

October 17, 2001

James E. Hoadley, PhD  
Nutrition Labeling & Programs Team  
Division of Nutrition Science & Policy  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C Street SW  
Washington DC 20204

RE: **Docket 01Q-0313; Health Claim Petition  
To Expand Oats Soluble Fiber and CHD Claim**

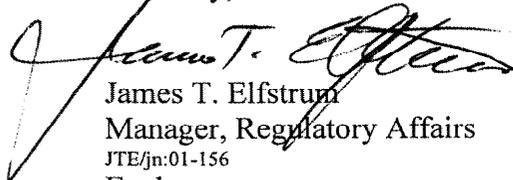
Dear Dr. Hoadley:

Please note the enclosed supplemental information, dated September 7, 2001, regarding the above referenced health claim petition for Oatrim (BetaTrim™) submitted by the Quaker Oats Company and Rhodia Inc. as petitioners.

This supplemental information responds to your request for additional details regarding the Gallaher Intestinal Contents Supernatant Viscosity Methodology contained in the petition specifically identified as "Study 1 & 2". Under the 1st tab you will note the complete studies described by Dr. Gallaher, dated January 14, 1999. Under the 2<sup>nd</sup> tab, you will note Study 3 explaining some of the results and additional information from Dr. Gallaher regarding his methodology.

I believe this responds to your request. If you have further questions regarding this matter, please call me at 609-860-4653.

Sincerely,



James T. Elfstrum  
Manager, Regulatory Affairs  
JTE/jn:01-156

Enclosure

cc: Priscilla Samuel  
Mark L. McGowan

01Q-0313

SUP 1

**DATE: September 7, 2001**

**OFFICE OF NUTRITION PRODUCTS, LABELING AND  
DIETARY SUPPLEMENTS  
CENTER FOR FOOD SAFETY & APPLIED NUTRITION  
FOOD AND DRUG ADMINISTRATION  
200 C STREET SW  
WASHINGTON, DC 20204**

**PETITIONERS: The Quaker Oats Company  
617 W. Main Street  
Barrington, IL 60010**

**&**

**Rhodia Inc.  
CN 7500, 259 Prospect Plains Road  
Cranbury, NJ 08512-7500**

**SUBJECT: OATRIM (BETATRIM™) HEALTH CLAIM  
PETITION**

**Petition to Expand the Oats Soluble Fiber and  
Coronary Heart Disease Health Claim, (21 CFR  
101.81)**

**Additional Reports  
Confidential**

Study 1 & 2

**Intestinal Contents Supernatant Viscosity of Rats  
Meal-Fed Oatrim™  
And  
It's Cholesterol Lowering Effect in Rats**

**A Research Proposal to the Quaker Oats Corporation**

by

**Daniel D. Gallaher, Ph.D.**

**Associate Professor**

**Department of Food Science and Nutrition**

**University of Minnesota**

**St. Paul, MN 55108**

**(612) 624-0746**

**FAX (612) 625-5272**

**Email: [dgallaher@che2.che.umn.edu](mailto:dgallaher@che2.che.umn.edu)**

**January 14, 1999**



## Background

It is well established that certain dietary fibers have cholesterol-lowering effects. Although the exact mechanism of this effect continues to be debated, most attention has focused on two physicochemical properties of dietary fiber as being responsible. These are viscosity and susceptibility to fermentation. Anderson's group has proposed that fiber fermentation is responsible for cholesterol lowering. Intestinal viscosity of oat fractions - March 1997.<sup>1</sup> This hypothesis is based on his findings that the short chain fatty acid propionate, a product of fiber fermentation, inhibits hepatic HMGCoA reductase, the rate limiting enzyme for cholesterol synthesis. However, a number of studies have failed to provide support for this hypothesis<sup>2</sup>, and consequently, many in the field do not believe that propionate production via fermentation is an important mechanism by which fiber lowers cholesterol concentrations.

In recent years our research group has focused on the role of viscosity as the characteristic of fiber responsible for cholesterol lowering. Using the non-fermentable fiber analog hydroxypropyl methylcellulose (HPMC), we have demonstrated in cholesterol-fed hamsters that increased viscosity of the intestinal contents is associated with reductions in both plasma and liver cholesterol. Further, this relationship was found to be predictable, as plasma cholesterol was inversely related to the log of intestinal contents viscosity. More recently we have found that HPMC consumption decreases cholesterol absorption in a viscosity-dependent manner. However, bile acid excretion is essentially unaffected. Studies in cholesterol-fed rats have resulted in similar results. That is, guar gum, two types of  $\beta$ -glucans, and HPMC all increased intestinal contents viscosity, reduced liver cholesterol concentrations, and reduced the efficiency of cholesterol absorption.

Thus, our conclusion from these studies is that increases in intestinal contents viscosity is the characteristic responsible for cholesterol reduction in fibers by fibers such as guar gum,  $\beta$ -glucans, and modified celluloses. Given this, it is reasonable to predict that consumption of foods that result in increases in intestinal viscosity would have a cholesterol lowering effect when consumed on a regular basis.

## Objective

The objective of this project will be to determine the intestinal contents viscosity of rats that have consumed a meal of one of three formulations of a  $\beta$ -glucan enriched product, Oatrim. In addition, the relative cholesterol-lowering effect of these three fractions will be assessed in cholesterol fed rats. The Oatrim formulations are to be provided by Quaker Oats, Inc.

## Experimental Design

### ***Intestinal Contents Supernatant Viscosity of Oatrim – Study 1***

Male Sprague-Dawley rats, weighing between 250-275 g will be used in the study. After receipt, rats will be adapted for 4 days to a semipurified diet (AIN-93G diet). Following an overnight fast, rats (10 per group) will be given meals of one of 3 different oatrim formulations, oatmeal, or cellulose.

The rats will be presented with a quantity to be determined by conversations with Quaker Oats. To the meal we propose to add 0.5g sucrose to improve palatability.

Rats will be allowed 2-3 hours to consume the meals. Any amount not consumed will be weighed so that we will know how much of the meal each rat consumed. At the end of the 2-3 hour period, the rats will be killed by exposure to ethyl ether, opened by a midline incision, and the small intestine removed. The intestinal contents will be carefully collected by finger stripping of the intestine. The contents will then be centrifuge at 50,000 x g for 30 minutes at room temperature and the supernatants collected for viscosity measurement.

Viscosity of the intestinal contents supernatants will be determined at 37° C using a Brookfield cone/plate viscometer. This instrument measures absolute viscosity, so values are directly comparable with values we have obtained in other experiments. Since viscosity of  $\beta$ -glucans is non-Newtonian (i.e. the viscosity changes with shear rate), we will measure viscosity at several shear rates for each sample and then extrapolate the viscosity readings of each sample to a common shear rate ( $23 \text{ s}^{-1}$ ) that will be used for all samples.

### ***Cholesterol Lowering Effect of Oatrim Formulations – Study 2***

Male Wistar rats, weighing between 50-75 g, will be housed individually in wire-bottom cages. Upon arrival, rats (10 per group) will be fed one of five diets (modified AIN-93G), containing 7.5 % dietary fiber from one of the following fiber sources:

- Cellulose
- Oatrim formulation 1
- Oatrim formulation 2
- Oatrim formulation 3
- Oatmeal

All diets will contain 0.25% cholesterol, a level we have demonstrated will elevate liver cholesterol concentrations significantly, but will allow easy detection of cholesterol lowering by hypocholesterolemic agents.

Rats will be fed the diets for 20 days. On day 18, cholesterol absorption will be determined by the fecal isotope ratio method. Briefly, radiolabeled cholesterol and  $\beta$ -

sitosterol (as a non-absorbable marker) are given by gavage (i.e. intubation into the stomach). Eight hours later, feces are collected for 24 hrs. By measurement of the ratio of cholesterol to  $\beta$ -sitosterol in the fecal lipid extract compared to what was given by gavage, cholesterol absorption can be accurately determined. The ratio is determined by counting the radioactivity in a liquid scintillation counter.

