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Protecting Dietary Vitamin C and High Oleic Oil in Feed and Its Effect on The Nutritional Profile of Goat Milk

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Abstract: Protecting dietary vitamin C and high oleic oil from ruminal degradation by formulating these nutrients within a formaldehyde-free feed supplement and its effects on the nutritional profile of goat milk were investigated. Two protein-oil gel supplements (PGS), both containing high oleic sunflower oil (oleic acid source) and ascorbyl palmitate (vitamin C source), one based on casein (C-PGS) and the other based on keratin (K-PGS), were prepared and included in lactating goat diets. Both increased unsaturated fat content in goat milk, but only feeding with C-PGS resulted in a significant increase. K-PGS increased vitamin C in goat blood serum but not in milk. Blood serum vitamin C concentration for protein-oil gel supplement diets and unprotected ascorbyl palmitate diet were similar, so protection of vitamin C was questionable. The two protein-oil gel supplements increased concentration of oleic acid in both blood serum and milk with a corollary decrease in hypercholesteremic fatty acids.

Key words: Feed Supplement, protected nutrient, vitamin C, high-oleic oil, goat, milk, ruminant

INTRODUCTION

Milk is an important source of protein, calcium, vitamins and other essential nutrients in people's diets, but it is low in vitamin C and high in saturated fatty acids. Dietary saturated fatty acids, particularly myristic (C14:0) and palmitic (C16:0), are hypercholesteremic and have been associated with increased incidence of arteriosclerosis. On the other hand, increasing levels of monounsaturated fatty acids such as oleic acid (C18:1n9) have health benefits because they lower serum levels of LDL-cholesterol and have no effect on the serum levels of HDL-cholesterol[1]. Vitamin C is also positively related to levels of HDL-cholesterol and it lowers total cholesterol levels in blood serum. It is therefore reasonable that feeding vitamin C and oleic acid (C18:1n9) to dairy cows can produce naturally healthier and nutritionally enhanced milk.

Vitamin C as a water-soluble antioxidant serves to stabilize highly reactive free radicals, thereby maintaining the structural and functional integrity of cells[1]. However, feeding vitamin C to dairy cows has not gained much attention in the dairy industry because of the pro-oxidant activity of ascorbic acid[2,3]. Unlike ascorbic acid, vitamin E (fat-soluble antioxidant) has been fed to ruminants for enhancing immune systems[4,5] and minimizing off-flavors in milk due to lipid oxidation[6,7]. Recent studies have also shown that high levels of ascorbic acid can also reduce the oxidized flavor in milk[2,8], but ascorbic acid is vigorously degraded in the rumen[9].

Oleic acid content of milk fat may be increased by 50% by feeding lipids rich in C18 fatty acids[10]. However, feeding fat to ruminant animals at levels above 5% of the dietary dry matter results in depressed digestion of other components of the diet and depressed intake by the animal[11]. In addition, microbes in the rumen hydrolyze and hydrogenate the dietary lipids depending on the matrix in which they are delivered into the rumen[12]. Modifying the fat source to protect it from rumen degradation eliminates these problems. The most effective way of protecting dietary fat for ruminants has been a formaldehyde-treated product[12]. However, formaldehyde-treated lipid supplements have not been approved in the U.S. because of concerns that formaldehyde will be transferred to the milk produced[13]. In work on the protection of nutrients, Lee[14-17] developed

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a high-energy, lipid-containing feed supplement, which was nearly as effective as formaldehyde-treated supplements. Lee's development is called protein-oil gel supplement (PGS) because it is suspected that protection of the lipids from degradation in the rumen is effected by their entrapment in a protein matrix. The new protein-oil gel supplement is treated with 2,3-butanedione, which is generally recognized as safe and is not a known carcinogen. PGS supplements may also offer protection from rumen degradation to a fat-soluble form of ascorbic acid.

The objective of this research was to determine if feeding ruminant animals with protein-oil gel supplements containing protected high oleic sunflower oil (oleic acid source) and ascorbyl palmitate (vitamin C source) results in increased levels of unsaturated fatty acids and vitamin C deposition in blood serum and milk. Dairy goats were used as test animals in this proof-of-concept study.

