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Effect of different levels of saturated solution of calcium chloride in the preparation of calcium salt of fatty acid and its effect on rumen protozoa, pH and ammonia nitrogen

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Abstract

Calcium salt of fatty acid was prepared by adding 3 different levels of saturated solution of calcium chloride ($CaCl_2$) to soybean oil. It was found that fat content was not significantly affected by calcium chloride. However, calcium chloride had significant effect on other parameters like dry matter, fat, ash and sodium. The highest calcium and fat was obtained when 3.5 parts $CaCl_2$ were added ($p<0.01$). It was also observed that "sun drying" and "drying at room temperature" had no significant effect on any parameter except dry matter content. Calcium salt of fatty acid had significant anti-protozoal effect ($p<0.01$) in sheep. Treated group showed reduced protozoal number without affecting the rumen pH and rumen ammonia nitrogen. It was also found that the number of rumen protozoa in rumen liquor was less at 12:00 p.m. than that at 3:30 p.m. when sheep were fed 3% calcium salt.

Key words: Calcium chloride, Calcium salt, Rumen ammonia nitrogen, pH and protozoa

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Introduction

The use of calcium salts as a feed supplement for dairy rations can be traced back as early as 1980. At that time, research conducted at the Ohio State University under the direction of Dr. Donald Palmquist demonstrated conclusively that synthesized calcium salts of unsaturated long-chain fatty acids (LCFAs) prevented digestibility problems when added to cultures of ruminal microbes. Later, *in vivo* studies at Ohio State were the first to feed preformed calcium salts to ruminants, showing their ruminal inertness (Palmquist and Jenkins,1982). The first commercial product, Megalac, was marketed in 1984, and since then, it has been fed to millions of cows throughout the world.

A number of fat supplements are available today for dairy nutritionists to use. These include various commodity fats such as tallow, choice white grease, yellow grease, lard, and animal-vegetable blends, as well as commercial fat supplements that are designed in some way to be "inert" in the rumen. The concept of an "inert fat" is preferable and should be interpreted as a fat supplement that has less effect on rumen fermentation than native fats. Another major advantage of commercial fats is that they are "dry" fats. Formation of calcium soaps of fatty acids is another common method of producing dry fats. Such products (such as Megalac) are easily handled and are more inert in the rumen than native fats.

There are three main strategies for producing inert fats. One is to increase the saturation of the fatty acids in the product so that the melting point is above the range of environmental temperatures. This decreases solubility and the potential interaction of fats with rumen microbes. The second is to complex the fatty acids with calcium to form calcium soaps. Because, a free fatty acid is required for biohydrogenation and interaction with microbes, tying up the fatty acids as soaps prevents this interaction to some degree. Finally, fats may be "encapsulated" in various ways to physically prevent interaction with the ruminal microbes. These can be non-nutritive compounds (sodium alginate) or formaldehyde treatment of proteins to prevent rumen access but allow intestinal access. Of these strategies, only the first two are in common use currently.

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Feeding saturated fats or Ca salts of long chain fatty acids may minimize any detrimental effects on ruminal fermentation of supplementing large amounts of fat to the diet of dairy cows. Saturated fatty acids are less likely to alter fermentation in the rumen than unsaturated fatty acids, because saturated fatty acids are less soluble, and therefore are less likely to adsorb onto bacteria (Chalupa et al. 1984). Saturated fatty acids also react more readily with metal ions to form insoluble salts of fatty acids (Jenkins and Palmquist 1982). Feeding preformed Ca salts of fatty acids (CaS) does not alter fermentation in the rumen because of their insolubility (Chalupa *et al.* 1984, and Chalupa *et al.* 1986), provided the pH of the rumen is maintained above 6.0 (palmquist. 1984 and palmquist *et al.* 1986). Liquefying a mixture of fatty acids high in saturated fatty acid content and spraying the mixture of fatty acids under pressure into a cooled atmosphere results in a dried prilled fatty acid supplement that is inert in the rumen and does not alter rumen fermentation (Grummer 1988).