Animals will be meal fed, then anesthetized 2-3 hours later with ethyl ether, blood collected by cardiac puncture, and the liver removed and immediately frozen on dry ice. Blood will be centrifuged and plasma collected and frozen until analyzed for total cholesterol. Intestinal contents will also be collected and contents viscosity determined as described above.

Liver lipids will be extracted by the method of Folch et al. Liver total cholesterol will be determined by the cholesterol oxidase/peroxidase method, as per our standard procedure.

### **Statistics**

Means and standard error of the mean will be calculated for each group. The data will then be tested for normal distribution and equality of variance. If the data do not pass these tests, the data will be analyzed using a nonparametric analysis of variance (AOV) and the medians indicated. Otherwise, standard one-way AOV will be used. If statistically significant differences are found by analysis of variance, then differences among specific groups will be determined using a multiple range test such as Student-Newman-Kuels test (or Dunn's test if a nonparametric AOV is used).

## Budget

## References

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<sup>1</sup> Wright, R.W., Anderson, J.W., and Bridges, S.R. (1990) Propionate inhibits hepatic lipid synthesis. *Proc. Soc. Exper. Biol. Med.* 190: 26.

<sup>2</sup> Topping, D.L. (1995) Propionate as a mediator of the effects of dietary fiber. In: *Dietary Fiber in Health and Disease*, D. Kritchevsky & C. Bonfield, eds., Eagan Press, St. Paul, MN.

# Diet Composition of Cholesterol Lowering Feeding Trial



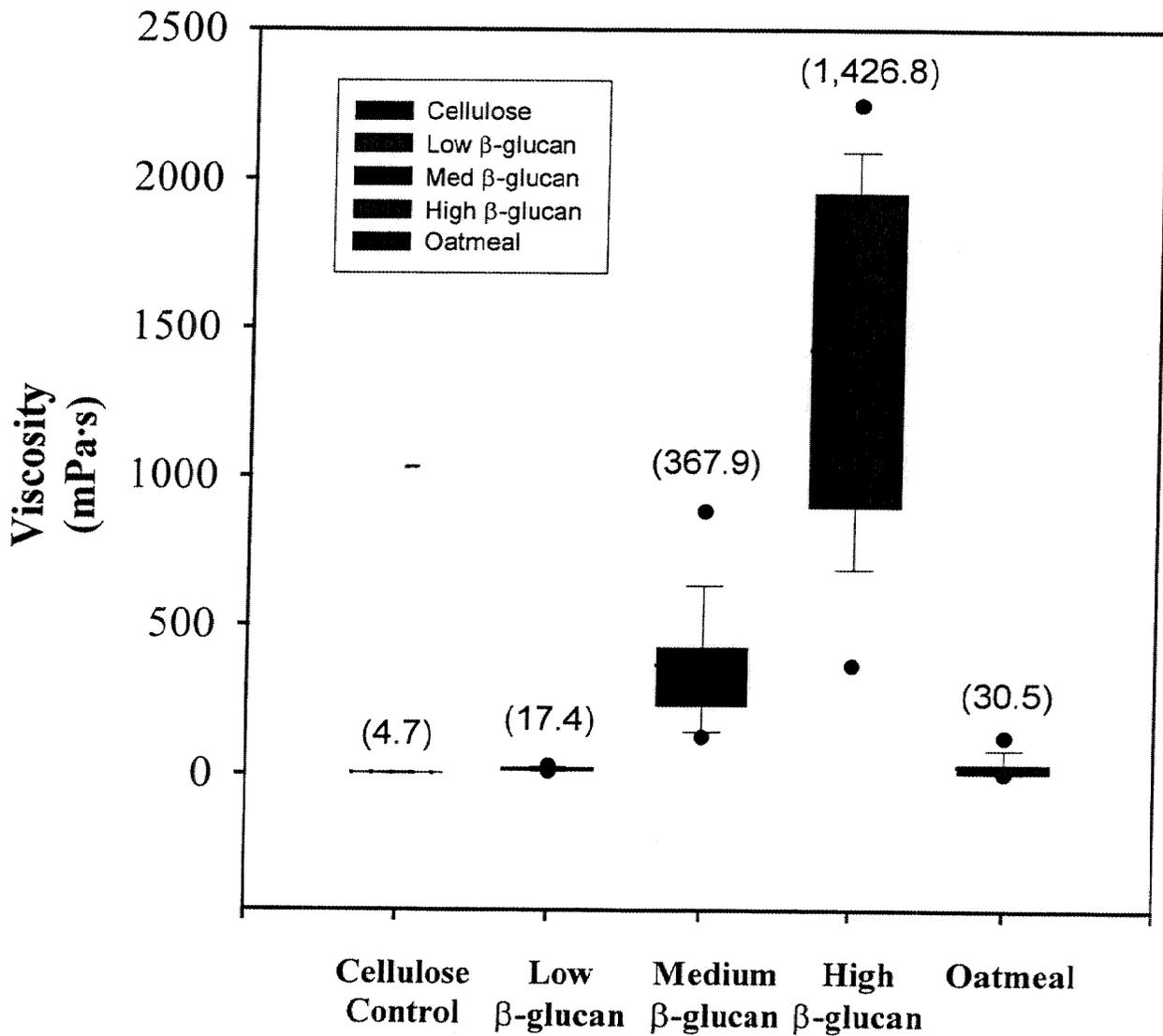


<b>% Carb.</b>	54.95	54.95	54.95	54.95	54.95	54.95	54.95
<b>% Protein</b>	20.0	20.0	20.0	20.0	20.0	20.0	20.0
<b>% Fat</b>	15.0	15.0	15.0	15.0	15.0	15.0	15.0
<b>% Fiber</b>	5.0	5.0	4.59	4.11	3.64	5.0	5.0

