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Sapindus rarak microparticles in feed and drinking water as a substitute for anticoccidials and antibiotics in broilers

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Abstract

This study compared the effects of *Sapindus rarak* microparticles (SRMs), provided through feed or drinking water, with commercial anticoccidial and antibacterial agents. The goal was to determine which would reduce *Eimeria* sp. oocysts and *Escherichia coli* populations and improve chicken performance. Three hundred seventy one-day-old Ross broilers were randomly assigned to seven treatment groups with replicates of nine birds each. The birds were reared for 28 days. The T1 group served as a control; T2 was provided anticoccidial/COXI at 2.5 g/L in the drinking water; T3 was fed antibacterial Zn-bacitracin at 0.5 g/kg feed; T4 and T5 were fed SRMs at 0.63 g/kg and 1.25 g/kg feed, respectively; and T6 and T7 were provided SRMs at 0.3 g/L and 0.63 g/L in the drinking water, respectively. Bodyweight, feed consumption, feed conversion, oocysts per gram faeces (OPGs), *E. coli* number, and mortality were recorded. The results showed no significant ($P > 0.05$) difference between the treatments on broiler performance, OPGs, and *E. coli* numbers. Mortality during the study was only 0.53%. Thus, SRMs provided at levels up to 1.25 g/kg of feed ANDWERE 0.63 g/L of drinking water were not significantly different from COXY and Zn-bacitracin in affecting broiler performance, and in reducing OPGs and *E. coli* numbers.

Keywords: antibacterial, chicken, Eimeria oocysts, Escherichia coli
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Introduction

Plant-based materials and plant extracts are common feed additives to improve animal performance. They are available in nature and used by traditionally for their medicinal properties. Many bioactive compounds in plant-based materials and plant extracts can enhance chicken production (Pasaribu, 2019). A recent publication by Zaker-Esteghamati *et al*. (2021) reported that several flavonoids and polyphenol compounds in *Cynara scolymus* and *Silybum marianum* extracts may have a positive effect on the immune system, enhancing chicken health and reducing feed cost. Many plants containing essential oils show antibacterial activity against pathogenic bacteria and affect chicken performance positively (Sinurat *et a*l., 2020; Amouei *et al*., 2021; Pasaribu *et al*., 2021).

Sapindus rarak (lerak), a species of soapberry, is a plant belonging to the Sapindaceae family that contains bioactive compounds called saponins. The saponin content of *S. rarak* pericarp is influenced by the particle size. Analysis of saponin with a smaller particle size of *S. rarak* pericarp resulted in higher saponin content. The *S. rarak* pericarp with a 75-µm particle size was found to contain 43.52% of saponins (Pasaribu *et al*., 2014a). Saponin in *S. rarak* is not a single compound. The structures of 20 saponins have been isolated and elucidated, all of which are triterpenoid saponins composed of a triterpene aglycone linked to one, two, or three saccharide chains of varying size and complexity (Hamburger *et al*., 1992; Grant *et al.*, 2003; Asao *et al*., 2009). Saponin in *S. rarak* can form stable foam, showing haemolytic and hypocholesterolemic properties (Asao *et al*., 2009; Wina, 2012; Pasaribu *et al*., 2014b). In addition, saponins exhibit antimicrobial, antifungal, and antiprotozoal properties (Cheeke, 2001; Wina *et al*., 2017). Saponins can kill protozoa because of their ability to bind cholesterol membrane components of protozoa. In relation to *Eimeria tenella,* which causes coccidiosis, *S. rarak* microparticles (SRMs) at a dose of 1.25 g/kg or 2.5 g/kg of feed could suppress the development of oocysts of *E. tenella* populations when chickens were infected with this parasite (Pasaribu *et al*., 2014b). Previous experiments conducted in chickens used SRMs as feed

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additives mixed in the feed. However, the use of SRMs in drinking water has never been reported. Since saponin is soluble in water, it was expected that the inclusion level of SRMs could be lower, and the effect of saponin to suppress *E. tenella* and pathogenic bacterial populations may be more effective than when it is mixed in the feed. A report by Zhai *et al*. (2014) showed that saponin of ginseng stems and leaves provided through drinking water led to a positive response to vaccination in chickens, whereas Chi *et al*. (2017) reported that the administration of tea saponins through drinking water increased antioxidant capacity in chicken. Therefore, in this study, SRMs with a size of 75 microns were provided to chickens by mixing them in feed and drinking water. The study aimed to compare the effectiveness of SRMs fed through feed and drinking water with commercial anticoccidial and antibacterial agents to reduce *Eimeria* sp. and *Escherichia coli* populations and to improve chicken health.