The main objectives of the study was to prepared calcium salt of fatty acids and then its effect on rumen pH, protozoa, and rumen ammonia nitrogen. A current interest in milk fat manipulation is to produce milk with higher proportions of unsaturated fatty acids. Milk with higher proportions of unsaturated fat is likely to be preferred by the consumer because of concerns about too much saturated fat in the diet. An increase in saturated fatty acid intake has been linked to a rise in cardiovascular disease. Dairy feeds that contain lipids with low susceptibility to ruminal biohydrogenation (e.g., oilseeds vs. free oil) can influence milk fatty acid composition. Milk from cows fed high oil corn, an oilseed has been shown to contain more unsaturated fatty acids than milk from cows fed Conventional Corn. Calcium salt of fatty acid is a protected fat and it can escape rumen biohydrogenation and for this reasons unsaturated fatty acids remain as it in the rumen. In this concern it was provided to the animal because, it thought that protozoa engulf rumen bacteria but bacteria is necessary for fiber digestion & in this way when there is less protozoal number there is more bacterial population and more fiber digestion will be occurred & thus milk production and fertility of the animal will be improved.

Materials and Methods

The experiment was conducted at the Animal Production Research Division, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka for a period of five months from 1st July to 1st December 2009. The chemical analyses of feed was done in the Animal Nutrition Laboratory of Bangladesh Livestock Research Institute (BLRI), analysis for Ca at the laboratory of soil Science Department of Bangladesh Agricultural University (BAU), Sodium (Na) at the Central Laboratory of Bangladesh Agricultural University (BAU) Mymensingh, fatty acid profile was determined at Institute of Food Science and Technology (IFST) University of Dhaka. Protozoa count of rumen liquor was performed at Rumen Microbiology Laboratory of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. Feeding trial was done at sheep and goat farm of BLRI.

Four parts of an aqueous solution of NaOH *(6M)* were added to five parts of soybean oil, and the hydrolysis of oil triacylglycerols was performed at 95 to 100^{o}C with continuous agitation and bubbling N_2. When no more oil was visible, the resulting blend was left to stand at 5^{o}C until Na soaped and solidified. The Na soaps were dissolved in hot water (95 to 100^{o}C) using a 1:5.6 ratio of soap to water, and a saturated solution of $CaCl_2$ at three different ratios of 2.5 parts, 3.5 parts and 4.5 parts of soap to water was added for salting out. A filter cloth was used to filter the Ca salts and tap water was used to remove residual NaOH and excess $CaCl_2$ was removed by following water. The Ca salts were finally dried both in air and sun, and kept at about –20^{o}C until used for feeding Chouinard et al. (1998).

Ten female sheep with an average live weight of 12.955±2.766 Kg were selected for the experiment. The experiment lasted for 15 days. The animals were divided into two groups of five sheep in each named control group T_0 (Live weight 12.92±3.02) and treatment group T_1 (live

weight 12.99±2.844). The animals were housed in individual pans.

During the trial period sheep belonged to control group T_o were offered Napier silage mixed with molasses *adlibitum* and concentrate mixture (i.e Wheat bran, rice polish and mustard oil cake) 1.5% of the live weight of the individual animal. Sheep belonged to treatment group T_1 were offered the same diet but this group was also offered 3% Calcium salt of fatty acid an additional or as replacement of NaCl on the basis of total concentrate mixture during the last five days of experiment. During the whole period clean drinking water was provided to all the animals.

Diets were offered two times daily. In the morning (8:00 am), animals were offered half of the concentrate mixture and in the evening (2:00 pm), the next half. In the last five days of experimental period 3% Calcium salt of fatty acid on the basis of concentrate mixture were given with the morning portion of concentrate mixture. Napier silage mixed with molasses was given only at 10:00 am daily. Each morning leftover feed samples were weighed to measure the feed intake. No refusals of concentrate mixture were found in any case.

Rumen liquor was collected by using saline pipe inserted in another wider pipe inserted through oesophagus and drawn by syringe. After collecting pH was measured and the liquor taken in 10ml test tube at a ratio of 1:9 rumen liquor and MFS (Methyl green-formalin-Sodium chloride) stain respectively for protozoal count.

