Production of FOS (Fructooligosaccharides) with fructosyltransferase enzymes and their effect on nutrient digestion in broilers

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Abstract. This study aims to determine the influence of FOS on microbial populations, enzyme activity digestive tract and nutrient digestion in broilers. The research's periods were (1) FOS production; (2) the effect of FOS on intestinal bacterial, amylase and protease activity; (3) a digestibility in-vivo test. The test involved 40 broilers, which were divided into four treatments: T0: control, T1: + FOS 2 gr/kg, T2 :+ FOS 4 g/kg and T3: + FOS 6 g/kg, using a complete random design with four replicates. The incubation results in relation to fructosyltransferase enzyme for 3 hours resulted in the production of FOS 52.07%. The addition of FOS at 4 g/kg increased the population of Bifidobacterial, and decreased that of E coli bacteria ($P<0.05$), while at a dose of 2 g/kg it increased the activity of amylase, proteases. At the FOS dose it had a noticeable effect on improving nitrogen digestibility, calcium and phosphor ($P<0.05$) but no effect on the digestibility of crude fibers and metabolic energy. The study conclusion is that at 3 h of incubation were converted to more than 50% to synthesize FOS. The addition of FOS to feed is demonstrated to have a positive effect on the performance of broilers.

1 Introduction

Some countries in the European Union prohibit the use of antibiotics as growth boosters in poultry, as they can cause negative effects on consumer health. For this reason, nutritionists are seeking alternative ingredients that can replace antibiotics to increase poultry production efficiently and produce healthy meat and eggs. Various ingredients have been used as growth boosters, including enzymes, probiotics, prebiotics and emulsifiers. Fructooligosaccharides (FOS) products are known as prebiotic ingredients in food (food additives), added to increase the population of bacteria that benefit bifidobacterial and suppress harmful bacteria (pathogens). FOS contain oligosaccharides that function as nutrients for bifidobacterial growth. The compound can be naturally extracted, but only in small amounts, from onions,

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garlic and dahlia tubers, while it is difficult to produce FOS from cane sugar or sucrose, which can be used to scale up the production of prebiotics [1].

FOS contain a linear chain of β-D-fructofuranose that binds to a 1,2 glycosidic bond. It is a term given as a fructose oligomer consisting of (GF2), (GF3) and (GF4), which bind to the fructosyl unit with glucose in the β-(2-1) bond. Ma et al. [2] state that FOS can be produced from sucrose with fructosyltransferase enzymes of microorganisms such as *Aureobasidium* and *Aspergillus niger* [3]. Fructose has the potential as a low-calorie sugar and is a dietary fiber food ingredient that can stimulate the growth of bifidobacterial in the digestive tract and suppress putrefactive compound content. The fructosyltransferase enzyme that can synthesize the fructose polymer from sucrose [1]. FOS is composed of sucrose, with 1-3 units of 1-kestose, nystose and fructosyl nystose fructose [3].

The ability to maintain the balance of the bacterial population is one of the factors to evaluate health status [4]. For example, the composition of both pathogenic and microbial organisms can be used as an indicator of the health status of livestock. The composition of bacteria can affect the passage rate and absorption of nutrients. A well-functioning digestive tract will be able to improve the efficiency of production performance [5]. The addition of functioning FOS is believed to affect the bacterial population will affect the enzyme content and the digestibility of nutrients. To date, there have been few studies on the effect of the addition of FOS on the nutrient metabolism process in poultry. Our study contends that FOS supplementation can improve nutrient digestion in poultry. The aim is to determine the production character of FOS from sucrose and its effect on intestinal microbes and nutrient digestibility in broiler chickens.

2 Material and Methods

2.1 Ethical approval

The care of the animals followed the relevant institutional and governmental guidelines for animal experimentation Politeknik Negeri Jember no: 052-PL17.4/PG/2025.

