

Thyroid Hormone and Blood Metabolites Concentrations of Broiler Chickens Subjected to Feeding Time Restriction

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ABSTRAK

Penelitian ini bertujuan untuk menguji pengaruh pembatasan waktu makan terhadap konsentrasi hormon tiroid dan metabolit darah ayam broiler selama periode pembatasan dan pemulihan. Sebanyak 400 ekor ayam broiler *unsexed* strain Lohmann umur 7 hari ditempatkan pada 5 perlakuan dengan menggunakan rancangan acak lengkap dan dianalisa menggunakan ANOVA. Perlakuan terdiri atas: ransum yang diberikan *ad libitum* (R-0); ransum yang disediakan 2 jam pada pagi hari dari pukul 08:00-10:00 dan 2 jam pada sore hari dari pukul 16:00-18:00 dari umur 7 hingga 14 hari (R-1) dan umur 7 hingga 21 hari (R-2); ransum disediakan 4 jam pada pagi hari dari pukul 08:00-12:00 dan 4 jam pada sore hari dari pukul 16:00-20:00 dari umur 7 hingga 14 hari (R-3) dan umur 7 hingga 21 hari (R-4). Setiap perlakuan memiliki ulangan sebanyak 4 dengan jumlah ayam sebanyak 20 ekor/unit. Konsentrasi hormon tiroid dan plasma albumin pada R-2 dan R-4 lebih rendah ($P < 0,05$) daripada R-0 selama pembatasan waktu makan dari umur 7 hingga 21 hari. Konsentrasi hormon tiroid dan metabolit darah tidak berbeda nyata diantara semua perlakuan selama periode pemulihan dari umur 21 hingga 42 hari. Disimpulkan bahwa konsentrasi hormon tiroid triiodotironin dan tiroksin (T_3 dan T_4) dan plasma metabolit darah (albumin) menurun selama pengaturan waktu makan 4 dan 8 jam/hari dari umur 7 hingga 21 hari, akan tetapi, kondisi demikian kembali normal selama periode pemulihan.

Kata kunci: broiler, hormon tiroid, metabolit darah, pengaturan waktu makan

ABSTRACT

The present study was conducted to determine the effect of feeding time restriction on thyroid hormone and blood metabolites concentration of broiler chickens during feed restriction and realimentation period. Four hundred of 7 days-old unsexed broiler chicken of Lohmann commercial strain were arranged in a completely randomized design with 5 treatments and analyzed based on ANOVA. The treatments were: chicks fed *ad libitum* as a control treatment (R-0); chicks had free access to feed during two periods of 2 h (08:00-10:00 and 16:00-18:00) from 7-14 d of age (R-1) and 7-21 d of age (R-2); chicks had free access to feed during two periods of 4 h (08:00-12:00 and 16:00-20:00) from 7-14 d of age (R-3) and 7-21 d of age (R-4). The treatments consisted of 4 replicates with 20 chicks each. During feeding time restriction from 7 to 21 d of age, thyroid hormone and albumin plasma concentration of R-2 and R-4 were lower ($P < 0.05$) than R-0. There were no statistical differences among the treatments on thyroid hormone and blood metabolites during realimentation period from 21 to 42 d of age. It is concluded that thyroid hormone triiodothyronine and thyroxine (T_3 and T_4) and blood metabolite (albumin) decreased during feeding time restriction for 4 and 8 h per d from 7 to 21 d of age and it returns to normal during realimentation period.

Key words: broiler, thyroid hormone, blood metabolite, feeding time restriction

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INTRODUCTION

Continuous genetic selection and improvement in nutrition have led to a very fast growth rate in modern broiler chicken strains. The early-life fast growth rate is accompanied by a number of problems, such as incidence of metabolic disorders and incidence of skeletal diseases (Olkowski *et al.*, 2008). These situations most commonly occur with broilers that consume feed *ad libitum*. In addition, under feeding *ad libitum*, some chicks may develop a habit of feed over consumption (Svihus & Hetland, 2001), and this condition does not always mean that broiler chicks are good status of health and welfare (Jang *et al.*, 2009). Therefore, restriction on feed availability through feeding time restriction may be a solution to these problems. Overwhelming evidence exists that feeding time restriction improves feed efficiency compared with *ad libitum* feeding (62% vs 58%), increased production index (329.04 vs 304.27), and decreased feed cost (6.42%) in production per kg body weight of broiler chickens (Svihus *et al.*, 2010; Azis *et al.*, 2011).

