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Implication of feed restriction during growth period on the growth hormone profiles and morphology ovary of quail hen (*Coturnix coturnix japonica)*

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi pengaruh pembatasan pakan pada profil hormon pertumbuhan dan morfologi ovarium selama periode pertumbuhan. Sebanyak 300 ekor burung puyuh umur empat belas hari dari digunakan pada penelitian ini. Program pembatasan pakan yang diberikan adalah pembatasan berdasarkan Energi Metabolisme pakan (ME) R1 = 2900 kkal / kg; R2 = 2800 kkal / kg dan pembatasan pakan kuantitatif P0 = 100% dari *ad libitum*; P1 = 90% dari *ad libitum* dan P2 = 80% dari *ad libitum* (n = 300). Setiap kelompok perlakuan (n = 50) terdiri dengan lima ulangan, masing-masing 10 ekor puyuh. Kadar hormon pertumbuhan ditentukan pada umur 28, 35, 42, dan 49 hari sementara morfologi ovarium diukur pada saat masak kelamin. Hasil penelitian menunjukkan bahwa pembatasan pakan terjadi penurunan hormon pertumbuhan dan *Insulin-like Growth Factor*-1 secara nyata (P<0.01) akan tetapi terjadi peningkatan setelah pakan diberikan lagi secara *ad libitum*. Tidak ada efek signifikan yang disebabkan oleh perbedaan kandungan energi metabolisme pakan. Jumlah folikel kuning telur besar (*large yellow follicle*, LYF) tidak berbeda antara puyuh yang diberi pakan *ad libitum* dan dibatasi 90% dari *ad libitum*, namun pembatasan pakan secara signifikan meningkatkan jumlah folikel kuning telur kecil (*small yellow follicle*, SYF). Dapat disimpulkan bahwa pembatasan pakan dapat diberikan hingga 90% *ad libitum* dengan EM 2900 kkal / kg selama periode starter (14 hingga 42 hari) tanpa mempengaruhi morfologi ovarium puyuh

Kata kunci: pembatasan pakan, growth hormone, Insulin-like Growth Factor-1, puyuh, morfologi ovary, folikel kuning telur

ABSTRACT

The aim of this research was to evaluate the effect of feed restriction on growth hormone profiles and ovarian morphology during the growth period. Three hundred 14-day-old quails were used. The quails were maintained on two feeding restriction programs: two dietary regimes based on metabolizable energy (ME), R1 = 2900 kcal/kg and R2 = 2800 kcal/kg, and 3 quantitative feed restriction diets, P0 = 100% *ad libitum*; P1= 90% *ad libitum* and P2 = 80%*ad libitum* (n=300). Each group (n=50) was processed with five replications, 10 birds in each replicate. The change in growth hormone was determined at 28, 35, 42, and 49 days of age, while ovarian morphology was determined at sexual maturity. The results indicated that feed restriction induced a significant increase in growth hormone and insulin-like growth factor-1 after re-feeding. There was no significant effect caused by the rationing of metabolizable energy. The number of large yellow follicles was not different between quails fed with

100% *ad libitum* and 90% *ad libitum*. However, feed restriction significantly increased the number of small yellow follicles. In conclusion, the feed could be restricted to 90% *ad libitum* with a 2900 kcal/kg ration of ME and fed during the starter period (14 to 42 days of age) without influencing ovarian morphology in quails.

Keywords: feed restriction, growth hormone, insulin-like growth factor-1, quail hen, yellow follicle

INTRODUCTION

Feed affects the body's metabolism and growth that is regulated or mediated by the endocrine system. Koyuncu (2013) explain that the main hormones involved with growth are growth hormone (GH), triiodothyronine (T3) and insulin-like growth factor 1(IGF-1). GH and IGF are known as the key hormones that affect growth, maturation, and metabolism. The hormones are influenced by nutritional factors and have been widely studied, especially in prosocial bird species (McNabb, 2006). These metabolic hormones are the most important factor in determining the level of hepatic poultry lipogenesis. The application of a feed restriction program on poultry farms primarily aims to reduce excess energy consumption which generally occurs when feeding is provided without restriction (*ad libitum*).

Laying chicken have an ability to consume 10-15% more energy than required (Busye *et al*., 2000) and excess energy is converted into body fat which results in faster growth and faster sex maturation. The excess energy consumed is automatically be converted into fat deposits in the body. The high-fat deposits in the body causes early sexual maturation and increases follicular development (Diaz and Anthony, 2013), whereas the reproductive conditions are not ready to support optimum egg production. Increased body fat deposition can cause a high incidence of metabolic disorders and high mortality rates (Saber *et al*., 2011; Sahraei, 2012). There is a negative correlation between body weight and reproductive traits. Thus, a rapid increase in body weight affects egg production, fertility and hatchability (Wariboko and George, 2015).

