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Research Article
Effect of Varying Level of Crude Protein and Energy on Insulin-like Growth Factor-I Expression Level in Indonesian Hybrid Chicken

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Abstract
Objectives: The aims of this study were to investigate the effect of crude protein and energy level in feed on insulin-like growth factor-I (IGF-1) expression level in a hybrid chicken (female Layer chicken × male Pelung chicken). **Methodology:** To study the correlation between feed type and IGF-1 expression level. Day-old chick (DOC) hybrids were divided into four groups and each group comprised 10 individuals. First group was fed with feed A [22% Crude Protein (CP) and 3100 kcal kg^{-1} Metabolizable Energy (ME)], second group was fed with feed B (20% CP and 3300 kcal kg^{-1} ME), third group was fed with feed C (21% CP and 2800 kcal kg^{-1} ME) and fourth group was fed with standard feed after starving for 3 days post hatching. Chickens were observed from post-hatch to the 7th day and weighed (at 0, 3 and 7 days) and five individuals were sacrificed at the 7th day for IGF-1 analysis. Total RNA was isolated from the liver for gene expression analysis. Real-time PCR was used to study the IGF-1 expression profiles of each group after being normalized with β-actin. **Results:** The highest body weight was recorded for group 1, followed by group 2, 3 and 4. The IGF-1 expression level was noticeably high for feed A, B and C groups by 4.82, 3.82 and 1.9 fold, respectively, compared with the starved group. The weight highly correlated with IGF-1 expression level (R^2 = 0.8). This moderate growth response to nutrition makes the hybrid chicken a suitable meat-type chicken. **Conclusion:** The result suggested that feed containing 22% CP and 3100 kcal kg^{-1} ME is a promising starter for the Indonesian hybrid chicken.

Key words: Metabolizable energy, crude protein, Indonesian hybrid chicken, insulin-like growth factor-I expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) was confirmed to be positively correlated to growth rate in chicken. Chicken IGF-1 hormone mainly play roles in cell proliferation and increasing the metabolism rate[1]. Hepatic IGF-1 expression is partly regulated by the nutrition quality and quantity is mediated by growth hormone (GH)[2]. Synergy between the genetic potential and nutrition intake determines the meat-type chicken performance[3].

Metabolizable Energy (ME) and Crude Protein (CP) levels are the two main variables that directly affect the cost of the feed. These parameters are important for developing an affordable feed that is also suitable for day-old chick (DOC) growth in Indonesia. Choosing the right dietary energy and protein source was important owing to the constant increase of feed demands in Indonesia[4]. Broiler chicken strains in Asia require 21% CP and 2800 kcal kg^{-1} ME[5], whereas the most cultivated local chicken in Indonesia, *Gallus gallus domesticus* var. Kampong was reported to require 19% CP and 2900 kcal kg^{-1} ME[6]. However, the requirement of the new Indonesian hybrid chicken remains unknown.

The Indonesian hybrid chicken was obtained by crossing female layer chicken with a male Pelung chicken. Pelung chicken was selected as the parental line because it is the heaviest local chicken in Indonesia[7]; layer chickens are widely accepted for mass egg production. The Indonesian hybrid chicken is expected to possess the growth performance trait and provide economic benefits. Here, IGF-1 expression level was used to evaluate the appropriate dietary protein and energy level for the Indonesian hybrid chicken using two-step real-time PCR.

MATERIALS AND METHODS

Feed formulation: All feed formulae (A, B and C) were prepared using similar ingredients and produced by the local poultry feed mill (Smart-G, Semarang, Indonesia). The difference in energy and crude protein levels among these formulae occurred because of the alteration in the amount of fat and protein sources. Feed A contained 3100 kcal kg^{-1} ME and 22% CP, feed B contained 3300 kcal kg^{-1} ME and 20% CP and feed C contained 2800 kcal kg^{-1} ME and 21% CP.