A number of variations of feed restriction including limiting the time of daily access to feed (Khetani *et al.*, 2009; Onbasilar *et al.*, 2009; Mohebodini *et al.*, 2009), removal of feed for up to 8 h a d or skip-a-day feeding, allowing birds to feed only once/h and feeding once every other day (Demir *et al.*, 2004; Khajali *et al.*, 2007; El-Fiky *et al.*, 2008; Benyi *et al.*, 2009, 2010; Boostani *et al.*, 2010; Saffar & Khajali, 2010), have been evaluated but results have been conflicting (Navidshad *et al.*, 2006; Cornejo *et al.*, 2007; Khetani *et al.*, 2009; Ghazanfari *et al.*, 2010). However, Mohebodini *et al.* (2009) on the respective research indicated that although intermittent feeding through free access to feed during four periods of 2 h (06:00-08:00, 12:00-14:00, 18:00-20:00, and 24:00-02:00) from 7-21 d of age had lower on body weight gain than control (436.3 vs 495.3 g), but had no effect on weight gain during realimentation period from 22-42 d of age. Such inconsistencies may relate to different feeding strategies applied which may affect the chick performance.

Controversy of growth phenomenon during realimentation period showed inconsistency result. These differences showed that growth is still a complex phenomenon due to the physiological aspects involved such as the role of thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) as the controlling metabolism and growth and condition of the blood metabolites as clinical indication is not widely disclosed. Blood profiling, initially used to detect subclinical disorders due to incorrect feeding, has recently been given more widely to evaluate the effects of different treatments on metabolic, nutritional and welfare conditions of animals (Bovera *et al.*, 2007). Thyroid hormone concentration is closely related to protein synthesis and energy production (Smith *et al.*, 2002), and that relate to the performance of growth and changes in energy metabolism of broilers (Zhan *et al.*, 2007). The other research, Jang *et al.* (2009) reported that the quantitative and qualitative feed restrictions during 7 d from 8-14 d decreased plasma protein and albumin concentrations of broiler chickens. However, Boostani *et al.* (2010) reported that feed availability for 8

h/d from 7-21 d did not influence plasma proteins and albumin concentrations of broiler chickens.

Feeding time restriction is a feed restriction schedule which chicks have daily free access to feed for 4 and 8 hours per day in specific time. It is an alternative lower intensity of early feed restriction (Zhang *et al.*, 2007) and it is less stressful (Susbilla *et al.*, 2003). Such conditions may be possible due to broilers eat to their maximal physical capacity (Bokkers & Koene, 2003) during the feed availability. Therefore, it is assumption that the feed restriction is not severe. There is a transient decrease in basal metabolic rate of feed restricted birds, leading to less energy required for maintenance (Rincon & Leeson, 2002; Tolkamp *et al.*, 2005). It is, however, not clear whether feeding time restriction can reduce thyroid hormone and blood metabolites concentrations. The objective of the current study was to determine the effect of feeding time restriction on thyroid hormone and blood metabolites concentration of broiler chickens during feed restriction and realimentation period.

MATERIALS AND METHODS

Birds, Feed, and Cage

A total of 400 unsexed Lohmann broiler chicks bought from commercial hatchery (PT Multi Breeder Adirama Indonesia Tbk, Lampung Indonesia), were used in current study. Chicks were fed commercial starter diet (crumble) from 7-21 d of age, and commercial finisher diet (pellet) from 22-42 d of age. The commercial feed purchased from Poultry Shop (manufacture by PT JAPFA Comfeed, Lampung Indonesia). The nutrient composition of commercial feed is presented in Table 1. All the chicks were reared in a conventional open-sided house with natural cyclic temperatures (minimum, 20 °C; maximum, 34 °C). The chicks were assigned to 20 floor pens with 20 chicks/pen (1 × w × h; 2 × 1.5 × 0.75 m).

