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Effects of Amino Acid Supplementation to a Low-Protein Diet on the Growth Performance and Protein Metabolism-related Factors in Broiler Chicks

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A low-protein (LP) diet may alleviate the environmental impact of chicken meat production by reducing nitrogen excretion and ammonia emissions. Thus, this study investigated the effect of a 15% reduced protein diet with or without amino acid (AA) supplementation on the growth performance of broiler chicks from 10 to 35 days of age and the underlying mechanism for loss of skeletal muscle mass. Thirty-six male broiler chicks were allocated to three experimental groups based on body weight: control, LP, and essential AA-supplemented LP (LP+AA). The body weight gain, feed conversion ratio, and weight of breast muscles and legs significantly decreased only in the LP group at the end of the feeding period. Plasma uric acid levels were significantly lower in the LP+AA group than those of the other groups. In the LP group, mRNA levels of microtubule-associated protein 1 light chain 3 isoform B were significantly higher in the *pectoralis major*, whereas those of atrogin-1, muscle RING-finger protein-1, and myoblast determination protein 1 were significantly higher in the *biceps femoris* compared to those in the control group. There were no significant differences in insulin-like growth factor 1 mRNA levels in the liver or skeletal muscle between groups. These findings suggested that supplementation with essential AAs ameliorated the impaired effects of an LP diet on growth performance in broiler chicks, and that the transcriptional changes in proteolytic genes in skeletal muscles might be related to the impaired effects of the LP diet.

Key words: chicken, feed efficiency, insulin-like growth factor, low-protein diet, myogenesis, proteolysis

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Introduction

Using a low-protein (LP) diet may alleviate the environmental impact of chicken meat production by reducing nitrogen excretion and ammonia emissions[1,2]. Supplementation with synthetic free amino acids (AAs) ameliorates the low growth performance of broiler chicks fed an LP diet[3,4]. For example, Belloir et al. have reported that supplementation with AAs reduces the dietary crude protein (CP) content by at least 17% in growing-finishing male broilers without impairing growth performance or meat quality[5]. Thus, formulating chicken rations with lower CP levels and supplementing with the required AAs would enable environmentally friendly and economical poultry production. However, the mechanisms underlying the restorative effects of dietary AAs on the growth performance of LP diet-fed broiler chicks have not yet been clarified.

Skeletal muscle mass is regulated by the balance between protein synthesis and degradation[6,7], and is governed by anabolic hormones, such as insulin-like growth factor (IGF)-1, and catabolic hormones, such as glucocorticoids[8–10]. For example, IGF-1 treatment promotes hypertrophy of C2C12 myotubes[11] and proliferation of chick embryonic myoblasts[12]. IGF-1 treatment significantly decreases the expression of atrogin-1, a regulatory enzyme of the ubiquitin-proteasome system, and increases intracellular protein levels in chicken embryonic myotubes[13]. IGF-1 also significantly decreases mRNA levels of microtubule-associated protein 1 light chain 3 isoform B (LC3B), an autophagy-related protein, in chicken embryonic myotubes[14]. The synthetic glucocorticoid dexamethasone significantly upregulates atrogin-1 mRNA levels in the *pectoralis major* (PM) muscles of chicks when administered intraperitoneally, as well as in chick embryonic myotubes *in vitro*[15]. Moreover, an LP diet may in-

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duce alterations in adrenocortical function in chicks and increase plasma corticosterone concentrations[16]. However, AAs protect against or alleviate dexamethasone-induced skeletal muscle atrophy in mammals[17,18]. In addition to circulating hormones, locally expressed IGF-1 may be involved in protein metabolism in chicken skeletal muscles. For example, the PM of heavy broiler chicks expresses high mRNA levels of IGF-1 and pro-myogenic genes, and low mRNA levels of anti-myogenic genes[19]. We suggest that IGF-1 in thigh muscles contributes to protein synthesis via the protein kinase B (Akt)/ribosomal protein 6 (S6) pathway in chicks[20]. Dietary methionine supplementation increases IGF-1 protein and mRNA levels in breast muscle and the relative weight of whole breast muscle in broilers with low hatching weights[21]. Therefore, circulating hormones and locally expressed IGF-1 may be involved in the impaired effects of a LP diet and the ameliorative effect of AA supplementation to an LP diet on the growth performance of broiler chicks.

