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Growth, Digestive Tract and Muscle Weights in Slow-Growing Broiler is not Affected by a Blend of Branched-Chain Amino Acids Injected into Different Sites of Egg

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Abstract

One hundred-fifty fertile eggs from 30-week-old Ross x Rhode Island Red (RIR) breeders as a factorial arrangement of 3 injection site (amnion, yolk sac or albumin) and 2 levels of BCAA (Control and BCAA, 0.2%) were arranged into 6 groups with 3 replicates. The solutions used *in ovo* feeding were aseptically prepared to contain 0% (C) or 0.2% BCAA (mixed within ratio of 2 L-leucine: 1 L-valine: 1 L- isoleucine) in 0.5% saline. At 18 d of incubation 1 ml of *in ovo* feeding solution was injected into the amnion (A), yolk sac (Y) or albumin (AL) of egg. Hence, there were six nutrition treatments; AC (n = 25), ABCAA (n = 25), YC (n = 25), YBCAA (n = 25), ALC (n = 25) and ALBCAA (n = 25). Upon hatch obtained birds were fed on standard broiler diets until slaughter (8 weeks of age). The YC had lower gizzard weight (P = 0.023) compared to other treatments. The interaction effect of factors on the gizzard weight was found significant (P < 0.025). The *in ovo* BCAA injection increased the gizzard weight of AL chicks while decreased that of A and Y birds. The crude fat content of thigh meat from Y birds was lower than those of A and AL broilers (P = 0.029). However, growth performance, muscles weights, digestive tract weight and length and abdominal fat, hearth, liver, weights are not affected by factors. The role of *in ovo* BCAA injection in slow-growing broiler needs further research.

Keywords: Poultry, nutrition, amino acids, growth performance, organ development

1. Introduction

In the commercial poultry, hatchery is an important factor to achieve maximum profit. Chicks hatched within a 36-48 hour period and hatching usually ends when caught out ratio of 95%. It means hatchlings withheld feed and water for up to 72 h depending on the time from hatching within the hatching window to placement on rearing farms. This period is associated with a reduced in initial body weight with respect to development of gastrointestinal tract (GIT), some organs and muscles, and thus, meat yield at market age (Mozdziak et al., 2002; Halevy et al., 2003; Bhuiyan et al., 2011). Therefore, the last period of incubation has become one of the critical periods for growth, developmental programming of digestive and metabolic organs and skeleton muscles and led to the development of *in ovo* feeding systems for poultry (Uni et al., 2005). It has been reported that, to reduce the negative effect of large hatching window and to increase performance, the use of some *in ovo* amino acid supplements (Tako et al., 2004; Kadam et al., 2008; Bakyaraj et al., 2011; Al-Daraj et al., 2012) are necessary in the last period of incubation. It is well known that leucine, isoleucine and valine, the branched-chain amino acids (BCAA), make up about one-third of muscle protein and promote muscle formation and development. The BCAA, especially leucine are thought to have effects on muscle protein synthesis apart from being a source of energy and may reduce the amount of proteolysis in the muscle and muscle damage that may occur under stress. Indeed, *in ovo* administration of BCAA, especially leucine and valine could accelerate embryo growth resulting in the acceleration of hatching time of chicks (Kita et al., 2015).

