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Evolving Suitable Method of Protection to Protect Sunflower Acid Oil Against Rumen Degradation

C. Sugumar and V. Balakrishnan
Department of Animal Nutrition, Madras Veterinary College,
Vepery, Chennai, Tamil Nadu, 600 007, India

Abstract: The sunflower acid oil was subjected to three methods of protection viz., Aldehyde treated protein encapsulated form, calcium soap and fatty acyl amides. The extent of rumen lipolysis was assessed by comparing the intensity and surface area of the free fatty acids zone on the chromatographic field between 24 h incubated and unincubated samples. The data originated from six measurements suggest that the calcium soaps offer best protection, as even after 24 h of incubation, the samples had the most desirable faint intensity and lower surface area of free fatty acids zone, which indicate that the calcium soaps undergo lowest metabolism in the rumen than its counterparts. Based on the intensity and surface area of the free fatty acids zone on the chromatographic field, calcium soaps of sunflower acid oil was selected as the potential protected fat in the rations of dairy cows.

Key words: Sunflower acid oil, rumen lipolysis, protected fat, calcium soap

INTRODUCTION

Dairy cows as a class of ruminants have evolved an extremely efficient digestive system, principally for utilizing poor quality forages found in natural diet. Although moderate levels of performance can be achieved from diets containing only forage, high producing dairy cows require additional energy and protein from supplementary sources. This is particularly true during early lactation, where appetite is usually reduced to such an extent that the cow cannot eat enough to satisfy nutrient requirements for high milk production.

Traditional sources of supplementing energy, high in starch can be rapidly fermented in the rumen leading to lowered pH (Jouany, 2006) which is detrimental to forage digesting bacteria. Fats can be used to formulate diets with very high-energy concentration, as they have the advantage of creating space within the diet, due to high energy content. In order to increase the energy density of concentrates and meet the fat requirement of crossbred and high producing animals, a non-conventional, cost effective and non-toxic source of fat is essential.

During refining of edible oils, free fatty acids are removed by treating them with sodium hydroxide and sulphuric acid. The free fatty acids thus removed by centrifugation along with variable amount of triglycerides and other minor fat-soluble constituents are termed acid oils. These are available at a cheaper price. These acid oils posses the potential to be used as a lipid source in ruminant rations. Though acid oils from various sources are available sunflower acid oil was chosen for this study as it is abundantly available in the study area (India).

However, feeding of free or unprotected fats beyond 3-4% level leads to reduction in microbial activity resulting in depression of fibre digestion in ruminants (Alexander *et al*., 2002). Hence, protection of fats is an alternative way of feeding fat to these animals. Therefore, techniques that would allow the use of fat in the form that is not harmful to microbes or retard digestion (Ramana Reddy *et al*., 2003) is gaining much importance.

Corresponding Author: Dr. V. Balakrishnan, Department of Animal Nutrition, Madras Veterinary Collage, Vepery, Chennai, Tamil Nadu, 600 007, India Tel: +91 44 25381506 Fax: 009144-25818836

While calcium salts was preferred to protect rapeseed oil (Kowalski, 1997), canolamide was found to be suitable for canola oil (Loor *et al*., 2002), Sutton *et al*. (1983) concluded protein encapsulated formaldehyde treatment was suitable to linseed oil and coconut oil. Keeping this concept in view, a study was conducted to chemically characterize the sunflower acid oil as well as to identify the suitable method of protection of acid oil against rumen degradation.

MATERIALS AND METHODS

Procurement of Samples

Sunflower acid oil samples were collected in airtight container containing 0.05% of butylated hydroxy anisole, from six different batches of processing from an oil mill and stored in cool and dark place.

Chemical Characterization of Sunflower Acid Oil

The samples were analyzed for their chemical composition viz., moisture, crude protein and total ash as per the method of AOAC (1995). The remaining part (i.e.,) 100- (Moisture+crude protein+total ash) was assumed as ether extract. The samples were also analyzed for their chemical characteristics viz., acid value, saponification number, unsaponifiable matters, peroxide value and free fatty acids content was carried out by adopting the method of AOAC (1995).

Preparation of Protected Forms of Sunflower Acid Oil

The sunflower acid oil was subjected to three methods of protection viz., Aldehyde treated protein encapsulated form was prepared by the method described by Sutton *et al*. (1983), calcium soaps was prepared by precipitation method as described by Alexander *et al*. (2002) and fatty acyl amides of sunflower acid oil were synthesized by mixing 100 g of sunflower acid oil and 100 g of ethanolamine as described by Loor *et al*. (2002). The final amide products were examined in thin layer chromatography as per Bilyk *et al*. (1991) to confirm the absence of triglycerides and free fatty acids, which indicated that the conversion of sunflower acid oil fatty acids to amide formation was complete.

