Mannan extract from palm kernel cake to control *Salmonella thypimurium* in broiler chickens

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Abstract

Palm kernel cake has a high content of mannan that has the potential to be used as a supplementary feed through an extracting process which functions to inhibit the colonization of bacteria that harm poultry. The purpose of this research was to examine the capability of mannan extract from palm kernel cake to control *Salmonella thypimurium* in broilers. The effect of mannan extract from palm kernel cake was done by agglutination, resistance and inhibitory tests in liquid medium. In vivo test was carried on 3-day old broilers which were orally infected with 10^5 CFU *Salmonella thypimurium*. Levels of mannan extract given were 0, 0.1, 0.2, 0.3 and 0.4%. The results showed that mannan extract from palm kernel cake had no bactericidal and bacteriostatic activity, but showed the agglutination ability when evaluated microscopically. The utilization of mannan extract from palm kernel cake at 0.3% and 0.4% showed significantly ($P<0.05$) higher number of *Salmonella thypmurium* (cfu/g) on feaces compared to the control (7.00 and 6.91 vs 6.69, respectively). Increasing level of mannan extract significantly ($P<0.05$) decreased the total number of bacterial colonies in caecum and the numbers of *Salmonella thypmurium* (cfu/g) at the levels of 0, 0.1, 0.2, 0.3 and 0.4% were 4.56; 4.51; 4.37; 4.13; 3.62, respectively. The utilization of mannan extract from palm kernel cake had no significant effect on caecal pH and the average daily gain of broiler chickens. It is concluded that mannan extract from palm kernel cake has the ability to reduce colony of *Salmonella thypimurium* in broilers so that it can be used as a feed supplement in broiler chickens diet.

Keywords : mannan extract, palm kernel cake, *Salmonella thypimurium*, broiler chicken.

Introduction

In the poultry industry, enteric disease gets a very important position to be considered because of its negative effect on the health of the gastrointestinal tract of poultry, and able to decrease productivity, increase mortality, preventive costs and contamination of poultry products for human consumption. Enteric disease can be caused by pathogenic bacterial infection. One of the bacterias that often attacks and contaminates the chicken is *Salmonella thypimurium* that can cause disruption or infection of the gastrointestinal tract of chicken (Ohl and Miller, 2001). Colonization of *Salmonella thypimurium* is mediated by receptor known as type I fimbriae and its attachment is sensitive to mannosa (Muller *et al.*, 1991).

Palm kernel cake is a by-product of palm oil processing which has the potential to serve as one of the raw materials in the

manufacturing of dietary supplements. One such feed supplement is prebiotic made by reforming the structure of palm kernel cake through the process of extracting the palm kernel cake into mannan-oligosaccharide using enzyme technology. Palm kernel cake has high fiber content and mannose components reaching 56.4% of the total palm kernel cake cell wall in the form of β-mannan bond (Daud *et al.,* 1993). Mannan oligosaccharide inhibits the process of attachment or colonization of *Salmonella thypimurium* bacteria in the gastrointestinal tract of broilers by blocking type 1 fimbriae of *Salmonella thypimurium* bacteria that specifically attaches to mannosa, prior to being excreted through the digestive tract (Baurhoo *et al.,* 2007).

The objective of this study was to examine the capability of mannan extract from palm kernel cake to control *Salmonella thypimurium* in broiler chickens.

Materials and Methods

In vitro test was conducted at Nutrition and Feed Science Laboratory and *in vivo* test was conducted in the Livestock Biology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Sumatra, Indonesia. Mannan extraction process of palm kernel cake was done according to the method described in a previous study using acetic acid and mannanase enzyme (Tafsin *et al.,* 2017).

Ability of Mannan Extract from Palm Kernel Cake to Control Salmonella thypimurium

Agglutination Test

Standardization of bacterial concentration was done by growing *Salmonella thypimurium* bacteria for 24 h using nutrient broth medium. The bacterial cells were harvested by centrifugation and suspended into Phosphate Buffer Saline (PBS, 0.05M, pH 7.2). The agglutination test was performed by adding 1 ml of bacterial suspension and 1 ml of the mannan extract suspension and left for 5 min and then agglutination was observed using a microscope (Spring *et al.,* 2000).