Materials and Methods

Ethical clearance for this research was granted by the Indonesian Centre for Animal Research and Development, Bogor, Indonesia, which all experimental procedures (No. Balitbangtan/Balitnak/A/03/2019).

The SRMs were prepared using the method described by Pasaribu *et al*. (2014b) and were mixed in the feed or drinking water, depending on the treatment. A total of 370 one-day-old Ross broilers were randomly divided into seven treatments, consisting of six replicates of nine birds each: T1 served as a control; T2 received anticoccidial/COXY 2.5 g/L drinking water; T3 received Zn-bacitracin 0.5 g/kg feed; T4 and T5 were supplemented with SRMs 0.63 g/kg and 1.25 g/kg feed, respectively; and T6 and T7 received SRMs 0.3 g/L and 0.63 g/L drinking water. The diet was provided and clean drinking water was available at all times. Bodyweight (BW) and feed intake (FI) were recorded weekly, and feed conversion ratio (FCR) was calculated. The birds were housed on husk material litter and reared for 28 days. The basal diet was the same for all treatments. The starter feed contained 22% protein and 3050 kcal/kg of metabolizable energy, and the grower feed contained 20% protein and 3100 kcal/kg of metabolizable energy (Table 1).

Table 1 Ingredients and nutrient composition of basal diets for feeding broiler chickens

Ingredients	Starter phase, %	Grower phase, %	Nutrient composition	Starter phase	Grower phase
Maize	54.34	64.00	Metabolizable energy, kcal/kg	3050	3100
DDGS	10.22	1.00	Crude protein, %	22.2	20.2
Soy bean meal	24.64	23.01	Crude fibre, %	3.78	3.92
CPO	2.00	4.57	Calcium, %	0.91	1.04
Sodium bicarbonate	0.10	0.10	Phosphorus, %	1.00	1.06
Meat bone meal	6.74	3.66	Available phosphorus, %	0.45	0.521
Calcium carbonate	0.55	0.62	Methionine, %	0.57	0.525
Dicalcium phosphate	0.57	0.50	Methionine + cysteine, %	0.98	0.930
Salt	0.20	1.31	Lysine, %	1.23	1.175
Vitamin premix[1]	0.03	0.03			
Mineral premix[2]	0.04	0.04			
DL-methionine	0.20	0.02			
L-lysine HCl	0.28	1.05			
Choline 70%	0.10	0.10			

DDGS: distillers dried grains with solubles, CPO: crude palm oil.
[1]Vitamin premix contained vitamins A, C, D_3, E, K_3, B_1, B_2, B_6, B_{12}, and niacin, pantothenic acid, folic acid,and biotin
[2]Mineral premix contained iron, copper, manganese, cobalt and iodine

Oocysts per gram measurements were recorded at the Veterinary Laboratory of Indonesian Research Institute for Animal Production, and *E. coli* measurements were made at the Bacteriology Laboratory of Indonesian Research Centre for Veterinary Science. The standard McMaster technique was used to determine OPG following Tampubolon (1996) with a few minor modifications. Briefly, one gram of faeces was put into a clean Erlenmeyer flask and mixed with 14 ml of a saturated salt solution until the mixture was homogenous. Then, the mixture was filtered with gauze and the filtrate was collected in a new beaker glass. The filtrate was mixed with 15 ml saturated salt solution until the mixture was homogeneous and then centrifuged at 1500 rpm. This was repeated several times by removing the supernatant. The solution was stirred until it was homogeneous. Finally, a sample was taken with a pipette and transferred to the McMaster chamber slide to calculate the total OPG of faeces, which consisted of the total number of oocysts in the two chambers multiplied by 100.

At the end of the trial, two birds from each replication (12 birds per treatment) were randomly slaughtered to collect their cecum contents. The amount of *E. coli* in the cecum was determined with the most probable number test according to Phillips (1993).

Statistical analyses of data on bodyweight, FI, FCR, and *E. coli* number were conducted via analysis of variance using the GLM procedure with the SAS general linear model software (SAS Institute Inc., Cary, North Carolina, USA). If the treatment effects were significant (P <0.05), Duncan's test was used to compare the means. Numerical values for OPG was calculated based on the average of each treatment.

Results and Discussion

The growth performance of broilers supplemented with various levels of SRMs through feed and drinking water are presented in Table 2. The BW, FI, and FCR of the broilers were not ($P < 0.05$) affected by the treatments. In this study, the chickens were not infected with *E. tenella,* therefore, signs of coccidiosis did not appear. Mortality during the study was only 0.53% with 2 of the 370 birds dying in Week 4, one each in treatment groups T4 and T7.

