Effect of Supplementing Sheep with Sunflower Acid Oil or its Calcium Soap on Nutrient Utilization

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ABSTRACT : Four adult rams (22.25±0.90 kg) were used in a 4×4 latin square design to evaluate the rations without (T_1) or with supplementation of sunflower acid oil at 5 (T_2), 10 (T_3) or calcium soap at 10% of dietary DM (T_4) on nutrient digestibility and balances of nitrogen, calcium and phosphorus. The basal ration contained 60 parts Brazilian napier grass hay and 40 parts concentrate mixture. The DM, CF, NDF and ADF digestibilities and nitrogen retention (g/d) decreased ($p<0.01$) by inclusion of sunflower acid oil at 5% of dietary DM. In addition, depression ($p<0.01$) in digestibilities of CP, nitrogen free extract (NFE), cellulose, hemicellulose, retention of calcium and phosphorus (g/d) were also observed with increasing the level of sunflower acid oil to 10% of dietary DM. The EE digestibility, total digestible nutrients (TDN) content and calcium retention (g/d) were significantly higher ($p<0.01$) for ration supplemented with calcium soap. It is concluded that sunflower acid oil supplementation in free form as low as 5% of dietary DM is deleterious to fibre digestion in sheep while as calcium soap, it can be fed up to 10% of dietary DM as an energy source without any adverse effect. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 9 : 1288-1293)***

Key Words : Sunflower Acid Oil, Calcium Soap, Nutrient Utilization, Sheep

INTRODUCTION

Sunflower acid oil, a byproduct obtained during refining of sunflower oil contains a high proportion of free fatty acids with variable amount of tryglycerides. This product is marketed at one-third the price of edible oils. The ME value of soap stock (acid oil) for ruminants was found at 30.54 MJ/kg (Fadel, 1999). Judicious use of energy value of fats for ruminants depend on level of supplementation. Inclusion of fat at higher level causes disturbances in rumen fermentation mainly by depressing fibre digestion (Devendra and Lewis, 1974). Several methods have been developed to make fat inert in the rumen environment (Scott and Ashes, 1993) so as to feed fat at higher levels without any adverse effect on fibre digestion. One of the approach to supply fat for ruminants is in the form of calcium soap (Synonym:Calcium salts of fatty acids). Research indicates that calcium soap is insoluble at normal rumen pH and thus inert towards fermentative digestion *in vitro* (Chalupa et al., 1984) and *in vivo* (Jenkins and Palmquist, 1984). According to Cronje and Oberholzer (1990) ingestion of unprotected fat by sheep in the form of cotton acid oil or fish soap stock at levels as low as 4% of the diet resulted in decrease in feed intake and eventual ruminal stasis. In contrast, no negative effect on feed intake and fibre digestion was observed when sheep were fed with canola acidulated fatty acids (acid oil) up to 10% level (Mir, 1988). It has been recognized that inhibition of rumen fermentation is severe when unprotected lipid contains a high proportion of free fatty acids rather than tryglycerides (Jenkins, 1993). Further, the stability of calcium soap in the rumen fluid is poor when it is based on unsaturated fatty acids (Sukhija and Palmquist, 1990). Sunflower acid oil contains a high proportion of free fatty acids with higher degree of unsaturation (Vila and Esteve-Garcia, 1996) as compared to other lipid sources. Hence, there is a need for information on the optimum level of inclusion of sunflower acid oil or its calcium soap in ruminant diets and their ultimate energy value for ruminants. The present study was undertaken to assess the effect of supplementing varying levels of sunflower acid oil or its calcium soap to the diets of Nellore brown sheep on nutrient digestibility and balances of nitrogen, calcium and phosphorus.

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MATERIALS AND METHODS

Procurement and storage of sunflower acid oil

Sunflower acid oil was obtained from M/S Tamil Nadu Edible Oils Limited, Chennai (India). It was stored in an airtight container in a cool dark place after addition of Butylated Hydroxy Anisole (BHA) at 0.05% level until used for feeding.

