Reproduction Performance of Post-Molting Tegal Ducks Given Cattle Reticulum Meal

Rosidi¹⁾*, T Yuwanta²⁾, Ismaya²⁾ and Ismoyowati¹⁾ ¹⁾Laboratory of Poultry Production, Faculty of Animal Science, Jenderal Soedirman University, Jl. Dr. Suparno 60, Purwokerto 53123, Central Java, Indonesia ²⁾Faculty of Animal Science, Gadjah Mada University, Jl. Fauna 3, Bulaksumur Yogyakarta 55281, Indonesia ^{*}Corresponding author email: ir.rosidi.mp@gmail.com; Telp: +6285526000066

Abstract. The objective of this research was to recognize the effect of cattle reticulum meal level as cholesterol source in feed on the quality of post-molting Tegal duck hatching eggs. Experimental method was exercised in this research, using 68-week old Tegal duck consisted of 60 female and 20 male, with cattle reticulum meal treatments (K) namely K_0 = 0% (control), K_1 = 1.43% (equaled to 0.371 g cholesterol), K_2 = 2.86% and K_3 = 4.29%. Each treatment consisted of three female and one male with 5 time repetition. The observed variables were estrogen level in blood serum, yolk cholesterol, fertility and hatchability. Data were subject to analysis of variance using Completely Randomized Design (CRD), followed by Honestly Significant Difference test (HSD). Result showed that the level of cattle reticulum meal had highly significant effect on fertility, significant effect on estrogen level, and non-significant effect on hatchability and yolk cholesterol. It was concluded that egg fertility could be maintained through the supplementation of cattle reticulum meal up to 2.86%, but it decreased at 4.29%, and that up to 4.29% level of cattle reticulum could not increase egg hatchability.

Key words: Fertility, hatchability, Tegal duck, cattle reticulum meal

Abstrak. Tujuan penelitian ini adalah untuk mengetahui pengaruh level tepung retikulum sapi sebagai sumber kolesterol yang digunakan dalam campuran pakan terhadap kualitas telur tetas itik Tegal setelah terjadi molting. Penelitian menggunakan metode eksperimen. Materi yang digunakan adalah itik Tegal umur 68 minggu sebanyak 60 ekor induk dan 20 ekor pejantan dengan perlakuan level tepung retikulum sapi dalam pakan (K), terdiri atas K₀= 0% (kontrol), K₁= 1,43% (setara 0,371 g kolesterol), K₂= 2,86% dan K₃= 4,29%. Setiap perlakuan terdiri atas 3 ekor itik induk dan 1 pejantan, yang diulang 5 kali. Peubah yang diamati adalah kadar hormon estrogen serum darah, kadar kolesterol kuning telur, daya tunas dan daya tetas telur. Data dianalisis variansi berdasarkan rancangan acak lengkap (RAL), dilanjutkan dengan uji Beda Nyata Jujur (BNJ). Hasil penelitian menunjukkan bahwa level retikulum sapi berpengaruh sangat nyata terhadap daya tunas dan berpengaruh nyata terhadap kadar hormon estrogen, berpengaruh tidak nyata terhadap daya tetas dan kadar kolesterol kuning telur. Kesimpulannya adalah bahwa daya tunas telur dapat dipertahankan dengan pemberian tepung retikulum sapi sampai dengan level 2,86%, namun menurun pada level 4,29% dan sampai dengan level 4,29% belum mampu meningkatkan daya tetas telur.

Kata kunci: Daya tunas, daya tetas, itik Tegal, tepung retikulum sapi

Introduction

Total average production of Tegal duck eggs from the total population within the first three month production was 70.5±10.01% and that of selected egg production with 30% selection intensity was 89.4±2.37% (Subiharta et al., 2010). Molting period of Tegal duck under traditional maintenance started from 17 months old, with 3-4 month molting period (Suswoyo, 1990). Molting is influenced by prolactin hormone which causes ovary regression as direct impact on gonad, while the indirect impact was by competing with progesterone produced by the ovary (Anwar and Savitri, 2005). Low progesterone level from ovary will induce negative feedback on hypothalamus and anterior hypophysis that constricts the release of gonadotropin produced by anterior hypophysis. According Hsu et al. (2000) in Ganaie and Shrivastava (2010) that in the pituitary, GnRH binds to the GnRH receptors on the gonadotropic cells to stimulate the release of FSH and LH to the circulation. The pulsatile secretion pattern of GnRH induces the cyclic release of LH and to a lesser extent of FSH. In female mammals, FSH induces follicle growth and subsequently estradiol and inhibin secretion by the granulose cells. After ovulation the luteinised granulosa and the theca cells start to produce the high levels of progesterone.

Very low gonadotropin hormone such as FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) will void follicle growth because both hormones are required in follicle growth and development, and egg oviposition (Anwar dan Safitri, 2005).

