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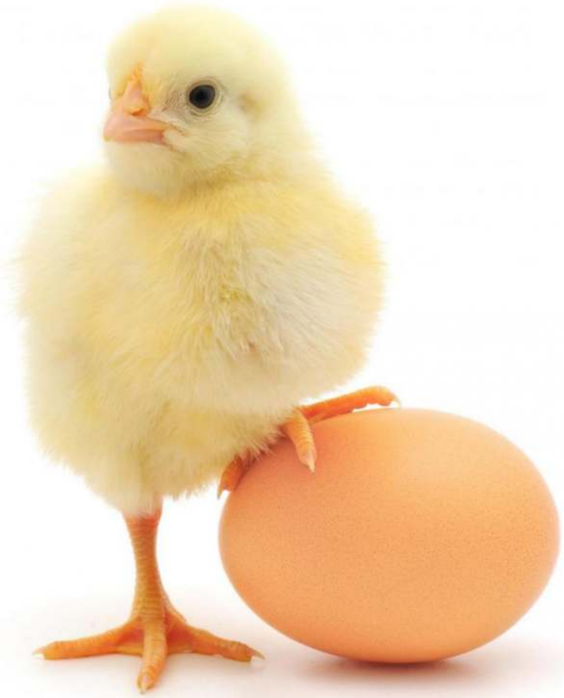

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Effect of Adding Chicory Fructans in Feed on Fecal and Intestinal Microflora and Excreta Volatile Ammonia

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Abstract: This study was conducted to evaluate the effectiveness of adding chicory fructans in feed on fecal ammonia and Microflora. Adding Raftifeed® to the diet reduced volatile ammonia and fecal pH in fresh fecal in the first four weeks of production. Reductions (P<0.05) in total aerobe and *E. coli* counts of fecal broiler were recorded for inulin or oligofructose treatment at the 4th week. Oligofructose increased the *lactobacilli* counts in the gizzard and small intestine contents of female broiler. Inulin or oligofructose supplementation reduced (P<0.05) total *Campylobacter* counts in the large intestine. The *Lactobacilli* counts of fecal in the female birds were also increased when the diets supplemented with either inulin or oligofructose. The *Campylobacter* count of the male birds and the *Salmonella* counts of the female birds were lower (P<0.05) in cecal contents for the chicory fructan supplemented birds.

Key words: Inulin, oligofructose, intestinal microflora, excreta, volatile ammonia

Introduction

One of the major concerns for the poultry industry is the threat of harmful bacteria associated with poultry products. *Campylobacter, Escherichia coli* and *Salmonella* are the primary food borne pathogenic microorganisms associated with poultry that lead to human illnesses. One proposed method to reduce pathogenic microorganisms is the popular and successful development of the competitive exclusion concept. Competitive Exclusion is the principle that prevents entry of one organism into a given environment because that space is already occupied by another organisms (Nurmi and Rantala, 1973). The competitive exclusion concept has lead to the development of commercial products known as probiotics. A probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotics contain single or multiple strains of lactic acid bacteria. They are part of the natural microflora of many animals, and they display antagonistic activity against pathogenic bacteria. Recent research and understanding of probiotics has lead to the development of prebiotics.

Prebiotics are not digested by the animal's digestive enzymes and affect the host beneficially by selectively stimulating the growth and/or metabolic activity of one or more either naturally present, or orally fed (probiotics) bacterial species in the intestine (Young, 1998). Normal intestinal microflora such as, *Lactobacillus spp.* or *Bifidobacterium spp* use inulin or oligofructose for fermentation more efficiently than other groups of bacteria. Bifidobacteria and lactobacilli are considered indicator organisms for a flora which allows good GI functioning of the host. These microorganisms produce short chain fatty acids and lactate on inulin and oligofructose, creating an acidic environment which suppresses the growth of putrefactive proteolytic bacteria.