Evaluation of Protection Against Rumen Degradation of Sunflower Acid Oil by Thin Layer Chromatography

The three protected forms of sunflower acid oil (100 mg) and un protected sunflower acid oil (100 mg) was evaluated by incubating (39°C) in rumen fluid (10 mL) for 24 h and evaluating the extent of rumen lipolysis (24 h) by measuring the free fatty acids production using thin layer chromatography as per the procedure described by Gulati *et al*. (1997). Un incubated samples (0 h) served as control to compare the extent of rumen lipolysis by 24 h of incubation.

At the end of 24 h 0.5 mL of 5 N HCl and 4 mL of distilled water were added to both unincubated and incubated tubes, shaken vigorously and allowed to stand for 2-4 h until the two phases were clearly distinguished. The lower organic phase was filtered to remove suspended solids. The filtrate was evaporated to dryness by keeping it in the oven at 60°C for 24 h. After drying, 2 mL of chloroform/methanol (2:1 v/v) was added. Accurately 20 μL of the mixture was spotted on silica gel F_{254} plate (aluminum sheet thickness 0.2 mm×10×20 cm) and placed in the solvent system made up of petroleum ether: diethyl ether: acetic acid (84/15/1 v/v/v). The separated lipids were visualized by keeping it in an iodine chamber.

The relative intensity and sizes of the free fatty acids spots in both the incubated and unincubated reaction mixtures were estimated semi-qualitatively by following a hundred point scale score for measuring the intensity of the free fatty acids spots and measuring the surface area of the spot to

determine the size of the free fatty acid zone. While one hundred points were assigned to the free fatty acids spot of the unincubated zero and unprotected fat, its relative points were assigned to other spots depending upon their intensity measured visually. The surface areas of the free fatty acids spots were calculated by formula:

$$\text{Surface area} = 3.14 \times a \times b$$

Where,

a = Longest radius,
b = Smallest radius.

Duplicate measurements for score and surface areas were carried out by two persons for six runs. The data obtained in different parameters were subjected to Complete Randomized Design (CRD) of statistical analysis as per the procedure of Snedecor and Cochran (1994).

RESULTS

Sunflower acid oil contained low level of moisture, crude protein and total ash. Sunflower acid oil has moderate level of acid value and free fatty acids necessitating use of anti-oxidant. A high saponification number of sunflower acid oil indicates its potential for converting into soaps. The peroxide value and unsaponifiable matters were at moderate level in sunflower acid oil (Table 1).

Protected forms of fat prepared from sunflower acid oil were evaluated by thin layer chromatography to find out the efficiency of protected fat. The relative intensity and size of free fatty acid zone present on the chromatographic field was used as the criteria of evaluation, as intensity and size of free fatty acid zone are inversely related to level of protection. The lower intensity and smaller size of the free fatty acid zone were considered to be better level of protection (Gulati *et al*., 1997).

Within the treatments of unprotected, aldehyde treated, calcium soaps and amide, the score for intensity as well as surface area at 24 h incubated samples were consistently higher than their respective 0 h samples (Table 2).

Table 1: Average chemical composition* of sunflower acid oil (% FMB) and important quality parameters (Mean±SE)

Components	Mean±SE
Moisture	0.74±0.05
Crude protein	0.28±0.02
Total ash	0.21±0.01
Ether extract	98.77±0.07
Acid value (mg KOH g^{-1})	94.18±1.34
Saponification number	186.32±1.31
Peroxide value (meq.oxygen kg^{-1})	8.74±0.36
Unsaponifiable matters (%)	8.63±0.21
Free fatty acid (as oleic acid)	52.61±0.75

*: Mean of six observations, FMB: Fresh Matter Basis

Table 2: Score for intensity and relative surface area of free fatty acids zone (Mean±SE*) measured from thin layer chromatography plate for various forms of protected and unprotected sunflower acid oil at 0 or 24 h of incubation

Parameters	Unprotected sunflower acid oil	Adehyde treated protein encapsulated oil	Calcium soaps of sunflower acid oil	Fatty acyl amides of sunflower acid oil
Intensity of the zone-0 h	100.00±5.30^{d}	85.00±3.65^{c}	7.50±2.81^{a}	45.00±8.06^{b}
Intensity of the zone-24 h	125.82±6.64^{c}	105.00±3.65^{bc}	35.00±7.42^{a}	85.00±2.58^{b}
Surface area (cm^2) of the zone-0 h	1.13±0.10^{c}	0.56±0.06^{b}	0.04±0.01^{a}	0.94±0.09^{c}
Surface area (cm^2) of the zone-24 h	2.92±0.18^{c}	1.57±0.11^{b}	0.71±0.08^{a}	1.41±0.13^{b}

*: Mean of six observations, Mean with different superscripts within a row differ significantly ($p<0.01$)

Across the treatments, the score for the intensity of free fatty acid zone of calcium soaps at 0 h was the best and significantly ($p<0.01$) different from rest. Similarly among 24 h of incubation, the intensity of free fatty acid zone of calcium soaps was the significantly ($p<0.01$) lower than the rest treatments. Fatty acyl amides of sunflower acid oil showed next significantly ($p<0.01$) better performance at 0 as well as at 24 h of incubation. Unprotected sunflower acid oil had the lowest values ($p<0.01$).

