

# Functional role of branched chain amino acids in poultry: a review

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**ABSTRACT** This review provides insight into the effects of the branched-chain amino acids (**BCAA**: leucine, isoleucine, and valine) on the growth, production performance, immunity, and intestinal health of poultry. Besides providing nitrogen substrates and carbon framework for energy homeostasis and transamination, BCAA also function as signaling molecules in the regulation of glucose, lipid, and protein synthesis via protein kinase B and as a mechanistic target of the rapamycin (**AKT-mTOR**) signaling pathway that is important for muscle accretion. The level of leucine is generally high in cereals and an imbalance in the ratio among the 3 BCAA in a low protein diet would produce a negative effect on poultry growth performance. This occurs due to the structural similarity of the 3 BCAA, which leads to metabolic competition and interference with the enzymatic degradation pathway. Emerging evidence shows that the inclusion of BCAA is essential for the proper functioning of the innate and adaptive immune system and the maintenance of intestinal mucosal integrity.

The recommended levels of BCAA for poultry are outlined by NRC (1994), but commercial broilers and laying hen breed standards also determine their own recommended levels. In this review, it has been noted that the requirement for BCAA is influenced by the diet type, breed, and age of the birds. Additionally, several studies focused on the effects of BCAA in low protein diets as a strategy to reduce nitrogen excretion. Notably, there is limited research on the inclusion ratio of BCAA in a supplemental form as compared to the ingredient-bound form which would affect the dynamics of utilization in different disease-challenged conditions, especially those affecting digesta passage ratio. In summary, this review encompasses the role of BCAA as functional AA and discusses their physiological effects on the productivity and health of poultry. The observations and interpretations of this review can guide future research to adjust the recommended levels of BCAA in feeding programs in the absence of subtherapeutic antibiotics in poultry.

**Key words:** branched chain amino acids, growth performance, immunity, microbiota, poultry disease

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## INTRODUCTION

Poultry needs protein for growth, egg production, immunity, enzymatic activity, tissue turnover, and much more (Beski et al., 2015). Similar to other essential amino acids (**AA**), supplemental branched-chain amino acids (**BCAA**) such as isoleucine and valine allow for a reduction in the crude protein (**CP**) in the diet, whereas leucine is generally found in higher amounts in the corn-based diet (NRC, 1994; Waldroup et al., 2002). A low CP diet is also expected to reduce the excess water consumption in birds that is often required to excrete higher N and could lead to wet litter and leg abnormalities (Francesch and Brufau, 2004; Shepherd and

Fairchild, 2010). However, a reduction in the CP of diets should be made only after accounting for the minimum requirements of essential AA. Besides it is to be noted that nonessential amino acids are also important, and low levels of such AA coupled with inadequate synthesis could affect physiological functions and performance (Hou et al., 2015). The three BCAA (leucine, isoleucine, and valine) also fall into the category of essential or indispensable AA. All 3 BCAA are structurally similar to branched-chain fatty acids and have a hydrophobic side chain. Leucine is 2-amino-4-methyl-pentanoic acid, isoleucine is 2-amino-3-methyl-pentanoic acid, and valine is 2-amino-3-methyl-butanoic acid (Adeva-Andany et al., 2017; Figure 1).

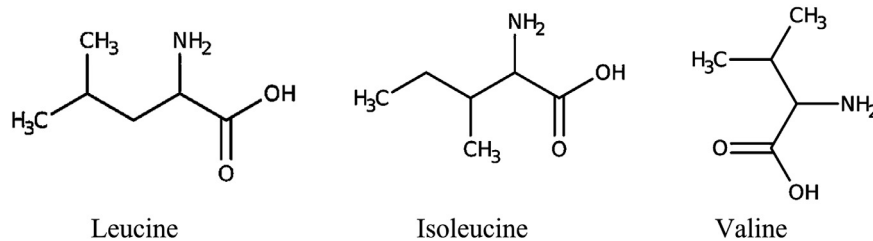
Unlike the other BCAA, valine is a limiting AA in the corn-soybean diet, and it is more susceptible to antagonism and enzymatic degradation than isoleucine in response to added leucine in the diets. Thus, valine must often be supplemented in a low CP diet (Nascimento et al., 2016). The three BCAA are structurally similar and are degraded first by branched-

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**Figure 1.** Structures of branched-chain amino acids with hydrophobic aliphatic side chain.

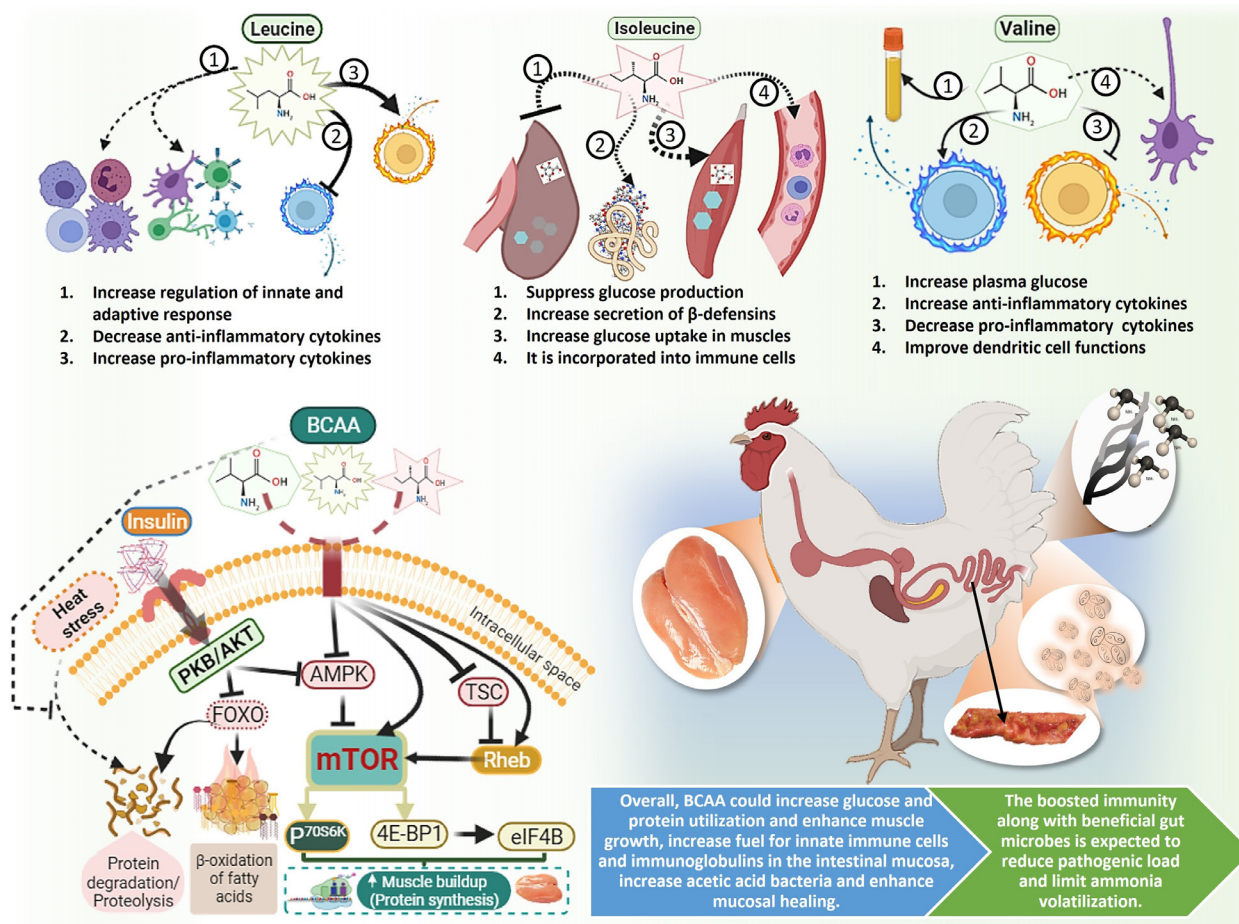
chain aminotransferase (**BCAT**) and then by branched-chain  $\alpha$ -keto acid dehydrogenase complex (**BCKD**), which causes irreversible catabolism to coenzyme A compounds (Brosnan and Brosnan, 2006). The stimulation of this enzymatic activity by one of the BCAA, most often leucine, leads to the catabolism of other BCAA, and thus the excess level of individual BCAA could lead to the degradation and deficiency of other BCAA present in the lower concentration. Allen and Baker (1972) observed that excess levels of leucine, and to the lesser extent isoleucine, impaired the growth of chicks and utilization of other BCAA, whereas excess valine did not have a major effect on leucine or isoleucine utilization.

The feed ingredients incorporated in poultry diets have disproportionate levels of leucine compared to other BCAA that could cause an imbalance in the BCAA ratio (Ospina-Rojas et al., 2020). When low CP diets are formulated by reducing the protein source and by increasing the cereals that are high in leucine, it increases the chance of antagonistic effects of leucine on other BCAA. A high level of leucine with an imbalance of other AA could aggravate AA degradation and impair muscle accretion in poultry. Considering the minimum requirements for individual BCAA, their optimum ratio in the low protein diet may not correspond to the proper ratio in a high protein or adequate protein diet. A high protein diet may be better utilized during the early growth phase of birds, and it would provide a higher concentration of limiting AA. This could prevent problems related to AA deficiency, but this high protein level may not ensure proper utilization of BCAA supplied in low concentration or other AA present in excess. An imbalance in dietary BCAA has been reported to negatively affect the broilers' performance (Ospina-Rojas et al., 2020), but the high leucine level may not show deleterious effects of antagonism of other BCAA when an adequate CP level is met by fulfilling the minimum requirement of isoleucine and valine (Waldroup et al., 2002).

