IJAS Iranian Journal of Applied Animal Science

In vitro Evaluation of Oil Releasing Extent from a Calcium Salt of Fatty Acids in Different Sites of Gastrointestinal Tract

Research Article

P. Peravian[1*], H. Mirzaei-Alamouti[1], M. Dehghan-Banadaky[2], H. Amanlou[1], M. Vazirigohar[2], H. Khalilvandi[3] and P. Rezamand[4]

[1] Department of Animal Science, University of Zanjan, Zanjan, Iran
[2] Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[3] Department of Animal Science, University of Urmia, Urmia, Iran
[4] Department of Animal and Veterinary Science, University of Idaho, Moscow Idaho 83844-2330, USA

Received on: 2 Jul 2020
Revised on: 8 Oct 2020
Accepted on: 15 Nov 2020
Online Published on: Sep 2022

*Correspondence E-mail: pyn.prvn@yahoo.com

© 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran

Online version is available on: www.ijas.ir

ABSTRACT

Calcium salts (CS) of fatty acids (FAs) are involved as fat sources in dairy nutrition. In this supplement dissociation of FAs and calcium is affected by the nature of fatty acids (saturated or unsaturated), so this process led to the release oil in the whole gastrointestinal tract. This study was designed to evaluate the oil releasing extent (ORE) from CS of FAs in a simulated culture of the gastrointestinal tract. Rumen fluid was collected from fistulated cows 4 hours after feeding and transferred to the laboratory. A simulation of the digestion environment at three sites (rumen, abomasums, and small intestine) pH (6.4, 1.2, 6.8) was used to incubate the rumen fluid. Treatments are represented by 1) CS of fish oil (CFO), 2) CS of flaxseed oil (CFL), 3) CS of herbal n-6 and n-3 (CHO) and 4) CS of fish and herbal n-3 (CFH). The ORE in the rumen was affected by treatments, with CS of flaxseed having the greatest rate of release (P<0.01). Differences between treatments in the abomasum were nonsignificant (P=0.1), however, the greatest ORE was in this part in comparison to other sections of the tract. The treatments did affect ORE in the small intestine (P<0.01). According to our results, CS of FAs supplied less than 200 mg/g ORE in the rumen which is acceptable and could be used with minimum adverse effects on rumen function.

KEY WORDS calcium salts, dairy nutrition, fatty acids, fish oil, flaxseed oil.

INTRODUCTION

The reason for adding fat in dairy cattle rations is based on their caloric value, which increases dietary energy density, and therefore, milk production, feed efficiency, and milk fat (Rabiee *et al.* 2012) and non-caloric effects, which include interactions with the immune system, inflammation, and reproduction (Silvestre *et al.* 2008). Findings of fat studies vary drastically for several reasons: the type and amounts of FAs (saturated or unsaturated); provided reaching the small intestine; supplying FAs; particle size of FAs; associative effects of the proportion of forage and other dietary ingredients and animal factors including production level, stage of lactation and parity (Rabiee *et al.* 2012; Vazirigohar *et al.* 2014; de Souza *et al.* 2018). Unsaturated FAs are toxic to some rumen microbes, so the majority of dietary lipids undergo rumen biohydrogenation producing a series of FAs intermediates that ultimately result in the production of saturated FAs. The rate of biohydrogenation depends on two main factors which are the nature of FAs in the rumen (e.g., n-6 or n-3) and rumen pH (Loor *et al.* 2004). For instance, in the case of n-3, low rumen pH leads to a reduction in the last step of biohydrogenation, so t11 C18:1 (trans-vaccenic acid) may accumulate in the rumen (Loor *et*