Insulin-like growth factor binding proteins (IGFBPs) are produced in the liver and skeletal muscles[22,23]. Hepatic IGFBPs are released in the bloodstream and function as carrier proteins of serum IGF-1 in mammals and chickens[23,24]. Supplementation with dietary valine and isoleucine significantly decreases IGFBP-1 mRNA levels in the liver of LP diet-fed pigs[25]. Jousse et al. have reported that AA limitation significantly induces IGFBP-1 expression in mammalian hepatoma cells[26]. Saxena et al. have found that an LP diet significantly increases IGFBP-2 mRNA levels in the liver of broiler chicks[27]. These findings suggest that dietary protein influences the function of IGF-1 by regulating hepatic and blood IGFBPs. Although the effect of dietary protein levels on IGFBP mRNA levels in skeletal muscle has not been investigated in chickens, mRNA levels of IGFBP-2 in breast muscle are increased by dietary leucine supplementation above recommended levels in 10-day-old broiler chicks[28]. In broiler chicks with high muscularity, IGFBP-2 mRNA levels are high in the PM[22]. Therefore, IGFBPs expressed in liver and skeletal muscles may be involved in the effects of AA supplementation on the growth performance of LP diet-fed broiler chickens.

In the present study, the effects of an LP diet and AA supplementation to the LP diet on various protein metabolism-related factors in the liver, skeletal muscle, and blood of broiler chicks were investigated. These findings suggested that transcriptional changes in proteolytic genes in skeletal muscles might be related to the adverse effects of an LP diet in broiler chicks.

Materials and Methods

Animals, experimental design, and diets

This study was approved by the Institutional Animal Care and Use Committee and performed according to the Kobe University Animal Experimentation Regulations (2021-11-02). One-day-old *Ross* 308 male broiler chicks were purchased from a local hatchery (Yamamoto Co., Ltd., Kyoto, Japan) and reared together using a commercial starter feed (22.0% CP and 3,050 kcal/kg metabolizable energy; Nichiwa Sangyo Co., Ltd., Kobe, Japan). Chicks were reared in battery cages with free access to feed and water under a 23 h/1 h light/dark cycle (23:00–24:00 dark), and the temperature was kept at 31 ± 2 °C during the first 7 days and then reduced gradually until reaching 25 ± 2 °C at 21 days of age. At 8 days of age, chicks were allocated based on body weight (BW) into three experimental groups of four replicates (12 chicks per group, 3 chicks per cage) and fed experimental diets from 10 days of age. Chicks in the control group received corn-soybean-based mash diets formulated to meet the nutrient recommendations of the breed[29] for the grower (10–24 days of age) and finisher (25–35 days of age) phases (Tables 1 and 2). AA content of each diet was calculated using data described in the Standard Tables of Feed Composition in Japan[30]. In a preliminary experiment, the detrimental effects of a 20% reduced protein diet were not ameliorated by AA supplementation. Therefore, a 15% reduced protein diet was used in this study. Either an isocaloric LP diet (LP, 85% CP) or LP supplemented with AAs (LP+AA) to meet only the essential AA (EAA) requirements were given in the other two groups. Feed intake (FI) of the replicates was measured every day and individual chick BW was measured every week.

Sampling and analysis of plasma biochemical parameters

At the end of the experiment and under carbon dioxide anesthesia, blood was collected from the carotid arteries of chicks and chicks were euthanized by decapitation. Plasma was separated immediately by centrifugation at 1,910 × g for 10 min at 4 °C and then stored at −80 °C until analysis. Plasma corticosterone and uric acid concentrations were measured using commercial kits (Corticosterone ELISA Kit, AssayPro LLC, MO, USA; Uric Acid C-Test, Code: 437-17301, Fujifilm Wako Pure Chemicals Co., Osaka, Japan). The weights of the breasts, legs, and livers were recorded. Then, the center pieces of the PM, BF, and liver were excised and immediately frozen in liquid nitrogen and stored at −80 °C for real-time polymerase chain reaction (PCR) analyses.

