Research Article

An Early Screening of Turmeric as an Antibiotic Growth Promoter Replacement and its Effects on the Reproductive Organs of Mojosari Ducks

Widiastuti Ardiansyah[1,3], Osfar Sjofjan[2*], Eko Widodo[2], Suyadi Suyadi[2], Danung Nur Adli[2]

[1]Faculty of Animal Science and Technology, University of Muhammadiyah Gorontalo, Gorontalo, 55281, Indonesia; [2]Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia; [3]Doctoral Student Lembaga Pengelola Dana Pendidikan (LPDP), Ministry of Finance, Republic of Indonesia on Faculty of Animal Science, University of Brawijaya, Malang 65145, Indonesia.

Abstract | This study aimed to determine the bioactive compound of turmeric and its effects on the reproductive organs of Mojosari ducks. A total of 500 grams of turmeric were prepared for the identification of bioactive compounds and flavonoids, while a total of 280 Mojosari ducks aged 20–28 weeks were used in the second step. The study comprised a descriptive analysis and a completely randomized design with six treatments and four replications with eight Mojosari laying ducks in each experimental unit. An extraction and maceration were employed during the early screening of the bioactive compound of turmeric. The treatments consisted of T_0 = basal diet, T_1 = basal diet + 0.2% turmeric, T_2 = basal diet + 0.8% turmeric, T_3 = basal diet + 0.2% turmeric + 0.1% probiotics, T_4 = basal diet + turmeric 0.8% + 0.6% probiotics, and T_5 = basal feed + bacitracin 0.01%. The data were analysed using Proc Means for multiple variates and one-way analysis of variance (ANOVA). The significant difference ($p < 0.05$) was applied if treatments were significant. The regression equation $y = 0.076x + 0.005$ was obtained with coefficient $r = 0.999$. The results on the reproductive appearance of the Mojosari ducks showed that the average weight of the ovaries, large white and small white follicles had a significant effect ($p < 0.05$). In conclusion, increasing the level of curcumin produced a linear increase in the number of bioactive compounds and the reproductive organs of Mojosari ducks.

Keywords | Bioactive compound, Follicle, Mojosari Duck, Reproductive Organ, Turmeric

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***Correspondence** | Osfar Sjofjan, Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia; **Email:** osfar@ub.ac.id
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INTRODUCTION

Ducks are a type of local waterfowl with a potential dual purpose, namely, to provide meat and eggs. Duck eggs comprise 19.35% of the 793,800 tonnes of eggs needed in Indonesia. In 2019 and 2020, duck egg production stood at 294 and 298 tonnes respectively, with an average duck egg production of 4% per year (Ditjenak, 2020). Various strains can be classified as local ducks, namely Tegal ducks (*Anas javanicus*), Alabio ducks (*Anas platurynchos*), Bali ducks (*Anas, sp*) and Mojosari ducks (Ismoyowati et al., 2022; FAO, 2016). However, the Mojosari duck is a candidate for development as a local duck and may potentially contribute animal protein to the people of Indonesia (Ismoyowati et al., 2022). Indonesia has developed either egg and meat from ducks (Sjofjan et al., 2021a). It has already satisfied the requirement set out by the Ministry of Agriculture of Indonesia. Mojosari ducks can lay their first eggs at the age of 24 weeks (6 months) and from the age of 7 months, egg production can reach approximately 70 to 80% (Ketaren, 2007). Duck also more adapted to digest fibre compare with other poultry (Adli et al., 2022).

In addition, Mojosari ducks are more disease resistant than other waterfowl. It has also been reported that Mojosari ducks are more resistant to the use of antibiotic growth promoters. Feed additives have generally been used in modern poultry farming to spur growth or increase livestock productivity and increase feed efficiency (Rahmat et al., 2021; Sjofjan and Adli, 2021). One type of feed additive used is herbal plants with components of bioactive substances such as curcumin, flavonoid polyphenols, and essential oils that can be used as a substitute for synthetic antibiotics (Toghyani et.al., 2011). Using turmeric as an additive has an important issue and related to its hormonal profile of poultry (Urom et al., 2018; Mazidda et al., 2020). Thus, herbal plants can improve the digestive tract, increase digestibility for weight gain, immunity, disease prevention, and decrease mortality and reproductive performance (Ardiansyah et al., 2022). Therefore, this study aimed to determine the bioactive compound of turmeric and its effects on the reproductive organs of Mojosari ducks.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was granted by the Animal Care and Use Committee, University of Brawijaya, No. 34-KEP-UB-2022.

