Scientific Research Publishing

Open Journal of Animal Sciences, 2018, 8, 432-438
http://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Analytical Evaluation of Omega 3 Fatty Acids Imbedded in Hydrophobic Starch in the Rumen

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How to cite this paper: Ballard, M. (2018) Analytical Evaluation of Omega 3 Fatty Acids Imbedded in Hydrophobic Starch in the Rumen. *Open Journal of Animal Sciences*, **8**, 432-438.
https://doi.org/10.4236/ojas.2018.84032

Received: September 4, 2018
Accepted: October 20, 2018
Published: October 23, 2018

Abstract

Two experiments were conducted to assess the value of hydrophobic starch as a method to encapsulate a supplement consisting of refined fish oil intended for use as a feed supplement for ruminant animals. In Study 1, the product was incubated *in vitro* for 24 hours. The entire media was analyzed to determine fatty acid composition. In Study 2, the test material was incubated for 0, 2, 4, 6, 8, 10, 12 and 24 hours in order to determine rate of loss of dry matter, as well as the fatty acid profile of the dry matter remaining at 24 hours. Results from Study 1 indicated that 61.1 % of the eicosapentaenoic acid (C20:5) and 75.3% docosahexaenoic acid (C22:6) were still intact after the 24 hour incubation period. In Study 2, 39.1% of the test material was solubilized in the 24 hour period. However, the losses in C20:5 and C22:6 fatty acids were less (25.32% and 27.90% respectively) indicating that the majority of the test product was protected against biohydrogenation. It was concluded that hydrophobic starch can be used to ruminally protected fish oil and to deliver C20:5 and C22:6 fatty acids past the rumen.

Keywords

Encapsulation Omega-3, Rumen-Protection

1. Introduction

Linoleic acid (C18:2) the first member of the omega 6 family and linolenic acid (C18:3), the corresponding first member of the omega 3 family, are considered to be essential fatty acids [1] as these two essential fatty acids can be elongated and desaturated to form the highly unsaturated fatty acids (HUFA). Omega 3 and omega 6 HUFA are the precursors of vital lipid mediators; the eicosanoids which in turn give rise to other important signaling compounds such as prostaglandins and leukotrienes [2]. Oddly enough the omega 3 and omega 6 fatty

acids are elongated and desaturated using the same enzyme systems, yet have apposing biological effects [3]. Because of this it is possible to have insufficient amounts of HUFA from one family if the enzyme system is overwhelmed by fatty acids from the other, with this generally occurring with the omega 3 family of fatty acids [4] [5].

Marine oils are rich sources of omega 3 fatty acids, most notably eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22: 6). The dietary provision of marine oils insures the availability of C20:5 and C22:6 in the diet for non-ruminant animals. However with ruminants, these fatty acids undergo biohydrogenation in the rumen. Kairenius *et al.* [6] recovered numerous trans- and cis2 20 and 22 carbon length fatty acids from abomasally cannulated cows that had received a diet supplemented with fish oil. However, only 1.13% of the C20:5 and 0.87% of the C22:6 fatty acids appeared after the rumen. Similarly, Shingfield *et al.* [7] reported that 93.1% - 96.7% of the C20:5 and 91.6% - 95.8% of the C22:6 were biohydrogenated in the rumen of steers.

A number of factors may influence the extent of biohydrogenation of fatty acids in the rumen. First, the fat must undergo lipolysis. Lipolysis rates may decline as dietary lipid increases, particularly if the source is high in unsaturated fatty acids which tend to be more harmful to rumen bacteria [8]. Rumen pH can likewise have an effect on the extent of biohydrogenation [9]. Fierez *et al.* [10] also noted that there are differences from lab to lab with respect to donor animal diets, adaptation of donor animals, amount of inoculum used in culture media and atmospheric gas that can result in some across laboratory variability. However, even under such varying conditions, the extent of biohydrogenation remains high, with little of these important fatty acids available to the animal to incorporate in tissues.