Based on research results of Busye *et al*. (2000), restrictions on feeding are followed by an increase in GH secretion which further increases metabolic effects towards the body tissues. Increased GH stimulates the liver to increase IGF-I secretion, obtained both through circulation and local production. Feed restriction programs cause a different response in hormone profiles that affect growth, and do not always cause an increase in GH concentration (El-far, 2014). Generally, IGF-1 concentrations decrease during feed restriction programs to regulate growth and body weight in chickens. However, after being returned to *ad libitum* feeding, there is an increase in IGF-1.

Feed restrictions, as a nutritional management approach to reduce feed costs or problems related to egg production in quail research, have been carried out which show the positive impact of qualitative and quantitative dietary restrictions on growth performance (Abbas *et al*., 2015). Prevous studies revealed that restricted feeding are critical for quail reproduction performance (Hassan *et al*., 2003; Fidan and Kaya, 2014; Wariboko and George, 2015), carcass quality (Azeem and Azeem, 2011), and spawning properties (Petek, 2006).

The relationship between GH concentration and quail ovarian morphology has not been clearly studied. The decrease in feed consumption could change the GH, IGF-I and other growth hormone concentrations. However information the effect of restricted feeding and its implication on quails growth hormone profile are scarce. Thus, it is assumed that there will be an effect on ovulation and egg production in poultry caused by limiting feed and *ad libitum* feed. The aim of this study was to evaluate the implications of feed restrictions involving different energy contents of feed on GH profiles and ovarian morphology at the sexual maturation period.

MATERIALS AND METHODS

Sampling and Feed Restriction Formulation

The livestock used as the sample were 14-day-old quail hens, obtained from the Jaya Mulya Livestock Farmers Group, Pare Kediri city. As many as 500 quails were reared from the age of one day to 14 days with no feed restriction (*ad libitum*). The cages used in this study were 40× 30× 30 cm, with a capacity to house 15 quails in each unit; up to 30 units were used.

Treatment with feed restrictions began in 14-day-old quails. Two types of feed that differed in energy content (2900 kcal/kg and 2800 kcal/kg) were given according to Altine (2016); these were provided at 90% and 80% of *ad libitum* feeding, plus 100% of *ad libitum* feeding as the control. Feed restriction was modified from Hertamawati (2006). Feed R1 contained 24% crude protein (CP) and 2900 kcal/kg metabolizable energy (ME), based on the standard requirements for quail hen's growth period from NRC (1994). Feed R2 contained 24% as CP and 2800 kcal/kg ME.

Experimental Design

The experiment was based on a nested completely randomized design consisting of two independent variables, feed ME factors consisting of two ME values (2900 kcal/kg and 2800 kcal/kg) and the second factor was the P factor or feed restrictions which consisted of three levels (100%, 90% and 80% as *ad libitum* feed) that nested in factor R. The feed restriction treatment was conducted from 14 to 42 days of age. Quails were divided into six treatments, as follows : R1P0 = ME 2900 Kcal/kg, *ad libitum feeding,* R1P1 = ME 2900 Kcal/kg, restricted feeding 90% *ad libitum*, R1P2 = ME 2900 Kcal/kg restricted feeding 80% *ad libitum*, R2P0 = ME 2800 Kcal/kg, *ad libitum* feeding, R2P1 = EM 2800 Kcal/kg , restricted feeding 90% *ad libitum*, R2P2 = EM 2800 Kcal/kg, restricted feeding 80% *ad libitum*. The quails were fed *ad libitum* feed after 42 days of age.

Data Collection

Observation of ovarian morphology was carried out when quails reached sexual maturation. It involved a surgical procedure and observation of reproductive organs by counting the number of ovarian follicles (Arora and Samples, 2011), including large yellow follicles (LYF) (> 1 cm), medium yellow follicles (MYF) (<0.5–1 cm) and small yellow follicles (SYF) (<0.5 cm). Blood sampling for hormone profiling analysis was carried out from the brachial vein on the wings before the quails reached egg-laying age (28 and 35 days of age), mature age (42 days of age) and during the period of egg laying (49 days of age). Blood was collected into an EDTA tube and immediately centrifuged for 20 minutes at 2500 rpm to separate the plasma. The GH and IGF-1 hormone levels were analyzed using the enzyme-linked immunosorbent assay (ELISA) and expressed in units of ng/ml. The measurement procedure was conducted according to guidelines of the ELISA Poultry GH Kit (Elabscience Catalog No: E-EL-Ch2116) and IGF-1 Kit (Elabscience Catalog No: E-EL-Ch0116).