Sensitivity Test / Resistance Test

Sensitivity test was done using paper discs known as Kirby - Bauer Method. Paper discs were surface layered with mannan extract. The concentration of mannan extract tested was 0%; 0.1%; 0.2%; 0.3% and 0.4% based on the total sugar content, and 50 µl of it were spread on the disc paper. Subsequent incubation was carried out for 24-48 h and observation was performed for the presence or absence of the formation of clear zone in the medium culture (Jawetz *et al.*, 2005).

Inhibitory Test in Liquid Medium

Test for *Salmonella thypimurium* bacteria in Nutrient Broth medium plus mannan extract with concentrations of 0, 0.1 0.2, 0.3, and 0.4% based on the total sugar content was performed. The initial colony count was 10^3 cfu, then the culture was incubated for 24 h at 37°C. Dilution in series using physiological NaCl and continued culture on petri dish using medium of Nutrient Agar was also followed. The number of colonies was calculated after incubation for 48 h at 37°C.

Ability of Mannan Extract from Palm Kernel Cake to Control Salmonella thypimurium in Broiler Chickens

This study used 96 1-day old Cobb strain broiler chickens which were infected orally with 10^5 cfu *Salmonella thypimurium* on the third day of the experiment. A Completely Randomized Design (CRD) with 6 treatments

and 4 replicates, each replicate consisted of 4 The treatments were:

$R0_A$: Infection *of S. thypimurium* without supplementation.
$R0_B$: Infection *of S. thypimurium* + antibiotics.
R1 : Infection of *S. thypimurium* + diet supplemented 0.1% mannan extract from palm kernel cake.
R2 : Infection of *S. thypimurium* + diet supplemented 0.2% mannan extract from palm kernel cake.
R3 : Infection of *S. thypimurium* + diet supplemented 0.3% mannan extract from palm kernel cake.
R4 : Infection of S thypimurium + Diet supplemented 0.4% mannan extract from palm kernel cake.

Colonization of Salmonella thypimurium Bacteria in Feaces

Feacal sampling was performed at 1 d after infection. The samples weighing 1 g each were suspended in advance by using 9 ml physiological NaCl, then 1 ml of the suspension was cultured in 9 ml nutrient broth medium and incubated for 24 h at 37°C, then continued on SS medium for 1 – 2 d in the incubator maintained at 37°C.

Colonization of Salmonella thypimurium Bacteria in Cecum

The process of taking the cecum was carried out on the 5th and 15th day after infection. The cecum was collected in a sterile tube and physiologic solution (NaCl 0.9 %) was added according to the weight of the cecum content (dilution 1:10) and homogenized and 0.5 ml of the cecum suspension was introduced into a diluent tube containing 4.5 ml of saline to obtain a dilution of 10^{-1}, then vortexed to homogeneous dilution. The process chickens was adopted for the study. continued on SS agar medium, by inserting 0.1 ml of the diluted sample into the petri dish, then incubated for 1 – 2 d in the incubator maintained at 37°C.

pH Content of the Cecum

The contents of the cecum were measured for pH using a pH meter (TOA ion meter IM40S) which had previously been calibrated with buffer pH 4 and pH 7. The content of diluted cecum was then homogenized by using vortex. The pH of the cecum was measured by dipping the pH meter electrode into the sample and the result was recorded.

Average Daily Gain of Broiler Chickens

The average daily gain of broiler chickens was obtained by calculating the difference between the final weight and the initial weight of the chicken and divided by the number of days the birds were on feed during the study.

