in regulating transfer of the methyl group to tetrahydrofolic acid. Therefore, a marked increase in choline requirement has been observed under conditions of folacin and/or vitamin B_{12} deficiency.

In choline deficiency, methionine furnishes methyl groups to combine with ethanolamine to form choline; in reverse, methyl groups from choline (via betaine) can unite with homocystine to form methionine (du Vigneaud et al., 1939). Therefore, dietary adequacy of both methionine and choline directly affect the requirement of the other. Other than exogenous sources of methyl groups from choline and methionine, methyl formation from de novo synthesis of formate carbons will be reduced by folate and/or vitamin B_{12} deficiencies. The requirement for choline increases when the amount of methionine in the diet is deficient (Zeisel and Blusztajin, 1994). They suggested further that the requirement for choline increases as energy and fat content of the diet increases.

Males are more sensitive to choline deficiency than females (Wilson, 1978). Excess dietary protein increases the young chick's choline requirement (McDowell,

1989). He also noted that diets rich in fat aggravate a choline deficiency and increase its requirement.

The need for choline presumably is lower for adults than for infants because substantial amounts of choline are used for phospholipid deposition in growing organs (Zesiel, 1990). Age and size of an animal also can affect the choline or betaine requirement. Choline is considered to be an essential and potentially growth limiting metabolite in young animals (Canty and Zeisel, 1994, and NRC, 1981). Choline supplementation increased ADG of calves (Gralak et al. 1998). Similarly, choline had a positive effect on growth rate of smaller and younger finishing (350 kg) steers (Bryant et al. 1999, and Bindel et al. 1998). In contrast, no benefit from added choline was detected with older and heavier (463 kg) finishing feedlot steers (Goodall and Brethour, 1999). This difference implies that younger and lighter animals have higher requirement for choline than older or heavier animals. Supplemental choline also may be required by pregnant and lactating animals. Zeisel et al. (1995) showed that during pregnancy and lactation a "typical" diet was not able to meet the extraordinary demand for choline during pregnancy and lactation in rat. Erdman et al. (1984) detected a beneficial effect from adding dietary free choline on production of 4% fat corrected milk and milk fat percentage of lactating dairy cows. Sharma and Erdman (1988b) observed that post-ruminal infusion of choline in dairy cows increased the fat percentage in milk. Erdman and Sharma (1991) have shown that ruminally protected choline chloride increased milk production. The concentration of choline in the diet and animal status can affect the choline requirement of animals. Puchala et al. (2000, unpublished data) indicated that

hair- producing animals catabolize choline at much faster rate than animals producing little or no hair. Therefore, haired animals have a higher requirement for choline.

Deficiency of choline

The most common signs of choline deficiency include poor growth, fatty livers, perosis, hemorrhaging tissue, and hypertension (McDowell, 1989) in non-ruminants. In addition, choline deficiency in poultry reduces egg production and increases chick mortality.

Deficiency in ruminants: The ability of ruminants to synthesize choline is well known. However, ruminal synthesis and /or an un-supplemented diet may not always supply enough choline to maximize performance of feedlot cattle. An apparent choline deficiency syndrome was produced with a synthetic milk diet containing 15% casein (Johnson et al., 1951). Within 6-8 days, calves developed extreme weakness, labored breathing, and were unable to stand. Supplementation with 260 mg of choline per liter of milk replacer prevented these deficiency signs. In growing animals, fatty liver and reduced growth rate are the most common choline deficiency symptoms. Morrow (1976) indicated that the use of 50 g of choline chloride per cow per day as a treatment method for fat cow syndrome. However, since no controls were used in these studies, it is not known whether dietary choline was an effective treatment. Choline deficiency results in increased serum free fatty acid concentrations, and reduces circulating triacylglycerols (Kuksis and Mookerjea, 1978). Impaired release of liver triacylglycerols in choline deficiency has been linked to a defect in either synthesis of apolipoprotein or attachment of triacylglycerols to apolipoprotein. Since liver triacylglycerols are released almost

uniformly in the form of lipoprotein, any defect in this step would result in an increased liver lipid content.

Table 1. Choline content of selected feedstuffs.

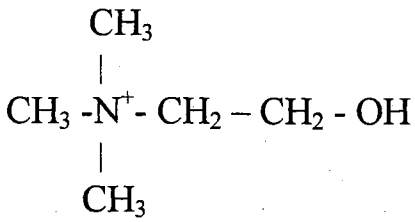
Feeds	Choline, mg/kg	Reference
Alfalfa meal	1401	2
Barley	990	2
Blood meal	695	2
Brewer's dried grain	1723	2
Corn	620	2
Corn gluten feed	1518	2
Corn gluten meal	330	2
Cotton seed cake (solv.extracted)	2685	2
Distiller's dried grains	1180	2
Fish meal, menhaden	3056	2
Hominy feed	1155	2
Meat and bone meal	1996	2,3
Oats	946	2
Peanut meal (solv. Extracted)	2396	2
Soybean (solv. Extracted)	2794	2
Sunflower (solv. Extracted)	3791	2
Wheat bran	1232	2
Wheat middlings	1439	2
Timothy hay	360	2

Source: [2]NRC, 1994; [3] NAS, 1971

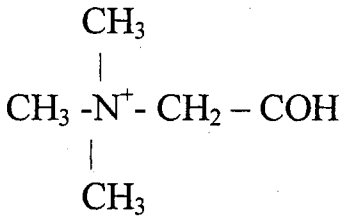
Table 2. Betaine content of common feed ingredients

Feeds	Betaine, mg/kg	Reference
Alfalfa, 17% dehydrated meal	3175-3850	1
Soybean meal	Below detection limit*	2
Fish meal	400	2
Meat meal	190	2
Canola meal	Below detection limit	2
Corn, ground yellow	Below detection limit	1
Milo, ground	Below detection limit	1
Rice	Below detection limit	2
Oats	590	2
Barley	730	2
Wheat, ground	1400	2
Wheat bran	2675	1
Wheat standard middling	2675	1
Beet molasses	6200	

* Average detection limits for betaine in feedstuffs is about 150 mg/kg
Source: 1 = Westberg, 1951: 2 = Vertanen, 1993



(a) Choline



(b) Betaine

Figure 1. Structure of choline and Betaine

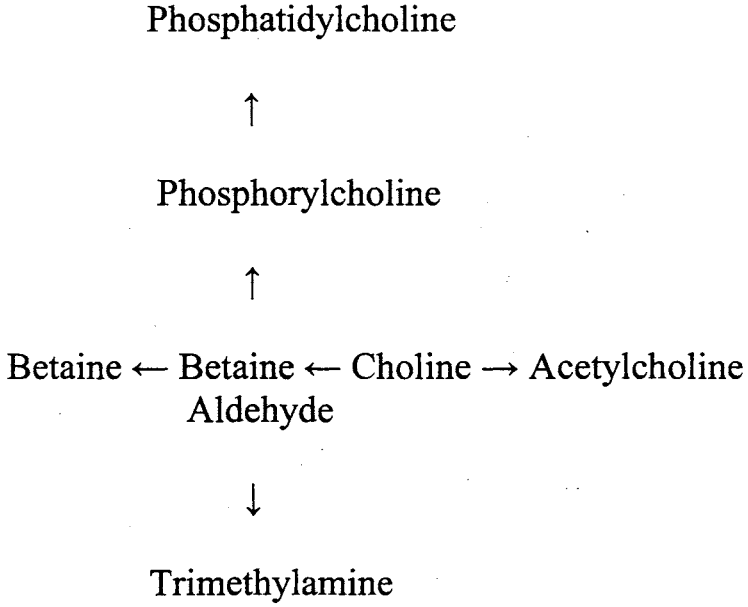


Figure 2. Metabolism of Choline

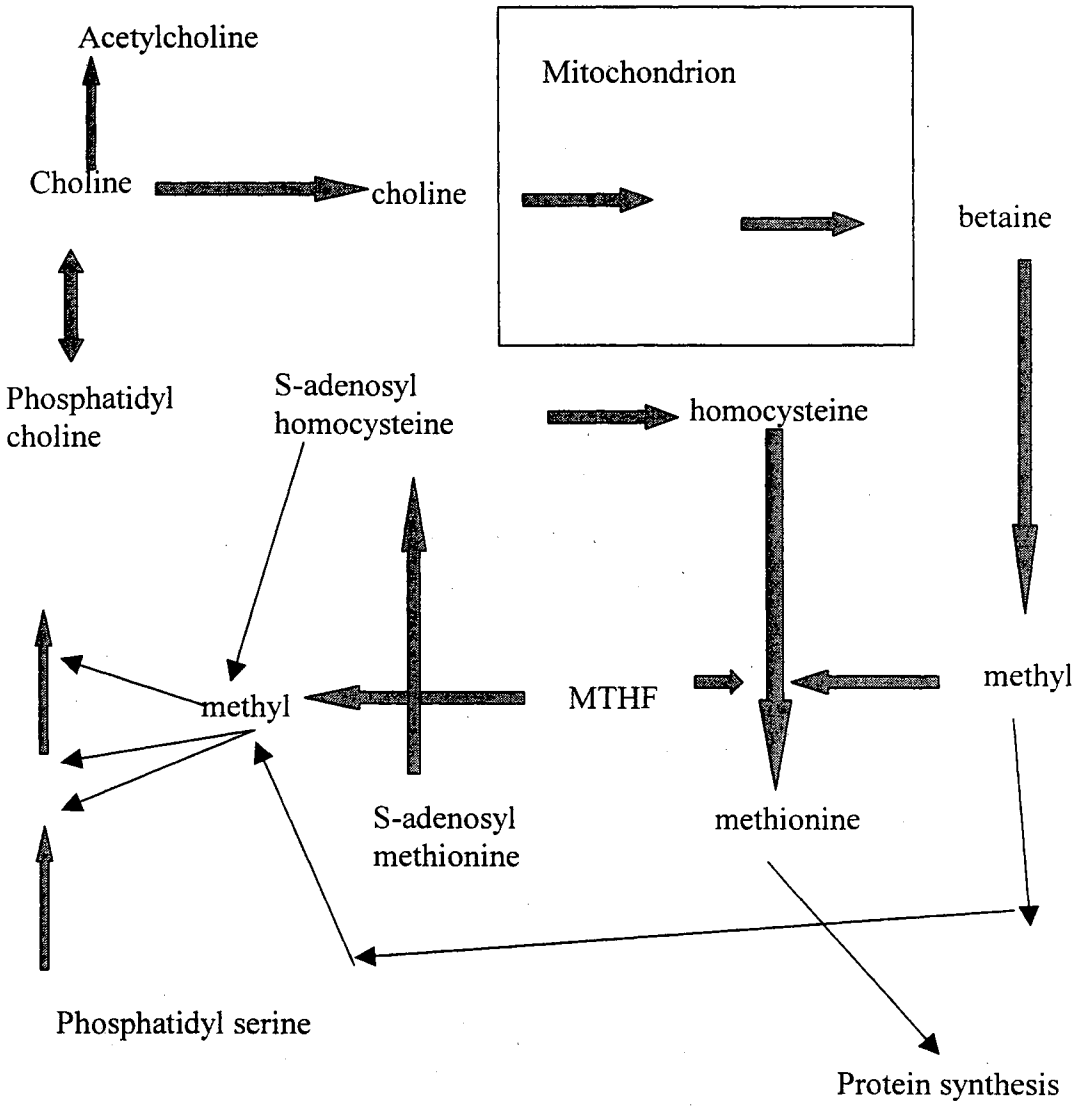


Figure 3. Metabolic relationship between choline, methionine and betaine

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CHAPTER III

EFFECTS OF RUMINALLY PROTECTED CHOLINE ON PRODUCTIVITY OF ANGORA GOATS

T. Shenkoru[12], F. N. Owens[2], R. Puchala[1], A. Goetsch[1], and T. Sahlu[1]

[1]E (Kika) de la Garza Institute for Goat Research, Langston University, OK

[2]Animal Science Department, Oklahoma State University, Stillwater, OK

Abstract

Twenty-five Angora wethers (29 ± 6 kg initial BW and > 1 yr of age) were used to evaluate effects of a ruminally protected choline chloride product (PC) on weight gain, mohair growth and characteristics, and concentrations of ruminal fluid and blood plasma constituents. Animals were randomly allocated to five treatments and given *ad libitum* access to a 55% concentrate diet (oat-based, 13% CP) for 90 d. Treatments included 0, 4, 8, and 12 g of PC per kg of diet DM (C, 1PC, 2PC, and 3PC, respectively) and 4 g of unprotected choline chloride per kg of diet DM (UPC). Dry matter intake, daily gain, mohair yield, and mohair staple length were not significantly affected by choline treatment

($P > 0.10$). However, mohair fiber diameter ($P < 0.05$; 31.1 vs. 35.2 μm) and the proportion of medullated fibers ($P < 0.07$; 0.27 vs. 0.47%) were lower for goats fed UPC than for goats fed 3PC. The proportion of kemp fiber increased linearly ($P < 0.002$) as PC increased and was greater ($P < 0.02$) for goats fed 3PC than for those fed UPC. Ruminal pH linearly decreased ($P < 0.01$) as the level of PC increased. The molar percentage of acetate increased and butyrate decreased linearly ($P < 0.07$) as level of PC increased, whereas the molar percentage of propionate was less for 1PC and 2PC than for C and 3PC (quadratic; $P < 0.07$). Plasma concentration of NEFA increased then declined as PC level increased. Triacylglycerol concentration was tended to be lower for 3PC than for UPC (17.2 vs. 22.1 mg/dL). Plasma concentration of methionine linearly increased ($P < 0.03$) as dietary PC increased. Although dietary supplementation of Angora wethers with PC did not influence ADG or mohair growth, effects on ruminal fermentation conditions, plasma concentrations of methionine and NEFA, and proportions of medullated and kemp fiber were detected that deserve further attention.