Saponins in *S. rarak* did not affect chicken bodyweight. This finding was consistent with that of several studies in which various saponins were used. Bera *et al*. (2019) reported that the provision of saponins from *S. mukorossi* at a dose of 100–200 mg/kg did not affect the BW gain of birds significantly between 1 and 42 days old. Similarly, saponins from *Acacia concinna* (Peptasan®) up to a dose of 1000 ppm had no effect on the BW of broilers with or without *Eimeria* spp (Sánchez-Hernández *et al*., 2019). A study by Ayoub *et al*. (2019) reported adding 0.5 to 1 mL of Yucca Plus® in drinking water did not affect chicken BW significantly compared with the control. Likewise, the administration of yucca-derived saponin products up to 500 mg/kg did not significantly affect BW of 28-day-old chickens with *Eimeria* compared with those that were not given these supplements (Oelschlager *et al*., 2019). In contrast, a recent publication showed that the provision of saponin mixture from *Yucca schidigera* and *Quillaja saponaria* led to a positive effect on the growth performance of broilers (Bafundo *et al*., 2021 and increased villus height and crypt depth, thereby enlarging the absorptive surface of the intestine, increasing nutrient absorption and nitrogen retention, and improving growth (Bafundo *et al*., 2021). The inconsistent effect of saponins on growth performance may be because of the complexity of saponins from different plant sources. The properties of each saponin are determined by its structure, type of sugar, point of attachment of the sugar, length of sugar that is attached, and number of sugar chains attached to sapogenins (Wina *et al*., 2017).

Reports found that saponins formed pores in the cell membrane and disrupted the cell's ion balance, resulting in lysis and ultimately cell death (Koczurkiewicz *et al*., 2015). In the digestive tract, saponins might increase the permeability of mucosal cells, resulting in decreased cell function with consequent impact on nutrient absorption (Onning *et al*., 1996). Damage to the intestinal mucosa affecting the nutrient absorption process might ultimately affect broiler performance. This study indicated that the administration of SRMs through feed at dosages of T5 in feed and T7 in drinking water were safe for boilers. Thus, *S. rarak* saponins may have less toxicity compared with saponins from other sources, although this hypothesis was not tested.

Table 2 Means of bodyweight, feed intake, and feed conversion ratio of broilers treated with *Sapindus rarak* microparticles mixed in their feed and drinking water

Treatments	Initial weight, g	Final weight, g	Feed intake, g	Feed conversion ratio, g/g
T1	43.36 ± 0.3	1240.5 ± 25.1	1656.8 ± 22.1	1.338 ± 0.0
T2	42.80 ± 0.4	1281.3 ± 16.2	1665.7 ± 10.6	1.301 ± 0.0
T3	42.88 ± 0.6	1213.7 ± 23.6	1588.7 ± 21.5	1.311 ± 0.0
T4	43.55 ± 0.5	1196.5 ± 28.2	1609.2 ± 37.0	1.348 ± 0.0
T5	42.87 ± 0.4	1248.7 ± 30.3	1597.0 + 23.0	1.282 ± 0.0
T6	42.82 ± 0.3	1167.8 ± 45.5	1606.5 ± 23.1	1.399 ± 0.1
T7	43.28 ± 0.5	1172.0 ± 29.5	1568.6 ± 28.8	1.341 ± 0.0
P-value	0.7639	0.2625	0.0862	0.5016

T1: No anticoccidial/antibiotic (negative control), T2: anticoccidial 2.5 g/L drinking water, T3: Zn-bacitracin 2.5 g/L drinking water, T4: *Sapindus rarak* microparticles (SRM) 0.63 g/kg feed, T5: SRM 1.25 g/kg feed, T6: SRM 0.3 g/L drinking water, T7: SRM 0.63 g/L drinking water, IW: initial weight, BW: bodyweight, FI: feed intake, FCR: feed conversion ratio.

Feed intake of broilers aged 28 days was not significantly different (*P* >0.05) between groups. However, there was a tendency towards reduced intake in SRM groups compared with the control (T1 or T2). It was expected that SRMs in drinking water would affect feed consumption negatively. However, the bitter taste of SRM saponins might affect the palatability of feed slightly, whether the saponin is provided through feed or drinking water. One study reported that the administration of SRMs up to 5 g/kg in feed reduced daily FI and growth performance significantly. However, a lower amount of SRMs up to 2.5 g/kg did not significantly decrease feed consumption (Pasaribu *et al*., 2014a, 2014b). Miah *et al*. (2004) reported that the provision of saponins with a concentration of 75 mg/kg of feed had no effect on FI in poultry. Saponins from *S. mukorossi* at a dose between 100 and 200 mg/kg did not affect the FI of birds aged 1-42 days significantly (Bera *et al*., 2019). Saponins sourced from *A. concinna* (Peptasan®) up to a dose of 1000 ppm did not affect FI in broilers with or without *Eimeria* spp (Sánchez-Hernández *et al*., 2019). Thus, the reduction in FI may be affected by the level, type and concentration of saponin in the source, and other bioactive compounds in the source.

