Use of Dietary Mannobiose to Reduce Gut Salmonella and Campylobacter in Broiler Chickens and Turkeys

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Abbreviated title: Mannobiose for pathogen control

Summary

Fresh poultry meat is the main risk factor for human Salmonellosis and Campylobacteriosis. Beta-1,4 mannobiose is a novel disaccharide that could bind Salmonella and Campylobacter and eliminate them from poultry gut. A series of studies have been conducted to evaluate the effect of dietary mannobiose on broiler chickens and turkeys. The first broiler study showed that 3 weeks after Salmonella challenge, adding mannobiose at 130 ppm reduced cecal *S. enteritidis* counts by 3.5 log CFU/g ($P < 0.01$). In a second trial with broilers challenged with *S. enteritidis*, 130 ppm of mannobiose was able to completely eliminate fecal Salmonella 19 days after ($P < 0.05$). The third broiler trial showed that mannobiose (130 ppm) was able to reduce cecal Campylobacter counts at day 41 by 1.6 log CFU/g ($P = 0.09$).

Campylobacter was also demonstrated in a turkey trial in which 20 ppm of mannobiose resulted in a reduction of cecal Campylobacter by 0.6 log CFU/g ($P = 0.07$). In all trials, mannobiose did not affect the growth performance of the birds ($P>0.05$). Therefore, mannobiose could be used as a novel additive in poultry diets to control Salmonella and Campylobacter.

Keywords: Mannobiose, Salmonella, Campylobacter, Chickens, Turkeys

Introduction

Campylobacteriosis and Salmonellosis remain as the first- and second-most commonly recorded zoonotic disease respectively in humans in the European Union and USA (EFSA, 2009; Altekruse et al, 2006). Studies have identified that broiler and other poultry meat are important sources of infection (Kimura et al, 2004). Control of Salmonella and Campylobacter in the poultry meat production has been proved to be a constant challenge.

Mannobiose

Mannose and mannose-based oligosaccharides have been investigated using poultry because of their inhibitory properties to the FimH adhesins present in enteric salmonellas (Kisiela et al, 2006). Beta- 1,4 mannobiose is a disaccharide and a potential inhibitor of FimH adhesion. In addition, mannobiose may modulate the immune responses of birds to infections related to Salmonella and other bacteria such as *E. coli* and Campylobacter.

The mannobiose product used in the studies was derived from a coconut meal enzymatic hydrolysate, which contained approximately 13% beta-1,4 mannobiose and only residual mannose (<3% mannose, Fuji Oil Inc, Japan). Mannobiose is more water soluble and more homogenous than the large mannose-containing molecules like manno-oligosaccharides. And it showed stronger *in vitro* yeast anti-coagulation activity than that from mannose. Besides, it was more resistant to bacterial degradation than mannose when incubated with intestinal flora (Fuji Oil Inc).

This article summarized the recent studies with mannobiose in poultry diets for control of Salmonella and Campylobacter. The studies have been conducted in the various research facilities in Japan and Canada.

Results and discussion

Experiment 1: Broiler chickens fed with hydrolyzed coconut meal high in mannose or mannobiose

This trial was conducted at a research facility of Fuji Oil Inc. in Japan (Toshimichi and Yokomizo, 2006). A total of 45 newly hatched broiler chicks were divided equally into three groups. Group 1 (control) was given a commercial broiler starter feed (Itochu Feed Mills Co., Ltd., Japan). Group 2 was fed a feed supplemented with 0.1% of hydrolyzed coconut meal with high mannose content (13.7%, providing 137 ppm mannose and a residual mannobiose of 6 ppm in feed). Group 3 was fed a feed supplemented with 0.1% of the hydrolyzed coconut meal with high mannobiose content (13%, providing 130 ppm mannobiose and a residual mannose of 10 ppm in feed). The chicks were initially raised for one week with free access to the respective feed and water. The birds were then orally inoculated with salmonella (*Salmonella enteritidis* HY-1 strain) by 2.27 x 10^7 CFU per chick at the end of week one. The count of salmonella (log) in the cecal content of the respective group was monitored at weeks one, two and three after Salmonella infection, by sacrificing 5 birds per treatment group weekly.

