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Fructooligosaccharides supplementation: effects on broiler chicken performance, intestinal morphology, microbial community, and stress indicators

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Abstract Feeding broilers with different amounts of fructooligosaccharides (FOS) under conditions of higher stocking densities and reused litter did not affect growth performance ($p>0.05$). However, FOS supplementation improved gut health by increasing villus height to crypt depth ratio. It also promoted the growth of *Lactobacillus* and reduced the incidence of *Escherichia coli*. The decrease in heterophils to lymphocytes ratio in FOS-supplemented group indicates a reduction in stress levels.

Keywords: Prebiotic, Gut morphology, Microbiota, Stress index, White blood cells

Introduction

Antibiotics are frequently used in broiler feed to prevent pathogenic microorganisms and support growth (Mehdi *et al*., 2018). However, antibiotic use can lead to resistant bacteria and residue risks, posing potential risks to both animals and consumers. To address this, broiler producers prioritize natural extracts that promote beneficial bacteria and inhibit harmful ones. Exports to the European Union are prohibited from utilizing antibiotics in animal production (Castanon, 2007). To avoid using antibiotics, animal nutritionists have developed alternative supplementation techniques. For example, prebiotics and probiotics can be used to support the growth of beneficial bacteria. Prebiotics serve as nourishment for the resident intestinal probiotics.

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They are defined as nondigestible ingredients by enzymes present in the digestive tract. In contrast, intestinal microorganisms can degrade them which beneficially affects the host (Kumar *et al*., 2019). The most studied prebiotics are fructooligosaccharides (FOS) classified as a carbohydrate type of biomolecule. FOS, which is naturally present in some high-fiber cereals, is fermented by bifidobacteria and lactobacilli to form short-chain fatty acids (SCFAs) such as lactic, butyric, and propionic acids (Besten *et al*., 2013). These acids play a role in reducing harmful bacteria like *Salmonella* and *Escherichia (E.) coli* (Bogusławska-Tryk *et al*., 2012). FOS supplementation improved broiler growth performance, as noted by Shang (2014). By increasing SCFAs and lactic acid production in the cecum, FOS helps to limit the proliferation of pathogens (Ricke, 2015), support the structural integrity of the intestine and improve immune defense (Xu *et al*., 2003; Emami *et al*., 2012). Furthermore, adding prebiotics to animal feed promotes the growth of the animals' natural microorganisms (Ricke, 2015). In the case of intensive raising broilers, they are often raised in conditions that cause stress, such as rearing in a high stocking density and using recycled litter to reduce production costs, supplemented prebiotics are necessary. According to limited information about supplemented prebiotics in raising broilers under stress conditions, the experiment of supplemented different levels of FOS was performed. Therefore, this research focused on investigating how FOS supplementation affected the growth performance, intestinal structure, microbial communities, and stress markers in broilers raised broilers under stress conditions.

Materials and methods

Animal ethics

The committee of animal treatment for scientific work at Kasetsart University at Kamphaeng Saen Campus (ACKU66-AGK-027) approved all animal experiments.

Animals, diets, treatments, and raising procedures

The experiment included 900 one-day-old male Ross 308 chicks reared for 38 days at the Animal Research and Development Center, Kasetsart University, Kamphaeng Saen Campus, Thailand. The chicks were randomly assigned to three treatments (T) with 10 replicates of 30 chicks each, housed in 1.45 × 1.95 m² pens. The study incorporated three phase-specific basal diets: starter for days 1–14, grower for days 15–24 and finisher for days 25–38. The

dietary composition is detailed in Table 1. Each treatment of chicks received the same diet but supplemented with different percentages of FOS for 1-14 days as follows: T1 was chicks fed without any supplement (control), T2 was the diet supplemented with 0.2 % FOS and T3 was the diet supplemented with 0.5 % FOS. The chicks were reared under stress with recycled litter and the rearing density was 11 birds per square meter, which surpassed the standard of 10 birds per square meter (Thema *et al*., 2022). They were reared in the evaporative cooling system house and fed *ad libitum* with water and feed.