Identification of bioactive component of curcumin

A total of 500 grams of turmeric rhizome simplicial was macerated using 70% ethanol solvent, then filtered and evaporated to obtain a thick extract. Second, 15 grams of viscous extract were partitioned using N-Hexane as the solvent. The hexane-insoluble sample was separated and repartitioned with the same solvent and repeated three times. Meanwhile, the insoluble sample was taken and re-evaporated until a thick extract was obtained. Furthermore, the sample was centrifuged and then filtrated. The obtained filtrate was analysed using UV-Vis spectrophotometry. Third, the samples were prepared by weighing 10 mg and then dissolving this in 10 mL of pro-analytical ethanol (96% ethanol purity) to produce a sample solution concentration of 1000 ppm, following which the solution was diluted to several concentrations (50 ppm – 100 ppm). A total of 250 microlitres of the curcumin sample was pipetted from 1000 ppm stock into a 5 ml volumetric flask. Pro-analytical ethanol was then added up to the volume limit mark to give a sample concentration of 50 ppm, and three replications of the sample were made. Next, the absorbance of the solution was measured at the maximum wavelength. The result was obtained using the following formula:

% percentage = Level of curcumin absorbance / level of maximum wavelength x 100%

Identification of flavonoid on turmeric

First, the samples were prepared by weighing and then dissolving in pro-analytical ethanol with concentrations of 50 ppm to 100 ppm. A total of 150 microlitres of the turmeric sample were pipetted from the stock of 1000 ppm to produce a sample concentration of 30 ppm; three replications of the sample were then made. Furthermore, the sample solution was reacted with AICI3 and sodium acetate such as standard quercetin, incubated for 30 minutes and the absorbance was measured at the maximum wavelength. A standard curve of quercetin was produced by carefully weighing 10 mg of standard quercetin. This was then dissolved in pro-analytical ethanol, placed into a 10 ml volumetric flask and the volume was made up to the mark. Third, quercetin dilution was undertaken to obtain final concentrations of 2, 4, 6, 8 and 10 ppm after being reacted with 10% AICI3 and other reagents. Fourth, the diluted quercetin was reacted with 100 l of 10% folic AICI3 and 1 ml of sodium acetate and the volume was made up in a 5 ml volumetric flask to form a yellow colour.

Finally, mixture (d) was incubated for 30 minutes and the absorbance was measured at the maximum wavelength. The result was obtained using the following formula:

% percentage = Level of curcumin absorbance / level of maximum wavelength x 100%

Experimental design

A total of 280 female Mojosari ducks (28 old-weeks with coevicient variation 9.22%) were used in an eight-week trial. In this experiment, the probiotic used was *α-Lactobacillus sp.* 1.4 x 10^{10} (RAPID) and the additive used was derived from turmeric (Curcuma longa) rhizomes harvested at 11 to 12 months. All of the ducks were given ad libitum access to water through adjustable nipple drinkers. Each treatment was randomised as part of a complete block design. The treatments were as follows: T0 = basal diet + 0% control, T1 = basal diet + 0.2% turmeric flour, T2 = basal diet + 0.8% turmeric flour, T3 = basal diet + 0.2% turmeric flour + probiotics 0.1%, T4 = basal feed + 0.8% turmeric flour + 0.6% probiotics, and T5 = basal feed + bacitracin antibiotic 0.01%. The formulated feed shown in Table 1. The formulated feed consisted yellow maize, maize bran, soybean meal, bone meal, meat meal, soy oil, mineral premix, vitamin premix, anti-oxidant, and canthaxanthin. Representative of the formulated feed were analyzed for metabolizable energy (Kcal/kg), crude protein (CP), crude fibre (CF), calcium (Ca), and phosphor (P) according to established procedures described by (AOAC, 2000). The composition of formulated feed showed in the Table 1.

