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IMMUNOLOGIC INHIBITION OF RUMEN LIPASE ACTIVITY IN VITRO

Anderson, R.C., Krueger, N.A., Edwards, H.D., S.L. Kronberg, Apić, B.Jelena, Božić, K.A., Harvey, R.B., Nisbet, D.J.[1]

SUMMARY: Diets high in saturated fats are associated with certain negative-health effects such as coronary heart disease. Ruminant-derived foods contain high proportions of saturated fats, a result of microbial biohydrogenation within the rumen which rapidly saturates and thus limits the availability of free unsaturated fatty acids for absorption and assimilation. Flaxseed is an attractive dietary supplement for ruminants because it contains high amounts of unsaturated fat, mainly alpha-linolenic acid (18:3n-3). However, the oil in unprocessed whole flaxseed may be largely unavailable for digestion throughout the total gastrointestinal tract whereas the oil in processed seeds may be too rapidly hydrolyzed to bypass ruminal biohydrogenation. Presently, we studied the *in vitro* hydrolysis of cracked and extruded flaxseed by mixed populations of ruminal microbes treated with IgY antibodies raised against whole cells of a lipase-expressing *Propionibacterium acnes*. Results from a general analysis of variance revealed that rates of free fatty acid accumulation were indeed lower ($P < 0.05$) following 24 h anaerobic incubation of 5 ml rumen fluid cultures supplemented with cracked flaxseed than with extruded flaxseed added at 4% wt/vol (30.40 versus 348.32 nmol ml^{-1} h^{-1}, respectively). Cultures incubated with extruded flaxseed exhibited a 46% slower rate of free fatty acid accumulation when treated with anti-*P. acnes* IgY antibody compared to untreated controls; however, antibody treatment did not significantly reduce rates of lipolysis in cultures incubated with cracked flaxseed. Inoculating a lipolytic *Propionibacterium avidum* into the mixed populations did not affect rates of free fatty acid accumulation.

Key words: Alpha linolenic acid, biohydrogenation, flaxseed, lipid metabolism, rumen.

Introduction

Health-promoting benefits of dietary n-3-polyunsaturated fatty acids for humans are well recognized [14]. Conversely, human diets that contain high amounts of saturated fats are associated with negative-health effects such as coronary heart disease [15]. Foods produced from ruminants contain high proportions of saturated fats, a result of biohydrogenation by bacteria within the rumen which rapidly saturates and thus decreases the availability of free unsaturated and polyunsaturated fatty acids for absorption and incorporation into meat and milk [6]. Consequently, there is considerable interest in developing strategies to enrich ruminant-derived foods with unsaturated and polyunsaturated fatty acids [1, 16]. Flaxseed is an excellent source of alpha linolenic acid (18:3n-3), an essential dietary precursor of longer chain omega-3-fatty acids in mammals [2] and supplementation of ruminant diets with whole flaxseed can potentially increase assimilation of n-3-polyunsaturated fatty acids in grass-fed heifers [9] as well as in hay and barley silage-fed cows [7]. In the latter study, however, concomitant increase in plasma concentrations of unsaturated, mono-unsaturated and saturated fatty acids was observed in the flax-fed cattle indicating that appreciable amounts of flaxseed oil had not escaped rumen lipolysis and biohydrogenation [7]. Processing technologies such as heat treatment and extrusion of flaxseed have been applied to increase the degree of protection of amino acids and flaxseed oil from rumen lipolysis and subsequent biohydrogenation but results from feeding studies have not necessarily shown efficacious improvements in amino acid protection [13] or in incorporation of alpha linolenic acid into meat and milk [5]. Considering that lipolysis is an absolute pre-requisite for rumen biohydrogenation, it seems reasonable to hypothesize that inhibition of ruminal lipolytic activity may be another way to promote ruminal bypass of dietary lipids for digestion and absorption in the proximal small intestine. Krueger and colleagues [10] reported that egg yolk (IgY) antibodies raised against certain lipolytic rumen bacteria inhibited rates of free fatty acid accumulation during in vitro culture of mixed populations of ruminal microbes and suggested that this may protect fats from rumen lipolysis and subsequent biohydrogenation. More recent work by

Ivited paper

[1]Robin C. Anderson, PhD, research microbiologist, Nathan A. Krueger, PhD, postdoctoral scientist, David J. Nisbet, PhD, research leader and Roger B. Harvey, DVM, PhD, veterinary medical officer, United States Department of Agriculture/Agricultural Research Service, College Station, Texas, USA. Holly D. Edwards, M.S., doctoral student, Department of Animal Science, Texas A&M University, College Station, TX, USA. Scott L. Kronberg, PhD, research animal scientist, United States Department of Agriculture/Agricultural Research Service, Mandan, North Dakota, USA. Jelena Apić, DVM, M.S., research assit., Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia. Aleksandar Božić, PhD, professor, Faculty of Agriculture, Novi Sad, Serbia.
Corresponding author: Robin C. Anderson, United States Department of Agriculture /Agricultural Research Service, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, 2881 F&B RD, College Station, Texas, USA; E-mail: Robin.Anderson@ars.usda.gov; Phone: +001 097 260-9317.