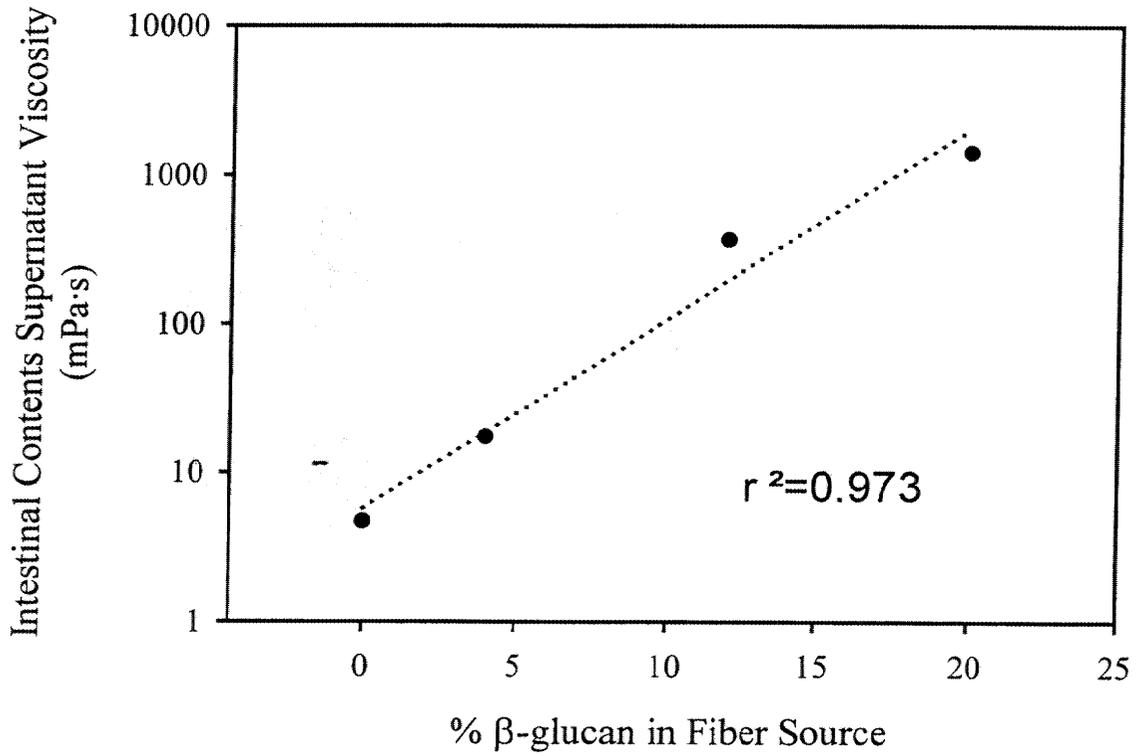
# Results of Meal Feeding Study



## Intestinal Contents Supernatant Viscosities After Meal-Feeding of $\beta$ -Glucan Concentrates



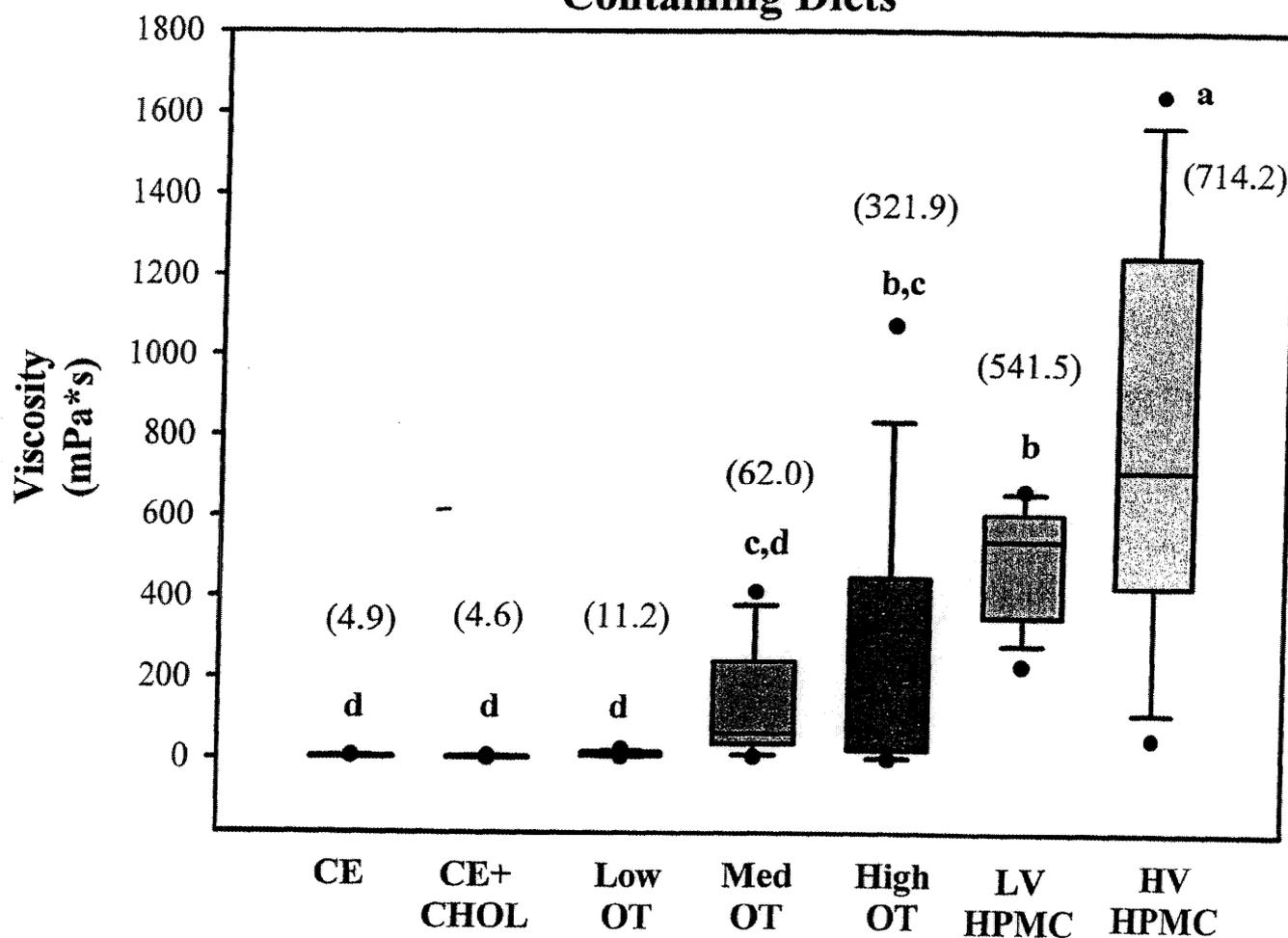
Correlation Between Dietary  $\beta$ -Glucan Concentration  
and Intestinal Contents Supernatant Viscosity  
in Rats Meal-Fed Oatrim (BetaTrim) of Different Viscosities



# Results of Cholesterol Lowering Feeding Trial



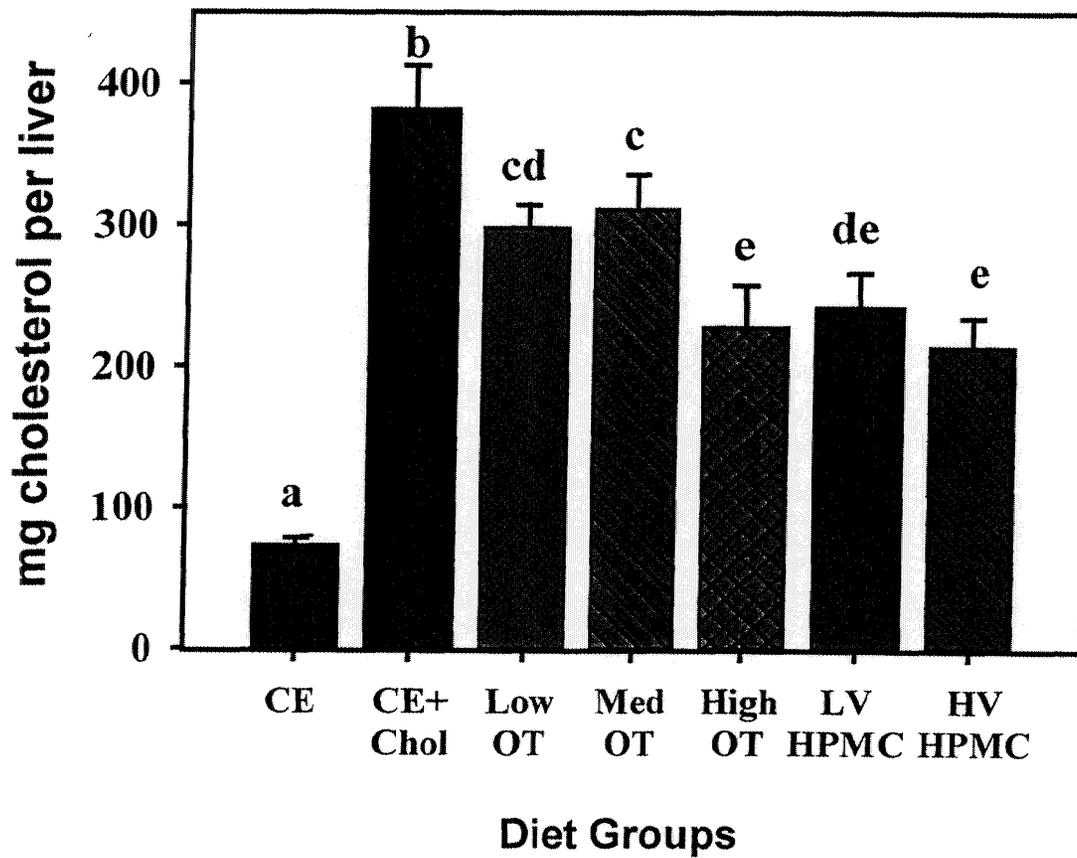
## Intestinal Contents Supernatant Viscosities After Meal Feeding of Oatrim (OT) or Hydroxypropylmethylcellulose (HPMC) Containing Diets



