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EFFECT OF DRINKING WATER SUPPLEMENTATION WITH EXTRACT FERMENTED *Tamarindus indica* BY *Saccharomyces spp* ON EGG PRODUCTION, FEED DIGESTIBILITY AND N-NH_3 CONTENT IN EXCRETA LAYING HENS

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ABSTRACT

The present experiment was conducted to determine effects of drinking water supplementation with extract fermented *Tamarindus indica* by *Saccharomyces spp* (*Tamarin-Pro*) on egg production, feed digestibilty, and N-NH_3 in excreta of laying hens up to 54 weeks old. A total of 240 laying hens with an average initial body weight of 1674.91±18.52 g were randomly allotted into four drinking water treatments. The dringking water treatments included: drinking with 2% *Tamarin-Pro* extract (A); drinking water with 4% *Tamarin-Pro* extract (B); drinking water with 6%*Tamarin-Pro* extract (C); and drinking water as a control (D), respectively. Result showed that the Tamarin-Pro drinking water treatment had improved feed digestibility compared with control treatments ($p<.05$). The egg mass in Tamarin-Pro treatments B and D were greater than that in control treatment ($p<.05$). Moreover, *Tamarin-Pro* extract decreased significantly ($p<.05$) N-NH_3 in excreta of birds. In conclusion, fermented Tamarindus indica by *Saccharomyces spp* (*Tamarin-Pro*) extract supplementation improved egg mass and nutrient digestibility in laying hens and decreased N-NH_3 in excreta of hens

KEYWORDS: *Tamarindus indica*, water extract, probiotics, amonia, feed digestibility.

INTRODUCTIONS

The trend of using phytochemical compounds from herbs in feed and drinking water has increased over the past two decades. The results of the study showed that the use of antibiotics in excessive feed would intensify the potential risks of increasing resistance in human pathogens. Bacterial resistance and antibiotic residues in animal products cause increasing concern in using antibiotics as a growth driver and ultimately result in a ban on the use of antibiotics in feed in most developed countries (Gheisar and Kim, 2018).

The herbal leaf fermentation process can increase the efficacy of the treatment of active ingredients and reduce the anti-nutritional effects on herbal leaves, thereby increasing the growth of poultry and the value of the benefits of herbal leaves (Ahmed *et al.*, 2016). Tanin is known to have a bitter or astringent taste which reduces palatability and hence food intake (Jansman, 1993). The fermentation process can reduce the concentration of tannic acid, which can reduce the adverse effects of tannin found in natural herbs on feed intake.

Supplementation of herbal plant extracts can improve taste and feed consumption, stimulate animal appetite, and then increase feed intake (Frankic *et al.*, 2009). As a result, increased feed intake can contribute to increased productivity of poultry. Different results regarding the effects of herbal extract additives on livestock production performance were reported in the study of Gheisar and Kim (2018) and Ahmed *et al.* (2017). Similar results were reported by Ahmed *et al.* (2016) that supplementation of herbal leaves (pomegranate, Gingko biloba and licorice) in natural or fermented forms, turned out to have no effect on growth, but reduced feed consumption and increased feed efficiency compared to controls (without herbal leaf supplementation).

The phytobiotic antioxidant function of herbal leaves is positively influencing food stability, improving egg quality, and prolonging the storage time of eggs (eggs are easily damaged if stored too long). In general, some results of the study show the positive effects of herbal phytochemical compounds on livestock performance (Gheisar and Kim, 2018; Lei *et al.*, 2018; Bidura *et al.*, 2017) and probiotics (Bidura *et al.*, 2019; Tufarelli *et al.*, 2017; Phuoc and Jamikorn, 2017; Mountzouris *et al.*,

2010). These conflicting results regarding growth performance responses to natural herbs, fermentation, or extractions can be caused by different herb species, concentrations of herbs, and methods of processing herbs (Windisch *et al.*, 2008; Hashemi and Davoodi, 2011; Embuscado 2015).

Recently, in Indonesia there has been a ban on the use of antibiotics, so probiotics and phytochemicals of herbal leaves have been suggested as the most beneficial alternative for livestock because of their beneficial effects (Abdelqader *et al.*, 2013). *Tamarindus indica* leaves have long been used by the community as herbal leaves with their phytochemical content, such as alkaloid, steroid, terfenoid, fenolik, flavonoid, dan tanin (Carvalho, 2019). Likewise, the yeast *Saccharomyces sp* has long been used as a "tape" fermentation inoculant, and can act as probiotics to improve feed digestibility and poultry performance, and reduce ammonia gas content in cages (Bidura *et al.*, 2012; 2019 and Ezema and Eze, 2015). Hasan *et al*. (2016) and Hasanuddin et al. (2017; Zhen et al., 2019) reported that that feed fermentation generally improves the bacterial ecology of the digestive tract and the immune response in chicks, therefore, becomes a new model for future strategies to control chicken disease.