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A dietary deficiency of valine or isoleucine decrease carcass protein and moisture contents, whereas it increase carcass fat or leucine deficiency has a harmful effect on body weight gain (Okumura et al., 1985). Ospina-Rojas et al. (2014) noted that a mix of valine and isoleucine improve the poultry performance and the breast meat yield. Also, leucine increases HMB (β-hydroxy-β-methyl-butyrate) formation (Kornasio et al.. 2009) and serum insulin-like growth factor 1 (GIF-1) concentration (Pedrosa et al., 2013). It has been indicated that among BCAA, only leucine is capable of activating translation initiation factors and stimulating protein synthesis in skeletal muscles (Anthony et al., 2000). Thus, studies have focused especially on HMB (Foye et al., 2006, 2007; Tako et al., 2004) or BCAA (Bhanja and Mandal, 2005; Kita et al., 2015) supplementation in *in ovo* solution. However, leucine is not only a source for HMB formation, but also is an AA having extra proteolytic effects. Therefore, BCAA, especially leucine are thought to have effects on muscle protein synthesis apart from being a source of energy and may reduce the amount of proteolysis in the muscle and muscle damage that may occur under stress. Researchers have shown that *in ovo* injection site may affect hatchability and body weight at hatch (Al-Murrani, 1982; Ohta et al., 1999). Therefore some studies were conducted to prefer the targeted injection site in embryonated eggs for to understand various physiological features associated with late embryonic development (Ohta and Kidd, 2001; Kop-Bozbay et al., 2013). It has been investigated the effect of the blend of BCAA (Bhanja and Mandal, 2005) or each of BCAA (Kita et al., 2015) on the hatchability traits and growth performance of fast-growing broilers. To our knowledge, the effect of *in ovo* administration of a mixture of BCAA (2 leucine: 1 valine: 1 isoleucine), which have an extra protein effect, into different sites of egg (amniotic cavity, yolk sac and albumin) on growth performance, carcass and some demand (muscle, blood etc.) and supply (heart, liver and intestine etc.) organs characteristics of slow-growing broilers has not been reported. The present study was conducted to describe the effects of *in ovo* administration of a mixture of BCAA (2:1:1) on growth performance, carcass weights, edible inner organs and gastrointestinal tract (GIT) characteristics in slow-growing broilers.

2. Material and Method

In Ovo Feeding Procedure

In this study, a total of 150 fertile eggs from 30-week-old slow-growing line Ross x Rhode Island Red (RIR) breeders (Yamak et al., 2014) improved at the University of Ondokuz Mayis, Agricultural Faculty Research and Application Farm were incubated under routine conditions (37.8 C and 60% RH) at the same Research Farm. At 18 days of embryonic incubation, eggs containing viable embryos were weighted individually and randomly divided into six groups with three replicates of 25 eggs each with an average egg weights. Then eggs were allocated to hatching trays into six experimental groups (according to a 3×2 factorial arrangement for three *in ovo* injection site (IOIS: amnion (A), yolk sac (Y) or albumin (AL)) and two *in ovo* solution (IOS: control; 5 g of NaCl/l (C)) or supplemented with 2 g BCAA/l in 5 g of NaCl/l (BCAA)). According to the experimental design, six treatments consisted of : (1) AC solution: 5 g of NaCl/l injected into the amnion, (2) ABCAA solution: 2 g BCAA/lt in 5 g of NaCl/l injected into the amnion , (3) YC solution: 5 g of NaCl/l injected into the yolk sac, (4) YBCAA solution: 2 g BCAA/lt in 5 g of NaCl/l injected into the yolk sac ,(5) ALC solution: 5 g of NaCl/l injected into the albumin ,(6) ALBCAA solution: 2 g BCAA/lt in 5 g of NaCl/l injected into the albumin. The positive control was not performed since it has been reported (Uni et al., 2005) that there was no difference between the hatchability traits from eggs untreated (positive control) and that from eggs injected 1 mL of 5 g/L NaCl (negative control). The BCAA used in this study were mixed within ratio of 2 L-leucine (Cas no: 61-90-5): 1 L-valine (Cas no: 73-32-5): 1 L-isoleucine (Cas no: 72-18-4) and thus, solution with BCAA at 2 g/l were assumed to contain up to about 1 g L-leucine which it was equal the HMB of %1 in a previous study (Tako et al., 2004). The injection place was sterilized with a solution of 75% ethyl alcohol prior to injection. Eggs were injected with 1 ml solution 27-gauge needle into amnion, yolk sac or albumin as described in detail by Uni et al. (2005). After the eggs were injected, the injection holes were sealed with liquid paraffin. Finally, all eggs were placed in relevant hatching trays.

Housing Condition and Sample Collection

Upon hatch all birds (126 chicks) were weighted, recorded and hatchability was calculated as a percentage of the number of fertile eggs set. The new hatchlings housed in floor pens with wood shavings. The pens of identical size (1 × 1.5 m) contained 1 plastic poultry drinker and 1 hanging tube feeder. The temperature was maintained at 32 °C at hatch and was subsequently gradually decreased to 25 °C by 7 days of age. During the experiment, all groups were subjected to the appropriate and similar management practices, as reported in the study of Yamak et al. (2014) for this genotype. All groups were fed *ad libitum* with commercial broiler chickens (Table 1).