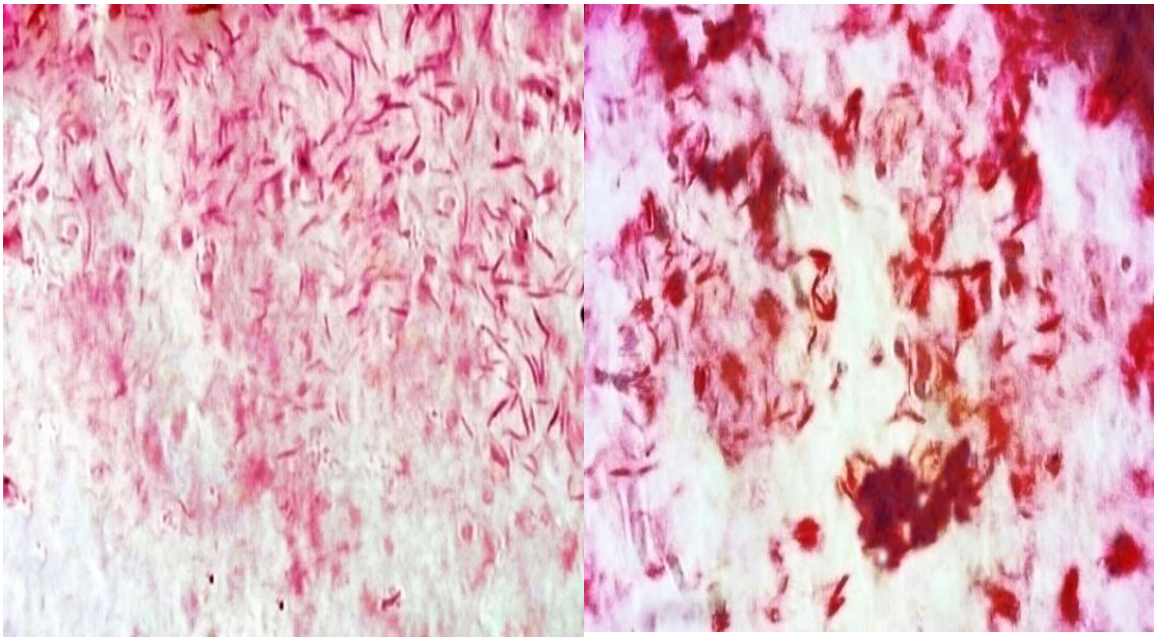
Results and Discussion

Ability of Mannan Extract from Palm Kernel Cake to Control Salmonella thypimurium

Agglutination Test

The result of agglutination test using mannan extract from palm kernel cake in *Salmonella thypimurium* bacteria through visual observation did not show clumping, but microscopic observation showed that there was a difference between control and treatment with mannan extract (Figure 1) with the difference seen due to the occurrence of *Salmonella thypimurium* accumulation on mannan extract as shown by the arrows on the colored darker part (Figure 1), while the *Salmonella*

thypimurium in the control group were seen to have spread evenly.



(a) (b)

Figure 1. The agglutination test results on *Salmonella thypimurium* microscopically, (a) Control; (b) *Salmonella thypimurium* + mannan extract from palm kernel cake.

The accumulation of *Salmonella thypimurium* bacteria showed that the content of mannan extract was capable of agglutinating *Salmonella thypimurium* bacteria. This indicated that there was activity in the form of attachment between the receptor of *Salmonella thypimurium* bacteria and the mannose component of palm kernel cake extract. The type of bacteria that inhibited attachment was gram-negative bacteria with a specific type 1- fimbriae mannose such as *Salmonella thypimurium* (Ofek *et al.,* 1977). Fimbriae type 1 is a receptor whose attachment is sensitive to mannose (Muller *et al*., 1991). Mannanoligosaccharides have the ability to adhere to mannose-specific lectins from gram-negative type 1 fimbriae bacteria such as *Salmonella* and *E. coli* (Baurhoo *et al.,* 2007). This study is consistent with Tafsin (2007) and Syahruddin *et al.* (2008) that the use of mannanoligosaccharide from palm kernel cake microscopically showed agglutination (clumping) of *Salmonella thypimurium.*

Resistance Test

The results of visual resistance test of *Salmonella thypimurium* bacteria on agar medium using disc paper added mannan extract with different concentrations (0 - 0.4 %) and antibiotic as control showed that the clearing zone formation gave negative results at each concentration of mannan extract. Based on this test, it was seen that the diameter of the clear zone formed by the antibiotic was relatively small while the formation of clear zone at each concentration of mannan extract gave negative result. Negative clear zone test at each concentration of mannan extract indicated

that mannan extract from palm kernel cake had little bacterial kill activity (bactericidal), so all *Salmonella thypimurium* bacteria tested were resistant to the mannan extract.

According to Pratiwi (2008) the small clear zone indicated a lower anti-bacterial activity, whereas large clear zones showed greater anti-bacterial activity. This might be caused by many factors other than the sensitivity of the test organism (bacteria), and might also be affected by the culture medium, incubation conditions (temperature, time and pH), velocity of the anti-bacterial compound diffused in the agar medium, concentration of the microorganism, concentration of the anti-bacterial compound and media composition (Schlegel and Schmidt, 1994).