Table 1. Nutrient composition of commercial starter (7-21 d) and finisher diet (22-42 d)*

Nutrient composition	Feed	
	Starter	Finisher
Dry matter (%)	87.18	88.64
Crude protein (%)	22.18	20.24
Ether extract (%)	6.12	7.06
Crude fiber (%)	3.31	3.83
Nitrogen free extract (%)	5.08	51.97
Ash (%)	5.49	5.54
Ca (%)	1.52	1.49
P (%)	0.77	0.65
NaCl (%)	0.36	0.28
GE (kcal/kg)	3924.00	3875.00

Note: *) Laboratory Feed Science and Technology, Bogor Agricultural University (2010).

Blood Sampling and Measurement of Parameter

On d 14 and 21 (end feed restriction) and 42 (end realimentation period), blood samples were collected from a brachial vein of 2 chicks randomly selected birds from each pen using disposable syringe (± 3 ml/chick). The blood samples were collected directly into testtube with anticoagulant ethylenediaminetetraacetic acid; EDTA (1.5 mg/ml). Thereafter, the blood samples were centrifuged at 3000 rpm for 5 min and plasma was harvested and kept frozen at -30 °C until analyzed. Hormone analyses (T_3 and T_4) on the plasma samples were performed by radioimmunoassay (RIA) using standard commercial kits according to the procedure of Izotop, Institute of Isotopes Co Ltd, (2010). The range of standard to made curve standard of T_3 concentration was from 0.1-100 nmol/dl. T_3 concentration was analyzed directly in the plasma with a volume 100 μ l. The range of standard to made curve standard of T_4 concentration was from 10-1000 nmol/dl. T_4 concentration was analyzed directly in the plasma with a volume 25 μ l. Each sample was in duplicate determination and radioactivity T_3 and T_4 were counted by gamma counter. Blood metabolite concentrations (plasma protein and albumin) were determined by enzymatic techniques using a commercial kit (ReiGed Diagnostics, Italy) according to the procedure of Gowenlock *et al.* (1988). The range of standard to made curve standard of plasma protein (0.1-10 g/dl) and albumin concentrations (0.11-6 g/dl) with a volume 10 μ l. Each sample was in duplicate determination and plasma protein and albumin concentrations measured by spectrophotometer (Spectronic Hitachi U-2001) with wavelength at 520 and 580 nm, respectively. All concentrations of plasma protein and albumin were in the range of standard. Globulin concentration was determined from the difference between the value of protein and albumin.

Treatments and Experimental Design

Broiler chickens had free access to feed from 1-6 d of age. On 7 d, all birds were weighed and randomly allocated to each of five experimental treatments. The treatments were: chicks fed *ad libitum* as a control treat-

ment (R-0); chicks had free access to feed during two periods of 2 h (08:00-10:00 and 16:00-18:00) from 7-14 d of age (R-1) and 7-21 d of age (R-2); chicks had free access to feed during two periods of 4 h (08:00-12:00 and 16:00-20:00) from 7-14 d of age (R-3) and 7-21 d of age (R-4). The treatments were arranged in a completely randomized design with 4 replications. All data were analyzed based on ANOVA and the significant differences between treatment means were determined by LSD based on testing at $P < 0.05$ (Steel & Torrie, 1991).

RESULTS AND DISCUSSION

Hormone Thyroid (T_3 and T_4) Concentrations

Stress induced by feed restrictions may reflect in dramatic change plasma biochemical. Previous studies in chickens showed that the feed restriction may modified the concentration of hormones that modulate energy metabolism and growth, such as hormone T_3 and T_4 (Decuyper *et al.*, 2005; Zhan *et al.*, 2007). Measurement of T_3 and T_4 hormone concentrations in blood plasma of broiler chickens carried out at the end of restriction period (14 and 21 d of age) and the end realimentation period (42 d of age). The concentrations of hormones T_3 and T_4 were presented in Table 2.