Prebiotics have shown the ability to decrease colonization of bacteria such as *E. coli*, and *Salmonella,* while increasing the growth of non-pathogenic microorganisms. Bailey (1991) studied the effect of oligofructose on *Salmonella* colonization of the chicken intestine, and reported that feeding the beta(2-1) fructan in the diet of chickens may lead to a shift in the intestinal gut microflora and in some cases reduce susceptibility of colonization. Chambers *et al.* (1997) reported that broilers fed crude oligofructose had higher *Salmonella* counts than all other broiler groups: whereas, broilers fed refined oligofructose had lower infections than control broilers. Although prebiotic Broil act has been successful in lowering levels of *Salmonella*, it was found to be ineffective against *Campylobacter* (Aho *et al*., 1992). Few if any reports concerning the effects of oligofructose structure on *Salmonella* and other harmful bacteria have been reported.

Ammonia is of great concern for the poultry industry. Some possible effects to birds are air sacculitis, viral infections, reduced production, and condemnation. Based on flock of 20,000 birds, costs for a farmer due to complications and losses because of high ammonia levels are approximately $320 (Lacey, 1991). When calculated with the approximate 7.6 billion birds produced in a year, this is a tremendous cost to farmers nationwide. In poultry farming, odor generation, emissions, and movement are very complicated. By lowering the ammonia levels in the chicken houses, harsh working conditions for farmers, along with respiratory difficulty in chickens, may be prevented. Ammonia has shown to damage the bird's respiratory tract. The best approach to controlling odor in poultry farming seems to be by eliminating the source of the odor rather than capturing the odor and treating it. An obvious way to reduce odors is to modify the diet fed to the animals. Since lactosucrose has shown potential in reducing ammonia odor, a diet containing oligofructose or inulin may just be a solution to this ammonia problem. Research on the potential of prebiotics for improving growth performance and reducing presence of pathogenic microorganisms and harmful ammonia emission in poultry farming are not only timely but significant contribution to the continued growth of the poultry industry. Therefore, the objective of this research was to study the effectiveness of adding oligofructose to the diet on fecal and intestinal microflora and excreta ammonia.

Materials and Methods

Birds and Diets: Ninety-eight chicks (day old chick) consisting of 48 male and 48 female, hatchery vaccinated (Mareks and IBDV), Ross x Ross strain, were obtained from Peco Farms in Gordo, Alabama. Birds were assigned to twelve 75x65x35 cm cages with four birds per cage. Four cages male and four cages female were randomly assigned to each of the following treatments:

1. Control feed,
2. Feed with 1.0% Raftifeed® IPF (inulin) which was obtained by extracting chicory roots with hot water. It is a beta (2-1) fructan with chain length varying between degree of polymerization (DP) 3 to 60 with average DP of 9.
3. Feed with 1.0% Raftifeed® OPS (oligofructose) which is a partial enzymatic hydrolysate of chicory inulin, and has a DP ranging between 3 and 8 (DP average of 4).

Inulin and oligofructose were produced by Orafti, N.V., Belgium. Birds were housed in a room with continuous lighting and maintained at a temperature of 32 °C initially. After two weeks, the temperature was reduced to 24 °C and where it remained for the rest of the feeding period. Chicks had free access to antibiotic-free feed with Starter diets contained 3150 kcal/kg ME and 21.31% crude protein (CP) and grower diets contained 3200 kcal/kg ME and 19.79% CP (Table 1) and water during the 6-week grow out period.

Table 1: Composition of experimental broiler basal diets

Ingredient	Starter	Grower
	% of diet	
Corn	57.55	61.72
Soybean	33.86	30.00
Poultry Fat	4.42	4.45
Dical P(Phosphor Sources)	1.78	1.65
Limestone (Ca sources)	1.34	1.16
NaCl	0.47	0.46
DL-Methionine	0.32	0.24
Vitamin/Mineral	0.25	0.25
L-Lysine HCL	0.01	0.07
Total	100.00	100.00

Starter diet: from 1 to 4 weeks of age (21.31% CP and 3150 kcal ME)

Grower diet: from 4 to 6 weeks of age (19.79% CP and 3200 kcal ME)

Analyses

Microbiological Analysis: Fresh fecal samples were collected by placing a 54 cm x 81 cm sterile aluminum foil on the bottom of each cage, 12 hr prior to the fecal collection. Fifty grams of fecal materials were removed from the replica cages of each treatment and placed into a sterile 50 ml centrifuge tube. Microbiological analyses were conducted at 2, 4, and 6 wk of broiler age.

