

## Effects of Branched-Chain Amino Acids on Growth Performance and Intestinal Development in Broiler Chickens: Postprint

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### Abstract

This experiment aimed to investigate the effects of branched-chain amino acids (BCAAs) on growth performance and intestinal development in broiler chickens. A total of 384 healthy 1-day-old Arbor Acres (AA) broiler chicks were selected and randomly divided into 4 groups with 6 replicates per group and 16 birds per replicate. Each group was fed experimental diets with BCAA levels of 3.04%, 3.93%, 4.82%, and 5.71%, respectively, with dietary leucine:isoleucine:valine ratios of 1.8:1.0:1.2 in all groups. Birds had ad libitum access to feed and water, and the experimental period lasted 21 days. The results showed that there were no significant differences in body weight or average daily gain among groups ( $P > 0.05$ ). However, the average daily feed intake of broilers in the 5.71% group was significantly lower than that in the 3.04% group ( $P < 0.05$ ), and the feed conversion ratio of broilers in the 4.82% and 5.71% groups was significantly lower than that in the 3.04% group ( $P < 0.05$ ). At 14 and 21 days of age, as dietary BCAA levels increased from 3.04% to 4.82%, the weight per unit length of duodenum and jejunum and the relative weight of jejunum in broilers gradually increased, and the weight per unit length of duodenum in the 4.82% group was significantly greater than that in the 3.04% group ( $P < 0.05$ ); however, when BCAA levels increased from 4.82% to 5.71%, the weight per unit length of duodenum and the relative weight of jejunum in broilers decreased significantly ( $P < 0.05$ ). At 14 and 21 days of age, the villus height of duodenum and ileum in the 4.82% group was significantly greater than that in the 3.04% group ( $P < 0.05$ ). At 10 and 14 days of age, the jejunal villus height in the 4.82% group was significantly greater than that in the 3.04% group ( $P < 0.05$ ); the small intestinal villus height in the 5.71% group was significantly lower than that in the 4.82% group ( $P < 0.05$ ). Additionally, there was no significant difference in jejunal crypt depth between the 5.71% and 3.04% groups ( $P > 0.05$ ); however, at 10 and 21 days of age, the jejunal crypt depth in the 4.82% group

was significantly greater than that in the 3.93% group ( $P < 0.05$ ). At 10, 14, and 21 days of age, the villus height/crypt depth (V/C) ratio in jejunum and ileum was lowest in the 5.71% group, with no significant difference from the 3.04% group ( $P > 0.05$ ); however, the V/C ratio in jejunum and ileum in the 3.93% group was significantly greater than that in the 5.71% group ( $P < 0.05$ ). In conclusion, under the conditions of this experiment, increasing dietary BCAA levels could reduce the feed conversion ratio of broilers; appropriate BCAA levels could promote intestinal growth and development in broilers; however, high BCAA levels significantly reduced average daily feed intake and simultaneously impaired small intestinal growth and development.

## Full Text

### Effects of Branched-Chain Amino Acids on Growth Performance and Intestinal Development of Broilers

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## Abstract

This experiment was conducted to investigate the effects of branched-chain amino acids (BCAAs) on growth performance and intestinal development in broilers. A total of 384 one-day-old healthy Arbor Acres (AA) male broilers were randomly allocated to four groups with six replicates per group and 16 birds per replicate. The groups were fed experimental diets containing 3.04%, 3.93%, 4.82%, and 5.71% BCAAs, respectively, with a leucine:isoleucine:valine ratio of 1.8:1.0:1.2. Birds had ad libitum access to feed and water throughout the 21-day experimental period. The results showed no significant differences in body weight or average daily gain among groups ( $P > 0.05$ ). However, average daily feed intake in the 5.71% group was significantly lower than in the 3.04% group ( $P < 0.05$ ), and the feed/gain ratio in the 4.82% and 5.71% groups was significantly lower than in the 3.04% group ( $P < 0.05$ ). At 14 and 21 days of age, the unit length weight of duodenum and jejunum and the relative weight of jejunum increased gradually as dietary BCAAs increased from 3.04% to 4.82%, with the 4.82% group showing significantly greater duodenal unit length weight than the 3.04% group ( $P < 0.05$ ). When BCAAs increased from 4.82% to 5.71%, both duodenal unit length weight and jejunal relative weight decreased significantly ( $P < 0.05$ ). Villus height in duodenum and ileum of the 4.82% group was significantly greater than in the 3.04% group at 14 and 21 days of age ( $P < 0.05$ ). Jejunal villus height in the 4.82% group was significantly greater than in the 3.04% group at 10 and 14 days of age ( $P < 0.05$ ), while villus height throughout the small intestine in the 5.71% group was significantly lower than in the 4.82%