MATERIALS AND METHODS

Protein-oil gel supplement preparation: Two dietary supplements containing high oleic sunflower oil and ascorbyl palmitate were prepared following the same procedure. They only differed in principal protein ingredient and method of dehydration after preparation. One supplement was based on casein (C-PGS) while the other was based on keratin (K-PGS). Protein-oil gel supplement production was started by first dissolving approximately 489 g of sodium caseinate (or keratin) along with 7.9 g of soy lecithin in a 0.1% (w/w) NaOH solution heated to 80°C in a Groen steam kettle (DI Foodservice Companies, Jackson, Miss., U.S.A.). This mixture was then transferred into a meat cutter (Cryovac, Inc., Duncan, S.C., U.S.A.) used as an alternative for a high-speed blender. In turn, high-oleic sunflower oil (1.6 kg), defatted soy flour (16.0 kg) and ascorbyl palmitate (3.1 g) were slowly added into the cutter and mixed with 489.0 g of 2,3-butanedione (Aldrich Chemical Company, Milwaukee, Wis., U.S.A.) till all ingredients were well blended. Defatted soy flour provided the balance of protein needed to form the protective gel structure of the supplement. The mixture from the cutter coalesced into a gel, which was then ground through a 0.625-cm screen. The ground casein gel supplement was dried in a convection oven at 50°C to about 10% wet basis while the ground keratin gel was simply dried under forced airflow at ambient temperature (~27°C) for 48 h. Both protein-oil gel supplements were stored at 4°C in sealed plastic storage bags for no longer than 3 weeks before being fed. Nutritional enhancement of a commercial feed (basal diet, BD) with these two supplements or with just ascorbyl palmitate comprised the test diets.

Moisture, total lipid and protein content as well as fatty acid composition of C-PGS and K-PGS were determined. Moisture content was determined by the AOAC vacuum oven drying method[18]. Total lipids content was determined through chloroform-methanol extraction[19]. The protein content of the basal diet and each gel supplement was determined by AOAC Kjeldahl analysis[18]. Fatty acid composition of each representative sample was determined by AOCS method of preparing fatty acid methyl esters (FAME) and analyzing these by gas chromatography (GC)[20], described below.

Feeding trial and sampling: A feeding trial was conducted to evaluate any improvement in milk composition resulting from feeding the supplements. The feeding trial also served as a means of subjectively assessing the effectiveness of protection from ruminal degradation of both fat and fat-soluble ingredients afforded by the developed process/product. Experimental procedures were conducted according to guidelines and approval of The University of Tennessee Animal Care and Use Committee. Only limited amounts of protein-oil gel supplements could be produced through the laboratory-scale production process developed, thus the feeding trial was constrained to using just three lactating goats (one Nubian, two Alpine; all in their second lactation and about 3 yr old) as experimental units. Goats were selected as test animals because they required only a limited amount of feed on a daily basis and there was more information on milk production in goats than in sheep in the U.S. Goats were allowed access to a 0.2 or 0.4 ha paddock containing a mixture of cool and warm season forages. Treatment diets were fed in a feed pail attached to a stanchion while the goats were milked twice daily at about 0730 and 1630. One-half of the daily allotment was fed at each milking. Feed refusals were collected after milking and returned to the laboratory for analysis to determine nutrient intake.

The goats were fed in six different periods of 11 days each in order to evaluate four diet treatments: 1) basal diet (BD), 2) basal diet plus supplemental ascorbyl palmitate (AP), 3) basal diet plus casein-based protein-oil gel supplement (C-PGS) and 4) basal diet plus keratin-based protein-oil gel supplement (K-PGS). Selection of an 11-d feeding period followed the protocol of Gulati *et al.*[21]. In each period, two treatments were tested. For the first seven days of each feeding period, each goat was fed 1818 g d^{-1} BD. During the last four days of a feeding period, the goats were fed a test diet (supplemented). Each goat received 1372 g d^{-1} BD supplemented with 410 g C-PGS, 384 g K-PGS, or 0.2 g ascorbyl palmitate during the different feeding periods. The amounts of added supplements were calculated to simultaneously meet two requirements: 1) provide the same amount of ascorbyl palmitate among the

AP, C-PGS and K-PGS diets and 2) provide the same level of dietary fat between the C-PGS and K-PGS diets. With PGS-supplemented diets, a total of 114 g of high-oleicsunflower oil was presented to each goat per day. Since BD was fed during each feeding period, no break between feeding periods was included in the experiment. This produced 18 observations for basal diet (all 6 periods x 3 goats) and 6 observations for each test diet (2 replicate periods x 3 goats).