Inhibitory Test in Liquid Medium

The results of inhibitory test in liquid medium to *Salmonella thypimurium* using nutrient broth medium plus mannan extract from palm kernel cake with concentration of 0 to 0.4% are shown in Figure 2. Based on the observation of the addition of mannan extract into a liquid medium containing 10^3 CFU of *Salmonella thypimurium* bacteria it was shown that *Salmonella thypimurium* bacteria still grew and bred well. This indicated that the mannan extract had little activity to suppress bacterial growth (bacteriostatic). The decrease in total *Salmonella thypimurium* colonies with the increasing concentration of mannan extract in the liquid medium nutrient broth was predicted from the clumping process of the bacteria, so that when cultured on the medium it caused the total number of colonies of *Salmonella thypimurium* bacteria to reduce. Clumping effects on the bacteria could be seen microscopically in the agglutination test.

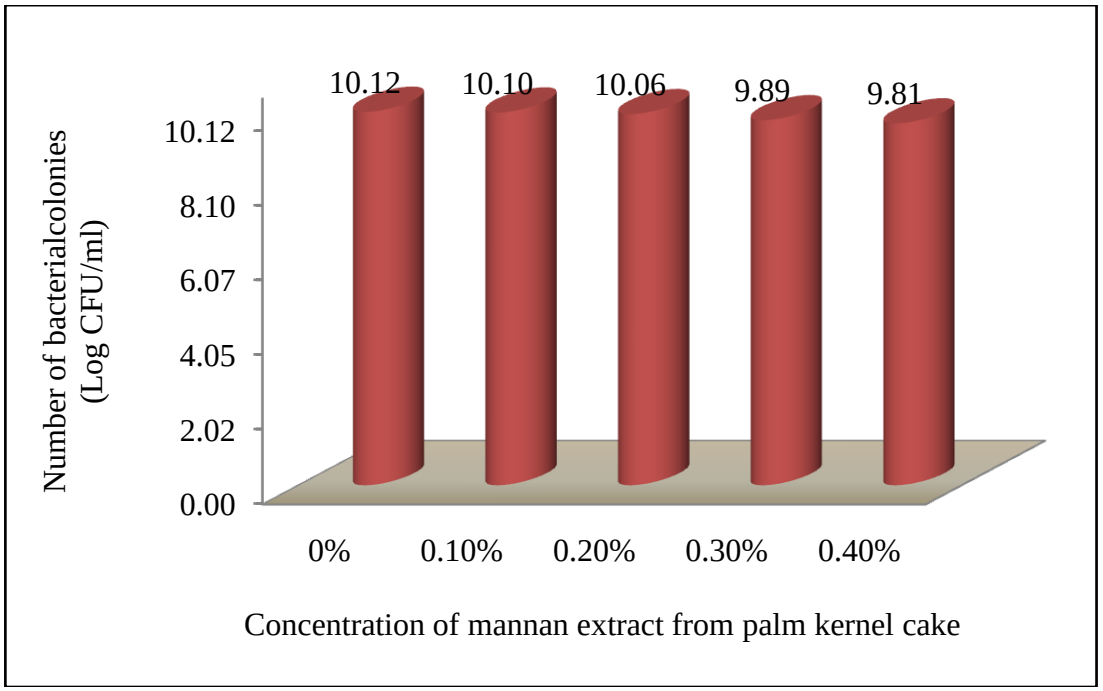


Figure 2. Effect of addition of mannan extract from palm kernel cake to *Salmonella thypimurium* bacterial colony on inhibitory test in liquid medium (log cfu/ml).

The decrease in the total number of bacterial colonies also indicated that *Salmonella thypimurium* could not use mannan extract from palm kernel cake as food for its growth. According to Tafsin (2007) that the use of mannan polysaccharide extract from palm kernel cake (0 - 4000 ppm) showed a decrease in the total number of *Salmonella thypimurium* bacterial colonies, as mannan polysaccharide content increased in the liquid medium.