Birds and feed were weighed as a pen at the beginning and at the end of experiment (from 1 to 56 d) for performance evaluation (feed intake, weight gain and feed conversion ratio (FCR)). In the end of the experiment, two birds (1 female, 1 male) from each replication with body weight within 1 standard deviation of the mean treatment weight (eight birds per treatment) were slaughtered to determine weights of whole GIT, abdominal fat, empty gizzard, heart and liver, and lengths of the whole GIT. Heart, liver, gizzard and gastrointestinal tract were carefully removed from the abdominal cavity then they were weighted. Length of extending GIT was measured and recorded. *Pectoralis* (PM) and *ilio tibialis muscles* (ITM) were weighed and recorded, because breast meat yield is becoming an important aspect of the poultry industry. Dry matter, protein, fat and ash contents were determined by AOAC-approved methods (AOAC, 1990). Before analysis, the breast and thigh samples obtained from each carcass were mashed using the electric mixer and then this mixed sample was analysed in duplicate for each bird. The protein and fat were expressed as percentages on a DM basis. All animal procedures were approved by the local Ethical Committee of Ondokuz Mayis University for Experimental Animals.

Table 1: Ingredients (g/kg as-Fed Basis) and Nutrient Composition of the Experimental Diet

Ingredients, g/kg	Starter (1 to 7 days)	Grower (8 to 28 days)	Finisher (29 to 56 days)
Corn	494.0	510.5	558.0
Soybean meal, 47%	310.0	288.0	228.0
Fullfat soybean	120.0	120.0	130.0
Vegetable oil	37.3	44.0	49.0
Limestone	10.0	10.0	9.0
Dicalcium phosphate, 18%	19.0	19.0	17.0
Sodium chloride	3.0	3.0	3.0
Vitamin-mineral premix[1]	2.5	2.5	2.5
L-lysine, 78%	0.6	0.3	0.5
DL-methionine, 99%	2.6	1.7	2.0
Soda	1.0	1.0	1.0
Calculated composition (%), kg KM			
Dry matter	88.00	88.35	90.80
Crude protein	26.28	22.12	20.78
Crude fat	7.22	8.04	8.38
Crude cellulose	2.98	3.05	2.91
Ash	5.76	6.04	6.15
Starch	35.72	37.77	38.75
Sugar	5.63	5.31	5.90
Metabolic energy, MJ/kg	13.03	13.03	13.03

[1]Vitamin-mineral: 12000 IU vitamin A; 2400 IU vitamin D3; 40 mg vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 25 mg niacin; 10 mg calcium-D-pantotenat; 5 mg vitamin B6; 0.03 mg vitamin B12; 0.05 mg D-biotin; 1 mg folic acid; 80 mg Mn; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se; 200 mg choline chloride.

Statistical Analyses

Data were analyzed using the GLM procedure (SPSS 21.0; SPSS Inc., Chicago, IL, USA). Pen means were used as the experimental unit for all analyses. All data were analyzed in a randomized block design as a factorial arrangement (3 injection site x 2 *in ovo* solution) of treatments. Because poults placed in mixed gender, the effect of gender on the examined features were not tested. All percentage data were converted to arcsines prior to analysis. When the F-test was significant, differences were compared using Tukey's multiple range tests.

3. Results and Discussion

The results of the present study indicate that *in ovo* BCAA injection into the amnion, yolk sac or albumin did not affect hatching and some performance characteristics of slow-growing broilers. No significant differences were noted in hatchability, body weight of chicks at hatch (Table 2), mortality, weight gain, feed intake and FCR between groups (Table 3). Similarly there was no significant differences slaughter among treatments in terms of carcass and muscle weights (Table 4) as well as the weights of abdominal fat and demands and supply organs (Table 5).