The commercially available 16% Milk Goat Enhancer Ration manufactured by the Tennessee Farmers CO-OP (Lavergne, Tenn., U.S.A.), procured retail, was used as the basal diet. Its composition is given in Table 1. From the measured feed intake and chemical composition of the basal diet and the protein-oil gel supplements, an average daily dietary supplement intake (g), fat intake (g), level of oleic acid (C18:1n9) intake (g) and ascorbyl palmitate intake (mg) were estimated.

Blood and milk samples were collected from each lactating goat on the seventh day (end of feeding BD) and on the eleventh day (end of feeding supplemented diets) for each feeding period. Blood was obtained from the jugular vein by venipuncture into 20-mL vacuum tubes. Serum was separated from the blood sample by centrifugation at 2,000 g for 20 min in a refrigerated centrifuge unit at 4°C (Model J6, Beckman Coulter, Inc., Fullerton, Calif., U.S.A.). Each blood serum sample was placed into a vial, flushed with nitrogen, sealed and then stored in the dark at -18°C until analyzed.

Table 1: Basal diet composition (16% Milk Goat Enhancer Ration)

Item	Composition
Ingredient composition (%)	
Crude protein (min)	16.00
Crude fat (min)	2.75
Crude fiber (max)	16.50
Calcium (min)	0.60
(max)	1.10
Phosphorous (min)	0.50
Salt (min)	0.60
(max)	1.10
Magnesium	0.35
Potassium	1.03
Sulfur	0.24
Minerals (ppm)	
Selenium (min)	0.47
Copper (min)	5.00
(max)	20.00
Zinc	191.56
Cobalt	0.56
Manganese	86.43
Iron	212.90
Iodine	1.42
Essential Vitamins (IU kg^{-1})	
Vitamin A	17.04
Vitamin D	1.50
Vitamin E	49.23

The three goats were milked twice daily, morning and evening, each day of the feeding trial. On sampling days, morning milk collection from each goat was set aside for analysis. This raw milk was pasteurized at 72°C for 15 s in a SafGard Pres-Vac Home Pasteurizer (The Schlueter Company, Janesville, Wis., U.S.A.). A 35-mL aliquot of the pasteurized milk was delivered into a polypropylene conical tube, flushed with nitrogen, sealed and stored in the dark at -18°C until analyzed. Skim milk was produced by separating milk fat from the remaining milk by centrifuging at 3,500 *g* for 20 min in a Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge at 4°C (Kendro Laboratory Products, Asheville, N.C., U.S.A.). The skim milk was also stored at -18°C in the dark until analyzed.

Blood serum and milk analysis: Ascorbic acid levels in the blood serum and skim milk samples from each goat were determined according to procedures of Omaye *et al.*[22]. A 1-mL sample of blood serum (or skim milk) was pipetted into a 15-mL conical tube, which contained 1-mL of ice-cold 10% (w/w) trichloroacetic acid (TCA). The conical tube contents were mixed thoroughly for 1 min using a Fisher Vortex Genie 2 (Fisher Scientific International, Inc., Hampton, N.H., U.S.A.) and centrifuged for 20 min at 3,500 *g* in an automatic refrigerated centrifuge at 4°C to separate ascorbic acid from the mixture. One-half mL of supernatant from the centrifuged mixture was transferred into a micro glass tube and 0.1-mL of 2,4-dinitrophenylhydrazine/thiourea/copper solution (containing 0.4 g thiourea, 0.05 g $CuSO_4 \cdot 5H_2O$ and 3.0 g 2,4-dinitrophenylhydrazine in 100-mL 9N H_2SO_4) was added. The contents in the micro glass tube were mixed for 30 s with the Fisher Vortex and incubated for 3 h at 37°C in a reciprocal shaking bath (Precision Scientific, Winchester, Va., U.S.A.) to form *bis*-2,4-dinitrophenylhydrazone. Subsequently, 0.75-mL of ice-cold 65% (w/w) H_2SO_4 was added to the incubated mixture and mixed for 1 min with the Fisher Vortex. The mixed solution was then allowed to stand at room temperature for an additional 30 min. Ascorbic acid content was then measured using a Shimadzu UV-Vis recording spectrophotometer equipped with a CPS controller (Model UV160, Shimadzu Scientific Instrument, Inc., Columbia, Md., U.S.A.). The absorbance of ascorbic acid in the sample was recorded at 250 nm against 5% (w/w) TCA blank on the UV-Vis spectrophotometer. Standard ascorbic acid solutions (0 to 20 μg mL^{-1}) were prepared in 5% (w/w) TCA and analyzed under the same conditions as the experimental samples. Linear calibration curves between absorbance and ascorbic acid concentration were developed and from these curves the concentration of ascorbic acid in blood serum and skim milk (μg mL^{-1}) samples was calculated.