Key words: Angora goats, Choline, Mohair, Productivity

Introduction

Choline is considered essential for young calves (Johnson et al., 1951). The adult ruminant's requirement for choline is assumed to be met by dietary sources plus synthesis in the rumen (Smith and Church, 1971) and liver (Bremer and Greenberg, 1961; Neill et al. 1979). However, Dyer et al. (1962) reported that daily gain of finishing steers was increased by 7, 10, and 21%, when 3, 1, and 4 g/d, respectively, of unprotected choline was added to the diets. Although, factors responsible for this effect are unknown, Puchala et al. (1997) observed that plasma methionine concentrations increased when a diet was supplemented with unprotected choline. In contrast, Wise et al. (1964) and Harris et al. (1966) detected no benefit from addition of unprotected choline to high concentrate diets fed to finishing beef steers. Differences in results among such experiments might relate in part to differences in choline requirements and to extensive ruminal degradation of choline added to the diet. To avoid rumen catabolism, products with choline protected from ruminal destruction have been developed. Research with steers in New Mexico (Galyean et al., 1997) and Kansas (Goodall and Brethour, 1999) has shown that adding 5 to 20 g of ruminally protected choline to the diet per day appreciably improved rate and efficiency of gain. Similarly, Erdman and Sharma (1991) found that diet supplementation with ruminally protected choline increased milk production of dairy cows. Effects of supplementing the diet with ruminally protected choline on performance of mohair-producing Angora goats have not been studied. The potential for a benefit from choline through increasing the supply of methionine was suggested by results of Puchala et al. (1997); methionine should be most beneficial to

sheep and goats because of the high S content of the animal fiber (protein) that they produce. The objective of this experiment was to determine effects of dietary supplementation with ruminally protected choline, on live weight gain, mohair growth and characteristics, and concentration of ruminal fluid and blood constituents.

Materials and Methods

Animals and Diets

Twenty-five Angora wethers (29 ± 6 initial BW and > 1 yr of age), housed individually in metabolic crates were used in this study. The experiment was conducted at the Oklahoma State University Animal Science Nutrition Laboratory. Animals randomly allocated to five treatments, were given *ad libitum* access to a 55% concentrate diet (oat-based, 13% CP) for 90 days. The totally mixed diets were similar in chemical composition except for their choline content (Table 1). Choline was added to each diet to supply 4, 8, and 12 g/d of the ruminally protected choline chloride product per goat was determined by assuming that DM intake would average 1 kg/d. The ruminally protected product was assayed to contain 25% choline, whereas choline content of the unprotected product (choline chloride) was 75%. Diets were designed to contain either 0, 4, 8, and 12 g of protected choline per kg of diet DM (Balchem, Slate Hill, NY) (C, 1PC, 2PC and 3PC, respectively) or 4 g of unprotected choline (UPC). This translates to intakes of 0, 1, 2, 3, and 3 g of choline daily for the five different diets if animals consumed 1 kg of feed each day. The ruminally protected product consisted of choline chloride coated with hydrogenated vegetable oils, corn flour, reduced iron, and a surfactant. The diet was formulated to provide adequate amounts of energy, CP, minerals and vitamins for

growing goats (NRC, 1981). Animals were fed once daily at 0800 and had free access to water. The experimental diets were offered free-choice for 90 days with the amount of feed provided each day being approximately 110% of the amount consumed during the preceding few days.

Feed Samples Collection and Analyses

The amount of feed offered and refused each day were recorded. Samples of feed and orts were obtained once weekly, composited across 30 d periods, and analyzed for DM, ash, N (AOAC, 1990), and NDF and ADF (filter bag technique; ANKOM Technology Corp., Fairport, NY, USA). Gross energy was determined with an adiabatic bomb calorimeter (Parr Instrument, Moline, IL).

Mohair Yield and Quality Evaluation

At the beginning and end of the experiment, each goat was shorn. From these samples, greasy and clean mohair weights were measured according to ASTM (1990) procedures. Fiber length and diameter, and the proportion of medullated and kemp were measured from samples sheared from skin patches taken at 30-d intervals from the same 100 cm^2 of skin each time. Mohair diameter was measured with a Peyer Texlab FDA 200, as described by Lynch and Michie (1976). Staple length was determined using an Agritest Staple Breaker System (Agritest, 1988) and medullated and kemp fiber were determined as described by ASTM (1990). When the diameter of the medulla was less than 60% of the total fiber diameter, the fiber was classified as a med fiber; when a fiber

had a medulla greater than 60% of the fiber diameter, it was classified as a kemp fiber (ASTM, 1990).

Ruminal pH and VFA

Ruminal samples were taken via stomach tube and blood samples were obtained at 30-d intervals during the study. Ruminal fluid was collected by inserting a tube with a strainer tip into the rumen and applying a mild vacuum with a 60-cc syringe. The first 20 to 30 mL of ruminal fluid obtained was discarded to reduce contamination with saliva. Ruminal pH was measured using a pH meter (SA-720, Orion Research, Boston, MA) immediately after sampling. Thereafter, 20 mL of fluid was collected for analysis. For VFA measurements, 1 mL of 25% (wt/wt) metaphosphoric acid was added to 4 mL of ruminal fluid, and the mixture was stored at -20°C until being analyzed with a gas chromatograph (Hewlett-Packard Co., Avondale, PA). In other tubes, 3 mL of ruminal fluid was combined with 2 mL of 4% (wt/vol) of trichloroacetic acid for ammonia analysis by the procedure of Broderick and Kang (1980).

Blood Samples Collection and Analyses

On days 0, 30, 60, and 90, jugular blood was obtained via venipuncture 2 h post-prandially. Blood samples were collected into 7-mL tubes containing either potassium oxalate and sodium fluoride, K_3 EDTA, or sodium heparin (Becton Dickinson, Vacutainer Systems, Ruthrerford, NJ). The tubes were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at 1,500 x g at 4°C for 20 min. Plasma aliquots were stored at -20°C until analyzed.

Blood Metabolites

Plasma glucose concentrations were analyzed colorimetrically using a Technicon Autoanalyzer II System(Technicon Instruments, Tarrytown, NY). Plasma urea N was determined as described by Chany and Marbach (1962). Non-esterified fatty acids (Wako Kit No. 990-75401, Wako Biochemical, Osaka, Japan), triacylglycerol (Wako kit no. 997-69801, Wako Biochemical), and cholesterol (Sigma Kits, No. 352, Sigma Aldrich) also were quantified. Plasma choline was determined using the enzymatic method described by Nie et al. (1993).

Plasma Amino Acids

For amino acids analysis, 0.45 mL of blood plasma was deproteinized using 0.05 mL of a 50% (wt/vol) 5-sulfosalicylic acid solution containing sarcosine and norvaline as internal standards. This mixture was vortexed, centrifuged (1,500 x *g*; 4°C; 10 min) and placed in sample vials. Amino acid analysis was performed with an AminoQuant system (Hewlett-Packard, San Fernando, CA) using precolumn derivatization with ortho-phthaladehyde and 9-fluorenylmethychloromate and UV detection.

Digestibility

A digestibility study was conducted during the last week of the trial using three animals per treatment. Total feces excreted were collected for 7 d using fecal bags. These bags were attached to the animals 3 days before collection started to allow time for adaptation. An aliquot (10%) of feces produced each day was dried at 60°C for 48 h, ground to pass a 1-mm screen and stored frozen until analyzed. Nitrogen content of feces

was determined according to AOAC (1990) procedures. A fresh 100-g sample of feces was dried for 24 h at 100°C to determine DM concentration.

Statistical Analysis

The analysis included the adoption of a covariance structure for the repeated measures. Where appropriate, Satterthwaites's approximation for the degrees of freedom was used (Littell et al. 1996).

For certain data (ADG, greasy and clean mohair, N intake, digestibility of feed, N retained, feed efficiency, and the ratio of acetate: propionate), values were analyzed using a completely randomized design and the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Non-orthogonal polynomial contrasts were used to determine the linear, quadratic, and cubic effects, and to contrast the response to UPC (1 g unprotected choline per kg diet) with that from 3PC (1 g protected choline per kg diet).

Results and Discussion

Performance Data

Average daily gain (ADG), feed efficiency, dry matter intake (DMI), DMI as percentage of body weight (BW), and DMI, in g/kg $BW^{0.75}$ were not significantly different for goats fed the different diets ($P > 0.10$, Table 2). However, goats receiving 2PC and 3PC diets tended to have to have slightly greater ADG (18.4 and 16.2%) than goats receiving no PC. Galyean et al. (1997) and Bindel et al. (1998) reported that ADG was increased by 11

and 8.6%, respectively, when finishing cattle received 5 g of supplemental protected choline per day. Likewise, Gralak et al. (1998) supplemented the diet of black and white lowland calves with 2.25% PC and reported that ADG during a 15-wk experiment was increased. However, Goodall and Brethour (1999) observed no difference in ADG when finishing feedlot steers were supplemented with 4.5 g/d PC. Bryant et al. (1999) also found no effect of adding 0.25% PC on ADG by Suffolk lambs wethers (90-d of age), although ADG was increased when 1% PC was included in the diet. Similarly, ADG of Alpine Doelings (17-wk of age) increased as dietary PC was increased (Puchala et al. unpublished data). The more limited ADG response to PC supplementation observed in our experiment may be due to the fact that goats in our experiment were older than the Alpine doelings used by Puchala et al. (unpublished data). Younger and (or) lighter animals may have a higher requirement for choline than do older and (or) heavier animals. Choline can be synthesized in the body using methyl groups derived from methionine. The reason for the differences observed among the different experiments also could be due to the fact that mature animals can synthesize and re-circulate adequate amounts of choline to maintain an endogenous choline pool. On the other hand, supply of methionine available in young animals should be lower than for older animals because protein synthesis, and thereby methionine use, is proportionally greater.

Although one report (Goodall and Brethour, 1999) suggested that DMI was increased in response to PC supplementation, we did not detect a significant effect of PC on DMI in our experiment.

Based on the concentration of choline in the protected product, actual intakes of choline for C, 1PC, 2PC, and 3PC averaged 0, 1.17, 2.14, and 3.26 g/d, respectively, while

goats fed unprotected choline consumed 3.18 g choline per day. The company (Balchem, Slate Hill, NY) that manufactured PC claimed that post ruminal digestion of this product should exceed 90% of delivery choline. Indeed, nylon bag degradability study (our unpublished observations) of the protected choline chloride product detected 7% ruminal degradation from dacron bags after 48 h of incubation. Furthermore, based on 42% fecal recovery of weight of material placed in mobile dacron bags, we would estimate a maximum of 56% postruminal disappearance of choline chloride reaching the duodenum. Based on these estimates, the quantity of supplemental choline from PC that should be available to absorb from the intestine would have been 0.61, 1.11, and 1.70 g/d for 1PC, 2PC, and 3PC, respectively.

