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IMPLICATION OF FEED RESTRICTION DURING THE GROWTH PERIOD ON THE GROWTH HORMONE PROFILES AND OVARIAN MORPHOLOGY IN QUAIL HENS

(*Coturnix coturnix japonica*)

ABSTRACT

This research aimed 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 Metabolize Energy (ME), R1 = 2900 kcal/kg and R2 = 2800 kcal/kg, and three 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 metabolizing 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. 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. The main hormones involved with growth are growth hormone (GH), triiodothyronine (T3) and insulin-like growth factor 1 (IGF-1) (Koyuncu, 2013). GH and insulin-like growth factors (IGF) are known as the essential hormones that affect growth, maturation, and metabolism that is influenced by nutritional factors and has been widely studied, especially in prosocial bird species (McNabb, 2006). These metabolic hormones are the most crucial 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 indefinitely (*ad libitum*).

Poultry has the ability to consume 10-15% more energy than required 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 cause early sexual maturation and increase 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-I 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-I.

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, 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 explicitly studied. Based on the results of a previous study, the decrease in feed consumption could change the GH, IGF-I and other growth hormone concentrations. 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. This study aimed to evaluate the implications of feed restrictions involving different energy contents of feed on GH profiles and ovarian morphology at the sexual maturation period.

METHODS

Sampling and Feed Restriction Formulation

The livestock used as the sample were 14-day-old quail hens (DOQ = day-old quail), 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; these were provided at 90% and 80% *ad libitum* feeding, plus 100% *ad libitum* feeding as the control. Feed R1 contained 24% as crude protein (CP) and 2900 kcal/kg metabolic 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 entirely 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 on the 14 days to the 42. The quails were fed *ad libitum* feed after 42 days of treatment.

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, 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 jugular vein on the wings before the quails reached the egg-laying age (28 days and 35 days), mature age (42 days) 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 diversity analysis with a nested design and continued with the smallest real difference test if there were differences.

Table 1. The feed formulation and calculated analysis of experimental diets offered to quails (1 to 42 days of age)

Variables	R1	R2		
Maize (%)	0.40	0.30		
Rice bran (%)	0.07	0.18		
Concentrate feed (%) (Comfeed Ltd.)	0.53	0.52		
Calculated analysis				
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	-
Metabolize energy (kcal/kg)	2916.56	2930**	2822.40	2830**

*proximate analysis

**estimation of gross energy

RESULTS AND DISCUSSION

Growth Hormone Profiles

The hormonal profiles associated with growth include GH and IGF-1 and levels of these after feed restrictions can be seen in Figure 1 and 2. The ANOVA test 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 gave *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 (R1P2 and R2P2), both with ME 2900 kcal/kg and ME 2800 kcal/kg ($p < 0.05$). There was also a decrease in GH levels on day 35 with *ad libitum* feeding. It was suspected to have quail passed through the growth period as Goddard et al. (1988) stated that plasma GH levels increase sharply between 3 and 4 weeks of age and then suddenly decrease to low levels in puberty and adulthood.

The ANOVA test 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* (R1P2 and R2P2). 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.

Table 2. The average and standard intersections of GH and IGF-1 profiles at 28, 35, 42 and 49 days of age with *ad libitum* feeding and limited

ME content (kcal/kg)	Feed restriction (% <i>ad libitum</i>)	28 days		35 days		42 days		49 days	
		GH	IGF-1	GH	IGF-1	GH	IGF-1	GH	IGF-1
2900	100	71.8±5.5 7 ^a	22.47±3.9 8 ^a	30.7±2.8 9 ^a	7.66±0.89 a	42.8±3.2 1 ^a	13.45±3.5 0 ^a	38.4±3.5 5 ^a	39.16±2.72 a
	90	34.7±1.9 5 ^b	16.10±4.9 6 ^b	25.7±4.5 8 ^a	20.40±1.1 2 ^b	22.7±3.5 2 ^b	24.32±3.9 0 ^b	37.9±3.5 0 ^a	78.15±3.35 b
	80	35.3±4.0 6 ^b	11.41±1.4 9 ^b	24.5±1.3 4 ^a	11.47±0.6 2 ^b	17.3±2.4 4 ^b	29.10±5.3 9 ^b	23.3±2.3 2 ^b	82.17±8.43 b

2800	100	59.2±2.8 7 ^a	14.02±3.5 5 ^a	28.5±5.1 4 ^a	12.75±2.8 8 ^a	50.4±1.1 6 ^a	19.95±6.1 3 ^a	70.5±5.4 4 ^a	43.13±9.21 a
	90	22.0±4.1 5 ^b	11.43±5.4 4 ^b	26.0±4.3 2 ^a	18.96±3.1 3 ^b	29.5±2.9 7 ^b	20.81±5.8 4 ^a	49.4±2.7 3 ^b	88.50±3.86 b
	80	28.2±7.5 2 ^b	11.48±1.6 6 ^b	15.6±1.0 8 ^b	9.25±1.33 a	13.3±2.0 7 ^c	18.99±3.1 1 ^a	26.8±2.6 1 ^c	56.12±2.63 a

a, b, c significant different notations (p < 0.05)