Statistical results showed that at 28 days FCR was not affected significantly by the treatments (*P* >0.05) (Table 2). Although statistically not significantly different, the FCR in T5 was numerically lower than T2 and T3. The difference in FCR was related to feed consumption. Feed intake to form 1 kg of chicken meat was 1282 g in the T5 treatment, 1338 g in T1, 1301 g in T2, and 1311 g in T3. This showed that the provision of SRMs at a dose of 1.25 g/kg was more efficient than COXY and Zn-bacitracin for broiler growth. Thus, the feed conversion in T5 was better than in T4, T6, and T7. Miah *et al*. (2004) reported that the provision of saponins at 75 mg/kg feed did not affect FCR significantly during growth in male broiler chickens between 22 and 42 days old. Saponins of *S. mukorossi* in birds did not significantly affect FCR (Bera *et al*., 2019). Provision of *A. concinna* containing saponins (Peptasan®) up to 1000 ppm did not affect the FCR in birds with *Eimeria* spp. (Sánchez-Hernández *et al*., 2019). The saponins from *Sapindus* sp matched COXY and Zn-bacitracin in improving broiler performance, and the provision of saponins at low doses was recommended.

Table 3 presents the number of OPG faeces collected every week. In all treatments, the number of oocysts was highest at the second week (14 days old). Although the birds were treated with anticoccidial or antibacterial agents or SRM, the life cycle of *Eimeria* sp remained the same, but the amount of OPG in each treatment was different. At the peak of growth (14 days), the amount of OPG in T4, T5, T6, and T7 was slightly lower than in T2. At 21 days, the number of OPGs in T6 was almost the same as T2 (COXY), whereas T4, T5, and T7 showed a decrease in OPG and were better than T3 (Zn-bacitracin).This indicated that SRM provided through a mixture of feed or drinking water could inhibit the development of OPG *Eimeria* spp in faeces.

Table 3 Average number of oocysts per gram of faeces from broilers treated with *Sapindus rarak* microparticles in feed and drinking water

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
T1	0.3	1.0	188.7	62.9	7.9
T2	0.3	0.5	156.8	14.0	4.0
T3	0.4	0.7	162.4	60.3	1.8
T4	0.6	0.9	154.9	50.3	1.9
T5	0.5	0.8	152.1	35.0	3.9
T6	0.4	0.4	150.4	17.7	1.0
T7	1.1	1.4	147.5	28.9	2.0

T1: No anticoccidial or antibiotic (negative control), T2: anticoccidial 2.5 g/L drinking water, T3: Zn-bacitracin 2.5 g/L drinking water, T4: 0.63 g/kg *Sapindus rarak* microparticles (SRM) on feed, T5: (1. 25 g/kg SRM in feed, T6: 0.3 g/L SRM in drinking water, T7: SRM 0.63 g/L in drinking water

In a previous experiment, Pasaribu et al. (2014b) reported that the administration of SRMs at a dose of up to 2.5 g/kg feed reduced the OPG of E. tenella in faeces. In this experiment, SRMs administered at a lower levels than in the previous experiment and provided through feed or drinking water also reduced the amount of OPG *Eimeria* spp in faeces. Sánchez-Hernández et al. (2019) reported that saponins from A. concinna at doses up to 1000 ppm could reduce oocysts in broilers infected with *Eimeria* spp Thus, saponins from various plants can damage oocysts of *Eimeria* spp Saponins might penetrate oocysts through the micropyle cap located at the polar end of the oocysts, damaging the sporocyte, which is characterized by the sporocyte having an irregular shape (abnormal) or shrunken in size (Pasaribu et al., 2014a), and inhibiting the growth of existing sporozoites. Although the difference was not significant, the number of OPGs was slightly lower when SRMs were provided through drinking water. In a study by Sander et al. (2019) where saponins were provided as vaccine adjuvants or immune stimulating complexes from native plants, saponins improve immunoglobulin G. Further studies are warranted to evaluate the effect of SRM provision in drinking water on chicken immunity against *E. tenella*.