Mohair Yield and Quality

Regression analysis indicated that fiber diameter increased by 1 μm for every 53 g increase in fleece weight produced during the 12 weeks trial. This compares quite well with a 1 μm increase in diameter for every 50 g increase in fleece weight reported by Bown et al. (1992) for Angora does. Mohair yield and staple length were not affected by treatment. However, both mohair diameter and the proportion of medullated fibers were lower (12% and 43%) for UPC than for 3PC ($P < 0.05$ and 0.07, respectively). Fiber kemp increased linearly ($P < 0.002$) as PC supplementation increased and was 112% greater ($P < 0.02$) for 3PC than for UPC. These results depict a negative effect of PC on mohair quality. Most kemp fibers appear chalky white after dyeing, whereas medullated fibers accept dye normally (Lupton, 1993). Most previous reports (Tiffany-Castiglioni,

1986; Lupton et al., 1991) have indicated that nutrient intakes have little or no impact on medullation or kemp prevalence.

Ruminal pH and VFA

Ruminal pH decreased linearly ($P < 0.01$) as PC intake increased (Table 4). Ruminal pH was 0.24 units greater for UPC than 3PC ($P < 0.02$). The concentration of total VFA first decreased but then increased as level of PC in the diet increased (quadratic; $P < 0.02$), with the total VFA concentration being 10 m*M* greater for 3PC than C. The molar percentage of acetate increased linearly ($P < 0.07$) as PC increased. The molar percentage of propionate first declined but then rose as PC level increased (quadratic effect; $P < 0.05$) but was similar for C and 3PC. The molar percentage of butyrate decreased linearly ($P < 0.05$) as the level of PC increased. The isovalerate molar percentage and the acetate to propionate ratio changed quadratically with PC supplementation, with the highest values for goats fed the 1PC diet. This observation contradicts findings by Erdman et al. (1984), Rumsey (1985), Sharma and Erdman (1988), each of which reported that dietary unprotected choline supplementation did not influence ruminal fermentation. In contrast, Swingle and Dyer (1970) noted that both total and individual VFA concentrations increased when diets were supplemented with unprotected choline.

Blood Plasma Metabolites

No significant differences were observed in plasma glucose, or cholesterol concentration (Table 5) although the concentration of non-esterified fatty acid (NEFA) tended to increase and then to decline as dietary PC increased (quadratic, $P < 0.10$). Sharma and Erdman (1989) reported that NEFA concentration was increased by PC supplementation and suggested that PC elevated the efficiency of utilization of triacylglycerol by tissues, thus increasing blood NEFA level. Dietary treatments had no significant impact on triacylglycerol concentration in this study, but plasma urea-N decreased then increased as dietary PC level increased (quadratic effect, $P < 0.08$).

Plasma choline concentration was not significantly different among treatments, although absolute values were lowest for goats receiving no supplemental choline (Table 5). Previously, supplementation of Alpine doelings with PC resulted in increased plasma choline concentrations (Puchala et al., 1995). However, Sharma and Erdman (1989) detected no change in plasma choline concentration in cows with abomasal infusion of up to 90 g/d of choline chloride. After observing the varied reports in the literature, Kerri et al. (1998) suggested that choline concentration in peripheral blood is not reliable as an indicator of choline absorption.

Plasma Amino acids

Among the amino acids, choline supplementation influenced blood plasma concentrations of only two amino-acids methionine and leucine (Table 6). Methionine concentration increased linearly ($P < 0.03$) as dietary PC increased. Others (Pesti et al.,

1981; Baker et al., 1983) have reported that choline, through supplying methyl groups for various reactions can spare methionine by reducing the need for s-adenosyl-methionine. Further, being a source of labile methyl groups, choline can supply labile methyl groups for converting homocysteine to methionine. Thereby, choline could increase the amount of methionine available to the animal. Jackson (1996) stated that conversion of homocysteine to methionine is limited because the homocysteine concentration in typical feedstuffs are very low. Some workers (Baker and Czarnecki, 1985; Puchala et al., 1995) have postulated that betaine, but not choline, can enhance conversion of homocystiene to methionine. The factors responsible for the linear increase in plasma leucine ($P < 0.07$) with the increasing dietary PC level are unclear.

Nitrogen intake increased quadratically ($P < 0.05$) as dietary PC level increased. Digestible N intake first increased but then decreased as dietary PC level increased (quadratic, $P < 0.10$) with the highest digestible N intake being for goats receiving the 1PC diet.

Conclusions

Although dietary supplementation of Angora wethers with PC did not influence ADG or mohair growth, PC had effects on concentration of ruminal fermentation products, plasma concentration of methionine and NEFA, and the proportions of medullated and kemp fiber in mohair. The increased NEFA concentration may reflect increased lipid turnover. The increased proportion of kemp fiber with increasing level of

PC depicts a negative effect of PC on mohair quality. How choline in the ruminally-protected form can affect ruminal metabolism or plasma methionine concentrations is not yet clear. Further studies are needed to detect the mechanisms of action of protected choline.

Table 1. Composition of experimental diets fed to Angora goat

Item	Treatment[a]				
	C	UPC	1PC	2PC	3PC
Ingredient	%DM				
Alfalfa hay	7.0	7.0	7.0	7.0	7.0
Ground corn	19.0	19.0	19.0	19.0	19.0
Ground oats	25.0	25.0	25.0	25.0	25.0
Soybean meal	9.0	9.0	9.0	9.0	9.0
Cottonseed hull	37.8	37.5	37.4	37.0	36.6
Unprotected choline	-	0.4	-	-	-
Protected choline	-	-	0.4	0.8	1.2
Trace minerals [b]	1.0	1.0	1.0	1.0	1.0
Limestone	1.0	1.0	1.0	1.0	1.0
Vitamin premix[c]	0.2	0.2	0.2	0.2	0.2
Chemical composition					
Ash, % DM	6.0	5.6	5.3	5.5	6.1
GE, Mcal /kg DM	4.1	4.1	4.2	4.1	4.2
CP, % DM	10.4	10.5	11.1	12.0	11.4
NDF, % DM	51.6	52.5	52.0	48.8	45.1
ADF, % DM	30.4	36.2	32.6	29.5	30.5

[a]C = no added choline chloride, UPC = 4 g unprotected choline chloride, 1PC = 4 g protected choline chloride per kg diet DM, 2PC = 8 g protected choline chloride per kg diet DM, 3PC = 12 g protected choline chloride per kg diet DM.

[b]Containing (percentage): NaCl 94 to 95; Mn > 0.2; Ferrous Fe > 0.16; Ferric Fe > 0.14; Cu > 0.033; Zn > 0.10; I > 0.007; and Co > 0.005.

[c]Each gram contained 2,200 IU of vitamin A, 2,200 IU of vitamin D_3, and 0.2 IU of vitamin E

Table 2. Effects of types and dietary level of choline chloride on average daily gain, dry-matter intake, and feed efficiency in Angora goat

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quadratic	Cubic	3PC vs UPC
No. of Animals	5	5	5	5	5					
Body weight[c]	32.6	31.4	32.8	32.6	32.6	3.08				
DMI, g/d	1,062	1,061	1,166	1,070	1,087	161	0.99	0.66	0.86	0.86
DMI, %BW	3.24	3.12	3.65	3.40	3.36	0.37	0.95	0.54	0.60	0.99
DMI, g/kg $BW^{0.75}$	77.8	78.5	87.9	78.1	79.7	6.51	0.99	0.96	0.97	0.98
ADG, g/d	84.1	81.2	77.7	99.6	97.7	14.2	0.35	0.85	0.41	0.44
Gain, g/kg DMI	79.9	79.3	66.0	95.1	89.8	11.9	0.28	0.75	0.17	0.56

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and 3PC

[c]Average body weight for 90 days, used to calculate DMI, % BW and DMI, g/ kg $BW^{.75}$

Table 3. Effects of types and dietary level of choline chloride on mohair yield and qualities in Angora goat

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quadratic	Cubic	3PC vs UPC
Clean mohair, kg*	1.90	1.61	1.48	1.85	1.63	0.19	0.53	0.78	0.21	0.96
Grease mohair, kg*	2.14	1.64	1.74	2.06	1.76	0.20	0.37	0.82	0.15	0.68
Mohair length, mm	25.1	23.8	26.0	26.1	25.1	1.32	0.83	0.45	0.89	0.24
Mohair diameter, μm	33.6	31.1	36.4	33.8	35.2	1.24	0.65	0.60	0.21	0.05
Medullated fiber	0.34	0.27	0.37	0.54	0.47	0.11	0.11	0.55	0.29	0.07
Kemp fiber	0.38	0.64	0.67	1.04	2.00	0.46	0.002	0.33	0.80	0.001

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and 3PC

*90-d growth

Table 4. Effects of types and dietary level of choline chloride on ruminal metabolites in Angora goats

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quad	Cubic	3PC vs UPC
Ruminal, pH	6.32	6.15	6.14	6.17	5.91	0.10	0.001	0.59	0.11	0.02
VFA, m*M*	61.7	62.1	51.6	57.6	71.5	6.66	0.10	0.02	0.70	0.17
Acetate, mol/100 mol	65.0	66.1	67.3	66.6	67.7	1.22	0.07	0.51	0.21	0.22
Propionate, mol/100 mol	20.1	19.7	17.1	18.0	19.7	1.50	0.95	0.05	0.49	0.96
Isobutyrate, mol/100 mol	0.69	0.80	0.89	0.76	0.64	0.12	0.45	0.08	0.31	0.20
Butyrate, mol/100 mol	12.1	11.1	11.7	11.9	10.4	0.60	0.02	0.10	0.13	0.22
Isovalerate, mol/100 mol	0.83	1.08	1.55	1.30	0.75	0.32	0.55	0.02	0.40	0.31
Valerate, mol/100 mol	1.25	1.15	1.28	1.22	1.04	0.18	0.26	0.36	0.85	0.53
A : P ratio[c]	3.30	3.50	4.10	3.80	3.50	0.27	0.80	0.05	0.38	0.99

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC = 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and C.

[c]Calculated as acetate(m*M*)/ Propionate (m*M*),

Table 5. Effects of types and dietary level of choline chloride on plasma metabolites in Angora goats

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quad	Cubic	3PC vs UPC
Glucose, g/d	96.5	90.3	99.0	96.5	93.4	6.80	0.58	0.57	0.84	0.65
NEFA, meq/L[c]	148	138	182	161	123	29.9	0.35	0.10	0.71	0.66
Total protein, g/L	88.1	71.2	77.7	83.1	74.4	9.85	0.26	0.90	0.35	0.76
Urea-N, mg/dL	19.3	16.7	16.7	17.4	19.9	1.96	0.70	0.08	0.83	0.12
Cholesterol, mg/dL	110	106	96.0	106	104	9.41	0.82	0.36	0.26	0.84
Triglyceride, mg/dL	14.5	22.1	17.7	15.2	17.2	4.88	0.61	0.73	0.64	0.14
Plasma choline, μM	4.03	5.39	5.44	5.88	6.12	0.64				

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC = 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and C

Table 6. Effects of types and dietary level of choline chloride on in plasma amino acids concentration (μmol/L) in Angora goats

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quad	Cubic	3PC vs UPC
Glutamine	140	144	153	132	155	9.25	0.46	0.47	0.01	0.25
Serine	158	176	155	141	163	14.9	0.92	0.28	0.30	0.34
Histamine	69.8	70.2	65.0	64.3	71.5	4.87	0.79	0.09	0.85	0.90
Glysine	946	1,074	987	979	990	71.1	0.23	0.70	0.74	0.28
Threonine	82.8	79.8	79.4	79.6	91.9	8.57	0.33	0.22	0.74	0.18
Alanine	243	237	236	220	238	16.6	0.56	0.32	0.43	0.99
Arginine	175	195	178	186	200	16.3	0.11	0.63	0.99	0.74
Tyrpsine	68.8	65.4	67.8	64.8	74.4	6.36	0.65	0.34	0.45	0.27
Valine	228	248	241	252	270	16.6	0.11	0.64	0.99	0.74
Methionine	15.1	15.3	15.8	17.4	18.7	1.78	0.03	0.78	0.89	0.07
Phenylalanine	49.2	48.6	49.8	53.9	54.4	3.54	0.11	0.88	0.55	0.17
Leucine	118	124	122	133	142	13.2	0.07	0.83	0.80	0.24

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC = 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and C.