group ( $P < 0.05$ ). Additionally, no significant difference in jejunal crypt depth was observed between the 5.71% and 3.04% groups ( $P > 0.05$ ), but crypt depth in the 4.82% group was significantly greater than in the 3.93% group at 10 and 21 days of age ( $P < 0.05$ ). The villus height/crypt depth (V/C) ratio in jejunum and ileum was lowest in the 5.71% group at 10, 14, and 21 days of age, showing no significant difference from the 3.04% group ( $P > 0.05$ ), while the V/C ratio in the 3.93% group was significantly greater than in the 5.71% group ( $P < 0.05$ ). These findings indicate that increasing dietary BCAAs level can reduce feed/gain ratio in broilers, and appropriate BCAAs levels promote intestinal growth and development, whereas excessive levels significantly decrease average daily feed intake and retard small intestinal development.

**Keywords:** branched-chain amino acids; broilers; growth performance; intestinal development

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Branched-chain amino acids (BCAAs) are neutral essential amino acids with branched aliphatic hydrocarbon chains on the  $\alpha$ -carbon that cannot be synthesized by animals, including leucine (Leu), isoleucine (Ile), and valine (Val). BCAAs play crucial physiological roles in enhancing oxidative energy supply, improving immunity, regulating lactation in female animals, promoting gluconeogenesis, stimulating protein synthesis, and inhibiting protein degradation. Research has shown that BCAAs participate in the glucose-alanine cycle, metabolizing to produce alanine and ketone bodies that provide substantial ATP for the body, and can promote secretion of certain hormones such as growth hormone and insulin. Consequently, BCAAs are vital for animal growth and development. Current research on BCAAs has primarily focused on pigs and mice, mainly investigating effects on skeletal muscle protein synthesis and lipid metabolism, with limited reports on poultry intestinal development. Additionally, many studies have demonstrated antagonistic interactions among BCAAs, where dietary imbalance can affect amino acid utilization efficiency. Therefore, maintaining BCAAs balance is potentially important for improving amino acid utilization. This experiment investigated the effects of BCAAs on broiler growth performance and intestinal development by adjusting dietary BCAAs levels while maintaining BCAAs balance, providing guidance for precise amino acid nutrition in poultry and a theoretical foundation for further research on the molecular mechanisms of BCAAs as functional amino acids.

### 1.1 Experimental Animals and Location

Healthy Arbor Acres (AA) male broilers were obtained from Beijing Huadu Broiler Company for a 21-day experiment. The feeding trial was conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences.

## 1.2 Experimental Design and Diets

A single-factor completely randomized design was employed. Three hundred and eighty-four one-day-old healthy AA male broilers with similar body weight were randomly divided into four groups with six replicates per group and 16 birds per replicate. Each group was fed experimental diets with BCAAs levels of 3.04%, 3.93%, 4.82%, and 5.71%, respectively, with a Leu:Ile:Val ratio of 1.8:1.0:1.2. Diets were formulated according to the Chinese “Feeding Standard of Broilers” (NY/T 33-2004). Diet composition and nutrient levels are presented in Table 1 .

## 1.3 Management

Birds were raised in cages following conventional management and immunization procedures recommended in the AA Broiler Management Manual. They had ad libitum access to feed and water, with 23 hours of lighting daily. The birds' mental state, appetite, and fecal condition were observed daily, and mortality was recorded.

## 1.4 Sample Collection

At 4, 7, 10, 14, and 21 days of age, one bird per replicate with body weight close to the average was selected, euthanized by jugular venous exsanguination, and dissected. The intestines were isolated for organ index determination, and approximately 1.5 cm segments from each small intestinal section were fixed in 4% formaldehyde solution for histological section preparation and evaluation of small intestinal morphological development.

### 1.5.1 Growth Performance

On the morning of day 21, birds were weighed after overnight fasting to record body weight (BW). Feed was withdrawn at 22:00 on the day before slaughter while water remained available. At 08:00 the following day, birds were weighed by replicate to record feed intake and calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G).

### 1.5.2 Digestive Tract Organ Indices

After slaughter and dissection, the duodenum, jejunum, and ileum were separated to measure length and weight, from which relative weight and unit length weight of each segment were calculated.