Table 1. Diet compositions of experimental diets

Ingredient (%)	Starter	Grower	Finisher
Corn	55.00	58.39	61.67
Soybean meal 48% CP	30.70	25.28	19.91
Full-fat soybean	8.00	10.00	12.00
Vegetable oil	1.84	2.41	2.98
Monocalcium phosphate	1.28	1.05	0.83
Limestone	1.13	1.03	0.92
Pellet binder (Pelex Dry)	0.30	0.30	0.30
Salt	0.05	0.08	0.12
Broiler vit/min premix	0.20	0.20	0.20
DL-Methionine	0.32	0.27	0.24
L-Lysine HCl	0.26	0.23	0.19
Sodium bicarbonate	0.34	0.28	0.23
Choline Chloride 60%	0.10	0.09	0.09
Antimold	0.20	0.20	0.20
Salinomycin 12%	0.06	0.06	0.06
Phytase	0.01	0.01	0.01
Metabolizable energy (kcal/kg)	3,000.00	3,100.00	3,200.00
Crude protein	23.00	21.50	19.50

Data collection

Production performance

The body weights of chickens were measured on days 1 and 38 to evaluate body weight gain (BWG) and average daily gain (ADG). Feed intake (FI) was assessed by recording the feed supplied and feed residues for each replicate, which was used to determine the feed conversion ratio (FCR).

Intestinal sampling and methods for measuring intestinal morphology

On day 38, intestinal morphology was assessed by sampling two birds per replicate. A 2 cm mid-jejunum section was fixed in 10% buffered formalin, paraffin-embedded, cut into 5 µm slices, and stained with hematoxylin and eosin (Iji *et al*., 2001). Microscopic analysis at 400x magnification with images

taken with a digital camera and processed with image analysis software allowed measurement of villus height (VH) — from the brush border membrane to the villi-crypt junction — and villus width (VW) — across the villi-crypt junction. The villus surface area (VSA) was calculated using the formula $(2\pi)(VW/2)(VH)$ (Sakamoto *et al*., 2000). Crypt depth (CD) was also assessed to determine the VH:CD ratio (Rahman *et al*., 2017).

Intestinal collection for measuring microbial community

On day 38, cecal digesta samples were collected from two randomly selected chickens per replicate to assess intestinal microorganisms. The spread plate technique (ISO 15214, 1998) was used for preparing suspensions, inoculation, incubation, and enumeration of *Lactobacillus*. *Salmonella* and *E. coli* were measured following ISO 6579-1 (2020) and Feng *et al*. (2002).

Blood sampling and methods for measuring types of white blood cells for stress indicators

On day 38, 3 ml venous blood samples were collected from two chickens per replicate into ethylenediaminetetraacetic acid (EDTA) tubes. White blood cells, including heterophils (H), lymphocytes (L), monocytes, eosinophils, and basophils, were counted from Wright-Giemsa-stained smears under 100x magnification using DP73 software. The H:L ratio was assessed as a stress marker (Gross and Siegel, 1983).

Statistical analysis

A completely randomized design was used in the study. Descriptive statistics summarized the data, and ANOVA (F-test) examined mean differences between treatments. The Duncan's New Multiple Range test compared means between groups. Data were analyzed with IBM SPSS Statistics version 26. The statistical model equation is:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} is the observed dependent variable, μ is the overall mean, τ_i is the effect of treatments, and ε_{ij} is the random error.

Results

Production performance

The results in Table 2 indicate that growth performance was not affected by FOS supplementation during the 38-day rearing period (P > 0.05).

Table 2. Performance of Broilers Supplemented with FOS

Trait	Control	FOS 0.2%	FOS 0.5%	Pooled SE	P-value
Initial weight (g)	42.85	43.09	43.20	0.1774	0.733
Body Weight (g)	2,978.83	2,988.18	2,985.12	26.5737	0.990
Body Weight Gain (g/b)	2,935.97	2,945.08	2,941.93	26.5964	0.991
Average daily gain (g/d)	77.256	77.495	77.411	0.7000	0.991
Feed Intake (g/b/d)	4,048.60	4,015.49	4,027.69	29.1932	0.944
FCR	1.379	1.363	1.369	0.0075	0.808

Intestinal morphology

FOS supplementation significantly influenced the intestinal morphology of broilers. The inclusion of 0.5% FOS notably increased jejunal VH:CD ratio (P<0.01), which could potentially improve nutrient absorption in the birds' intestines.

