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Research Article
Histological Analysis of Breast Muscle Growth in Young Hybrid Chickens in Response to Different Dietary Energy-Protein Ratios

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Abstract

Objective: The aim of this study was to analyze the increase in body weight and growth of breast muscle in hybrid chickens in response to varied dietary energy-protein ratios. **Materials and Methods:** Four different starter diet formulae were given to one-day-old hybrid chickens (DOCs) for 14 days. One hundred twenty one-day-old hybrid chickens were randomly allotted into four diet groups with 3 replicate pens consisting of 10 DOCs each. The first group was given starter diet I containing 25.1% crude protein (CP) and 3500 kcal kg^{-1} metabolizable energy (ME), the second group was given starter diet II (22% CP; 3200 ME), the third group was given starter diet III (22.7% CP; 3400 ME) and the fourth group was fed starter diet IV (18% CP; 3200 ME) during a 14-day observation period. Body weight was assessed in chickens post hatching and at 3, 7 and 14 days of age. Muscle weight and size and the cross-sectional area of the myofibers were assessed at 7 and 14 days of age. **Results:** Body weight, muscle weight and area and myofiber area of the hybrid chickens fed starter diets III and IV were significantly lower than those of chickens fed diets I and II. **Conclusion:** A hybrid chicken starter diet with an ME-CP ratio of approximately 139-145 is optimal for maximizing body weight and breast muscle growth.

Key words: Breast muscle, crude protein, energy-protein ratio, hybrid chickens, metabolizable energy

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

INTRODUCTION

Indonesia has the fourth highest population in the world and its people have a strong preference for chicken-based dishes. Recently, local breeds that have inadequate adult body weight and productivity have been crossbred with commercial chicken breeds. The hybrid chickens produced from crosses with the Bangkok and Layer breeds are commonly called *Jawa Super* chickens and are a profitable strain in Indonesian chicken breeding[1]; these hybrids are morphologically similar to local chickens but have a higher growth rate. Hybrid chickens can reach 1,000 g by three months of age when reared under conditions that comply with the standards for the welfare of broiler chickens.

Chicken breed quality and feed efficiency are two of the main factors that influence the success of the poultry industry. Good feed efficiency is achieved by quality feed stuff formulation, resulting in increases in chicken body weight with minimum feed intake[2]. In the chicken industry, there are three types of diet according to the growth period: starter, grower and finisher. The main differences between these diet types are the crude protein and energy levels. Formulated crude protein content, which has an important role in chicken growth performance and muscle development, is essential.

Dietary protein and energy are essential for optimal chicken growth, particularly in the starter diet for day-old chickens (DOCs). In DOCs, excess metabolic energy and protein are provided to stimulate growth, whereas in adults, metabolizable energy is mainly used for metabolism and protein content, providing amino acids as the source of protein regeneration. The utilization level of these components during the starter period determines the success of the next step of growth. Dietary protein and energy for optimal growth are different in temperate and tropical zones due to body thermoregulation, whereas in cold areas, broilers require additional energy to compensate for heat loss at low temperatures[3]. The energy-protein ratios in the starter diet for Broilers crossbred with Kampung in Asia are reported to be 142 and 119[4]. Increasing the crude protein (CP) percentage in feed, which contributes to energy production, is very costly; therefore, appropriately formulated nitrogen content becomes crucial[2,5].

Previous studies in *Kampung Super* (Pelung crossed with Layer) and *Jawa Super* (Bangkok crossed with Layer) showed a positive correlation between feed quality and muscle performance[1,2]. Muscle development in commercial poultry is paramount, as it is the most edible part of chicken. Breast muscle (BM), or pectoral muscularis, is the largest muscle in chickens. In view of the size of this muscle, BM is commonly used in the poultry industry as the developmental parameter and poultry is slaughtered when the BM has reached a certain size and weight. The original function of the BM is to support wing locomotion and it contributes to 10% of the total muscle mass[6]. After genetic selection, however, the BM contributes to up to half of the total muscle mass[7].