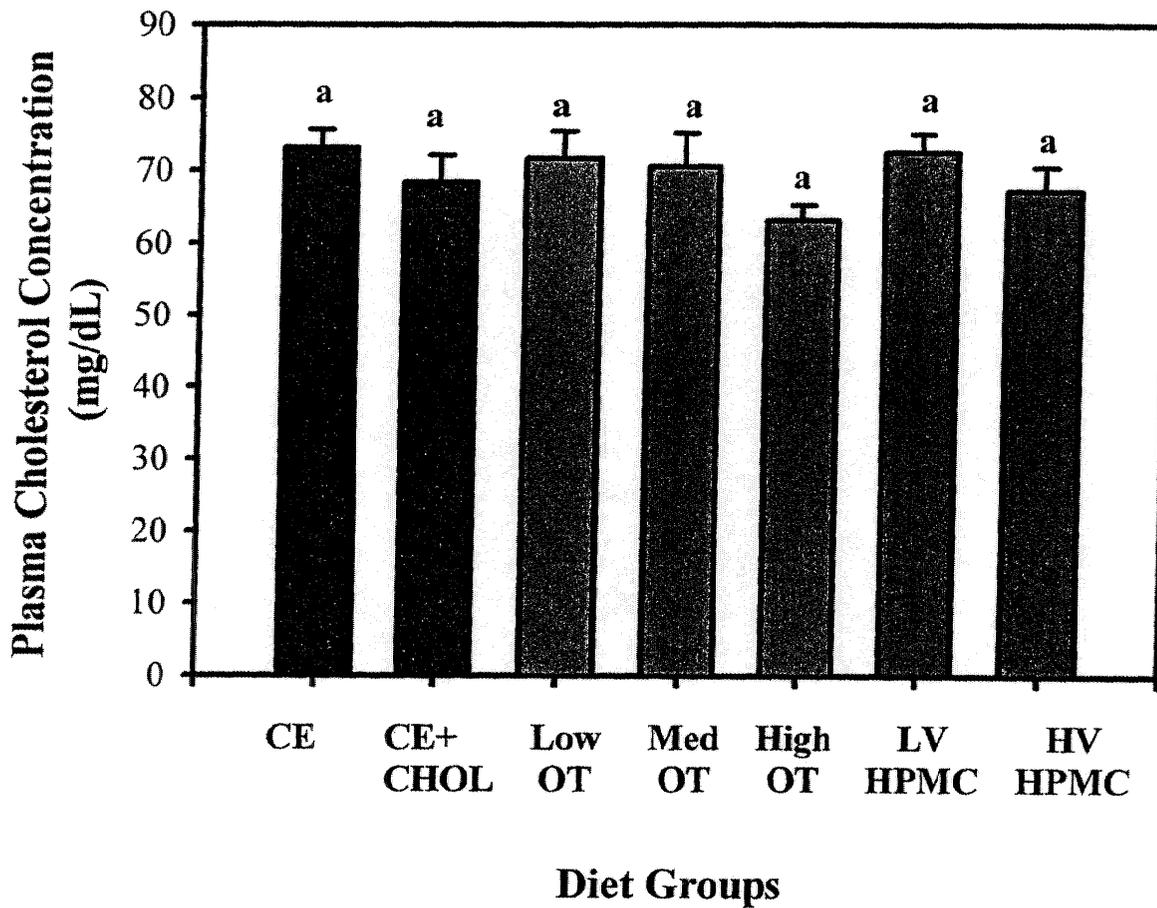
CE=Cellulose control  
 CE+CHOL=Cellulose+cholesterol  
 Low OT=Low Oatrim  
 Med OT=Medium Oatrim  
 High OT=High Oatrim  
 LV HPMC=Low viscosity HPMC  
 HV HPMC=High viscosity HPMC

### Diet Groups

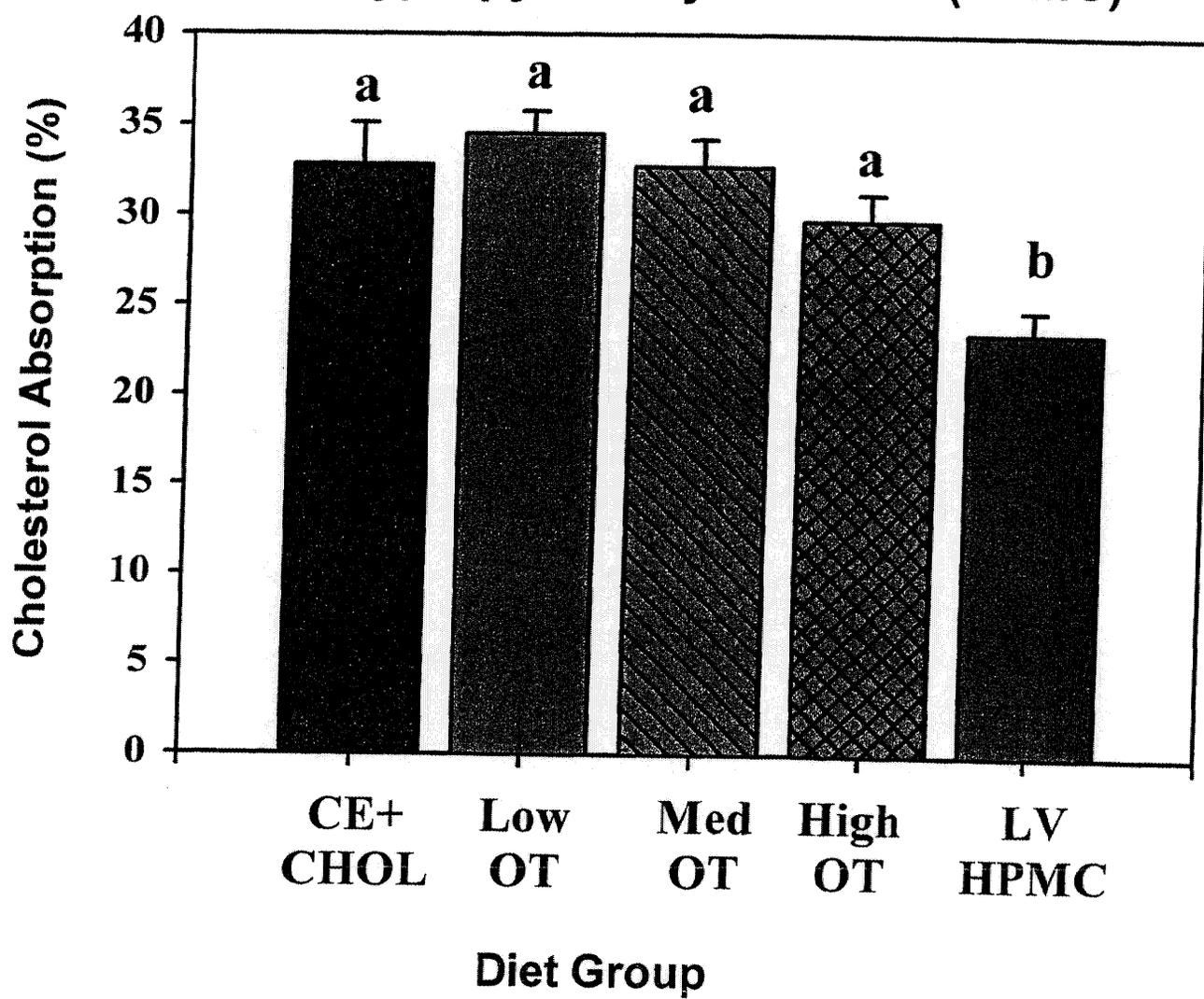
**Total Liver Cholesterol in Rats Fed  
Oatrim (OT)  
or Hydroxypropyl Methylcellulose (HPMC)**