Reproductive organs

At the end of rearing, duck samples were taken (one for each experimental unit) before the ducks were slaughtered

Table 1: Ingredient of *MOJOSARI* duck diets (20-28 weeks)

Ingredients (% as is basis)						
	T_0	T_1	T_2	T_3	T_4	T_5
Yellow Maize	50.00	50.00	50.00	50.00	50.00	50.00
Maize bran	15.00	15.00	15.00	15.00	15.00	15.00
Soybean	20.00	20.00	20.00	20.00	20.00	20.00
Bone meal	7.00	7.00	7.00	7.00	7.00	7.00
Meat meal	4.00	4.00	4.00	4.00	4.00	4.00
Soy oil	2.00	2.00	2.00	2.00	0.90	1.99
Custom Mineral premix*	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix**	0.50	0.50	0.50	0.50	0.50	0.50
Antioxidant***	0.20	0.20	0.20	0.10	0.00	0.20
Canthaxanthin	0.30	0.08	0.00	0.10	0.00	0.30
Probiotics	0.00	0.00	0.00	0.10	0.60	0.00
Curcuma longa flour	0.00	0.20	0.80	0.20	0.80	0.00
Bacitracin	0.00	0.00	0.00	0.00	0.00	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition						
ME (Kcal/kg)	2700.00	2700.00	2700.00	2700.00	2700.00	2700.00
Crude Protein (CP)	18.30	18.30	18.30	18.30	18.30	18.30
Crude fibre (CF)	4.00	4.00	4.00	4.00	4.00	4.00
Calcium (Ca)	0.60	0.60	0.60	0.60	0.60	0.60
Phosphorus (P)	0.40	0.40	0.40	0.40	0.40	0.40
Proximate composition (Wet chemical analysed)						
ME (Kcal/kg)	2721.03	2734.02	2678.01	2712.01	2713.22	2713.01
Crude Protein (CP)	18.56	18.21	18.11	18.20	17.93	19.01
Crude fibre (CF)	4.01	4.32	4.01	4.03	4.11	4.12
Calcium (Ca)	0.60	0.60	0.60	0.60	0.60	0.60
Phosphorus (P)	0.40	0.40	0.40	0.40	0.40	0.40

**: Vitamin A, 6000IU, Vitamin D3, 1000IU, Vitamin E, 10mg, Vitamin K3, 1.5mg, Vitamin B1, 5mg, Vitamin B2, 2.5mg, Vitamin B6 0.5mg,Vitamin B12, 2.0mg, niacin, 5.5mg, pantothenic acid, 0.2mg, betaine, 30mg.

*: Iron, 12.50mg, copper, 3mg, manganese, 37.5mg, zinc, 31.32mg, iodine, 5mg and selenium 0.0625mg***Carrier was $CaCo_3$

Table 2: Classification of ovary

Classification	**Color**	**Min. Diameter**	**Max. Diameter**	**Total follicle**
Big yellow follicle	Kuning	10 mm		7-9
Small yellow follicle	Kuning	5 mm	10 mm	5-15
Big white follicle	Putih	2-5 m	5 mm	5-15
Small white follicle	Putih	< 2 mm	1 mm	>1.000-12.000

Table 3: Reproduction organs of *MOJOSARI* duck

Parameters	**T0**	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Weight of ovary	35.25	42.33	44.00	49.00	55.24	30.38	1.24
Total of yellow big follicle	2.00	2.25	3.50	6.00	6.25	2.25	0.78
Total of yellow small follicle	1.50	2.75	4.00	8.50	9.00	1.25	0.82
Total of white big follicle	5.00	8.50	19.00	10.50	12.50	8.75	3.70
Total of white small follicle	145.00	137.50	187.50	300.00	237.50	20.00	36.96

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and their ovaries collected. Ovarian samples were observed for the number of follicles. The classification of the number of ovarian follicles refers to Wheeler and Fields (1993) and is presented in Table 2.

Data analysis

Prior to the statistical analysis, using the general linear model (GLM) was carried out using SAS OnDemand for Academics (ODA, Cary, NC, USA). The results were presented as the standard error of the mean (SEM). Moreover, the probability values were calculated using the least significant difference test. The following model was used:

$Y_{ij} = \mu + T_i + e_{ij}$

Where Yij was the parameter observed, μ was the overall mean, Ti was the effect level of α-Lactobacillus sp. and Curcuma longa flour, and eij was the error number value. The linear and quadratic effects of adding α-Lactobacillus sp. and Curcuma longa (i= T0 = basal diet + 0% control, T1 = basal diet + 0.2% turmeric flour, T2 = basal diet + 0.8% turmeric flour, T3 = basal diet + 0.2% turmeric flour + probiotics 0.1%, T4 = basal feed + 0.8% turmeric flour + 0.6% probiotics, T5 = basal feed + bacitracin antibiotic 0.01%).