Hormone T_3 and T_4 concentrations of feed restricted broilers (R-1, R-2, R-3 and R-4) did not differ with the broilers fed *ad libitum* (R-0) at 14 days of age. However, concentrations of T_3 and T_4 in the restricted broilers of R-4 and R-2 had significantly lower ($P < 0.05$) than those *ad libitum* broilers (R-0) at 21 d of age. During this period, T_3 in R-2 was lower ($P < 0.05$) than R-4, and there was no statistical differences between the R-2 and R4 on T_4 concentration. These findings showed that the feeding time restriction for 7 d did not decrease circulating of T_3 and T_4 . These findings accordance with findings Khajali *et al.* (2007) also reported that there were no difference between feed restricted broilers through skip-a-day feeding at age 9, 11, and 13 d with control on T_3 (3.21 vs 3.47 ng /ml) and T_4 (62.11 vs 68.06 ng /ml) at 28 d of age. However, the thyroid hormone (T_3 and T_4) concentrations decreased when feed restriction was applied for 14 d from 7-21 d of age. This fact is in agreement with

Table 2. The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in the plasma of broilers

Hormone	Age (d)	Treatments				
		R-0	R-1	R-2	R-3	R-4
T_3 (nmol/l)	14	1.87 \pm 0.38	1.46 \pm 0.80	1.48 \pm 0.49	1.45 \pm 0.14	1.45 \pm 0.05
	21	1.29 \pm 0.14 ^a	1.23 \pm 0.05 ^a	0.72 \pm 0.12 ^c	1.22 \pm 0.11 ^a	0.96 \pm 0.11 ^b
	42	0.60 \pm 0.17	0.63 \pm 0.17	0.89 \pm 0.27	0.92 \pm 0.27	1.01 \pm 0.75
T_4 (ug/dl)	14	3.89 \pm 0.77	4.70 \pm 0.53	5.05 \pm 0.31	5.04 \pm 1.50	5.10 \pm 0.42
	21	5.59 \pm 0.66 ^a	5.21 \pm 0.28 ^a	4.07 \pm 0.52 ^b	5.31 \pm 0.40 ^a	4.16 \pm 0.24 ^b
	42	6.19 \pm 0.37	5.67 \pm 1.14	5.43 \pm 0.81	5.70 \pm 1.28	5.65 \pm 0.61

Note: Means in the same row with different superscript differ significantly ($P < 0.05$). Chicks fed *ad libitum* (R-0); chicks had free access to feed during two periods of 2 h (08:00-10:00 and 16:00-18:00) from 7-14 d of age (R-1) and 7-21 d of age (R-2); chicks had free access to feed during two periods of 4 h (08:00-12:00 and 16:00-20:00) from 7-14 d of age (R-3) and 7-21 d of age (R-4).

the findings of Zhan *et al.* (2007) that the concentration of hormones T_3 (1.7 vs 2.1 ng/ml) and T_4 (55.3 vs 60.5 ng/ml) in feed withdrawal for 4 h/d (14:00 to 18:00) from 1-21 d of age were lower than the control.

Evaluation of plasma biochemistry in bird allows the identification of metabolic alterations due to a realm of factors, including genetic type, husbandry conditions, age, physiological state and pathological (Harr, 2002; Gayathri *et al.*, 2004; Jurani *et al.*, 2004; Alonso-Alvarez, 2005). Changes of blood thyroid hormone concentrations are an indirect measure of the changes in thyroid gland activity and circulating thyroid hormones can be considered as indicators of the metabolic and nutritional status of the animals (Todini, 2007; Todini *et al.*, 2007).

The changes in plasma thyroid hormones concentration in feed restricted broilers likely result from a shift in the balance between deiodination of T_4 by hepatic D1 and T_3 degradation by hepatic D3 deiodinases (Darras *et al.*, 2000; Ryens *et al.*, 2002). The other report, Gyorffy *et al.* (2009) found that during energy restriction, T_3 concentration is lowered due to a decreased T_4 activation and increased T_3 inactivation. Furthermore, they show that hepatic type-I deiodinase (D1) is not affected by energy restriction, however, hepatic D2 is decreased on both transcriptional and enzyme activity levels. The thyroid hormones, including T_3 and T_4 , are recognized as the key metabolic hormones of the body, with T_3 being the most functionally active form. The majority of circulating T_3 is derived from the deiodination of T_4 in nonthyroidal tissues such as the liver and kidney (Smith *et al.*, 2000). Decrease in activity monodeiodinase during feed restriction is part the changes of T_3 concentration. Therefore, it was supposedly that metabolic rate decreased during feed restriction causes hormonal activity associated with energy metabolism and growth declined. This result is in line with observation of Azis *et al.* (2011) who found that growth performance of broilers decrease during feed restriction. This might be caused by the low basal metabolism and thus allows the organism to spare energy by decreasing basal metabolism (Hornick *et al.*, 2000). Zhang *et al.* (2007) summarized that concentration of thyroid hormones were closely correlated with growth performance and shift the energy metabolism of broilers.