Avian muscle develops in the embryonic stage and enlarges at the early post hatching stage[8,9]. During the embryonic phase, the paraxial mesoderm generates somites, which further develop into skeletal muscles[9]. Skeletal muscles start to develop and become mature because of myoblast proliferation before the chicken hatches[10] but myofiber numbers continue to increase in the early post hatching stage[11]. BM requires adequate protein and energy levels to develop during the growing period[5,12,13]. At the early post hatching stage, muscle hypertrophy results from the accumulation of protein and nuclei that originate from the proliferation and fusion of satellite cells[14]. The aim of the present research was to reveal the most appropriate feed content to achieve optimum weight gain and BM growth of hybrid chickens.

MATERIALS AND METHODS

Chicken feeding: Chickens were raised using a semi-intensive housing procedure and under standard management conditions. All chickens were given *ad libitum* access to food and water.

During the 14-day observation period, the treatment groups were fed four different formulae. Diet I contained 25.1% crude protein (CP) and 3500 kcal kg^{-1} metabolizable energy (ME), diet II contained 22% CP and 3200 ME, diet III contained 22.7% CP and 3400 ME and diet IV contained 18% CP and 3200 ME (Table 1).

Table 1: Nutritional value of diets I, II, III and IV derived from the proximate test

Code	Crude protein (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Water (%)	Calcium (%)	Phosphorus (%)	ME (kcal kg^{-1})	Ratio of energy: crude protein
Diet I	25.1	5	6.1	5	9.2	0.9	0.7	3500	139.44
Diet II	22	5.3	6.2	5	10.2	0.9	0.7	3200	145.45
Diet III	22.7	4.8	6.7	5	9.5	0.9	0.7	3400	149.78
Diet IV	18	5	6.5	6	10.5	0.9	0.7	3200	177.78

ME: Metabolizable energy

Ethical approval: The experimental design used in this research was approved by the Gadjah Mada University Animal Ethical Committee (Integrated Research and Testing Laboratory; Laboratorium Penelitian dan Pengujian Terpadu; LPPT) with certificate number 00052/04/LPPT/IV/2017.

Experimental design and sample preparation: One hundred twenty one-day-old hybrid chickens fed four different feed formulae were randomly distributed into four groups. Each treatment was replicated 3 times with 10 DOCs in each replicate pen. Group 1 was given starter diet I, group 2 was given diet II, group 3 was given diet III and group 4 was given diet IV for 14 days post hatching. All chickens were weighed at 3, 7 and 14 days of age. Five chickens from each group were slaughtered and the breast muscle was detached; the sinistral section was used to assess the BM weight and the dextral part was used to measure the muscle area (Fig. 1).

Myofiber area measurement: The dextral BM was sliced into 3x3-mm pieces and fixed in Bouin's solution. The muscle samples were dehydrated using a graded alcohol series before being cleared using toluol. The samples were then infiltrated with paraffin and embedded in paraffin blocks. The paraffin-embedded BM samples were cut into 6-µm-thick sections using a rotary microtome and then fixed on microscope glass, where they were deparaffinized and rehydrated using xylol. A second set of BM samples was hydrated using a graded alcohol series, immersed in Ehrlich hematoxylin and then rinsed in running water. The samples were dehydrated to 70% alcohol before being immersed in Eosin Y and then further dehydrated in 96% alcohol. Excess alcohol was removed using filter paper and the samples were immersed in xylol and preserved on glass slides[15]. The cross-sectional area of myofibers was measured using ImageJ micrometer software. The myofiber area from the pectoralis muscle was calculated by choosing five views of the fasciculus in the histological slides. Ten myofibers were chosen from each fasciculus to measure the area of myofibers using ImageJ software.

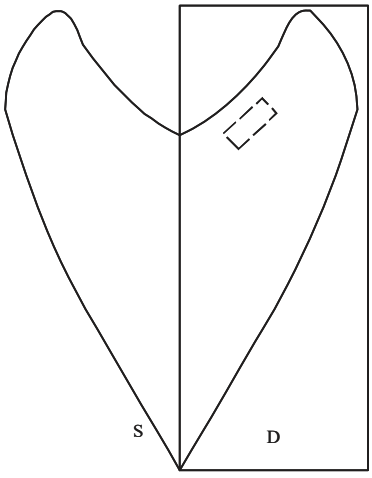

Fig. 1: Representation of chicken breast muscle
The detached sinistral (S) part was used to measure muscle weight and the dextral (D) part was used to estimate breast area. Tissue in the rectangles defined by solid and dashed lines was used to measure muscle area and myofiber area, respectively.