In a broiler study, Konashi et al. (2000) found that a 50% deficiency of each of the 3 BCAA reduced the bodyweight of 24-day-old birds by one-fourth compared to the control, whereas birds fed a diet 50% deficient in sulfur-containing AA were reduced in weight by almost one-third. The authors also reported that the relative weight of the thymus and the bursa of Fabricius was significantly depressed relative to the control in a low BCAA diet, suggesting the critical role of BCAA in lymphoid organ development and immune response in

broilers. In addition to being utilized in various metabolic pathways, BCAA regulate gene expression and signal transduction for protein synthesis (Efeyan et al., 2012; Bai et al., 2015). The combined amount of all three BCAA is known to account for one-third of total muscle protein, and they play a vital role in preventing proteolysis under heat stress (Kop-Bozbay and Ocak, 2015) and promoting muscle formation and development. Busquets et al. (2000) concluded that BCAA inhibit lysosomal proteolysis of muscle in the short term and most likely reduce ATP-dependent proteolysis of skeletal muscle in the long term in rats. Besides the effect of BCAA on muscle protein turnover, their depletion also leads to increased  $\beta$ -oxidation of fatty acids through the AMPK-mTOR-FoxO1 pathway (Bai et al., 2015; Figure 2). Among all BCAA, leucine has been reported to be more potent in stimulating protein synthesis through the mTOR pathway and plays a greater role in immune function (Li et al., 2007; Wu, 2010). In addition to promoting immune function, Mattick et al. (2013) reported that BCAA could reduce oxidative stress by removing reactive oxygen species. The utilization of feed ingredients, the overall growth performance, and the immune status of poultry are also affected by the population of bacteria and other microorganisms residing in their gut, and they are commonly referred to as gut microbiota or gut microbiome (Kiarie et al., 2013; Singh and Kim, 2021). Therefore, evaluating the effects of BCAA on the gut microbiota diversity of poultry is also critical before making a recommendation of the BCAA ratio in the diet. However, the analysis of the gut microbiota is still an evolving field of study in poultry, and there is limited information of BCAA role on gut microbiota diversity in poultry. It has been known that microbiota interact with both host and diet and vary with species type, age, location, and feed ingredients etc. (Pan and Yu, 2014). However, some similarity could be expected in microbiota-diet interaction in poultry, pigs, and mice because these species are hind-gut fermenters, and most of the microbial population from a donor species can be heritably colonized and reproduced in gnotobiotic animals (Turnbaugh et al., 2009; Heinritz et al., 2013; Kiarie et al., 2013).

Besides the 3 limiting AA (methionine, lysine, and threonine) in corn and soybean meal (**SBM**) diets for poultry (Ojano-Dirain and Waldroup, 2002; Singh et al., 2019), BCAA can also become limiting, and there is debate among researchers about exactly what should be considered the fourth and onward essential AA in



**Figure 2.** Functions of individual and mixed branched-chain amino acids on gut health functions, and schematic model of hypothesized mechanism of regulation of mTOR signal pathway.  $\uparrow$ , increase or induction;  $\downarrow$ , decrease or inhibition. The dotted lines represent expected pathways. BCAA, branched chain amino acid; mTOR, mammalian target of rapamycin; AMPK, AMP-activated protein kinase; TSC, tuberous sclerosis complex; Rheb, ras homolog enriched in brain; FOXO, forkhead box transcription factors; PKB/AKT, protein kinase B; P70S6K, phosphoprotein 70 ribosomal protein S6 kinase; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; eIF4B, eukaryotic translation initiation factor 4B.

poultry diets. The results from previous research are inconsistent, and there is support for either valine or isoleucine (Kidd et al., 2004; Corzo et al., 2009, 2010). Some researchers believe they should be co-limiting based on the dietary CP concentration and ingredient composition of the feed (Maynard et al., 2020). Furthermore, the essential AA requirements and their composition in the feed are presented either on a total basis or digestible basis or occasionally as a ratio to lysine by different researchers. The ileal digestibility of protein and AA in poultry is presented either as apparent ileal digestibility (AID) or as standardized ileal digestibility (SID). Previously AID has been defined as the digestibility of AA that does not consider the recovered endogenous AA in the flow of digesta whereas SID is estimated by correcting for the endogenous losses (Erdaw et al., 2017). Because the digestibility of AA varies depending on the composition of the diet, declaration of the digestible percentage of AA and their ratio to lysine would be more realistic for the comparison of AA ratio, but such information is limited in few available poultry research literature. This review comprehensively summarizes the effects of BCAA on performance, nutrient absorption, immunity, and gut microbiota of poultry

and provides the comparative requirements of BCAA in commercial guides for broilers, laying hens, and breeders along with those recommended by NRC (1994). Based on the current information about the requirements of BCAA, this review also describes the existing research gap and provides a direction for future research to update the recommended levels of BCAA for different breeding conditions.

## REQUIREMENTS AND RATIOS OF BCAA FOR GROWTH AND MEAT YIELD IN BROILERS

The effects of different concentrations and ratios of BCAA have been observed on the growth performance and carcass yield of broilers (Table 1). The commercial broiler nutrient specifications are updated frequently with the release of new strains and with changes in feeding schedules (Aviagen, 2019; Cobb-Vantress, 2020c), whereas the NRC (1994) guidelines for these AA are on a total basis and for 2 to 6 wk feeding phases (Table 2). The recommendations for BCAA are outlined for poultry based on the minimum CP requirements of the birds.

**Table 1.** Summary of inclusion level and effects of branched-chain amino acids in poultry in different experimental settings from the recent studies conducted within the latter half of the past decade.

BCAA level, basis, and design	Poultry age and type, diet type, energy level, and crude protein level	Performance response (FI, ADG, FCR, Digestibility, Egg production, meat yield)	Other physiological responses	References
<b>BCAA:</b> Val (65, 75 and 85 %), Ile (58, 66 and 74%), Leu (110, 130 and 150%) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> 3 level (-1, 0, +1), 3 factor Box-Behnken	<b>Age:</b> 22–35 d <b>Poultry:</b> Male & female; Lohman Indian River broilers <b>Diet:</b> Corn, SBM, peanut meal; Crumbled pellet <b>ME:</b> 3,100 kcal/kg <b>CP:</b> 18.13–19.46%	<b>↔Val, ↓Ile, Leu in female:</b> ↑BWG <sup>a</sup> , ↓FCR <sup>a</sup> ↑Val (Female: ↑ breast meat <sup>a</sup> ) ↑Ile, ↓Val, Leu (Male: ↑Carcass <sup>b</sup> ) ↑Ile (F: ↑breast meat <sup>a</sup> )		(Kidd et al., 2021)
<b>BCAA:</b> Leu: Ile: Val (0:0:0; or 1:0.25:0.25; or 1:0.25:0.75) <b>Basis:</b> Total, per kg diet <b>Design:</b> 2 × 3 factorial CRD	<b>Age:</b> 1–42 d <b>Poultry:</b> Mixed sex Ross 308 <b>Diet:</b> Corn, SBM; BCAA added in low CP diet; Mash <b>ME:</b> St: 3,050 kcal/kg Gr: 3,200 kcal/kg <b>CP:</b> St: 22% vs 20%, Gr:20%	<b>BCAA:</b> ↔ (BW, FCR, carcass) <sup>NS</sup> <b>BCAA (1:0.25:0.25):</b> ↓breast yield <sup>a</sup>		(Kop-Bozbay et al., 2021)
<b>BCAA:</b> Val (65, 75, and 85), Ile (58, 66, and 74), Leu (110, 130, and 150) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> Box-Behnken design	<b>Age:</b> 15–34 d <b>Poultry:</b> Male Cobb MV × 500 fast feathering broilers <b>Diet:</b> Corn, SBM, peanut meal <b>ME:</b> 12.97 MJ/kg <b>CP:</b> 18.1%	↑Leu: ↑FCR <sup>NS</sup> ↑Ile × ↓Val: ↓FCR <sup>a</sup> ↓Ile × ↑Val: ↓FCR <sup>a</sup>		(Maynard et al., 2021)
<b>BCAA:</b> Leu (ED-7: 34.5 μmol/500 μl per egg in yolk sac) <b>Basis:</b> Amount per egg <b>Design:</b> In ovo, heat challenge	<b>Age:</b> 29–44 d <b>Poultry:</b> Chunky broiler, post hatch male <b>Diet:</b> Commercial <b>ME:</b> 3,100 kcal/kg <b>CP:</b> 22%	↑BWG in chronic heat stress <sup>a</sup>	↑Leu, Ile in liver ↓Leu, Ile in plasma	(Han et al., 2020)
<b>BCAA:</b> 0 or 2 g blend of Leu: Ile: Val (3:1:2) <b>Basis:</b> Total per kg <b>Design:</b> 2 × 3 Factorial CRD	<b>Age:</b> 0–7 d <b>Poultry:</b> Turkey (Hybrid Converter) <b>Diet:</b> Corn, SBM, fish meal, sunflower meal <b>ME:</b> 12 MJ/kg <b>CP:</b> 23.94%	<b>2g BCAA:</b> ↑BW <sup>ab</sup> , ↑FI of 48 h delayed fed, ↓FI of 72 h delayed fed, ↑proventriculus and gizzard, liver, pancreas, and pectoralis muscle wt <sup>a</sup>		(Kop-Bozbay and Ocak, 2020)
<b>BCAA:</b> Val (75–78%) and Ile (67–69%) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> RCBD	<b>Age:</b> 0–48 d <b>Poultry:</b> Ross 708 broilers <b>Diet:</b> Corn, SBM, peanut meal, animal protein blend <b>ME:</b> 3,000–3,200 kcal/kg <b>CP:</b> 22–24.5%	<b>Supplemental BCAA:</b> ↑FI, breast meat, carcass, fat pad <sup>b</sup> ↓nitrogen excretion <sup>a</sup>		(Lee et al., 2020)
<b>BCAA:</b> Ile (66–99%, phase 1 and 2) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> CRD	<b>Age:</b> Phase1:20–27 wk, Phase 2: 28–46 wk <b>Poultry:</b> Shaver white hens <b>Diet:</b> Corn, SBM, wheat, DDGS, pork meal <b>ME:</b> 2,800 kcal/kg <b>CP:</b> 18 & 16% vs. 16 & 14%	↑Ile: ↑hen-day egg production and restored as high CP control <sup>a</sup> <b>90% Ile</b> had greater proportion of highest egg wt. <sup>NS</sup>		(Parenteau et al., 2020)
<b>BCAA:</b> Leu (6 mmol/kg) <b>Basis:</b> per kg body wt. <b>Design:</b> Oral administration	<b>Age:</b> 0–7 d <b>Poultry:</b> Local broiler <b>Diet:</b> Commercial <b>ME:</b> 3,050 kcal/kg <b>CP:</b> 23.5%		<b>Encapsulated Leu:</b> ↑Plasma glutamic oxaloacetic transaminase (GOT) at 4 h	(Erwan et al., 2020)
<b>BCAA:</b> Val (0.70, 0.75, 0.80, 0.85, 0.90, and 0.95%) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> CRD	<b>Age:</b> 8–21 d <b>Poultry:</b> Female Cobb 500 broilers <b>Diet:</b> Corn, SBM <b>ME:</b> 2,991 kcal/kg <b>CP:</b> 19%	↑Val: ↑BWG, ↓FCR <sup>a</sup>		(Amirdahri et al., 2020)
<b>BCAA:</b> Val (-0.8%) <b>Basis:</b> Digestible, lysine 100% <b>Design:</b> Deletion assay	<b>Age:</b> 0–14 d <b>Poultry:</b> Male and Female Cobb MV × 500 broilers <b>Diet:</b> Corn, SBM <b>ME:</b> 12.76 MJ/kg <b>CP:</b> 20.3 vs. 18.7%	↓Val: ↓BWG (-82g) in males <sup>b</sup>		(Maynard et al., 2020)
<b>BCAA:</b> Val (+0.2 to 1.2%) <b>Basis:</b> Digestible, per kg <b>Design:</b> 2 × 3 Factorial CRD	<b>Age:</b> 0–42 d <b>Poultry:</b> Ross 308 broilers <b>Diet:</b> Corn, SBM <b>ME:</b> 2,860 kcal/kg <b>CP:</b> 100%, 90% and 85% of (St-21.7%, Gr-20%, Fn-18%)	+ ↑Val in low CP restored growth in starter and grower phase + ↑Val in low CP diet ↑breast muscle <sup>a</sup>	+ ↑Val in low CP diet ↑VH:CD ratio <sup>a</sup>	(Allameh and Toghiani, 2019)
<b>BCAA:</b> Leu (Ed-7: 35 and 70 μmol/egg) <b>Basis:</b> Amount per egg <b>Design:</b> In ovo, heat challenge	<b>Age:</b> 0-10 d post hatch <b>Poultry:</b> Male broilers <b>Diet:</b> Commercial <b>ME:</b> 12.6 MJ/kg <b>CP:</b> 23%	↔ (BWG, FI) <sup>NS</sup>	↑Leu: ↓rectal temperature, plasma thyroxine at hatch <sup>b</sup> ; ↓rectal temperature in heat stress birds <sup>a</sup>	(Han et al., 2019)
<b>BCAA:</b> Blend Leu: Val: Ile (0.2% BCAA (3:1:2) in 0.9% normal saline in the yolk sac) <b>Basis:</b> Amount per egg <b>Design:</b> In ovo at Ed-22	<b>Age:</b> Ed-24 to hatch <b>Poultry:</b> Turkey (Hybrid Converter)	↑BCAA: ↓hatchability, embryo wt <sup>a</sup> , ↑BW, breast ms wt at hatch <sup>a</sup>		(Kop-Bozbay and Ocak, 2019)
				(Liu et al., 2018)