al. 2004). Because of the adverse effect of unprotected FAs on rumen environment through altering the direct pathway of rumen biohydogenation and alteration of the FAs profile in the fore-stomach, as well as sensitivity to oxidation during storage of unprotected FAs, methods should be used to protect poly unsaturated fatty acids in the rumen (Chilliard *et al.* 2007). Protection methods include either encapsulation of unsaturated FAs inside a microbial-resistant shell (such as lipid encapsulation) or modification of the FAs structure through blocking carboxyl group (such as CS or fatty amides) to resist microbial enzymes (Dewhurst *et al.* 2013). According to the type FAs, type of protection led to challenging and various results on biohydrogenation (Carroll *et al.* 2006). In some studies, CS of FAs had minimum protection for herbal n-3 FAs and therefore change the rate of oil release and consequently, rumen biohydrogenation (Baldin *et al.* 2018) or sodium hydroxide, formic acid or ammonium tetraformate reduced the rate and extent of biohydrogenation in compare of formaldehyde protection (Sinclair *et al.* 2005). Because of the commercial availability of calcium salt research has used this form of protection in assessing the flow of FAs in the duodenum, (Fievez *et al.* 2007). Therefore in this experiment, we hypothesized that in calcium salt of fatty acids, the nature of the fatty acids affects the linkage between calcium and fatty acid, and this, therefore, affects the rate of oil released at different sites of gastrointestinal tract. The objective of this study was *in vitro* evaluation of the extent of oil released from different calcium salts of fatty acids in three simulated sites of the gastrointestinal tract.

MATERIALS AND METHODS

Preparation of the simulated gastrointestinal tract

Measuring oil releasing in different sites of the gastrointestinal tract was conducted according to Kosaraju *et al.* (2009). Treatments were 1) CS of fish oil (CFO), 2) CS of flaxseed oil (CFL), 3) CS of herbal n-6 (corn and canola oil) and herbal n-3 (flaxseed oil) (CHO) and 4) CS of fish and herbal n-3 (flaxseed oil) (CFH). Oil was placed into a sealed glass-lined vessel equipped with mixing blades and an argon blanket. After heating to 30 ˚C, an appropriate calcium source to provide molar proportions of calcium and fatty acids were added and thoroughly mixed. The vessel was then resealed and the mixture was stirred and heated until the saponification reaction has occurred. During the saponification reaction a light smoke was released and the material formed a yellow toffee-like material. It was then cooled inside the vessel to 25 ˚C under the argon blanket before emptying from the vessel and being processed into small granules of a dry, free-flowing calcium salt. The profile of FAs in various CS is shown in Table 1.

To measure ORE in rumen, rumen fluid was collected from two early lactations rumen-cannulated Holstein dairy cows (BW= 680±20 kg, 40% forage diet, ration formulated according to NRC, 2001 software) from both central and ventral sites of rumen for four hours after the morning feeding. To minimize the effect of day and time of incubation, sampling was performed on two consecutive days. The rumen fluid was filtered by cheese-cloth (0.5 mm), placed in anaerobic bottles, and incubated under constant CO_2 gas. The rumen simulative environment was prepared by shaking batch culture (39 ˚C with 1 rcf for 24 h) plus 6 bottles for mixing of CS of FAs and rumen fluid. Three bottles were considered as blanks for correction of ORE from other sources of rumen fluid such as free FAs which were in the diet. McDougall buffer was used for controlling pH of rumen fluid to 6.4. To each bottle 1.5 g CS of FAs and 50 ml rumen fluid were added with the constant flow of CO_2 and kept anaerobic for 24 h in a batch culture. After incubation, two separate phases were made in each bottle. To measure ORE, the chloroform phase named the low phase was extracted by Folch (chloroform/methanol (2/1)) method two times. After drying with N_2 gas in the oven (65 ˚C), differences in weight which is weight of samples before and after drying were calculated and adjusted in line with the blank.
The extent of oil release in the abomasum was measured by preparing pepsin (SIGMA life science) and adding 2 g sodium chloride and 3.2 g pepsin to 500 mL of HCL 370 cc/lit of distilled water. The pH was set to 1.2. Six bottles were used for mixing. Each bottle was given 1.5 g CS of FAs and 50 mL of solution and kept anaerobic for 3 h in a batch culture. After incubation, two phases were extracted from each bottle. To measure ORE in the abomasum the ether phase named the high phase was extracted by petroleum solution (1/1) twice. After drying with N_2 gas in the oven (65 ˚C), differences in weight which are weight of samples before and after drying were calculated and corrected to the blank.