2.2 Experiment I: FOS production

The fructosyltransferase enzyme is produced using enzymes from *Aspergillus niger* grown in a 10 ml solution containing 5 % sucrose, with yeast powder 2%, K2HPO4 0.5%, NaNO3 0.2%, MgSO47H2O 0.05%, KCl 0.05% and pH 5.0, which were sterilized at a temperature of 120 ºC for 30 minutes, then shaking at 180 rpm was performed for 24 hours at a temperature of 30 ºC. One tube was transferred to a 500 ml Erlenmeyer with a 200 ml sterile growing media composition, then shaking was also performed for 24 hours at a temperature of 30 ºC. transferred back to several growing media for 72 hours at a temperature of 30 ºC. The media and mycelia were then separated by centrifugation at 6000 rpm for 20 minutes so that supernatant was produced as a crude enzyme.

Determination of the value of the enzyme activity was made with an enzyme solution of 2 ml added to 10 ml of sucrose, with a concentration of 10%, and with a pH 5 McIlvaine buffer as a substrate at a temperature of 50 ºC for 15 minutes. Product analysis was conducted with HPLC (high performance liquid chromatography), with the standards of glucose, fructose, sucrose, 1-kestose (GF2) and nystose (GF3). HPLC analysis used a column size of 300 x 7.8 mm Carbohydrate Bandopax, Phase of motion: Methanol : H2O = 60 : 40 , injection volume : 20 µl, Detector: Refractive methanol index kec 1 ml/min. Pump: M 6000A recorder integrator Hewlett Packard type 3380 A, HPLC. HPLC Waters associates ALC/GPC. One

unit of activity of the enzyme fructosyltransferase (Ut): the amount of enzyme that converts µmol fructose transferred in the reaction, while the enzyme fructofuranosidase (Uf): the amount of mM of sucrose required for the reaction.

2.3 Experiment II: Microbe population and enzyme activity

Twenty broiler chickens aged 14-28 days were divided into four treatments: T0= control, T1= + FOS 2 g/kg, T2= + FOS 4 g/kg; and T3=+ FOS 6 g/kg. Samples taken at the time of treatment for 14 days taken from the feed experiment. The samples were taken from the small intestine, from the distal duodenum to the ileocecal, and cecum parts were collected in container cups with the addition of CO2 gas, tightly closed, then put into an ice thermos until analyzed. 10 g were blended with the addition of CO2 in 90 ml of ADS (anaerobic dilution solution) solution, then dissolved in series in another glass. The medium used by Wilkins Chalgren agar for the total anaerobes, MRS (Lactobacillus), reinforced clostridial agar for the *Bifidobacterium* and Mac Conkey No 2 for the *Escherichia coli*. The chickens were slaughtered; each treatment was then taken from the small intestine at the age of 28 days and then immediately stored at a temperature of -20 ºC until analyzed. Crude enzyme was extracted with 1 ml of 1mM HCl for 1 hour at a temperature of 4ºC and centrifuged at 3000 rpm. The substrate was extracted for use in the protease analysis, amylase [6].

The proteases were analyzed using azo casein substrates, amylase using water-soluble starch Amylase activity is expressed in units defined as the number of enzymes that hydrolyzed 1 micromole of substrate per minute. The enzyme activity test was conducted by reacting the enzyme on the substrate for 1 hour at a temperature of 37ºC. The reducing sugars of the mixture were then measured using the Nelson-Somogyi combination.

2.4 Experiment III: Digestibility test treated by feed-supplemented FOS

Twenty broiler chickens aged 30 days with a weight of 1.7 kg were used for the metabolic experiments. They were placed in individual metabolic cages for the feed digestibility test, with four replicates to measure endogenous N, a feed group that was randomly placed from four treatments, namely: T0= feed without FOS (control); T1 = feed with FOS 2 g/kg; T2= feed with FOS 4 g/kg; and T3= feed with FOS 6 g/kg. The basalt feed used for the broiler feed in the growth phase was 14-28 days old, with a protein content of 21%, energy of 2,800-3000 kcal/kg, Ca content of 1%, and P of 0.6%. The composition of the ration consisted of corn, wheat pollard, soybean meal, fishmeal, bone meal, bran and premix. The feed produced was tested for its chemical composition, including dry matter, ash, crude fiber, protein, Ca and P, and energy content was measured with a calorimeter bomb. The procedure involved the chickens being raised for 5 days, then fasted 24 hours of treatment and endogenous. After 24 hours of treatment the chickens were fed 30 grams while endogenous remained fasted. Excreta were collected after 24 hours, then weighed and placed in a freezer for 24 hours. They were then dried at 60 ºC for 24 hours and ground, ready for use in the energy analysis.

