Effect of In Ovo Administration of Branched-Chain Amino Acids on Embryo Growth and Hatching Time of Chickens

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The effect of various branched-chain amino acids (BCAA: isoleucine, leucine, valine) on embryo growth and the hatching time of fertilized eggs of chickens was examined. Before the onset of egg incubation, one of BCAA was injected into fertilized eggs. The amount of each BCAA administrated into eggs was equal to 1% of each amino acid exits in the egg. On day 14 of incubation, the weight of embryos was measured. On day 21, the hatching time was recorded, and body weight of chicks at birth was measured. The in ovo administration of BCAA increased the weight of whole embryo compared to the control. Compared to the control, the in ovo administration of leucine and valine significantly accelerated the hatching time. There were no significant differences in body weight of newly hatched chicks among all treatments. It was concluded that the in ovo administration of BCAA, especially leucine and valine, could accelerate embryo growth resulting in the acceleration of hatching time of chicks.

Key words: branched chain amino acids, embryo growth, isoleucine, hatching time, leucine, valine


Introduction

To examine the influence of nutrients on the embryo growth of chickens, the in ovo administration of various nutrients has been conducted. The injection of amino acid mixture solution into the yolk sac at day 0 of incubation resulted in lowered hatchability. Whereas the same treatment on day 7 of incubation showed the same hatchability to that of the control group (Ohta et al., 1999). It was also reported that chick weight relative to initial egg weight was higher in eggs injected with amino acid mixture solution as compared to eggs injected with water (Ohta et al., 2001).

It has been well known that leucine, which is one of branched-chain amino acids (BCAA), has an anabolic effect on protein metabolism by increasing the rate of protein synthesis (Alvestrand et al., 1990; Nair et al., 1992; Blomstrand et al., 1997). Leucine can directly activate the signaling pathway of mammalian target of rapamycin complex 1 (mTORC1) (Dodd and Tee, 2012). In chickens, we also reported that leucine could stimulate protein synthesis of chicken embryo myoblasts (Oki et al., 2007). Compared to leucine, the information about the influence of other BCAA, isoleucine and valine, on embryo growth of chickens has been limited. The aim of this study, therefore, was to examine the influence of various BCAA on embryo growth and the hatching time of chickens.

Materials and Methods

Eighty fertilized eggs of Single Comb White Leghorn chickens were purchased from a local hatchery (Koiwai Farm Co., Ltd, Shizukuishi, Iwate, Japan). Egg were divided into 4 groups (n=20). Before the onset of egg incubation, one of BCAA (isoleucine, leucine, valine) was injected into the egg. The blunt end of eggs was sterilized with 70% ethanol prior to incubation, and the small hole was made at the blunt end. A 1-mL disposable syringes attached with a 27-gauge needle were used for injection into the eggs. The solution was injected beneath the air cell. The amount of each BCAA administrated into eggs was equal to 1% of each amino acid exits in the egg, which was based on the research studied by Ohta et al. (2001). They injected the mixture of amino acids including 2.71 mg of isoleucine, 4.53 mg of leucine and 4.25 mg of valine that almost corresponded to approximate 1% of these amino acids existing in whole eggs (Standard Tables of Food Composition in Japan, 2007). Amino acids were dissolved in Dulbecco’s phosphate buffered saline (DPBS), and the volume of amino acid solution injected into the egg was 0.42 mL. The concentrations of isoleucine, leucine and valine were 62, 100 and 85 mM, respectively.
group, DPBS was injected into the egg. The small hole of eggs was sealed up with the cellophane tape. Thereafter, eggs were started to incubate at 37.5 °C. On day 14 of incubation, 10 eggs in each treatment were weighed. Embryos were removed from the amniotic fluid, separated from the yolk sac. They were rinsed in DPBS, wiped with paper towel, and weighed. On day 21, the hatching time of remaining eggs was recorded, and body weight of chicks at birth was measured. Animal care was in compliance with applicable guidelines from the Iwate University Policy on Animal Care and Use.

Statistical analysis of data was performed by one-way ANOVA using the General Linear Model Procedures of SAS (SAS/STAT version 6) (SAS Institute, 1999). After ANOVA, Duncan’s multiple range test was performed to compare each treatment and control. Differences between means were considered to be significant at $P < 0.05$.

**Results**

Embryo weight at 14 days of incubation is represented in Fig. 1. The *in ovo* administration of BCAA increases the weight of whole embryo compared to the control (DPBS). There are no significant differences in embryo weights among BCAA.

The hatching time of chicks at birth is shown in Fig. 2. The time when the first chick hatched from eggs is regarded as time 0. The hatching time indicates the difference between time 0 and time when a chick in each treatment hatched. The *in ovo* administration of valine and leucine significantly accelerates the hatching time of chicks compared to that of the control chick.

Body weight of chicks at birth is represented in Fig. 3. There were no significant differences among all treatments.

**Discussion**

In the present study, amino acids injected into eggs were dissolved in DPBS. Physiological saline has traditionally been assumed to be a good carrier for injected nutrients. However, potassium chloride has the potential to be used more effectively than sodium chloride. Sodium dihydrogen phosphate has also the potential of adding additional phosphate, allowing for the increased concentration and subsequent storage in high energy compounds (McGruder *et al.*, 2011).

BCAA have been recognized as deleterious to chicks when fed in excess (D’Mello and Lewis, 1970). On the other hand, 100% excess of dietary leucine (2-times of leucine requirement) did not affect growth performance in chicks (Smith and Aurtic, 1978). In the present study, the amount...
of each BCAA administrated into eggs was equal to 1% of each amino acid exits in the egg, which did not seem to affect negatively to growth performance of chicken embryos.

Since the establishment of primary cell culture system, it has been well recognized that fetal calf serum (FCS) was required to grow and maintain animal cells. In avian species, FCS was also used for normal growth of fibroblasts and myoblasts derived from chicken embryos (Kita et al., 1996; Kita and Okumura, 2001). The growth promoting effects of FCS was due to the existence of various growth factors, e.g. insulin-like growth factor-I (IGF-I) etc. Previously, we have reported that chicken IGF-I could be available for healthy growth of chicken embryo myoblasts (Kita and Okumura, 2001). Furthermore, we also demonstrated that skeletal muscles of chicken embryos were the major site of IGF-I production (Kita et al., 2000). In the present study, as represented in Fig. 1, body weight of chicken embryos in fertilized eggs administrated with BCAA was significantly heavier than that in eggs injected with DPBS. Additionally, as shown in Fig. 2, the hatching time of chicken embryos was accelerated by administrating with BCAA. Anthony et al. (2000) reported that leucine could stimulate skeletal muscle protein synthesis, and in the present study if BCAA made skeletal muscles larger than that in the control group, BCAA could accelerate the growth and hatching time of chicken embryos via increasing IGF-I production in skeletal muscles. In addition, although it was reported that, in the skeletal muscle of neonatal pigs, leucine reduced the microtubule-associated protein 1 light chain 3 (LC3) II, which is the important marker of macroautophagy (Suryawan and Davis, 2014), the relation of BCAA to autophagy has not been clarified in the muscle of chicken embryos. These issues would be elucidated in the future.

References