Preparation of calcium soap

Calcium soap from sunflower acid oil was prepared by precipitation method (Garg, 1998) with minor modifications. The exact procedure used to prepare calcium soap is as follows.

1. Hundred grams of acid oil was mixed to one litre of water and stirred vigorously for 5 minutes.
2. Two hundred ml of 11 per cent sodium hydroxide was added.
3. The contents were heated and stirred until the fatty acids were dissolved completely.
4. While hot, the resulting blend was slowly added with 200 ml of 20% calcium chloride solution.
5. The calcium soap formed was separated and washed with tap water.
6. Excess water was removed by squeezing the calcium soap through muslin cloth.
7. Finally, the calcium soap was air dried in a dark room and stored at subzero temperature until used for feeding.

Ration formulation

Four grower rations were formulated without (T_1) or supplementation with 5 (T_2), 10% (T_3) of Sunflower acid oil or its calcium soap at 10% of dietary DM (T_4) to assess the nutrient utilization in Nellore brown sheep. The basal ration contained 60 parts Brazilian napier grass hay (Chaffed) and 40 parts concentrate mixture (ground nut cake, 42; ground maize, 35; deoiled rice bran, 20 and mineral mixture with salt 3% plus Vit. AB_2, B_3 @ 30 g/q) with 14 per cent CP.

Metabolism trial

A metabolism trial was conducted using 4 adult Nellore brown rams (22.25±0.90 kg) in a 4×4 latin square design. A 14 day preliminary period and 7 day collection period were followed. The rams were housed in individual pens (2 m×1 m) during preliminary period and were shifted to metabolism cages 2 days prior to collection period. The rations were offered twice daily at 8:00 h and 14:00 h in equal proportions throughout the experimental period. Rams were fed with concentrate mixture first followed by Brazilian napier grass hay. Animals in T_2 received approximately 11.25 g of acid oil per 100 g of concentrate mixture, while animals in T_3 and T_4 received 22.5 g of acid oil and 22.5 g of calcium soap per 100 g of concentrate mixture respectively. The fat supplements were mixed with concentrate mixture daily at the time of feeding. Roughage to concentrate ratio was maintained by reducing or increasing the quantity of both roughage and concentrate offered proportionately as per previous day intake throughout the experimental period. Total faeces voided for 24 h was collected and an aliquot of 10% was composited in polythene bags and frozen. Daily urine output was measured and an aliquot of 5% was composited in glass bottles with few drops of hydrochloric acid and preserved at 4°C. Representative samples of feed and feed refusals if any were composited and preserved in polythene bags for further analysis.

Chemical analysis

The free fatty acid (as oleic acid) and iodine value (Hanus method) of sunflower acid oil were determined (AOAC, 1995). Concentrate mixture, Brazilian napier grass hay and faeces were analyzed for proximate composition (AOAC, 1995) and cell wall constituents (Goering and Van Soest, 1970). Ether extract was analyzed after acid hydrolysis (Wiseman et al., 1992). Samples of fresh faeces and urine were analyzed for nitrogen content (Kjeldahl method, AOAC, 1995). Calcium in calcium soap, calcium and phosphorus in feed and faeces were estimated by the method suggested by Talapatra et al. (1940). Calcium and phosphorus in urine were analyzed by using the method of Ferrow and Ham (1957) and Fiske and Subba Raw (1925) respectively.

Statistical analysis

The data were subjected to analysis of variance (Snedecor and Cochran, 1989) and the means were tested for significance by latin square design.

RESULTS AND DISCUSSION

Chemical composition of concentrate mixture

Fat supplements were added to concentrate mixture without replacing a specific ingredient. Hence the concentrations of OM, CP, CF, NFE, phosphorus and all cell wall constituents linearly decreased as the levels of sunflower acid oil or calcium soap increased in the concentrate mixture (Table 1)

Intake, digestibility and retention of nutrients

Both roughage and concentrate intake were significantly lower ($p<0.05$) in rams fed ration T_3 than those fed T_1, T_2 or T_4 (Table 6). This could be attributed negative effects of sunflower acid oil on digestibility of nutrients. However, no change in dry matter intake was observed when sheep were fed with canola acidulated fatty acids at 10% level (Mir, 1988). This variation might be due to variation in quality of roughage used in that study rather than the type of fat.