However, the YC had lower gizzard weight ($P < 0.05$) compared to other treatments. The interaction effect of factors on the gizzard weight was found significant ($P < 0.05$). The *in ovo* BCAA injection increased the gizzard weight of AL chicks while decreased that of A and Y birds. The crude fat content of thigh meat from Y birds was lower ($P < 0.05$) than those of A and AL broilers (Table 6). Hatchability and body weight at hatch may affect *in ovo* amino acid injection depending on injection site. Previous studies demonstrated that *in ovo* amino acid injection at 7 d into the yolk (Al-Murrani, 1982), air cell (Ohta et al., 1999), chorioallantoic membrane or into the amniotic cavity (Ohta and Kidd, 2001) decreased hatchability. In our study BCAA injected into the amnion, yolk sac or albumin at 18 d of incubation did not, however, reduce hatchability or studied performance characteristics of birds. The disappearance between our study and previous studies (Al-Murrani, 1982; Ohta et al., 1999, 2001) may be resulted in injection time (7th vs. 18th day of incubation), as reported by Bhanja and Mandal (2005). The albumen is transferred into the amniotic cavity and yolk sac (Ohta et al., 1999) after the second week of incubation. Thus, it can explain why AL groups were ineffective on studied parameters. Although there was no clear effect of the mixture of BCAA on mortality of slow-growing broilers, because the mortality was within the accepted limit for all groups. The growth performance of slow-growing broilers in the present study was similar to standards of experimental birds of the strain (Yamak et al., 2014). In general, no differences in body weight gain, feed intake or feed to gain ratio were observed in slow-growing broilers from eggs injected the BCAA blend into different sites. These findings are agreement with a previous study (Bhanja and Mandal, 2005) using the BCAA blend in fast-growing broilers.

Table 2: Hatchability (%) and Hatching Weight (HW, g/chick) of Slow-Growing Broiler from Eggs Injected Branched-Chain Amino acids into Different Sites

Site	Solution	Egg weight (EW, g)	Hatchability	HW	HW/EW
A	C	56.9	95.2	45.6	80.3
	BCAA	58.7	90.3	46.3	79.0
Y	C	55.4	100.0	45.7	82.9
	BCAA	56.5	90.3	45.2	80.0
AL	C	55.6	90.5	45.3	81.5
	BCAA	56.8	86.9	44.5	78.4
Site					
	A	57.8	92.8	46.0	79.6
	Y	56.0	95.1	45.5	81.4
	AL	56.2	88.7	44.9	80.0
Solution					
	C	55.9	95.2	45.6	81.6
	BCAA	57.3	89.2	45.3	79.1
SEM		0.50	2.06	0.45	0.78
Mean effects of factor					
	Site	-	NS	NS	NS
	Solution	-	NS	NS	NS
	Site x Solution	-	NS	NS	NS

A: Amnion; Y: Yolk sac; AL: Albumin; C: Control; BCAA: Branched-chain amino acids; SEM: standard error of the mean, NS: $P > 0.05$.

The effect *in ovo* feeding on the poultry performance varies depending on many factors. These may be considered the content of *in ovo* solution, site of injection, line of breeders, breeding age, egg weight, the storage conditions for egg, hatchling's chronological and biological age and incubation conditions (Ohta and Kidd, 2001; Bhanja and Mandal, 2005; Shafey et al., 2012; Kop-Bozbay et al., 2013; Schulte-Drüggelte, 2015). The body weight at hatch, hatchability, FCR and GIT traits in the present study was comparable to that obtained by Bhanja and Mandal (2005), who used a mixture of leucine, isoleucine and valine of about 1.75:1.00:1.23 percent in amino acid injected birds. Likewise, Kita et al. (2015) founded that there was no significant differences in body weight at hatch among *in ovo* injection of each BCAA. In the present study, the fact that the BCAA blend did not affect negatively the studied variables indicate that the dose of BCAA blend was not a level that would cause a harmful effect on these parameters, because excess BCAA in diet has detrimental effect to chicks (D'Mello and Lewis, 1970).

Table 3: The Body Weight Gain (BWG, g/bird/day) and Feed Intake (FI, g/bird/day), feed Conversation Rate (FCR, g feed:g gain) and Mortality (%) of Slow-Growing Broiler from Eggs Injected Branched-Chain Amino acids into Different Sites

Site	Solution	BWG	FI	FCR	Mortality
A	C	51.7	87.4	1.69	0.00
	BCAA	49.8	81.9	1.65	6.64
Y	C	51.0	86.9	1.71	0.00
	BCAA	45.9	84.0	1.86	9.53
AL	C	46.0	90.5	1.99	0.00
	BCAA	50.0	88.8	1.79	0.00
Site					
	A	50.8	84.6	1.67	3.33
	Y	48.4	85.4	1.78	4.76
	AL	48.0	89.7	1.89	0.00
Solution					
	C	49.6	88.3	1.79	0.00
	BCAA	48.6	84.9	1.77	5.40
SEM		1.27	1.07	0.491	0.051
Mean effects of factor					
Site		NS	NS	NS	NS
Solution		NS	NS	NS	NS
Site x Solution		NS	NS	NS	NS

SEM: standard error of the mean. NS: P>0.05. The abbreviations are as in Table 2.