(continued)

BCAA: Leu (10, 20 and 40 mM) Basis: Cell culture Design: In vitro LPS challenge 0, 0.5, 1 and 2 µg/mL	Time: 2h Poultry: SPF chicken embryo small intestine		<u>↑Leu</u> : ↓SIgA; <u>40mM Leu</u> : restored mTOR and p70s6k phosphorylation, suppressed NF-κB, p38 MAPK and JNK phosphorylation, ↓IL-6 <sup>a</sup>	(Viana et al., 2017)
BCAA: Ile (55.61, 67, 73, and 79%) Basis: Digestible, lysine 100% Design: CRD	Age: 15–35 d Poultry: Unsexed meat-type quails Diet: Corn, SBM ME: 3,050 kcal/kg CP: 17.1%	<u>Ile</u> : quadratic response on BWG <sup>a</sup> , FI ↔ NS		
BCAA: Leu (1.0, 1.2, 1.4, 1.6, or 1.8%), Val (0.52, 0.67, 0.82, 0.97, or 1.12%) Basis: Digestible, per kg Design: 5 × 5 factorial CRD	Age: 21–42 d Poultry: Cobb 500 male broilers Diet: Corn, SBM, wheat bran, MBM ME: 3,125 kcal/kg CP: 16%	<u>Leu &amp; Val</u> : quadratic effect on FCR <u>↑Leu &amp; val</u> : ↓abdominal fat <sup>a</sup> <u>1.13 and 1.24% Leu</u> increased breast ms yield, while <u>0.71% Val</u> increased thigh ms yield	<u>↑Leu</u> : ↓triglycerides and β-hydroxybutyrate <sup>a</sup>	(Ospina-Rojas et al., 2017)
BCAA: Val (+0.1% (0-21d) to 0.2% (22–42 d) than NRC (1994)) Basis: Total, per kg Design: CRD	Age: 0–42 d Poultry: Ross 308 male broilers Diet: Corn, SBM, hazelnut kernel meal ME: 3,000–3,100 kcal/kg CP: 22–20%	<u>Val</u> : ↔growth parameters <sup>NS</sup>	<u>Val</u> : ↔spleen and bursa (NS) 1%(+0.1%) <u>Val</u> : ↑ thymus development	(Kaplan and Yildiz, 2017)
BCAA: Leu, Ile, Val: (35, 21 and 29 µmol) / 500 µL sterile water Basis: Amount per egg Design: In ovo, Ed-7, heat challenge	Age: Ed-7– d 6 Poultry: Chunky broiler eggs Diet: Commercial ME: 12.55 MJ/kg CP: 23%	<u>Leu</u> : ↑FI, BWG <sup>a</sup> <u>Ile, Val</u> : no significant response	<u>Leu</u> : ↓body temperature at hatch <sup>a</sup>	(Han et al., 2017)
BCAA: Leu(1.16, 1.94, and 2.73%); Ile & Val in the same ratio as the standard Basis: Total, per kg Design: 2 × 3 factorial, aflatoxin challenge	Age: 0–20 d Poultry: Ross 708 male broilers Diet: Corn, SBM ME: 3,184 kcal/kg CP: 22.07-22.55%	<u>↑BCAA</u> : ↑BWG, FI, G:F, breast ms <sup>a</sup> , ↓ileal digestibility of nitrogen and 9 AA <sup>a</sup> ↑BCAA: ↑villus height <sup>b</sup>		(Chen et al., 2016)
BCAA: Val (0.77), Ile (0.67) Basis: Digestible, lysine coefficient Design: RCBD	Age: 0–42 d Poultry: Cobb 500 male broilers Diet: Corn, SBM ME: 12.39 MJ/kg CP: 18.4 to 22.4%	<u>Val &amp; Ile</u> in this ratio maintained competitive growth performance		(Miranda et al., 2015)
BCAA: Val (72 vs. 77%), Ile (63 vs. 69%) Basis: Digestible, lysine 100% Design: CRD	Age: 14–28 d Poultry: Cobb 500 female broilers Diet: Corn, SBM ME: St: 2,900 kcal/kg; Gr: 3,075 kcal/kg CP: Gr: 18.7 vs. 20%	<u>BCCA</u> : ↔growth parameters <sup>NS</sup>	<u>↓BCAA</u> : ↓hepatic lipogenic genes <sup>a</sup> , ↑hepatic mitochondrial β-oxidation genes <sup>a</sup> , ↑phosphorylation of AMPKα <sup>a</sup> , ↓phosphorylation of mTOR and P70S6K <sup>a</sup>	(Bai et al., 2015)
BCAA: Leu: Val: Ile (2:1:1) 0.2% in 0.5% saline (2 g/5 g NaCl in either yolk sac or amnion or albumin) on Ed-18 Basis: Amount per egg Design: In ovo, 3 × 2 factorial arrangement	Age: 1–56 d Poultry: Broiler eggs from Ross × Rhode Island Red breeders Diet: Corn, SBM, full fat soybean ME: 13.03 MJ/kg CP: St:26.28, Gr:22.12, Fn:20.78%	<u>BCCA, in ovo</u> : ↔growth performance <sup>NS</sup> <u>BCAA (in albumin)</u> : ↑gizzard wt. <u>BCAA (in amnion or yolk sac)</u> : ↓gizzard wt.		(Kop-Bozbay and Ocak, 2015)
BCAA: Each BCAA 1% of their level in egg (Ile:2.71 mg, Leu: 4.53 mg, Val: 4.25 mg) dissolved in Dulbecco's PBS making 62, 100 and 85mM, respectively. Basis: Amount per egg Design: In ovo before incubation	Age: Ed: 1–21 Poultry: Layer eggs from Single Comb White Leghorn	<u>Each BCAA</u> : ↑Embryo wt. <u>Leu, Val</u> : ↓hatch time <u>BCCA</u> : ↔BW at hatch <sup>NS</sup>		(Kita et al., 2015)
BCAA: Val (0, 1, 2, 3, and 4 g) Basis: Total, per kg Design: CRD	Age: 40–47 wk Poultry: HyLine Brown layers Diet: Corn, SBM, peanut meal, wheat bran ME: 11.31 MJ/kg CP: 16.16%	<u>Val</u> : ↔egg production parameters and FCR <sup>NS</sup> <u>Val</u> : quadratic response on FI, high at 2 g/kg	<u>Val</u> : quadratic response of serum glucose peaking at 2 g/kg <u>Val (2 g/kg)</u> : ↑serum triiodothyroxine	(Azzam et al., 2015)

Column3: Underlined texts are treatment conditions and texts without the underline are responses.

↔ Represents no difference. ↑ Represents increased or high or upregulated. ↓ Represents decreased or low or downregulated.

Abbreviations: BCCA, branched-chain amino acids; Leu, leucine; Ile, isoleucine; Val, valine; FI, feed intake; ADG, average daily gain; FCR, feed conversion ratio; BW, body weight; BWG, body weight gain; SBM, soybean meal; DDGS, distillers dried grains with solubles; MBM, meat and bone meal; NS, not significant; ME, metabolizable energy; CP, crude protein, Ed, embryonic day; CRD, completely randomized design; RCBD, randomized block design; St, starter; Gr, grower; d, day; h, hour; wk, week; SPF, Specific-pathogen-free; LPS, lipopolysaccharides; SIgA, secretory immunoglobulin A.

<sup>a</sup>Denotes significant response.

<sup>b</sup>Denotes response with trend.