The DM digestibility significantly decreased ($p<0.01$) as the levels of sunflower acid oil in the ration increased (Table 2). Similar findings were reported by Davidson and Woods (1960) for DM digestion when sheep were fed corn cobs based rations supplemented with corn oil or fatty acid mixtures at 5% level. Added fat as calcium soap did not influence digestibility of DM significantly in the present investigation. Garg (1998) observed similar results for DM digestibility when sheep were fed rations supplemented with bypass fat produced from mixed vegetable oil at 7 and 14% level.

Decrease in protein digestibility when corn oil was

Table 1. Chemical composition of concentrate mixtures, Brazilian napier grass hay and calcium soap[a]

Attribute	T_1 (Control)	T_2 (Acid oil at 5% of dietary DM)	T_3 (Acid oil at 10% of dietary DM)	T_4 (Calcium soap at 10% of dietary DM)	Brazilian napier grass hay	Calcium soap
Proximate composition						
Dry matter	91.0	91.3	91.4	91.2	91.6	97.7
Organic matter	89.8	90.7	91.7	88.0	87.8	-
Crude protein	25.3	22.5	20.7	20.6	6.9	-
Ether extract	3.3	12.7	20.6	18.0	1.7	86.1
Total ash	10.2	9.3	8.3	12.0	12.2	13.5
Crude fibre	8.3	7.6	7.0	7.0	32.5	-
Nitrogen free extract	52.8	47.7	43.5	42.3	46.8	-
Minerals						
Calcium	0.95	0.93	0.87	2.5	0.50	7.8
Phosphorus	0.93	0.89	0.82	0.83	0.26	-
Cell wall constituents						
NDF	43.1	38.7	35.2	35.1	71.3	-
ADF	17.8	15.9	14.5	14.5	48.7	-
ADL	4.0	3.6	3.2	3.2	6.0	-
Hemicellulose	25.3	22.8	20.6	20.6	22.6	-
Cellulose	11.2	10.1	9.2	9.1	38.3	-

[a]On dry matter basis except for dry matter.

Table 2. Effect of supplementing varying levels sunflower acid oil or calcium soap on nutrient digestibility in Nellore brown rams

Attribute	T_1 (Control)	T_2 (Acid oil at 5% of dietary DM)	T_3 (Acid oil at 10% of dietary DM)	T_4 (Calcium soap at 10% of dietary DM)	SEM
Dry matter**	62.9^{a}	61.2^{b}	54.2^{c}	63.1^{a}	0.31
Crude protein**	72.9^{a}	71.4^{b}	68.1^{c}	74.1^{a}	0.42
Ether extract**	63.1^{a}	74.1^{b}	61.5^{a}	71.7^{b}	1.1
Nitrogen free extract	71.7^{a}	68.9^{a}	65.2^{b}	69.5^{a}	0.74
Crude fibre**	52.0^{a}	47.4^{b}	34.4^{c}	52.6^{a}	0.78
NDF**	55.4^{a}	51.4^{b}	41.7^{c}	57.1^{a}	0.74
ADF**	48.6^{b}	44.2^{b}	31.7^{c}	50.2^{a}	0.68
Hemicellulose**	65.9^{ab}	61.8^{b}	56.8^{c}	67.1^{a}	0.89
Cellulose**	63.2^{ab}	58.5^{b}	46.5^{c}	64.8^{a}	0.92

[a,b,c] Values in the rows bearing different superscripts differ significantly. ** $p<0.01$.

