FINAL REPORT
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DEVELOPMENT OF A TALLOW SUPPLEMENT FOR CATTLE RATIONS AS A DELIVERY VEHICLE FOR BIOACTIVE LIPIDS

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Lay Overview of Project and Goals:
Animal fats, such as tallow, are an important byproduct of the rendering industry and obtaining maximum value from their disposal is a challenge to the industry. Tallow continues to be a feed ingredient for dairy cattle diets, which remains a major avenue for obtaining income from the disposal of this fat byproduct. However, the value of tallow as a livestock feed ingredient could be enhanced considerably by application of novel materials processing technology. One of these potential applications is combining tallow fatty acids with other nutraceuticals for enhanced post-ruminal delivery of required nutrients, such as omega fatty acids. The intent of this project is to convert tallow from a simple high energy source to a rumen-protected fat source with improved handling qualities, enhanced nutritional value, and higher economic value. The approach of this project is to combine tallow with varying concentrations of omega fatty acids and convert the mixture to calcium salts. The hypothesis is that calcium salts of tallow containing the added fatty acids will provide protection from ruminal biohydrogenation beyond that observed for calcium salts of the pure omega fatty acids.

Objective (s):

a) Increase the amount of tallow that can be utilized in rations of dairy cattle by developing a value-added tallow calcium salt product that is capable of protecting essential nutrients from ruminal degradation.
b) Improve the handling characteristics of tallow by their conversion to calcium salts.
c) Determine the optimum combination of tallow and fish oil needed to provide maximum protection of omega-3 fatty acids (DHA and EPA) from microbial destruction in batch in vitro cultures and continuous cultures.

Progress Overview:

Introduction

Fish oil contains a high amount of unsaturated, bioactive omega-3 fatty acids including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Supplementation of DHA has been associated with physiological benefits in many species. In dairy cows, DHA supplementation has been shown to alter the fatty acid composition of some parts of the reproductive system and PGF2a secretions, which may, in turn, improve reproductive efficiencies. To increase DHA intake, DHA can be included in dairy rations either as a

component of fish meal or as a fat supplement. When DHA is injected into the duodenum more DHA is transferred to milk than when DHA is injected into the rumen, indicating that DHA is hydrogenated in the rumen . Feeding DHA can modify the ruminal environment and decrease dry matter intake, milk production, and milk fat production as well as alter fatty acid profiles.

Some have reported in vitro rates of DHA biohydrogenation. They reported that lipolysis and biohydrogenation both occurred in ruminal batch cultures, but that increasing levels of fish oil decreased the percent of both lipolysis and biohydrogenation at 24h. Lipolysis rates fell from 83% to 58% and biohydrogenation rates decreased from >90% to <30% as fish oil increased from 12.5mg to 125mg per culture. Others also found that DHA disappeared from ruminal batch cultures inoculated with Holstein rumen fluid. They reported the percentage of DHA that disappeared from cultures decreased from 60% to 7% as DHA level in cultures increased from 1% to 4% of the diet. Both of these studies indicate that high levels of DHA inhibit the conversion of DHA into less toxic compounds.

Several in vivo studies have also examined biohydrogenation of DHA. When fish oil was fed to lambs, diet concentrations reached 1.9g/kg DM DHA and 3.9g/kg DM EPA. They calculated biohydrogenation from duodenal flow of fatty acids and found 72% of DHA and 78% of EPA were hydrogenated. In dairy cows, DHA levels in milk decreased from control (0.06%) when fish oil was supplemented at 160g/d (0.17%) but increased when fish oil was supplemented at 320g/d (0.43%). The varied rates of biohydrogenation rates of DHA that have been reported (0 to >90%) indicate that level of DHA supplementation and a variety of dietary and external factors may be involved in regulating biohydrogenation of DHA.

MATERIALS AND METHODS

Experiment 1

The following treatments were examined in batch in vitro cultures.

1. Tallow (control)
2. Tallow with 5% added safflower after initial reaction so there is free oil in the product
3. Tallow with 10% added safflower after initial reaction so there is free oil in the product
4. Tallow+0.5% fish oil
5. Tallow+1.0% fish oil
6. Tallow+0.5% fish oil with 5% safflower after initial reaction so there is free oil in the product
7. Tallow+0.5% fish oil with 10% safflower after initial reaction so there is free oil in the product
8. Tallow+1.0% fish oil with 5% safflower after initial reaction so there is free oil in the product
9. Tallow+1.0% fish oil with 10% safflower after initial reaction so there is free oil in the product

The rumen in vitro design was adapted from Sayre and Van Soest (1972) and AbuGhazaleh and Jenkins (2004b). For each set of batch cultures, a novel collection of rumen fluid was taken from one of two lactating, ruminally-fistulated Holstein cows being fed 36.5 kg/d of TMR (56.5% corn silage, 3.7% alfalfa hay, 3.7% rye grass and oat haylage (ensiled hay) mix, and 36% grain mix by weight). The rumen fluid was strained through two layers of cheesecloth, and then transported to the lab in a sealed and insulated container. At 0 h, 8 ml of media, 0.4 ml reducing solution, and 2 ml of rumen fluid were added to screw-capped culture tubes [15cm x