The population of *E. coli* was not different (*P* >0.05) between the groups (Figure 1). Although statistically not different, T4 and T7 reduced total *E. coli* by 12.3% and 4.3%, respectively, compared with T1. Although T3, T5, T6, and T7 did not reduce the population of *E. coli*, T2 was numerically better than all other treatments. Saponin in SRMs suppressed the growth of *E. coli*. Other saponins have been reported to possess antibacterial properties against *E. coli*. A study by Ayoub *et al*. (2019) showed that the administration of Yucca Plus liquid® could reduce the population of *E. coli* in intestinal broilers. Likewise, Wang & Kim (2011) stated that the number of *E. coli* decreased with the provision of *Yucca shidigera* extract compared with the control. The ethanolic extract of *S. mukorossi* fruit pericarp also showed antibacterial activity against *E. coli* (George & Shanmugam, 2014). An in vitro experiment by Arabski *et al*. (2012) found that the presence of saponins of *Quillaja saponaria* at concentrations of 1–12 µg/mL did not inhibit the growth of *E. coli*. Arabski *et al*. (2012) assumed that the concentration of saponins affects its activity to suppress *E. coli* growth. Saponin inhibits *E. coli* growth through its ability to bind cell membrane components, thereby forming pores in the cell membrane. These pores disrupt the ion balance of the cell and lead to the loss of intracellular constituents and leakage of nucleic acids and proteins, resulting in the lysis and malfunction of the cell membrane, causing cell death (Koczurkiewicz *et al*., 2015, Dong *et al*., 2020). It was indicated that saponins of SRMs could also damage the cell membrane of *E. coli,* causing the population to decrease. Therefore, SRM provision at the dose of 0.63 g/kg of feed or per litre of drinking water was recommended because it could still reduce the population of *E. coli* by approximately 10.9% and 4.1%, respectively. SRM activity against *E. coli* growth in this broiler experiment was an important finding. It is useful for SRM application at farm level because *E. coli* contamination of drinking water and litter occurs frequently in broiler farms.

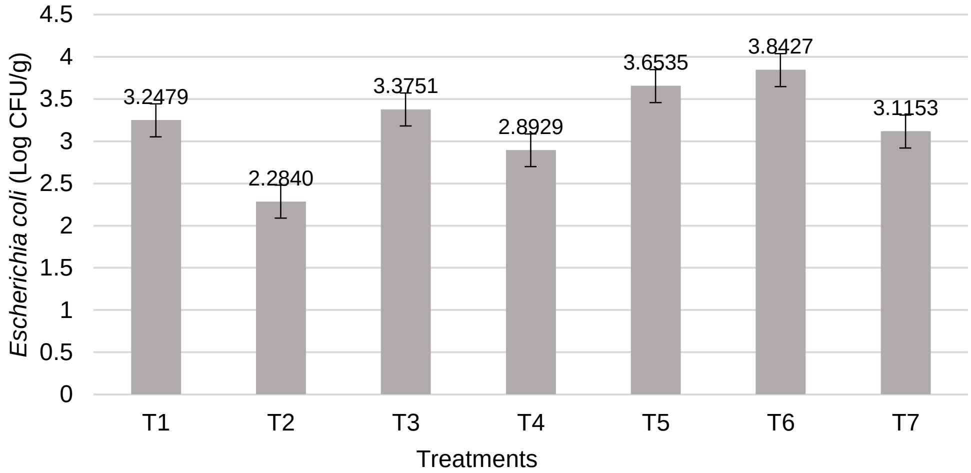

Figure 1 Number of *Escherichia coli* in the cecum of broiler chickens treated with *Sapindus rarak* microparticles

T1: No anticoccidial or antibiotic (negative control), T2: anticoccidial 2.5 g/L drinking water, T3: Zn-bacitracin 2.5 g/L drinking water, T4: 0.63 g/kg *Sapindus rarak* microparticles (SRM) on feed, T5: (1. 25 g/kg SRM in feed, T6: 0.3 g/L SRM in drinking water, T7: SRM 0.63 g/L in drinking water

Conclusion

Sapindus rarak microparticles administered at levels up to 1.25 g/kg in feed or 0.63 g/L in drinking water could be used as an anticoccidial and antibacterial agent for broiler chickens. Its performance is similar to that of COXY and Zn-bacitracin as a dietary supplement.

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Author's Contribution

TP and EW designed and conducted the experiment. TC participated in the collection of the data. TP and TW analysed and interpreted the data and wrote the manuscript. All authors have read and approved the manuscript.

Conflict of Interest

There was no conflict of interest in this article.

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