Total blood serum lipids were extracted by a modification to the procedure of Bright, *et al.*[23] that did not require addition of butylated hydroxytoluene to $CHCl_3$ for the extraction. Meanwhile, total lipids were also extracted from milk by a modified method of Melton *et al.*[19]. This latter modification was a miniaturization of the original procedure. Sample amount was reduced from 50 to 2.0 mL with solvents and other chemicals needed reduced accordingly. Fatty acid composition of extracted lipids from blood serum and milk samples was determined by first preparing FAME from the total lipids extracted according to AOCS procedures[20]. FAME were analyzed with a Shimadzu GC-9A gas chromatograph equipped with a Shimadzu AOC-9 automatic injection system (Shimadzu Scientific Instruments, Inc., Columbia, Md., U.S.A.). A 0.25-mm i.d., 60-m long fused silica SP-2380 capillary column (Supelco, Inc., Bellefonte, Pa., U.S.A.) was used to separate the methyl esters that were then detected with a flame ionization detector. An injection temperature of 270°C was used and the column temperature was programmed from 50°C (2 min) to 250°C at 4°C/min. Helium was used as the carrier gas with a flow rate of 50 mL/min and a split ratio of 30:1. Identification of individual FAME in the samples was accomplished by matching the retention time of the sample peak with that of the standard peak (Alltech Associates, Inc., Deerfield, Ill., U.S.A.). The relative weight percentages of each FAME (C4:0-C22:6) in each sample were calculated using their corrected areas.

Statistical Analysis: The feeding trial was a randomized incomplete block design, blocked on goat and feeding period. Statistical analyses were performed using the SAS Mixed Procedure[24] for each dependent variable including total lipids in milk, concentration of ascorbic acid in blood serum and skim milk and concentrations of individual fatty acids in serum and milk. Each of the dependent variables was analyzed with a model including dietary treatment ($n = 4$) and blocking effects of goat ($n = 3$), period ($n = 3$) and their interaction. The pooled block by treatment interaction term was used as experimental error. Significant differences among treatment least-squares means were separated by Fisher's Least Significant Difference at the 5% significance level.

RESULTS AND DISCUSSION

Chemical composition of feed components: The weight percentage concentrations of moisture, fat and protein in BD, C-PGS and K-PGS are given in Table 2. On a dry matter basis, C- and K-PGS had 32.4 and 29.5% protein and 30.5 and 39.2% fat, respectively. However, BD contained only 16.8% protein and 4.7% fat. Major fatty acids present in BD and the PGSs are also shown in Table 2. Palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6) and linolenic (C18:3n3) acids constituted most of the fatty acids in BD and each supplement: 95.0% in BD, 94.4% in C-PGS, 94.0% in K-PGS, respectively.

Goat feed/nutrient intake estimation: Average intake levels by the goats were estimated based on the diet amounts presented to each goat per day minus the residual feed in order to estimate in turn the oleic acid and vitamin C intakes. In estimating intake levels, it was assumed that BD and the supplements are homogenously blended. Calculated intake levels of BD and supplemented diets are presented in Table 3. Goats consumed an average of 81.7% of the C-PGS and 75.6% of the K-PGS in the diets offered. Goats fed the basal diet only or the AP diet had less fat intake than goats fed either the C-PGS or K-PGS diet. Goats fed C-PGS or K-PGS diets had total daily intakes of 144.2 or 141.5 g fat/head, respectively, while goats fed BD or the AP diets had total daily intakes of 70.5 and 58.2 g fat/head, respectively. Oleic acid intake also differed greatly among treatment diets (Table 3). Goats receiving PGS ingested in excess of 84 g oleic acid/head daily compared to less than 20 g of oleic acid for goats fed BD or the AP diet. This large difference is due to the high oleic acid content (86.9% of the total lipid) of the high oleic sunflower oil incorporated into the protein-oil gel supplements. Estimated daily intake of ascorbyl palmitate for each goat in mg/head/d based on the formulation of each dietary supplement is also presented in Table 3.