However, a proper ratio of BCAA is required for ideal utilization, otherwise the BCAA in excess of the limiting AA would be deaminated and used for energy, rather than for protein synthesis. Chen et al. (2016) reported that increasing leucine from 1.88 to 2.73% of the diet with fixed concentrations of isoleucine at 59% and valine at 69% of leucine in the diet increased body weight gain (BWG) of broilers by 45%, and improved their feed intake, feed efficiency, and breast muscle weight during

a 20-d study period. Lee et al. (2020) fed broilers from d 0 to 48 with supplemental BCAA (L-valine and L-isoleucine) in peanut meal and animal protein blend added diet to meet the same ratio as in a corn-SBM diet (valine, 75–78% of lysine; isoleucine 67–69% of lysine). This BCAA supplementation in the diet with CP reduced by 1.62% maintained broilers BWG and feed conversion ratio (FCR) and reduced litter nitrogen content by 8.5%. The processing of these birds resulted in

**Table 2.** Recommendations for intake and feed composition of the branched-chain amino acids for the major commercial breeds and other poultry birds.

Poultry	Branched-chain amino acids recommendation by age of birds										References			
Cobb 500 broilers	Digest.	0–8 d	9–18 d	19–28 d	>29 d	Digest.	0–8 d	9–18 d	19–28 d	>29 d	(Cobb-Vantress, 2018)			
	Val/kg, %	0.89	0.85	0.76	0.73	Ile/kg, %	0.77	0.72	0.67	0.64				
	Val: Lys	73	75	75	75	Ile: Lys	63	64	65	66				
Cobb 700 broilers	Digest.	0–14 d	15–27 d	28–38 d	39–51 d	Digest.	0–14 d	15–27 d	28–38 d	39–51 d	(Cobb-Vantress, 2020c)			
	Val/kg, %	0.93	0.87	0.83	0.78	Ile/kg, %	0.83	0.78	0.72	0.68				
	Val: Lys	74	75	77	78	Ile: Lys	66	67	67	68				
Cobb 500 & 700 breeders fast and slow feathering	Digest.		0–28 d	29–105 d		Digest.	106 d –1 egg	1 egg–266 d	>267 d	Male >168 d	(Cobb-Vantress, 2020a, 2020b)			
	Val/kg, %		0.67	0.45		Val/kg, %	0.47 0.48 <sup>b</sup>	0.50 0.47 <sup>a,b</sup>	0.47 0.45 <sup>a,b</sup>	0.38				
	Val: Lys		72	75		Val: Lys	75	75	75	75				
	Ile/kg, %		0.64 0.63 <sup>b</sup>	0.42		Ile/kg, %	0.44	0.46 0.44 <sup>a,b</sup>	0.44 0.42 <sup>a,b</sup>	0.40				
Ross 308 and Ross 708 broilers	Digest.	0–10 d	11–24 d	25–39 d	40–46 d	Digest.	0–10 d	11–24 d	25–39 d	40–46 d	(Aviagen, 2019)			
	Val/kg, %	0.96	0.87	0.78	0.75	Ile/kg, %	0.86	0.78	0.7	0.66				
	Val/Lys	0.75	0.76	0.76	0.78	Ile/Lys	0.67	0.68	0.69	0.69				
	Leu/kg, %	1.41	1.27	1.12	1.06									
	Leu/Lys	1.10	1.10	1.10	1.10									
Ross 308 breeders fast feathering	Digest.		0–21 d	22–42 d	43–105 d	Digest.	106 d– 5% eggs	>5% eggs to 224 d	225–350 d	> 351 d	Males >175 d	(Aviagen, 2021)		
	Val/kg, %		0.81	0.72	0.6	Val/kg, %	0.57	0.64	0.6	0.56	0.47			
	Val/Lys		0.81	1.00	1.15	Val/Lys	1.16	1.03	1.07	1.08	1.34			
	Leu/kg, %		1.20	1.03	0.82	Leu/kg, %	0.79	0.95	0.90	0.86	0.66			
	Leu/Lys		1.20	1.43	1.58	Leu/Lys	1.61	1.53	1.61	1.65	1.89			
	Ile/kg, %		0.7	0.58	0.47	Ile/kg, %	0.44	0.52	0.5	0.49	0.41			
Ross 308 breeders slow feathering	Digest.		0–21 d	22–35 d	36–105 d	Digest.	106 d– 5% eggs	>5% eggs to 245 d	246–350 d	> 351 d	Males (Lay-house)	(Aviagen, 2021)		
	Val/kg, %		0.71	0.63	0.44	Val/kg, %	0.45	0.56	0.53	0.51	0.37			
	Val/Lys		0.75	0.94	0.85	Val/Lys	0.87	0.93	0.95	0.98	0.84			
	Leu/kg, %		1.11	0.83	0.69	Leu/kg, %	0.72	0.94	0.9	0.86	0.52			
	Leu/Lys		1.17	1.24	1.33	Leu/Lys	1.38	1.57	1.61	1.65	1.18			
	Ile/kg, %		0.62	0.55	0.4	Ile/kg, %	0.41	0.5	0.48	0.45	0.34			
Ross 708 breeders	Digest.		0–28 d	29–133 d		Digest.	134 d to 5% eggs	>5% egg to 280 d	281–350 d	> 351 d	Males >175 d	(Aviagen, 2021)		
	Val/kg, %		0.8	0.64		Val/kg, %	0.6	0.62	0.59	0.58	0.47			
	Val/Lys		0.84	1.05		Val/Lys	1.07	1.03	1.04	1.07	1.34			
	Leu/kg, %		1.2	0.84		Leu/kg, %	0.79	0.9	0.86	0.85	0.66			
	Leu/Lys		1.26	1.38		Leu/Lys	1.41	1.50	1.51	1.57	1.89			
	Ile/kg, %		0.68	0.5		Ile/kg, %	0.45	0.52	0.49	0.47	0.41			
Hy-line W-36 layers	Digest.	0–21 d	22–42 d	43–84 d	85–105 d	Digest.	106– 119 d	120–245	246–385	386–504	505–595	>596	(Hy-line, 2020)	
	Ile/kg, %		0.74	0.71	0.65	0.57	Ile/kg, %	0.62	0.67	0.62	0.59	0.55		0.52
	Ile/Lys		0.70	0.72	0.74	0.75	Ile/Lys	0.79	0.80	0.81	0.80	0.79		0.79
	Val/kg, %		0.76	0.73	0.69	0.61	Val/kg, %	0.66	0.74	0.68	0.65	0.61		0.57
	Val/Lys		0.72	0.74	0.78	0.80	Val/Lys	0.85	0.88	0.88	0.88	0.87		0.86
Hy-line W-80 layers	Digest.	0–21 d	22–42 d	43–84 d	85–105 d	Digest.	106– 119 d	120–259	260–336	337–434	435–532	>533	(Hy-line, 2019)	
	Ile/kg, %		0.74	0.71	0.65	0.57	Ile/kg, %	0.62	0.67	0.62	0.59	0.55		0.52
	Ile/Lys		0.70	0.72	0.74	0.75	Ile/Lys	0.79	0.80	0.81	0.80	0.79		0.79
	Val/kg, %		0.76	0.73	0.69	0.61	Val/kg, %	0.66	0.74	0.68	0.65	0.61		0.57
	Val/Lys		0.72	0.74	0.78	0.80	Val/Lys	0.85	0.88	0.88	0.88	0.87		0.86

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**Table 2** (Continued)

Poultry	Branched-chain amino acids recommendation by age of birds											References
Hy-line brown layers	Ile/kg, %	0.72	0.67	0.62	0.51	0.58	0.62	0.57	0.54	0.53	0.52	(Hy-line, 2018)
	Ile/Lys	0.71	0.71	0.74	0.75	0.78	0.78	0.78	0.77	0.78	0.79	
	Val/kg, %	0.74	0.69	0.66	0.55	0.63	0.7	0.64	0.61	0.59	0.58	
	Val/Lys	0.73	0.73	0.79	0.81	0.85	0.88	0.88	0.87	0.87	0.88	
	Digest.	0–21 d	22–42 d	43–84 d	85–105 d	106–119 d	120–259 (1st egg to peak–2%), 98 g/d	260–336 (Peak–2% to 90%), 110 g/d	337–434 (89–85%), 110 g/d	435–532 (84–80%), 109 g/d	>533 (<80%), 108 g/d	
Hy-line brown parents stock	Ile/kg, %	0.71	0.66	0.61	0.45	0.56	0.67	0.58	0.57	0.56	0.55	(Hy-line, 2016)
	Ile/Lys	0.70	0.72	0.74	0.75	0.78	0.80	0.79	0.80	0.80	0.80	
	Val/kg, %	0.73	0.68	0.64	0.48	0.61	0.74	0.64	0.62	0.61	0.6	
	Val/Lys	0.72	0.74	0.78	0.80	0.85	0.88	0.88	0.87	0.87	0.87	
	Digest.	0–21 d	22–42 d	43–84 d	85–112 d	113–126 d	127–252 (1st egg to peak–2%), 103 g/d	253–378 (Peak–2% to 84%), 111 g/d	379–441 (83–77%), 111 g/d	442–525 (<77%), 112 g/d		
Lohman LSL-Lite /brown lite layers and parent stock	Ile/kg, %	0.75	0.63	0.58	0.54	0.61	0.62	0.56	0.54	0.51	(Lohmann-breeders, 2019, 2020)	
	Ile/Lys	0.70	0.72	0.73	0.75	0.78	0.78	0.78	0.78	0.78		
	Val/kg, %	0.77	0.65	0.62	0.58	0.66	0.70	0.63	0.61	0.57		
	Val/Lys	0.72	0.74	0.78	0.81	0.85	0.88	0.88	0.88	0.88		
	Digest.		Layers 0–21 d	0–56 d	57–199 d	200–5% eggs	Parent Stock 0–21 d	28–56 d	57–126 d	127–5% eggs		
Immature Leghorn type chickens	White-egg layer					Brown-egg layer						(NRC, 1994)
	Total	0–6 wks	6–12 wks	12–18 wks	18 wks–1 egg	0–6 wks	6–12 wks	12–18 wks	18 wks–1 egg			
	Ile/kg, %	0.60	0.50	0.40	0.45	0.57	0.47	0.37	0.42			
	Ile/Lys	0.71	0.83	0.89	0.87	0.71	0.84	0.88	0.86			
	Leu/kg, %	1.10	0.85	0.70	0.80	1.00	0.80	0.65	0.75			
	Leu/Lys	1.29	1.42	1.56	1.54	1.25	1.43	1.55	1.53			
	Val/kg, %	0.62	0.52	0.41	0.46	0.59	0.49	0.38	0.43			
	Val/Lys	0.73	0.87	0.91	0.88	0.74	0.88	0.90	0.88			
Turkey, 90% DM- total basis	Male	0–4 wks	4–8 wks	8–12 wks	12–16 wks	16–20 wks	20–24 wks	Breeders			(NRC, 1994)	
	Female	0–4 wks	4–8 wks	8–11 wks	11–14 wks	14–17 wks	17–20 wks	Holding	Laying hens			
	Ile/kg, %	1.10	1.00	0.80	0.60	0.50	0.45	0.40	0.50			
	Ile/Lys	0.69	0.67	0.62	0.60	0.63	0.69	0.80	0.83			
	Leu/kg, %	1.90	1.75	1.50	1.25	1.00	0.80	0.50	0.50			
	Leu/Lys	1.19	1.17	1.15	1.25	1.25	1.23	1.00	0.83			
	Val/kg, %	1.20	1.10	0.90	0.80	0.70	0.60	0.50	0.58			
	Val/Lys	0.75	0.73	0.69	0.80	0.88	0.92	1.00	0.97			
Broilers, Pekin ducks, Japanese quail; 90% DM, total basis	Broilers					White Pekin ducks		Japanese quail (Coturnix)				(NRC, 1994)
	Total	0–3 wks	3–6 wks	6–8 wks		0–2 wks	2–7 wks	Breeding	Starter + grower	Breeding		
	Ile/kg, %	0.80	0.73	0.62		0.63	0.46	0.38	0.98	0.90		
	Ile/Lys	0.73	0.73	0.73		0.70	0.71	0.63	0.75	0.90		
	Leu/kg, %	1.20	1.09	0.93		1.26	0.91	0.76	1.69	1.42		
	Leu/Lys	1.09	1.09	1.09		1.40	1.40	1.27	1.30	1.42		
	Val/kg, %	0.90	0.82	0.70		0.78	0.56	0.47	0.95	0.92		
	Val/Lys	0.82	0.82	0.82		0.87	0.86	0.78	0.73	0.92		