Ability of Mannan Extract from Palm Kernel Cake to Control Salmonella thypimurium on Broiler Chickens: Colonies of Salmonella thypimurium bacteria in Feaces and Cecum of Broiler Chickens

The results of observations on the number of bacterial colonies (log cfu/g) in the feaces and content of cecum are presented in Table 1. There was significant effect of the treatments on the number of colonies in the feaces on day 1 after infection. The use of palm kernel cake extract with concentration of 0.3% and 0.4% significantly increased the number of *Salmonella thypmurium* bacterial colonies expelled by feaces compared to the control. This indicated that the rate of *Salmonella thypmurium* expenditure on chicken feaces was higher due to the use of mannan extract with increased concentration mixed in the broiler chicken diet.

Table 1. The use of mannan extract from palm kernel cake against the number of *Salmonella thypimurium* bacterial colonies in the feaces and cecum of broiler chickens (log cfu/g).

Treatment	Days after infection		
	Feces	Cecum	
	1 day	5 day	15 day
R0A	6.69 ^{b} ± 0.08	5.77 ^{b} ± 0.05	4.56 ^{a} ± 0.05
R0B	6.70 ^{b} ± 0.11	5.89 ^{a} ± 0.09	4.49 ^{ab} ± 0.02
R1	6.80 ^{ab} ± 0.11	5.56 ^{c} ± 0.06	4.51 ^{ab} ± 0.03
R2	6.80 ^{ab} ± 0.19	5.51 ^{cd} ± 0.02	4.37 ^{b} ± 0.10
R3	7.00 ^{a} ± 0.14	5.89 ^{a} ± 0.02	4.13 ^{c} ± 0.07
R4	6.91 ^{a} ± 0.14	5.46 ^{d} ± 0.03	3.62 ^{d} ± 0.22

^{a,b}Means in the same column having different superscripts are significantly different (P<0.05)

The results of the use of mannan extract showed positive result in the control of *Salmonella thypmurium* bacteria. The ability of mannan extract to control *Salmonella thypmurium* was due to the presence of mannose components of mannan extract from palm kernel cake used. The use of D-mannose was also effective in preventing *Salmonella* colonization (Oyofo *et al.*, 1989). *Salmonella thypmurium* is a bacteria always looking for attachment to simple sugar mannose or carbohydrates that have mannose content such as mannan-oligosaccharides, because some *Salmonella* bacteria have a receptor known as type 1 fimbriae and attachment is sensitive to mannose (Muller *et al*., 1991). The attachment of *Salmonella thypmurium* bacteria to mannan-oligosaccharides belonging to the group of fibers and non-digestible carbohydrates cause *Salmonella thypimurium* bacteria to be excreted through the feaces. According to Baurhoo *et al.* (2007) that mannan-oligosaccharide has the ability to adhere to mannose-specific lectins from gram-negative type 1 fimbriae pathogens such as

Salmonella and *E. coli* which are then excreted from the gastrointestinal tract.

Based on the results of the cfu on the cecum, there was highly significant effect of treatments on the decrease *of Salmonella thypmurium* bacterial colony when observed on days 5 and 15 after infection. The use of mannan extract from palm kernel cake with a concentration of 0.4% in the ration significantly reduced the number of *Salmonella thypmurium* bacterial colonies in chicken cecum compared with control and treatment with mannan extract at concentration of 0.1% - 0.3%. This indicated that mannan extract was able to decrease the number of *Salmonella thypmurium* bacterial colonies in the cecum along with increased concentration of palm kernel cake extract added in the ration. Tafsin (2007) reported that the use of mannan-polysaccharide showed a faster process of *Salmonella* removal at concentration of 2000 - 4000 ppm at infection rate 10^4 CFU. The incidence of *Salmonella thypmurium* was not recovered on the 15th day after infection. The high rate of excretion from *Salmonella thypmurium* bacteria due to the use of mannan extract also affected the decrease in the number of *Salmonella thypmurium* bacterial colonies in chicken cecum from days 5 to 15 after infection. Additionally this caused the *Salmonella thypmurium* bacteria to be attached to the mannose contained in the mannan-oligosaccharide and excreted through the feaces because mannan-oligosaccharide has the ability to adhere to mannose-specific lectins from gram-negative type 1 fimbriae pathogens such as *Salmonella* and *E. coli* which are then excreted from the gastrointestinal tract (Baurhoo *et al.*, 2007).