Vitamin c and fatty acids in blood serum and milk: Least squares means of vitamin C levels in the blood serum and skim milk samples are presented in Table 4. The highest levels of vitamin C in blood serum was found in goats fed the K-PGS diet followed in turn by the C-PGS diet, AP diet and BD. Data shows that blood serum vitamin C level of goats fed the K-PGS diet, which contained protected ascorbyl palmitate, was significantly different from the level of vitamin C in the blood serum of goats fed only BD ($p<0.05$). However, the difference between vitamin C levels in the blood serum of goats fed the PGS supplement diets and the AP diet, which had unprotected ascorbyl palmitate, were not significant. Nonetheless, there appears to be some protection of the fat soluble form of ascorbic acid (ascorbyl palmitate) by its inclusion in the protein-oil gel supplements, specifically K-PGS. This result from this limited testing suggests further development of the protein-oil gel supplement could make it an acceptable delivery system

for nutraceuticals or other dietary elements that promote ruminant animal health. Although the C-PGS and the AP diets did not differ in vitamin C levels from goats fed only BD ($p>0.05$), feeding these two diets increased vitamin C levels in goat blood serum to the point where they were not significantly lower than levels in the blood serum of goats fed the K-PGS diet.

While there were some differences in vitamin C levels in blood serum of goats resulting from treatment diets, no significant differences were found in the vitamin C levels in skim milk samples (Table 4). Evidently, concentration of vitamin C in milk was not affected by feeding either protected or unprotected forms of vitamin C. These results were similar to what has been previously reported in studies about feeding vitamin C to dairy cows[25].

Average total fat concentrations in whole milk for the tested diets are also presented in Table 4. There were no differences ($p>0.1$) in total fat content of whole milk samples from goats fed AP, C-PGS, or K-PGS diets. However, compared to goats fed BD, goats fed the C-PGS diet had tended to increase ($p<0.1$) total milk fat. Normally, when unprotected dietary fat is fed in excess of 5% of the ruminant diet, rumen microbial activity is reduced and fiber digestibility is decreased. This negatively affects the mammary glands and eventually results in reduced milk fat content[26]. Even when calcium salts of high oleic sunflower oil were fed to Holstein dairy cows as a supplement, milk fat content decreased linearly with increasing levels fed[13]. When high levels of dietary fat are protected from rumen degradation, this effect is reversed[10,12]. In this study, milk fat levels increased from 3.34% for goats fed BD to the highest 4.55% for goats fed the C-PGS diet (Table 4) but differences were not at a statistically significant level. Feeding C-PGS provided an average of 92.2 g of protected dietary fat to each goat per day (Table 3).

Fatty acid composition of blood serum: Fatty acid composition of blood serum lipids from goats fed the treatment diets is shown in Table 5. Seven saturated fatty acids: lauric (C12:0), myristic (C14:0), pentadecylic (C15:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0) and eicosanoic (C20:0) acids) in the blood serum were identified by GC. No significant difference was found between the mean concentration of lauric acid in blood serum lipids from goats fed BD and those fed the C-PGS diet or the K-PGS diet. Regarding the medium- and long-chain saturated fatty acids (C14:0 and C20:0), goats fed BD, AP diet and K-PGS diet had higher ($p<0.05$) blood serum levels of pentadecylic acid and margaric acid than those fed the C-PGS diet. Goats ed BD had higher ($p<0.05$) blood serum levels of palmitic acid (C16:0) than goats fed the C-PGS diet. Goats fed the AP diet or the K-PGS diet had blood serum palmitic acid concentrations that were not different from those of goats fed BD.