It is interesting to study the use of medicinal properties of phytochemicals combined with probiotics. Phytochemical and probiotic compounds, both show antimicrobial effects, antioxidants, anti-inflammatory, boost growth and improve feed efficiency. Therefore this study was conducted to examine the use of *Tamarindus indica* leaf extract fermented by probiotics *Saccharomyces spp* (*Tamarin-Pro*) on drinking water to egg production, feed digestibility, and ammonia levels in excreta of laying hens

MATERIAL AND METHODS

Experimental design, animals, housing and diets

A total of 240 laying hens with an average initial body weight of 1674.91±18.52 g were randomly allotted into four drinking water treatments. The dringking water treatments included: drinking water with 2% *Tamarin-Pro* extract (A); drinking water with 4% *Tamarin-Pro* extract (B); drinking water with 6% *Tamarin-Pro* extract (C); and drinking water as a control (D), respectively. All hens were housed in an environmentally controlled room with forced ventilation. Each pen was equipped with a nipple drinker and a varalon feeder. Laying hens were provided with free access to drinking water and feed throughout experimental period. All chickens were given commercial feed specific for laying hens containing 2.750 kcal/kg of Metabolizable Energy (ME); 18% of CP; 3.5% of Ca; and available phosphor of 0.45%.

The laying hens were randomly divided into four groups: A: drinking water with administration of 2%*Tamarin-Pro* water extract; B: with the administration of 4% *Tamarin-Pro* water extract; C: with the administration of 6%*Tamarin-Pro* water extract; and D: drinking water without administration of *Tamarin-Pro* water extrac, repectively. Each treatment consisted of five replicate pens with 10 birds were randomly assigned to each pen at 150×70×45 cm (length×width×height). Each experimental diet was in mash form and the birds had free access to feed and water throughout the experiment.

Preparation of natural and fermented herbs

The leaves of *Tamarindus indica* are washed, dried in air and powdered. For solid fermentation, *Tamarindus indica* leaf powder is mixed with *Saccharomyces spp* at $3.7x10^7$ colony forming units (cfu)/g. After that, the mixture is immersed in distilled water to maintain a moisture concentration of 40%. The hydrated herb is then fermented at 37^0C. After fermentation for 72 hours, the fermented sample is mixed with distilled water 1: 1 (v: v) and it is meserated for 24 hours. Then filtered to get *Tamarindus* leaf water extract which has been fermented with *Saccharomyces spp* as a probiotic source. This water extract is then referred to as ***Tamarin-Pro*** extract.

Live performance

Continuous lighting and access to feed and water was provided throughout the experiment. The birds were weighed at the commencement (40 weeks of age) and the end (56 weeks of age) of the experiment. Eggs were collected daily and egg production was expressed on a hen-day basis (% hens day). Individual egg weights were recorded then used to calculate mean egg weight for all experimental period. The total egg mass was calculated by multiplying egg weights by egg production. Feed intake was measured on a cage basis (hen) every week. Daily feed intake per bird was calculated on a cage total feed intake basis for the entire experimental period and for the number of days in all the period. Feed conversion ratio (kg of feed/kg of eggs mass) for the all period was calculated on a cage basis from egg production, egg weight, and feed consumption.

Retention and excretion of nutrients

In order to determine the nutrient digestibility (dry matter and organic matter digestibility) value of the diet: The amount of diet used was 100 g, this amount as based on preliminary assays with laying hens consumption of ration. All the birds were deprived of feed for 24 h to ensure that their alimentary canals were empty from feed residues. They were then force-fed with the specific amount of diets (all treatments). Stainless steel funnel with 40 cm stem was used in *force feeding technique* (Bidura *et al*., 2019). Water was available *ad libitum* during the experimental period. The total excreta were collected in plastic trays. The excreta samples were frozen, allowed coming to equilibrium with the atmospheric moisture, weighed, and ground through a 1 mm sieve. Samples of excreta and diets were subjected to appropriate analysis to determine dry matter (DM) and organic matter (OM), respectively. Dry matter (DM) and

organic matter (OM), and ash determinations were done according to the Assocciation of Official Analytical Chemists (2005). All assays were conducted in triplicate.