At the end period of realimentation (42 d of age), T_3 and T_4 concentrations in all feed restricted broilers were no differences ($P>0.05$) than those *ad libitum* broilers. Gonzales *et al.* (1998) reported that quantitative restriction (20% *ad libitum*) from 8-21 d were not differences with control in T_3 (1.30 vs 1.32 ng/ml) and T_4 (9.46 vs 8.91 ng/ml) at 42 d of age. In addition, Zhan *et al.* (2007) reported that the concentration of T_3 (1.1 vs 1.0 ng/ml) and T_4 (63.3 vs 65.7 ng/ml) in feed withdrawal for 4 h/d (14:00 to 18:00) from 1-21 d of age were not differences than the control during the period of realimentation from 21-62 d of age. It suggests that degree of feed restrictions applied was not severe because there was a very fast adaptive response with small and transient alterations in T_3 and T_4 concentrations during the period of realimentation. This might be caused by increased activity of the enzyme deiodinase type III (D3) during the realimentation period would recover concentrations of T_3 hormone (Darras *et al.*,

2000; Gyorffy *et al.*, 2009). Furthermore, Gyorffy *et al.* (2009) explained that hypothalamic feedback was not involved in the changes in serum T_3 and T_4 concentrations. These findings indicate that T_4 and T_3 concentrations of the feed restricted broiler were normalized and remain as the value of *ad libitum* broilers during realimentation period. These results accordance with findings Azis *et al.* (2011) that feed restricted broilers during starter period (7-21 d of age) had normal growth during realimentation period (21-42 d of age) so that compensatory growth and normal body weight could be achieved at 42 d of age.

Blood Metabolites Concentration

Plasma albumin concentration of feed restricted broilers (R-2 and R-4) were lower ($P<0.05$) than R-0, R-1, and R-3 at 21 d of age, and there was difference between R-2 and R-4 on the concentration of albumin during this period (Table 3). These findings indicate that plasma albumin concentration decreased due to feeding time restriction for 4 or 8 h/d during 7-21 d of age. Decrease of plasma albumin concentration during feed restriction associated with thyroid hormone status. About 50% of T_3 is associated with albumin, and 75% of T_4 is attached to albumin (Butler, 1983). These results in line with El-Fiky *et al.* (2008) that feed availability for 4 h/d with two periods of 2 h (7:00 to 9:00 and 12:00 to 14:00) decreased plasma albumin concentration of broiler (2.10 vs 2.63 g/dl) at 35 d of age. The other report, Jang *et al.* (2009) found that plasma albumin concentrations of broiler subjected to feed restrictions (70% *ad libitum*) from 8-14 d of age is lower than the control at 14 d of age (0.82 vs 1.26 g/dl). Nevertheless, these findings differ with Demir *et al.* (2004) that feed withdrawal for 16 h/d from 13-21 d of age was not influence plasma albumin of broilers at the end feed restriction (123 vs 117 g/dl). These differences in findings may be due to the differences in the intensity of feed restriction, time of feed restriction, age of initiation feed restriction, and the availability of feed for birds.

At the end period of realimentation (42 d of age), the concentrations of plasma albumin in all feed restricted broilers were no differences ($P>0.05$) than those *ad libitum* broilers. These findings indicate that plasma albumin concentrations of the feed restricted broiler were normalized and remain as the value of *ad libitum* broilers during realimentation period. Result of the present study was in agreement with the results of El-Fiky *et al.* (2008) and Boostani *et al.* (2010) who also show that plasma albumin concentrations were not affected by feed availability for 4 h/d (2.03 vs 2.14 g/dl) at 49 d of age, and feed availability for 8 h/d (1.74 vs 1.78 g/dl) at 42 d of age.