$$TME = \frac{(FI \times GEf) - (Eks \times GE\ Eks) - (E\ Endo \times GE\ Endo)}{(FI \times GEf)} \quad (1)$$

Ca digestibility

$$Ca = \frac{(FI \times Ca\ r) - (Eks \times Ca\ eks) - (E\ Endo \times Ca\ Endo)}{(FI \times Ca\ r)} \quad (2)$$

P digestibility

$$P = \frac{(FI \times P\ r) - (Eks \times P\ eks) - (E\ Endo \times P\ Endo)}{(FI \times P\ r)} \quad (3)$$

Nitrogen retention (NR)

$$NR = (FI \times N\ r) - (Eks \times N\ eks) - (E\ Endo \times N\ Endo) \quad (4)$$

$$Nitrogen = protein / 6{,}25$$

FI = Feed intake (g), GEf = Gross energy diet (Kkal/kg)
Ca r = Calcium %
P r = Phosphor %
N r = Nitrogen
Eks = Feces (g)
GE Eks = Gross energy of feces (kkal/kg)
Ca eks = Ca Feces %
P eks = P Feces %
N eks = N Feces
E Endo = Endogenous feces (g)
Ca endo = Ca endogenous %
P endo = P endogenous %
GE Endo = Gross energy endogenous feces (kcal/kg)

2.5. Statistical analysis

The experimental design aimed to determine the effect of different dosage treatments on, the amount of *Bifidobacterium* and *E coli*, the digestibility of protein nutrients, crude fiber, metabolic energy and the digestibility of minerals Ca and P, using a complete random design (CRD). If there was a noticeable difference, the Tukey test was continued.

3 Results and discussion

3.1 FOS production

The FOS compounds formed can be seen in Figure 1. The length of fermentation affected the production of FOS synthesis. At an incubation time of 60 -180 min the detected compounds were kestose, nystose, glucose and fructose. A gradual increase in the amount of FOS indicated an increase in the activity of the fructosyltransferase enzyme corresponding to the length of the incubation duration. The amounts of FOS formed during the substrate's reaction with the enzyme over four consecutive time points—0, 1, 2, and 3 hours—were 0%, 19.3%, 34.6%, and 52.07%, respectively, which were accompanied by a decrease in sucrose content at the 1-hour reaction time by 47% and at the 3-hour reaction time to 27.5%, or a decrease of 42%. This shows the occurrence of the transfer activity of fructose groups to form polyfructose. The transfer requires hydrolysis and synthesis time so that new compounds are formed. Todero et al. [5] reported an effective time for FOS synthesis of 48-72 hours using *Aspergillus theromutatus*.

According to Liu et al. [3], the optimal production of FOS is achieved after a reaction of 40 hours at pH 7.6 and temperature of 33 ºC . Diaz et al. [7] found that fructosyltransferase activity was highest in *Basidium pullulans* at a temperature of 55-60 ºC, as in enzymes from another strain, *Aspergillus japonicus.* FOS synthesis is influenced by fructosyltransferase activity. According to Mao [8], formation of FOS is optimal at 2-4 hours of enzyme

incubation. The transfructosylation reaction involves the breakdown of the glycosidic beta 2-1 bond and the transfer of the fructosyl group from the donor to the acceptor (other than water). The physical chemical composition of commercial products for some FOS and products oligosaccharide components is 55% [9].