### 1.5.3 Small Intestinal Morphology

Fixed tissues were processed through dehydration, embedding, sectioning, baking, xylene dewaxing, hydration, staining, and mounting before microscopic observation. Professional image analysis software Image-Pro Plus 7.0 was used for data measurement, including villus height and crypt depth. Five fields of

view were measured per slide and averaged as the final result. Villus height was defined as the vertical distance from the intestinal gland opening to the villus tip, while crypt depth was the vertical distance from the crypt opening to the crypt base. The villus height/crypt depth ratio (V/C) was calculated.

## 1.6 Statistical Analysis

Experimental data were analyzed using one-way ANOVA with LSD test in SPSS 19.0 statistical software. Multiple comparisons were performed using LSD method for factors with significant F-test results. Data were expressed as mean  $\pm$  standard deviation, with  $P < 0.05$  as the criterion for significant difference.

### 2.1 Effects of BCAAs on Broiler Growth Performance

As shown in Table 2, body weight and average daily gain in the 4.82%, 3.93%, and 5.71% groups were greater than in the 3.04% group, but differences among groups were not significant ( $P > 0.05$ ). Average daily feed intake and feed/gain ratio decreased gradually with increasing BCAAs levels. Specifically, average daily feed intake in the 5.71% group was significantly lower than in the 3.04% group ( $P < 0.05$ ), and feed/gain ratio in the 4.82% and 5.71% groups was significantly lower than in the 3.04% group ( $P < 0.05$ ).

#### 2.2.1 Effects of BCAAs on Small Intestinal Length at Different Ages

Table 3 shows that at 7 days of age, total small intestinal length and ileal length in the 3.04% group were significantly greater than in the 3.93% and 5.71% groups ( $P < 0.05$ ), with total small intestinal length in the 5.71% group significantly less than in the 4.82% group ( $P < 0.05$ ). Duodenal length in the 3.04% group was significantly greater than in the 5.71% group ( $P < 0.05$ ). At 10 days of age, total small intestinal length and jejunal length in the 4.82% group were significantly greater than in the 5.71% group ( $P < 0.05$ ). No significant differences were observed among groups at other ages for total small intestinal length or segment lengths ( $P > 0.05$ ).

#### 2.2.2 Effects of BCAAs on Relative Weight of Small Intestine at Different Ages

Table 4 indicates that at 4 days of age, no significant differences existed among groups in total relative small intestinal weight ( $P > 0.05$ ), though duodenal relative weight in the 4.82% group was significantly greater than in the 3.93% group ( $P < 0.05$ ). At 7 days of age, total relative small intestinal weight in the 5.71% group was significantly lower than in the 3.04% and 3.93% groups ( $P < 0.05$ ), and duodenal relative weight was significantly lower than in the other three groups ( $P < 0.05$ ). At 10 days of age, jejunal relative weight in the 4.82% group was significantly greater than in the 3.04% and 5.71% groups ( $P < 0.05$ ). At 14 days of age, total relative small intestinal weight in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ), with jejunal relative weight

lower than in the 3.93% and 4.82% groups ( $P < 0.05$ ), while the 3.04% group had significantly lower jejunal relative weight than the 4.82% group ( $P < 0.05$ ). Ileal relative weight in the 5.71% group was significantly lower than in the 3.04% group ( $P < 0.05$ ). At 21 days of age, total relative small intestinal weight and jejunal relative weight in the 4.82% group were significantly greater than in the 5.71% group ( $P < 0.05$ ), but not significantly different from the 3.04% and 3.93% groups ( $P > 0.05$ ). No significant differences were observed among groups for duodenal and ileal relative weights ( $P > 0.05$ ).

### 2.2.3 Effects of BCAAs on Unit Length Weight of Small Intestine at Different Ages

Table 5 shows that at 4 days of age, duodenal unit length weight in the 4.82% group was significantly greater than in the 3.04% and 3.93% groups ( $P < 0.05$ ). At 7 days of age, ileal unit length weight in the 3.93% group was significantly greater than in the 3.04% and 4.82% groups ( $P < 0.05$ ). At 21 days of age, duodenal unit length weight in the 4.82% group was significantly greater than in all other groups ( $P < 0.05$ ). No significant differences were observed among groups for jejunal unit length weight at any age ( $P > 0.05$ ), though a similar trend to duodenum was observed, with unit length weight decreasing as BCAAs level increased from 4.82% to 5.71%.