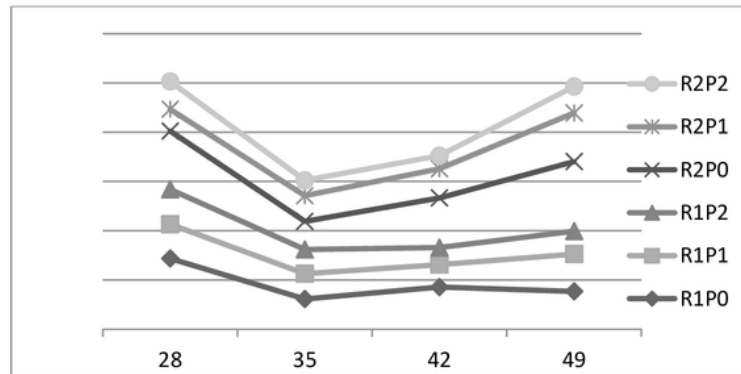
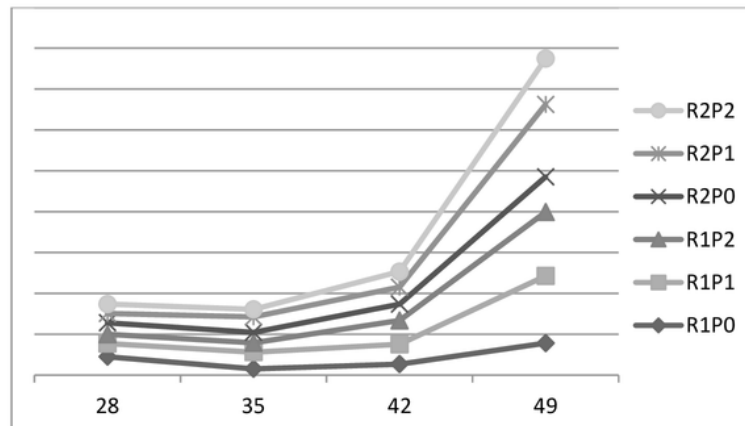


Figure 1. The GH hormone profiles in quail blood samples at the age of 28, 35, 42 and 49 days with *ad libitum* and limited feeding



The IGF-1 hormone profiles in quail blood samples at the ages of 28, 35, 42 and 49 days with *ad libitum* and limited feeding

Ovarian Morphology

Ovarian morphology at sexual maturity after feed restriction showed a large number of large yellow ⁵ follicles (LYF), medium yellow follicles (MYF) and small yellow follicles (SYF) (Table 3).

The ANOVA results indicated that 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 an ME content of 2800 kcal/kg. However, feed containing ME 2900 kcal/kg did not decrease the amount of LYF. This result was different to the findings of Diaz and Anthoni (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 Anthon, 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 the 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.

Table 3. Average of ovarian morphology values and standard intersection during the sexual maturity period with *ad libitum* and limited feeding

ME content (kcal/kg)	Feed restriction	Body weight at sexual maturity (g)	Large yellow follicle (LYF)	Medium yellow follicle (MYF)	Small yellow follicle (SYF)
2900	<i>Ad libitum</i>	187.4 ± 8.85	3.40 ± 0.55 ^a	2.80 ± 0.84	18.60 ± 7.23 ^a
	90% <i>ad libitum</i>	172.4 ± 8.62	3.20 ± 0.45 ^a	2.80 ± 0.45	25.20 ± 3.77 ^b
	80% <i>ad libitum</i>	169.6 ± 14.57	2.80 ± 0.45 ^b	2.80 ± 0.45	21.20 ± 2.28 ^b
2800	<i>Ad libitum</i>	185.4 ± 15.13	3.60 ± 0.55 ^a	2.40 ± 0.55	20.40 ± 4.72 ^a
	90% <i>ad libitum</i>	177.2 ± 12.32	3.20 ± 0.45 ^a	2.40 ± 0.55	28.40 ± 7.50 ^b
	80% <i>ad libitum</i>	174.4 ± 10.60	2.60 ± 0.55 ^b	2.60 ± 0.89	20.40 ± 1.14 ^a

^{a, b} significant different notations (p < 0.05)

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 this 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 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

In conclusion, the feed restrictions on quail hens could reduce the GH and IGF-1 levels. There is no difference in the number of LYF 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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