The reaction mechanism with sucrose as a substrate can act as a donor or as an acceptor, so that 1 mole of glucose and 1 mole of 1-kestose (DP3) are formed simultaneously, indicating a disproportional reaction mechanism. In the synthesis process 1-kestose (DP3) acts as a substrate, with sucrose and nystose (DP4) produced; nystose (DP4) subsequently acts as a substrate, with ketose (DP3) and fructofuranosyl-nystose (DP5) formed.

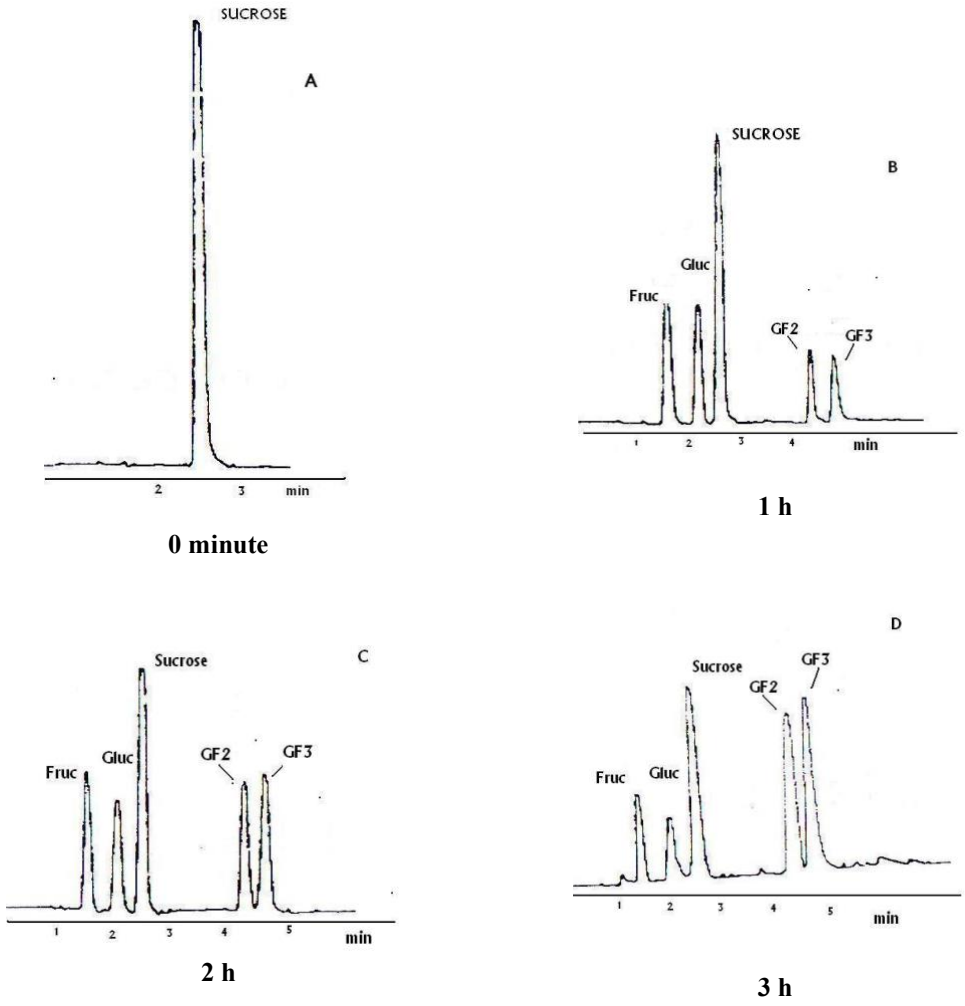

Fig. 1. FOS production with different incubation times.