Determination of N-NH_3 concentration in excreta

The determination of N-NH_3 levels in excreta was carried out using the Conway dish diffusion as follows: 1 ml of supernatant sample on the left side of the Conway dish, 1 ml of saturated Na_2CO_3 solution on the right bulkhead, 1 ml of H_3BO_3 2% indicated BCG+MR on the middle cup, then the vaseline conway cup is closed tightly, shake it slowly, until the supernatant with Na_2CO_3 solution is completely mixed, then left for 24 hours at room temperature, then titrate using a 0.005N H_2SO_4 solution, until the end point titration. N-NH_3 levels can be calculated as follows: mM N-NH_3 = (titration volume x N H_2SO_4 x 1,000)

Statistical analysis

All data were analyzed with ANOVA to determine the differences among treatments. If differences were found, then further analysis was performed with Duncan's multiple range test.

RESULTS

The results showed that initial weight, final body weight, number of eggs, feed consumption, feed conversion ratio, digestibility of dry matter, organic matter, and ammonia levels in excreta in group A (drinking water with 2% extract of *Tamarin-Pro*), B (drinking water with 4% extract of *Tamarin-Pro*), C (drinking water with 6% extract of *Tamarin-Pro*), and D (drinking water without *Tamarin-Pro* extract as control), respectively were presented as in Table 1. The total number of eggs and feed consumption for 56 days of research on the treatment of Tamarin-Pro extract through drinking water did not show a significant difference (P>0.05) with control (D).

Table 1. Effects of supplementation Tamarin-Pro level on drinking water on egg production, feeds digestibility and N-NH_3 in laying hens.

Variables	Groups[1]				SEM[2]
	A	B	C	D	
Initial body weight (g)	1670.35a[3)]	1675.08a	1679.31a	1668.74a	19.052
Final body weight (g)	1716.92a	1762.07a	1708.94a	1754.63a	32.904
Egg mass (g/bird/8weeks)	3163,01ab	3183,51a	3217,4a	3033,80b	42,046
Egg production (egg/bird/8weeks)	49,98a	50,18a	50,42a	49,00a	0,641
Feed consumption (g/bird/8weeks)	7265,61a	6888,54a	6912,26a	6963,48a	109,825
Feed conversion ratio (feed consumption/egg mass)	2,29a[3]	2,14b	2,15b	2,29a	0,017
Dry matter digestibility (%)	75,04b	78,59a	78,92a	74,81b	0,252
Organic matter digestibility (%)	77,09b	80,77a	81,02a	76,9b	0,389
N-NH_3 (m.Mol)	22,27b	21,66b	23,34b	42,47a	2,376

Note

1. The dringking water treatments included: drinking water with 2% *Tamarin-Pro* extrac (A); drinking water with 4% *Tamarin-Pro* extract (B); drinking water with 6% *Tamarin-Pro* extract (C); and drinking water as a control (D), respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different (P<0.05)

The administration of *Tamarin-Pro* water extract on drinking water, namely in group B and C chickens, was significant (P<.05) higher each: 4.93% and 6.05% than controls (without *Tamarin-Pro* extract). The average value of the feed conversion ratio (FCR) in the control chicken group (D) was 2.29/head (Table 2). The average FCR value in group A chickens was 0.18% not significant (P>.05) lower than the control. However, the average FCR values in the chicken group B and C, were: 5.59% and 6.28% significantly different (P<0.05) lower than the control (D).

Observations on the digestibility of feed dry matter turned out to increase significantly (P<.05) in the presence of 4% and 6% *Tamarin-Pro* extract in drinking water, ie 5.05% and 5.49% higher than controls. Similarly, the organic matter digestibility of feed in group B and C chickens was: 5.03% and 5.36% higher than the control (D).

The content of N-NH3 in chicken excreta treated with *Tamarin-Pro* extract, namely chickens in Groups A, B, and C, were decreased significantly (P<.05) lower by 47.56%; 48.99%; and 45.04%, rather than controls or chicken in Group D.

DISCUSSION

The total number of eggs and feed consumption for 56 days of research on the treatment of fermented Tamarindus indica extract by yeast *Saccharomyces spp* (*Tamarin-Pro*) through drinking water did not show a significant difference with control (D). This is due to the fact that the metabolic energy content and dietary nutrients for the four treatments are the same. Reported by Bidura *et al.* (2017); Ekayuni *et al.* (2017) and

Sanchez *et al*. (2005) which shows that herbal extracts do not have toxic effects or do not contain factors that limit intake which are opposite to absorption of nutrients.