The extent of oil release in the small intestine was measured by preparing Pancreatin (MERCK); 6.8 g KPO_4 diluted in 250 mL distilled water, 77 mL NaOH 0.2N and 500 mL distilled water and 10 g Pancreatin added to form a solution. After adding HCL and NaOH, the pH was 6.8. Six bottles were used. In each bottle 1.5 g CS of FAs and 50 mL of this solution were added and kept anaerobic for 3 h in a batch culture. Steps to measure fat extraction in the small intestine were similar to those used for the abomasum.

Statistical analysis

This experiment was analyzed as a complete randomized design with four treatments (different CS of FAs) and six replicates (bottles of calcium salt).

Table 1 Fatty acid profile of different calcium slats (g/kg DM)[a]

FA, g/kg DM	Treatments			
	CFO	CFL	CHO	CFH
C12:0	40.0	10.0	40.0	25.0
C14:0	10.0	10.0	10.0	10.0
C16:0	200.0	100.0	280.0	150.0
C16:1	50.0	0.0	30.0	25.0
C18:0	150.0	70.0	50.0	110.0
C18:1	250.0	210.0	260.0	230.0
C18:2	110.0	180.0	300.0	145.0
C18:3	50.0	420.0	30.0	235.0
C20:5	70.0	0.0	0.0	35.0
C22:6	70.0	0.0	0.0	35.0

[a] Profile of fatty acid in supplements measured by gas chromatography method.
CFO: calcium salt of fish oil; CFL: calcium salt of flaxseed oil; CHO: calcium salt of herbal n-6 (corn and canola oil) and herbal n-3 (flaxseed oil) and CFH: c alcium salt of fish and herbal n-3 (flaxseed oil).

Statistical analysis was performed using PROC GLM of SAS (2003). Data are reported as means ± standard error of means (SEM). Different days for sampling were not significant so this effect was removed from the statistical model, means differences were compared by Tukey test, and data were analyzed by the statistical model:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

Where:
Y_{ij}: rate of oil releasing.
T_i: effect of treatment.
e_{ijk}: random error.

RESULTS AND DISCUSSION

Mean ORE from the various CS of FAs in different sites of the gastrointestinal tract is shown both in Table 2 and Figure 1. As it was shown there were differences among fat sources in the rumen. The calcium salt of flax seed (CFL) had the highest extent of oil release compared with other treatments whereas the lowest extent was for calcium salt of herbal oil (CHO) (P<0.01). In the abomasum there were no differences among treatments (P=0.1). The greatest ORE was observed for the CHO and the lowest was for the CFO. In comparison with another site in gastrointestinal tract, the greatest extent of oil was released in the abomasum. Differences among treatments in the small intestine were significant; the greatest ORE was for the CFL and the lowest was for the CHO (P<0.01).

The nature of the FAs, the amount ingested, the rumen-protection method, and the rumen environment can all alter rumen biohydrogenation. Two types of n-3 included herbal sources (rich in α-linolenic acid) and marine sources [rich both in α-linolenic acid and long-chain FAs (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) probably influence the rates of rumen biohydrogenation.

Fish oil sharply decreased the rate of biohydrogenation through inhibition of the last step of biohydrogenation, which results in the high production of t11 C18:1 (Givens and Shingfield, 2006). In a study by Khalilvandi *et al.* (2013), rates of biohydrogenation and ORE were compared in FAs with different protection methods. The authors reported that n-3 of fish oil had the lowest rate of biohydrogenation and ORE in the rumen with all protection methods. In a recent study, among three sources of FAs (oleic acid (OA), linoleic acid (LA), α-linolenic acid (ALA)), ALA of linseed had the greatest biohydrogenation (947 g/kg) in comparison to OA (856 g/kg) and LA (898 g/kg) (Baldin *et al.* 2018). As observed in our study (Table 2 and Figure 1), the calcium salt of flaxseed had the highest release in the rumen compared with that of other treatments. Therefore, it could be speculated that CS of flaxseed seems to be more sensitive to biohydrogenation than the other sources tested. In another study, crude linseed oil (18:3 n-3) was compared with whole seed, and different protection methods including formaldehyde, formaldehyde + sodium hydroxide, formic acid, or ammonium tetra format + xylose regarded as fiber sources (Sinclair *et al.* 2005). In addition, crude fish oil was compared with fish oil encapsulated with saturated fat. The authors concluded that biohydrogenation of n-3 in linseed oil and whole linseed was rapid and extensive, while xylose or formaldehyde offered little protection. Sodium hydroxide, formic acid, or ammonium tetraformate reduced the rate and extent of biohydrogenation (Sinclair *et al.* 2005).