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Table 2 (Continued)

Poultry		Branched-chain amino acids recommendation by age of birds		References	
Other research recommendations	Poultry	Basis and BCAA	Age	Requirement	
	Shaver White	Digestible, Ile/Lys, %	20–46 wk	82–88	(Parenteau et al., 2020)
	Female Cobb 500	Digestible, Val/Lys	3 wk	0.78	(Amirdahri et al., 2020)
	Unsexed meat-type quails	Digestible, Ile/Lys, %	2–5 wk	67	(Viana et al., 2017)
	Cobb 500 male broilers	Digestible, Leu/kg, %	3–6 wk	0.86 & 1.15	(Ospina-Rojas et al., 2017)
	Ross 308 male broiler	Digestible, Val/kg, %	3–6 wk	0.86 & 1.19	(Kaplan and Yildiz, 2017)
		Total, Val/kg, %	1–3 wk	1%	(Nascimento et al., 2016)
	Male Cobb700 broilers	Digestible, Val/kg, %	3–6 wk	0.82%	
			0–8 d	1.028	
			9–21 d	0.905	
			22–42 d	0.853	
			Breast ms	0.826	
			(22–42 d)		

Abbreviations: BCAA, branched-chain amino acids; Digest-, digestible; Ile, isoleucine; Lys, lysine; Leu, leucine; Val, valine

<sup>a</sup>Cobb 500 slow feathering.

<sup>b</sup>Cobb 700 fast and slow feathering; wks, weeks; d, days; ms, muscle.

higher breast fillet, increased total white meat, and greater fat pad weight than in BCAA groups in the above study. Both valine (+0.075 to 0.15%) and isoleucine (+0.075 to 0.15%) supplementation to a negative control diet (0.73% isoleucine and 0.86% valine) decreased the FCR in broilers raised until d 21, and their effect was additive until the 0.15% inclusion level (Corzo et al., 2009). The authors also observed a higher body weight and increased plasma total protein and albumin in response to L-valine addition alone, and a further improvement in these parameters was recorded for supplementation of both L-valine and L-isoleucine until their additional inclusion of 0.15%. A higher inclusion of valine-containing ingredients is essential, as the diet with valine deficiency can lead to impaired growth performance along with abnormality in feathers and legs in chicken (Amirdahri et al., 2020). The requirement for valine needs to be met before any further improvement can occur with isoleucine supplementation. Hence, valine could be regarded as the fourth limiting AA in chicken raised on a corn-SBM-based diet (Corzo et al., 2009; Berres et al., 2010).

Corrent and Bartelt (2011) reviewed several pieces of literature on AA use in broilers and reported that the relative concentration of isoleucine should be 70 to 80% of lysine and valine to be around 80% of lysine as a frequently used inclusion level in most of the studies. The authors also discussed that the ratio of leucine to lysine was variable across most of the study reports, but it was higher in the starter phase and lower in the finisher phase and mostly remained around 130%. When Corzo et al. (2008) reduced SID of isoleucine from 0.71 to 0.58% in heavy broilers from d 35 to 54, their BWG was reduced by 5%, and the FCR increased by 6%. The increase in the inclusion of isoleucine from 0.58 to 0.66% in this study reversed the poor performance but the addition of arginine (having a higher CP equivalency) to the low isoleucine diet did not reverse the drop in performance, suggesting that it was isoleucine specific and not dependent on nonessential N. Thus, the authors concluded that the inclusion of isoleucine above the theoretically marginal level of 0.58% SID is essential in a 2% meat and bone meal corn-SBM diet. In a broiler study where test diets were fed in the finisher phase (d 28–42), the negative control with reduced isoleucine and valine levels by 1 g/kg each had depressed BWG that improved when valine and isoleucine were added back in, but BWG was more responsive to valine (Corzo et al., 2010). However, the breast yield was more responsive to isoleucine, whereas the gain to feed ratio and reduction in abdominal fat responded better to a combined supplementation of isoleucine and valine. The breast meat yield in Ross × Hubbard male broilers has also been found to be reduced in response to an isoleucine deficient diet (Kidd et al., 2000), indicating that isoleucine is important for body composition modulation and meat yield in broilers. Corzo et al. (2004) reported that 0.73% inclusion of valine in Ross × Ross 308 males was optimum for growth and carcass weight during the 56-d trial period. Supplementation of 50% more isoleucine in an



isoleucine deficient (isoleucine 0.51%) diet from 3 to 8 wk in male broilers produced growth performance similar to that of a positive control (PC) diet containing balanced AA supplied via SBM and blood cells (Kidd et al., 2004). A low isoleucine diet (0.64%) has also been reported to depress the growth performance and deteriorated the feed efficiency of cockerels (Peterson × Hubbard) raised for d 0 to 16 even when a 5% additional other AA (except arginine and lysine) was added to the diet (Park and Austic, 2000). These authors used piecewise regression to determine that isoleucine needed to be between 0.63 and 0.65% of the diet (3.28–3.38% of CP). They noticed that the isoleucine imbalance was further aggravated by the addition of other AA in large amounts except supplemental arginine and lysine in the diet. The AID of isoleucine/lysine percentage was found to be ideal around 66% for d 7 to 21 and 68% for d 30 to 43 in male Cobb broilers, and the response of added isoleucine was quadratic in the starter phase in the study of (Tavernari et al., 2012). Therefore, proper inclusion levels and ideal ratios of isoleucine to other AA, preferably other BCAA and lysine, are required for better performance and good breast meat yield in meat birds even after meeting the requirements of major limiting AA. The requirements for valine and isoleucine are also interdependent; however, their optimal levels are mostly affected by the concentration of leucine in the diet.

Among BCAA, leucine is especially known to stimulate protein synthesis (Deng et al., 2014; Bai et al., 2015), but additional leucine can also reduce the plasma concentration of isoleucine and valine, so the optimal amount of leucine in the diet should be determined based on the types of ingredients used in the feed. The addition of 0.5% leucine in the broiler diet has been observed to increase the carcass weight by 9% when the birds were fed experimental diets from 3 to 6 wk of age in a study by Erwan et al. (2008), and the same level of leucine addition for the same age broiler decreased fat deposition in another study by Erwan et al. (2011). Feeding encapsulated leucine as an oral bolus injection (6 mmol/10 mL kg<sup>-1</sup> body weight) in 7-day-old broilers reduced plasma valine and isoleucine in a recent study (Erwan et al., 2020). Moreover, feeding 3.06% of leucine for 7 d in 1-wk-old broilers receiving low levels of isoleucine and valine (~0.8% each) reduced growth performance, whereas increasing the dietary levels of isoleucine and valine (~1.2%) restored the growth parameters, suggesting a toxic effect of leucine if added to low CP diet with imbalanced BCAA ratios (Farran et al., 2003). Antagonism among these BCAA in chicks has been confirmed (Smith and Austic, 1978). In a study conducted 3 decades earlier in 3-wk-old broilers by Farran and Thomas (1990), it was observed that feeding low CP (18.4%) or high CP (20.6%) did not affect the performance as long as the diets contained the required amount of essential AA. The authors concluded that the inclusion of leucine, isoleucine, and valine should be 1.16, 0.80, and 0.90%, respectively at 3,200 kcal of metabolizable energy per kilogram diet for

optimum growth performance for 3-wk-old birds. In this study, only 4 replicates were used per treatment, thus low statistical power, but it provides a direction for targeting better growth performance of chickens even at lower CP diets. Dietary supplementation of 1.5 times higher leucine (1.635%) along with 1.5 times higher isoleucine (1.095%) and valine (1.230%) in a broiler diet from d 18 to 28 in the study of Imanari et al. (2008) increased free glutamate (the taste-active component in meat) in the meat soup by 30% and increased the score of taste intensity in the meat during sensory evaluation. In a recent study by Kidd et al. (2021), the increase in dietary leucine in the female Lohman Indian River broiler diet impaired growth performances that were only offset by increasing the level of dietary isoleucine, suggesting that these females were sensitive to the dietary BCAA ratio changes. In the previous study, increasing the level of isoleucine while decreasing levels of leucine and valine improved carcass yield in female broilers with the best response attained when leucine:lysine and valine:lysine was 129 and 75, respectively. Moreover, the authors noted that increasing isoleucine and valine provided higher breast meat yield in broilers, but additional leucine caused lower yield.