RESULTS AND DISCUSSION

Identification of bioactive components

The major bioactive components in turmeric include curcuminoids. As one of these curcuminoids, curcumin has a yellow-orange colour in an acidic environment. In wet conditions, however, the colour changes to red. This bioactive compound is insoluble in water but soluble in ethanol and acetone (Sawant and Godghate, 2013). The analysis revealed absorbance and curcumin concentrations of 0.414% and 5.855 ppm, respectively. Compared with Stanojevic et al. (2017) the curcumin content was only 10.92%. The turmeric consisted of curcumin at 0.197% and 2.356%, respectively. The higher the concentration used, the darker the yellow colour produced.

The regression equation y = 0.076x + 0.005 was obtained with coefficient r = 0.999 (Figure 1). Several factors affected the bioactive compounds such as nutrient content and chemical content (Azizah et al., 2014). Routine standard solutions were used since the majority of the flavonoids found most often in plants are in the form of glycosides such as quercetin 3-rutinoside (Azizah et al., 2014). Flavonoids are phenolic compounds; therefore, their colour will change when added to a base or ammonia so that they can be easily detected on a chromatogram or in solution (Harborne, 1987). This shows that the average level of flavonoid water extract is 30.206%. To determine the flavonoid content of the aluminium chloride method, the measurement was based on the formation of colour and complexes between the aluminium chloride and the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom. Heating simplicia by mixing magnesium (Mg) and hydrochloric acid (HCl)5N and then filtering produces flavonoids; these create a red filtrate that can then be drawn by amyl alcohol (Priyanka., 2015). The total flavonoid levels were determined using 13 UV-Vis spectrophotometry because flavonoids contain a conjugated aromatic system so that they show strong absorption bands in the ultraviolet and visible spectrum regions (Harborne, 1987).

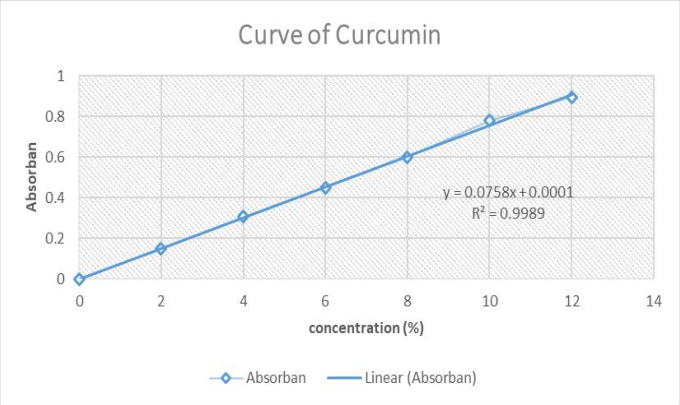

Figure 1: Regression linear of curcumin concentration

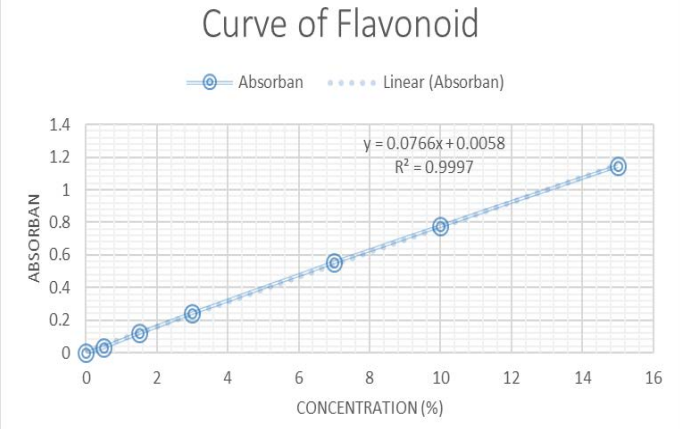

Figure 2: Regression linear of floavonoid concentration

Based on the calibration curve in Figure 9, the regression equation y = 0.076x + 0.005 was obtained (Figure 2). The correlation coefficient r = 0.999 gives a number close to 1, which indicates a very high correlation between absorbance and compound content and shows a relationship between the two. A small amount of simplicia powder in a test tube was heated in a water bath and then filtered. A solution of iron (III) chloride reagent was then added to the filtrate. The presence of phenolic compounds is indicated by the occurrence of a green-blue black to black colour (Priyanka et al., 2015). The heating temperature does not affect the phenol content produced in the extraction process but can reduce its anti-bacterial properties (Gupta et al., 2015). The phenolic compounds found in turmeric can dissolve lipids from cell membranes because they contain