2cm (experiment 1) or 15cm x 2.5cm (experiment2)] containing 100 mg of substrate. Acids are produced by ruminal microorganisms and accumulate in batch cultures over time, which can interfere with microbial activities including decreased biohydrogenation (Martin and Jenkins, 2002). In this study, the media used was buffered with 1g/L ammonium bicarbonate and 8.75g/L sodium bicarbonate in an effort to maintain the pH and prevent any negative effects of low pH, including preventing a decrease in *trans*-18:1 production AbuGhazaleh and Jenkins (2004b). The base substrate was composed of alfalfa pellets (50%), ground corn (48%), soybean meal (22%), soybean hulls (24%), dicalcium phosphate (2.7%), trace mineral salt (1.2%), and sodium bicarbonate (1.6%), all ground through a 0.5 mm screen. The 0 h cultures were acidified with 6N HCl immediately prior to the addition of ruminal microorganisms, and then stored at -15°C to prevent biohydrogenation. The remaining cultures were flushed with CO_2, capped, and incubated at 39°C. At 6, 24, and 48 h sampling times, the appropriate cultures were acidified with 6N HCl and stored at -15°C.

Experiment 2

Whole ruminal contents were collected from a ruminally-fistulated Holstein cow two hours after being fed a 50% forage/50% concentrate diet. All surgical and animal care protocols were approved by the Clemson University Animal Care and Use Committee. Within 20 minutes of collection, large particles were removed from the whole ruminal contents by filtration through two-layers of cheesecloth, and the filtrate containing the microbial population was transferred immediately to the laboratory in a sealed container. With constant stirring, the filtered ruminal inoculum (approximately 750 mL) was then added to a dual-flow continuous culture fermenter that was modified in construction and operation from the design described by Teather and Sauer (6). The main modifications were a reconfigured overflow sidearm that angled downward at approximately 45º to facilitate emptying, a faster stirring rate (45 rpm) that still allowed stratification of particles into an upper mat, a middle liquid layer of small feed particles, and a lower layer of dense particles, and a higher feeding rate (60 g/d). The culture was maintained for ten days (seven days for adaptation and the last three days for sampling).

A buffer solution (7) was delivered continuously using a peristaltic pump to achieve a 0.10/h fractional dilution rate. Buffer pH was titrated each day with sufficient 6N NaOH or 3N HCl to maintain a pH of 6.5. The fermenter was continuously infused with CO_2 at a rate of 20 mL/min to maintain anaerobic conditions. The temperature of the fermenter was held at 39ºC by a circulating water bath. Culture samples were taken for volatile fatty acid (VFA) analysis on the last day at 0 (before the 0800 feeding), and at 2 and 4 h after feeding.

After freeze-drying, sodium methoxide and methanolic HCl were added to the batch culture tubes for direct tranesterification of fatty acids to methyl esters (9). An internal standard (2 mg heptadecanoic acid) also was added at the start of methylation to quantify fatty acid masses. Quantities of individual fatty acids present in the cultures were determined on a Hewlett-Packard 5890A gas chromatograph equipped with P-2380 fused silica capillary column (100 m × 0.25 mm) with 0.2 μm film thickness (Supelco Inc., PA). The conditions used were initially 140 ºC for 3 min with a ramp of 3.7 ºC per min up to 220 ºC holding for 20 min. Helium was used as the carrier gas at 20 cm/s.

RESULTS

Experiment 1

1	2	3	4	5	6	7	8	9	10
Control	Tallow 1%FO 10%SFO	Tallow	Tallow 5%SFO	Tallow 10%SFO	Tallow .5% FO	Tallow 1%FO 5%SFO	Tallow .5%FO 10%SFO	Tallow .5%FO 5%SFO	Tallow 1%FO
Fatty acids, mg 0h									
0.93	1.56	1.35	1.2	1.53	1.03	1.19	1.28	1.32	0.98
1.74	2.2	2	1.92	2.19	1.73	1.86	1.97	1.99	1.76
0.12	0.15	0.16	0.1	0.17	0.12	4.33	0.14	0.17	0.13
0.43	1.48	1.15	0.89	1.39	0.66	0.7	0.97	1.04	0.52
0.53	0.19	0.82	2.74	0.81	0.52	0.52	0.61	0.79	0.56
0.11	0.07	0.26	0.39	0.1	0.1	0.11	0.41	0.12	0.11
0.046	0.043	0.06	0.066	0.033	0.04	0.045	0.056	0.059	0.042
Fatty acids, mg 48h									
1.04	0.34	0.23	0.19	0.13	0.03	0.35	0.32	0.38	0.22
2.67	3.02	2.67	2.65	2.49	2.39	3	3.03	3.03	2.83
0.22	0.34	0.11	0.18	0.22	0.24	0.27	0.26	0.28	0.26
0.14	0.42	0.25	0.3	0.31	0.28	0.36	0.36	0.47	0.33
0.06	0.09	0.08	0.09	0.07	0.07	0.09	0.11	0.14	0.11
0.027	0.029	0.025	0.033	0.024	0.029	0.013	0.03	0.03	0.028
nd	nd	0.023	0.033	0.011	0.036	0.142	0.116	0.117	0.107