Table 7. Effects of types and dietary level of choline chloride on nitrogen metabolism during metabolism trial in Angora goats

	Treatment[a]						Contrast[b] and probability			
Item	C	UPC	1PC	2PC	3PC	SE	Linear	Quad	Cubic	3PC vs UPC
Intake, g/d	15.3	18.0	25.4	21.3	17.5	3.04	0.87	0.05	0.31	0.90
Fecal output, g/d	3.84	5.72	5.54	6.03	5.37	0.67	0.12	0.11	0.98	0.72
Digestibility, %[a]	74.1	69.1	77.0	69.8	67.5	5.43	0.30	0.63	0.86	0.98
Digestible N intake, g/d	11.5	12.3	19.8	15.2	12.1	3.13	0.85	0.10	0.33	0.97
Expected digestibility, %[c]	61.2	61.4	63.1	65.0	63.6					

[a]C = no added choline chloride, UPC = 4 g/d unprotected choline chloride, 1PC = 4 g/d protected choline chloride, 2PC = 8 g/d protected choline chloride, 3PC = 12 g/d protected choline chloride

[b]Linear, quadratic and cubic effects of level of protected choline, including UPC and C.

[c]Calculated as N digestibility (%)= (%CP*0.9 - 3)/ %CP as described by NRC (1996)

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CHAPTER IV

Betaine supplementation for enhancing mohair production by Angora goats

T. Shenkoru[1,2], F. N. Owens[2], R. Puchala[1], T. Sahlu[1], and A. Goetsch

[1]E (Kika) de la Garza Institute for Goat Research, Langston University, Langston, OK
[2]Animal Science Department, Oklahoma State University, Stillwater, OK

Abstract

Twenty-five Angora wethers (20 ± 2 kg initial BW and approximately 7 mo of age) were used to evaluate effects of ruminally protected betaine (PB) on ADG, mohair growth and characteristics, and concentrations of ruminal fluid and plasma constituents. Animals randomly allocated to five treatments were given *ad libitum* access to a 53% concentrate diet (15% CP) for 84 d beginning in September. Diets were supplemented with 0.0, 0.2, 0.4, and 0.6% of PB (C, 2PB, 4PB, and 6PB, respectively), or with, 0.6% unprotected betaine (UPB). Linear and quadratic effects ($P < 0$ .002, and < 0.03, respectively) in ADG with supplemental PB were detected. ADG was substantially greater with 6PB than UPB (82 vs 46 g/d). Feed efficiency also increased linearly ($P <$ 0.02) as dietary PB concentration was increased.

Mohair greasy (unclean) weight tended to increase quadratically ($P < 0.10$) with PB supplementation. Ruminal pH increased linearly ($P < 0$ .01) with increased level of PB in the diet while ruminal ammonia N responded quadratically being higher ($P < 0.05$) for UPB than 6PB. Total molar concentration of ruminal VFA also responded quadratically ($P < 0.05$) to dietary supplementation of PB whereas the molar percentage of isobutyrate, and isovalerate decreased ($P < 0.06$) as the amount of PB in the diet was increased. Plasma concentration of glucose first increased but then declined as PB level was increased (quadratic effect, $P < 0.02$). Plasma NEFA and glycine responded cubically. Intake of digestible N and retention of N increased linearly ($P < 0.001$, $P < 0.006$, respectively) with increasing PB level. In conclusion, supplementing the diet with protected betaine increased DMI, ADG, feed efficiency, greasy mohair production, digestible N intake and N retention by growing goats.

Key words: Angora goat, Betaine, Mohair, Productivity

Introduction

Betaine (trimethyl glycine) is a product of choline oxidation and a source of labile methyl groups (Odle, 1996). Betaine, choline, and methionine are nutritionally interrelated. Since betaine is an intermediate in choline metabolism, one might expect that betaine could replace choline in its roles in fat transport and metabolism in the liver (Bock et al., 2000). Spared choline in turn could be used in lecithin synthesis, which is used for fat transport (Fernandez et al., 1998). Another potential lipotropic activity of betaine is its conversion to acetate in the rumen (Mitchell et al., 1979). Betaine can donate its methyl group to remethylate homocystine to methionine. Betaine is more efficient than choline in donating methyl to remethylate homocystine to methionine. Donated methyl groups also can be used directly for other methylations, thereby sparing methionine. The possibility of using betaine to replace some dietary methionine is attractive because betaine is less costly than methionine.

Although betaine shares some functions with choline, independent physiological functions are possible. Bender (1984) reported that the dehydrogenation of choline to betaine requires an unusual type of dehydrogenase. At high levels of choline supplementation, this dehydrogenase could be a limiting step in choline metabolism and utilization. However, there is little published research on effects of supplementing diets with betaine. Hence, the objectives of this study were to determine the effects of levels of dietary inclusion unprotected betaine or of ruminally protected betaine on live weight gain, mohair growth and characteristics, and on concentrations of various ruminal fluid and blood constituents.

Materials and Methods

Animals and Diets

Twenty-five castrated Angora goats (20 ± 2 kg initial BW and 7 mo of age) were housed individually in metabolism crates for this trial. The experiment was conducted at the Oklahoma State University Animal Science Nutrition Laboratory. Animals were randomly allocated to five treatments and were given ad *ad libitum* access to a 53% concentrate diet (15% CP) for 84 d beginning in September. Treatments included no added betaine (C), 0.6% of unprotected betaine in the dietary DM (UPB), and 0.2, 0.4, and 0.6% of ruminally protected betaine in dietary DM (2PB, 4PB, and 6PB, respectively). The betaine content of the ruminally protected product, assayed by its producer, Finnsugar Bioproduct, Inc. Schaumburg, IL, was 60%. This translates to intakes of 0, 1.2, 2.4, 3.6, and 3.6 g of betaine daily for the five different diets if animals consumed 1 kg of feed each day. The lipid-coated product consisted of a mixture of feed grade betaine and calcium stearate. Each diet was mixed completely, and feed sorting by goats was minimal. Diets were formulated to contain adequate amounts of CP, energy, vitamins, and minerals for growing goats (NRC, 1981; Table 1). Diets were fed once daily at 0800 and water was available *ad libitum*. Diets were offered free-choice for 84 d at approximately 110% of the amount disappearing during the preceding few days. Before initiation of the experiment, goats were allowed to adapt to their diets for 2 wk. Animals were weighed every 28 d after a 16 h period of feed and water withdrawal.

Feed Sample Collection and Analyses

Daily feed intake was measured and feed and ort samples were collected daily and composited by week. Feed, feces, and urine were analyzed for DM, N (AOAC, 1990),

and NDF (filter bag technique; ANKOM Technology Corp., Fairport, NY). Gross energy was determined with an adiabatic bomb calorimeter (Parr Instrument, Moline, IL).

Mohair Yield and Quality Evaluation

Goats were shorn at the beginning and end of the experiment; mohair was weighed and evaluated for greasy weight according to ASTM (1990) procedures. Fiber growth and characteristics were measured for mohair samples sheared from the same 100 cm^2 patch of skin at 28-d intervals. Mohair diameter was measured with a Peyer Texlab FDA 200 instrument (Siegfried Peyer AG CH-8832, Wollerau, Switzerland) as described by Lynch and Michie (1976). Fiber staple length was determined using an Agritest Sample Breaker System (Agritest, 1988), and medullated and kemp fibers were quantified as described by ASTM (1990).

Ruminal pH and VFA

Ruminal samples were taken via stomach tube and blood was sampled at 28, 56 and 84 days after the trial began. The first 20 to 30 mL of ruminal fluid obtained was discarded to reduce contamination with saliva. Ruminal pH was measured using a pH meter (SA-720, Orion Research, Boston, MA) immediately after sampling. Thereafter, 20 mL of ruminal fluid was collected for analysis. For VFA measurements, 1 mL of 25% (wt/wt) metaphosphoric acid was added to 4 mL of ruminal fluid and the mixture was stored at -20°C until being analyzed with a gas chromatograph (Hewlett- Packard Co., Avondale, PA). In other tubes, 3 mL of ruminal fluid was combined with 2 mL of 4% (wt/vol) of trichloroacetatic acid for ammonia analysis by the procedure of Broderick and Kang (1980).

Plasma Amino acids and Metabolites

Blood samples were taken via jugular venipuncture 2 h after feeding on days 28, 56, and 84. Blood was collected into 7-mL tubes containing potassium oxalate, sodium fluoride, K_3 EDTA, or sodium heparin (Becton Dickinson, Vacutainer Systems, Rutherford, NJ). Tubes were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at 1,500 x *g* at 4°C for 20 min. Plasma aliquots were stored at -20°C until analyzed. Plasma glucose concentration was analyzed colorimetrically using a Technicon Autoanalyzer II System (Technicon Instruments, Tarrytown, NY). Plasma urea N was determined as described by Chaney and Marbach (1962). For amino acids analysis, 0.45 mL of plasma was deproteinized using 0.05 mL of a 50% (wt/vol) 5-sulfosalicylic acid solution containing sarcosine and norvaline as internal standards. The mixture was vortexed, centrifuged (1,500 x *g*; 4°C; 10 min), and placed in vials. Amino acid analyses were performed on an Amino Quant system (Hewlett-Packard, San Fernando, CA) using precolumn derivatization with ortho-phthaladehyde and 9-fluorenylmethylchloromate and UV detection. Non-esterified fatty acids (Wako kit no. 990-75401; Wako Biochemical, Osaka, Japan), triglycerides (Wako kit no. 997-69801; Wako Biochemical), and cholesterol (Sigma kit, Procedure No. 352; Aldrich Company Ltd., Dorset, UK) also were quantified.

Digestibility

A digestibility trial was conducted during the last week of the experiment using three animals per diet. Fecal bags were used to collect total feces over a 7-d period; total urine output was collected as well. Fecal bags were attached to the animals 3 d before

collection began to allow time for adaptation. A 10% aliquot of daily fecal excreted was dried at 60°C for 48 h, ground to pass 1-mm screen, and stored frozen until analyzed. Also, a fresh 100-g sample of feces was taken and dried for 24 h at 100°C to determine DM concentration. Each urine container contained 100 mL of 0.1 N H_2SO_4 to ensure that the pH remained less than 3 to avoid loss of ammonia. Urine volume was measured with a graduated cylinder. Ten percent of the daily urine output was retained and stored at –20°C until analyzed. Dry matter content of urine was determined by freeze-drying.

Statistical Analysis

Data for ADG, greasy and clean mohair, N intake, N retention, digestibilities, efficiency of feed conversion, and the ratio of acetate:propionate, were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

For data with repeated measures, analysis used PROC MIXED (Littell et al. 1996); sources of variation included treatment, animal within treatment, time, treatment by time interaction, and residual error. If the treatment by time interaction was significant, simple effects of treatment means at each time were analyzed using the SLICE option for the LSMEANS statement. Non-orthogonal polynomial contrasts were used to determine the linear, quadratic, and cubic responses to concentration of PB, and to test response to ruminal protection of a single level (6PB vs UPB).

Results and Discussion

Performance Data

There are no published data available concerning the effect of betaine on the growth and mohair production of Angora goats. Consequently, our results will be compared with responses reported from trials with other ruminant species.

Dry matter intake was not affected by betaine supplementation; this agrees with results from some experiments with other ruminant species. However, Loest et al. (1998) reported that DMI increased linearly as feed grade betaine was added to the diet of finishing steers. Dry matter intake, expressed as a percentage of body weight, was 17% greater ($P < 0.05$) for goats fed 6PB than for goats fed UPB. The gain to feed ratio also was increased linearly ($P < 0.02$) as a level of PB in the diet increased, and the ratio tended to be lower ($P < 0.06$) for UPB than for 6PB.

Both linear and quadratic effects ($P < 0$ .002 and 0.03, respectively) of adding PB on ADG (Table 2) were detected. Average daily gain was 79% greater ($P < 0.01$) for goats fed 6PB than goats fed UPB. This result is in agreement with the findings of Puchala et al. (1999) who reported that feeding ruminally protected betaine increased growth rate of Angora kids fed a 9% CP diet. However, Loest et al. (2000), using crossbred-beef heifers, Goodall et al. (1999) using finishing steers, and Fernandez et al. (1998) using lambs, detected no benefits in ADG from betaine supplementation.

Mohair Yield and Quality

Mohair greasy weight tended to decrease and then to increase (quadratic response, $P < 0.10$) as dietary PB level was increased; however, the quantity of clean mohair was

not influenced by treatment ($P > 0.10$; Table 3). Both greasy and clean mohair weight tended to be numerically greater (21 and 15% more) for goats fed 6PB than for goats fed UPB. A treatment by period interaction for mohair length was detected, but that interaction appears to be due to the relative magnitude of treatment differences rather than to the direction of differences. Mohair length was 10% greater ($P < 0.05$) for 6PB than UPB.

The diameter and medullation of fiber were not affected by dietary treatment, although an unexplained tend for a cubic response ($P < 0.10$) in the percentage of kemp was detected.