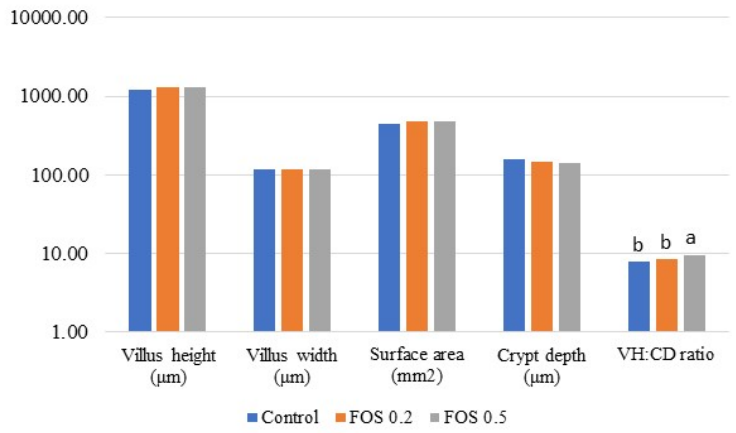

Figure 1. Jejunal morphology of broilers as influenced by FOS supplementation
[a,b] Means in column with distinct superscripts differ significantly (P<0.01).

Microbial community

As shown in Table 3, FOS supplementation influenced the intestinal microbiota of broilers. FOS supplementation tended to reduced *E. coli* populations (P<0.05). Additionally, *Lactobacillus spp*. significantly increased compared to control (P<0.01). However, FOS supplementation did not affect the populations of *Salmonella spp.* in the cecum.

Table 3. The effect of FOS supplementation on broiler intestinal microbiota (log CFU/g)

Trait	Control	FOS 0.2	FOS 0.5	Pooled SE	P-value
Salmonella spp.	N/A	N/A	N/A	-	-
Escherichia coli	7.373[a]	6.707[b]	6.802[b]	0.1103	0.027
Lactobacillus spp.	7.532[b]	7.952[a]	8.174[a]	0.0815	0.002

[a,b] Means in column with distinct superscripts differ significantly.

Stress indicators

Table 4 shows the impact of FOS supplementation on the broilers' white blood cell profiles. There were no significant variations in monocytes, eosinophils, and basophils between the experimental and control groups (P>0.05). Notably, there were enhancements in H, L, and H:L ratio among treatments (P<0.01). L counts were significantly higher in broilers supplemented with FOS compared to control group (P<0.01). Furthermore, FOS-supplemented group had lower H levels and H:L ratio, suggesting lower stress levels than the control group (P=0.000).

Table 4. Effect of FOS supplementation on the percentage of white blood cells in broilers at 38 days of age

Trait	Control	FOS 0.2	FOS 0.5	Pooled SE	P-value
Heterophil	39.20[a]	37.10[b]	36.10[b]	0.3797	0.001
Lymphocyte	41.40[b]	45.10[a]	46.90[a]	0.6656	0.001
H:L ratio	0.947[a]	0.823[b]	0.770[b]	0.2139	0.000
Monocyte	12.30	10.85	10.60	0.3276	0.680
Eosinophil	2.80	2.80	2.65	0.1773	0.929
Basophil	4.50	4.45	4.56	0.1707	0.757

[a,b] Means in column with distinct superscripts differ significantly (P<0.01).

Discussion

This study found that FOS supplementation had no impact on broiler growth performance. In contrast, Xu *et al*. (2003) observed higher weight gain in broilers fed 0.4% FOS compared to controls. On the other hand, Mikkelsen *et al*. (2004) found reduced body weight in broilers supplemented with 0.5% FOS, which may be due to increased gas production in the intestines. This result is consistent with Shang (2014), who reported that 1% FOS supplementation had adverse effects such as diarrhea and gas formation due to FOS fermentation in the gut, which ultimately lowered productivity. Broiler growth performance in this study may have been influenced by factors such as age, sex, health status, environmental conditions, and FOS supplementation level (Yang *et al*., 2009), regardless of supplementation levels.

