EFFECT OF DRINKING WATER SUPPLEMENTATION WITH EXTRACT FERMENTED Tamarindus indica BY Saccharomyces spp ON EGG PRODUCTION, FEED DIGESTIBILITY AND N-NH₃ 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 digestibility, and N-NH₃ 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 drinking 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-NH₃ 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-NH₃ 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). Tannin 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 Hasanuuddin 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 drinking 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 varaloon 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 extract, respectively. 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 3.7x10^3 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°C. After fermentation for 72 hours, the fermented sample is mixed with distilled water 1: 1 (v: v) and it is mersated 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 exact 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 Association of Official Analytical Chemists (2005). All assays were conducted in triplicate.

**Determination of N-NH₃ concentration in excreta**
The determination of N-NH₃ 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 Na₂CO₃ solution on the right bulkhead, 1 ml of H₂BO₃ 2% indicated BCG+MR on the middle cup, then the baseline Conway cup is closed tightly, shake it slowly, until the supernatant with Na₂CO₃ solution is completely mixed, then left for 24 hours at room temperature, then titrate using a 0.005N H₂SO₄ solution, until the end point titration. N-NH₃ levels can be calculated as follows: mM N-NH₃ = (titration volume x N H₂SO₄ 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 Tamarind-Pro), B (drinking water with 4% extract of Tamarind-Pro), C (drinking water with 6% extract of Tamarind-Pro), and D (drinking water without Tamarind-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 Tamarind-Pro extract through drinking water did not show a significant difference (P>0.05) with control (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 (Tamarind-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

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**Table 1. Effects of supplementation Tamarind-Pro level on drinking water on egg production, feeds digestibility and N-NH₃ in laying hens.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>1670.35a¹</td>
<td>1675.08a</td>
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<tr>
<td>Final body weight (g)</td>
<td>1716.92a</td>
<td>1762.07a</td>
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<tr>
<td>Egg mass (g/bird/8weeks)</td>
<td>3163.01ab</td>
<td>3183.51a</td>
</tr>
<tr>
<td>Egg production (egg/bird/8weeks)</td>
<td>49.98a</td>
<td>50.18a</td>
</tr>
<tr>
<td>Feed consumption (g/bird/8weeks)</td>
<td>7265.61a</td>
<td>6888.54a</td>
</tr>
<tr>
<td>Feed conversion ratio (feed consumption/egg mass)</td>
<td>2.29²</td>
<td>2.14b</td>
</tr>
<tr>
<td>Dry matter digestibility (%)</td>
<td>75.04b</td>
<td>78.59a</td>
</tr>
<tr>
<td>Organic matter digestibility (%)</td>
<td>77.09b</td>
<td>80.77a</td>
</tr>
<tr>
<td>N-NH₃ (mMol)</td>
<td>22.27b</td>
<td>21.66b</td>
</tr>
</tbody>
</table>

**Note**
1. The drinking water treatments included: drinking water with 2% Tamarind-Pro extract (A); drinking water with 4% Tamarind-Pro extract (B); drinking water with 6% Tamarind-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 Tamarind-Pro water extract on drinking water, namely in group B and C chickens, was significant (P<0.05) higher each: 4.93% and 6.05% than controls (without Tamarind-Pro extract). The average value of the feed conversion ratio (FCR) in the control group (D) was 2.29/head (Table 2). The average FCR value in group A chickens was 0.18% not significant (P>0.05) 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<0.05) in the presence of 4% and 6% Tamarind-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 Tamarind-Pro extract, namely chickens in Groups A, B, and C, were decreased significantly (P<0.05) lower by 47.56%; 48.99%; and 45.04%, rather than controls or chicken in Group D.
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; Embuscardo, 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 hens. 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, 1995). 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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