In our study, ORE in the rumen was affected by the nature of FAs. Probably in treatments 1 and 4 calcium salt was a suitable protection method in comparison with that flaxseed (CFL). Calcium salt of flaxseed did not seem to restrain ORE, so perhaps another protection method should be considered for this type of n-3 FAs.

Table 2 Oil releasing extent in different site of simulation gastrointestinal tract (GIT; in mg/g)

Site of GIT	Treatments				SEM	P-value
	CFO	CFL	CHO	CFH		
Rumen	156.4[b]	199.3[a]	111.1[c]	148.0[b]	5.3	< 0.01
Abomasum	870.2	930.9	948.4	944.0	21.8	0.10
Small intestine	68.4[b]	81.4[a]	51.7[c]	79.9[a]	2.8	< 0.01

CFO: calcium salt of fish oil; CFL: calcium salt of flaxseed oil; CHO: calcium salt of herbal n-6 (corn and canola oil) and herbal n-3 (flaxseed oil) and CFH: c alcium salt of fish and herbal n-3 (flaxseed oil).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

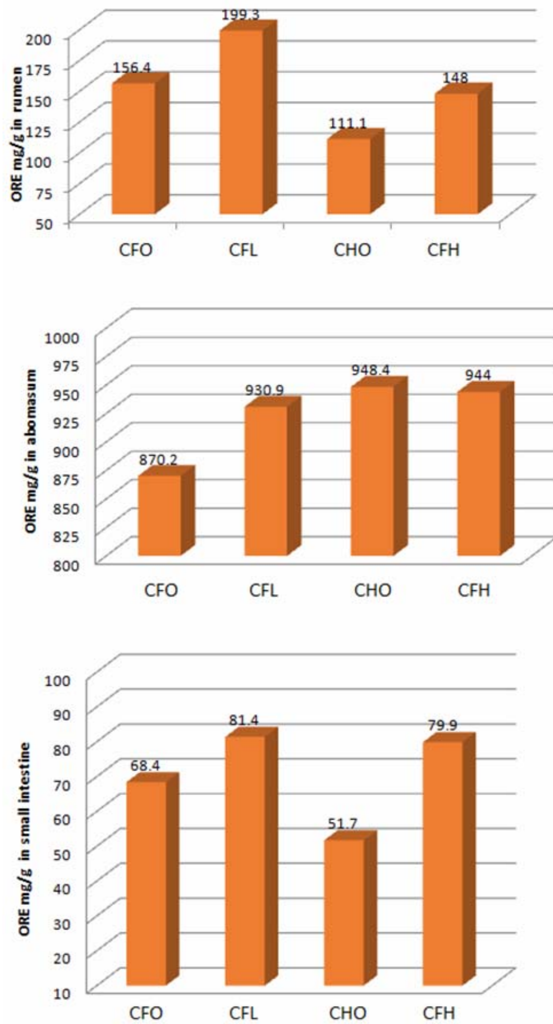

Figure 1 Oil releasing extent in different site of simulation gastrointestinal tract (GIT; in mg/g)

As was expected, low pH in the abomasum led to dissociation of the linkage between carboxyl group of FAs and calcium, resulting in the highest ORE being in the abomasum compared with the other sites of the gastrointestinal tract (Table 2 and Figure 1). In the small intestine, it is assumed that high pH and pancreatic enzyme activity reduce the rate of dissociation of the carboxyl group of FAs and calcium (Kosaraju *et al.* 2009), which could explain the lower rate of ORE at this site in our experiment.

CONCLUSION

Generally, protection of fatty acids with calcium could be an alternative and safe method against biohydrogenation in rumen so it may diminish the detrimental effect of fatty acids on microorganisms. Based on our results, the nature of the FAs in calcium salt besides the site of gastrointestinal tract could alter the extent of an oil release. Probably regarding form of protection (calcium salt), herbal n-3 fatty acid seems more sensitive to oil releasing extent in rumen than marine n-3. Due to the low pH in abomasums in comparison to other site of gastrointestinal tract, the greatest rate of oil is released in this site.