-OH groups that can disrupt and affect the integrity of the cytoplasmic membrane, causing cell lysis and inhibiting ATP-ase binding on bacterial cell membranes (Harborne, 1987). Essential oils with anti-bacterial properties generally contain hydroxyl (OH) and carbonyl functional groups. Phenol derivatives interact with bacterial cells through an adsorption process involving hydrogen bonds. At low levels, phenol protein complexes are formed with weak bonds and immediately undergo decomposition, followed by phenol penetration into cells, and cause precipitation and protein denaturation. High levels of phenol lead to protein coagulation and cell membrane lysis (Infante, et al., 2014).

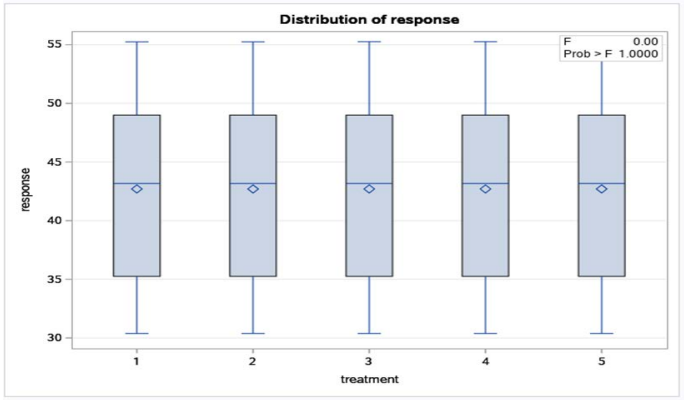

Figure 3: Distribution response of weight of ovary

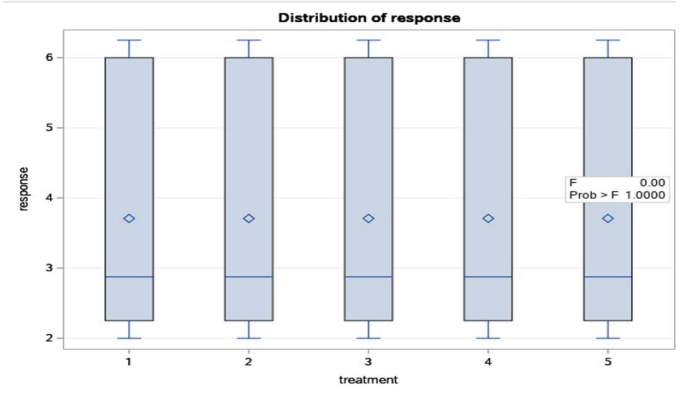

Figure 4: Distribution response of total of yellow big follicle

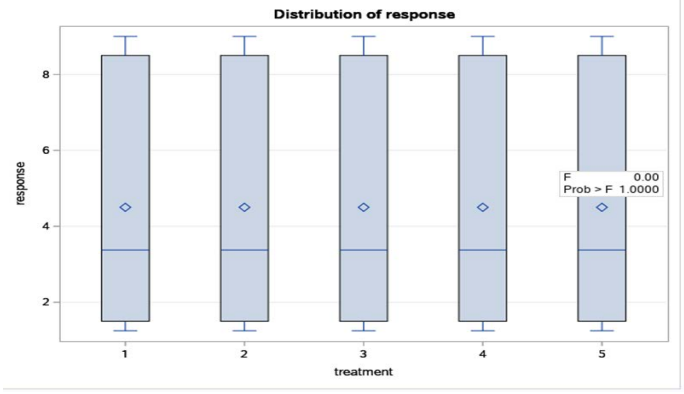

Figure 5: Distribution response of total of yellow small follicle

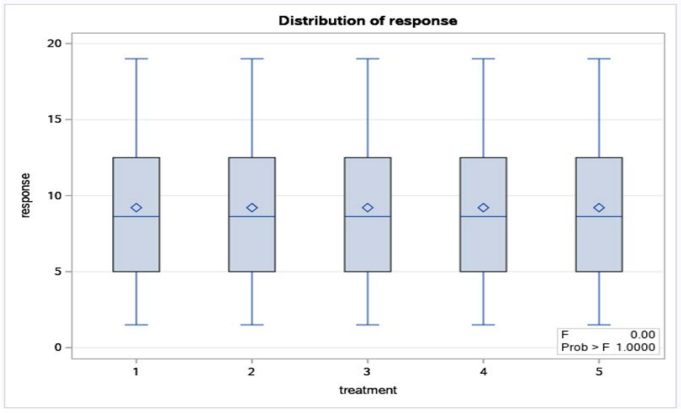

Figure 6: Distribution response of total of white big follicle

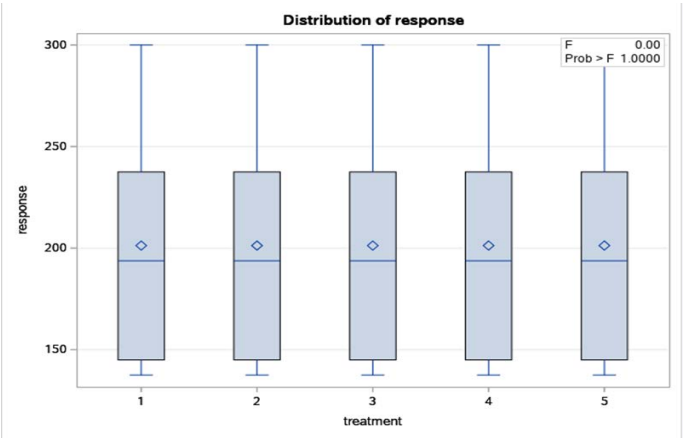

Figure 7: Distribution response of total of white small follicle

Reproductive organs

The results on the reproductive appearance of the Mojosari ducks showed that the average weight of the ovaries, large white and small white follicles had a significant effect (p <0.05) on the T4 treatment, while the number of large and small yellow follicles had a significant effect (p < 0.01) compared with treatments T2 and T3 (Figure 3). The average weight of the ovaries of the Mojosari ducks during maintenance can be seen in Table 3. The T4 treatment had the heaviest weight compared to the other treatments (55.24 g), followed by T3, T2, T1, T0 and T5 at (49.00 g), (44.00 g), (42.33 g), (35.25 