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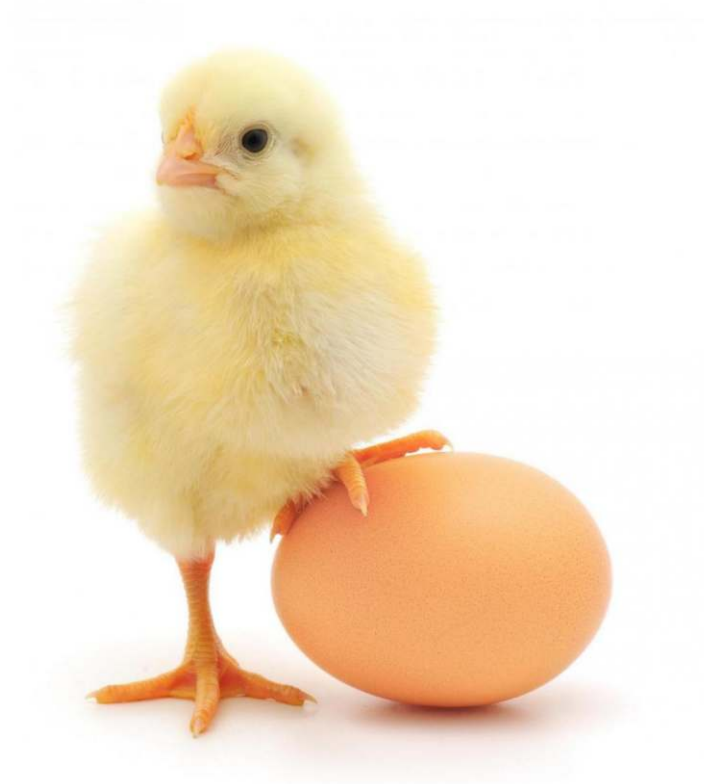

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Research Article
Effects of Oral Administration of Encapsulated-Leucine on Amino Acid and Plasma Metabolite Profiles in Broiler Chicks During the Starter Phase

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Abstract
Background and Objective: An abundant studies show that leucine (Leu) acts as an anabolic agent that stimulates skeletal muscle growth in human as well as in animals. However, the effect of encapsulated leucine (CL) supplementation on growth performance of broiler chickens has not been evaluated. Therefore, the aim of this study was to determine initial metabolic responses to CL supplementation on food intake, plasma metabolites and branched chain amino acid concentration in 7-day-old broiler chicks. **Materials and Methods:** A total of 24 chicks were randomly assigned to the following treatments: (1) Control 4 h (C), (2) Free leucine 4h (L) and (3) Encapsulated-leucine 4h (CL). After 6 h of fasting, chicks were given a bolus of oral injection of distilled water, free leucine (6 mmol/10 mL kg^{-1} BW) or encapsulated-leucine (6 mmol/10 mL kg^{-1} BW). Immediately after the injection, chicks were allowed free access to a commercial starter diet for 1 h. Blood collections were obtained 4 h after the oral injection. Food intake, total glucose, total cholesterol, triacylglycerol, plasma leucine levels and the activity of glutamic oxaloacetic transaminase (GOT) were measured. **Results:** Food intake, glucose, total cholesterol and triacylglycerol levels were not affected by Leu supplementation. At 4 h, GOT levels were greater ($p<0.05$) in the CL group than that of the Leu groups. At 4 h, although plasma Leu levels were similar in all groups, Valine levels were lower ($p<0.005$) in the C and CL groups than that of the Leu group and isoleucine levels were lower ($p<0.001$) in the CL group than that of the C and Leu groups. **Conclusion:** The results of this study suggest that an oral administration of CL caused prolonged leucine-induced anabolic effects that may be beneficial for growth. Our observations also pave the way for studying the long-term effects of CL supplementation on performance of broiler chicks.

Key words: Broiler chicks, leucine, encapsulated-leucine, food intake, glucose, total cholesterol, triacylglycerol, glutamic oxaloacetic transaminase

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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

Feeding is one of the regulators of whole-body protein deposition in growing animals[1]. Postprandial events such as the rise in anabolic hormones (insulin) and growth factors, along with sufficient substrates such as amino acids, contribute to major physiological processes, including protein turnover (protein synthesis and degradation), resulting in a net whole-body protein deposition[2]. Studies show that some amino acids-for example, leucine (Leu) not only serves as substrates for protein synthesis but also can act as an anabolic agent[3]. Similar to insulin, Leu can stimulate skeletal muscle protein synthesis via the mTORC1-dependent activation of signaling components[4]. For this reason, leucine supplementation has been widely studied to improve protein deposition in various species, including broiler chickens[5].