Real-time PCR

Real-time PCR analysis was performed as previously described[31–33]. Total RNA was extracted from tissues using Sepazol-RNA I Super G (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from total RNA using Rever Tra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co. Ltd, Osaka, Japan). mRNA levels were quantified for each primer using TB Green Premix Ex Taq II (Tli RNaseH Plus; Takara Bio Inc., Otsu, Japan) according to the supplier's recommendations based on a relative standard curve using the Thermo Scientific Piko Real Real-Time PCR System (Thermo Fisher Scientific Oy, Vantaa, Finland). Primer sequences for IGF-1, IGF1R[31], IGFBP-1–3[32], atrogin-1, muscle RING-finger protein-1 (MuRF-1)[33], myoblast determination protein 1 (MyoD), myogenin (MyoG), and ribosomal protein S17 (RPS17)[34] may be found in previous studies. The following primer sequences were used for LC3B (NM_001031461) and glucocorticoid receptor (GCR, NM_001037826): LC3B sense, 5'-TCC GAG ATC AGC ATC CAA CT-3′, LC3B antisense, 5'-CAC CAT GCT GTG

Table 1. **Ingredients and nutrient compositions of the experimental diets**

Ingredient (%)	10–24 days			25–35 days		
	Control	LP	LP+AA	Control	LP	LP+AA
Yellow corn, ground	55.01	65.19	66.14	58.92	68.54	69.35
Soybean meal (CP 45%)	33.7	25.05	22.65	28.82	20.7	18.55
Fish meal (60%)	3	3	3	3	3	3
Soybean oil	4	2.6	2.9	5	3.6	3.9
Calcium carbonate	0.85	0.85	0.85	0.85	0.85	0.85
Dicalcium Phosphate	1	1.1	1.1	1	1.1	1.1
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin and mineral mix[1]	1.91	1.91	1.91	1.91	1.91	1.91
DL-Methionine	0.23	-	0.35	0.2	-	0.32
L-Lysine, HCL	-	-	0.27	-	-	0.27
L-Threonine	-	-	0.13	-	-	0.1
L-Valine	-	-	0.11	-	-	0.09
L-Isoleucine	-	-	0.15	-	-	0.14
L-Arginine	-	-	0.14	-	-	0.12
Total	100	100	100	100	100	100
Calculated composition						
CP (% in diet)	21.5	18.3	18.3	19.5	16.6	16.6
CP (% of requirement[2])	100	85	85	100	85	85
ME (Kcal/kg diet)	3110	3110	3110	3211	3205	3207
ME (% of requirement[2])	100	100	100	100	100	100

LP, low-protein diet; LP+AA, amino acid supplemented low-protein diet.
1 Supplied the following per kg diet:vitamin A, 47,200 IU; vitamin D3, 11,800 IU; vitamin E, 71 mg; vitamin K3, 11.6 mg; vitamin B1, 5 mg; vitamin B2, 15.8 mg; vitamin B12, 0.02 mg; pantothenic acid, 23.8 mg; niacin, 35 mg; pyridoxine, 7.8 mg; biotin, 0.17 mg; folic acid, 2.7 mg; choline, 550 mg; copper, 11 mg; zinc, 110 mg; iron,112 mg; manganese, 112 mg; selenium, 0.10 mg; iodine, 1.1 mg; cobalt,0.11.
2 Ross Broiler Nutrition Specifications[29]

TCC GTT C-3'; GCR sense, 5'-CAT GAA CCT CGA AGC TCG CAA G-3,' and GCR antisense, 5'-ACC TCC AGC AGT GAC ACC AG-3'. mRNA levels of target genes were normalized to those of RPS17.

Data analysis

All data were statistically analyzed using the Tukey–Kramer method ($P < 0.05$) and Stat View 5.0 software (SAS Institute Inc., NC, NSA).

Results

The initial body weight of chicks was as follows: control, 205 ± 1.7 g; LP, 205 ± 0.7 g, and LP+AA, 208 ± 1.6 g. As shown in Table 3, BW gain (BWG) significantly decreased and the feed conversion ratio (FCR) significantly increased in the LP group throughout the experimental period compared to those in the control group. A significant decrease in BWG and an increase in FCR were observed in the LP+AA group in the first week (10–17 days of age) compared to those in the control group. Total feed intake was significantly higher in the LP+AA group than that in the control group.

As shown in Table 4, weights of the body, breast muscle, and legs significantly decreased in the LP group. There was no significant difference in liver weight and plasma corticosterone levels between groups, whereas plasma uric acid concentrations were significantly lower in the LP+AA group than those of the other groups.