Results and Discussion

Effect of drying process and different level of Calcium Chloride ($CaCl_2$) in the preparation of calcium salt of fatty acid shown in Table 1. It was found that 3.5 parts ($CaCl_2$) and air dry give the best result. 3.5 parts ($CaCl_2$) and sun dry have significant effect on Ca (15%) and DM (87.06%) content ($p<0.01$) and ($p<0.05$) respectively. 3.5 parts ($CaCl_2$) has no significant effect on either drying process in terms of fat (40.39% for air dry and 40.07% for sun dry) content. In this study the procedure adopted by Chouinard et al. (1998) was followed. Calcium salt of fatty acid. According to Chouinard *et al*. (1998) the prepared Calcium salt of fatty acid should contain 92-93% fat, 6-7% Ca and <1% Na and Cl. Less fat content of Calcium salt of fatty acid in our study may due to wash out of most of the fat during filtration. Ca content in this study was almost double which may due to increased concentration of Calcium Chloride ($CaCl_2$) solution and Na and Cl content was almost similar to Chouinard et al. (1998).

Table 2 shows feed intake of the experimental animal. It was found that there was no significant effect of Dry Matter (DM), Crude Protein (CP), Organic Matter (OM) and Acid Detergent Fiber (ADF) intake among treated group and control group.

Table 3 shows that effect of calcium salt of fatty acid on rumen pH and ammonia nitrogen. No significant difference was observed for rumen pH and rumen ammonia nitrogen contents between the treated groups.

Table 1. Effect of different levels of saturated solution of calcium chloride ($CaCl_2$) and drying process in the preparation of calcium salt of fatty acids.

Compo-sition	Combination (Mean±SD)						Sig. level
	2.5 parts x Air Dry	2.5 parts x Sun Dry	3.5 parts x Air Dry	3.5 parts x Sun Dry	4.5 parts x Air Dry	4.5 parts x Sun Dry	
DM	83.99^{d}±9.25	84.71^{c}±7.55	87.06^{b}±5.47	89.08^{a}±7.58	83.21^{d}±8.29	89.27^{a}±6.89	**
Fat	38.17±6.11	40.03±3.85	40.39±3.14	40.07±4.74	39.63±4.15	40.23±5.32	NS
Ash	14.90±1.05	14.74±0.85	14.43±0.99	15.51±1.15	14.79±1.27	15.66±1.13	NS
Ca	16.09^{b}±1.22	16.09^{c}±1.09	15^{d}±1.41	17.27^{a}±1.25	15.63^{d}±0.98	16.63^{b}±1.13	*
Na	0.70±0.04	0.80±0.03	0.80±0.05	0.70±0.02	0.70±0.07	0.70±0.09	NS

*Means with different superscript in the same row differed significantly; *, p<0.05; **, p<0.01; NS, non-significant*

Table 2. Feed intake (Mean±SE) of the animals when calcium salt of fatty acid was offered as dietary supplement

Parameters	Control Group*	Treated Group**
DM (g/d)	363.69±30.604	365.32±28.846
CP (g/d)	162.46±12.071	160.32±11.251
OM (g/d)	1326.40±97.151	1312.65±91.056
ADF (g/d)	607.41±43.462	598.01±40.685

**, with Ca salt of fatty acid; **, without Ca salt of fatty acid*

Yang and Varga (1989) found 56.1, 48.1, and 60.0 ($x10^3$ cell/ml) rumen protozoa and 6.14, 6.07 and 6.04 rumen pH by feeding concentrate mixture 1 times, 2 times and 3 times daily which was slightly high in the present research. It was observed that calcium salt of fatty acid significantly reduce the rumen protozoal number without affecting the rumen pH and ammonia nitrogen. Rumen liquor were collected 2 times daily and found that first collection gave less number of protozoa compared to 2^{nd} collection. In the treated group rumen protozoal number was found to be 53.63±1.29 at 12:00 PM and 81.15±0.89 at 3:30 PM, and in the control group protozoal number was found to be 141.22±0.50 at 12:00 P.M and 157.96±1.96 at 3:30 PM. A severe decrease in protozoal numbers was observed with all three of the unsaturated C_{18} acids particularly by linolenic ($C_{18:3}$) and linoleic ($C_{18:2}$) acids. Several medium-chain saturated and long-chain unsaturated FA were found to exert dramatic effects on ruminal fermentation. Capric and lauric acids application completely eradicated ruminal protozoa, (Hristov *et al.* 2004). Machmuller and M. Kreuzer (1999) found a strong inhibitory effect of coconut oil (included at 3.5 and 7.0% of dietary DM) on ruminal ciliates, which was partially responsible for a substantial decrease in methane production.