added to cottonseed hulls based basal diet (Brooks et al., 1954) or corn oil or fatty acid mixtures supplementation at 5% levels (Davidson and Woods, 1960) were reported. However, in the present investigation, CP digestibility was not affected by acid oil addition at 5% level while acid oil supplementation at 10% of dietary DM significantly ($p<0.01$) reduced digestibility (Table 2). Bayourthe et al. (1994) observed that nitrogen (N) digestibility was not affected by calcium soap (60% unsaturated fatty acids) addition up to 12% of the diet in sheep which is in accordance with the findings of present study for calcium soap supplementation at 10% of the dietary DM. However, linear decrease in CP content of rations in response to fat supplement also reflected in its DCP content (Table 6). The DCP content was significantly lower ($p<0.01$) for rations T_2, T_3 or T_4 as compared to T_1. The N retention (g/d) was also significantly lower ($p<0.01$) in rams fed ration T_2 or T_3 than those fed control ration. Lower N intake with decrease in digestibility appears to be the cause for lower N retention in rams fed ration T_3.

The CF, NDF and ADF (Table 3) digestibilities decreased ($p<0.01$) as the levels of sunflower acid oil in the ration increased, and more severe at 10% level. The digestibilities of cellulose and hemicellulose were significantly lower ($p<0.01$) in rams fed ration T_3 than those fed T_1, T_2 or T_4. Depression in digestibility of cellulose (Czerkawski 1966), CF (Macleod and Buchnan-Smith,1972) and ADF (Jenkins and Palmquist, 1984) was reported in both sheep and cattle due to acid oil supplementation at 3% of the diet. The mechanism by which dietary fat alters fibre digestion is still unclear. Devendra and Lewis (1974) summarized five theories to explain decrease in fibre digestion.1. Physical coating of fibre by fat. 2. Shortage of calcium due to formation of insoluble complexes with long chain fatty

Table 3. Nitrogen utilization in Nellore brown rams fed rations supplemented with sunflower acid oil or calcium soap

Attribute	T_1 (Control)	T_2 (Acid oil at 5% of dietary DM)	T_3 (Acid oil at 10% of dietary DM)	T_4 (Calcium soap at 10% of dietary DM)	SEM
Metabolic body weight (W $kg^{0.75}$)	10.7	10.7	10.8	10.9	0.09
Nitrogen intake					
g/d*	16.7^{a}	13.8^{b}	13.0^{b}	16.7^{a}	0.72
g/W $kg^{0.75}$/d*	1.6^{a}	1.3^{b}	1.2^{b}	1.5^{a}	0.06
Nitrogen excretion (g/d)					
Faecal	4.5	4.0	4.1	4.3	0.22
Urinary	5.9	5.0	4.9	5.4	0.27
Total	10.3	9.0	9.0	9.7	0.49
Nitrogen retention					
g/d**	6.3^{ab}	4.8^{bc}	4.0^{c}	7.0^{a}	0.35
g/W $kg^{0.75}$/d**	0.59^{a}	0.44^{b}	0.37^{b}	0.63^{a}	0.03
Per cent of intake*	36.7^{ab}	34.2^{b}	30.5^{b}	41.3^{a}	1.8
Per cent of absorbed**	51.2^{ab}	48.4^{ab}	44.7^{b}	55.4^{a}	1.4

[a,b,c] Values in the rows bearing different superscripts differ significantly. * $p<0.05$. ** $p<0.01$.

Table 4. Effect of supplementing sunflower acid oil or calcium soap on calcium balance in Nellore brown rams

Attribute	T_1 (Control)	T_2 (Acid oil at 5% of dietary DM)	T_3 (Acid oil at 10% of dietary DM)	T_4 (Calcium soap at 10% of dietary DM)	SEM
Calcium intake (g/d)**	5.0^{b}	4.3^{b}	4.1^{b}	11.1^{a}	0.55
Calcium excretion (g/d)					
Faecal**	3.2^{b}	3.0^{b}	4.2^{b}	7.5^{a}	0.35
Urinary**	0.21^{b}	0.26^{b}	0.21^{b}	0.59^{a}	0.05
Total**	3.4^{b}	3.3^{b}	4.4^{b}	8.1^{a}	0.39
Calcium retention (g/d)**	1.6^{b}	1.0^{b}	-0.31^{c}	3.0^{a}	0.23

[a,b,c] Values in the rows bearing different superscripts differ significantly. ** $p<0.01$