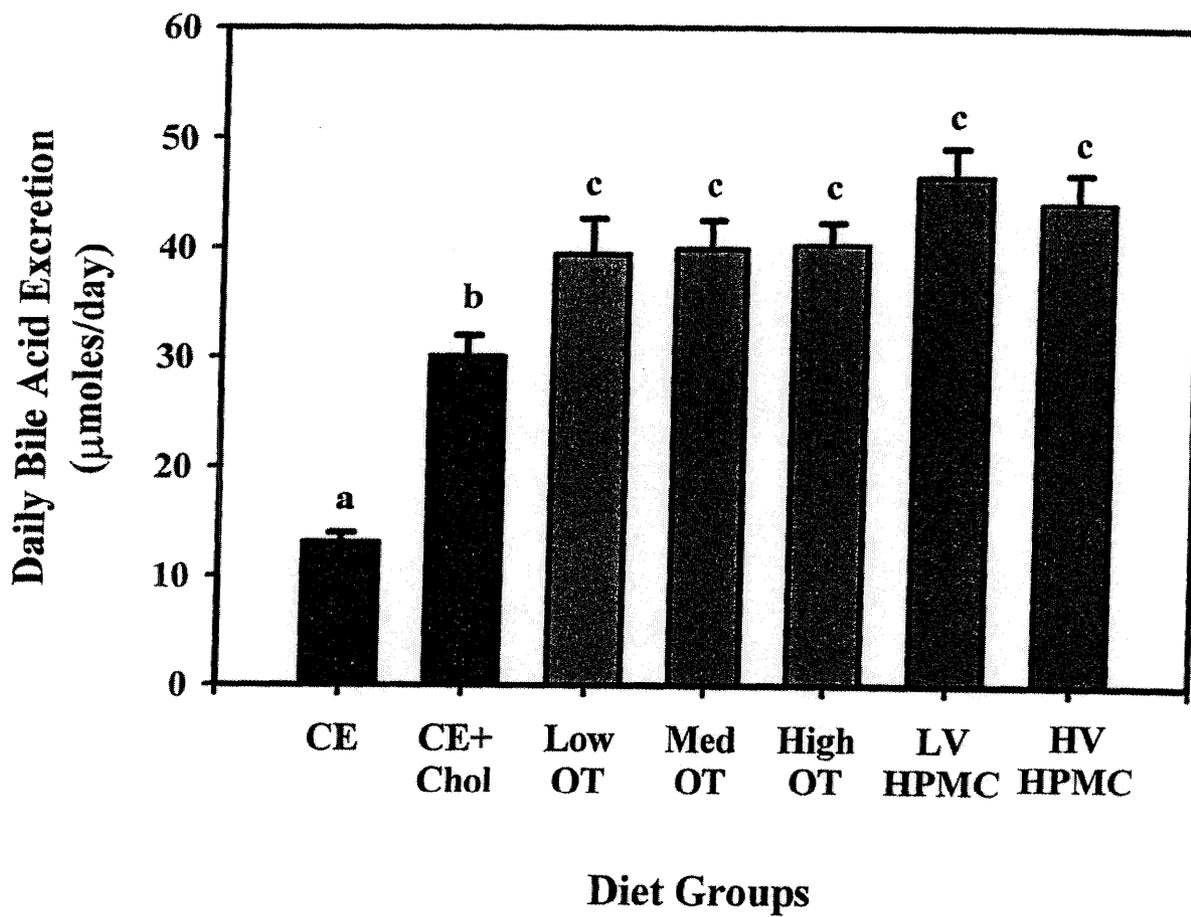
**Plasma Cholesterol Concentration  
in Rats Feed Oatrim (OT)  
or Hydroxypropyl Methylcellulose (HPMC)**



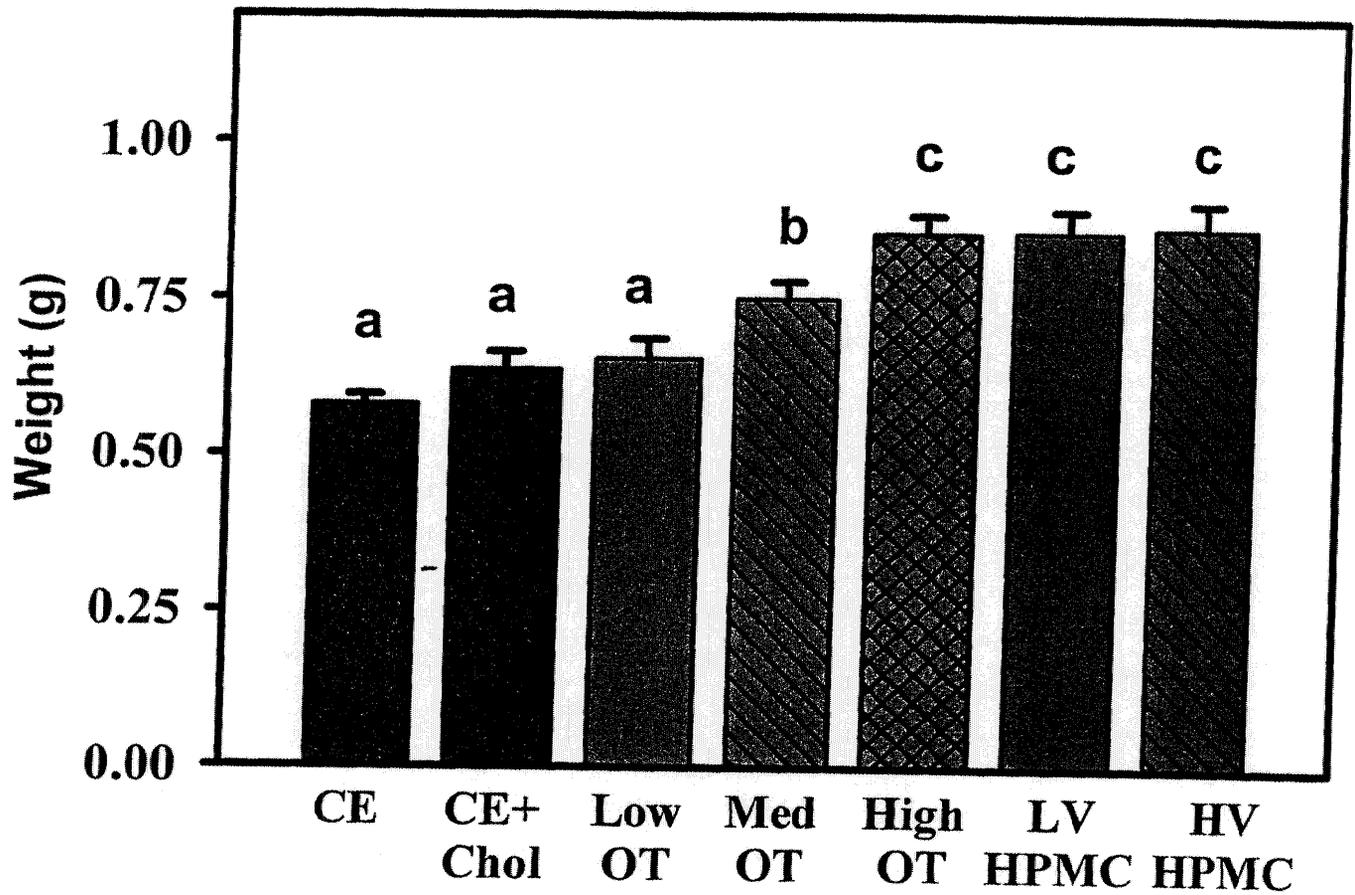
### Cholesterol Absorption in Rats Fed Oatrim (OT) or Hydroxypropyl Methylcellulose (HPMC)