In a study with female Cobb broilers from d 15 to 35 by Maynard et al. (2020), it was observed that low CP (19%) diets with reduced isoleucine (0.78 vs. 0.82% in the PC diet) and leucine (1.57% vs. 1.66% in the PC diet) had 6% higher wing weight compared with those birds fed a diet low in valine (0.87% vs. 0.91% in the PC diet). The authors did not find an effect for reduced BCAA on other meat qualities such as woody breast and carcass weight of female broilers. Feed intake was higher in the low isoleucine and leucine groups compared to the low valine group in the previous Maynard et al. (2020) study, and this could have provided variable levels of AA per bird. The authors, in that study experimented with male Cobb broilers, reported that the low isoleucine group had better FCR than the low valine or leucine groups, but the feed intake was not different between treatments. Maynard et al. (2020) also studied the effect of valine and leucine levels in feed on the carcass traits of male Cobb broilers and reported that the low isoleucine group had a higher carcass weight than the low valine group. The reduced isoleucine and leucine-fed male broilers also had a 6.6% higher wing weight when compared to low valine-fed male broilers. Leeson and Summers (2005) found that isoleucine and valine needed to be 79 and 89% of lysine, respectively. Miranda et al. (2014) observed that maintaining ratios of valine to lysine of 0.77 and isoleucine to lysine of 0.67, respectively on a digestible basis without restricting the CP level produced the best performance and low-fat deposition in broilers raised for 6 wk. The authors suggested that a reduced CP diet should be supplemented with both isoleucine and valine to maintain a similar performance but in their study, they did not recommend the inclusion level of these BCAA. In a study by Miranda et al. (2015), the authors compared the effect of a synthetic source of isoleucine and valine with that

supplied through the feed ingredients, but did not find any difference in the growth performance of broilers. Ospina-Rojas et al. (2014) studied the effect of supplementing valine, isoleucine, and valine + isoleucine in broilers fed negative control (NC) diet with reduced CP (−3%), valine (−15%), and isoleucine (−17%) until 6 wk of age to equal the level of these AA in the control diet. The authors noted that FCR was only improved numerically by supplemental valine and isoleucine, whereas BWG was increased by 11% by valine + isoleucine, and serum ammonia was reduced by around 30% by valine, isoleucine, or a combination when compared with NC. In another study by Ospina-Rojas et al. (2017) in broilers, different levels of leucine and valine were fed from d 21 to 42. The interaction between leucine and valine was observed on feed intake and BWG, and a quadratic effect with minimal point estimated to be 1.19% for leucine and 0.86% for valine was noted for FCR. In the same study, the authors reported that breast yield was the highest at 1.13% leucine. Pectoralis major fiber diameter was increased by the diet containing 1.24% leucine, and thigh yield was the highest for 0.71% valine in the diet, which would warrant the consideration of leucine level for increasing valine to optimize performance. The authors also reported that the addition of leucine linearly decreased the serum concentrations of triglycerides and  $\beta$ -hydroxybutyrate, whereas the abdominal fat decreased linearly with the increasing levels of both leucine and valine. This observation is intriguing because the increase in the bodyweight of birds in response to leucine and valine supplementation would be expected to increase more muscle weight than the deposition of adipose tissue. In contrast, in a recent study by Kop-Bozbay et al. (2021) in Ross broilers from d 16 to 42, the BCAA blend did not improve growth performance. Pastor et al. (2013) used a nonlinear regression model to determine the ideal lysine: leucine: isoleucine: valine in male broilers during d 10–20 and d 25–35 and concluded that the demand for leucine and valine is higher during the later growth phase, so they increased the optimum ratio from 100:94:55:65 in the starter phase to 100:106:56:72 during the finisher phase. The existing research literature and the recommended breed standards state that lower concentrations of BCAA are required for optimal growth of the birds as they age; however, the ratios of BCAA to lysine increase during the later feeding phases (Allameh and Toghyani, 2019; Aviagen, 2019; Lee et al., 2020; Cobb-Vantress, 2020c).

## REQUIREMENTS AND EFFECTS OF BRANCHED-CHAIN AMINO ACIDS IN LAYING HENS

BCAA are expected to play an important role in egg production in layers, as hepatic yolk-lipoprotein production via fatty acid metabolism in the liver is regulated by BCAA (Macelline et al., 2021). The commercial layer nutrient specification guides for Hy-line and Lohman

breeds do not include recommendations for leucine inclusion, whereas NRC (1994) mentions leucine requirements in layers and breeders (Table 2). The requirements for BCAA vary by the growth period and laying status and have been reported either as digestible, total or as a percentage of lysine for laying hens (Table 2).

Recently, Macelline et al. (2021) reviewed the AA requirements in laying hens and reported that there were inconsistencies in the isoleucine requirements, but valine requirements were 614 mg/bird daily on a total basis and 532 mg/bird on a digestible basis, and dietary valine intake was correlated positively with feed intake. Machlin (1955) studied the effect of leucine in Rhode Island Red and Single Comb White Leghorn pullet's performance and indicated that the leucine requirement should not be higher than 1% of a diet formulated at 16% CP. When blood meal was used as the main protein source, Miller et al. (1954) determined that 0.53% L-isoleucine was required for the maintenance and egg production in Single Comb White Leghorn pullets, but the CP and apparent metabolizable energy (AME) values of the diet used were not reported. Parenteau et al. (2020) studied the effect of adding isoleucine to a low CP (2% less than control in each phase) diet in Shaver white hens from wk 20 to 46, and the increasing percentage of isoleucine to lysine restored the hen-day egg production loss (1st phase 3.3% and 2nd phase 1.5%) due to low CP diet compared with adequate CP diet. Moreover, 90% isoleucine to lysine content in the above study produced a higher proportion of large eggs (56 g–63 g), and the authors observed that the optimum response was generated when isoleucine was supplemented to reach 82 to 88% of lysine content on SID basis in the diet. Interestingly, the concentration of isoleucine in the diet could be tweaked to produce large size eggs at the expense of hen-day egg production and offset the negative effects of low CP on hens' live performance.

Peganova and Eder (2003) found that an interaction existed between isoleucine and valine + leucine inclusion in laying hen diets, where an increase in isoleucine (0.57–1.15%) at the lowest levels of valine + leucine (0.63 and 0.72%, respectively) reduced egg mass and BWG, while, at high valine + leucine (1.01 and 1.15%, respectively), high isoleucine only caused small depression in performance. In an earlier study in laying hens by Peganova and Eder (2002), maximum nitrogen retention was achieved by having isoleucine amounts between 0.43 and 0.57%, whereas concentrations above 0.8% reduced body weight and those above 1.0% decreased egg mass. When Dong et al. (2016) supplemented digestible isoleucine to increase from 0.54 to 0.94% in a low CP (14%) diet, no difference was observed in laying performance or mucosal and immune parameters. The requirements for isoleucine, leucine, and valine for maintenance broiler breeder hens were determined to be 94, 52, and 155 mg/kg<sup>0.75</sup>/d, respectively, on a metabolic weight basis and 329, 172, and 546 mg/kg CP/d, respectively, on a metabolic protein basis in a study by Sakomura et al. (2015) using a linear regression model for protein intake vs. AA intake. Lima et al. (2018)

estimated that the efficiency of valine and isoleucine utilization by 30-wk-old broiler breeders to be 0.70 and 0.66%, respectively, where the daily intake was estimated to be 803 mg/d for valine and 708 mg/d for isoleucine using a broken line model. In a recent laying hen trial from 33 to 41 wk, valine was added at different levels from 0.59 to 0.79% where the feed intake was around 100 g/d/bird (Jian et al., 2021a). The authors reported that the egg mass, laying rate, and trypsin activity in the duodenum, and AA transporter gene expression levels were increased with increasing valine, whereas the Haugh unit, eggshell thickness, egg yolk color, and immune parameters decreased. In a study by Azzam et al. (2015) in laying hens, increasing the L-valine concentration in the diet by 29% (9 g/kg vs. 7 g/kg) increased serum albumen by 4% when compared to the control diet. The authors did not observe any variation in the immunoglobulin concentration in response to L-valine supplementation, while serum glucose and triiodothyronine increased by 44 and 25%, respectively, when L-valine was supplemented at 2 g/kg compared with the control diet. The birds in this study tolerated L-valine up to 1.12% inclusion, but there was no difference in egg production or serum metabolites. Bregendahl et al. (2008) recommended isoleucine and valine as 79 and 93% of lysine for 28- to 34-wk-old layers. The AA requirements specified by NRC (1994) for the same age layers are higher for both isoleucine (94% of lysine) and valine (101% of lysine).

### REQUIREMENTS OF BRANCHED-CHAIN AMINO ACIDS IN OTHER POULTRY SPECIES

There have been some reports in the literature about the essential AA requirements in poultry species other than chicken, but the information about the BCAA requirement and their optimum ratios are limited. Turkey and ducks are also reared on commercial poultry feed for meat production and further knowledge about the optimum BCAA inclusion ratio would help to increase the productivity and feed efficiency of these birds. In the limited work that has been done, L-valine supplementation study was conducted in a graded level up to 1.27% by Timmler and Rodehutscord (2003) in 0 to 21 d White Pekin ducks fed a basal diet containing 0.68% valine, 18% CP, and 2,990 kcal ME/kg, and no effect was observed. However, the authors determined that 0.7% valine was optimal to achieve 95% protein accretion. The study of BCAA has also been reported in breeder Japanese quail by Hanafy and Attia (2018) where the authors determined 18% CP and 0.2% L-valine was optimal for better growth performance and egg productivity of the quail. In a study by Kop-Bozbay and Ocak (2020) in turkey poults, the BCAA blend of L-leucine, isoleucine, and valine in the ratio of 3:1:2 was fed to 1-wk-old birds that were either fed immediately or delay-fed after 48 or 72 h. The result showed that the BCAA blend tended to increase BWG

in all groups and enhanced pectoral muscle growth in delay-fed birds.