Table 5. Effect of supplementing sunflower acid oil or calcium soap on phosphorus balance in Nellore brown rams

Attribute	T_1 (Control)	T_2 (Acid oil at 5% of dietary DM)	T_3 (Acid oil at 10% of dietary DM)	T_4 (Calcium soap at 10% of dietary DM)	SEM
Phosphorus intake (g/d)*	3.9^{a}	3.3^{b}	3.1^{b}	4.1^{a}	0.21
Phosphorus excretion (g/d)					
Faecal	2.2	1.9	2.3	2.7	0.09
Urinary	0.11	0.11	0.09	0.12	0.01
Total	2.3	2.0	2.4	2.8	0.15
Phosphorus retention (g/d)**	1.6^{a}	1.2^{ab}	0.71^{b}	1.9^{a}	0.15
Per cent of intake**	40.6^{a}	38.5^{a}	22.8^{b}	44.3^{a}	1.9
Percent of absorbed*	93.1^{a}	92.4^{a}	87.7^{b}	93.6^{a}	1.2

[a,b] Values in the rows bearing different superscripts differ significantly. * $p<0.05$. ** $p<0.01$.

acids. 3. Inhibition of rumen microbes by surface-active effects of fatty acids. 4. Reduced microbial activity or modification of microbial population due to toxic effects of oil. 5. Reduction in the proportion of rumen isobutyric and isovaleric acids. Jenkins (1993) stated that unsaturated fatty acids and a free carboxyl group inhibited fermentation more than saturated fatty acids and fatty acid derivatives like calcium soap. In our study, sunflower acid oil contained higher proportion of free fatty acids (58.22%) with higher degree of unsaturation (Iodine value: 112.78). Further, calcium content of the ration was low. Hence supplemental acid oil even at 5% of dietary DM significantly decreased CF, NDF, and ADF digestibilities. However, fibre digestion might not have affected if the basal ration contained hay rich in calcium like alfalfa (Mir, 1988) rather than Brazilian napier grass hay.

In vitro studies have shown that for given pH, the percentage of dissociation of calcium soap is higher when they are based on unsaturated fatty acids rather than saturated fatty acids (Sukhija and Palmquist, 1990). Hence, most of the commercially available calcium soaps are prepared from oils rich in saturated fatty acids. In the present investigation calcium soap used was prepared from highly unsaturated sunflower acid oil. However, fibre

Table 6. Nutritive value and plane of nutrition of rations supplemented with varying levels of sunflower acid oil or calcium soap

Attribute	T_1 (Control)	T_2 (Acid oil @ 5% of dietary DM)	T_3 (Acid oil @ 10% of dietary DM)	T_4 (Calcium soap @ 10% of dietary DM)	SEM
Nutritive value					
Digestible crude protein (%)**	10.4^{a}	9.4^{b}	8.4^{c}	9.2^{b}	0.09
Total digestible nutrients (%)**	61.7^{b}	60.9^{b}	58.3^{c}	65.4^{a}	0.60
Plane of nutrition					
DM intake (g/d)					
Roughage*	437.4^{a}	370.2^{ab}	336.0^{b}	430.1^{a}	20.1
Concentrate*	292.0^{a}	247.0^{ab}	225.2^{b}	290.1^{a}	13.8
Total* (including supplement)	729.4^{ab}	648.0^{b}	617.3^{b}	792.1^{a}	35.1
DCP intake (g/d)*	75.9^{a}	60.8^{b}	51.7^{bc}	73.0^{ab}	3.7
TDN intake (g/d)*	450.6^{ab}	394.8^{bc}	360.2^{c}	518.6^{a}	23.0

^{a,b,c} Values in the rows bearing different superscripts differ significantly. * $p<0.05$. ** $p<0.01$.

digestion was not influenced ($p<0.01$) by inclusion of calcium soap at 10% of dietary DM. Similar findings were also observed by Doreau et al. (1993) when calcium soap prepared from rape seed oil was used. It appears that unsaturated soaps will be stable in the rumen fluid if sufficient level of roughage is used in the ration to maintain normal rumen pH.