Statistical analysis: Weight data and breast muscle performance were analyzed using one-way ANOVA followed by Duncan's test at the 5% confidence level.

RESULTS AND DISCUSSION

Numerous factors can influence muscle development and thus contribute to the final quality of the meat in chickens reared for human consumption. Prolonged genetic selection has yielded highly muscular Broiler and Layer chickens. However, selection also alters the endocrine system, which thus requires specific nutritional intake[11,16]. Similarly, hybrid chickens have different nutritional requirements from their parents. The current study shows that the energy-protein ratio in feed is an effective measure of post hatching muscle growth. Thus, the feed was formulated specifically for the new hybrid chickens.

Feed nutrition value: All starter diet formulae were given to the chickens from post hatching to 14 days of age in crumble form. Before the treatment, a proximate test was performed at the Center for Veterinary Services (Balai Besar Veteriner), Ministry of Agriculture (ISO 9001:2008), Wates District, Indonesia. Table 1 shows the ratios of ME to CP for diets I, II, III and IV, which were 139, 145, 150 and 178, respectively.

Energy-protein ratio effect on chicken body weight: DOCs are important experimental subjects for feed analysis, since their endocrine systems are sensitive to nutrition alteration. Chickens, especially those subjected to prolonged genetic selection, possess effective nutrient digestion. Moreover, broiler growth yield is closely related to dietary nutrition quality and quantity, especially CP and ME, since both items are essential to most bodily metabolic processes. As a result of protein breakdown, amino acids are absorbed, which is most effective at an early age due to the high levels of trypsin and

Table 2: Hybrid chicken body weight (g) at post hatching and 3, 7 and 14 days with administration of diet I, II, III and IV

	Treatments				
Variables	Age	Diet I	Diet II	Diet III	Diet IV
Body weight (g)	Post hatching	34.50±3.37^{ns}	32.70±3.30^{ns}	34.50±3.74^{ns}	34.20±3.32^{ns}
	day 3	49.30±5.67^{ns}	47.90±3.17^{ns}	45.70±4.37^{ns}	44.50±3.77^{ns}
	day 7	75.90±6.29^{b}	75.80±4.75^{b}	69.20±8.65^{ab}	62.20±5.84^{a}
	day 14	132.80±11.60^{bc}	142.90±9.78^{c}	118.50±16.20^{ab}	102.90±14.61^{a}
Feed intake (g day^{-1})		11.63±1.09	14.34±0.60	13.22±1.18	13.89±3.59
Weight gain (g day^{-1})		6.57±1.6	7.21±2.27	5.55±1.68	4.56±1.2
FCR		1.82±0.3	2.11±0.6	2.59±1.07	3.05±0.03

$^{a-c}$Values within rows without a common superscript are significantly different ($p \leq 0.05$), ^{ns}Not significant; values are Means±SE

Table 3: Growth yield of measured breast muscle of hybrid chickens at 7 and 14 days of age

Day	Variables	Diet I	Diet II	Diet III	Diet IV
	Muscle[1] weight (g)	2.53±0.42^{bc}	3.03±0.47^{c}	2.19±0.55^{b}	1.21±0.21^{a}
7	Muscle[1] area (cm^2)	8.65±0.27^{ab}	10.87±1.6^{b}	8.87±1.56^{ab}	6.38±1.38^{a}
	Myofiber CSA[2] (μm^2)	1.69±0.27^{a}	3.24±1.48^{b}	1.47±0.48^{a}	1.21±0.30^{a}
	Muscle[1] weight (g)	5.29±0.30^{c}	5.49±0.46^{c}	4.36±0.60^{b}	2.60±0.46^{a}
14	Muscle[1] area (cm^2)	16.43±0.1^{c}	15.65±0.2^{bc}	13.97±0.0^{b}	10.77±0.0^{a}
	Myofiber CSA[2] (μm^2)	2.32±0.17^{c}	3.48±0.28^{d}	1.62±0.56^{b}	1.21±0.53^{a}

$^{a-d}$Values within rows without a common superscript are significantly different ($p \leq 0.05$), Values are means±SE, [1]Muscle: Pectoralis muscularis, [2]CSA: Cross-sectional area

other protease enzymes. Enzyme secretion decreases in older birds[17,18]. After detecting significantly different growth yields among the groups on days 7 and 14, chickens were immediately slaughtered to confirm muscle growth at the histological level.