At 6 wk, the digestive tract contents were also sampled and analyzed microbiologically. Total aerobes, *Coliform/ E. coli.*, *Salmonella* were enumerated by using plate counts agar (Difco, Detroit, MI), 3 M *E. coli* Petri-Film (3M, St. Paul, MN) and Brilliant Green Agar (Difco, Detroit, MI), respectively. Plates were incubated at 30 °C for 24 hrs. *Lactobacilli* counts were enumerated by using MRS Agar (Difco) and incubated in an anaerobic chamber at 37 °C for 48 hrs. *Campylobacter* Agar kits with Blaser supplement (Difco, Detroit, MI) in a microaerophilic system (5% oxygen, 10% carbon-dioxide, balance nitrogen, Bacton Dickenson, Cockeysville, MD) were used to enumerate the *Campylobacter* count after incubating the plates at 42 °C for 24 hrs.

Volatile Ammonia: Volatile ammonia contents of the fecal material were measured weekly for 6 wk using a Kitagawa Toxic Gas Detector (Kitagawa, Matheson, MJ) and ammonia detecting tubes (0-20 ppm capacity, Matheson, NJ). Two hundred grams from each of the fecal materials were collected and placed into a 500 ml beaker and covered with plastic wrap. The beakers were incubated at 37 °C for 60 min prior to the measurement.

Fecal pH: The pH of fecal content was measured weekly using a pH meter (Model LS, Sargent-Welch Co., Springfield, NJ). Two hundred grams each of the fecal material were placed into 500 ml beakers and brought to the lab for pH measurements.

Fecal Moisture Content: Fecal moisture contents were determined weekly according to the method as

Table 2: Effect of dietary supplementation of inulin and oligofructose on broiler fecal microflora at 14, 28, and 42 days of broiler age

Microbial Group	Bird Age	Male			Female		
		Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
		Log cfu/g					
MRS Media							
(*Lactobacilli*)	14d	9.54a	9.70a	9.72a	9.53a	9.85a	9.74b
	28d	9.12ab	9.30b	8.81a	9.29a	9.46a	9.34a
	42d	9.19ab	9.07a	9.35b	9.02a	9.27b	9.23b
Total Aerobes							
	14d	9.80a	9.74a	10.03a	9.66ab	9.59a	9.97b
	28d	8.94b	8.96b	8.35a	8.97a	9.40b	8.65a
	42d	9.15a	8.82a	9.19a	9.13a	8.99a	9.27a
E.coli/Coliform							
	14d	8.66a	8.99b	9.71c	8.77ab	8.99b	8.21a
	28d	8.89b	8.49ab	8.28a	9.06b	8.61ab	8.18a
	42d	8.85a	8.31a	8.46a	8.89a	8.43a	8.85a
B.G. Media							
	14d	7.73a	7.44a	7.75a	7.46a	7.68ab	7.99b
	28d	7.80a	7.55a	7.29a	7.85a	7.12a	7.44a
	42d	9.01a	8.63a	8.54a	8.94ab	8.42a	9.01b
Campylobacter							
	14d	3.95a	4.18a	4.26a	4.03a	4.15ab	4.58b
	28d	4.25a	3.96a	3.72a	4.32a	4.24a	4.53a
	42d	5.51a	5.44a	5.36a	5.92a	5.53a	5.61a

Each mean represents four observations. Mean of each sex group within a row not followed by a common letter are significantly different (P<0.05).

described in AOAC (AOAC, 1990). Five grams of fecal material were measured into aluminum dishes and dried in an oven at 96 ^{o}C for 3 hr. Readings were taken after samples were cooled in a desiccator.

Statistical Analysis: The design for this experiment was a Completely Randomized Design (CRD) with four replications. Data were analyzed with the Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS Institute, 1990). When significant differences (P<0.05) were detected, the least significant difference (LSD) test was used to separate the mean values (Steel and Torrie, 1980).

