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

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## Background

It is established that food products containing the carbohydrate polymer β-glucans, such as oat bran, can reduce serum cholesterol in humans and serum and liver cholesterol in animal models. However, many food sources of β-glucans contain a relatively low concentration of β-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 β-glucans would be desirable in order to make consumption of sufficient β-glucan to have a cholesterol-lowering effect much easier.

BetaTrim™ is a β-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 b-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 β-glucans in the contents supernatant were assayed, and the viscosity was also expressed per unit of β-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 β-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.

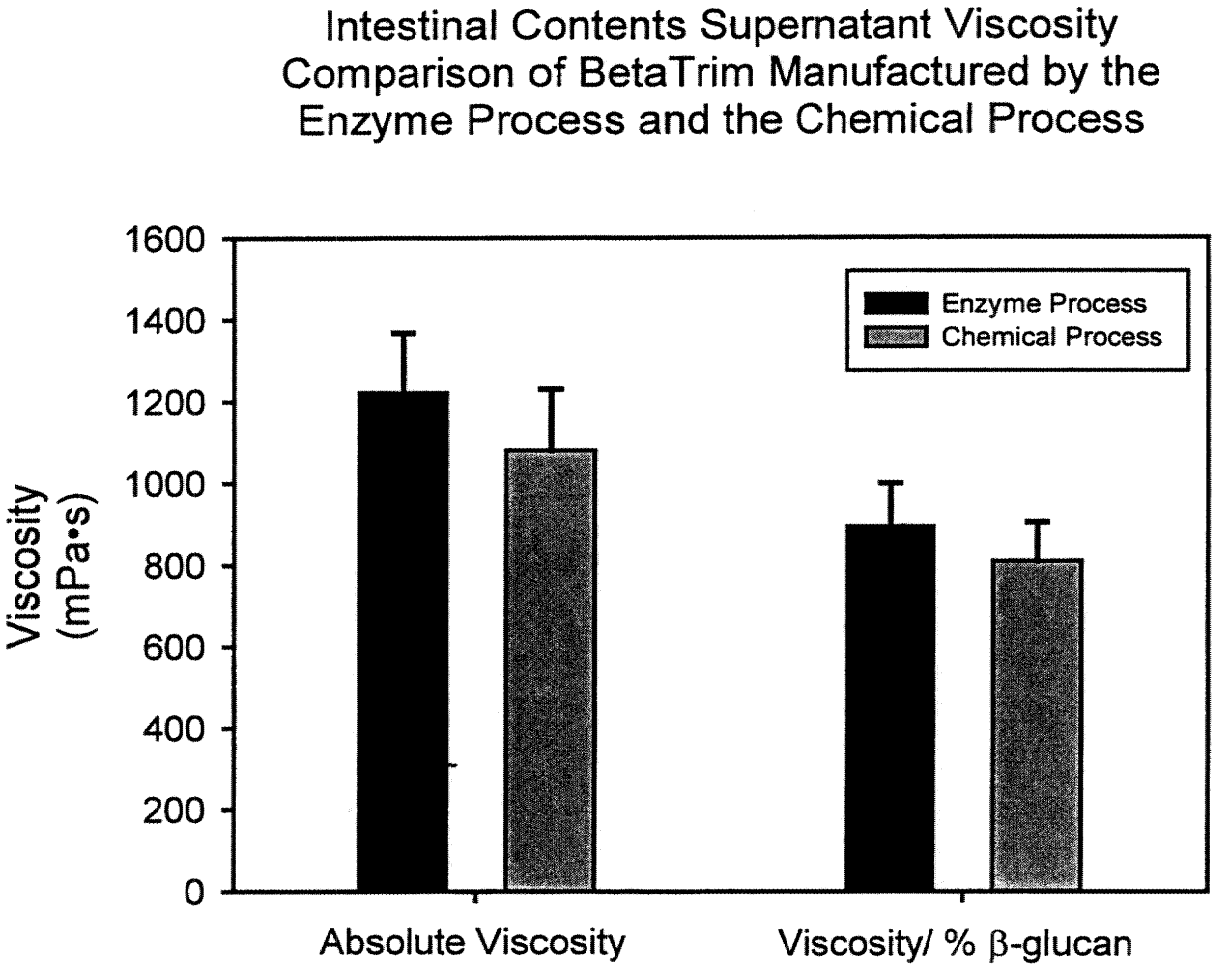
Table 1. Composition of modified AIN-93G diet

| 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) | $4.61 \pm 0.24$ | $3.84 \pm 0.28$ | 0.053 |
| Intestinal contents (g) | $3.23 \pm 0.20$ | $3.51 \pm 0.10$ | 0.23 |
| Contents supernatant (g) | $1.27 \pm 0.12$ | $1.45 \pm 0.07$ | 0.26 |

**Figure 1**




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