Follicle growth and development must be maintained through some efforts, one of which is stimulating FSH and LH by gonadotropin hormone precursor as in feed cholesterol. Cholesterol biosynthetic and metabolic pathways contain several branching points towards physiologically active molecules, such as coenzyme Q, vitamin D, glucocorticoid and steroid hormones, oxysterols, or bile acids (Rezen et al., 2010). Gonad produces sexual hormones and organizes reproduction function. Sexual hormones in female duck produced in ovary are estrogen and progesterone, while in male duck produced by testis called androgen, the most well-known and important is testosterone to increase egg fertility. Natural cholesterol is the dominant component of sterol compound in organ structure of human and animal including poultry with the innate biological function. Particularly in eggproducing poultry, some cholesterol was deposited naturally in the yolk as the supply for embryo growth outside the hen because it is estimated that enzyme for cholesterol synthesis does not exist during embryonal period (Sutton et al., 1984), so that eventually it will increase egg hatchability.

Cholesterol is derived from two sources, food or feed intake and biosynthesis in the liver. Cholesterol absorbed daily from the gastrointestinal tract, called exogenous cholesterol, an even greater amount formed in the cell body called endogenous cholesterol (Guyton and Hall, 2006). On average, cholesterol content in the blood of ducks fed different forms of feed was 181.22±7.06 mL dL-1 (Ismoyowati and Sumarmono, 2011).

Three types of fat in food or feed are saturated fat, MUFA (monosaturated fatty acid), and PUFA (polyunsaturated fatty acid). The expensive 95% cholesterol synthesis calls for the alternative cholesterol source, one of which is cattle reticulum containing 258.92 mg/g cholesterol (Rosidi, 2011). Cattle reticulum is plentiful in slaughter house as byproduct or waste. Cattle reticulum is considered inedible in foreign country and made into meal for feed. Indonesians consume cattle reticulum, but they recently cut it down due to the high cholesterol content. It is a prospect for farmers to utilize cattle reticulum as cholesterol-enriched feed. Accordingly, it is essential to conduct a research on utilizing cholesterol in cattle reticulum for duck feed to improve the reproduction performance of Tegal duck.

Cholesterol is a starting material for the biosynthesis of steroid hormones; these fat soluble, low molecular weight substances play diverse and important physiological functions. There are five major classes of steroid hormones: testosterone (androgen), estradiol (estrogen), progesterone (progestin), cortisol/ corticosterone (glucocorticoid), and aldo sterone (mineralocorticoids) (Payne and Hales, 2004). That will stimulate follicle formation, growth and development, and the continuation of ovulation process to improve egg fertility. Egg hatchability is therefore subject to improvement because FSH can control fatty acid deposition in the yolk and follicle development (in female). The secreted LH can

stimulate ovary development, stigma ovum rupture, progesterone and steroid secretion and ovulation (in female). It is obvious that cholesterol has a significant role to increase production and duck egg quality, however the exact amount of cholesterol intake to produce hormones is still unidentified. The objective of this research was to recognize the effect of cattle reticulum meal level as cholesterol source in feed on the quality of post-molting Tegal duck hatching eggs.

Materials and Methods

This research administered 68-week-old (17 months) Tegal duck, 60 female and male, based on report by Suswoyo (1990) that molting period in Tegal duck starts at 17 months old (68 weeks). Estrogen hormone test kit was used to analyzed estrogen level in blood. Treatment feed, composed of cattle reticulum meal, corn, rice bran, soybean cake and "Mineral Ayam B12", was based on the calculation of feed composition table by NRC (1999) and analysis result of Laboratory of Cattle Feed Science, Soedirman Jenderal University. Feed composition and nutrient of treatment feed is presented in Table 1.

Experimental research was conducted in Completely Randomized Design according to Steel and Torrie (1980). Cattle reticulum meal as treatment (K) consisted of K_0 = 0% (control), K_1 = 1.43% (equaled to 0.371 g cholesterol), K_2 = 2.86% and K_3 = 4.29%. Each treatment consisted of three female and one male with 5 time repetition.

The observed variables were serum estrogen on blood, yolk cholesterol, fertility and egg hatchability. Blood sample was taken at four week post-molting from one duck in each treatment through wing vena (vena axillaries). Five ml of blood was taken using syringe, positioned at 45° angle for \geq one hour until serum was formed on the blood, then stored in micro tube for estrogen analysis. The measurement of hormone level used radioimmunoassay (RIA) method in National Nuclear Energy Agency (BATAN) Jakarta, yolk cholesterol with Liebermen-Burchard method (Astuti, 1997), while fertility and hatchability with calculation of North and Bell (1990). The obtained data were subject to analysis of Variance according to Steel and Torrie (1980), proceeded by honestly significant difference test for the significant result (P<0.05)