Lanier and Corl [11] noted that, in spite of the need for a reliable source of supplemental HUFA, a practical vehicle to provide these fatty acids was not available. A number of rumen protected nutrients, such as amino acids, have been successfully developed, and these largely rely upon either a lipid matrix or a lipid coating. Lipid coatings are not logical choices for HUFA, as the HUFA would mix with the coating, reduce melting point of the final product, and severely reduce the effectiveness of the coating. While calcium salts of fatty acids generally reduce the biohydrogenation of fatty acids, they do not adequately protect C20:5 and C22:6 [12]. An alternative approach is to use a hydrophobic starch to protect the lipid from lipolysis and solubilization in the rumen. This research involved determining the extent of protection that can be provided by a product prepared from fish oil surrounded by a hydrophobic starch matrix.

2. Materials and Methods

The product evaluated in these investigations (Salmate, the Ballard Group, Cincinnati, OH, USA, **Table 1**) consisted of refined fish oils, vitamin E and hydrophobic starch. The product is made by flash freezing micro particles of the

Table 1. Guideline composition of the product tested.

Item	Value
Particle size, uM	<50
Lipid, %	45
Starch, %	55
C20:5 (EPA), % of fatty acids	7.3
C22:6 (DHA), % of fatty acids	10.9
Bulk density, g/ml	0.493

oil/vitamin E mixture, suspended in air fluidized bed spray drying technique, with the resulting particles less than 50 uM in diameter [13].

Because of the small particle size, rumen in sacco testing was not possible, as the particles were smaller than the pore openings in the bags. Therefore two independent laboratories with two different testing procedures were engaged to assess the integrity of the matrix with respect to rumen protection of HUFA.

2.1. Study 1

This experiment was conducted at the Atlantic Dairy and Forage Institute, New Brunswick, Canada. Rumen fluid was obtained from 2 non-lactating cows, filtered through 3 layers of cheese cloth and flushed with carbon dioxide gas. Six 250 Erlenmeyer flasks/treatment, containing 30 ml of strained rumen fluid and 30 ml of McDougall's buffer solution [14] were adjusted to pH 7.0 and maintained at 37 degrees in a shaking water bath. Treatments were 250 mg wheat starch/flask (control) and 250 mg/flask of the test product. Flasks were stoppered and a one-way gas release valve was attached.

After 24 h of incubation, two 10 ml aliquots of fluid were pipetted from each flask while samples were being stirred on a magnetic stirrer under a stream of carbon dioxide gas. These subsamples were freeze-dried, methylated, and fatty acid methyl esters were extracted with hexane [15]. Gas chromatography was used to determine fatty acid profiles. The fatty acid C13:0 was added as an internal standard.

2.2. Study 2

This experiment was conducted at Sana Dam Pars Research and Development, Tehran, Iran. Rumen fluid was collected from two rumen cannulated sheep maintained on an all forage diet. Fresh rumen fluid was homogenized in a blender for 1 minute under carbon dioxide, then filtered through 4 layers of cheesecloth. The rumen fluid filtrate was added to incubation bottles containing the test product, flushed with carbon dioxide gas and placed in a water bath at 37 degrees. Flasks (two per time period) were removed at 0, 2, 4, 6, 8, 12 and 24 h, and dry matter recovery was determined. Rate of loss of dry matter was determined using the procedure of Orskov and McDonald [16]. Fatty acid composi-

tion of the original material and the fatty acid composition of samples incubated for 24 hours were determined using the same methods as outlined in Study 1.

3. Results

3.1. Study 1

This trial provided information on the extent of biohydrogenation of the test product after incubation for a period of 24 h. Control samples, containing starch were evaluated to determine if any omega 3 fatty acids were generated by the rumen microbes in the absence of the fish oil additive.

The fatty acid profiles of the original sample, as well as the samples containing starch alone or the Salmate product are provided in Table 2. Results indicate that no omega 3 fatty acids were produced in the rumen fluid used in the study, and the only source was therefore of test sample origin. These results showed that 61% and 75% of the C20:5 and C22:6 fatty acids remained protected after 24 hours.