3.2 Gastrointestinal bacterial population, pH and enzyme activity in chicken feed containing FOS

The bacterial population can be seen in Table 1. The increase in the *Lactobacillus* population became effective at the addition of 2 g/kg. FOS plays a role in providing a source of energy for bacterial growth; a higher dose increased the population of Lactobacillus bacteria compared to controls without supplementation. On the other hand, the population of *E coli* bacteria fell in line with the addition of FOS. This is thought to be related to the increase in *Lactobacillus* producing more organic acids. Such acids lower the pH, so they tend to be lower than the controls. This will suppress the growth of *E coli*, meaning the total population will decrease. These results are not in accordance with a previous finding that the addition of 2 g/kg has no effect on the bacterial population in the digestive tract [10]. In addition, other studies on chickens have found that addition of 3.75 g/kg has no effect on performance [11]. As seen in Table 1, activity increased with the addition of 4 g/kg FOS prebiotics. FOS contain a Beta 2:1 fructosyl group resistant to hydrolyzation by digestive enzymes for intestinal microbial fermentation, which can increase the population of total bacterial. A number of *in vivo* studies have shown that the development of beneficial bacteria can suppress the development and reduce the level of the toxic compounds ammonia and phenol, with consequent health improvements.

Table 1. Bacteria population in intestinal tract

Parameter CFU/ml/(Log)	T0	T1	T2	T3	SEM
TPC	6.36	6.41	6.34	6.39	0.03
Lacctobacillus	5.64[b]	5.84[a]	5.94 [a]	5.95 [a]	0.05
Echercia coli	4.96[a]	4.80[b]	4.82 [b]	4.80 [b]	0.21
pH	5.50[b]	5.1[a]	5.4 [b]	5.4 [b]	0.10

Notes: TPC: total plate count; CFU = colony forming unit, SEM: standard error mean
T0: -FOS; T1: +FOS 2g/kg; T2: +FOS 4 g/kg; T3: +FOS 6 g/kg. Different superscripts within the same row indicate a significant difference (P<0.05).

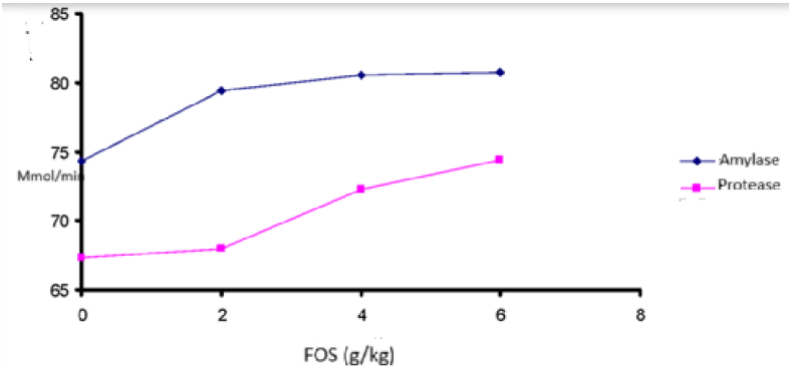

Fig. 2. Protease and amylase activity on chicken intestines.

As shown in Figure 2, the addition of FOS can increase enzyme activity**.** It also functions as an inducer of enzyme production, including glycolytic enzymes, which can increase the hydrolysis of insoluble carbohydrate polymers (fibers) and prevent the growth of pathogenic

bacteria such as *Salmonella*. The addition of oligofructose can also affect the activity of enzymes in the digestive tract. Xu et al [10] found that the addition of oligofructose affects the secretion of digestive enzymes and increases the activity of the enzymes amylase and trypsin. The addition of prebiotics will improve health status and will facilitate metabolic processes in the body, and will consequently also affect the performance of nutrient absorption [12].

The addition of FOS at levels of 2 and 4 g/kg increased the absorption of P from phytic acid in grain-containing feeds, or by 27% compared to the control feeds. This increase will in turn raise the available P content, allowing it to increase the digestion of P. This will reduce the percentage of P excreted through feces. The increase in P digestibility is due to the effect of the production of organic acids produced by *Lactobacillus*, thus lowering the pH in the digestive tract, or it may be caused by the increase in the metabolic system of nutrient absorption in the digestive tract. This will increase the digestibility of P, which will then raise the solubility of phytic acid in feed into dissolved P products and the proliferation of epithelial cells, thus improving the digestibility of P. Phytate is highly stable and can bind to valence ions such as Ca, Co, Cu and Fe and decrease the availability of minerals for livestock [9]. At low pH, the stability of phytate will be lowered, so it will release 2 and 3 valence ions.