g) and (30.38 g), respectively. The average ovary weight in this study aligned with that reported by Hafez (2000), who found that the duck ovary, which resembles a bunch of grapes, weighed between 40 and 60 grams during the egg-laying period. High egg weight indicates a high amount of yolk and egg white. The larger the egg yolks and egg whites, the greater the nutrients available for embryo development, resulting in a higher hatching weight. The factors that influence the weight of day-old-ducks (DOD) include feed and egg quality (Yousefi and Karkodi, 2007).

The variation in ovarian weight in this study might be due

to the age of the ducks at the egg-laying stages. Ketaren (2007) found that when ducks first began to lay eggs, they produced a low egg weight. In a surprising finding, Nalbandov (1990) reported that only two birds had ovulated follicles that could be recognised macroscopically approximately 90 days after ovulation. Meanwhile, 6-month-old ducks showed an increase in both ovaries and follicles for secreting the enzymes and proteins needed to lay eggs. The proteins produced were ovalbumin, globulin lysosomes, avidin, flavoprotein, and ovomucoid whites. Moreover, Suprijatna and Natawihardja (2005) found that the protein level of the feed had a significant effect on the relative weight of the ovaries, oviducts, the number of follicles entering the maturation phase, and body weight during laying ducks' early phase. Several hormones are responsible for egg production, including progesterone. Progesterone acts on the releasing factor hormone in the hypothalamus. This prompts the release of luteinising hormone (LH) from the anterior pituitary, which in turn leads to the release of a mature yolk from the ovary. In contrast, the yolk descends through the oviduct (Suprijatna and Natawihardja, 2005).