Edwards [4] demonstrated that pure cultures of a ruminal *Propionibacterium avidum* isolate expressed considerably more lipase activity than pure cultures of other ruminal lipolytic bacteria and thus may contribute appreciably to rumen lipolysis. The objectives of this experiment were to assess the potential protective effect conferred on flaxseed by processing and by treatment with specific IgY antibodies generated *ex situ* against a related ruminal lipase-producing *Propionibacterium* spp. on rates of ruminal lipolysis during incubation of mixed populations of ruminal bacteria in vitro.

Materials and Method

IgY Antibody preparation.

Whole cells of a ruminal lipolytic *Propionibacterium acnes* [11] were harvested by centrifugation (10,000 x g, 20 min) from cultures grown to late stationary phase in the basal medium of Kim et al. [8] supplemented with olive oil, linseed oil or corn oil. Cells were washed 3 times with 5 ml of anaerobic dilution solution [3], mixed with TiterMax® Gold Adjuvant (Sigma-Aldrich, USA) and injected (100 µg of protein) at six different locations intramuscularly to laying hens. Each bacterial cell suspension was administered to 3 laying hens to ensure that a sufficient quantity eggs could be produced. Booster shots of 100 µg of cell protein in ultrapure water were administered at 3 and 6 weeks post initial injection. Purification of IgY antibodies from eggs collected one week following the final booster injections was accomplished using Eggcellent™ Chicken IgY Purification Kits (Pierce Kit 44918, Rockford, IL). Purified antibody was stored at -80°C until use.

Determining effectiveness of anti-lipolytic bacterial IgY antibodies.

The effectiveness of the developed antibodies was tested against lipolytic activity expressed by mixed populations of rumen bacteria during in vitro incubation of freshly collected ruminal fluid (4 ml/tube) in 18 x 150 mm crimp top tubes preloaded with 10 g of 4 mm glass beads (added as a solid support matrix to promote interfacial activation), 1 ml anaerobic dilution solution and with or without 0.2 g cracked or extruded flaxseed. The cracked and extruded flaxseed originated from the same source and contained 35 to 36.5% lipid. Additions of 0.1 ml of an overnight culture of another rumen lipolytic bacterium, *Propionibacterium avidum* [4, 11] and 0.7 ± 0.13 mg of anti-*P. acnes* IgY were added to respective treatment tubes by syringe injection. Free fatty acid accumulations were measured colorimetrically [12] on samples extracted and analyzed during 24 h of incubation. Test for effects of substrate type (cracked versus extruded flaxseed), bacterial treatment (with or without *P. avidum* inoculation) or antibody treatment (with or without anti-*P. acnes* IgY) on rates of free fatty acid accumulation were accomplished using a general analysis of variance with a Tukey's separation of means.

Results and Discussion

Contrary to our expectations, rates of free fatty acid accumulation did not differ ($P = 0.26$) between cultures inoculated without or with *P. avidum* (168.21 versus 210.49 nmol free fatty acid ml^{-1} h^{-1}, respectively; SEM = 24.22). However, *P. avidum* is a normal inhabitant within mixed populations of ruminal bacteria and it is possible that our inoculations may have provided too few cells to be of consequence to numbers within the endogenous population. Rates of free fatty acid accumulation by mixed populations of ruminal microbes were greater ($P < 0.05$) when incubated with extruded flaxseed than with cracked flaxseed (Table) thus indicating that oil in the cracked flaxseed may be largely unavailable for ruminal digestion but whether the oil within the cracked flaxseed would be available for intestinal digestion is not apparent. Conversely, too rapid hydrolysis of free fatty acids from extruded flaxseed would be undesirable as this would make unsaturated fatty acids available for biohydrogenation. Moreover, accumulation of free fatty acids within the rumen can inhibit digestive processes such as cellulolysis and fiber digestion [6]. Rates of free fatty acid accumulation were reduced in cultures treated with anti-*P. acnes* IgY compared to cultures incubated without antibody treatment, but significance was only achieved in cultures incubated with extruded flaxseed (Table).

Table 1. Effects of flaxseed processing and anti-*P. acnes* IgY treatment on ruminal lipolysis by mixed populations of ruminal microbes in vitro

	Rates of free fatty acid accumulation (nmol ml^{-1} h^{-1})	
	Untreated	Anti-*P. acnes* IgY-treated
Cracked flaxseed	30.40^{c}	8.68^{c}
Extruded flaxseed	348.32^{a}	187.39^{b}
P value	0.03	
SEM	33.43	

[a,b,c]Values with unlike superscripts differ ($P < 0.05$).

Conclusion

Result from the present study provide support to our hypothesis that strategies inhibiting ruminal lipase activi may be an effective way to protect flaxseed oil from subsequent biohydrogenation. Further studies are warranted examine the effect of anti-*P.acnes* IgY on rumen lipolysis in vivo.

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