Similar to the intensity of zone, measurement of surface area of the free fatty acid zones reveals that calcium soaps at 0 h of incubation as well as at 24 h of incubation had significantly ($p<0.01$) lowest surface area than rest treatments (Table 2).

DISCUSSION

The results of this study are comparable to the reported values for constituents in sunflower acid oil (Alexander *et al*., 2002). The moisture level below 2.5% is desirable as higher moisture content permits the formation of rust, which will accelerate autocatalytic (non enzymatic) oxidative rancidity. Moisture in the presence of high levels of free fatty acids and high temperature will also promote autocatalytic hydrolysis of glycerides (Gunstone *et al*., 1994). Thus, with low level of moisture (0.74%), sunflower acid oil do not pose threat for oxidative rancidity.

The insoluble impurities like hair, fleshing grease, phospholipids, trace metals and protein as contaminants or adulterants may be associated with many blend compounds. Insoluble impurities cause not only the fall in the energy value of oil/fat, but also leads to blockage in feed mill equipments like fat spray nozzles (Howard, 1984). The negligible quantity of crude protein and total ash in the sunflower acid oil indicate that the study material is unadulterated and free from contaminants.

The observations of the present investigation were comparable to the reported acid value, Saponification number (Alexander *et al*., 2002). The higher observed saponification number might be due to the presence of higher proportions of short chain fatty acids in acid oils which might be the consequence of degradation of polyunsaturated fatty acids during the refining process of crude oil by heating acid oxidation (Wiseman *et al*., 1992). The mean peroxide value of 8.74 was within the reported range of 1.61 to 10.11 (meq oxygen kg^{-1}) for sunflower acid oil (Vila and Esteve-Garcia, 1996b). Peroxide value is the measure of peroxides contained in the oil. The degree of oxidation resulting from processing has often been assessed in terms of peroxide value. Vila and Esteve-Garcia (1996b) have reported that inclusion of sunflower acid oil having peroxide values up to 10.11 meq.oxygen kg^{-1} did not cause any deleterious effect when added in the poultry ration at 10% level. The sunflower acid oil values obtained in the current study were below this level and hence, could be considered to be safe. More over the sunflower acid oil is mixed with antioxidants upon arrival to the laboratory to prevent further oxidation.

Unsaponifiable matters refer to the material, which is soluble in petroleum ether but does not react with sodium or potassium hydroxide to form soaps. This includes wide variety of compounds such as sterols, pigments, fat-soluble vitamins, fatty alcohols, fatty-fatty esters (condensation products), waxes, mineral oils, pesticides etc. Unsaponifiable matters contribute very little to the energy value of feed fat. The unsaponifiable matters estimated in the current study were similar to the value reported by Wiseman *et al*. (1992).

The mean Free Fatty Acid (FFA as oleic acid) level of sunflower acid oil recorded in the present study were lower than the value (58.22%) reported by Alexander *et al*. (2002) and (57.885%) Vila and Esteve-Garcia, (1996a). However, lower values (38.80%) were reported by Wiseman *et al*. (1992). The variation in FFA content might be due to the difference in the method of processing and refining of

crude oils. Free fatty acids refer to the fatty acids not esterified to glycerol. In whole fats, presence of high levels of free fatty acids may be an indication of improper storage and/or handling of fat. Hydrolysis may occur as either enzymatic lipolysis during storage or prior to rendering or as autocatalysing hydrolysis. The latter is often associated with oxidative rancidity. Hence, addition of anti-oxidants is recommended to all feed fats to prevent rancidity particularly in the presence of high levels of free fatty acids.

Consistently higher score recorded for 24 h incubated samples than their respective 0 h samples might be due to the rumen metabolism and relative dissociation of samples in the rumen fluid.

The data suggest that the calcium soaps offer best protection, as even after 24 h of incubation, the samples had the most desirable faint intensity and lower surface area of free fatty acids zone, which indicate that the calcium soaps undergo lowest metabolism in the rumen than its counterparts.

Based on the intensity and surface area of the free fatty acids zone on the chromatographic field, calcium soaps of sunflower acid oil was selected as the potential protected fat to be used as concentrated energy source in the rations of dairy cows.

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