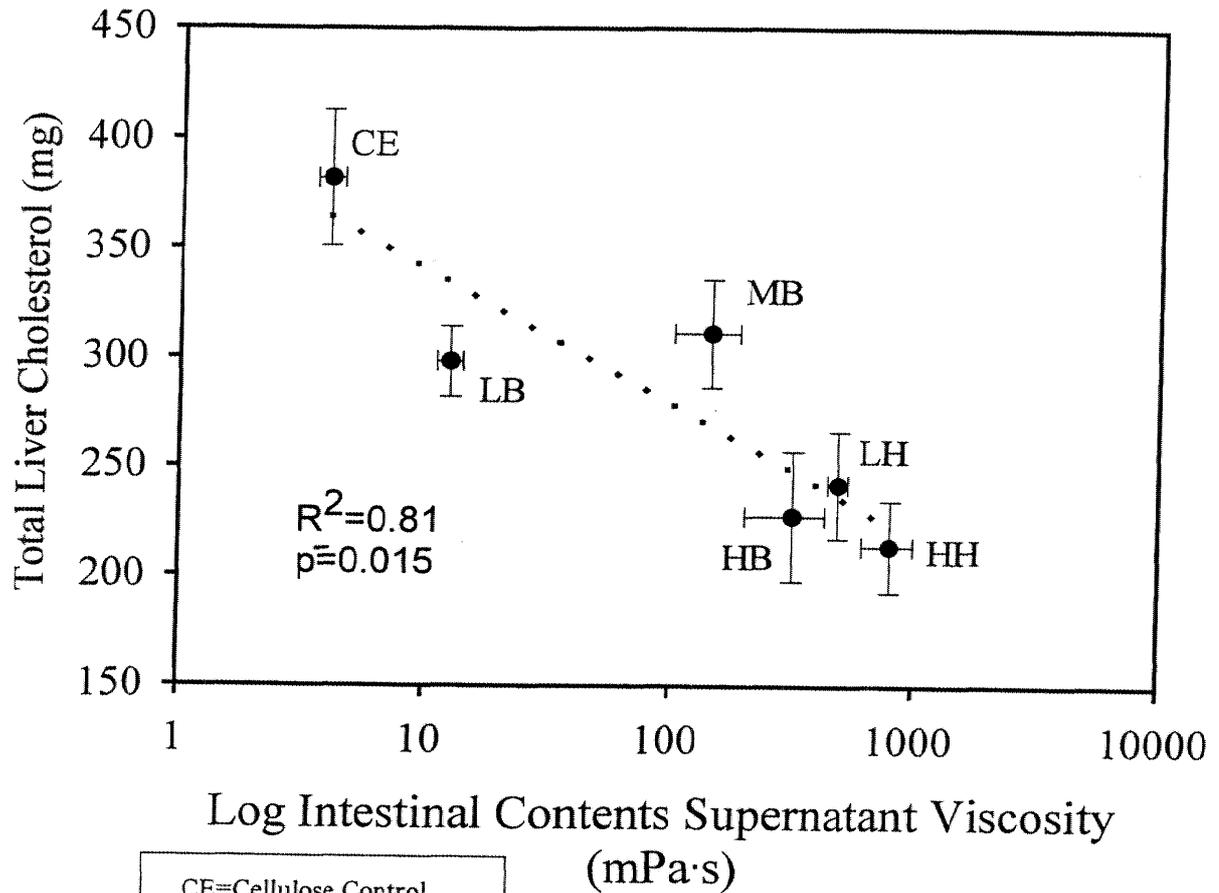
## Daily Fecal Bile Acid Excretion in Rats Fed Oatrim (OT) or Hydroxypropylmethylcellulose (HPMC)



# Cecal Wall Weight in Rats Fed Oatrim (OT) or Hydroxypropyl Methylcellulose (HPMC)



## Correlation Between Total Liver Cholesterol and the Logarythm of Intestinal Contents Viscosity



CE=Cellulose Control  
LB=Low  $\beta$ -Trim  
MB=Medium  $\beta$ -Trim  
HB=High  $\beta$ -Trim  
LH=Low-Viscosity HPMC  
HH=High-Viscosity HPMC

# Conclusions



## Conclusions

### *Oatrim Meal Feeding Study*

Rats were meal-fed a diet containing cellulose, Oatrim at 4, 12, or 20% concentration or oatmeal. The intestinal contents supernatant viscosity progressively increased from the low to high concentration of Oatrim. Oatmeal meal-feeding yielded a viscosity only slightly higher than that of the low Oatrim group.

There was an extremely high correlation ( $r^2=0.97$ ) between the intestinal contents supernatant viscosity and the % of  $\beta$ -glucan in the fiber source fed when considering the cellulose and Oatrim groups.

The results indicate that intestinal contents supernatant viscosity is linearly related to the quantity of  $\beta$ -glucans in the meal; the greater the concentration of  $\beta$ -glucans in the meal consumed, the greater will be the supernatant viscosity.

### *Cholesterol Lowering Feeding Trial*

The results completed to date indicate the following conclusions regarding the effect of Oatrim on cholesterol lowering and parameters related to this, as follows:

- Oatrim increases intestinal contents supernatant viscosity in relation to its concentration in the diet, similar to the results found in the meal feeding trial. Thus, the enhancement of supernatant viscosity is not lost with continuous consumption.
- All dietary levels of Oatrim were effective in lowering total liver cholesterol. The reduction did not follow a linear relationship with dietary concentration of Oatrim; however, the highest dietary level of Oatrim produced the greatest cholesterol lowering.
- Considering all dietary groups used in the study, there was a high and statistically significant correlation between total liver cholesterol and the log of supernatant viscosity.
- Plasma cholesterol concentrations were not altered by any dietary treatment, as expected. At the level of dietary cholesterol used, plasma cholesterol in the rat is highly resistant to change.
- Oatrim at any level did not alter cholesterol absorption. This is an unexpected result, as previous studies with other viscous fibers had demonstrated an inverse relationship between viscosity and cholesterol absorption.
- Oatrim increased fecal bile acid excretion. However, excretion was the same regardless of the dietary concentration of Oatrim.
- Correlations between supernatant viscosity and the  $\beta$ -glucan concentration in the supernatant for rats fed meals of whole oat fractions (determined in a previous study for Quaker Oats) and for the Oatrim groups were determined. The slope of the

regression line for the Oatrim group was much lower than that for the whole oat fractions. This strongly suggests that the molecular weight of the  $\beta$ -glucans in the Oatrim is substantially less than in whole oat fractions, suggesting degradation of  $\beta$ -glucans in the production of Oatrim.

It is clear that Oatrim is effective in reducing cholesterol levels. The mechanism by which it does so, however, is not yet entirely clear. Changes in cholesterol absorption appear not to be involved. Although enhanced bile acid excretion may be a part of the mechanism, the lack of a correlation between cholesterol lowering and bile acid excretion suggests that other factors are involved.

The estimation of cholesterol synthesis (by measurement of HMGCoA reductase) and bile acid synthesis (by measurement of cholesterol 7 $\alpha$ -hydroxylase) may explain more fully the observed cholesterol lowering effect of Oatrim.