The concentration of total protein, globulin and albumin/globulin ratio (A/G) in all feed restricted broilers were no differences ($P>0.05$) than those *ad libitum* broilers at the end of feed restriction and realimentation period. These results indicate that the plasma total protein and globulin have not changed due to feed restriction. Although there were indications of intake protein decreased (12.77 vs 15.04 g/bird/d) during feed restriction from 7-21 d of age (Azis *et al.*,

Table 3. The concentrations of total protein, albumin, globulin and albumin/globulin ratio (A/G) in the plasma of broilers

Blood metabolites	Age (d)	Treatments				
		R-0	R-1	R-2	R-3	R-4
Total protein (g/dl)	14	3.12±0.56	3.21±0.16	2.97±1.06	2.76±0.43	2.56±0.90
	21	4.61±0.49	4.35±0.91	4.77±0.26	3.92±0.32	3.86±0.58
	42	5.19±0.38	5.07±0.27	5.01±0.22	5.03±0.33	5.02±0.18
Albumin (g/dl)	14	1.70±0.17	1.62±0.07	1.64±0.06	1.63±0.09	1.63±0.04
	21	1.62±0.11 ^a	1.70±0.09 ^a	1.48±0.03 ^b	1.63±0.09 ^a	1.41±0.03 ^b
	42	1.73±0.04	1.82±0.08	1.86±0.10	1.77±0.14	1.70±0.05
Globulin (g/dl)	14	1.42±0.50	1.59±0.20	1.33±1.06	1.13±0.44	0.93±0.89
	21	2.99±0.53	2.65±0.95	2.45±0.32	3.14±0.23	2.45±0.58
	42	3.46±0.39	3.26±0.29	3.15±0.12	3.26±0.47	3.32±0.22
A/G Ratio	14	1.32±0.46	1.04±0.17	2.09±1.51	1.74±1.01	1.79±0.32
	21	0.55±0.11	0.70±0.23	0.52±0.04	0.61±0.10	0.60±0.15
	42	0.51±0.06	0.56±0.07	0.60±0.02	0.56±0.12	0.52±0.05

Note: Means in the same row with different superscript differ significantly ($P < 0.05$). Chicks fed *ad libitum* (R-0); chicks had free access to feed during two periods of 2 h (08:00-10:00 and 16:00-18:00) from 7-14 d of age (R-1) and 7-21 d of age (R-2); chicks had free access to feed during two periods of 4 h (08:00-12:00 and 16:00-20:00) from 7-14 d of age (R-3) and 7-21 d of age (R-4).

2011), however, it would not decrease protein reserves in the body. Plasma protein was an indication of protein reserve in the body that can reflect the ability of the chicken store "reserve" protein even after the animal has reached its maximum capacity for depositing tissue. The importance of such reserve in resisting the stresses of caloric restriction (Leveille & Sauberlich, 1961). Contrary to this finding, Jang *et al.* (2009) found that total protein concentrations of broiler subjected to feed restrictions (70% *ad libitum*) from 8-14 d of age was lower than the control at 14 d of age (1.96 vs 3.26 g/dl), however, it had similar level (2.76 vs 2.12 g/dl) at the end realimentation period (35 d of age). This finding indicate that feeding time restriction had lower intensity of feed restriction, so that there was not any change reserve protein during feed restriction. The other reports, the feed restriction through feed availability for 8 h/d during 7-21 d of age (Boostani *et al.*, 2010), and skip-a-day for 6 d during 8-18 d (Bratte, 2011) were not influence plasma protein concentrations at age of 42 d (3.76 vs 3.89 g/dl) and 56 d (5.60 vs 6.40 g/dl).

CONCLUSION

Thyroid hormone (T_3 and T_4) and blood metabolite (albumin) concentrations decreased during feeding time restriction for 4 and 8 h per d from 7-21 d of age, but any influence was not observed on thyroid hormone and blood metabolites concentrations during realimentation period.

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