Data Analysis

Data were analyzed using one-way ANOVA with a nested design and continued with Duncan's multiple range test if there were differences (SPSS 21).

RESULTS AND DISCUSSION

Growth Hormone Profiles

The hormonal profiles associated with

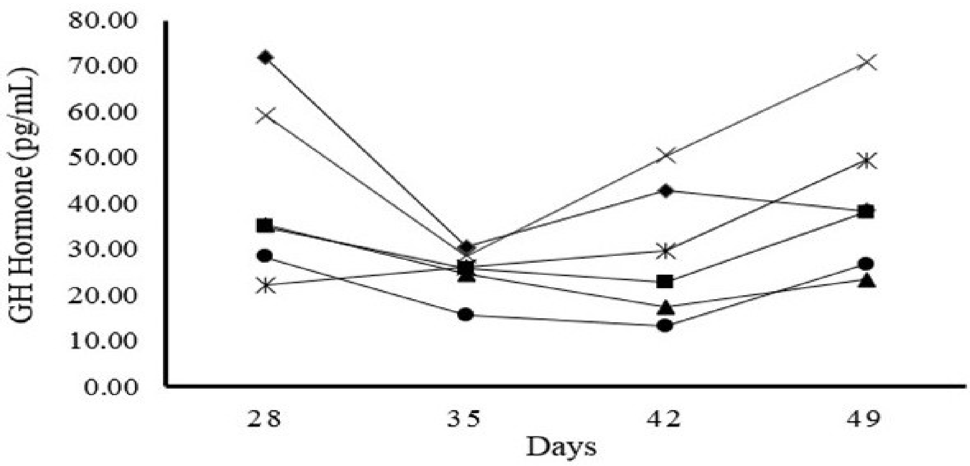

Figure 1. The GH Hormone Profiles in Quail Blood Samples at the Age of 28, 35, 42 and 49 Days with *ad libitum* and Restricted Feeding (R1P0 -♦- ; R1P1-■-; R1P2 -▲-; R2P0-×-; R2P1-*-; R2P2-●-)

growth include GH and IGF-1and levels of these after feed restrictions can be seen in Figure 1 and 2. The results showed that the treatments involving feed restriction and different feed energy contents produced a significant effect (P<0.01) on GH levels on days 28, 35, 42 and 49 (Table 1). The GH levels showed a decrease on day 35 with both limited feeding and *ad libitum* feeding, but increased at 49 days of age because at that time the feed given *ad libitum* (Figure 1). The GH reduction with feed restriction was also reported in the study of Busye *et al*. (2000) which stated that the decrease in GH levels occurred due to a decrease in the number of GH receptors in the liver of poultry that is given limited feed, indicating a decrease in GH secretion. The greatest GH hormone increase was on day 49 with the treatment of 80% *ad libitum* feed restriction, both with ME 2900 kcal/kg and ME 2800 kcal/kg (P<0.05).

Figure 2 present IGF-1 hormone profile. The results showed that the feed restriction treatment and different energy contents also affected the IGF-1 hormone levels significantly (P<0.01) at the ages of 28, 35, 42 and 49 days. The IGF-1 hormone levels were decreased at 35 days and then increased at 42 days. The highest increase occurred when the feed was provided *ad libitum* again (aged 49 days). The highest increase in IGF-1 (P<0.05) occurred in the quails feeding at 80% *ad libitum*. The GH works indirectly on growth through increased IGF-I by stimulating the liver to increase IGF-I secretion, through the circulation and local production (Hrabia, 2015). In this study, there was a proven mechanism by which GH levels increased on day 49, followed by an increase in IGF-1 levels. The decrease in IGF-1 in Peking ducks on a feed restriction program was also reported by El-Far (2103). Farhat and Chavez (1999) proved that IGF-1 concentrations were influenced by poultry strains, feed protein contents, sex, feed restrictions and poultry selection criteria.

Ovarian Morphology

Ovarian morphology at sexual maturity after feed restriction showed a large number of large yellow follicles (LYF), medium yellow follicles (MYF) and small yellow follicles (SYF) as showed at Table 3.