Ruminal pH and VFA

Ruminal pH increased linearly ($P < 0.01$) as dietary concentration of PB increased (Table 4). This contrasts with our previous experiment with choline in which pH decreased as more protected choline was included in the diet. Reasons for this difference in response to choline and betaine are unclear though the carrier as well as the test compound may be involved. Ruminal ammonia N concentration changed quadratically ($P < 0.05$) as level of PB increased, with ammonia being greater for 2PB than C but then decreased as the further PB was added. Ammonia N in the rumen was 37% greater ($P < 0.05$) for goats fed UPB than for goats fed 6PB. The concentration of total VFA in ruminal fluid also responded quadratically, increasing when 0.2% of PB was supplemented but decreasing as more PB was added (quadratic, $P < 0.05$). The molar percentages of acetate, propionate, butyrate, and valerate and acetate to propionate ratio were not significantly altered by dietary treatment ($P > 0.20$). However, the proportions

of the branched VFA, isobutyrate and isovalerate tended to decrease linearly ($P < 0.06$) as PB level of the diet was increased.

Blood Metabolites

Blood plasma glucose increased as the dietary concentration of PB was increased (quadratic, $P < 0.02$) with blood glucose being greater for moderate levels (2PB and 4PB) than with the extremes (C and 6PB; Table 5). Urea-N and triacylglycerol concentrations in the blood plasma were not affected by treatments. The blood plasma concentration of NEFA changed cubically ($P < 0.01$) as the dietary level of PB was increased. A deficiency of betaine could affect lipid metabolism in several different ways. It could affect lipid transport through its use in the synthesis of carnitine, a compound essential for the transport of fatty acids across the mitochondrial membrane for oxidation. According to Yamamoto et al. (1996), inhibition of mitochondrial fatty acid oxidation causes elevations in both triacylglycerols and cholesterol. However, as we detected no increase in plasma triacylglycerols concentration, betaine probably was not reducing fatty acid oxidation in our trial.

Plasma Amino acids

Concentrations of essential amino acids in blood plasma remained largely unchanged in our experiment. However, responses in blood plasma concentrations of glycine, serine, and tyrosine were detected (Table 6). Glycine concentration responded cubicly ($P < 0.06$), with the highest concentration for goats fed the 4PB diet. Serine concentration in blood plasma responded to the dietary PB level both quadratically ($P <$

0.02) and cubically ($P < 0.02$), with its greatest concentration again being with the 4PB diet. Failure of supplemental PB to alter plasma methionine concentration was surprising. Several factors might be involved. First, one may have a deficiency of homocysteine to be methylated by betaine to form methionine (Finkelstein et al. 1982, and Zeisel, 1992). Baker and Czarnecki (1985) using young chicks and rats, concluded that betaine enhanced conversion of homocysteine to methionine. However, Jackson (1996) suggested that conversion of homocysteine to methionine may be irrelevant with typical diets because the concentration of the substrate, homocysteine, in natural proteins fed to animals are very low. Second, methionine supply and utilization both may have been increased, with an increased supply being used for lean tissue synthesis and mohair synthesis, as reflected by increased mohair length. This would prevent its accumulation in plasma. Third, the methyl group produced during betaine oxidation might have been recruited for other metabolic functions such as synthesis of phosphotidylcholine, creatine, carnitine, and for regulation of DNA activity (Lobley et al., 1996).

Digestibility

Nitrogen intake initially decreased but then tended to increase (linear and quadratic effects, $P < 0.07$) as the level of PB in the diet was increased (Table 7). Nitrogen intake was 68% greater for 6PB vs UPB ($P > 0.01$). This can be ascribed largely to 17% greater DMI of the 6PB than the UPB diet. Fecal N output decreased then increased (quadratic $P < 0.002$) as PB level was increased; fecal N was highest for C and lowest for 2PB. The apparently digested N intake increased linearly ($P < 0.001$) as PB level increased, with highest value noted for 6PB. Typically, digestibility of N increases as dietary N

concentration increases, with expected values for these 5 diets, based on the NRC (1996) equation (DP= (0.9* (CP-3))/CP) being 70, 70, 72, 73, and 71%. This suggests that N digestibility was lower than expected for diets C and UPB. Apparent N digestibility increased linearly ($P < 0.005$) with increasing level of PB supplementation. Digestibility of N was markedly (13.4%) greater ($P < 0.03$) for the 6PB than the UPB diet. Urinary N excretion was similar among treatments. However, nitrogen retention increased linearly ($P < 0.006$) as dietary PB level increased. Nitrogen retention was 3.5 g/d lower ($P < 0.01$) for goats fed UPB than those fed the 6PB diet. Why PB should increase N digestibility is unclear.

Conclusions

Supplementing the diet with ruminally protected betaine increased ADG, feed efficiency, greasy weight, digestible N intake and the amount N retained. The relatively minor but significant effects of supplemental betaine on ruminal fermentation and blood constituents probably reflect the small differences observed in DMI rather than direct effects of betaine. No responses in blood plasma concentrations of methionine were detected. If this effect of betaine on ADG, greasy mohair yield and feed efficiency is a consistent response with typical diets fed to goats, supplemental betaine should have economic value as a feed additive.

Table 1. Composition of experimental diet fed to Angora goats

Item	Treatment[a]				
	C	UPB	2PB	4PB	6PB
Ingredients	---------- %DM----------				
Alfalfa	10.9	10.9	10.9	10.9	10.9
Ground corn	19.0	19.0	19.0	19.0	19.0
Ground oats	20.0	20.0	20.0	20.0	20.0
Soybean meal	15.0	15.0	15.0	15.0	15.0
Cotton seed hulls	33.0	33.0	32.8	32.6	32.4
Trace mineral salts premix[b]	0.50	0.50	0.50	0.50	0.50
Calcium carbonate	0.90	0.90	0.90	0.90	0.90
Vitamin premix[c]	0.20	0.20	0.20	0.20	0.20
Betaine	—	0.60	—	—	—
Protected betaine product	—	—	0.20	0.40	0.60
Composition[d]					
Crude Protein, % DM	13.4	13.4	13.2	13.2	13.6
Gross energy, Mcal/kg DM	4.10	4.10	4.10	4.00	4.10
NDF, % DM	43.0	41.9	40.3	41.9	42.0

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Containing (in percentages) NaCl: 94 to 95; Mn > 0.2; ferrous Fe, > 0.16; Ferric, > 0.14; Cu, > 0.33; Zn, > 0.10; Iodine, > 0.007; Cobalt, > 0.005.

[c]Each gram contained 2,200 IU of vitamin A; 1,200 IU of vitamin D_3, and 0.2 IU of vitamin E. [d]measured values

Table 2. Effect of types and dietary level of betaine on average weight gain, feed intake, and feed efficiency in Angora goat

Item	Treatment[a]						Contrast[b] and probability			
	C (1)	UPB (2)	3PB (3)	4PB (4)	6PB (5)	SE	Lin	Quad	Cub	2 vs 5
No. of animals	5	5	5	5	5					
Body weight[c]	23.9	24.6	22.0	25.2	26.6	1.30				
DMI, g/d	784	760	682	847	879	86.6	0.12	0.28	0.16	0.18
DMI, % BW	3.20	3.00	3.10	3.30	3.60	0.19	0.11	0.28	0.57	0.05
DMI, g/kg $BW^{0.75}$	66.2	64.4	58.8	70.6	74.8	4.40	0.13	0.29	0.16	0.19
Gain, g/kg DMI	58.0	63.0	50.0	69.0	95.0	11.8	0.02	0.16	0.71	0.06
ADG, g/d	45.0	45.8	28.6	56.0	82.0	8.80	0.002	0.03	0.27	0.01

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine. in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Quad = quadratic contrast; Cub = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected betaine.

[c]Average body weight for 90 days, used to calculate DMI, % BW and DMI, g/kg $BW^{.75}$

Table 3. Effects of types and dietary level of betaine on mohair yield and qualities in Angora goats

Item	Treatment[a]					SE	Contrast[b] and probability			
	C (1)	UPB (2)	2PB (3)	4PB (4)	6PB (5)		Lin	Q	Cub	2vs5
Greasy, kg	1.62	1.51	1.36	1.44	1.82	0.17	0.74	0.10	0.33	0.79
Clean, kg	1.30	1.30	1.20	1.20	1.50	0.14	0.32	0.17	0.88	0.30
Length, mm	24.1	23.8	24.4	24.2	26.2	0.05	0.10	0.31	0.50	0.05
Diameter,μm	27.9	27.7	27.6	25.9	27.3	0.99	0.27	0.27	0.18	0.64
Kemp, %	0.40	0.30	0.50	0.30	0.30	0.13	0.14	0.43	0.10	0.90
Med, %	0.20	0.10	0.20	0.20	0.20	0.06	0.42	0.87	0.52	0.15

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Q = quadratic contrast; Cub = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected betaine.

Table. 4. Effects of types and dietary level of betaine on ruminal pH, NH_3 N (mg/100 mL), VFA (m*M*), and individual fatty acids (mol/100 mol) in Angora goats

	Treatment[a]						Contrast[b] and probability			
Item	C (1)	UPB (2)	2PB (3)	4PB (4)	6PB (5)	SE	Lin	Quad	Cub	2 vs 5
pH	6.30	6.40	6.20	6.40	6.50	0.06	0.01	0.16	0.10	0.21
NH_3	23.2	25.9	29.3	22.8	18.9	3.30	0.08	0.05	0.16	0.05
VFA	74.9	75.1	84.9	77.4	71.8	5.45	0.36	0.05	0.27	0.54
Acetate	65.8	65.1	65.5	64.5	64.9	1.33	0.43	0.88	0.83	0.92
Propionate	19.6	21.5	19.9	20.9	21.2	1.53	0.32	0.99	0.81	0.84
Butyrate	11.5	10.6	11.6	11.9	11.2	1.04	0.76	0.55	0.70	0.60
Isobutyrate	0.61	0.53	0.55	0.49	0.51	0.06	0.06	0.38	0.66	0.77
Isovalerate	1.01	0.97	0.94	0.79	0.79	0.13	0.06	0.68	0.58	0.18
Valerate	1.50	1.40	1.50	1.50	1.50	0.09	0.97	0.97	0.71	0.31
A:P ratio[a]	3.40	3.10	3.30	3.20	3.10	0.22	0.33	0.98	0.96	0.83

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 %protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Quad = quadratic contrast; Cub = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected betaine.

[a]A:P = ratio of acetate (m*M*) / propionate (m*M*)

Table 5. Effects of types and dietary level of betaine on plasma glucose (mg/dL) Urea N(mg/ dL) NEFA (meq/L) Triglyceride (mg/dL) metabolites in Angora goats

Item	Treatment[a]					SE	Contrast[b] and probability			
	C (1)	UPB (2)	2PB (3)	4PB (4)	6PB (5)		Lin	Quad	Cub	2 vs 5
Glucose	34.9	35.1	39.1	41.1	34.7	2.17	0.89	0.02	0.53	0.88
Urea-N	25.3	24.6	22.2	24.2	22.1	1.45	0.27	0.73	0.16	0.24
NEFA	280	266	244	283	238	19.6	0.17	0.73	0.01	0.17
Trigly[c]	23.2	23.3	22.2	25.1	24.6	2.92	0.58	0.94	0.58	0.75

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Quad = quadratic contrast; Cub = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected Betaine.

[c]Trigly = Triglyceride

Table 6. Effect of types and dietary level of betaine on plasma amino acid concentration (μmol/L) in Angora goat

Item	Treatment[a]						Contrast[b] and probability			
	C (1)	UPB (2)	2PB (3)	4PB (4)	6PB (5)	SE	Lin	Quad	Cub	2 vs.5
Alanine	237	234	243	245	261	11.2	0.31	0.38	0.22	0.51
Arginine	206	190	197	198	198	14.9	0.62	0.64	0.78	0.44
Glutamine	329	326	322	338	331	27.1	0.82	0.99	0.57	0.87
Glysine	698	760	694	814	719	64.6	0.32	0.26	0.06	0.43
Histidine	51.2	52.5	53.7	52.9	51.5	5.0	0.99	0.51	0.84	0.80
Isoleucine	43.5	43.3	45.0	44.3	42.6	3.9	0.63	0.29	0.85	0.68
Leucine	56.7	55.2	59.1	57.0	55.9	6.2	0.72	0.51	0.63	0.82
Lysine	104	101	106	103	102	11.6	0.72	0.75	0.76	0.95
Methionine	25.7	27.1	28.4	28.5	26.3	3.3	0.84	0.25	0.98	0.78
Serine	208	239	219	274	209	25.3	0.41	0.02	0.02	0.14
Tryosine	74.8	71.4	83.3	79.2	67.3	7.8	0.13	0.01	0.77	0.39
Valine	183	185	187	175	181	17.5	0.58	0.88	0.25	0.63
Threonine	101	98.7	101	100	97.7	10.0	0.74	0.83	0.99	0.92

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Quad = quadratic contrast; Cub = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected betaine.