### IN OVO INJECTION OF BCAA ON EMBRYO DEVELOPMENT AND HATCHABILITY

Besides the importance of providing specific nutrients to birds post-hatch, in ovo feeding techniques are also evolving, thus adequate nutrients and bioactive compounds can be provided to the late-term embryos, potentially resulting in better growth performance post-hatch. Kita et al. (2015) injected about 1% leucine and isoleucine, which were equivalent to 453 mg leucine and 271 mg isoleucine in egg, in ovo beneath the air sac of eggs before incubation and reported that it accelerated the embryo growth and reduced the hatching time of chicks. In ovo feeding of L-leucine has been reported to improve thermotolerance and increase BWG of broilers during heat stress (Chowdhury et al., 2020; Han et al., 2020; Table 2). Compared to isoleucine or valine, in ovo injection of 35  $\mu$ mol leucine/500  $\mu$ L of sterile water in the yolk sac of the embryo on d 7 significantly reduced the body temperature at hatching and improved chicks' weight until 5 d of age by Han et al. (2017). Moreover, Han et al. (2019) reported that in ovo injection of L-leucine on the seventh day of embryogenesis reduced plasma thyroxine and rectal temperature immediately after hatching, increased rectal temperature in post-hatch neonatal chicks, and again reduced rectal temperature in 10-day-old heat-stressed male broiler chickens. The reduced body temperature at hatch may be due to the inability of the hatched chicks to maintain temperature homeostasis, but the increased rectal temperature of the neonatal chicks is suggestive of a developing thermoregulatory function associated with a higher metabolic rate in leucine administered groups. The reduction in the rectal temperature of the heat-stressed birds indicates that the in ovo leucine administration could increase the thermotolerance of chicks (Han et al., 2017). However, Zeitz et al. (2019) did not find any significant effect of leucine supplementation on protein synthesis or muscle degradation pathways when leucine was added up to 60% more than the breeder's recommendation (1.59 and 1.43 and 1.35% in the starter, grower, and finisher phases, respectively) for broilers. In ovo injection of leucine, isoleucine and valine at a dosage range of 4 to 7 mg in 0.5 mL sterile water resulted in a 21% increase in 1st-wk body weight along with an increase in humoral and cell-mediated immunity compared with control in a previous study in broilers (Bhanja and Mandal, 2005). In addition, in turkey poults when a 0.2% BCAA blend of leucine, valine and isoleucine in 3:1:2 ratio was injected in ovo into the amnion, the hatchability was depressed by more than 9%, but the poult quality was improved by 2.5%, and hatching weight was increased by 13%

compared with the normal saline injected control in a similar *in ovo* study (Kop–Bozbay and Ocak, 2019).

## EFFECTS OF BCAA ON INTESTINAL DEVELOPMENT, IMMUNITY, AND MICROBIOTA

For better growth and laying performance of poultry, proper development and functioning of their gastrointestinal organs associated with the immune cells, gut integrity, and balanced intestinal microbiota are indispensable (Singh and Kim, 2021; Singh et al., 2021a). Despite the acceptance of the significant role of BCAA in modulating immunity and gut health, no specific mechanism for its interaction has been described in the existing literature. Furthermore, in poultry, studies on the application of BCAA to target immunity, microbiota, and overall gut health have been nominal. The effects of BCAA supplementation would be expected to cause similar responses in poultry as those observed in pigs or mice (Wesney and Tannock, 1979; Zhang et al., 2018). Nevertheless, further studies are warranted to ascertain the effects of BCAA on the gut-health parameters of poultry, as there are inherent differences in the immunity and microbiota of poultry and pigs and other monogastric species.

Some of the research in human and *in vitro* models have determined that BCAA are transported via sodium independent neutral AA transporters or solute carriers and are expressed in several subfamilies in the brain, spleen, liver, skeletal muscles, intestine, and kidneys (Hayashi and Anzai, 2017; Bonvini et al., 2018). Regarding the oxidation, deamination, and transamination of BCAA for energy supply and other physiological functions of various cells and tissues, Bonvini et al. (2018) have published a comprehensive review. To summarize, BCAA transamination occurs in the presence of BCAA aminotransferase (BCAT) and yields branched-chain  $\alpha$ -keto acids (BCKAs) as well as irreversible oxidative decarboxylation of BCAA occurs and is catalyzed by the branched-chain  $\alpha$ -keto acids dehydrogenase (BCKD). Based on the pathways and generation of final metabolites, leucine is considered ketogenic, valine is glucogenic, and isoleucine can be both glucogenic and ketogenic. When BCAA are fermented, valine is converted to isobutyrate, leucine to 2-methyl-butyrate, and isoleucine to isovalerate as branched-chain fatty acid (BCFA) metabolites which are not known to have major toxic effects (Smith and Macfarlane, 1998; Apajalahti and Vienola, 2016). Intestinal mucosa has both BCAT and BCKD, the liver is low in BCAT, skeletal muscle known as the major site for BCAA, oxidation regulates BCAA via BCKD, and immune cells have a high level of both BCAT and BCKD and increase the uptake of BCAA mainly during S phase of the cell cycle.

### Role of BCAA on Immunity

In the liver, among BCAA, mainly valine stimulates the lymphogenesis of granular and agranular

lymphocytes as well as increases natural killer (NK) cells (Monirujjaman and Ferdouse, 2014). The ample supply of BCAA is also a stimulant of the mucosal secretory immunoglobulin A that reduces pathogenic multiplication in the lamina propria, and BCAA play an important role as a fuel source for the white blood cells, proinflammatory cytokines like IL-12, and dendritic cell function to boost both innate and adaptive immune responses (Ma et al., 2018; Nie et al., 2018). In a cell culture study, where the mesenchymal cells were cultivated in BCAA supplemented media, BCAA increased the proliferation of the mesenchymal cells, decreased p-FkB/NFkB and increased p-STAT-3/STAT-3 gene expression ratios, decreased IL-6 and TNF- $\alpha$ , and increased anti-inflammatory mediators (Sartori et al., 2020). It has been noted during *in vitro* studies BCAA are incorporated into proteins utilized to synthesize RNA and DNA, and oxidized for cellular functions by immune cells (Calder, 2006). These reports support the fact that BCAA have both anabolic and immunomodulatory effects. Furthermore, supplementation of BCAA in a low CP diet (CP-17%) for piglets to meet the standard BCAA requirement decreased plasma urea and intraepithelial lymphocytes number and increased villus height in the duodenum and immunoglobulin A compared with the protein-restricted diet (Ren et al., 2015). An elevated level of intraepithelial lymphocytes may not be desirable in normal functioning as it indicates ongoing inflammation in the mucosa (Cheroutre et al., 2011). In an *in vitro* study using intestinal tissue of a 19-d chicken embryo, Liu et al. (2018) explored the effect of leucine supplementation in lipopolysaccharide (LPS) treated tissues and observed that the LPS induced inflammatory response was downregulated by leucine through the phosphorylation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). An extra 0.3% supplementation of BCAA along with arginine and cysteine in a low dose (25  $\mu$ g/kg) LPS-challenge to weaned piglets partially reversed the stress biomarkers of inflammation and hormonal change exacerbated by the LPS challenge (Prates et al., 2021). The serum cortisol in the BCAA + arginine + cysteine treated groups was reduced from 3.5  $\mu$ g/dL to 1.7  $\mu$ g/dL at d 10, whereas the haptoglobin was reduced from 19.84 mg/dL to 13.59 mg/dL, and Insulin-like Growth Factor-1 was increased from 144  $\mu$ g/L to 231  $\mu$ g/L at d 35 compared with the control group in the above study. When valine was increased from 6.4 to 8.65 g/kg in broilers reared from 3 to 6 wk, Thornton et al. (2006) did not find any changes in the immune organs or antibody titers and concluded that the marginal valine deficiency would not compromise the immunity as the requirement for immunity may be less than the bird's growth requirements. These results imply that valine is essential for the growth and physiological functions; but its ratios to other AA, specifically the other BCAA, are crucial for an optimal response. In general, adequate availability of AA is essential for the synthesis of

cytotoxic molecules, polypeptides, immunoglobulins, cytokines, and more, as well as for chemical communications and functioning of both the innate and adaptive immune systems (Calder, 2006; Li et al., 2007). The adequate resource of BCAA is also necessary for immune cells to synthesize their nucleic acids and proteins as well as provide a resource for the dividing cells (Zhou et al., 2018).

### **Effects of BCAA on Intestinal Development and Nutrient Transport**

All kinds of poultry require longer villi to generate higher surface area for the better absorption of nutrients. Due to the interaction with food and gut microbes as well as senescence, there is a regular shedding of villus epithelial cells, which can be substantial during certain disease conditions (Singh and Kim, 2021). The lost cells of the villus are replenished from actively dividing crypts and during the villus atrophy, the crypt depth increases, thus the villus: crypt ratio is a good indicator of intestinal health (Jeurissen et al., 2002; Wang et al., 2020; Singh et al., 2021b). Supplemental leucine (1.37–2.2% of diet) in the broiler diet considerably increased the villus height in the jejunum and ileum and villus: crypt ratio in the duodenum, jejunum, and ileum in broilers, thus validating the role of leucine in intestinal development (Chang et al., 2015). In a broiler chicken study, Allameh and Toghyani (2019) reported that the supplementation of valine in low CP diets to reach up to 90% of digestible valine in a PC diet (0.9% in starter, 0.79% in grower and 0.71% in finisher) increased the villus height of the jejunum and ileum by 29 and 17%, respectively, as well as the number of goblet cells in the jejunum and ileum by 12 and 9%, respectively. The authors found that valine supplementation did not affect the immune response but increased protein accretion and improved intestinal morphology of broilers. During the transamination process BCAA could also supply the amino groups for the synthesis of other AA, especially glutamate and aspartate, which are recognized as major fuels for the small intestinal mucosa for intracellular protein turnover and nutrient transport (Zhou et al., 2018). The addition of leucine may not always be accompanied by the growth and multiplication of intestinal cells if the ratio of leucine to isoleucine and valine is imbalanced (Coëffier et al., 2011; Suryawan et al., 2013).

The metabolism of BCAA provides a carbon skeleton and nitrogen for other AA, especially glutamine, and thus supports immune activity (De Simone et al., 2013). The increased supply of BCAA in the brain restricts the high accretion of glutamate and prevents this neurotransmitter from reaching a toxic level (Yudkoff, 2017). Moreover, alanine and glutamine generated by transamination of BCAA prevent the accumulation of ammonia in the muscles and transport it to the liver and kidney for further metabolism and excretion (Coqueiro et al., 2018).