It was articulated that total Leu content in diets was a primary driver for muscle anabolic responses[6]. Therefore, it was a common practice to supplement the diets by adding free Leu in the normal or restricted diets[7]. Using this methodology, Leu has been proven to be an effective supplement that improves growth performance in broiler chickens[5]. Nonetheless, a study demonstrated that when Leu was administrated 2 h after feeding, higher levels of plasma Leu can be maintained, resulting in greater muscle protein synthesis in rats[8]. This study suggests the possibility that extending the anabolic action of Leu throughout the day can result in greater muscle protein deposition in broiler chickens.

Encapsulation technology has been used for decades to improve milk production[9]. In dairy cows, where methionine and lysine are the limiting essential amino acids, encapsulation of these amino acid can protect them from ruminal degradation, allowing these AA to be utilized for milk production[9]. In broiler chickens, encapsulation can be used to facilitate time-release absorption of amino acids along the gastro intestinal tract[10]. Since free or crystalline amino acids are rapidly absorbed in the small intestines[11], this technology will allow the slow release of amino acids in the plasma and maintain desirable levels for the duration of the time[10]. This approach is particularly important to maintain high concentration of plasma Leu for a particular duration that may prolong the anabolic effect of Leu on muscle protein deposition.

Improving growth performance that can reduce overall cost of broiler chicken production is a pivotal issue in the poultry industry. Leu supplementation is one of dietary approaches that can potentially improve broiler chicken production. To the best of our knowledge, supplementation of encapsulated-leu (CL) in the diet of broiler chickens is a new and novel concept in feeding practices. In this early-stage approach, we aim to evaluate the effect of a bolus supplementation of encapsulated-leu on food intake, plasma metabolites and branch chain amino acid (BCAA) profiles of 7-day old broiler chicks.

MATERIALS AND METHODS

Animals, housing and diets: A total of 24 one-day-old broiler chicks (*Gallus gallus domesticus*) were purchased from a local hatchery (CharoenPokphand Jaya Farm Ltd, Pekanbaru, Indonesia) and housed in a wooden cage ($50\times35\times33$ cm) in a group at a constant temperature of 30 ± 1 with continuous lighting. Feed (metabolizable energy: >3.050 kcal kg^{-1}, protein: >23.5%: commercial starter diet, 311-VIVO, Pokphand Tbk, Medan, Indonesia) and water were provided *ad libitum*. One day before the experiment, 24 chicks (6 days old) were weighed and distributed into six cages ($20\times25\times25$ cm) with four chicks in each cage. Chicks were assigned to 3 treatment groups on the basis of their body weight in order to produce uniform groups. Both of free-leucine- and free-coated leucine were a gift from King Techina Group, China.

Administration of Leu- or CL: Following an acclimatization period with individual rearing for 24 h, chicks were randomly selected and divided into three groups, each group consisting of 8 chicks. The birds were provided with *ad libitum* access to the diet during the whole experimental period. On the day of the experiment, each chick (7 days old) was orally administered either a solution of Leu or CL for the treatment groups, or distilled water (C) for the control group, via an elastic plastic needle on a small syringe. Chicks received oral administration at a dose of 6 mmol kg^{-1} body weight of Leu- or CL for 240 min. The criterion of dose selection was based the findings of our previous report[12].

Measurement of feed intake: Food intake (at 240 min) was determined by subtracting the amount of feed consumed from a pre-weighed feeder.

Blood collection and analysis of amino acid concentration and plasma metabolites: At 240 min after administration of Leu or CL, all birds in each group (n 8 per group) were sacrificed and their bloods were collected for analysis of amino acid and plasma metabolites. Blood was collected through jugular vein into heparinize deppendorf tubes and centrifuged at 5, 000×g for 15 min. Plasma was stored at -20°C until assay; triacylglycerol (TG), glucose (GLU), total cholesterol (TCHO) and total glutamic oxaloacetic transaminase (GOT) in the plasma were measured with Microlab 300 (Vital Scientific, Netherland) as per the manufacturer's instructions.