To identify the mechanisms underlying the effects of the LP diet and AA supplementation, myogenesis- and proteolysis-related gene expression in the PM and BF were analyzed. mRNA levels of LC3B were significantly increased in the PM of the LP group, whereas mRNA levels of atrogin-1, MuRF-1, and MyoD were significantly increased in the BF (Fig. 1).

The expression levels of IGF-1 and its related genes in the liver, PM, and BF are shown in Fig. 2. Only IGFBP-2 mRNA levels in the BF were significantly higher in the LP group than that in the other groups. Therefore, hepatic and skeletal muscle IGF-1-related genes may not be involved in the significant differences in growth and skeletal muscle mass between groups, at least under the present experimental conditions.

Discussion

In this study, EAA supplementation in a 15% reduced CP diet from 10 to 35 days of age restored the decreases in growth performance and meat production observed in the LP group. Chrystal et al. have reported that a 14% reduced CP diet supplemented with EAA maintains the BWG and does not influence FI or FCR

Table 2. **Calculated composition of essential amino acids in the experimental diets**

Nutrient	10–24 days			25–35 days		
	Control	LP	LP+AA	Control	LP	LP+AA
Percentage in diet						
Methionine	0.60	0.34	0.67	0.55	0.31	0.61
Methionine + Cystine	1.00	0.68	0.99	0.91	0.63	0.92
Lysine	1.41	1.16	1.29	1.26	1.02	1.17
Threonine	0.94	0.80	0.88	0.85	0.73	0.78
Valine	1.11	0.95	1.01	1.01	0.87	0.91
Arginine	1.60	1.33	1.38	1.44	1.18	1.23
Isoleucine	0.97	0.81	0.90	0.87	0.72	0.81
Leucine	2.11	1.87	1.78	1.95	1.73	1.65
Tryptophan	0.29	0.24	0.23	0.26	0.21	0.20
Percentage of requirement[1]						
Methionine	118	66	130	117	67	131
Methionine + Cystine	101	69	100	100	69	101
Lysine	109	90	100	109	88	101
Threonine	107	91	100	110	93	100
Valine	111	95	101	113	96	101
Arginine	117	97	101	118	97	100
Isoleucine	109	91	102	108	89	100
Leucine	148	132	126	154	136	130
Tryptophan	139	115	107	138	113	105

1 Ross Broiler Nutrition Specifications[29]

Table 3. **Growth performance of broiler chicks from 10 to 35 days of age**

Parameter		Days of age				
		10-17	17-24	24-31	31-35	Total
Weigt gain (g)	Control	351.1±10.5^{a}	464.8±20.1^{a}	580.1±40.2^{a}	295.6±36.2	1691.5±68^{a}
	LP	269.1±6.6^{c}	364.8±7.3^{b}	443.8±4.7^{b}	272.9±2.6	1350.6±17.5^{b}
	LP +AA	311.4±4.5^{b}	460.3±8.6^{a}	578±13.2^{a}	337.1±15.3	1686.8±29^{a}
Feed intake (g)	Control	433.7±9.4	640.1±18^{ab}	916.6±35.1	509.5±39^{b}	2499.8±62.4^{b}
	LP	402.2±16.4	602.3±24.9^{b}	951.6±19.6	578.6±15.3^{ab}	2534.8±55.3^{ab}
	LP +AA	429.2±7.8	675.9±6.3^{a}	983.4±21.8	613.8±13.6^{a}	2702.2±27.3^{a}
FCR (g/g)	Control	1.24±0.01^{b}	1.38±0.05^{b}	1.60±0.14^{b}	1.76±0.11^{b}	1.48±0.05^{b}
	LP	1.50±0.05^{a}	1.65±0.04^{a}	2.14±0.03^{a}	2.12±0.06^{a}	1.88±0.02^{a}
	LP +AA	1.38±0.03^{a}	1.47±0.02^{b}	1.70±0.03^{b}	1.83±0.06^{ab}	1.60±0.02^{b}

Values are mean ± SEM for four replicates in each group. Groups with different superscripts in the same column are significantly different ($P < 0.05$).