3.2. Study 2

This trial measured the rate of loss of dry matter from the test product (Table 3) during incubation. The results showed that at least 62.1% of the total sample would be expected to leave the rumen intact. The fatty acid composition of the sample before and after incubation is provided in Table 4. Although this calculation underestimates the test material that escapes the rumen by not including any of the "b" fraction from Table 2 that may escape the rumen, it still suggests that a substantial portion of the unsaturated fatty acids were protected by the coating material. In particular, the omega 3 fatty acids (C18:3, C20:5 and C22:6) largely remained in the fractioned destined to escape fermentation.

Table 2. Fatty acid composition (% of total fatty acids) of the media, test material, and samples of wheat starch and test product after 24 h of incubation *in vitro*.

Fatty Acid, %	Pre-incubation	Post Incubation		Remaining, %[1]
	Test	Control	Test	
C14:0	6.39	2.13	5.41	84.67
C15:0	0.43	1.85	0.43	100.65
C16:0	16.68	24.75	18.99	113.88
C16:1	7.75	0.00	6.74	87.03
C18:0	3.64	46.27	4.93	135.26
C18:1	23.47	0.00	31.21	132.94
C18:2	5.18	2.48	3.03	58.47
C18:3	0.82	0.00	0.96	117.22
C20:5	7.31	0.00	4.47	61.12
C22:6	10.90	0.00	8.23	75.31

[1]Postincubation test/pre-incubation test × 100.

Table 3. Rates of disappearance of test material *in vitro*[1].

Parameter	Value
a, %	12.0
b, %	37.9
c, %	62.1
r, portion/hour	0.073

[1]"a" is initial solubility at zero time, "b" the quantity that will potentially disappear at rate r/hour and "c" is the fraction of non-soluble dry matter will escape the rumen [16].

Table 4. Rates of disappearance of test material *in vitro*[1].

Fatty Acid, %	Initial	Final	Corrected[a]	Remaining, %[2]
C14:0	4.70	4.32	2.68	57.08
C16:0	18.34	16.32	10.13	55.26
C16:1	5.50	4.20	2.61	47.42
C18:0	5.21	6.68	4.15	79.62
C18:1	16.73	17.05	10.59	63.29
C18:2	17.30	18.33	11.38	65.80
C18:3	3.95	4.60	2.86	72.32
C20:5 (EPA)	7.90	9.50	5.90	74.68
C22:6 (DHA)	11.80	13.70	8.51	72.10

[1]Corrected for recovered dry matter (final fatty acids × 62.1/100); [2]Corrected fatty acids/initial fatty acids × 100.

4. Discussion

When fish oils are provided to ruminant animals, the 20:5 and 22:6 fatty acids have been demonstrated to be extensively biohydrogenated and therefore unavailable for metabolism by the ruminant animal. Shingfield *et al.* [17] determined that only 0.9 and 0.6 g of C20:5 and C22:6 were recovered at the duodenum in cows consuming 43.5 and 27.7 g of these two fatty acids. Similarly, *in vitro* studies have shown that C22:6 is completely hydrogenated [18], but can become toxic to rumen microbes at high concentrations, resulting in reduced lipolysis [18]. This is consistent with the findings of Dohme *et al.* [19].

Two differing approaches were taken in these experiments to determine the extent to which unsaturated fatty acids, and in particular the important omega 3 fatty acids are biohydrogenated. In the Study 1, aliquots of the total incubation media were analyzed. This method quantified the total fatty acids present. Saturated fatty acids increased with biohydrogenation of corresponding unsaturated fatty acids. However, unsaturated fatty acids were not fully biohydrogenated. In Study 2, only the solid residue remaining after incubation was analyzed. Any fatty acids in solution were not taken into account, and the results would be more likely to underestimate total fatty acids remaining. However, the results

also showed that protection was afforded by the hydrophobic starch matrix, and unsaturated fatty acids would be expected to leave the rumen intact.

5. Conclusion

Although different approaches were taken by the two independent laboratories, the results of these two experiments indicate that between 60% - 75% of the C20:5 and 70% to 75% of the C22:6 fatty acids in the test product would be expected to escape fermentation, and that the test product can reliably be used to supply omega 3 fatty acids to ruminants.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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