The monounsaturated fatty acids: myristoleic acid (C14:1n5), *trans*-palmitoleic acid (C16:1t), palmitoleic acid (C16:1n7), elaidic acid (C18:1t), oleic acid (C18:1n9) and eicos-9-enoic (C20:1n9) acid were all detected in the goat blood serum samples. Differences ($p<0.05$) were found between levels of all monounsaturated fatty acids, except eicos-9-enoic acid, in the blood serum lipids of goats fed BD and those fed the C-PGS diet. Goats fed the K-PGS diet had blood serum levels of these same monounsaturated fatty acids that were not significantly different from those of goats fed BD or the C-PGS diet. The highest concentration of a monounsaturated fatty acid in the blood serum was measured for oleic acid in blood serum of goats fed the C-PGS diet. Moreover, mean concentrations of myristoleic, *trans*-palmitoleic, palmitoleic and elaidic acids in the blood serum of these goats were lower than those in goats fed BD ($p<0.05$). Among the polyunsaturated fatty acids, linoleic (C18:2n6), linolenic (C18:3n6), ã-linolenic (C18:3n3), arachidonic (C20:4n6), eicosa-5,8,11,14,17-pentaenoic (C20:5n3), docosa-7,10,13,16,19-pentaenoic (C22:5n3) and docosa-4,7,10,13,16,19-hexaenoic (C22:6n3) acids were all identified. No significant differences were found among concentrations of polyunsaturated fatty acids in blood serum of goats fed the different treatment diets.

In summary, C-PGS diet increased oleic acid levels in blood serum while decreasing C14- C17 fatty acids as well as elaidic acid (C18:1t) relative to levels in BD fed goats. These results were also observed in goats fed the K-PGS diet but not at a statistically significant level. It has been reported that reduction of elaidic acid levels is an indication that unsaturated fatty acids are being protected from ruminal biohydrogenation[10,27]. Elaidic acid is an intermediate fatty acid generated by ruminal hydrogenation[10,28], but it is also produced from *cis*-oleic (C18:1n9) acid by microbial biohydrogenation in the rumen[29]. Since the elaidic acid level in the blood serum of goats fed the C-PGS diet was lower than that of goats fed BD, the protein-oil gel supplement must be protecting oleic acid from ruminal degradation. Research has also shown that feeding unprotected high oleic sunflower oil increased the concentration of stearic acid (C18:0) compared to a basal diet[13]. Considering that hydrogenation to stearic acid (C18:0) is the limiting step in the conversion of unsaturated fatty acids to saturated fatty acids[10,27], an unchanged level of stearic acid compared to a basal diet treatment would be another indication that some level of protection from ruminal degradation is being provided. The level of stearic acid was not changed by PGS diets in this study; therefore, the protein-oil gel supplements are protecting the oleic acid to some extent.

Table 2: Fat and protein analysis of the basal diet procured and the casein and keratin protein-oil gel supplements produced

		Protein-oil gel supplements	
Item	Basal diet	Casein[1]	Keratin[2]
Macro-composition[3] (% w.b.)			
Moisture	8.88	9.81	19.80
Protein	15.26	29.20	23.61
Fat	4.28	27.53	31.40
Fatty acid (%)			
Palmitic (C16:0)	18.62	3.77	4.13
Stearic (C18:0)	2.57	3.79	3.92
Oleic (C18:1)	23.50	84.57	83.99
Linoleic (C18:2)	49.72	5.51	5.40
Linolenic (C18:3)	3.18	0.81	0.82

[1]Ground and oven dried (50°C) casein gel containing high oleic sunflower oil and ascorbyl palmitate, [2]Ground and air dried (27°C) keratin gel containing high oleic sunflower oil and ascorbyl palmitate, [3]Weight % wet basis

Table 3: Estimated mean daily intake of ascorbyl palmitate, fat and oleic acid from the basal diet and dietary supplements fed to lactating goats

		Supplemented diets		
Item	Basal diet	AP	C-PGS	K-PGS
Ascorbyl palmitate intake (mg/goat)	---	199	186	146
Intake from basal diet				
Basal diet intake (g/goat)	1738	1301	1199	1173
Fat intake[1] (g/goat)	70	58	52	50
Oleic acid (C18:1) intake[1] (g/goat)	18	14	12	12
Intake from protein-oil gels				
Gel supplement intake (g/goat)	---	---	335	291
Fat intake[2] (g/goat)	---	---	92	91
Oleic acid (C18:1) intake[2] (g/goat)	---	---	80	79

[1]Calculated intake based on fat level and composition of basal diet, [2]Calculated intake based on fat level and composition of protected lipid gel.