Table 4. **Body and tissue weights and blood parameters of 35-day-old broiler chicks**

	Control	LP	LP+AA
Final body weight (g)	1896.4±68.4^{a}	1555.2±18.1^{b}	1894.9±30.4^{a}
Breast muscles weight (g)	261.0±14.2^{a}	180.3±3.5^{b}	266.2±6.5^{a}
Legs weight (g)	389.8±11^{a}	315.8±6.2^{b}	390.0±7.7^{a}
Liver weight (g)	34.1±1.3	33.8±0.4	38.6±1.8
Plasma Uric acid (mg/dL)	4.1±0.2^{a}	4.1±0.2^{a}	2.8±0.3^{b}
Plasma Corticosterone (ng/mL)	3.15±0.21	3.23±0.19	3.19±0.34

Values are mean ± SEM for four replicates in each group. Groups with different superscripts in the same row are significantly different ($P < 0.05$).

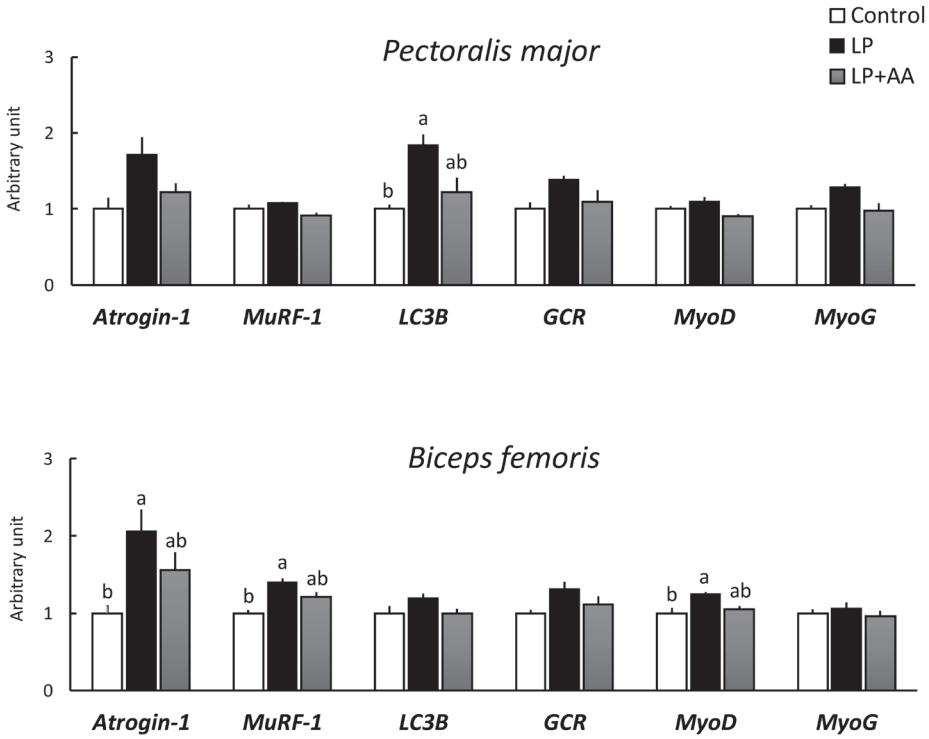

Fig. 1. **Effects of amino acid supplementation to a low-protein diet on mRNA levels of protein metabolism-related factors in skeletal muscle of broiler chicks.**

Data are expressed as means ± the standard error of the mean (SEM) of four replicates in each group. Groups with different letters are significantly different ($P < 0.05$). LP, low-protein; LP+AA, low-protein+amino acids; MuRF-1, muscle RING-finger protein-1; LC3B, microtubule-associated protein 1 light chain 3 isoform B; GCR, glucocorticoid receptor; MyoD, myoblast determination protein 1; MyoG, myogenin.

in broiler chicks between 14 and 35 days of age[35]. Supplementation of EAA to 10% reduced CP diets in broilers from 11 to 35 days of age[36] or 21 to 35 days of age[5] results in the recovery of growth performance and meat production. Thus, the current experimental conditions appeared to be comparable to those of previous studies and were desirable for clarifying the mechanisms underlying the effects of an LP diet and EAA supplementation in broiler chicks.