Animals and experimental procedures: The experiments involving animals in this project were approved by the Universitas Gadjah Mada ethics committee. Female Layer chickens were obtained from a commercial egg producer and Pelung chickens were obtained from a university farm breeder. Naturally mated in $5\times5\times5$ m^3 pens, the chickens were given free access to feed and water. Eggs were collected for artificial incubation by a machine. The DOC hybrid chickens were divided into four groups (n = 10 per group). The first group (G-1) was fed with feed A, the second group (G-2) was fed with feed B, the third group (G-3) was fed with feed C and the fourth group (G-4) was starved for 3 days post-hatching before being fed with feed A. Chickens were given free access to water and feed. At 0, 3 and 7 days of age, chickens in all the groups were weighed. Five chickens from each group were randomly sampled for slaughtering to obtain liver samples at 7 days of age.

RNA isolation and cDNA synthesis: Total RNA isolation was directly performed after liver sampling using a commercial kit (Total RNA Mini Kit (Tissue), Geneaid, South Korea). Total RNA concentrations were measured by Qubit 2.0 Fluorometer (Invitrogen, USA). Total cDNA was produced using oligo (dT) primers by reverse transcription of 1 μL total RNA using a cDNA synthesis kit (Revert aid, Thermo Fisher Scientific, USA). Total RNA was subjected to DNase treatment before being reverse transcribed (DNase I, RNase-free Thermo Fisher Scientific). Total cDNA was subjected to quantification by real-time PCR using a commercial qPCR kit (Kapa Sybr Fast qPCR kits, USA) on CFX 96 thermal cycler (Bio-Rad Laboratories, USA). The IGF-I cDNA was amplified by specific primers (Forward: 5-TGG CCT GTG TTT GCT TAC CTT-3 and Reverse: 5-TAC GAA CTG AAG AGC ATC AAC CA-3) and β-actin (Forward: 5-CCC CAT GCC ATC CTC CGT CTG-3 and Reverse: 5-CCT CGG GGC ACC TGA ACC TCTC-3)[8].

All specific primers were obtained from IDT (Singapore). The cycling procedure comprised a denaturation step (95°C for 3 min) and 40 cycles of amplification (denaturation at 95°C for 3 sec and annealing-extension at 60°C for 30 sec). The melting temperature was 75°C for IGF-1 primer and 84°C for β-actin primer. Each sample was run in triplicates. The gene expression level was estimated by comparing the Ct value of each group with that of the starved group (Ct)[9].

Data analysis: Body weights and IGF-1 expression variance in each group because of the feed type were analyzed by one way ANOVA ($p<0.05$), followed by Tukey's test. Correlation between body weights and IGF-1 expression level was evaluated by Pearson's correlation test[10]. All data obtained were analyzed using SPSS 13[11].

RESULTS

Effects of ME and CP on total weight: The feed content was derived using the proximate test and calorimeter bomb (Table 1). Proximate test results did not reveal any difference in feed quality between the formulated and commercial feeds.

The effect of varying CP and ME levels on the total weight of Indonesian hybrid chicken in each group is shown in Table 2. The weight gain of G-1 and G-2 was higher than that of G-3 and G-4 at the 3rd and 7th days ($p<0.05$). Feed intakes during 7 days in each feed group were not statistically different ($p<0.05$).

Effects of feed type on IGF-1 expression level: The IGF-1 expression level in the 7 days old hybrid chicken is shown in Fig. 1. The graph exhibits the highest gene expression level in G-1, followed by G-2 and G-3. The gene expression level in G-3 was higher than that in G-4, although both groups showed similar total weight.

Correlation between total weight and IGF-1 expression level: Correlation between gene expression and total weight of 7 days old chick after being subjected to Pearson's correlation test showed that the total weight of the Indonesian hybrid chicken was highly correlated with IGF-1 expression level (Fig. 2) ($R^2 = 0.8$). The heavier chicken showed a higher IGF-1 expression level ($p<0.05$).