Table 7. Effects of types and dietary level of betaine on nitrogen metabolism during the metabolism trial in Angora goat

Item	Treatment[a]						Contrast[b] and probability			
	C (1)	UPB (2)	2PB (3)	4PB (4)	6PB (5)	SE	Lin	Quad	Cubic	2 vs.5
Intake, g/d	14.1	10.3	10.2	14.8	17.3	1.56	0.07	0.07	0.17	0.01
Fecal excretion, g/d	6.70	4.10	3.40	4.30	5.00	0.49	0.08	0.002	0.06	0.20
Digestibility, %[c]	51.6	57.2	67.0	70.4	70.7	3.80	0.005	0.07	0.61	0.03
Digestible N intake, g/d	7.40	6.00	6.90	10.4	12.2	1.27	0.009	0.38	0.33	0.006
Urinary excretion, g/d	5.10	4.10	4.70	5.50	6.80	0.82	0.13	0.31	0.90	0.04
Retention, g/d	2.30	1.90	2.10	5.00	5.40	0.78	0.006	0.71	0.16	0.01

[a]C = no added betaine; UPB =0.6% unprotected betaine in the diet; 2PB = 0.2 % protected betaine in the diet; 4PB = 0.4% protected betaine in the diet; 6PB = 0.6% protected betaine in the diet.

[b]Lin = linear contrast; Quad = quadratic contrast; Cubic = cubic contrast; 2 vs. 5 = unprotected betaine versus 12 g protected betaine

[c]Apparent digestibility

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Chapter V

RUMINALLY PROTECTED BETAINE OR CHOLINE FOR LACTATING ALPINE GOATS

T. Shenkoru[1,2], F. N. Owens[2], R. Puchala[1], A. Goetsch[1], and T. Sahlu[1]

[1]E (Kika) de la Garza Institute for Goat Research, Langston University, OK

[2]Animal Science Department, Oklahoma State University, Stillwater, OK

Abstract

Twenty-four Alpine does and 24 doelings were used to evaluate the effect of supplementing the diet with protein or ruminally protected betaine or choline on lactation and milk composition. Doelings and does (6 of each per diet) were allotted randomly to four diets consisting of 50% concentrate and 50% forage. Diets containing 15% CP were supplemented with ruminally protected betaine (B) at 3% of DM, ruminally protected choline (C) at 3% of DM, no added methyl donors (N), or crude protein to increase CP to 18% (H). Compared with does, doelings gained more weight but produced less milk. The higher protein diet increased DMI by does and decreased fat percentage and milk yield early in the trial. Addition of betaine as a methyl donor increased DMI by doelings, decreased milk production by does, decreased fat yield late in the trial, and decreased the

acetate:propionate ratio in the rumen. Supplementing with either choline or betaine increased plasma concentrations of non-esterified fatty acids and insulin. In conclusion, supplementing the diet with betaine increased DMI of doelings but had no effect on milk production; supplemental betaine reduced milk production by does. Adding protein sources with high ruminal escape failed to increase milk production of either does or doelings Alpine goats fed a 15% CP diet.

Key words: Alpine goat, Betaine, Choline, Milk production and composition

Introduction

Betaine, an oxidation product of choline, may replace choline by providing labile methyl groups (Bock et al., 2000). Many studies have investigated the effects of choline on ruminant performance (Neill et al., 1979; Dawson et al., 1981; Sharma and Erdman, 1988a, 1988b, 1989; Erdman and Sharma, 1991). Erdman et al. (1984) indicated that adding unprotected choline to the diet of dairy cows increased production of 4% fat-corrected milk and milk fat percentage. Postruminal infusion of choline in dairy cows also has increased the percentage of fat in milk (Sharma and Erdman, 1988a; Grummer et al., 1987). Likewise, Erdman and Sharma (1991) detected increased milk yield and milk fat concentration when a dairy diet was supplemented with ruminally protected choline. These changes may reflect the fact that choline is directly involved in hepatic metabolism of lipoproteins (Lombardi, 1971) and transport of triglycerides from the liver (Lombardi et al., 1968). However, in other experiments, ruminally protected choline has failed to affect either fat concentration or milk yield (Piva et al. 1993). To date, effects of dietary choline on lactational performance of dairy goats have not been investigated. Because of differences in fat composition between dairy cows and goats, effects of choline may differ with species. Similarly, no research has examined the impact of ruminally protected betaine on ruminant milk production. In a recent experiment (Puchala et al., unpublished data), feeding ruminally protected betaine increased growth rate of Angora kids fed a 9% CP diet to the growth rate noted with kids fed a diet containing15% CP. Conversely ruminally protected betaine did not affect growth rate of kids fed a 15% CP diet. Perhaps this interaction was due to the addition of methyl groups, which were used for homocysteine remethylation and carnitine synthesis. Therefore, the objectives of this

study were to investigate the effects of feeding ruminally protected betaine or ruminally protected choline on lactational performance of Alpine does and doelings fed a diet containing a moderate concentration of crude protein.

Materials and methods

Animals and Diets

Twenty-four Alpine does and 24 Alpine doelings were used. From kidding in April, until mid-May, animals were maintained on pasture and milk production was monitored. These 48 experimental animals were selected from larger group based on milk production, body weight, and health. The experiment was conducted at Langston University. During the 3-wk covariate period and the 16-wk experimental period, animals resided in pens equipped with Calan® gate feeders (American Calan, Inc., Northwood, NH, USA). The covariate period lasted from mid-May through early June; during this time, the control (N) diet was available free choice, with daily feed supply being approximately 110% of the amount of feed that disappeared during the preceding few days. Feed was dispensed once daily in the morning at approximately 0800. Animals were milked twice daily at 0500 and 1700. Milk was sampled on the last day of the covariate period at both milkings; samples were subjected to standard DHIA analysis. Body weight was determined at the beginning and end of the covariate period. Based on milk production during the covariate period and body weight, does and doelings were allocated to the four treatments, with 12 animals per treatment, 6 of each parity (doeling and does).

Diets contained 50% concentrate and 50% forage, with equal amounts of forage coming from cottonseed hulls and alfalfa hay. Diets were formulated to contain adequate amount of CP, energy, vitamins, and minerals for lactating goats (NRC, 1981; Table 1). Three diets contained approximately 15% CP (DM basis) whereas one diet contained approximately 18% CP. Of the 15% CP diets, one was a control (N), one contained ruminally protected betaine (B), and one contained ruminally protected choline (C). The dietary inclusion level of ruminally protected betaine and choline was 3% of DM. At the mean DMI for goats in this trial (2.60 kg/d), daily intake of betaine from ruminally protected betaine was 40 g/d and of choline from ruminally protected choline was 35 g/d. These are higher than the 30 g/d feeding rate used in recent experiments with goats (Puchala et al., unpublished data). The 18% CP diet (H) contained neither added betaine nor choline; the additional CP was provided by feather, blood, and fish meals, with each of these high-bypass protein sources adding about one percentage unit of CP to the basal diet.

Residual feed was weighed daily, samples were taken weekly, and orts were removed and discarded; composite samples of orts were formed for each 28-d period. Feed and weigh back samples were analyzed for DM and CP (AOAC, 1990), and NDF (filter bag technique; ANKOM Technology Corp., Fairport, NY).

Milk Yield and Compositions

Morning and evening milk yield were recorded each day. Milk samples were taken on the last day of weeks 4, 8, 12, and 16 from both the morning and evening milkings; BW also was determined on the last day of these weeks. Milk samples were analyzed in

the DHIA laboratory of Langston University, for protein (N x 6.38), lactose, fat, and total solids (Multispec, Wheldrake, NY, USA).

Ruminal pH and VFA

Ruminal fluid samples were taken via stomach tube every 4 weeks at the same time as blood was sampled. Ruminal fluid pH was measured using a pH meter (SA- 720, Orion Research, Boston, MA) immediately after sampling. Thereafter, 20 mL was collected for VFA and ammonia N analysis. For VFA measurements, 1 mL of 25% (wt/wt) meta-phosphoric acid was added to 4 mL of ruminal fluid (Gas chromatograph; Hewlett Packard Co., Avondale, PA). For ruminal ammonia N measurement, 3 mL of ruminal fluid was collected in a separate tube containing 2 mL of 4% (wt/vol) of trichloroacetatic acid, this mixture was centrifuged and analyzed by the procedure of Broderick and Kang (1980).

Plasma Amino Acids, Metabolites and Hormones

Blood was collected via jugular venipuncture into two 7-ml vacutainer tubes containing, either sodium heparin or potassium oxalate plus sodium fluoride (Becton Dickinson, vacutainer system, Rutherford, NJ). Tubes were immediately chilled on ice, transported to the laboratory, and centrifuged at 1,500 x *g* at 4 °C for 20 min. Plasma aliquots were stored at -20°C until analyzed. Plasma glucose was analyzed colorimetrically using a Technicon Autoanalyzer II System (Technicon Instrument, Tarrytown, NY). Plasma urea N was determined by the method of Chaney and Marbach (1962).

Plasma hormones were analyzed using commercially available kits from ICN Biomedicals, Inc. (Costa Mesa, CA, USA) for insulin (Cat. No. 06.06B254221), total triiodothyronine (T_3; Cat. No. 06B25422), and total thyroxine (T_4; Cat. No. 06 06B263676). The inter-assay CV averaged 10.3% for insulin, 6.6% for T_3, and 5.9% for T_4. Plasma free amino acid concentration was determined using an amino-Quant system (Hewlett Packard, Waldbron, Germany). Blood plasma also was assayed for NEFA (Wako kit no. 990-75401; Wako Biochemical, Osaka, Japan), triacylglycerols (Wako kit no. 997-69801; Wako Biochemical), and cholesterol (Sigma kit, Procedure No. 352; Aldrich Co. Ltd. Dorset, UK).

Data Analysis

For variables measured at more than one time, data were analyzed using PROC MIXED (Littell et al., 1996), with sources of variation being treatment, parity, time, and their interaction. If the treatment by parity or treatment by time interaction was significant ($P < 0.05$), simple effects of treatment and parity means were analyzed using the SLICE option for the LSMEANS statement (SAS, 1990). Degrees of freedom of the pooled error term were calculated by using the Satterthwait's approximation. If the treatment by time interaction was not significant ($P > 0.05$), the main effect means were separated by least significant difference procedures. Means comparison among the groups were carried out using the following contrasts: contrast I, effect of 15% CP vs. 18% CP (N vs. H); Contrast II: methyl donors vs. the control (B + C vs. N); Contrast III: Betaine vs. choline (B vs. C); Contrast IV: doeling vs. doe.

Results

Dry matter intake

The effect of dietary treatment on DMI differed with parity (interaction, $P < 0.05$). Dry matter intake was greater ($P < 0.05$) for does fed the higher protein diet but not altered by addition of methyl donors to the diet. In contrast, for Doelings, addition of a bypass methyl donor (mean B and C) increased DMI by 19.5% ($P < 0.05$).

Dry matter intake, when expressed as a percentage of body weight (DMI, % BW) was not significantly altered by diet.

Average Daily Weight Gain

Doelings gained more ($P < 0.001$) weight than does (80 vs. 15 g/d). Matched with lower milk yield for Doelings, this indicates that Doelings were partitioning more of their nutrients and energy to body weight gain than to milk synthesis.

Milk Yield and Composition

Milk yield: Dietary treatment and parity again interacted ($P < 0.02$) to affect milk yield. For Doelings, milk yield was not altered by dietary treatments. But for does, milk yield was decreased ($P < 0.05$) by 13 % by addition of methyl donors. Within these methyl donors, milk yield was 22% greater ($P < 0.05$) with supplemental choline than with supplemental betaine, due primarily to decreased milk production for does fed betaine during the 12th and 16th week of the trial. Milk yield was greater ($P < 0.02$) for does than for Doelings.