In this study, the LP diet significantly decreased BWG without any significant changes in FI compared to that of the control diet. In addition, changes in skeletal muscle weight and proteolytic gene expression in the LP group might suggest the induction of skeletal muscle proteolysis. Therefore, it is possible that reduced protein intake, and not energy intake, is one of the causes of impaired growth and loss of muscle mass in the LP group.

As shown in the LP+AA group, BWG and the FCR recovered with AA supplementation, although this group showed the highest feed consumption. Notably, the effects of AA supplementation on FI in the LP diet differed depending on experimental conditions. For example, a 15.5% reduced CP diet supplemented with EAAs increases FI without affecting BWG in broiler chicks from 1 to 17 days of age[37]. A 14% reduced CP diet supplemented with EAAs does not affect the BWG or FI of broiler chicks from 14 to 35 days of age[35]. Moreover, the BW and FI of broiler chicks are not influenced by feeding a 17% reduced CP diet supplemented with EAAs from 10 to 28 days of age[38]. In the present study, BWG decreased and the FCR increased during the first week (10–17 days of age) in the LP+AA group; however, these changes were not detected during the second and third weeks (17–24 and 24–31 days of age). Subsequently, FI increased in the LP+AA group during the remaining four days (31–35 days of age). These results suggested that changes in metabolism occurred during the experimental period in the LP+AA group and influenced growth performance during each growing period.

In the LP group, mRNA levels of LC3B were significantly higher in the PM, whereas those of atrogin-1 and MuRF-1 were significantly higher in the BF. These changes were accompanied by decreased muscle weight. However, no significant difference in these mRNA levels was detected between the control and LP+AA groups. There is evidence that AAs regulate proteolytic systems and preserve skeletal muscle mass in chickens, as well as in mammals. For example, oral administration of leucine or