Results and Discussion

Effects of Dietary Supplementation of Inulin and Oligofructose on Broiler Microflora

Fecal Microbial Contents: In general, regardless of the treatment, no differences (P>0.05) in total aerobe, *Lactobacilli, Salmonella, and Campylobacter* counts were found at 2, 4, and 6 wk of age for the male birds (Table 2). However, significant reductions in total aerobe and *E. coli* (P<0.05) counts were recorded between the oligofructose treatment and the control at the 4th week.

Francis *et al.* (1978) and Fuller (1977) reported that *Lactobacillus* products added to poultry diets significantly decreased of coliform counts in the cecal small intestine and crop. Another study by Orban *et al.* (1997) studied the effect of the prebiotic, oligosaccharide, on coliform in the cecal of broilers and it was also determined that there was a reduction in coliform count.

For female birds, the effects of inulin and oligofructose on fecal microflora were not consistent. In general, there were increases in *Lactobacilli* counts, especially after 6 wk of feeding. Patterson *et al.* (1997) reported that anaerobically enumerated *lactobacilli* concentration of cecal content in kestose-treated birds was increased sevenfold compared to the control. It is of interest to note that, for the first two weeks, the *Salmonellae* counts in the fecal material were higher for the birds fed a diet containing oligofructose.

Gizzard Microbial Contents: In general, no difference (P>0.05) in total aerobe counts, *Lactobacilli, Salmonella, E. coli/coliform*, and *Campylobacter* were found in the gizzard contents among the broilers (Table 3). However, supplementing diets with oligofructose increased the *Lactobacilli* counts in the gizzard contents. The chicory fructans were shown to increase *Lactobacillus spp*. and *Bifidobacterium* in previous work by Mitsukoa *et al*., 1987; Hidaka *et al*., 1986.

Table 3: Effect of dietary supplementation of inulin and oligofructose on microflora of gizzard content at 42 days of broiler age

Microbial Group	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
	Log cfu/g					
MRS Media (*Lactobacilli)*	6.37a	7.06ab	7.31b	7.16b	6.68a	7.86c
Total Aerobes	7.16a	7.61a	7.41a	7.48a	7.62a	7.73a
*E.coli/*Coliform	3.40a	3.50a	4.22a	3.15a	4.11b	3.64ab
B.G. Media	2.42a	3.32ab	3.65b	2.79a	3.72a	2.86a
Campylobacter	2.48a	2.64a	2.92a	2.46a	3.07a	2.93a

Each mean represents four observations. Mean of each sex group within a row not followed by a superscript letter are significantly different (P<0.05).

Table 4: Effect of dietary supplementation of inulin and oligofructose on microflora of small intestine content at 42 days of broiler age

Microbial Group	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
	Log cfu/g					
MRS Media (*Lactobacilli)*	8.91ab	8.66a	8.99b	8.01a	8.62ab	8.89b
Total Aerobes	9.16a	9.03a	8.90a	9.20a	9.03a	9.24a
*E.coli/*Coliform	8.30a	7.50a	7.36a	8.25b	7.74a	8.27b
B.G. Media	8.34a	7.69a	7.81a	8.14a	7.81a	8.13a
Campylobacter	3.55a	3.70a	3.19a	3.78a	3.58a	3.54a

Each mean represents four observations. Mean of each sex group within a row not followed by a superscript letter are significantly different (P<0.05).

Small Intestine Microbial Contents: In general, regardless of the treatment, no differences (P>0.05) in total aerobes, *E. coli/coliform, Salmonellae,* and *Campylobacter* counts were recorded among the birds. Again, oligofructose supplementation increased the *Lactobacilli* counts in the small intestine (Table 4). Patterson *et al.* (1997) reported that anaerobically enumerated *Lactobacilli* concentration in kestose-treated bird were 7-fold higher when compared to those of the controls.