The research discovered that adding FOS to the diet impacted the structure of the jejunum, increasing the VH:CD ratio. This means the villi in the intestine are longer, and the crypts are shallower, creating a larger surface area for absorbing nutrients. A higher VH:CD ratio indicates a healthy intestine with efficient nutrient absorption, minimizing energy wastage. This leads to more efficient nutrient absorption, reduced energy requirements, improved feed conversion ratios, lower feed costs, and enhanced economic efficiency. Xu *et al.* (2003) found that adding 0.4% FOS to broiler diets increased ileal villus height and reduced crypt depth in both the jejunum and ileum, which ultimately led to a higher VH:CD ratio in 49-day-old broilers compared to those without FOS supplementation. Similarly, Shang *et al*. (2015) found that broilers receiving 0.5% FOS had increased ileal villus height relative to those fed diets with or without antibiotics, even though no significant difference in the VH:CD ratio was observed. Xu *et al*. (2003) explained that a decreased VH:CD ratio indicates shorter villi and larger crypts, suggesting a greater need for new tissue and a higher tissue turnover rate, which increases nutrient maintenance requirements. Inadequate nutrient absorption triggers and increases in gastrointestinal secretion, leading to diarrhea, decreased disease resistance, and reduced animal efficiency due to shorter villi and larger crypts. These changes are more likely caused by FOS improving intestinal microbial ecology rather than directly affecting intestinal tissue.

This study demonstrated that FOS supplementation decreased the pathogenic *E. coli* population and increased beneficial *Lactobacillus spp*. *Lactobacillus* in the intestines provides several benefits, such as improving digestion and nutrient absorption (Bjerrum *et al*., 2006). It supports the immune system by producing antimicrobial substances and stimulating immune responses, thereby reducing the risk of infections (Klasing, 2007). By lowering

the gut pH through lactic acid production, *Lactobacillus* creates an environment less hospitable to harmful pathogens (Corr *et al*., 2007). It also aids in preventing and treating diarrhea, reduces inflammation, and helps maintain a healthy gut flora balance.

Surrayai and Khalaifah (2022) found that young broilers, aged 3 and 5 days, fed with FOS supplementation exhibited similar levels of intestinal microorganisms, including lactic acid bacteria and *E. coli*. Since the intestines of young chickens are still developing and vulnerable to pathogenic bacteria, FOS can be beneficial by selectively promoting the growth of beneficial microbes like lactic acid bacteria while inhibiting harmful pathogens. Furthermore, FOS supplementation is known to enhance broiler growth performance. In a similar study, Akbaryan *et al.* (2019) compared the effects of resistant starch, FOS, and zinc bacitracin (ZnB) on caecal *Lactobacillus* and coliform bacteria in 35-day-old broilers. They found that the number of *Lactobacillus* was higher in broilers fed 0.4% FOS than in the other experimental groups. But Coliform counts were no different from broilers fed with 1 and 2% resistant starch but lower counts than the control groups. The ZnB-supplemented group had lower Coliform counts than the FOS-supplemented group.

Broilers supplemented with FOS in this study showed a reduced stress response, reflected by a lower H:L ratio compared to the control group. Consistent with Shang *et al.* (2015), who reported that broilers supplemented with 0.5% FOS had lower heterophil counts compared to those fed without antibiotics (virginiamycin and monensin). These results indicate that FOS supplementation in broiler diets may contribute to a reduced susceptibility to *Salmonella* colonization.

In conclusion, no effect on growth performance was observed in broiler chickens fed with 0.2% and 0.5% FOS under conditions of high stocking density and recycled litter. However, supplementation at the 0.5% level improved jejunal morphology by promoting the growth and expansion of intestinal epithelial cells, leading to an increased VH:CD ratio. In addition, FOS 0.5 % increases *Lactobacillus* spp, reduces *E. coli*. FOS supplementation reduces the H:L, a stress susceptibility indicator in broilers.

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