Table 1. Salmonella counts from the cecal content collected at week 1, 2, and 3 after Salmonella challenge

	Week 1 (log CFU/g)	Week 2 (log CFU/g)	Week 3 (log CFU/g)
Control	1.84	2.00	4.34 [a]
Mannose, 137 ppm	1.84	2.22	2.47 [ab]
Mannobiose, 130 ppm	2.74	1.60	0.80 [b]

Means within a column with different letters differ ($P < 0.01$).

The dietary treatment had no impact to the growth performance of the birds. The analysis of the weekly cecal Salmonella counts showed that by week 3, birds fed a feed with 130 ppm mannobiose resulted in a significant reduction compared to birds fed the control diet (-3.5 log, $P < 0.01$)) or 137 ppm mannose (-1.7 log, $P > 0.05$). This study indicated that mannobiose was more effective than mannose in binding and eliminating Salmonella from chicken gut.

Experiment 2: The influence of mannobiose or mannose on Salmonella colonization in broiler chickens

This study was conducted at the broiler research facility of University of Guelph (Agunos et al, 2007). The purpose was to investigate the effects of beta-1,4 mannobiose supplemented feeds on the kinetics of *Salmonella enterica* serovar *enteritidis* (SE) in broilers. D-Mannose was used for comparison. Both mannobiose (130 ppm) and mannose (137 ppm) were provided by using the same test materials as in Exp. 1. One hundred and twenty (120) day-old broiler chicks were used. All placed birds were Salmonella-free. The test diets were only fed for the first two weeks after hatching to investigate any protection against SE infection in growing birds and any immunomodulatory functions in the gut. After this period, birds were inoculated orally with 2×10^7 CFU SE/ml. Shedding of SE was monitored by faecal collection and culture every two days starting on the day after infection up to 19 d post-infection.

Table 2. Fecal Salmonella counts in the test birds

Days post-infection	Control CFU/g	Mannose CFU/g	Mannobiose CFU/g
1	8.38 [a]	5.73 [b]	6.36 [b]
4	8.51	8.26	7.55
7	5.70 [a]	5.62 [a]	2.26 [b]
10	6.49 [a]	6.30 [a]	3.50 [b]
13	6.30 [a]	2.83 [b]	2.00 [b]
16	5.55 [a]	3.99 [a]	0.00 [b]
19	6.03 [a]	3.04 [b]	0.25 [c]

Means within the same row with different letter differ ($P < 0.05$).

The test articles showed no significant impact on the growth performance of the birds. The fecal Salmonella count was summarized in Table 2. Seven (7) days after infection, birds fed mannobiose had significantly lower SE counts compared to the control or birds fed mannose which was not different from the control. The same result was found at day 10. By day 13, both mannobiose and mannose reduced SE significantly. By day 16, no SE was found in the feces of the birds fed mannobiose, while birds fed mannose still had high counts of SE in the feces. By the end of the

experiment, birds fed with mannobiose only showed very low counts of SE (0.25 log CFU/g), compared with the control (6.03 log CFU/g, $P < 0.05$), or birds with mannose (3.04 log CFU/ml, $P < 0.05$). Adding mannose also showed significant SE reducing effect. The present study indicates that feeding a diet supplemented with mannobiose during the first two weeks after hatching reduced susceptibility to SE infection. In addition, supplementing the diet with mannobiose or mannose increased intestinal IgA production and improved SE clearance by acting as immunomodulatory agents that prevented intestinal pathology (Agnus et al, 2007).

Experiment 3: Mannobiose supplementation on Campylobacter colonization in broiler chickens.

This study was conducted at Broiler Research Facility of Nutreco Canada (Han et al, 2007). The purpose was to investigate the possibility of supplementing mannobiose on the Campylobacter counts in broiler chickens. 180 Ross x Ross day-old broiler pullets were fed a commercial medicated starter diet (control, Nutreco Canada) or a diet containing 130 ppm mannobiose provided by a hydrolyzed coconut meal. There were 6 pens (15 birds/pen) in each treatment. The diets were fed from day 0-13. On Day 14, all birds were fed common medicated starter, grower and finisher feeds until day 41. The birds had free access to feed and water during the experiment. On days 14, 21, 28, and 41, two (2) birds per pen were randomly selected, euthanized and contents of one cecum was collected and enumerated for *Campylobacter* spp. The result was shown in Table 3.