Although it was expected that *in ovo* injection of the BCAA individually (Kita et al., 2015) or blend (Bhanja and Mandal, 2005) would stimulate the growth performance of broilers due to the may be related to the reported properties of the HMB and IGF-1 (Moore et al., 2005; Kornasio et al., 2009; Pedrosa et al., 2013), research on leucine, isoleucine and valine and/or blend of these yield contradicting results (Bhanja and Mandal, 2005; Kita et al., 2015). However, the results of the present study are in agreement with previous observations that indicated a mixture of BCAA that did not affect body weight gain, feed intake or feed efficiency in fast-growing broilers (Bhanja and Mandal, 2005). The lack of effects of BCAA blend used in the present study can firstly be explained by the fact that the fast-growing birds were better able to perform with commercial basal diet due to the fact that nutrient requirements increase depending on growth rate (Sarica et al., 2009; Yamak et al., 2014), and also they may be better able to digest the basal diet due to the development of the digestive tract and organs (Sarica et al., 2009; Baéza et al., 2015). In fact, the effect of BCAA bland was not significant on the dressing percentage, the relative weights of the whole GIT, pancreas and edible inner organs, including gizzard at slaughter age (data not shown). Bhanja and Mandal (2005) noted that the BCAA are critical for the growth of chicken embryo.

Therefore, the second reason for the lack of effects of BCAA blend may be related to the nutrients, especially protein content of egg. It has been reported that there is a role for BCAA beyond involvement in protein synthesis or extra-protein effects of BCAA in the low-protein diet on the growth performance (Ospina-Rojas et al., 2014). Therefore, our results indicate that the nutrient contents of eggs used in the present trial was in ideal levels, which could not affect the hatchability, chick quality and the degree of growth promotion. It is known that well-nourished, healthy chicks do not respond to *in ovo* supplements (Schulte-Drüggelte, 2015) and the degree of limiting protein synthesis of these amino acids depend on the ratios and antagonistic relationship between each of these amino acids (Dozier III et al., 2011; Burnham et al., 1992) and the protein content and quality of poultry diets (Corzo et al., 2009, 2010, 2011; Ospina-Rojas et al., 2014). Indeed, Burnham et al. (1992) reported that in case of diet isoleucine content is below the needs of poultry, the high levels of leucine and valine adversely affect the weight gain. Relatively large excesses of leucine and of valine, or of both do not depress growth, because if the dietary content of isoleucine is sufficient to meet the requirements of the broiler (Burnham et al., 1992; Ospina-Rojas et al., 2014). These suggestions indicate that each of amino acid in our BCAA blend was not below the needs of embryo or not excess any one of these amino acids.

Table 4: The Weight of Slaughter, Carcass and Muscles (g/birds) of Slow-Growing Broiler from Eggs Injected Branched-Chain Amino Acids into Different Sites

Site	Solution	Slaughter	Carcass	*Pectoralis Muscle*	*Iliotibialis Muscle*
A	C	2651	1948	478.6	165.8
	BCAA	2500	1838	415.9	172.2
Y	C	2423	1794	412.8	157.2
	BCAA	2349	1723	404.9	145.5
AL	C	2344	1730	430.7	151.1
	BCAA	2459	1806	429.4	165.3
Site					
	A	2576	1893	447.2	169.0
	Y	2386	1759	408.9	151.3
	AL	2401	1768	430.1	158.2
Solution					
	C	2473	1824	440.7	158.0
	BCAA	2436	1789	416.7	161.0
SEM		54.45	43.5	13.36	5.13
Mean effects of factor					
	Site	NS	NS	NS	NS
	Solution	NS	NS	NS	NS
	Site x Solution	NS	NS	NS	NS

PM: pectoralis muscle; ITM: iliotibialis muscle. SEM: standard error of the mean. The abbreviations are as in Table 2. NS: $P > 0.05$.