In addition, the presence of organic acids in the form of acetic acid contained in the supernatant from the extraction of palm kernel cake can also affect the number of *Salmonella thypimurium* bacteria in the cecum. This is closely related to the decrease of intestinal pH and cecum into acidic condition so that the growth of pathogenic bacteria including *Salmonella thypimurium* that could inhibit and otherwise non-pathogenic microbes can live well. According to Byrd *et al*. (2001) that the administration of organic acids in drinking water could decrease the number of pathogenic bacteria such as *Salmonella* and *Campylobacter*. Nugroho (2014) stated that the addition of acetic acid with a level of 0.7 % produced the number of *Salmonella sp*. and *E*. coli to be less in the gastrointestinal tract than the administration of acetic acid with levels of 0.25% and 0.5%.

pH Cecum

The results of observation on the 15th day after infection on pH of cecum due to treated different concentrations of mannan extract from palm kernel cake are presented in Figure 3. Control treatments (R0A and R0B) had higher pH than the diet treatments supplemented by palm kernel extract. Indirectly, extract of palm kernel cake provided benefits by lowering the pH of the gut so as to reduce colonization of pathogenic bacteria in the gut (Pudjiatmoko *et al.*, 2014). This could be seen in the pH of cecum of chickens fed ration of mannan extract with concentration of 0.2% and 0.4% been acidic. This decrease in the pH of the cecum had a close relationship with the number of bacterial colonies in the cecum, because in addition to decreasing the pH of cecum given mannan extract with 0.2% and 0.4% concentrations the extract also caused a decrease in the number of *Salmonella thyipimurium* bacterial colonies in the cecum. In addition, a decrease in the pH of the cecum accompanied by a decrease in the number of colonies of *Salmonella thypimurium* bacteria in cecum was also due to the use of acetic acid in extracting the

palm kernel cake into a simpler material in the form of a supernatant so that the acetic acid content present in the supernatant could have caused a decrease in the pH of the cecum. The acidic condition of the caecal pH could have suppressed *Salmonella thyipimurium* growth by damaging the *Salmonella* flagella, causing *Salmonella thyipimurium* to become motile and ultimately lysing. Rofiq (2003) stated that *Salmonella typhimurium* had three antigens and one of them was H antigen (flagella). *Salmonella* flagella could be damaged when acid and alcohol were added.

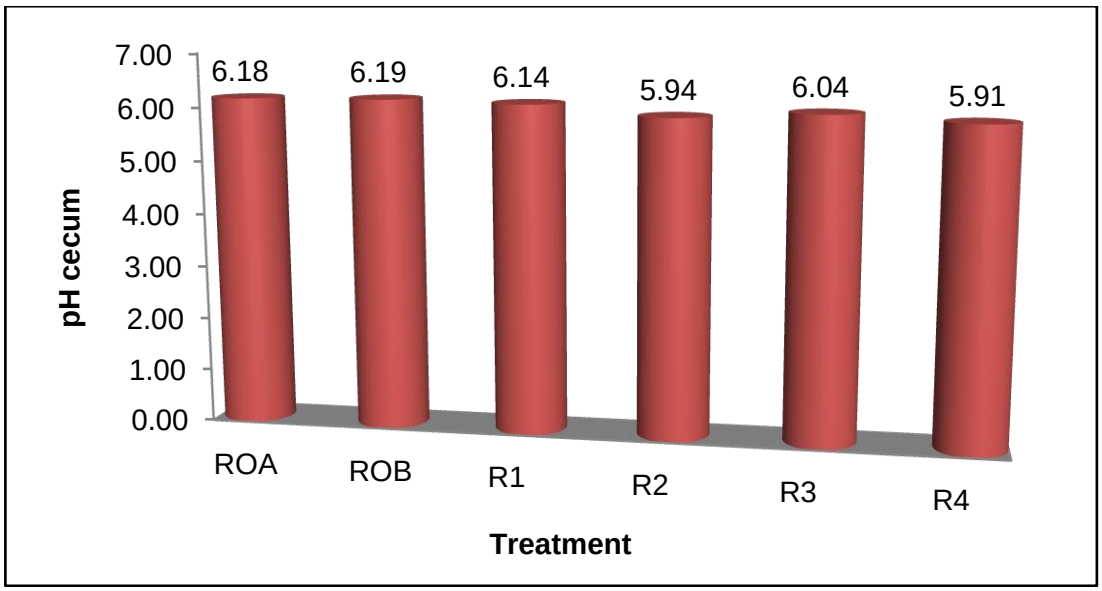


Figure 3. The use of mannan extract from palm kernel cake on pH of cecum.