## Addendum Report: Intestinal Adaptation to Viscous Materials in the Diet

### Background

Results of a number of studies using the viscous polymer hydroxypropyl methylcellulose (HPMC) suggested that the intestinal contents supernatant viscosity measured after consumption of a single meal containing HPMC was greater than after the animals had been fed the HPMC-containing diet for a number of weeks. That is, with prolonged feeding of a viscous polymer, the contents supernatant viscosity was lower than after a single meal containing the polymer. This suggested an adaptation to the presence of a viscous polymer in the diet.

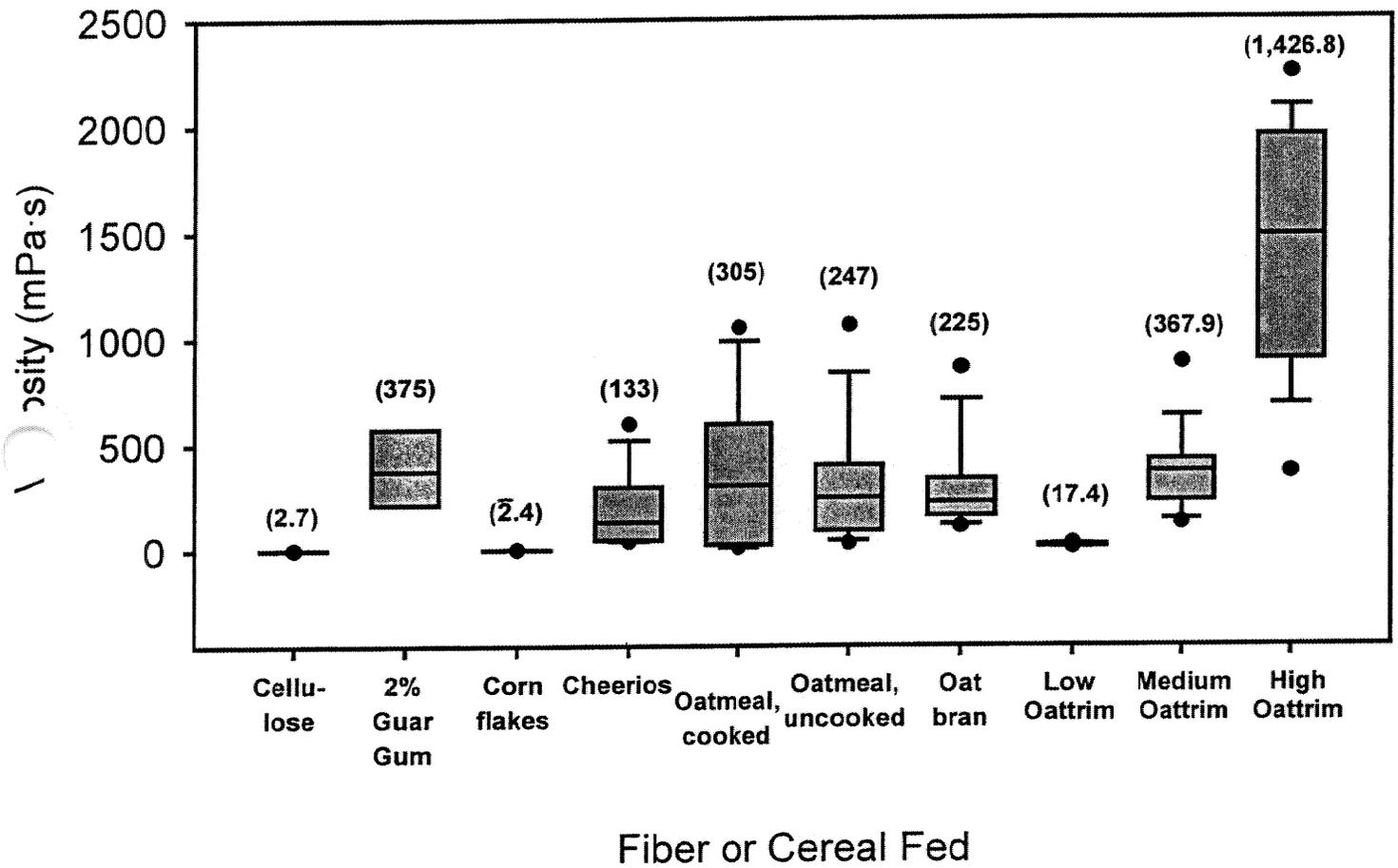
Recently, we were able to test this possibility directly, using a  $\beta$ -glucan-rich material (BetaTrim™) as the source of the viscous polymer. Two experiments were conducted in which the same three BetaTrim™-containing diets were fed to rats. The diets contained either 4, 12, or 20% BetaTrim™. In one experiment, the rats were adapted to the cellulose-based AIN-93G diet for 5-7 days. Rats were then unfed overnight, and given a 5 g meal the following morning. The meal consisted of the AIN-93G diet diluted 1:10 with one of the three BetaTrim™ preparations (containing 4, 12, and 20% beta-glucan); that is, in each case BetaTrim™ was 10% of the diet. Rats were given two hours to eat the meal, then killed, small intestinal contents collected, and viscosity of the supernatant determined by our standard method. In the second experiment, rats were fed 10% BetaTrim™-containing diets (4, 12, and 20% BetaTrim™) for 28 days. They were then unfed overnight, given a 5 g meal of their respective diet the following morning, killed two hours later, and contents supernatant viscosity measured as just described.

The results, shown below, indicated that for each BetaTrim™ diet, rats that had been adapted to the BetaTrim™ had a much lower contents supernatant viscosity than rats who had only been given a single BetaTrim™-containing meal. The difference was most dramatic for the 20% and 12% BetaTrim™-containing diets.

Feeding Regime	Contents Supernatant Viscosity (mPa·s)		
	4% BetaTrim™	12% BetaTrim™	20% BetaTrim™
Single Meal	17.4	367.9	1,426.8
28 Day Feeding	11.2	62.0	321.9

The results suggest strongly that rats adapt to the presence of viscous polymers in the diet by reducing the viscosity within the small intestine. I speculate that this occurs by delaying the rate of stomach emptying; that is, after time, the same diet is emptied from the stomach into the intestine at a much slower rate. This presents a caution to those studying the effects of viscosity on various physiological effects, such as cholesterol lowering. One must consider whether the animals were given only a single meal of the viscous polymer or adapted to the diet containing the viscous polymer in order to allow a proper comparison with published studies.

## Intestinal Contents Supernatant Viscosity of Rats Meal-Fed Fibers or Different Cereals



**Study 3**

**NOTE**

The preliminary results on "Oatrim (BetaTrim™) Contents Supernatant Viscosity Enzyme vs. Acid/Base Processes" showed a P-value =0.43, reported in the Quaker-Rhodia Petition on page 27, figure 4. The final study report which is hereby enclosed (following pages) shows P values=0.51 and P=0.56 for absolute viscosity and viscosity/% beta-glucan respectively (Study Report, page 4). Despite a minor difference in the P-values, the conclusions based on these results remain unchanged. In that, contents supernatant viscosity is not significantly different for Oatrim (BetaTrim™) processed by enzyme vs. acid/base processes.

# **Intestinal Contents Supernatant Viscosity Produced by BetaTrim™: A Comparison of BetaTrim™ Produced By the Enzymatic and Chemical Processes**

Daniel D. Gallaher, Ph.D.  
Department of Food Science and Nutrition  
University of Minnesota  
St. Paul, MN 55108

## **Background**

It is established that food products containing the carbohydrate polymer  $\beta$ -glucans, such as oat bran, can reduce serum cholesterol in humans and serum and liver cholesterol in animal models. However, many food sources of  $\beta$ -glucans contain a relatively low concentration of  $\beta$ -glucans, requiring consumption of a large amount of the food in order to have the desired effect. Therefore, a food product or ingredient that contains a greater concentration of  $\beta$ -glucans would be desirable in order to make consumption of sufficient  $\beta$ -glucan to have a cholesterol-lowering effect much easier.