Analysis of amino acids: Plasma amino acids concentrations were determined with HPLC (ICI instrument/SHIMADZU SCL-10A / SHIMADZU CBM 20A)

Statistical analysis: For all parameters, a one-way analysis of variance (ANOVA) was applied. The Tukey test was done as a posthoc test. Differences of $p<0.05$ were considered statistically significant. Values were presented as means±SEM. Statistical analysis was carried out using the commercially available package Stat View (Version 5, SAS Institute, Cary, USA,[13]). All data in each group were first subjected to a Thompson rejection test to eliminate outliers ($p<0.05$) and the remaining data were used for the analysis among groups.

RESULTS

Food intake and plasma metabolites: Oral administration of both Leu and CL did not significantly alter food intake (data not shown). As shown in Table 1, at 4 h plasma levels of Glu and TCHO were not affected by the administration of Leu or CL. However, the activity of GOT was significantly higher [$F(2,17) = 5.80$, $p<0.05$)] in the CL group than that of the Leu groups.

BCAA concentration: Four hours after oral Leu administration, we determined the plasma BCAA concentrations (Fig. 1). The plasma Leu levels were similar in all groups, indicating that they have reached the basal levels of this amino acid. On the other hand, valine levels were significantly lower in the CL and C groups [$F(2,15) = 10.62$ ($p<0.005$)] than that of the Leu group. In a slightly different trend, isoleucine levels were significantly lower in the CL and C group [$F(2,16) = 12.33$ ($p<0.001$)] than that of the Leu groups.

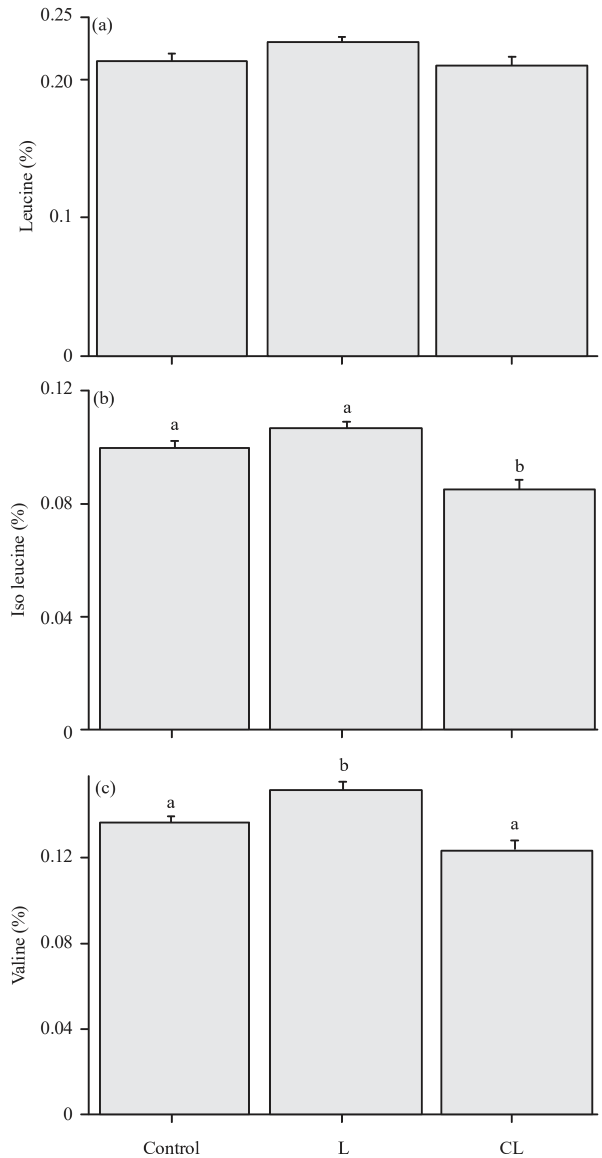

Fig. 1(a-c): Effects of encapsulated-leucine or leucine on leucine, valine and iso leucine concentration in chicks

The number of chicks used in each group ranged between 5-8. Values are Means±SEM.