Results and Discussion

Post-Molting Estrogen Level of Tegal Duck

The average post-molting estrogen level was 5.18±3.5 nmol/l ranging from 1.1 nmol/l to 12.19 nmol/l. Analysis of Variance Result (Table 1) showed that cattle reticulum meal had highly significant effect (P<0.01) on estrogen hormone. Tukey Honestly Significant Difference (HSD) showed the level of that cattle reticulum meal among treatments produced significantly different estrogen levels (P<0.05) (Table 2). Estrogen level results of K₀ was 1.54±0.37 nmol/l, K_1 was 3.54±0.25 nmol/l , K_2 was 5.15±1.11 nmol/l and K₃ was 10.50±1.25 nmol/l. Relation between cattle reticulum meal and estrogen level was highly significant (P<0.01) as shown in cubic equation Y = 1.54 + 2.49X - $1.11X^2 + 0.24X^3$ (Fig. 1), with coefficient of determination (R²)= 79.19%.

Figure 1 shows that cholesterol is very essential in estrogen synthesis, in which the higher reticulum meal level, the more cholesterol content, and the higher estrogen produced. Cholesterol is the common estrogen precursor, 17β hydroxidehidrogenase activity will convert androstenedion into testosterone that is not the dominant production in ovary. Testosterone would undergo demetallation at C19 and the process of aromatization into estradiol as the most estrogen secreted by ovary. Estradiol also increased to considerable amount from androstenedion trough estone. Estriol is peripheral metabolit from estone and

Feed composites (%)	K _o	K ₁	K ₂	K ₃
Corn	29.70	31.07	32.64	34.71
Ricebran	42.00	40.50	38.80	36.50
Cattle reticulum	0	1.43	2.86	4.29
Soybean cake	21.30	20.00	18.70	17.50
Chicken mineral B12	7.00	7.00	7.00	7.00
Total	100	100	100	100
Nutrient content :				
ME (kcal/kg)	2765.64	2771.82	2778.56	2785.73
Protein (%)	17.31	17.32	17.31	17.32
Fat (%)	5.73	5.99	6.24	6.41
Cholesterol (g)	0	0.37	0.74	1.11
Crude fiber (%)	6.93	6.88	6.81	6.68
Ca (%)	3.52	3.51	3.51	3.51
Р (%)	1.05	1.05	1.05	1.05

Table 1. Treatment feed composition and nutrient content

Cholesterol content in K₁ was based on cholesterol demand from feed (20%) by feeding 150 g/duck/day, and the multiples for feed K₂ and K₃. Feed was made iso-protein and iso-energy. K_0 = 0% reticulum, K_1 = 1.43% reticulum, K_2 = 2.86% reticulum, K_3 = 4.29% reticulum.

Table 2. Result of analysis of variance and HSD test of cattle reticulum level on post-molting duck performance

Cattle reticulum level				Cignificance
K ₀	K1	K ₂	K ₃	Significance
1.54 ^ª	3.54 ^b	5.15 [°]	10.50 ^d	*
20.81	19.93	21.53	21.11	NS
96.88 ^ª	100.00 ^ª	93.33 ^a	75.00 ^b	**
50.00	55.83	50.00	60.00	NS
	K ₀ 1.54 ^ª 20.81 96.88 ^ª 50.00	K ₀ K ₁ 1.54 ^a 3.54 ^b 20.81 19.93 96.88 ^a 100.00 ^a 50.00 55.83	Cattle reticulum level K_0 K_1 K_2 1.54^a 3.54^b 5.15^c 20.81 19.93 21.53 96.88^a 100.00^a 93.33^a 50.00 55.83 50.00	Cattle reticulum level K ₀ K ₁ K ₂ K ₃ 1.54 ^a 3.54 ^b 5.15 ^c 10.50 ^d 20.81 19.93 21.53 21.11 96.88 ^a 100.00 ^a 93.33 ^a 75.00 ^b 50.00 55.83 50.00 60.00

Values bearing different superscript at the same row shows significant (P<0.05). $K_0 = 0\%$ reticulum, $K_1 = 1.43\%$ reticulum, $K_2 = 2.86\%$ reticulum, $K_3 = 4.29\%$ reticulum. * = Significant, ** highly significant significant, NS = not significant



Figure 1. Correlation between cattle reticulum meal level and estrogen level

estradiol, not of ovary secretion. Estrogens are synthesized from androgens in premenopausal ovary and in extra ovarian tissue including fat, muscle, liver and the breast by the conversion of cholesterol which is made by the adrenal gland in post menopausal females (Atoum et al., 2012). Estrogens induce cellular changes through several different mechanisms. Central to these mechanisms is the protein to which estrogens bind, the estrogen receptor (ER). In the "classical" mechanism of estrogen action, estrogens diffuse into the cell and bind to the ER, which is located in the nucleus (Deroo and Korach, 2006). Jayachitra et al. (2012) also stated that cholesterol is an important precursor molecule for the synthesis of vitamin D and steroid hormones. Including the adrenal gland hormones cortisol and aldosterone as well as the sex hormones progesterone, estrogens, and testosterone, and their derivatives.