Table 3. Mannobiose supplementation on the cecal Campylobacter counts in broilers

	Day 14 Log CFU/g	Day 21 Log CFU/g	Day 28 Log CFU/g	Day 41 Log CFU/g
Control	2.76	3.25	2.28	2.61
Mannobiose	2.92	2.58	1.65	1.03
P value	ns	ns	ns	0.09

The birds in the control and test groups had similar cecal Campylobacter counts on day 14 or day 21. By day 28, birds fed mannobiose tended to have lower Campylobacter counts than the control. By day 41, mannobiose supplementing

resulted in a reduction of 1.6 log CFU per g compared to the control birds (P = 0.09). This study indicated the potential to use mannobiose in broiler diets as a means for reducing gut Campylobacter colonization. However, the Campylobacter reducing capacity of mannobiose was not as pronounced as its Salmonella reducing capacity, as demonstrated in the previous studies. Further study may be needed to understand the mechanism.

Experiment 4: Supplementing mannobiose or yeast beta glucan on Campylobacter colonization in turkeys

This study was conducted at Turkey Research Facility of Nutreco Canada (Han, 2006, unpublished data). The purpose was to investigate the possibility of supplementing mannobiose on the Campylobacter counts in turkeys. A total of 720 turkey poults were assigned to treatments at arrival. A randomized complete block design was used. There were 6 blocks in the study, each comprised of 4 pens. Pens within block were randomly and equally assigned to the treatments. There were approximately 30 poults per pen and each pen within a block had birds of similar initial bodyweight. A commercial feed formulation program was followed for starter, grower and finisher stage (Nutreco Canada). The feeds contained monensin sodium at 99 ppm (0 – 63 days of age) and 66 ppm (63-77 days of age) but no antibiotic growth promotants. Four dietary treatments were used in the study, including a control, a group with 20 ppm mannobiose (from hydrolyzed coconut meal), a group with 40 ppm mannobiose, or a group with 40 ppm yeast beta glucan (Progressive Bioactives, Canada). Treatment diets were introduced on Day 0 and fed continuously until Day 21. The treatment diets were re-introduced on Day 42 and fed continuously to Day 63. Common feeds were fed between treatment periods according to the feeding schedule. The trial terminated on Day 111. Two birds per pen were randomly selected at Day 111 for collection of cecal contents.

The growth performance of the birds was not affected by different diets. The dietary effect on cecal Campylobacter counts was presented in Table 4. At the end of the trial (day 111), the turkeys treated with 20ppm mannobiose had significantly less Campylobacter counts in the cecal content, compared to those in the control (5.74 vs. 5.13 CFU/g, P = 0.07). Adding 40ppm mannobiose also resulted in a numerical reduction of the counts but was not significant compared to the control (5.74 vs. 5.22 CFU/g, P > 0.1). Yeast beta glucan had no impact on the bacterial counts.

Table 4. Dietary treatments on the cecal Campylobacter counts at the end of the trial

	Cecal Campylobacter log CFU/g
Control	5.74 [a]
Mannobiose 20 ppm	5.13 [b]
Mannobiose 40 ppm	5.22 [ab]
Yeast beta glucan 40 ppm	5.51 [ab]
P value	0.07

Means with the column with different letters differ (P < 0.07).

The trial confirmed what had been found in broiler chickens that adding mannobiose would result in a lower number of Campylobacter in gut. Unlike broiler trials that using high levels of mannobiose (130 ppm mannobiose), only 20 or 40 ppm was used in the turkey trial. Even though mannobiose was intermittently administered in diets from D0 to 21 and again D43 to 63, the effect of mannobiose lasted to the end of the trial. So the results indicated that intermittent inclusion mannobiose could be an effective and economical way to reduce intestinal Campylobacter.

Conclusions

1. Supplementing mannobiose during the early stage of broiler production was able to effectively reduce gut Salmonella.

2. Supplementing mannobiose in broiler diets was able to effectively reduce gut Campylobacter.

3. Addition of dietary mannobiose also showed the potential to reduce gut Campylobacter in turkeys.

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