Starter diets with variable CP and ME contents were given to four groups of one-day-old hybrid chickens. Hybrid chickens (Table 2) fed diets I and II were heavier than those fed diets III and IV over the 14 days of observation. This result is similar to the findings of other studies on feed research that show a positive correlation between CP content elevation and growth rate in chickens[3-5]. Twenty-five percent CP and 3500 kcal kg^{-1} ME in diet I (ratio of 139) provided chickens with more than the growth nutritional requirement, since the growth yield from diet I was similar to that obtained from 22% CP and 3200 of ME in diet II (ratio of 145). In fact, diets II and III, containing similar ME-CP ratios, generated significantly different growth yields, indicating specific dietary requirements of hybrid chickens. The average body weight of chickens fed diets III and IV was lower than the average for the diet I and diet II groups (Table 2). The high ME-CP ratios in diets III (150) and IV (178) were not suitable for hybrid chickens because they were ineffective for chicken growth. The consequences of an excessive energy-protein ratio were low efficiency and diminishing nutrition values because of the limited absorption ability of the gut.

In tropical Southeast Asia, the optimal dietary ME-CP ratio for Broiler chickens was previously reported as 142, while the optimal ratio for Kampung chickens was 119[3]. Climate (wind, temperature and humidity) influenced nutrition absorption and chicken growth by inducing the birds to strive for stable body homeostasis. Heat loss or production to maintain body temperature consumed energy that was intended for growth.

Performance of breast muscle: After the feed trial, five chickens from each group were slaughtered following the recommendations of the ethical committee and their breast muscles were detached to measure muscle weight, muscle area and myofiber area. The growth yield detected in each muscle sample was used to confirm nutrient utilization.

The myofiber area at 14 days was higher in the first two groups than in the latter two groups (Table 3 and Fig. 2). Diets I (2.32±0.17 μm^2) and II (3.48±0.28 μm^2) were sufficient for muscle growth performance, while diets III (1.62±0.56 μm^2) and IV (1.21±0.53 μm^2) had inadequate protein and ME content. Breast muscle is the most valuable part of the chicken carcass as a consequence of its massive size[11,16,19]. Muscle tissue enlargement comes from myofiber elongation and dilatation until 15 weeks of age[20]. Thus, appropriate nutrition is paramount during the proliferation of satellite cells and their merging into myofiber units, as these processes require a considerable amount of energy and amino acid building blocks[21].

In this study, myofiber dilatation and elongation contributed to muscle weight gain from 7-14 days in all groups, particularly in chickens fed diet II. From these results,