The feed restriction treatment had a significant effect on the number of LYF and SYF (P<0.05), but had no effect on the number of MYF. Feed restriction at 80% *ad libitum* significantly decreased the amount of LYF (P<0.05) at sexual maturity with feed with a ME

Table 1. Composition and Calculated Analysis of Experimental Diets Offered to Quails from 14 to 42 Days of Age

Variables	R1		R2	
Maize (%)	0.40		0.30	
Rice bran (%)	0.07		0.18	
Concentrate feed (%)[1]	0.53		0.52	
	Calculated analysis			
	R1		R2	
Protein (%)	24.01	23.6*	24.01	23.9*
Fat (%)	4.95	3.3*	5.66	3.9*
Crude fiber (%)	5.28	2.8*	5.77	3.1*
Ca (%)	1.37	-	1.34	-
P(%)	0.76	-	0.90	-
Metabolizable Energy (kcal/kg)	2916.56	2930**	2822.40	2830**

*proximate analysis
**estimation of gross energy
[1]Product of Comfeed Ltd.

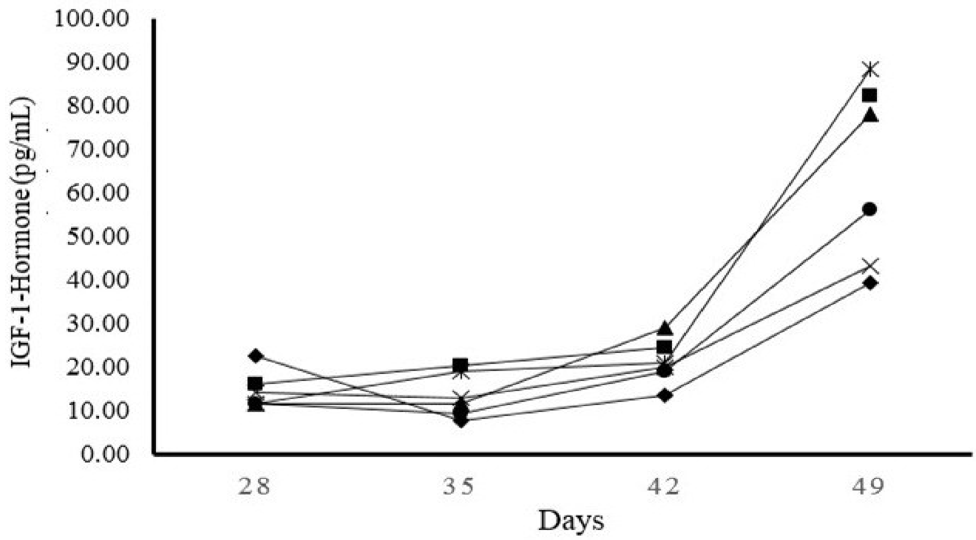

Figure 2. The IGF-1 hormone profiles in quail blood samples at the ages of 28, 35, 42 and 49 days with *ad libitum* and restricted feeding (R1P0 -♦- ; R1P1-■-; R1P2 -▲-; R2P0-×-; R2P1-*-; R2P2-●-)

content of 2800 kcal/kg. However, feed containing ME 2900 kcal/kg did not decrease the amount of LYF. This result was different to findings of Diaz and Anthony (2013) who reported that feed restriction reduced the number of follicles by 50%.

The highest average LYF value was with *ad libitum* feeding and ME feed content of 2900 kcal/kg and 2800 kcal/kg as much as 3.40 ± 0.55 and 3.60 ± 0.55, respectively. The number of LYF was less than the number of follicles reported by Kashmiri and Samples (2011) who found 4.2 follicles in quails with a body weight of 120–130 g and 5.4 follicles in quails with a body weight of 150–160 g. The optimum number of LYF was related to the length of the spawning period. *Ad libitum* feeding in quails did not result in excessive follicular development (more than 5 LYF) as has occurred in broiler chickens (Renema *et al*., 1997).

Body weight during the sexual maturity period is also associated with an increase in LYF quantity. It has been suggested that ovaries in quails with a higher body weight are more active compared to those with lower body weight, due to gonadotropin activity and an increase in steroidogenic transcripts (Diaz and Anthony, 2013). This was supported by Hocking (2009) who showed a linear relationship between body weight and follicle number in poultry with high body weight compared to low body weight in the sexual maturity period. This was suggested to be a weakness in feed restriction programs or limited energy consumption which results in weight loss that then affects body composition and reduces the number of follicles in the ovary.