BetaTrim™ is a  $\beta$ -glucan-enriched food ingredient that appears to be effective in lowering serum cholesterol in humans and liver cholesterol in animal models. BetaTrim™ has traditionally been manufactured from oat flour or oat bran by an enzymatic digestion of starch, a process referred to hereafter as the enzyme process. Recently, however, a chemical method of starch hydrolysis has been utilized to produce BetaTrim™, hereafter referred to as the chemical process. However, strong acids can also hydrolyze non-digestible carbohydrate polymers (i.e. dietary fibers), reducing or eliminating their native viscosity. The viscosity of a dietary fiber is strongly associated with its cholesterol lowering ability. Therefore, it was unknown whether the BetaTrim™ produced by the chemical process would be equivalent to BetaTrim™ produced by the enzymatic process, which is believed not to affect the native viscosity of the  $\beta$ -glucans in the oat flour or bran.

## **Objective**

The objective of this study was to determine whether the intestinal contents supernatant viscosity of rats fed BetaTrim™ manufactured by the chemical process was equivalent to the viscosity produced by BetaTrim™ manufactured by the enzymatic process.

## **Experimental Design**

Male Wistar rats were used in the study. There were two treatment groups, with 10 animals in each group. Upon arrival, the rats were adapted to a modified semipurified AIN-93G powder diet for 14 days (Table 1). Following an overnight fast, the rats were presented with a 5.0 g meal of one of two BetaTrim™-containing diet. The BetaTrim™ meals contained 4.5 g of the AIN-93G diet and 0.5 of the BetaTrim™, produced by either the enzymatic process or chemical process.

The rats were allowed 2 hours to consume and digest the meals. The rats were then killed by exposure to ethyl ether. The spillage and amount left in the food cup were weighed to determine how much of the meal each rat consumed. The small intestines were removed and the intestinal contents were collected by finger stripping the intestine. The contents were centrifuged at 20,000 X g for 45 minutes at 30° C. The supernatants were collected for viscosity measurement. The viscosities of the intestinal supernatants were determined at 37° C using a Brookfield-Wells coneplate viscometer. SigmaStat (Jandel Scientific) was used to analyze the data.

## **Results**

Final body weight of the rats was  $258 \pm 5.8$  g. As shown in **Table 2**, there was a trend for rats given the enzyme process BetaTrim™ diet to consume more of the last meal than those given the chemical process. However, neither the intestinal contents weight nor the weight of the intestinal contents supernatant weight differed between the two groups.

**Figure 1** shows that the intestinal contents supernatant viscosity did not differ between rats fed the enzyme process BetaTrim™ and the chemical process BetaTrim™ ( $p=0.51$ ). The concentration of  $\beta$ -glucans in the contents supernatant were assayed, and the viscosity was also expressed per unit of  $\beta$ -glucans. Contents supernatant expressed in this manner also did not differ between the two processes ( $p=0.56$ ).

## **Conclusions**

The objective of this experiment was to determine whether the method of manufacture of a  $\beta$ -glucan-enriched product, BetaTrim™, affected the viscosity produced within the small intestine of rats. The interest in viscosity stems from the strong association between intestinal contents supernatant viscosity and cholesterol lowering demonstrated in animal models, as well as the association between viscous fibers and cholesterol lowering in humans.

The clear conclusion from this study is that the two processes, the enzymatic and the chemical, produce a BetaTrim™ product that yields an equivalent contents supernatant viscosity. That is, both products are the same in their ability to increase small intestinal viscosity in rats. Consequently, both products would be predicted to have the same cholesterol lowering capability.

Table 1. Composition of modified AIN-93G diet

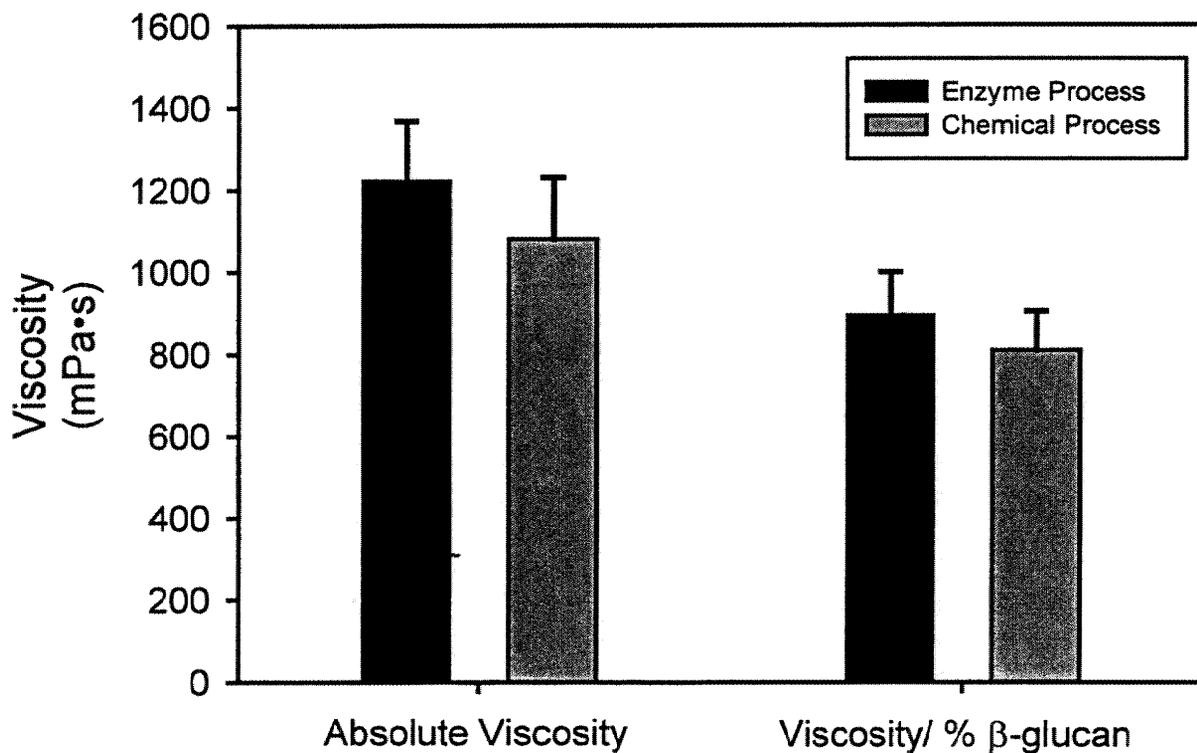
Constituent	% of diet
Cornstarch	34.70
Casein	20.00
Dextrinized cornstarch	11.52
Sucrose	8.73
Corn oil	15.00
Cellulose	5.00
Mineral mix	3.50
Vitamin mix	1.00
L-Cystine	0.30
Choline bitartrate	0.25
BHT	0.001

Table 2. Last meal, intestinal contents, and contents supernatant weights

Parameter	Enzyme process	Chemical Process	P value for difference
Last meal consumed (g)	4.61 ± 0.24	3.84 ± 0.28	0.053
Intestinal contents (g)	3.23 ± 0.20	3.51 ± 0.10	0.23
Contents supernatant (g)	- 1.27 ± 0.12	1.45 ± 0.07	0.26

**Figure 1**

**Intestinal Contents Supernatant Viscosity  
Comparison of BetaTrim Manufactured by the  
Enzyme Process and the Chemical Process**



There are no significant differences between the processes for viscosity ( $P = 0.51$ ) or viscosity/ %  $\beta$ -glucan ( $P = 0.56$ ).