The presence of least significant difference indicates/shows that the T4 ovary was significantly different (p < 0.05) among the treatments (Figure 6). This may reflect the fact that the ovary is still in the active growth phase. This aligns with Hafez (2000), who found that the size of the ovary depends on its reproductive status; thus, at the reproduction phase, the ovary will enlarge to a diameter of approximately 5 cm. In contrast, during the active phase, it is small, with a diameter of around 5 mm. The large yellow and small yellow follicles in the T0 treatment showed significantly different results (p <0.01) compared with T3 and T4 (Figure 4 and 5). This may indicate a higher number of large and small yellow follicles with the addition of turmeric and probiotics compared to the other treatments. Table 2 indicates that the average number of large yellow follicles for T3 and T4 were (6.00) and (6.25), respectively. This finding aligns with Suprijatna and Natawihardja (2005), who reported that female ovaries usually consist of 5–6 developing follicles with large yellow (yolk) and small white follicles (immature follicles). The number of small yolk follicles depends on the feed and pituitary hormones. Olszanska and Stepinska (2000) found that the small yellow follicles are rich in egg yolk content with an approximate size of 5–10 mm. First, the ovaries receive blood supply from the ovary vessels, which consist of Reno lumbar vessels. Second, the ovaries consist of blood vessels that contain macroscopic arteries and veins. Ultimately, the ovarian arteries divide into specific branches and generally separate the blood vessels into follicular stalks (Suprijatna and Natawihardja, 2005).

The presence of least significant difference indicates that the number of large white follicles from T0 to T4 showed a significant difference (p <0.05), while the number of small white follicles from T0 to T3 was also significantly different (p <0.01) (Figure 7). The variation in the average number of small white follicles in this study was due to the influence of hormones produced from each ovulation. In Mojosari ducks, the largest follicle is close to the ovulatory size. In accordance with this condition, the amount of blood flowing into the vascular system was compared with that in the smaller follicles. First, the level of gonadotropin hormone began to reduce at the smallest follicle. Second, during the sufficient phase, the follicle began to grow rapidly; however, this did not start the ovulation process (Nalbandov, 1990). Follicle development takes place in line with the amount of follicle stimulation hormone (FSH) from the anterior pituitary gland. First, the ovary begins to secrete both oestrogen and progesterone. Second, oestrogen and ovarian hormones stimulate growth in the oviduct. Third, the blood profiles increase during egg production, the pubic bones stretch, and the cloaca enlarges (Suprijatna and Natawihardja, 2005). The female reproductive system comprises one ovary and one oviduct. The ovary is located on the left of the body cavity and consists of five to six follicles, a pair of yellow follicles, and a small white follicle (Suprijatna and Natawihardja, 2005). There are two stages to the ovulation process in ducks. It begins when follicles enter the ovulation stage with the support of gonadotropic hormones and then enter the follicular circulation system (Nalbandov, 1990).

The development level of the subepithelial glands is related to the secretion of egg white. The gland is well differentiated, which indicates the amount of egg white secretion. This finding is in line with Lu et al. (2003), who reported that the magnum gland will secrete the egg white proteins ovalbumin, lysozyme, Ovo transferrin, and ovomucin. Yuriwati et al. (2016) identified three types of local ducks that are famous in Indonesia: Magelang ducks, Tegal ducks and Pengging ducks. Sexual maturity is reached by the age of approximately six months, by which time cell differentiation in the magnum is complete. Moreover, it has been reported that steroid hormone assists in egg formation. An increase in the number of follicles reflects the total amount of oestrogen produced. A high level of prolactin hormone will lower the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Latifa and Sarmunu, 2008). The bioactive compound had a positive impact on the reproductive organs of Mojosari ducks. However, the differences and specific effects of curcumin depend on the doses and the type of target organs in ducks.

CONCLUSIONS

Intestinal length and weight were unaffected by the ad-

dition of antibiotics, probiotics and herbs. However, the height and width of the villi gave the best results with the T_4 treatment, namely the addition of 0.8% turmeric and 0.6% probiotics. The best hen day production, feed egg ratio, and egg weight were in the T_4 treatment. In addition, T_4 can suppress mortality of Mojosari ducks.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

Since there is limited information of an early screening of turmeric as an antibiotic growth promoter replacement and its effects on the reproductive organs of Mojosari ducks. Several substances such as curcumin, flavonoid, and polyphenols were having an important effect as phyto genic.

AUTHOR'S CONTRIBUTION

WA conceptualization, drafting the original manuscript, collecting data, OS conceptualization, providing materials, EW conceptualization, supervision, SS conceptualization, supervision, revise the manuscript, DNA drafting manuscript, revise grammatically, creating illustration, and validation

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