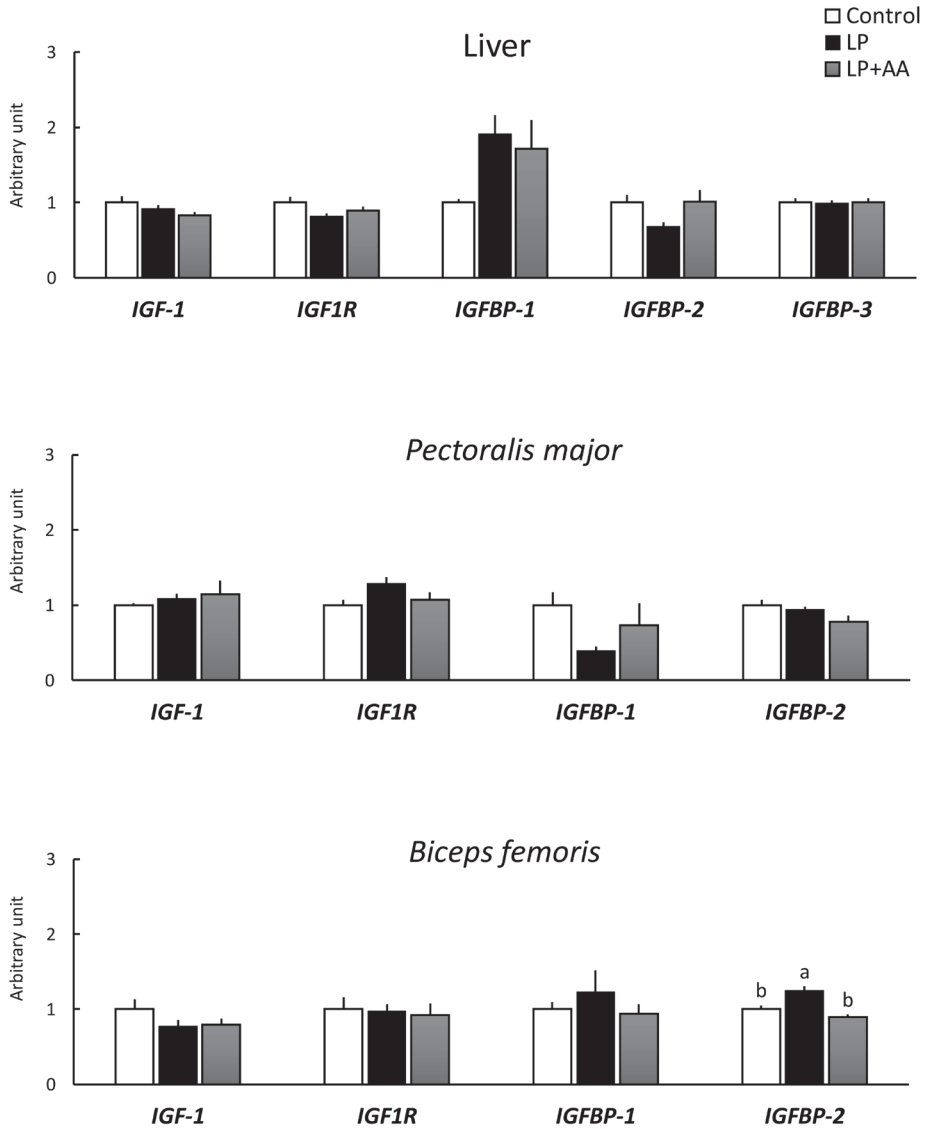

Fig. 2. **Effects of amino acid supplementation to a low-protein diet on mRNA levels of insulin-like growth factor 1 (IGF-1) and IGF-1 related genes in the liver, *pectoralis major*, and *biceps femoris* muscles of broiler chicks.** Data are expressed as means ± the standard error of the mean (SEM) of four replicates in each group. Groups with different letters are significantly different ($P < 0.05$). LP, low-protein; LP+AA, low-protein+amino acids; IGF-1, insulin-like growth factor 1; IGF1R, insulin-like growth factor 1 receptor; IGFBP, insulin-like growth factor binding protein.

isoleucine suppresses myofibrillar proteolysis *in vivo* and *in vitro* by downregulating the ubiquitin-proteasome pathway in chick skeletal muscle[39]. L-arginine treatment suppresses protein levels of atrogin-1 and MuRF-1 and increases muscle fiber diameter in primary chicken myoblast cultures[40]. In cultured chick myotubes, non-essential AA supplementation decreases mRNA levels of LC3B and atrogin-1[14]. These findings suggested that skeletal muscle proteolysis might be induced in the LP group and that dietary AAs attenuated the changes in both PM and BF in the LP+AA group.

Protein malnutrition increases plasma corticosterone levels in rats[41] and chickens[42]. Glucocorticoids regulate protein metabolism in chickens[43]. The GCR is an intracellular receptor that conveys glucocorticoid signals and is implicated in the induction of catabolic responses in skeletal muscles of mammals[44,45]. Therefore, plasma corticosterone levels and skeletal muscle expression of GCR were compared between groups. However, in the present study, no significant differences were observed in plasma corticosterone or GCR mRNA levels in the PM or BF between groups. Considering that metabolic changes

might have occurred during the feeding period in the LP+AA group, the corticosterone-induced proteolytic pathway might be temporarily upregulated in the first week of the feeding period. Further studies are required to clarify the effects of an LP diet over a short period in broiler chickens.

In the LP group, MyoD mRNA levels were significantly higher in the BF. MyoD and MyoG play important roles in the regeneration of adult skeletal muscle and remodeling of damaged fibers to restore fiber integrity and function[46]. Ishido et al. have proposed that high expression of MyoD in myonuclei and satellite cells in skeletal muscle under neurotrophy contributes to protection against apoptotic cell death and enhances myoblast proliferation in rats[47]. Therefore, the increased gene expression of MyoD in the BF of the LP group might be an adaptive mechanism that accelerates muscle regeneration.

In this study, the PM, but not the BF, showed significantly higher levels of LC3B mRNA, suggesting that the autophagic lysosomal system was induced in the PM, but not in the BF. The PM is mainly composed of glycolytic muscle fibers[48,49], whereas the BF, the largest skeletal muscle in the thigh, is composed of both glycolytic and oxidative muscle fibers[50]. A comparison of autophagy (GFP-LC3 localization) between different muscle types reveals that autophagic rates in glycolytic muscles are more upregulated than those in oxidative muscle after 24 h starvation in mice[51]. Thus, the different responses in LC3B gene expression might be attributed to different fiber types.