DISCUSSION

These results indicate that suitable ME and CP levels for optimum growth of the Indonesian hybrid chicken was 3100 kcal kg^{-1} and 22% (protein:energy ratio, 141). Energy requirement of the hybrid chicken did not match the recommendation for the Indonesian local chicken[6], which was 2700 kcal kg^{-1} or that of the Layer chicken, which was 2900 kcal kg^{-1} [12]. The difference in energy requirements occurred owing to higher metabolism of the hybrid chicken than that of the local pure breed chicken (Kampong chicken); the hybrid chicken needed more energy because of its higher growth rate[13]. Energy and protein requirement of the hybrid chicken were similar to those of the Super chicken (offspring of male Bangkok chicken and female Layer chicken) at 7 days of age[14]. This similarity was observed because of the same female parent, but the hybrid chicken from the Pelung sire seemed to weight more than the hybrid chicken from the Bangkok sire[14]. Disparity in growth performance between the fed group and G-4 indicated

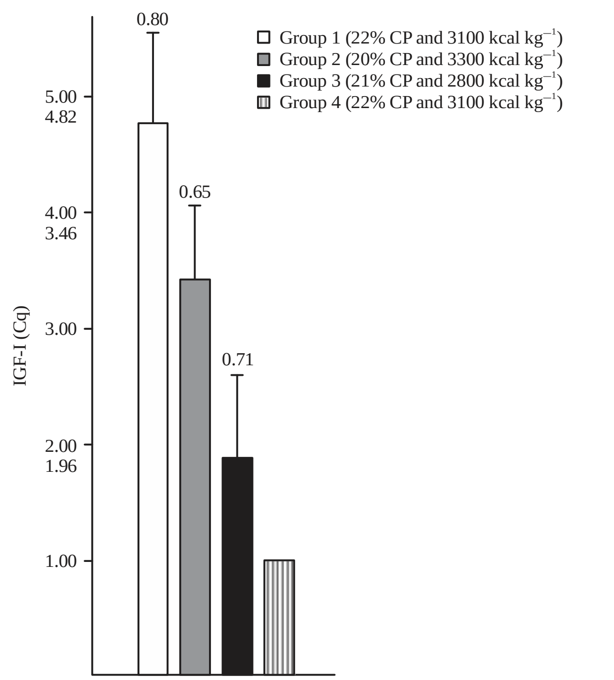

Fig. 1: Relative IGF-1 expression level in the liver of the 7 days old Indonesian hybrid chicken after feeding (n = 5) and administration of normalized β-actin compared with that in the liver of the starved chicken. Standard deviation is mentioned on each bar

Table 1: Proximate test on feed formula

Parameters	ME (kcal kg^{-1})	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Dry matter (%)	Nitrogen free extract (%)	Protein:Energy
Feed A	3100	21.84	5.48	3.81	5.60	94.42	63.26	147
Feed B	3300	20.19	7.66	2.51	6.68	89.41	62.95	165
Feed C	2800	21.13	4.08	3.25	16.95	88.10	54.59	133

Table 2: Total weight of the Indonesian hybrid chicken in each feed group

Parameters	G-1 (Feed A)	G-2 (Feed B)	G-3 (Feed C)	G-4 (Feed A)+starved
Post-hatch weight (g)	32.0±0.95	33.6±0.6	32.8±0.73	32.6±0.60
3 days weight (g)	35.2±0.80	34.8±0.8	31.8±0.73^{b}	28.0±0.44^{a}
7 days weight (g)	54.0±0.54	53.2±2.0	40.0±1.00^{b}	40.0±0.70^{b}

^{a}Significant compared with groups 1, 2 and 3 ($p<0.05$), ^{b}Significant compared with groups 1 and 2 ($p<0.05$)