Feed restriction at 90% *ad libitum* in feed with ME of 2900 kcal/kg (R1) and 2800 kcal/kg (R2) increased the number of SYF compared to *ad libitum* feeding ($P < 0.05$). The lowest SYF number was found in quails with *ad libitum* treatment due to the higher body weight gained at sexual maturity than with feed restriction treatment, so that ovarian development was not optimal. This finding was supported by Arora and Samples (2011) who stated that a decrease in the number of SYF in quails with a higher body weight receiving feed containing ME of 2800 kcal/kg showed the number of follicles that were not different from *ad libitum* treatment, presumably due to the level of restriction and feed energy levels inhibiting follicular development.

The changes in IGF-1 hormone levels due to nutritional differences have been described in mammals and poultry. The decrease in hormone levels associated with the growth was due to dietary restrictions and energy restrictions (Kita *et al*., 1996; McMurtry, 1997). In this study, the GH and IGF-1 levels were also decreased due to the feed restrictions and increased after the feed provision was returned to *ad libitum* (Table 2). Feed restrictions at 90% *ad libitum* produced the same number of follicles as *ad libitum* feeding, which is economically more efficient in the use of

Table 2. Means for the Effect of *ad libitum* and Restricted Feeding on GH and IGF-1 Profile of Quails (pg/mL) at 28, 35, 42 and 49 Days of Age

ME Content (kcal/kg)	Feed Restriction (% *ad libitum*)	28 days		35 days		42 days		49 days	
		GH	IGF-1	GH	IGF-1	GH	IGF-1	GH	IGF-1
2900	100	71.8±5.57 [a]	22.47±3.98 [a]	30,7±2,89 [a]	7.66±0.89 [a]	42.8±3.21 [a]	13.45±3.50 [a]	38.4±3.55 [a]	39.16±2.72 [a]
	90	34.7±1.95 [b]	16.10±4.96 [b]	25,7±4,58 [a]	20.40±1.12 [b]	22.7±3.52 [b]	24.32±3.90 [b]	37.9±3.50 [a]	78.15±3.35 [b]
	80	35.3±4.06 [b]	11.41±1.49 [b]	24,5±1,34 [a]	11.47±0.62 [b]	17.3±2.44 [b]	29.10±5.39 [b]	23.3±2.32 [b]	82.17±8.43 [b]
2800	100	59.2±2.87 [a]	14.02±3.55 [a]	28,5±5,14 [a]	12.75±2.88 [a]	50.4±1.16 [a]	19.95±6.13 [a]	70.5±5.44 [a]	43.13±9.21 [a]
	90	22.0±4.15 [b]	11.43±5.44 [b]	15,6±1,08 [a]	18.96±3.13 [b]	29.5±2.97 [b]	20.81±5.84 [a]	49.4±2.73 [b]	88.50±3.86 [b]
	80	28.2±7.52 [b]	11.48±1.66 [b]	26,0±4,32 [a]	9.25±1.33 [a]	13.3±2.07 [c]	18.99±3.11 [a]	26.8±2.61 [c]	56.12±2.63 [a]

[a-c] Mean values with different superscripts within the same column differ significantly ($P<0.05$)

Table 3. Means for the Effect of *ad libitum* and Restricted Feeding on Ovarian Morphology during the Sexual Maturity Period

ME Content (kcal/kg)	Feed Restriction (% *ad libitum*)	Body weight at sexual maturity (g)	Yellow Follicle		
			Large	Medium	Small
2900	100	187.4± 8.85	3.40±0.55 [a]	2.80±0.84	18.60±7.23 [a]
	90	172.4± 8.62	3.20±0.45 [a]	2.80±0.45	25.20±3.77 [b]
	80	169.6±14.57	2.80±0.45 [b]	2.80±0.45	21.20±2.28 [b]
2800	100	185.4±15.13	3.60±0.55 [a]	2.40±0.55	20.40±4.72 [a]
	90	177.2±12.32	3.20±0.45 [a]	2.40±0.55	28.40±7.50 [b]
	80	174.4±10.60	2.60±0.55 [b]	2.60±0.89	20.40±1.14 [a]

[a, b] Mean values with different superscripts within the same column differ significantly (P<0.05)

feed. The feed restrictions on quails improved egg production due to the increase in levels of GH and IGF-1 hormones after the feed restriction program, and ovary morphology composition was mainly can be seen from the number of SYF at the period of sexual maturity.

CONCLUSION

The feed restrictions on quail hens reduce the GH and IGF-1 levels. There is no difference in the number of large yellow follicles with up to 90% *ad libitum* restriction feeding. The feed restriction programs can be applied with 90% *ad libitum* feed restrictions with feed containing ME of 2900 kcal/kg during the starter period.

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