Although apparent fat digestibility may increase linearly with increasing intake the more general relationship is quadratic (Palmquist, 1994). In our study, except ration T_3 other fat supplemented rations (T_2 and T_4) showed higher ($p<0.01$) EE digestibility (Table 2). It might be due to higher digestibility of added fat than the lipid component of the basal diet (Grummer, 1988). In the present study, supplementation of acid oil at 5% of dietary DM significantly ($p<0.01$) increased EE digestibility, which is in accordance with Davidson and Woods (1960). The digestibility of EE was significantly ($p<0.01$) higher for ration T_4 compared to ration T_3 even though the fat intake was relatively similar in rams fed both rations. Wu et al. (1991) attributed lower ruminal biohydrogenation and subsequent higher unsaturated fatty acids in intestinal chyme when calcium soap was fed. In general, unprotected fat that passes through rumen are prone to lipolysis and biohydrogenation. In the present investigation intake of sunflower acid oil at 10% of dietary DM might have led to extensive biohydrogenation of linoleic acid. Andrews and Lewis (1970) reported that the digestibility of fatty acid increases as unsaturation increases. Hence, the higher digestibility of ration T_4 than T_3 might be due to the lower ruminal biohydrogenetion and availability of large proportion of long chain unsaturated fatty acids in the small intestine for absorption when added as calcium soap in ration T_4. However, apparently lower digestibility of EE for ration T_4 than ration T_2 might be related to level of intake. Bayourhte et al. (1994) also observed apparently lower EE digestibility when sheep were fed calcium soaps at 12% of the diet compared to those animals fed at 6% of the diet.

The TDN content was significantly higher ($p<0.01$) for ration T_4 than that of T_1. This could be attributed to higher intake and digestibility of added fat in T_4. Because of poor digestibility of all organic nutrients, the ration T_3 had lower ($p<0.01$) TDN content. Lower DM intake ($p<0.05$) further contributed to low TDN intake in rams fed ration T_3.

Calcium excretion (g/d) through faeces was higher than the intake in rams fed ration T_3 leading to a negative balance. Tillman and Breathour (1958) also observed decrease in apparent and true digestibility as well as decrease in calcium retention when sheep were fed 7.5% supplemental maize oil. It might be due to precipitation of calcium as insoluble soaps in GI tract and subsequent excretion through faeces. Increased calcium intake through calcium soap resulted in higher retention of calcium in rams fed ration T_4.

Phosphorus retention expressed as percent of intake ($p<0.01$) or percent of absorbed ($p<0.05$) was lower in rams fed ration T_3 than those fed rations T_1, T_2 or T_4. These findings are in agreement with the observations of Tillman and Brethour (1958) who added corn oil to the diet. However, Palmquist (1991) observed decreased digestibilities of phosphorus with the higher levels of fat in the ration irrespective of source of fat (fatty acid or calcium soap).

CONCLUSION

Supplementation of sunflower acid oil at 5% of dietary dry matter to sheep receiving 60% Brazilian napier hay and 40% concentrate as a basal diet decreased fibre digestibility. Severe depression in fibre digestion and negative calcium balance were observed as the level of sunflower acid oil increased to 10% of dietary dry matter. The interaction between sunflower acid oil, fibre digestion and calcium retention shows that it is prudent to use basal diet rich in calcium when higher levels of unprotected fat supplementation is warranted. The fibre digestion was not influenced by supplementation of calcium soap prepared from sunflower acid oil at 10% of dietary dry matter. It

indicates that calcium soap prepared from unsaturated oil can be used at higher levels without any adverse effect on fibre digestion. When roughage part constitutes 60% of basal diet. Further. calcium soap supplementation enhanced ether extract digestion. total digestible nutrients content of the ration and calcium retention.

It is concluded that calcium soap prepared from sunflower acid oil can be fed to sheep at 10% of dietary dry matter as a source of energy without affecting fibre digestibility. Supplementation of sunflower acid oil as such even at 5% of dietary dry matter to sheep as an energy supplement did not appear beneficial.

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