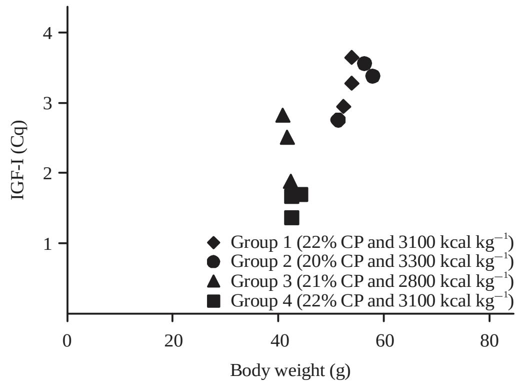

Fig. 2: Correlation between the total weight of the 7 days-old Indonesian hybrid chicken and fold change in IGF-1 expression level

that growth hormone sensitivity to nutrition was high in the hybrid chicken i.e., the growth hormone will increase the weight of the hybrid chicken with increased dietary intake. Similar results were reported for broiler chickens[7], but a greater contrast was observed between the fed and starved groups with respect to weight gain. Starved broiler chickens at 5-7 days of age could catch up performance at harvest. However, this did not occur in the Indonesian hybrid chicken at first 3 days post-hatching. The ability of the hybrid chicken to compensate for the weight loss after starving was not well supported because they did not have as responsive an endocrinal system as the broiler chicken[15].

The effect of dietary energy on the performance of growing birds depends on the capacity of the bird to alter feed intake for meeting changing demands with respect to their calories[16]. Unequal capacity in each strain was because the broiler chicken was formed after long genetic selection, whereas, the Indonesian hybrid chicken was an F_1 resulting from the Pelung and layer chicken. The Indonesian hybrid chicken was suggested to be more heterozygous than the broiler chicken.

Dietary proteins and energy content influence the IGF-1 mRNA abundance in rat liver[17]. The nitrogen source from the digested protein in the feed is required for new protein synthesis[2]. The nitrogen source and amino acid trigger GH-IGF-1 system activation via neurotransmitters[18,19]. Dopamine and other neurotransmitter influence GHRH secretion in the hypothalamus during the absence of somatostatin (somatotropin-releasing inhibitory factor)[19]. Furthermore, circulating GH in the liver induces IGF-1 expression[18]. High protein in the feed increases IGF-1 mRNA levels. The fold change value of G-1 suggested that feed A dietary energy accommodated growth that allowed high expression of IGF-1 in the liver. The G-2 that shows similar weight gain at 7 days of age exhibits lower fold change of IGF-1. Therefore, the fold change in G-3 was the lowest, indicating that this CP and ME combination did not accommodate tissue development. Compared with feeds A and B, feed C did not have enough energy for sufficient body metabolism. Low ME in Isonitrogenous diet treatment resulted in low growth performance in broiler chickens[12]. Feed C had a high protein level but did not improve weight gain of the chicken likely owing to the lower ME. Sufficient energy derived from glucose, fatty acid and other energy sources drives the metabolism of the whole body.

The growth rate of G-4 was higher than that of G-3, but the gene expression level of G-4 was lower than that of G-3 because the re-feeding program 3 days post-hatching in G-4 prompted a very low gene expression even when the best feed was administrated. Low gene expression indicated that optimum gene expression was not observed if administration of the feed was delayed. Weight gain between G-3 and G-4 was not statistically different because of the involvement of extra-hepatic IGF-1 mRNA expression in the target tissue. In contrast, after 3 h of re-feeding, IGF-1 mRNA increased to 3-fold of that before starving in broiler chickens but decreased after 7 h nearly to that before starving. Feed containing adequate protein drives the processes of the whole body in rats[17].

Dietary energy and IGF-1expression level were highly correlated with total weight in 7 days-old chicks ($R^2 = 0.8$). We suggest using 22% CP and 3100 kcal kg^{-1} ME feed for the Indonesian hybrid chicken in the starter phase.

SIGNIFICANCE STATEMENTS

New hybrid chickens, obtained by crossing female layer with male Pelung chicken (the Indonesian native chicken), were evaluated to be positioned as meat-type in Indonesia. Here we attempted to develop a starter-feed formula that fulfilled the hybrid chicken growth requirement.

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