ACKNOWLEDGEMENT

This research was financially supported by the University of Zanjan and University of Tehran, Iran.

REFERENCES

Baldin M., Rico D.E., Green M.H. and Harvatine K.J. (2018). Technical note: An *in vivo* method to determine kinetics of unsaturated fatty acid biohydrogenation in the rumen *J. Dairy Sci*. **101,** 1-9.

Carroll S.M., DePeters E.J. and Rosenberg M. (2006). Efficacy of a novel whey protein gel complex to increase the unsaturated fatty acid composition of bovine milk fat. *J. Dairy Sci*. **89,** 640-651.

Chilliard Y., Glasser F., Ferlay A., Bernard L., Rouel J. and Doreau M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European J. Lipid Sci. Technol*. **109,** 828-855.

De Souza J., Preseault C.L. and Lock A.L. (2018). Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. *J. Dairy Sci*. **101,** 172-185.

Dewhurst R.J. and Moloney A.P. (2013). Modification of animal diets for the enrichment of dairy and meat products with omega-3 fatty acids. Pp. 257-287 in Food Enrichment with Omega-3 Fatty Acids. C. Jacobsen, N. Skall Nielsen, A. Frisenfeldt Horn and A.D. Moltke Sørensen, Eds. Woodhead Publishing Ltd, Cambridge, United Kingdom.

Fievez V., Vlaeminck B., Jenkins T., Enjalbert F. and Doreau M. (2007). Assessing rumen biohydrogenation and its manipula-

tion *in vivo*, *in vitro* and *in situ*. *European J. Lipid Sci. Technol*. **109,** 740-756.

Givens D.I. and Shingfield K.J. (2006). Optimizing dairy milk fatty acid composition. Pp. 252-280 in Improving the fat content of foods. C.M. Williams and J. Buttriss, Eds. Woodhead Publishing Ltd, Cambridge, United Kingdom.

Khalilvandi H., Dehghan-banadaki M. and Ghafarzadeh M. (2013). *In vitro* assessment of different protection methods of fish oil. *J. Rumin. Res*. **4,** 1-28.

Kosaraju S.L., Weerakkody R. and Augustin M.A. (2009). *In vitro* evaluation of hydrocolloid-based encapsulated fish oil. *Food Hydrocol*. **23,** 1413-1419.

Loor J., Ueda J., Ferlay K., Chilliard A.Y. and Doreau M. (2004). Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage: Concentrate ratio and linseed oil in dairy cows. *J. Dairy Sci*. **87,** 2472-2485.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC., USA.

Rabiee A.R., Breinhild K., Scott W., Golder H.M., Block E. and Lean I.J. (2012). Effect of fat additions to diets of dairy cattle on milk production and components: A meta-analysis and meta-regression. *J. Dairy Sci*. **95,** 3225-3247.

SAS Institute. (2003). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Silvestre F.T., Carvalho T.S.M., Crawford C., Santos J.E.P., Staples C.R. and Thatcher W.W. (2008). Effects of differential supplementation of calcium salts of fatty acids (CSFAs) to lactating dairy cows on plasma acute phase proteins and leukocyte responses: Phagocytic and oxidative burst, CD62L and CD18 expression and cytokine production. *J. Biol. Reprod*. **2008,** 1-158.

Sinclair L.A., Cooper S.L., Chikunya S., Wilkinson R.G., Hallett K.G., Enser M. and Wood J.D. (2005). Biohydrogenation of n-3 polyunsaturated fatty acids in the rumen and their effects on microbial metabolism and plasma fatty acid concentrations in sheep. *J. Anim Sci*. **81,** 239-248.

Vazirigohar M., Dehghan-Banadaki M., Rezayazdi K., Krizan S.J., Nejati-Javaremi A. and Shingfield K.J. (2014). Fat source and dietary forage to concentrate ratio influences milk fatty acid composition in lactating cows. *Animal*. **8,** 163-174.