Post-molting yolk cholesterol of Tegal duck

Average post-molting yolk cholesterol was 20.84±1.48 mg/g, ranging from 18.48 mg/g to 23.06 mg/g. Result of this study (Table 2) showed that cattle reticulum meal did not significantly affect (P>0.05) blood cholesterol. Supplementation of 4.29% cattle reticulum meal could not increase yolk cholesterol. It was different from Sutton et al. (1984) that Particularly in egg-producing poultry, some cholesterol was deposited naturally in the yolk as the supply for embryo growth outside the hen because it is estimated that enzyme for cholesterol synthesis does not exist during embryonal period. Hammad et al. (1996) reported that additional 0%-5% cholesterol resulted in naught correlation between yolk and plasma cholesterol observed and measured at the age of 10, 14 and 18 weeks old, followed by 2, 6 and 10 weeks of cholesterol-enrich feed intake. However, yolk cholesterol was higher in high fat content than in low fat content at 10 and 14 weeks old. Moreover, supplementation of high fat on quail ration would increase yolk cholesterol in 10 and 14 week old quail. Fat is one nutritional substance as energy source and vitamin dissolvent. Fat consisted of saturated fatty acid (SAFA), trans fatty acid (TFA) and unsaturated fatty acid namely polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA). The difference is assumed to derive from different age between quail and duck when the treatment was given, namely at productive age in quail and at molting in duck.

Post-Molting Egg Fertility of Tegal Duck

Average post-molting Tegal duck egg fertility was 91.30±11.30 ranging from 66.67% to 100%. Results of this study (Table 2) showed that level of cattle giblet meal (reticulum) had highly significant effect (P<0.01) on egg fertility. Result of honestly significant difference test (Tukey HSD) demonstrated that 0% cattle reticulum meal (K_0) showed 96.88% egg fertility, relatively similar to 1.43% in K_1 (100%) and 2.86% in K_2 (93.33%), but significantly different (P<0.05) with 4.29% in K_3 (75.00%). Correlation between cattle reticulum meal level and egg fertility was significant (P<0.01) as described in square equation Y = 96.78 + 6.19X - 2.62X² (Fig. 2), with coefficient of determination (R²)= 60.18 %.

The difference of post-molting egg fertility between K_0 , K_1 , K_2 and K_3 was due to different estrogen production (Table 1), in that higher reticulum meal level caused higher hormone estrogen and lower FSH secretion. Low FSH caused its main function in stimulating ovum formation and follicle development unable to serve continually. This distracted fertilization, in that the higher reticulum meal level, the lower egg fertility. Bliss et al. (2010) that the gonadotrope cell of the anterior pituitary plays a particularly critical role within this system as the intermediary between the hypothalamic gonadotropin-releasing hormone (GnRH) signal and the germ cell reservoirs and steroid hormone productivity of the gonads. Expression of the GnRH receptor (GnRHR) is a defining characteristic of the gonadotrope. Binding of



Figure 2. Correlation between cattle reticulum level and post-molting egg fertility

GnRH to its receptor triggers a complex array of intracellular signal transduction events within the gonadotrope; these signaling cascades orchestrate the overall physiological response of these cells to GnRH stimulation, culminating in the synthesis and release of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH).

Post-Molting Egg Hatchability of Tegal Duck

Average post-molting egg hatchability was 53.96±8.15 %, ranging from 40.00 to 70.00 %. Result of this study (Table 2) showed that level of cattle reticulum meal did not significantly affect (P>0.05) egg hatchability due to nonsignificant difference on yolk cholesterol among treatment. Sutton et al. (1984) reported that particularly in egg-producing poultry, some cholesterol was deposited naturally in the yolk as the supply for embryo growth outside the hen because it was estimated that enzyme for cholesterol synthesis does not exist during embryonal period so that eventually it would increase egg hatchability. However, since yolk cholesterol among treatments was not significantly different, increasing cattle reticulum supplementation up to 4.29% has yet able to increase egg hatchability. Besides, low FSH was unable to control fatty acid deposition in yolk was absent because. It was in line with Widodo (2010) that generally fat in feed was digested in small intestine by the aid of bile salt into glycerol and fatty acid, then circulated through blood vessel and collaborated with FSH (Folicle Stimulating Hormone) to form yolk in ovary.

Conclusion

Egg fertility could be maintained by supplementing 2.86% cattle reticulum meal in feed, but decreased at 4.29%, and that up to 4.29% level of cattle reticulum could not increase egg hatchability.

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