Table 2. Nitrogen retention, Metabolisable Energy (ME) with the addition of FOS prebiotics (*in-vivo*) in broiler chickens.

Parameter	T0	T1	T2	T3	SEM
Nitrogen retention	0.34^{b}	0.50^{a}	0.50^{a}	0.53^{a}	0.63
ME (Kcal/kg)	2.903	2.958	2.926	2.731	200

Notes: SEM: standard error mean; T0: -FOS; T1: +FOS 2g/kg; T2: +FOS 4 g/kg; T3: +FOS 6 g/kg
Different superscripts within the same row indicate a significant difference ($P<0.05$).

In *in vivo* tests with metabolic cages, it was shown that the addition of FOS in broiler chickens at doses of 2, 4 and 6 g/kg increased nitrogen retention (Table 2). This increase in nitrogen retention is likely attributable to the prebiotic compound of FOS, which enhance the growth of beneficial intestinal microbiota. Improved microbial balance can stimulate better nutrient digestion and absorption, thereby reducing nitrogen losses and allowing the chickens to utilize dietary protein more efficiently. FOS are known to selectively stimulate the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* [13], these bacteria play a crucial role in improving gut health and nutrient absorption.[14].

Table 3. Absortion of Ca and P with the addition of FOS prebiotics (*in-vivo*) in broiler chickens.

Parameter	T0	T1	T2	T3	SEM
Crude fibre	0.13	0.16	0.50	0.17	0.07
Calcium (Ca)	0.61	0.71	0.70	0.68	0.04
Phosphor (P)	0.38^{b}	0.48^{a}	0.49^{a}	0.41^{ab}	0.06

Notes: SEM: standard error mean; T0: -FOS; T1: +FOS 2g/kg; T2: +FOS 4 g/kg; T3: +FOS 6 g/kg.
Different superscripts within the same row indicate a significant difference ($P<0.05$).

The addition of FOS will affect the pH and the *Lacctobaccillus* bacteria population in the digestive tract, increasing the P released from phytat, phytate bound to the mineral phosphorus which binds to proteins. The addition of FOS will increase the absorption of protein nitrogen, together with that of amino acids. but it is not accompanied by an increase in the digestion of Ca and P. It is possible that such concentrations may not lead to the proliferation of epithelial cells which can increase the absorption of nutrients; Xu et al. (2002) found that an increase in the population of *Lactobacillus* will inhibit damage to the villi and microvilli caused by Escherichia coli, which will affect the secretion of digestive enzymes.

The addition of FOS did not affect the metabolic energy of the broiler livestock. The polysaccharide component used as an energy source was not affected by the addition of FOS oligosaccharides, although the phytate content of the feed, in addition to the binding protein groups, also binds starch through Ca ions with fatty acids. The increase in the *Lactobacillus* population did not sufficiently affect the true metabolic energy (TME). In the control feeds with reduced Ca absorption, it was shown that increased Ca minerals excreted with the addition of FOS could increase Ca absorption by up to 13% (Table 3). The increase in minerals is due to the release of the Ca bond bound to phytic acid in the feed. Phytate is reactive to Ca ions, so a decrease in the pH of the digestive tract will increase the hydrolysis of minerals bound to it. Obianwuna et al. [12] found that 1 mole of phytic acid can bind to 3-6 mol of Ca ions in the form of insoluble phytate at the pH of the small intestine in the digestive tract. This is in accordance with the findings of Wang et al. [15], that the addition of fructooligosaccharides at various degrees of polymerization increases the Ca content in bones and also the availability of Ca and Mg minerals, accompanied by decreases in the Ca content of feces

4 Conclusion

It can be concluded that sucrose substrates with fructosyltransferase enzymes can produce FOS conversion of up to 52% during 3 hours of fermentation. The addition of FOS to feed can increase protein digestibility, so it has the potential to improve livestock performance.

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