Large Intestine Microbial Contents: Supplementing diets with inulin or oligofructose significantly reduced (P<0.05) total *Campylobacter* counts in the large intestine. The *Lactobacilli* counts in the female birds were increased when the diets were supplemented with either inulin or oligofructose (Table 5). Stern (1994); Mead *et al.* (1996); Schoeni and Wong (1994) reported that the probiotic strain bacteria were effective in reducing the colonization of *Campylobacter* within the intestine of poultry. Morishita *et al.* (1997) claimed that a probiotic containing *Lactobacillus acidophilus* and *Streptococcus* were capable of reducing *Campylobacter* loads by 70%. However, Aho *et al.* (1992) reported that a commercial probiotic, Broil act, was ineffective against *Campylobacter*.

Cecal Intestine Microbial Contents: Among the microflora tested, the *Campylobacter* count of the male birds and the *Salmonella* counts of the female birds were lower for the inulin and the oligofructose supplementation (Table 6). Dietary chicory fructans reduced cecal colonization by *Salmonella typhimurium* (Choi *et al.,* 1994), counts of *S. typhimurium*, and chicken of market age (Waldroup *et al.*, 1993). Bailey (1991) reported that chickens treated with 0.75% oligofructose had a four-fold reduction in the level of *Salmonella* in the cecal.

The reduction of *Campylobacter* and *Salmonella* might be due to increase in cecal acidity. Corrier *et al.* (1990) reported that dietary lactose increased cecal acidity and influenced intestinal flora involved in control of enteropathogens in poultry. Lower *Salmonella* was believed to be achieved by stasis caused by low pH or elevated levels of volatile fatty acids.

Fecal Volatile Ammonia: The volatile ammonia contents of the fresh fecal from the week-old chicks were very low and the readings increased with age. The volatile ammonia from fresh fecal sample peaked around the 4^{th} and 5^{th} week of age, regardless of the treatment (Table 7). Adding oligofructose to the diet reduced (P<0.05) the volatile ammonia in fresh feces in the first four weeks of production. However, no significant (P>0.05) reduction was observed during the 5^{th} and 6^{th} weeks. Little or no effect on volatile ammonia

Table 5: Effect of dietary supplementation of inulin and oligofructose on microflora of large intestine content at 42 days of broiler age

Microbial Group	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
	Log cfu/g					
MRS Media (*Lactobacilli*)	9.18a	9.35a	9.22a	8.97a	9.34b	9.40b
Total Aerobes	9.22a	9.10a	8.90a	8.93a	9.40b	9.49b
E.coli/Coliform	8.85a	7.90a	8.12a	8.60b	8.08a	8.73a
B.G. Media	8.53a	8.22a	7.82a	8.92a	8.04a	8.83a
Campylobacter	5.32b	4.36ab	3.69a	5.37b	3.78a	4.12a

Each mean represents four observations. Mean of each sex group within a row not followed by a superscript letter are significantly different (P<0.05).

Table 6: Effect of dietary supplementation of inulin and oligofructose on microflora of cecal intestine content at 42 days of broiler age

Microbial Group	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
	Log cfu/g					
MRS Media (*Lactobacilli*)	9.18a	9.00a	9.32a	8.95a	9.07a	9.00a
Total Aerobes	8.59a	8.35a	8.29a	8.89a	8.47a	8.70a
E.coli/Coliform	7.99a	7.96a	7.65a	8.69b	7.57a	7.71a
B.G. Media	8.26a	7.79a	7.86a	8.65b	7.56a	7.71a
Campylobacter	5.29b	4.18ab	3.30a	4.43a	4.14a	4.07a

Each mean represents four observations. Mean of each sex group within a row not followed by a superscript letter are significantly different (P<0.05).

Table 7: Fecal volatile ammonia concentration (ppm) of broiler as effected by inulin and oligofructose supplementation

Week	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
1	0.25a	0.33b	0.33b	0.21a	0.42ab	0.59b
2	7.63b	3.13a	3.13a	7.50b	4.13a	2.00a
3	3.63b	5.88b	1.25a	4.38b	5.13b	1.38a
4	14.25b	11.25b	3.50a	10.00b	13.75b	2.93a
5	7.12a	7.50a	5.00a	12.88a	10.50a	9.38a
6	4.13a	2.75a	3.88a	2.56a	2.13a	2.50a

Each mean represents four observations. Mean of each sex group within a row not followed by a common letter are significantly different (P<0.05).

content was observed for the inulin treatment.