***Milk fat percentage and yield*:** Time by treatment interactions were detected for milk components. During week 4, crude protein supplementation and addition of methyl donors to the diet decreased ($P< 0.05$) milk fat percentage by 27% and 17%, respectively. During subsequent weeks, milk fat production was 12 to 17 % lower ($P < 0.05$) with choline than with supplemental betaine.

Yield of milk fat was decreased by 25% by added protein during week 4, whereas during week 16, methyl donor supplementation decreased ($P < 0.05$) fat yield by 21%.

***Milk Protein percentage and Milk protein Yield*:** During week 4, milk protein percentage was decreased ($P < 0.05$) by added protein by 11% but no differences during subsequent weeks in protein yield were detected.

***Lactose Percentage*:** During week 4, lactose percentage was increased ($P < 0.05$) by added protein (7%) or by added methyl donors (8%) due primarily to a low lactose concentration for milk produced by goats fed control diet. Differences during other weeks were not significant.

***Total solids Percentage*:** Total solids percent was lower ($P < 0.05$) for milk from goats fed supplemental choline than from goats fed supplemental betaine.

Rumen pH and VFA

Ruminal pH was not affected by treatments (Table 4). Ruminal NH_3 N was increased ($P < 0.05$) by addition of either protein (35%) or methyl donors (21%) to the diet. Total VFA concentrations were not affected ($P > 0.85$) by dietary treatments, but the addition of methyl donors to the diet increased the molar proportion of acetate ($P <0.05$) while decreaseing the percentage of propionate and valerate leading to an

increased ($P < 0.05$) acetate:propionate ratio. Virtually all of these changes can be attributed to supplementation with betaine, as the response in each case was greater with betaine than with choline supplementation.

Blood metabolites and hormones

The plasma concentration of glucose was not affected by dietary treatment (Table 5). Matching effects on ruminal ammonia, blood plasma urea N was greater ($P < 0.05$) with added protein or with added methyl donors though plasma urea N concentration was lower with choline than with betaine supplementation. Non-esterified fatty acid concentration in plasma was not significantly altered for doelings fed various diets, but for does, supplementation with methyl donors increased NEFA by 32%. Concentrations of triiodothyroxine (T_3) and thyroxine (T_4) were not affected by treatment. But numerically, goats fed betaine tended to have the highest concentrations both of these hormones. A parity effect ($P < 0.04$) on insulin concentration of plasma was detected (62 vs. 76 μ IU/mL for doeling and does, respectively) insulin was increased ($P < 0.05$) by a 32% by supplementing the diet with methyl donors.

Plasma amino acids

The concentrations of amino acids are presented in Table 6. Compared with plasma from does, plasma from doelings had higher ($P < 0.05$) concentrations of alanine, glycine, luecine, isolucine, methionine, lysine, serine, threonine, valine, and tyrosine. Arginine, luecine, lysine, valine, and tyrosine concentrations in plasma were increased ($P < 0.05$) by 20 to 37% with supplementation with crude protein whereas isoleucine and phenylalanine were lower with choline than with betaine supplementation. Methionine

concentration, though not significantly affected by supplements , was numerically least with choline supplementation

Discussion

The higher ADG and lower milk yield for doelings than for does reflects greater partitioning of nutrients by doelings than by does toward lean tissue and protein growth than to milk synthesis. Surprisingly, plasma concentration of both insulin and NEFA were higher for does than doelings. Does had lower plasma concentrations of alanine, glycine, isoleucine, methionine, lysine, and threonine than doelings, perhaps reflecting greater use of these amino acids by the mammary gland.

Increasing the dietary crude protein concentration from 15 to 18% increased DMI of does and increased lactose content of milk while decreasing fat percentage and fat yield early in the trial (week 4). Added protein increased ruminal ammonia and plasma urea nitrogen concentrations, as expected, while increasing plasma concentrations of arginine, leucine, lysine, valine, and tyrosine, prsumably due to an increased dietary supply of these amino acids.

Addition of methyl donors to the diet had numerous effects, many of which can be attributed to effects of betaine due to the significant difference between choline and betaine in responses. Specifically, methyl donor supplementation, in cases where betaine is primarily responsible for the effect, were apparent: decreased milk production by does, decreased fat yield during week 16, increased rumninal acetate to propionate ratio, decreased propionate, valerate, and increased plasma urea N concentrations. The increased acetate concentration of ruminal fluid supports the suggestion of Mitchell et al.

(1979) and is additional potential route for betaine to exert lipotropic activity. A high acetate to propionate ratio that may result in increased metabolic heat loss has been blamed as one cause for caloric inefficiency (Van Soest, 1994). According to MacRae and Lobley (1986) when the acetate:propionate ratio is high, propionate may be insufficient for gluconeogenesis and glucogenic amino acids may be required to fill the gap. Use of glucogenic amino acids for glucose synthesis will divert them from protein synthesis, increase the catabolism of metabolizable protein, and increase urea excretion. However, the efficiency at which acetate isutilized may depend on the the supply of NADPH, which in turn depends on glucogenic sources (Black et al. 1987). In our study, ruminal NH_3 N of betaine supplemented animals was not markedly elevated, but plasma urea N still was markedly greater for goats fed betaine than for goats fed the control diet or supplemented with choline where VFA ratios were not altered.

Supplementing the diet with either methyl donor, where response to choline was not statistically different from the response to betaine, include stimulation of DMI by doelings, greater lactose concentrations in milk during week 4, and higher plasma concentrations of non-esterified fatty acids and of insulin. Although no data are available on Alpine goats with regard to milk fat synthesis and excretion, the higher NEFA suggests that betaine increased fat mobilization; this seems contrary to previous research in feedlot cattle (Bock et al., 2000; Goodall and Brethour, 1999; Loest et al., 1998) where supplementation with betaine tended to increase back fat thickness. Any increase in fat mobilization and decrease in fat reserves by does may retard breeding performance; this concern warrants further research.

Conclusions

Supplementing a 15 % protein with rumen escape betaine increased DMI of doelings, but had no effect on their milk production. In contrast, supplemental betaine reduced milk production by does. Whether these responses can be attributed fully to the increased acetate:propionate ratio of ruminal contents or to additional metabolic effects (increased insulin and non-estereified fatty acids in plasma) is not certain. Increasing crude protein from 15% to 18% by adding protein sources with high in ruminal escape increased DMI by does but failed to increase milk production even though plasma concentrations of many amino acids were increased. This suggests that 15% CP is adequate to support milk production for both age groups of lactating Alpine goats.

Table 1. Composition of diets consumed by Alpine goats

	Treatment[1]			
Item	N	B	C	H
Ingredients	---------- % DM ----------			
Cottonseed hulls	25.0	25.0	25.0	25.0
Alfalfa hay	25.0	25.0	25.0	25.0
Ground corn	32.5	28.8	28.8	28.6
Soybean meal	11.0	11.7	11.7	10.8
Blood meal	0.00	0.00	0.00	1.31
Fish meal	0.00	0.00	0.00	1.75
Feather meal	0.00	0.00	0.00	1.31
Dried molasses	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.33	1.36	1.36	1.03
Limestone	0.72	0.70	0.70	0.60
Vitamin premix[2]	0.50	0.50	0.50	0.50
Trace mineral salt[3]	0.70	0.70	0.70	0.70
Magnesium oxide	0.30	0.30	0.30	0.30
Protected betaine	0.00	3.00	0.00	0.00
Protected choline	0.00	0.00	3.00	0.00
Composition[4]				
CP, %	15.0	15.0	15.0	18.0
NE_L, Mcal/kg	1.49	1.43	1.43	1.47
Ca, %	1.00	1.00	1.00	1.00
P, %	0.50	0.50	0.50	0.50

[1]N = Control; B= Betaine; C = Choline; H = High protein

[2]Contained 2,200 IU vitamin A, 1,200 IU vitamin D_3, and 2.2 IU vitamin E per gram.

[3]Contained 95 to 98.5% NaCl and at least 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.011% Co, 0.007% I, and 0.005% Zn; [4]Calculated values

Table 2. Effects of dietary inclusion of betaine and choline on DMI, ADG, and milk yield in Alpine does and doelings

Item	Parity	Treatment[a]				SE	Contrast[b] and probability						
		N	B	C	H		I	II	III	IV	V	VI	VII
No. of animals		12	12	12	12								
Body weight[C]		48	47	45	49	3.9							
DMI, kg/d	Doeling	2.15	2.68	2.46	1.98	0.10	NS[d]	*	NS	NS	-	19.5	-
	Doe	2.13	2.51	2.24	2.49	0.10	*	NS	NS	NS	16.9	11.5	-
DMI, % BW	Mean	4.43	5.57	5.20	4.58	0.51	NS	NS	NS	NS	3.39	21.6	-
ADG, g/d	Doeling	80.7	62.5	84.9	93.5	10.9	NS	NS	NS	*	15.9	-8.67	-
	Doe	26.1	6.11	7.62	19.8	10.9	NS	NS	NS		-24.1	-73.7	-
Milk yield, kg/d	Doeling	2.08	2.01	2.18	2.15	0.13	NS	NS	NS	*	-	-	-
	Doe	3.45	2.68	3.27	3.54	0.13	NS	*	*		-	-13.8	22.01

[a]N = Control; B= Betaine; C = Choline; H = High protein

[b]Contrast I = 15 vs. 18% CP; Contrast II = Methyl donors vs. control; Contrast III = Betaine vs. choline; IV = doeling vs. doe; V = (18% CP/15% CP)*100; VI = (Methyl donors/ control)*100; VII = (choline/betaine)*100

[C]Average body weight for both parity combined for the whole experimental period

[d]NS = Not significant ($P > 0.10$)

Table 3. Effects of dietary inclusion of betaine and choline on concentration of milk fat, lactose, and total solids in Alpine does and doelings

		Treatment[a]					Contrast[b] and probability						
Items	wk	N	B	C	H	SE	I	II	III	IV	V	VI	VII
Fat, %	4	3.45	3.01	2.70	2.51	0.133	*	*	ns[c]	ns	-27.3	-17.3	-
	8	2.72	2.83	2.33	2.66	0.133	ns	ns	*	ns	-	-	-17.7
	12	2.56	2.84	2.43	2.55	0.133	ns	ns	*	ns	-	-	-14.4
	16	3.04	3.30	2.89	3.11	0.133	ns	ns	*	ns	-	-	-12.4
Protein, %	4	2.93	2.69	2.71	2.62	0.104	*	ns	ns	ns	-10.6	-	-
	8	2.87	2.83	2.79	2.89	0.104	ns	ns	ns	ns	-	-	-
	12	2.92	2.91	2.89	2.97	0.104	ns	ns	ns	ns	-	-	-
	16	3.11	3.29	3.17	3.15	0.104	ns	ns	ns	ns	-	-	-
Lactose, %	4	3.62	3.93	3.87	3.89	0.071	*	*	ns	ns	7.46	7.73	-
	8	3.90	3.88	3.99	3.92	0.071	ns	ns	ns	ns	-	-	-
	12	3.80	3.80	3.88	3.85	0.071	ns	ns	ns	ns	-	-	-
	16	3.99	3.92	3.97	4.02	0.071	ns	ns	ns	ns	-	-	-
Fat yield, g/d	4	82.1	78.8	69.9	61.8	6.39	*	ns	ns	ns	-24.7	-	-
	8	87.8	77.7	77.1	78.5	6.39	ns	ns	ns	ns	-	-	-
	12	86.0	67.6	73.4	77.1	6.39	ns	ns	ns	ns	-	-	-

	16	78.4	57.5	66.8	71.3	6.39	ns	*	ns	ns	-	-20.7	-
Protein, g/d	4	81.3	76.9	78.2	68.6	9.9	ns	ns	ns	ns	-	-	-
	8	87.0	75.6	85.5	86.9	9.9	ns	ns	ns	ns	-	-	-
	12	85.1	65.9	82.3	85.5	9.9	ns	ns	ns	ns	-	-	-
	16	77.6	56.2	74.4	79.0	9.9	ns	ns	ns	ns	-	-	-
Total solids		10.1	10.2	9.77	10.0	0.09	ns	ns	*	ns	-	-	-4.22

[a]N = Control; B= Betaine; C = Choline; H = High protein

[b]Contrast I = 15 vs. 18% CP; Contrast II = Methyl donors vs. control; Contrast III = Betaine vs choline; IV = doeling vs. doe; V = (18% CP/15% CP)*100; VI = (Methyl donors/ control)*100; VII = (choline/betaine)*100

[C]ns = Not significant ($P > 0.10$)

Table 4. Effects of dietary inclusion of betaine and choline on ruminal pH, VFA, individual fatty acids (mol/100 mol) in Alpine does and doelings

	Treatment[a]					Contrast[b] and probability						
Item	N	B	C	H	SE	I	II	III	IV	V	VI	VII
pH	6.59	6.64	6.65	6.69	0.05	ns	ns	ns	ns	-	-	-
VFA,mM	71.2	70.0	68.4	68.6	2.36	ns	ns	ns	ns	-	-	-
Acetic	67.3	70.4	67.1	67.7	0.41	ns	*	*	ns	-	2.15	-4.69
Propionic	19.5	17.2	19.0	18.9	0.42	ns	*	*	ns	-	-7.18	10.5
Butyric	9.45	9.10	10.4	9.80	0.48	ns	ns	ns	ns	-	-	-
Isobutyric	1.13	1.12	1.19	1.05	0.06	ns	ns	ns	ns	-	-	-
Valeric	1.57	1.28	1.46	1.46	0.04	ns	*	*	ns	-	-12.7	14.1
Isovaleric	1.15	0.97	1.02	1.09	0.11	ns	ns	ns	ns	-	-	-
A:P	3.52	4.20	3.66	3.68	0.095	ns	*	*	ns	-	11.7	-12.9
Ammonia N	21.1	25.7	25.3	28.4	1.73	*	*	ns	ns	34.6	20.9	-

[a]N = Control; B= Betaine; C = Choline; H = High protein

[b]Contrast I = 15 vs. 18% CP; Contrast II = Methyl donors vs. control; Contrast III =Betaine vs. choline; IV = doeling vs. doe; V = (18% CP/15% CP)*100; VI = (Methyl donors/ control)*100; VII = (choline/betaine)*100; [c] ns = Not significant ($P > 0.10$)

Table 5. Effects of dietary inclusion of betaine and choline on plasma urea N (mg/dL), glucose (mg/dL), NEFA (meq/L), T_3 (nmol/L), T_4 (noml/L) and insulin.