Henderson (1973) reported inhibition of the growth of several ruminal bacteria by $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$ FA. At low concentrations (from 0.01 to 0.1 g/L), $C_{10:0}$, $C_{12:0}$, and $C_{18:1}$ FA had a stimulatory effect, but at higher concentrations also decreased bacterial growth.

Table 3. Effect of Calcium salt of fatty acids on rumen pH and ammonia nitrogen

Compo-sition	Time (PM)	Mean±SE	
		Control group	Treated group
Rumen pH	12.00	6.31±0.070	6.26±0.54
	3.30	6.37±0.090	6.34±0.55
RAM (mol/L)	12.00	147.68±1.61	166.55±1.47
	3.30	169.75±1.23	175.45±1.03
RP ($x10^3$ cell/ml)	12.00	141.22^a±0.50	53.63^b±1.29
	3.30	157.96^a±1.96	81.15^b±0.89

RAM, rumen ammonia nitrogen; RP, rumen protozoa; Means with different superscript in the same row differed significantly (p<0.05)

In contrast, Maczulak et al. (1981) found no effect of $C_{16:0}$ or $C_{18:0}$ FA, but $C_{18:1}$ dramatically inhibited the growth of certain cellulolytic strains. Shingfield et al. (1983) reported that coconut oil (high in C_{12} to C_{14} saturated FA) possessed stronger antiprotozoal properties than did linseed oil (high in C_{18} unsaturated FA), and that both oils produced changes in volatile fatty acid (VFA) proportions that are typically associated with decreased protozoal numbers in the rumen. In contrast, Newbold and Chamberlain (1988) found a stronger antiprotozoal effect of C_{18} unsaturated acids (supplied as linseed oil) than was exerted by saturated C_{12} to C_{14} acids (from coconut oil). Pantoja et al. (1994) observed that the efficiency of microbial protein synthesis in the rumen increased linearly with the degree of unsaturation in dietary fat for dairy cows. As well, ruminal protozoal populations were shown to decrease linearly with increasing the degree of un-saturation of dietary fats (Oldick and Firkins, 2000). Rapeseed oil, which is high in unsaturated FA, effectively decreased ruminal ammonia and butyrate concentrations and also increased the efficiency of microbial protein synthesis in the rumen, although protozoal numbers were not significantly decreased (Tesfa, 1993).

Medium-chain saturated FA not only decreased or eradicated ciliate populations, but also inhibited (to different degrees, depending on chain length) bacterial growth, proteolysis, and deamination. Thus, the effects of these FA on ruminal ammonia concentration seem to result both from inhibition of protozoal growth and from inhibition of

bacterial activities. Lipids are hydrolyzed extensively in the rumen by microbial lipases, releasing long-chain fatty acids that may inhibit bacterial activity. Among long-chain FA, unsaturated ones are more antimicrobial than saturated ones (Harfoot and Hazlewood, 1997).

Conclusion

Calcium salt of fatty acid was prepared by adding 3 different levels saturated solution of $CaCl_2$ and also different drying methods (air and sun drying) were measured. It was found that 3.5 parts $CaCl_2$ gave the best result in both drying process. Thus it could be said that calcium salt of fatty acid can be prepared by applying 3.5 parts $CaCl_2$ and by both drying process. Calcium salt of fatty acid significantly reduced protozoal number without affecting the rumen pH and rumen ammonia nitrogen. Therefore, when there is less protozoal number higher the bacterial population and thus higher the fiber digestion and at the same time no alteration of rumen environmental condition.

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