Terada *et al.* (1994) reported that the cecal concentration of ammonia, phenol and cresol were decreased on Day 62 of lactosucrose consumption, and on Day 62 of lactosucrose consumption, environmental ammonia and the odor of chicken cecal were greatly reduced. Reducing crude protein (CP) from 215 g/kg (21.5% CP) to 196g/kg (19.6% CP) caused equilibrium ammonia gas concentration and litter N to decline by 31 and 16.5%, respectively (Ferguson *et al.*, 1998). The change in free ammonia content between the 5th and 6th week might be due to the change of diet.

Fecal pH and Moisture: In general, fecal pH values were lower (P<0.05) for the oligofructose treated broilers during the first 3 to 4 weeks of age, but this difference disappeared during the 5-6 weeks of age, regardless of the bird sexes (Table 8). Earlier, Chambers *et al.* (1997) reported that mean cecal pH of broiler fed rations with added oligofructose or lactose derivatives were lower than those of control broilers. This treatment difference for cecal pH disappeared within one week of withdrawal of carbohydrates from 5-wk-old broilers.

Unlike other parameters, generally, no difference

Table 8: Broiler fecal pH as effected by inulin and oligofructose supplementation

Week	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
1	4.88a	5.10b	5.15b	5.70b	5.55ab	5.38a
2	7.40b	7.05b	6.06a	7.50b	7.13b	6.65a
3	7.35b	7.20b	6.60a	7.52c	7.13b	6.33a
4	7.60b	7.35b	6.63a	7.05a	7.13b	6.95a
5	7.13a	7.28a	6.73a	7.15a	7.00a	6.85a
6	7.00a	6.58a	6.83a	6.68a	6.25a	6.43a

Each mean represents four observations. Mean of each sex group within a row not followed by a common letter are significantly different (P<0.05).

Table 9: Effect of inulin and oligofructose supplementation on fecal moisture content

Week	Male			Female		
	Control	Inulin	Oligofructose	Control	Inulin	Oligofructose
	%					
1	69.86a	68.48a	69.86a	71.85a	73.50a	71.22a
2	79.36a	78.54a	79.21a	79.79a	79.41a	80.67a
3	79.36a	79.17a	80.28a	78.44a	81.06a	83.09a
4	81.30b	75.97a	73.35a	80.33a	78.49a	79.75a
5	82.32a	80.57a	80.09a	85.22b	80.14ab	78.49a
6	81.45a	81.74a	82.03a	82.12a	82.42a	82.17a

Each mean represents four observations. Mean of each sex group within a row not followed by a common letter are significantly different (P<0.05).

(P>0.05) in moisture contents among the fecal samples was observed throughout the entire experiment. During the 4th week and 5th week, inulin and oligofructose treated broilers had a lower fecal moisture contents (Table 9).

Conclusions: Oligofructose added to the diet reduced the volatile ammonia, and fecal pH in fresh fecal during the first four weeks of production. Little or no effect on volatile ammonia content or fecal pH were observed for the inulin treatment. No differences (P>0.05) in moisture contents among the fecal samples were observed throughout the entire experiment. During the 4th week and 5th week, inulin and oligofructose treated broilers had lower fecal moisture contents. Significant reductions in total aerobe and *E. coli* (P<0.05) counts were recorded between the oligofructose treatment and the control at the 4th week for male birds. Supplementing diets with oligofructose increased the *Lactobacilli* counts in the gizzard, and small intestine contents for female broiler. Supplementing diets with inulin or oligofructose significantly reduced (P<0.05) total *Campylobacter* counts in the large intestine. The *Lactobacilli* counts in the female birds were increased when the diets were supplemented with either inulin or oligofructose. Among the microflora tested, the *Campylobacter* count of the male birds and the *Salmonella* counts of the female birds were lower in cecal contents for the inulin and the oligofructose supplemented birds.

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