Table 1: Effect of orally L or CL administration on plasma metabolites in chicks for 240 min of the experimental period

	6 mmol per kg body weigh		
	Control 4 h	Leucine 4h	Coated Leu 4 h
Glucose (mg dL^{-1})	229±16	209±8	206±10
Total cholesterol (mg dL^{-1})	356±31	258±44	297±32
GOT (g dL^{-1})	308±7[ab]	277±11[b]	335±16[a]
Triacylglycerol (mg dL^{-1})	292±37[a]	241±21[ab]	166±20[b]

Values are means±SEM. The number of samples used for analysis was 6-8

DISCUSSION

The result on feed intake obtained in this study was consistent with a previous study conducted by Erwan *et al*.[5] who revealed that there was no significant effect of Leu on feed intake when supplemented at level 0.5% in standard diet of broiler chickens. Furthermore, it was revealed that dietary Leu and peripheral injections of Leu had no effect on food intake in Djungarian hamster[14]. Furthermore, it was revealed that the 4% excess of Leu in diet contained 23% protein and 3,130 kcal ME kg^{-1} diet had no significant effect on food intake, body weight gain and gain/feed of broiler chicks[15]. Moreover, supplementation of 4% Leu in a diet containing 20% protein (CSBM) also had no deleterious effects on growth of pigs[16]. Penz *et al*.[17] reported that addition of 1.6% Leu to the diet without added niacin did not affect body weight gain but D'Mello and Lewis[18] revealed that addition of 2.9% Leu to the diet of broiler chicken decreased body weight gain[17]. The results of present study also suggest that oral administration of Leu seemed to have similar effects on food intake in pig, chicks and hamster[16-18]. Likewise, Williams *et al*.[19] demonstrated no significant differences in feed intake when diet of lactating cows was supplemented by encapsulated methionine.

In term of plasma metabolites, this finding is consistent with Ospina-Rojas *et al*.[20] who reported that dietary supplementation of Leu did not influence serum Glu in broiler chickens. Additionally, Koch *et al*.[14] revealed that dietary Leu and peripheral injections of leu had no effect on glucose in Djungarian hamster. Even though several studies revealed that BCAA influence the glucose metabolism[21,22]; however, serum glucose concentrations were not affected.

However, the activity of GOT was significantly higher [$F(2,17) = 5.80$ $p<0.05$)] in the CL group than that of the Leu groups. The activity of GOT, along with lactate dehydrogenase (LDH) and creatine phosphokinase (CK) are markers of tissues damage caused by chronic heat stress[23]. Furthermore, Imaeda[24] stated that increased serum LDH and GOT activities are associated with sudden death syndrome (SDS). Higher activity of GOT is also considered an important bioindicator of liver damage in broiler chickens[25]. To the best of our knowledge, Leu supplementation had no effect on the tissue damage, liver function and SDS. Furthermore, in this study, the levels of GOT activity were within normal range and comparable to the findings of Xie *et al*.[23]. Taken together, we anticipate that encapsulated-leucine supplementation will not have detrimental effects on overall chicken health. Plasma TG concentration significantly decreased by CL supplementation compared with the control group ($F(2,19) = 6.37$ $p<0.05$). Even though Leu did not affect TG compared to control but the decreasing trend was observed. The reduction in TG after dietary supplementation of Leu was reported by Ospina-Rojas *et al*.[20]. The reduction in TG by CL supplementation indicated that CL might have higher ability to activate hypothalamic POMC neurons by mTOR pathway.

The plasma Leu levels were similar in all groups, indicating that they have reached the basal levels of this amino acid. On the other hand, valine levels were significantly lower in the CL and C groups [$F(2,15) = 10.62$ ($p<0.005$)] than that of the Leu group. In a slightly different trend, isoleucine levels were significantly lower in the CL and C group [$F(2,16) = 12.33$ ($p<0.001$)] than that of the Leu groups. In this study, the reduced levels of valine and isoleucine could be due to an antagonistic action of Leu[26]. Thus, our results suggest that oral administration of encapsulated-leucine prolonged the leucine circulation compared to free Leu supplementation, as well as the control treatment. Consequently, higher levels of plasma Leu will stimulate anabolic signaling factors that influence metabolic outcome, including protein synthesis[27].

CONCLUSION

In conclusion, four hours after the bolus administration of encapsulated-leucine, there was no effect on food intake, Glu and TCHO levels. However, in the CL group, the activity of GOT was greater than that of the other group but the activity levels were in the normal range. Even though at 4 h the plasma Leu were similar in all groups, the antagonistic effect of leucine appeared due to the fact that both valine and isoleucine levels were significantly reduced by CL treatment. We speculate that long-term administration of CL will improve growth performance of broiler chicks during the starter period. Currently, this study is in progress.

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