The third reason may be the extra-protein effect of the BCAA blend (Ferrando et al., 1995; Anthony et al., 2000). There are lots of researches related to the extra-protein effect of the BCAA and/or a role for BCAA in protein synthesis (Anthony et al., 2000; Ospina-Rojas et al., 2014), although these effect and roles of the BCAA blend were not determined in the present study. Accordingly, the results with respect to growth performance show that either the level of the leucine, isoleucine or valine supplied from BCAA to eggs in the present study has no enough beneficial effect on embryonic development or thus, chick weight at hatch or it was not such a level that would cause a beneficial effect on these parameters. This confirms the propositions of Bhanja et al. (2004) and Bhanja and Mandal (2005). Ospina-Rojas et al. (2014) noted that supplementation of the mixture ratio of valine and isoleucine into starter diet could improve the performance and breast meat yield of broiler and with such an additional could be saved 2% protein content in diet. These results may explain that why not increased muscle weight with *in ovo* BCAA feeding in the research. Moreover the amount of used BCAA in the present study determined according to known positive impact on mentioned properties level of HMB. However, the amount of HMB production level (5% of the leucine amount) obtained from leucine metabolism is only 0.005%. Thus, the level of HMB provided with leucine remained below the levels that show this effect in the present study.

Table 5: Gastrointestinal Weight (GITW) and Length (GITL), Abdominal fat (AF), gizzard, Heart and Liver Weight of Slow-Growing Broiler from Eggs Injected Branched-Chain Amino Acids into Different Sites

Site	Solution	GITW	GITL	AF	Gizzard	Heart	Liver
A	C	218.17	214.92	37.92	37.67	18.83	57.42
	BCAA	200.00	212.75	44.42	36.25	18.08	53.58
Y	C	184.83	204.67	31.92	31.17	16.92	48.75
	BCAA	185.92	205.92	44.58	30.75	15.92	50.08
AL	C	196.42	205.50	41.58	30.92	16.08	49.58
	BCAA	197.75	208.00	41.92	40.92	17.08	52.08
Site							
	A	209.08	213.83	41.17	36.96a	18.46	55.50
	Y	185.38	205.29	38.25	30.96b	16.42	49.42
	AL	197.08	206.75	41.75	35.92a	16.58	50.83
Solution							
	C	199.81	208.36	37.14	33.25	17.28	51.92
	BCAA	194.56	208.89	43.64	35.97	17.03	51.92
SEM		3.61	3.71	2.70	1.06	1.22	1.26
Mean effects of factor							
	Site	NS	NS	NS	*	NS	NS
	Solution	NS	NS	NS	NS	NS	NS
	Site x Solution	NS	NS	NS	*	NS	NS

SEM: standard error of the mean. The abbreviations are as in Table 2. [a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

4. Conclusion

The results of the present study indicated that *in ovo* BCAA injection into the amnion, yolk sac or albumin did not affect hatching and in general, studied parameters of slow-growing broilers and also that no differences in studied variables were observed in slow-growing broilers from eggs injected the BCAA blend into different sites. To identify optimal compositions of *in ovo* BCAA feeding should be taken into account the relationships between leucine and isoleucine and/or valine in the future research. The effect of the supplementation of BCAA on the compounds or metabolites such as HMB, and IGF-1 related to programming of muscle development in newly hatched poultry should also be investigated. In addition, dietary quantity of protein and energy are likely to be important in the assessment of responses to BCAA supplementation.

Table 6: The Nutrient Contents of Pectoralis Muscle and Iliotibialis Muscle of Slow-Growing Broiler from Eggs Injected Branched-Chain Amino Acids into Different Sites

		Pectoralis muscle				*Iliotibialis muscle*			
Site	Solution	DM	Ash	EE	CP	DM	Ash	EE	CP
A	C	28.56	1.53	3.81	23.21	25.37	1.52	4.09	19.76
	BCAA	26.11	1.50	3.27	21.35	25.30	1.38	3.96	19.97
Y	C	27.63	1.42	3.59	22.61	24.97	1.50	4.26	19.21
	BCAA	27.29	1.49	3.84	21.96	25.48	1.52	3.37	20.59
AL	C	28.03	1.41	4.02	22.60	26.94	1.40	4.45	21.09
	BCAA	27.09	1.36	3.89	21.84	22.82	1.40	5.19	16.22
Site									
	A	27.33	1.52	3.54	22.28	25.34	1.45	4.03a	19.86
	Y	27.46	1.46	3.71	22.29	25.22	1.51	3.81b	19.90
	AL	27.56	1.38	3.96	22.22	24.88	1.40	4.82a	18.65
Solution									
	C	28.07	1.46	3.81	22.81	25.76	1.48	4.27	20.02
	BCAA	26.83	1.45	3.67	21.72	24.53	1.43	4.17	18.93
SEM		0.41	0.03	0.20	0.36	0.44	0.03	0.17	0.42
Mean effects of factor									
	Site	NS	NS	NS	NS	NS	NS	*	NS
	Solution	NS	NS	NS	NS	NS	NS	NS	NS
	Site x Solution	NS	NS	NS	NS	NS	NS	NS	**