Table 4: Effect of supplemented diets on concentrations of vitamin C in blood serum and skim milk and fat in whole milk

			Supplemented diets (*n* = 6)			
Item	Basal diet(*n* = *18*)	SE	AP	C-PGS	K-PGS	SE
Blood serum vitamin C ($\mu g\ mL^{-1}$)	7.88[b]	1.05	8.96[ab]	9.50[ab]	11.12[a]	1.40
Skim milk vitamin C ($\mu g\ mL^{-1}$)	14.18	2.33	12.96	17.28	15.13	3.72
Whole milk Total fat (%)	3.34a	0.46	3.58a	4.55a	4.01a	0.61

[1]Least-squares means in a row with a common letter are not significantly different at the 5% level.

Fatty acid composition of milk: The effect of the dietary treatments on the fatty acid composition of milk fat is shown in Table 6. Both C-PGS and K-PGS diets decreased ($p<0.05$) levels of saturated fatty acids ranging from caproic (C6:0) to tridecanoic (C13:0) in comparison to levels in milk of goats fed BD. Interestingly, similar results have been obtained by feeding hay or corn silage diets to which unprotected high oleic sunflower oil had been added[10,28]. Such diets lowered the saturated fatty acids from caprylic (C8:0) to palmitic (C16:0) acids with increasing butyric (C4:0) and stearic (C18:0) acids[10,28]. In this study, concentrations of stearic (C18:0) and uncosanoic (C21:0) acids were significantly higher in milk samples from goats fed the PGS supplemented diets than those fed BD.

Table 5: Least-squares means of fatty acid concentrations[1,2] in goat blood serum lipids as affected by diet treatments

			Supplemented diets (*n* = 6)			
Fatty acid	Basal diet(*n* = *18*)	SE	AP	C-PGS	K-PGS	SE
C12:0	0.56	0.10	0.39	0.55	0.49	0.12
C14:0	1.05	0.13	1.22	0.59	0.75	0.26
C14:1n5	0.30[a]	0.01	0.34[a]	0.18[b]	0.26[ab]	0.02
C15:0	0.59[a]	0.01	0.62[a]	0.44[b]	0.54[ab]	0.03
C16:0	14.74[a]	0.26	15.06[a]	12.49[b]	14.48[a]	0.36
C16:1t	0.85[a]	0.03	0.93[a]	0.48[b]	0.85[a]	0.05
C16:1n7	0.87[a]	0.04	0.87[a]	0.58[b]	0.72[ab]	0.06
C17:0	0.78[a]	0.02	0.81[a]	0.59[b]	0.66[ab]	0.04
C18:0	20.53[b]	0.28	21.11[ab]	21.48[ab]	22.55[a]	0.56
C18:1t	1.85[ab]	0.11	2.34[a]	1.22[b]	1.95[ab]	0.19
C18:1n9	15.91[b]	0.82	16.53[b]	24.50[a]	18.97[ab]	1.58
C18:2-isomers[3]	0.55	0.04	0.51	0.47	0.52	0.08
C18:2n6	31.02	0.93	27.89	28.93	28.93	1.64
C18:3n3	1.51	0.20	1.74	0.96	1.61	0.35
C20:0	0.47	0.03	0.49	0.50	0.52	0.03
C20:1n9	0.29	0.03	0.27	0.17	0.29	0.05
C21:0	0.30	0.10	0.35	0.28	0.82	0.20
C20:5n3	0.59	0.07	0.62	0.47	0.37	0.10
C22:5n3	0.98	0.12	0.97	0.77	0.87	0.17
C22:6n3	0.48	0.08	0.48	0.39	0.47	0.12

[1]Wt % of fatty acid methyl esters., [2]Least-squares means in a row with a common letter are not significantly different at the 5% level., [3]Tentatively identified as isomers of linoleic acid (C18:2n6).

Table 6: Least-squares means of fatty acid concentrations[1,2] in goat milk fat as affected by diet treatments