In essence, AA are the building blocks of peptides that are produced endogenously and can have antimicrobial effects. The epithelial cell lining in the gut produces broad-spectrum antimicrobial peptides including defensins and cathelicidins to avert the continuous challenges of microbial invasion (Gallo and Hooper, 2012). During a collective in vitro study with porcine intestinal epithelial cells and an in vivo swine study by Ren et al. (2016), it was observed that BCAA, most potently isoleucine, stimulated  $\beta$ -defensins possibly by activating the Sirt1/ERK/90RSK signaling pathway. The BCAA ratio of leucine: isoleucine: valine ranging from 1:0.25:0.25 to 1:0.75:0.75 supplemented in protein-restricted diet (CP-17%) in pigs have been shown to elevate the AA transporter expression, decrease muscle protein degradation genes, elevate muscle free AA concentration, and reduce serum urea nitrogen content (Duan et al., 2016). Added BCAA in a low protein diet (CP 17% vs. CP 20.9% in the control) to have equal levels of leucine, isoleucine, and valine in weanling piglets showed such supplementation increases  $\text{Na}^+$ -neutral AA exchanger and cationic AA transporter along with the improvement in the mucosal histomorphology (Zhang et al., 2013). The villus height and villus: crypt ratio has been noted to increase in response to leucine supplementation in suckling piglets (Sun et al., 2015). The authors also reported the upregulation of ATB<sup>0+</sup> protein, a known AA transporter that transports 18 of the 20 proteogenic AA along with other leucine transporters in the jejunum of the leucine-supplemented piglets. It implies that the supplemental leucine in a proper ratio would increase the absorption of several AA in the intestine.

### **Effects of BCAA on the GIT Microbiome**

The information on the effect of BCAA on the intestinal microbiota is still very limited and further research is necessary. In an in vitro cultivation study using pig intestinal digesta, Dai et al. (2010) included BCAA along with other AA and stated that these AA could be utilized by bacteria for their protein synthesis, and thus these BCAA could regulate the intestinal bacterial diversity. van der Wielen et al. (2002) conducted an in vitro study using lactate-fermenting bacterium (strain G17<sup>T</sup>) isolated from the ceca of 31-day-old broilers and this bacterium possessing similarity to *Clostridium propionicum* (93<5%) and *Clostridium neopropionicum* (93<5%) showed slow and moderate growth with L-valine, L-leucine, and L-isoleucine in the substrate. In piglets with a severe CP restriction (CP 14% vs. 20%) in a recent study by Yin et al. (2020), increasing the BCAA in the standard diet improved the abundance of *Gammaproteobacteria*, *Lactobacillales*, and *Aeromonadales* that were altered by the CP restriction. In another study on weaned nursery piglets by Spring et al. (2020), pigs fed low CP (CP 22% vs. 13–14%) were supplemented with all 3 BCAA to reach the standard level of the control diet and this addition favored the colonization of the beneficial gut bacterial population. The

authors reported that the BCAA addition to the low CP diet increased the population of *Paludibacteraceae* and *Synergistaceae* and decreased the abundance of *Streptococcaceae*, *Oxyphotobacteria\_unclassified*, *Pseudomonadaceae*, and *Shewanellaceae* in the feces compared with control. *Paludibacter* in the *Paludibacteraceae* family is known to ferment carbohydrates to produce acetate and butyrate. [Apajalahti and Vienola \(2016\)](#) have reported that *Lactobacillus spp.* and *C. perfringens* require leucine and isoleucine for their growth whereas *Escherichia coli* do not depend on these AA and thus the variability in the supply of such AA could favor certain groups of bacteria in their competitiveness against others in the GIT of poultry. [Jian et al. \(2021b\)](#) studied the effect of supplemental valine fed to 33-wk-old laying hens where basal control diet contained 0.59% total valine and the supplemented diet had 0.79% valine. The authors reported that supplemental valine did not affect alpha and beta diversity of cecal microbiota, but it decreased the relative abundance of *Fusobacterium*, *Aeriscardovia*, *Anaerobiospirillum*, *Aerococcus*, *Corynebacterium*, and *Campylobacter*, and increased the relative abundance of *Oribacterium*, and *Frisingicoccus*. Such outcomes suggest that the addition of BCAA could have a generally beneficial effect on gut microbiota and in turn improve growth. From the perspective of microbiota modulation in poultry, adjusting the BCAA levels in the diet need to be explored with more focus in light of the void created due to the constraints on the use of antibiotics growth promoters.

## EFFECTS OF BCAA ON SKELETAL DEVELOPMENT IN BROILERS

[Farran and Thomas \(1992\)](#) studied the effects of valine and a BCAA-deficient diet on the male broiler reared for 3 wk and reported that bone ash and bone calcium were lower in valine deficient (0.63%) birds than in control or BCAA-deficient birds. In this study, the fractional excretion of calcium was 3 times higher in valine-deficient-fed broilers than that in valine-supplemented birds. The authors also stated that a proper ratio of available BCAA is required for osteoblastic activity and the disruption in this ratio by lower valine could have increased osteoclastic activity leading to a bone abnormality in valine deficient groups. However, in a recent study by [Amirdahri et al. \(2020\)](#) in female broilers, none of the tibia-related parameters were altered by various valine: lysine ratios. Thus, not only the deficiency of some or all of the BCAA could have negative effects on the health and performance of birds but maintaining their optimum ratio would also have a critical role in the metabolic activity of birds.

## FUTURE BCAA POULTRY RESEARCH

There is a piece of increasing evidence that the selective permeability of intestinal mucosa for nutrients may be altered in the disease state and could become more

permeable to toxins and pathological bacteria that can cause further complications ([Bischoff et al., 2014](#)). The intestinal permeability also increases during the *Salmonella* challenge in broilers ([Prado-Rebolledo et al., 2017](#)). The requirements for BCAA may change during such states, not only for the repair of the intestinal integrity but also for the proliferation of immune cells and antimicrobial secretions. Because enteric infections increase the endogenous loss of AA, and supplemental AA are found to be essential to improve intestinal integrity and gut permeability ([Bortoluzzi et al., 2020](#); [Castro et al., 2020](#)), further research with BCAA is warranted to understand how these AA requirements change especially in laying and meat-type chickens and turkeys. Regarding the utilization of BCAA during poultry disease state, these are among the AA with the greatest reduction in the AID during *Eimeria* infection ([Parker et al., 2007](#)). The reduction of villus absorptive surface and downregulation of brush border nutrients transporters during infection could contribute to the reduction of digestibility of BCAA. During coccidiosis, intestinal mucosal integrity is affected which leads to increased paracellular permeability and may show variability in the absorption of nutrients and affect nitrogen retention ([Teng et al., 2021](#)). However, [Rochell et al. \(2016\)](#) reported an elevation in the plasma concentration of BCAA in response to *Eimeria* infection, which is intriguing and needs further research to ascertain how these AA are utilized during infections and whether a change in their ratio would be beneficial to the animal. As discussed earlier, to better understand the antagonistic effect of high leucine or plasma concentrations of isoleucine and valine, extensive experimentation would be required where poultry are grown in both disease challenged and unchallenged states on different levels (low to high) of either of BCAA while keeping the level of other 2 BCAA constant. It is also essential to note that reducing CP in the diet has been reported to reduce the production of butyric acid production by the microbiome, and butyric acids are used as fuel for the colonocytes as well as stimulate host defense systems ([Hilliari et al., 2020](#)). The authors also noted that the subclinical necrotic enteritis caused by co-infection with *Clostridium perfringens* and *Eimeria* caused a decrease in the CP digestibility and feeding of higher AA levels led to a faster recovery from the disease challenge.

Besides disease challenge, heat stress in poultry is expected to increase the need for BCAA due to the increased muscle protein catabolism. The provision of BCAA increases the body weight in chronically heat stressed birds and decreases rectal temperature in young birds. For poultry produced in tropical climates, it would be essential to evaluate if a high-density diet containing a higher level of BCAA would be effective in maintaining the growth of the birds when feed restrictions or low feed intake might reduce the amount of nutrients available for the birds as compared with the recommended level. Even the requirement for digestible BCAA needs to be evaluated based on diet types and disease status. Supplemental unbound BCAA may be

utilized differently than those bound in the ingredient structure. It has been previously reported that a low CP diet fortified with AA does not perform as well as a high CP diet with AA and proteins supplied via ingredients, due to an adverse effect on feed intake (Waldroup et al., 2002). The SBM used in poultry feed has been the main source of CP, essential AA and BCAA. Improper processing techniques, fiber content, and CP levels of the SBM might influence BCAA utilization. The requirements and recommendations of BCAA during different infections like coccidiosis, salmonellosis, and necrotic enteritis are poorly defined in the current literature. The change in the requirements of BCAA along with other nutrients is a matter of debate. Further assessment of BCAA during disease challenge is proposed based on the current understanding of deteriorated mucosal barrier function, intestinal permeability, and increased need for AA by the immune system to fight infections. In recent years, laying hen farming has gradually transitioned from conventional cages to cage-free systems. This change in rearing environment is expected to expose hens to several disease challenges including coccidiosis. Moreover, due to the limitation on the inclusion of antibiotics in feed, the requirements for BCAA need to be redefined. Likewise, further investigations will be necessary to determine the optimum ratio and requirement levels for BCAA in laying hens affected by clinical or subclinical diseases, causing disruptions of transporters involved in AA uptake for egg formation.

## CONCLUSIONS

BCAA are among the key regulators of protein synthesis, and their optimal ratio is essential to induce nutrient sensors to signal myocyte proliferation and differentiation leading to muscle growth and development. Accumulating data also highlight the important role of BCAA in sustaining the immune response in poultry, however, the current dietary inclusion recommendations may not be sufficient during increased disease challenge due to new restrictions on antibiotic growth promoters. The relationship between BCAA ratio and breast muscle myopathies has not been well-explored and needs further research. The role of BCAA on intestinal integrity, nutrient transporters and gut microbiome has been explored, but the requirements for BCAA have not been standardized on such bases. In poultry diets formulation, different feed ingredients should be selected carefully to ensure a proper ratio of BCAA as corn and distiller's dried grain with solubles tend to have higher leucine to other BCAA content; blood meal is low in isoleucine, whereas SBM and meat and bone meal have a more balanced ratio of BCAA (NRC, 1994). Moreover, the digestible level of amino acids should be obtained for calculating their ratio as the supplemental unbound amino acids may not be utilized similar to protein-bound amino acids in the diet. Further research would be necessary to understand the effects of the range of BCAA ratios in poultry feeding that would optimize inclusion

levels of major feed ingredients with variable BCAA contents.

## DISCLOSURES

There is no conflict of interest.

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