SEM: standard error of the mean. The abbreviations are as in Table 2. DM: Dry matter, EE: Ether extract, CP: Crude protein, [a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

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5. Reference

Al-Daraji, H.J., Al-Mashadani, A.A., Al-Hayani, W.K., Al-Hassani, A.S., & Mirza, H.A. (2012). Effect of in ovo injection with L-arginine on productive and physiological traits of Japanese quail, S. Afr. J. Anim. Sci., 42, 139–145.

Al-Murrani, W.K. (1982). Effects of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl, Br. Poult. Sci., 23, 171–174.

Anthony, J.C., Yoshizawa, F., Anthony, T.G., Vary, T.C., Jefferson, L.S., & Kimball, S.R. (2000). Leucine stimulates translation initiation in skeletal muscle of post-absorptive rats via a rapamycin-sensitive pathway, J. Nutr., 130:2413–2419.

AOAC. (1990). Official methods of analysis. Association of official analytical chemists, IAC, Arlington, Virginia, 22001.920.153.928.950.

Bakyaraj, S., Bhanja, S.K., Majumdar, S., & Dash, B. (2011). Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens, J. Sci. Food Agric., 92, 313–320.

Baéza, E., Gondret, F., Chartrin, P., Le Bihan-Duval, E., Berri, C., Gabriel, I., Narcy, A., Lessire, M., Métayer-Coustard, S., Collin, A., Jégou, M., Lagarrigue, S., & Duclos, M. J. (2015). The ability of genetically lean or fat slow-growing chickens to synthesize and store lipids is not altered by the dietary energy source, Animal, doi:10.1017/S1751731115000683.

Bhanja, S.K., Mandal, A.B., & Goswami, T.K. (2004). Effect of in ovo injection of amino acids on growth, immune response, development of digestive organs and carcass yield of broiler. Indian J. Poult. Sci., 39, 212–18.

Bhanja, S.K., & Mandal, A.B. (2005). Effect of in ovo injection of critical amino acids on preand post-hatch growth, immunocompetence and development of digestive organs in broiler chickens, Asian-Austral. J. Anim. Sci., 18, 524–531.

Bhuiyan, M.M., Gao, F., Chee, S.H., & Ij, P.A. (2011). Minimising weight loss in new broiler hatchlings through early feeding of simple sugars, Anim. Prod. Sci., 51, 1002–1007.

Burnham, D., Emmans, G.C., & Gous, R.M. (1992). Isoleucine requirements of the chicken: The effect of excess leucine and valine on the response to isoleucine, Br. Poult. Sci., 33, 71–87.

Corzo, A., Loar II, R.E., & Kidd, M.T., 2009. Limitations of dietary isoleucine and valine in broiler chick diets, Poult. Sci., 88: 1934-1938.

Corzo, A., Dozier III, W.A., Loar, R.E., Kidd, M.T., & Tillman, P.B., 2010. Dietary limitation of isoleucine and valine in diets based on maize, soybean meal, and meat and bone meal for broiler chickens, Br. Poult.Sci., 51: 558–563.

Corzo, A., W.A. Dozier, III, L. Mejia, C.D. Zumwalt, M.T. Kidd, & P.B. Tillman. (2011). Nutritional feasibility of L-valine inclusion in commercial broiler diets. J. Appli. Poult. Sci., 20, 284-290.

D'Mello, J.P.F., & Lewis D. (1970). Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine and valine. Br. Poult. Sci., 11, 313-323.

Dozier III, W.A., Corzo, A., Kidd, M.T., Tillman ,P.B., & Branton ,S.L. (2011). Determination of the fourth and 5th limiting amino acids of broilers fed diets containing maize, soybean meal, and poultry by-product meal from 28 to 42 days of age, Br. Poult. Sci., 52, 238–244.

Ferrando, A.A., Williams, B.D., Stuart, C.A., Lane, H.W., & Wolfe, R.R. (1995). Oral branched-chain amino acids decrease whole-body proteolysis, J. Parenteral Enteral Nutr., 19, 47–54.