			Supplemented diets (*n* = 6)			
Fatty acid	Basal diet(*n* = *18*)	SE	AP	C-PGS	K-PGS	SE
C4:0	8.84	0.46	9.34	7.38	7.67	0.83
C6:0	6.17[a]	0.26	5.96[ab]	4.64[b]	4.94[ab]	0.50
C8:0	5.48[a]	0.21	4.98[ab]	3.99[b]	4.19[ab]	0.42
C10:0	16.8[a]	0.57	14.99[ab]	11.77[b]	12.32[b]	1.12
C11:0	0.32[a]	0.02	0.31[a]	0.24[a]	0.21[a]	0.04
C12:0	6.67[a]	0.20	5.80[ab]	4.67[b]	4.94[b]	0.40
C13:0	0.08[a]	0.01	0.06[ab]	0.05[b]	0.05[ab]	0.01
C14:0	8.14	0.18	8.04	7.49	7.74	0.35
C14:1n5	0.22	0.01	0.26	0.21	0.25	0.02
C15:0	0.61	0.02	0.66	0.58	0.64	0.04
C16:0	21.62	0.67	22.23	19.34	20.66	1.32
C16:1t	0.33[ab]	0.02	0.40[ab]	0.28[b]	0.42[a]	0.04
C16:1n7	0.58	0.04	0.59	0.51	0.53	0.06
C17:0	0.54	0.03	0.54	0.48	0.58	0.04
C18:0	5.75[c]	0.46	6.61[bc]	9.13[ab]	10.18[a]	0.81
C18:1t	1.39[a]	0.08	1.41[a]	0.69[b]	1.52[a]	0.14
C18:1n9	12.53[c]	0.66	13.90[bc]	25.11[a]	18.90[b]	1.26
C18:2-isomers[3]	0.38[b]	0.02	0.38[ab]	0.38[ab]	0.45[a]	0.04
C18:2n6	1.77	0.08	1.70	1.86	1.82	0.15
C18:3n3	0.65[ab]	0.06	0.85[a]	0.45[b]	0.83[a]	0.08
C18:3n6	0.15[b]	0.01	0.17[ab]	0.19[a]	0.20[a]	0.01
C20:0	0.15	0.03	0.16	0.13	0.20	0.03
C20:1n11	0.12	0.03	0.05	0.04	0.06	0.06
C21:0	0.07[b]	0.01	0.09[ab]	0.12a	0.12[a]	0.01
C22:5n3	0.05	0.01	0.05	0.05	0.05	0.01

[1]Wt % of fatty acid methyl esters., [2]Least-squares means in a row with a common letter are not significantly different at the 5% level., [3]Tentatively identified as isomers of linoleic acid (C18:2n6).

The higher levels of oleic acid in the two PGS diets changed concentration of unsaturated fatty acids in milk samples. Both PGS diets resulted in an increase in oleic acid in milk ($p<0.05$). Feeding goats the C-PGS diet doubled oleic acid levels in milk from BD-fed goats. Goats fed the K-PGS diet had mean oleic acid levels in milk that was 51% over levels in milk of goats fed just BD. Apparently, both C-PGS and K-PGS offered protection to oleic acid from microbial degradation in the rumen, but protection by C-PGS was more effective. As was the case with blood serum, concentration of elaidic acid (C18:1t) was decreased significantly in milk from goats fed the C-PGS diet compared with that from goats fed the other diets. This is a further indication that unsaturated fatty acids incorporated in C-PGS may be protected from ruminal biohydrogenation.

CONCLUSIONS

Both protein-oil gel supplements described here increased the unsaturated fat content in milk, but only feeding with C-PGS resulted in a significant increase compared to levels for goats fed BD. C-PGS doubled the C18:1n9 level in BD-fed goat milk from 12.5 to 25.1% while K-PGS increased C18:1n9 to 18.9%. K-PGS significantly increased vitamin C in blood serum of goats compared with that of goats fed BD (8.0 vs. 11.4 $\mu g\ mL^{-1}$), but these higher levels did not translate into the milk produced. Considering that vitamin C levels in blood serum of goats fed the protein-oil gel supplements were not significantly different from that of goats fed a ration to which ascorbyl palmitate was just directly added, the degree of protection of the ascorbyl palmitate afforded by the protein-oil gel is questionable. The two protein-oil gel supplements increased oleic acid content in both blood serum and milk with a corollary decrease in hypercholesteremic fatty acids (lauric, myristic, palmitic and elaidic acids).

This limited study has clearly demonstrated that a formaldehyde-free formulations described here for protection of dietary lipids from ruminal degradation have promise for protecting unsaturated fatty acids and possibly at-soluble ietary ngredients. However, a larger -scale verification experiment on dairy cattle is recommended to determine the extent of protection provided, especially for fat-soluble components (e.g., ascorbyl palmitate). Furthermore, decreasing the production cost for protein-oil gel supplements is desirable for commercial success. It might be possible to replace the more costly proteins (casein and defatted soy flour) in the gel supplement with food processing by-products such as chicken feathers (keratin).

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