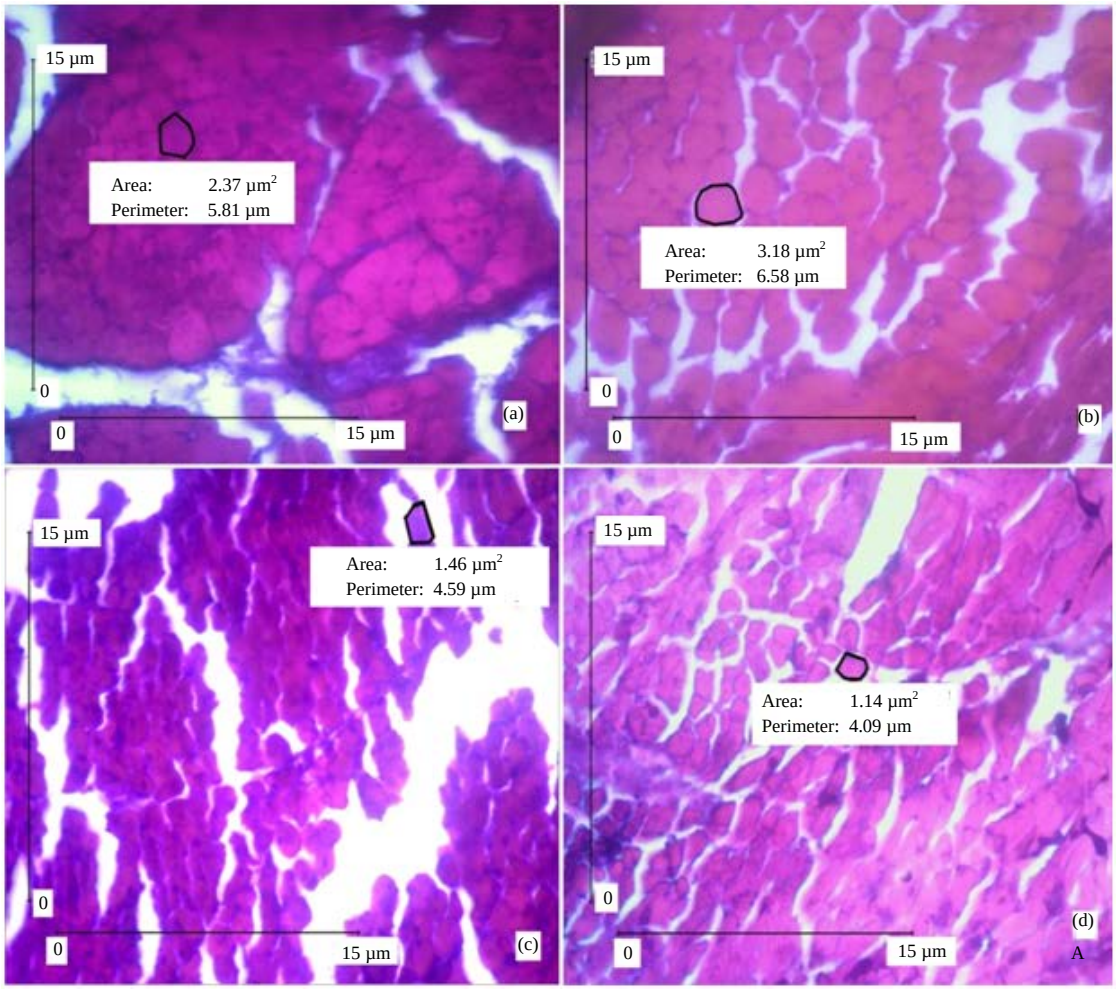

Fig. 2(a-d): Histological microscopy of hybrid chicken BM from each group. Selected myofibers are marked with a black border. (a) Diet I group, (b) Diet II group, (c) Diet III group, (d) Diet IV group

the development of the myofiber appears to be important for muscle growth and affected by nutrition. In chickens, BM is composed of type IIB myofibers alone[10,21]. Type IIB (fast-twitch glycolytic) myofibers are the most sensitive to nutritional alteration.

Naturally, myofibers vary in diameter and length, ranging from less than a hundred microns to a few hundred microns and from a few millimeters to a few centimeters, depending on age, strain and nutritional status[22]. The present research showed differentiation among groups of the same age. Specifically, the energy-protein ratio contributed to myofiber diameter, since one myofiber unit is constructed from a single multinucleated muscle cell. Adequate metabolic fuel generated from Adenosine triphosphate (ATP) and protein building blocks from the protein content of feed increase myofiber protein accumulation. Since myofibers lack the ability to divide, muscle repair and maintenance depend on satellite cells and vascularization, which require a substantial amount of energy[22,23].

CONCLUSION

In hybrid chickens post hatching, early feeding with an energy-protein ratio of approximately 139-145 was important for body mass gain and breast muscle growth.

SIGNIFICANCE STATEMENT

This study assessed the most appropriate feed energy-protein ratios that would be beneficial for growth of a new Indonesian chicken line. The results also reveal a prospective mating scheme for the mass production of light meat-type chicken lines.

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