Foye, O.T., Uni, Z., & Ferket, P.R. (2006). Effect of in ovo feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys, Poult. Sci., 85, 1185–1192.

Foye, O.T., Ferket, P.R., & Uni, Z. (2007). The Effects of in ovo feeding arginine, β-hydroxy-β-methyl-butyrate, and protein on jejunal digestive and absorptive activity in embryonic and neonatal turkey poults, Poult. Sci., 86, 2343–2349.

Halevy, O., Nadel, Y., Barak, M., Rozenboin, I., & Sklan, D. (2003). Early posthatch feding stimulates satellite cell proliferation and skeletal muscle growth in turkey poults, J. Nutri., 133, 1376–1382.

Kadam, M.M., Bhanja, S.K., Mandal, A.B., Thakur, R., Vasan, P., Bhattacharyya, A., & Tyagi, J.S. (2008) Effect of in ovo threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens, Br. Poult. Sci., 49, 736–741.

Kita, K., Ito, K.R., Sugahara, M., Kobayashi, M., Makino, R., Takahashi, N., Nakahara,H., Takahashi K., & Nishimukai M. (2015). Effect of in ovo administration of branched-chain amino acids on embryo growth and hatching time of chickens. J. Poult. Sci., 52, 34-36.

Kop Bozbay, C., Konanç, K., Ocak, N., & Öztürk, E. (2013). The effects of in ovo injection of propolis and injection site on hatchability, hatching weight and survival of newly-hatched chicks (In Turkish), 7. Ulusal Hayvan Besleme Kongresi, 26–27 Eylül, Ankara.

Kornasio, R., Riederer, I., Butler-Browne, G., Mouly, V., Uni, Z., & Halevy, O. (2009). Betahydroxy-beta-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways, Biochim. Biophys. Acta, 1793, 755–763.

Moore, D.T., Ferket P.R., & Mozdziak, P.E. (2005). The effect of early nutrition on satellite cell dynamics in the young turkey, Poult. Sci., 84, 748–756.

Mozdziak, P.E., Walsh, T.J., & McCoy, D.W. (2002). The effect of early nutrition on satellite cell mitotic activity, Poult. Sci., 81, 1703–1708.

Ohta, Y., Tsushima, N., Koide, K., Kidd, M.T., & Ishibashi, T. (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks, Poult. Sci., 78, 1493–1498.

Ohta, Y., & Kidd, M.T. (2001). Optimum site for in ovo amino acid injection in broiler breeder eggs, Poult. Sci., 80, 1425–1429.

Okumura, J., Mori, S., & Muramatsu, T. (1985). Relationship between food consumption and energy and nitrogen utilisation by chicks given varying amounts of standard and leucine-, isoleucine- and valine-deficient diets, Br. Poult. Sci., 26, 519–525.

Ospina-Rojas, I.C., Murakami, A.E., Do Amaral Duarte, C.R., Eyng, C., Lopes De Oliveira, C.A., & Janeiro, V. (2014). Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases, Br. Poult. Sci., 55, 766–773.

Sarica, M., Karacay, N., Ocak, N., Yamak, U., Kop, C., & Altop A. (2009). Growth, slaughter and gastrointestinal tract traits of three turkey genotypes under barn and free-range housing systems, Br. Poult. Sci., 50, 487–494.

Schulte-Drüggelte, R. (2015). The importance of quality nutrition and management on the breeder farm, International Hatchery Practice, 29,6,25-26.

Shafey, T.M., Alodan, M.A., Al-Ruqaie, S.I.M., & Abouheif, M.A. (2012). In ovo feeding of carbohydrates and incubated at a high incubation temperature on hatchability and glycogen status of chicks, S. African J. of Anim. Sci., 42, 3.

Tako, E., Ferket, P.R., & Uni, Z. (2004). Effects of in ovo feeding of carbohydrates and B-hydroxy-B-methylbutyrate on the development of chicken intestine, Poult. Sci., 83, 2023–2028.

Uni, Z., Ferket, P.R., Tako, E., & Kedar, O. (2005). In ovo feding improves energy status of late-term chicken embryos, Poult. Sci., 84, 764–770.

Yamak, U.S., Sarica, M., & Boz, M.A. (2014). Comparing slow-growing chickens produced by two- and three-way crossing with commercial genotypes. 1. Growth and carcass traits, European Poultry Science (Archiv für Geflügelkunde), 78, 1-11.