There was no significant difference among treatments on cecal pH on day 15 after infection. According to Spring *et al.* (2000) that the use of mannan extract from palm kernel cake did not affect pH of cecum, Volatile Fatty Acid (VFA), as well as population of *Lactobacillus* and *Bifidobacteria* bacteria. According to Tafsin (2007) the use of *mannan polysaccharides* from palm kernel cake could affect the pH of cecum.

Average Daily Gain of Broiler Chickens

The effects of mannan extract treatments on average daily gain (ADG) of broiler chickens are presented at Table 2. Administration of mannan extract in ration with concentration 0.4% had higher ADG and chicken body weight higher than the control group and lower mannan extract concentration (0.1% - 0.3%). This indicated that the addition of mannan extract at 0.4% was able to overcome the adverse effects of inhibition of chicken growth due to pathogenic microbes (*Salmonella thypimurium*) in the digestive tract associated with increased excretion of *Salmonella thypimurium* bacteria in feaces, able to reduce the occurrence of colonization of *Salmonella thypimurium* bacteria in cecum. Tafsin (2007) reported that the use of mannan-polysaccaride at 3000 ppm was found to overcome the adverse effects of growth inhibition due to pathogenic microbes (*Salmonella thypimurium*) in the digestive tract.

Table 2. The effect of mannan extract from palm kernel cake on average daily gain of broiler chickens on day 18th.

Treatment[2]	Average daily gain[1] (g/day/bird)	Final body weight[1] (g/bird)	CV final body weight of chickens (%)
R0A	15.38 ± 1.78	327.50 ± 32.02	9.8
R0B	16.91 ± 1.83	355.00 ± 33.17	9.3
R1	15.39 ± 2.13	327.50 ± 38.62	11.8
R2	16.48 ± 0.29	347.50 ± 5.00	1.4
R3	16.78 ± 0.95	352.50 ± 17.08	4.8
R4	17.48 ± 0.55	365.00 ± 10.00	2.7

[1]Means ± SD; Not significantly different between treatments (P>0.05)

[2]R0 : Infection *of S. thypimurium* without supplementation.
$R0_B$: Infection *of S. thypimurium* + antibiotics.
R1 : Infection of *S. thypimurium* + diet supplemented 0.1% mannan extract from palm kernel cake.
R2 : Infection of *S. thypimurium* + diet supplemented 0.2% mannan extract from palm kernel cake.
R3 : Infection of *S. thypimurium* + diet supplemented 0.3% mannan extract from palm kernel cake.
R4 : Infection of S thypimurium + Diet supplemented 0.4% mannan extract from palm kernel cake.

There was no significant difference among treatments on average daily gain of broiler chickens and final body weight on day 15 after infection. This showed that the addition of mannan extract in the ration with concentration of 0.1% - 0.4% was not able to significantly increase average daily gain of broiler chickens. According to Ma *et al.* (2006) that the use of mannanoligosaccharides in broilers did not show the effect on the chicken. Shafey *et al.* (2001) stated that giving 0.3% mannanoligosaccharides did not increase daily body weight gain of chickens.

Conclusion

Microscopically, the result of agglutination test *in vitro* showed positive results with the occurrence of *Salmonella thypimurium* bacteria agglutinating with mannan extract from palm kernel cake. Mannan extract was found to have no bacterial kill activity (bactericidal) or suppress bacterial growth (bacteriostatic). The use of mannan extract from palm kernel cake was able to increase the release of *Salmonella thypimurium* bacteria through the feaces and decrease the number of bacterial colonies in the cecum. However, it did not affect the caecal pH and the average daily gain of broiler chickens. In the infusion of 10^5 CFU *Salmonella thypimurium*, the use of mannan extract from palm kenel cake with concentration of 0.4% in the ration was effective in decreasing the number of *Salmonella thypimurium* bacterial colonies in the cecum.

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