The extract of fermented herbal products has not been able to increase egg production and feed consumption. According to Shivaramaiah *et al.* (2011), among these directly given microbes, their survival through digestion, growth in the digestive tract, and excretion through feces. *Tamarin-Pro* supplementation in drinking water has no significant effect on egg production. The same thing was reported by Davis and Anderson (2002) that there was no increase in egg production in laying hens added with probiotic bacteria, including *Lactobacillus* and *Bacillus.* This is also in accordance with the study of Kalavathy *et al.* (2009) who reported that probiotic supplementation had no significant effect on egg production in chickens. Egg production in chickens fed probiotic bacteria and yeast did not differ significantly from control chickens aged 40 to 52 weeks (Balevi *et al*., 2001). Similarly, the results found by researchers, such as Ahmed *et al*. (2017) finding herbal leaf flour has no effect on feed consumption. Similar things Paguia *et al*. (2014) found that the supply of *Moringa* leaf flour did not affect the consumption of rations.

These conflicting results regarding growth performance responses to naturally fermented or extracted herbs are caused by different types of herbs, concentrations of herbs, and herbal processing methods (Windisch *et al.*, 2008; Embuscado, 2015). Decrease in feed consumption can be attributed to the presence of phytate, tannins, and saponins, in herbal leaves which all can reduce feed consumption, due to their unpleasant nature and binding to nutrients (Anhwange *et al.*, 2004).

The use of *Tamarindus* leaf extract fermented by probiotics (*Tamarin-Pro*) on the drinking water were significantly increased egg mass and feed efficiencies in group B and C chickens. *Tamarin-Pro* leaf water extract can be used as an effective feed supplement for laying poultry to improve feed efficiency (Akhouri *et al*., 2013; Sanchez *et al.*, 2005). The main way of action of this active ingredient is the inhibition of microbial pathogens and endotoxins in the chicken intestine and increasing pancreatic activity, producing metabolism and better utilization of nutrients (Windisch *et al.*, 2008; Grashorn, 2010).

Digestion of dry matter and organic matter in hens groups C and D, was higher than the control. As recommended by Hernandes *et al*. (2004), that plant extract supplements can improve nutrient digestibility in the digestive tract of poultry. Herbal extracts (Garlic) can increase the activity of pancreatic enzymes and microenvironmental conditions for better utilization of nutrients in mice (Ramakrishna *et al.*, 2003).

Plants are rich in various secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. These active compounds in the digestive tract of chickens can help absorb nutrients. As reported by Adibmoradi *et al*. (2006), that herbal active compounds in garlic can increase villous height and crypto depth, and reduce epithelial thickness and the number of villous cells in the duodenum, jejunum, and poultry ileum. Increased height of villi, as well as the thickness of epithelium and cup in the duodenum, jejunum and ileum will increase nutrient uptake (Nusairate, 2007). The results of the study by Bidura *et al.* (2017) found that the administration of Sauropus leaf extract in drinking water can significantly improve feed efficiency in laying hens.

The administration of *Tamarindus* leaf extract fermented by probiotics (*Tamarin-Pro*) in drinking water, namely in Group B and C hens, significantly reduced ammonia gas content in chicken excreta compared to Group D (control). *Tamarin-Pro* contains yeast *Saccharomyces spp* as a source of probiotics in drinking water drunk by chicken can reduce the concentration of ammonia gas in chicken excreta. The use of probiotic microbes in poultry is reported to be able to suppress urease enzyme activity and can reduce the amount of uric acid in the digestive tract of chickens, because uric acid has been used as a microbial protein (Chiang and Hsieh, l995). According to Nguyen *et al*. (2019), the largest increase in growth performance, nutrient digestibility, faecal bacterial enumeration, and harmful gas emissions in weaned pigs was obtained when a probiotic mixture was added to feed as much as 0.3%. The same results were obtained when fermented probiotics in dry form were used as chicken daily feed (Lokman *et al*., 2015).

It is assumed that feed fermentation generally improves the bacterial ecology of the digestive tract and the immune response in chicks, therefore, becomes a new model for future strategies to control chicken disease (Hasan *et al*., 2016). Probiotics can maintain beneficial intestinal microflora, increase host resistance to enteric pathogenic bacteria, such as *Salmonella* and *Campylobacter species*, and produce a healthy digestive environment in poultry digestive systems, feed conversion, and higher poultry performance (Vila *et al.*, 2009; Mountzoris *et al*., 2010).

Supplementation of probiotics into rations significantly increases weight gain and decreases N-NH3 levels in excreta of poultry (Chen *et al.*, 2009; Roni *et al*., 2014; Puspani *et al*., 2014). The same was reported by Bidura *et al.* (2014), that supplementation of Saccharomyces spp culture isolated from Bali cow feces by as much as 0.20% in diets can improve performance and reduce ammonia levels in broiler excreta.

CONCLUSION

We conclude that supplementation of fermented *Tamarindus indica* by *Saccharomyces spp* (*Tamarin-Pro*) extract on drinking water positively changed egg

performance and feed digestibility of laying hens, but decreased N-NH3 in hens excreta.

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Conflict of Interest Declaration

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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