		Treatment[a]					Contrast[b] and probability						
Item	Parity	N	B	C	H	SE	I	II	III	IV	V	VI	VII
Urea N		20.7	24.9	21.7	26.5	0.63	*	*	*	ns	28.0	12.6	-12.9
Glucose		49.1	50.3	49.8	48.8	1.09	ns[d]	ns	ns	ns	-.061	1.93	-0.99
NEFA[c]	Doeling	119	99.1	128	105	17.6	ns	ns	ns	*	-	-	-
	Doe	134	184	179	114	17.6	ns	*	ns		-	35.5	-
Triiodothyronine		194	220	190	157	13.8	ns	ns	ns	ns	-	35.5	-
Thyroxine		3.74	4.14	3.93	3.62	0.19	ns	ns	ns	ns	-	-	-
Insulin		57.2	78.4	72.4	67.2	6.53	ns	*	ns	ns	-	31.6	-

[a]N = Control; B= Betaine; C = Choline; H = High protein

[b]Contrast I = 15 vs. 18% CP; Contrast II = Methyl donors vs. control;Contrast III = Betaine vs choline; ; IV = doeling vs. doe; V = (18% CP/15% CP)*100; VI = (Methyl donors/ control) *100; VII:(choline/betaine)*100

[c]NEFA = non esterified fatty acid

[d]ns = Not significant ($P > 0.10$)

Table 6. Effects of dietary inclusion of betaine and choline on plasma amino acid concentrations (μmol/L) in Alpine goat

	Treatment[a]					Contrast[b] and probability							
Item	N	B	C	H	SE	I	II	III	Doeling	Doe	V	VI	VII
Alanine	159	146	130	156	9.94	ns[C]	ns	ns	163*	134	20.4	-	-
Arginine	231	252	231	278	14.4	*	ns	ns	258	239	-	-	-
Glutamine	171	156	167	155	14.9	ns	ns	ns	157	167	-	-	-
Glycine	506	441	467	500	32.4	ns	ns	ns	548*	409	-	-	-
Histidine	60.0	59.0	51.5	65.6	4.10	ns	ns	ns	62.9	55.1	29.0	-	-
Leucine	131	153	124	169	10.3	*	ns	ns	157	132	-	-	-19.3
Isoleucine	85.7	103	83.1	99.4	6.50	ns	ns	*	100*	85.5	-	-	-
Methionine	29.4	30.2	24.4	29.2	2.00	ns	ns	*	31.3*	25.3	31.5	-	-
Lysine	108	123	98.9	142	9.87	*	ns	ns	131*	105	-	-	-24.5
Phenlyalanine	35.1	43.3	32.7	42.4	3.10	ns	ns	*	40.2	36.5	-	-	-
Serine	112	98.4	91.8	111	7.50	ns	ns	ns	114*	92.8	-	-	-
Threonine	71.6	80.1	63.0	86.1	6.20	ns	ns	ns	83.0*	67.4	-	-	-
Valine	180	218	173	246	16.1	*	ns	ns	224	184	36.7	-	-
Tyrosine	68.1	79.3	74.1	83.3	4.50	*	ns	ns	81.4*	71.0	22.3	-	-

[a]N = Control; B= Betaine; C = Choline; H = High protein; [b]Contrast I = 15 vs. 18% CP; Contrast II = Methyl donors vs. control; Contrast III =Betaine vs. choline; IV = doeling vs. doe; V = (18% CP/15% CP)*100; VI = (Methyl donors/ control) *100; VII = (choline/betaine)*100 ; [d]ns = Not significant ($P > 0.10$)

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Chapter VI

Summary

Scope of these Studies

A total of 98 goats were used in these studies. Responses of goats to supplemental dietary ruminally protected choline or betaine were measured in terms of body weight gain, feed efficiency, mohair yield and quality, milk yield and composition. The effects of dietary choline or betaine were evaluated at four levels 1) animal performance 2) ruminal fermentation 3) plasma metabolites 4) plasma amino acids and nitrogen utilization.

Findings and Conclusions

In the first experiment, 25 wether Angora goats (29 ± 6 kg initial BW, and > 1 yr of age) were used to evaluate body growth, feed efficiency, mohair yield and quality. Goats were given *ad libitum* access to a 55 % concentrate diet (oat based, 13 % CP) for 90 days. The diets contained 0, 4, 8, and 12 g of protected choline chloride product per kg of diet DM (C, 1PC, 2PC, and 3PC, respectively), 4 g unprotected choline chloride product per kg of diet DM (UPC). Choline supplementation did not significantly affect feed intake or body weight gain. However, growing goats supplemental 2PC and 3PC numerically had greater ADG (18.4 and 16.2 %) more than goats fed the unsupplemented diet. Mohair yield and fiber length were not affected by treatment. However, mohair quality (mohair

diameter, kemp and medullated fiber percent) was adversely affected by choline supplementation. Ruminal pH linearly decreased as dietary PC supplementation was increased. Goats fed unprotected choline had higher ruminal pH values than goats fed 3PC. Total VFA first decreased but then increased as the level of PC increased (quadratic response, $P < 0.02$). Acetate increased linearly, while butyrate decreased linearly with addition of protected choline, and quadratic effects on isovalerate and the A:P ratio were detected. No effects on plasma glucose and cholesterol were detected. Non-esterified fatty acids increased then declined as the level of PC increased (quadratic response; $P < 0.10$). Methionine concentration of plasma linearly increased when PC level in the diet increased. These results suggested that dietary supplementation of Angora wethers with PC did not affect ADG or mohair growth, but supplemental PC altered ruminal fermentation conditions and the plasma concentration of methionine.

In the second experiment, twenty- five castrated Angora goats (20 ± 2 kg initial BW and 7 months of age) were used to evaluate effects of ruminally protected betaine on ADG, ruminal fermentation end products, and mohair quality and production. Goats were given *ad libitum* access to a 53 % concentrate diet (15 % CP) for 90 days beginning in September. Treatments included no added betaine (C), 0.6% of unprotected betaine (UPB) in the dietary DM, and 0.2, 0.4, and 0.6% of protected betaine in dietary DM (2PB, 4PB and 6PB, respectively).

Added PB had linear and quadratic effects on ADG with ADG being greater for goats receiving ruminally protected than free betaine (45.8 vs. 82 g/d). Feed efficiency also was improved linearly by PB supplementation but was poorer for goats fed unprotected betaine than for goats fed protected betaine.

Uncleaned (greasy) mohair yield increased quadratically with dietary supplementation of PB; yield of clean mohair was not affected by treatment; numerically, clean mohair yield was highest for the 6PB treatment, being 15 % above that of goats fed the unsupplemented diet. Fiber diameter and medulated fiber were not affected by supplementation of PB but fiber kemp prevalence responded cubicly to an increased dietary supply of PB.

Plasma glucose increased linearly with supplementation of PB. Plasma concentration of NEFA was affected (cubic response; $P < 0.01$) by added PC. Plasma urea-N and triacylglycerol were not affected by treatment.

Ruminal pH increased linearly to an increased level of PB in the diet. Ruminal ammonia-N showed a quadratic effect and was higher for UPB than 6PB. The molar concentration of VFA in ruminal fluid first increased but then declined as PB level was increased. Glycine responded cubicly while serine concentration was affected by dietary PB in both a quadratic and cubic fashion, with the greatest concentration for the 4PB diet. Intake of digestible N intake was increased as the level of dietary PB increased.

Based on these results, protected betaine was more beneficial for improving ADG and feed efficiency than unprotected betaine, the slight increase in clean mohair yield should prove economically beneficial.

In the third experiment 24 Alpine does and 24 Alpine doelings were used to evaluate responses to dietary supplementation with ruminally protected betaine or choline on lactational performance. The animals were randomly allocated to four treatments and given free choice access to a 50% concentrate 50% forage diet. Three diets contained approximately 15% CP, and one contained 18% CP. One of the 15% CP diets did not

contain added ruminally protected betaine or choline, one contained ruminally protected betaine and the other included ruminally protected choline; ruminally protected betaine and choline were added as 3% of DM. The 18% CP diet did not contain added choline or betaine. Supplementing the 15% protein with rumen escape betaine increased DMI of Doelings, but had no effect on their milk production; supplemental betaine reduced milk production by does. Whether these responses can be attributed fully to the decreased acetate:propionate ratio of ruminal contents or to other metabolic effects (increased insulin and non-estrified fatty acids in plasma) are not certain. Increasing CP from 15% to 18% by adding protein sources with high ruminal escape increased DMI by does but failed to increase milk production even though plasma concentrations of many amino acids were increased. This suggests that 15% CP is adequate to support milk production for both age groups of lactating Alpine goats.

Significance of the Studies

Rate and efficiency of growth of young Angora goats were improved by supplementing diets with ruminally stable betaine. For maximum animal performance, betaine must be protected to by pass the rumen and increase supply of this supplement.to the intestines. In a separate degradability experiment, the amount choline or betaine reaching the small intestine from the ruminally protected forms was quite small. Hence, technology for coating for ruminal protection of these compounds requires some improvement. From our experience and others research results, young animals exhibited more response to choline or betaine supplementation than older animals.

Limitations and Future Outlook

We did not measure choline, betaine and homocystine contents of the diets we fed. To define modes of action, it would have been desirable to have included measurements of enzymes and intermediate products involved in metabolism of choline and betaine. Some direct measurements of postruminal supply and availability with various types of diets also would have helped to quantify "requirements" more closely. In vitro measurements would have helped to determine whether ruminal VFA responses were due to short-term effects on microbial species already present in the rumen or long-term changes in species prevalence.

VITA

Teshome Shenkoru

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF BETAINE AND CHOLINE SUPPLEMENTATION ON MOHAIR AND MILK PRODUCTION BY GOATS

Major Field: Animal Nutrition

Biographical:

Personal Data: Born in Addis Ababa, Ethiopia, 29 January, 1956, the son of Shenkoru Meketa and Tenagne Mergia.

Education: Graduated from Addis Ababa University, Ethiopia, June 1982, received Bachelor of Science degree Animal Production; received Master of Science degree from Alemaya University of Agriculture, Ethiopia in June 1987; completed the requirements for Doctor of Philosophy Degree at Oklahoma State University in August, 2001.

Professional Experience: Dairy Herd Manager and Instructor at Ambo Agricultural College, Ethiopia 1972 - 1977; Worked for the Department of Animal Science at Alemaya University of Agriculture as Assistant Professor from March, 1977 to January, 1997; Visiting Scholar, in USA, 1995.

Professional Organization: Ethiopian Society of Animal Production: Ethiopian Feed Resource Net Work; Ethiopian Teachers' Association; Oklahoma State University Animal Science Graduate Student Association.