In the present study, the LP diet resulted in different changes in mRNA levels of proteolytic genes, such as atrogin-1, MuRF-1, and LC3B, between the PM and BF groups (Fig. 1). However, the reasons for these different responses remain unclear. Site-specific responses to nutrients and hormones have been reported in chicken skeletal muscles. For example, the first exogenous nutrient significantly suppresses mRNA levels of atrogin-1 in the *sartorius*, but not in the PM of newly hatched chicks[52]. Intraperitoneal injection of clenbuterol significantly suppresses mRNA levels of atrogin-1 in the *sartorius*, but not in the PM, in neonatal chicks[53]. The capacity for protein synthesis, estimated from the RNA:protein ratio, is significantly suppressed in the BF, but not in the PM, in broiler chickens by pair-feeding[54]. Therefore, it is likely that chicks maintain protein homeostasis by transcriptionally regulating protein metabolism-related genes in skeletal muscle in a site-specific manner.

IGF-1 produced in the liver plays an important role in chicken growth[55]. IGF-1 in the thigh muscles may contribute to protein synthesis via the Akt/S6 pathway in chicks[20]. An extremely deficient LP diet (CP 12.15%) suppresses growth and decreases plasma IGF-1 concentrations in broiler chicks compared to those fed a high protein diet (CP 21.58%)[56]. A reduction in soybean protein from 20% to 5% in the diet significantly suppresses growth and decreases plasma IGF-1 concentrations in layer chicks[57]. However, in the present study, no significant difference was observed in IGF-1 mRNA levels in the liver, PM, or BF between groups. These results suggested that neither circulating nor skeletal muscle IGF-1 was involved in the changes in growth or skeletal muscle mass induced by LP or EAA-supplemented LP diets, at least under the present experimental conditions.

In the LP group, IGFBP-2 mRNA levels were higher in the BF. Kita et al. have reported that a 5% soybean protein diet significantly increases gizzard IGFBP-2 mRNA levels and significantly decreases brain IGFBP-2 mRNA levels compared to that of a 20% soybean diet in layer chicks[57]. Therefore, transcriptional regulation of IGFBPs is likely tissue-dependent in chickens. Wang et al. have reported that IGFBP-2 knockdown significantly increases MyoD mRNA levels in chicken myoblasts; however, in the present study, both IGFBP-2 and MyoD mRNA levels are increased in the BF of the LP group[58]. Thus, the upregulation of IGFBP-2 expression in the LP group might not play an important role in the suppression of skeletal muscle growth.

In chickens, ammonia produced by AA catabolism is excreted in the form of uric acid. The LP+AA group showed a significant decrease in plasma uric acid concentration compared to those of the control and LP groups, although the plasma uric acid concentration did not significantly change in the LP group. Feeding diets with 11%–13% reduced protein from 1 to 48 days of age do not influence the plasma uric acid concentrations or decrease BW in broilers[59]. Feeding diets with 23%–27% reduced dietary protein from 1 to 21 days of age decrease the BWG and blood uric acid concentration in broilers[60]. Hence, diets with more than 23% reduced CP might influence both the BWG and plasma uric acid levels in broiler chicks. Abou-Elkhair et al. have reported that 5%–10% reduced dietary CP supplemented with EAAs from 1 to 35 days of age decreases serum uric acid levels and improves growth in broiler chicks compared to that broiler chicks fed the normal diet[61]. These results and the current findings suggest that the addition of EAAs might suppress AA catabolism without affecting BWG in broiler chicks.

In this study, corn and soybean meals were the major feed ingredients. Further studies are required to examine the effects of other feed ingredient-based LP diets and LP+AA diets in broiler chicks. For example, wheat, sorghum, distillers, dried grains with soluble corn gluten meal, and rapeseed meal may all be used as feed ingredients. However, soybean meal is the major dietary protein source in animal feed throughout the world[62]. Therefore, these findings provide essential information that contribute to future research.

In summary, the effects of a 15% reduced LP diet, with or without EAA supplementation, were examined on growth performance and protein metabolism-related factors in skeletal muscle of broiler chicks. These findings suggested that changes in mRNA levels of proteolytic genes in skeletal muscle might be related to the impaired effects of the LP diet in broiler chicks.

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Author Contributions

Asmaa S. El-Far and Kazuhisa Honda designed the experiments; Asmaa S. El-Far and Maho Kamiya conducted the experiments and analyzed the data; Asmaa S. El-Far drafted the manuscript; Takaoki Saneyasu and Kazuhisa Honda supervised the experiments and edited the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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