Statisical comparisons of treatments (* denotes differences at P < 0.05)

1 vs 2	2 vs 3,4	3 vs 4	2 vs 5,6	5 vs 6	2 vs 7,8	7 vs 8	2 vs 9,10	9 vs 10
*				*			*	
*				*			*	
*							*	
			*					
*	*		*		*		*	
*								

Experiment 2

	CON		TAL		TAL-FO		
	5.5	6.5	5.5	6.5	5.5	6.5	SEM
18:0[b]	66.1	246.9	117.6	276.7	88.9	202.9	26.0
C18:1[ab]	161.7	193.4	296.2	376.9	240.7	336.7	26.2
18:2[b]	192.6	215.5	209.9	240.8	198.0	258.4	23.5
18:3[b]	23.8	27.9	26.1	28.1	24.3	30.2	2.4
EPA[a]	-0.03	0.11	-0.18	0.02	8.0	5.4	0.72
DHA[a]	0.0	0.0	0.0	0.0	5.3	5.6	0.38
total FA[ab]	979.8	1334.5	1490.7	1853.4	1350.1	1792.4	114.8

[a]fat effect (P < 0.05)
[b]pH effect (P < 0.05)
[c]fat*pH interaction (P < 0.05)

DISCUSSION

My final thought on the project was that protection value from Ca salts of tallow were pH sensitive and that might be the reason I did not see positive results previously. Therefore, I made one final attempt with partial funding assistance from Virtus Nutrition (no additional funds were requested from ACREC) to examine how tallow-fish oil combinations might accentuate ruminal escape of

omega-3 fatty acids. The experiment was not proposed in the original project description and required more time for analysis of all the samples. The main goal was to evaluate how the tallow-fish oil combinations were affected by ruminal pH. Treatments were arranged as a 3 x 2 factorial to examine three fat treatments (CON with no added fat; TAL with added calcium salts of tallow; TAL-FO with calcium salts of tallow and fish oil) and 2 pH (6.5 and 5.5). Fermentations were run in continuous culture for a total of 10 days, with the first 7 days for adaptation to the diet and the last 4 days for sample collection. After each 10-day period is complete, diets will be fermented again in a different fermentor until three replications have been completed. The statistical design will be a randomized block design with a 3 x 2 factorial arrangement of treatments.

Significance to the rendering industry:
Three limitations of tallow hamper its growth in dairy rations compared to the commercial rumen-inert fat sources; 1) it is classified as a rumen-active fat source that can interfere with ruminal fermentation at higher inclusion levels, 2) the solid or semi-solid physical form of tallow makes it more difficult to transport and mix using conventional on farm equipment, and 3) tallow lacks any nutritional quality that distinguishes it from other high energy fat sources. Strategies are needed to increase tallow usage in dairy rations, where the growth potential for fat ingredients continues to grow. If only ten percent of U.S. milking cows (approximately 1 million cows) consumed an additional .5 pound of tallow each day for 100 days of lactation, receipts from tallow would increase over 7 million dollars annually.

The intent of this project is to convert tallow from a simple high energy source to a rumen-protected fat source with improved handling qualities, enhanced nutritional value, and higher economic value. Past research suggests that the fatty acid profile of tallow might make it an excellent carrier for other nutrients, such as some essential amino acids, choline, or selected omega fatty acids known to have metabolic and physiologic benefits in dairy cattle. For instance, reproductive performance of dairy cattle has been improved by feeding additional omega-3 or omega-6 fatty acids to lactating dairy cows (Petit, 2003). To be effective, the fatty acid supplement added to the diet must provide protection from ruminal biohydrogenation for effective absorption and transport to body tissues. If this project is successful, the value of tallow in dairy rations could be enhanced by using it as a protector of nutrients to enhance animal performance and health. The results confirmed that Ca salts did provide protection of DHA and EPA from biohydrogenation, but unexpectedly, this was only true for the lower level of fish oil supplementation. Higher levels of fish oil in the Ca salt matrix had higher losses, probably meaning that a higher proportion of the fish oil was exposed to the outer surface and readily accessible to microbial enzymes.

Intellectual Property Development? No disclosures were submitted on the project pending the outcome of this experiment showing the tallow-fat combination with the most rumen protection.

Virtus Nutrition (Corcoron, CA) donated their time and resources to synthesize the tallow products used for testing, and provide partial financial assistance.