### 2.3.1 Effects of BCAAs on Small Intestinal Villus Height at Different Ages

Table 6 presents the effects on duodenal mucosal development. At 4 days of age, duodenal villus height in the 4.82% group was significantly greater than in the 3.93% and 5.71% groups ( $P < 0.05$ ), while the 5.71% group was significantly lower than the 3.04% group ( $P < 0.05$ ). At 7 and 10 days of age, duodenal villus height in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ). At 14 and 21 days of age, duodenal villus height in the 4.82% group was significantly greater than in other groups ( $P < 0.05$ ). At 14 days of age, the 3.93% group had significantly greater duodenal villus height than the 3.04% group ( $P < 0.05$ ), while at 21 days of age, the 5.71% group was significantly lower than the 3.93% group ( $P < 0.05$ ).

For jejunal mucosal development, at 4 days of age, jejunal villus height in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ). At 7 days of age, the 4.82% group had significantly greater jejunal villus height than the 5.71% group ( $P < 0.05$ ). At 10 days of age, the 4.82% group showed significantly greater jejunal villus height than other groups ( $P < 0.05$ ). At 14 days of age, jejunal villus height in the 4.82% group was significantly greater than in the 3.04% and 5.71% groups ( $P < 0.05$ ). At 21 days of age, the 5.71% group had significantly lower jejunal villus height than other groups ( $P < 0.05$ ). No significant differences were observed among other groups at the same ages ( $P > 0.05$ ). Notably, at 10, 14, and 21 days of age, jejunal villus height showed a similar trend,

with the 5.71% group having the lowest values and villus height increasing as BCAAs level rose from 3.04% to 4.82%.

For ileal mucosal development, at 4 and 7 days of age, ileal villus height in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ). At 10 days of age, ileal villus height in the 3.93% and 4.82% groups was significantly greater than in the 5.71% group ( $P < 0.05$ ), but not significantly different from the 3.04% group ( $P > 0.05$ ). At 14 and 21 days of age, ileal villus height in the 3.93% and 4.82% groups was significantly greater than in the 3.04% and 5.71% groups ( $P < 0.05$ ), with no significant difference between the 5.71% and 3.04% groups ( $P > 0.05$ ). No other significant differences were observed among groups at the same ages ( $P > 0.05$ ).

### 2.3.2 Effects of BCAAs on Small Intestinal Crypt Depth at Different Ages

Table 7 shows that for duodenal mucosal development, at 7 days of age, duodenal crypt depth in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ), while the 4.82% group was significantly lower than the 3.93% group ( $P < 0.05$ ) but not significantly different from the 3.04% group ( $P > 0.05$ ). At 14 days of age, duodenal crypt depth in the 5.71% group was significantly lower than in the 3.93% group ( $P < 0.05$ ). At 21 days of age, the 3.93% group had significantly lower duodenal crypt depth than the 5.71% group ( $P < 0.05$ ), with no other significant differences among groups ( $P > 0.05$ ).

For jejunal mucosal development, at 4 days of age, jejunal crypt depth in the 3.93% and 4.82% groups was significantly lower than in the 3.04% group ( $P < 0.05$ ). At 10 days of age, jejunal crypt depth in the 4.82% group was significantly greater than in the 3.04% and 3.93% groups ( $P < 0.05$ ), and the 5.71% group was significantly greater than the 3.93% group ( $P < 0.05$ ). At 21 days of age, the 3.93% group had significantly lower jejunal crypt depth than other groups ( $P < 0.05$ ), with no other significant differences ( $P > 0.05$ ). Additionally, at 10, 14, and 21 days of age, jejunal crypt depth showed a consistent trend, being highest in the 4.82% group, followed by the 5.71% group, and lowest in the 3.93% group.

For ileal mucosal development, at 4 days of age, ileal crypt depth in the 5.71% group was significantly lower than in other groups ( $P < 0.05$ ), with no other significant differences ( $P > 0.05$ ). At 7 days of age, ileal crypt depth in the 4.82% group was significantly greater than in the 3.04% group ( $P < 0.05$ ), with no other significant differences ( $P > 0.05$ ). At 14 and 21 days of age, ileal crypt depth tended to increase with BCAAs level, and at 14 days of age, the 5.71% group was significantly greater than the 3.04% group ( $P < 0.05$ ).

### 2.3.3 Effects of BCAAs on Small Intestinal V/C Ratio at Different Ages

Table 8 shows that in the duodenum, at 4 and 7 days of age, the V/C ratio in the 4.82% group was significantly greater than in the 3.93% group ( $P < 0.05$ ), with no other significant differences ( $P > 0.05$ ). At 10, 14, and 21 days of age, no significant differences were observed among the 3.04%, 3.93%, and 4.82% groups ( $P > 0.05$ ), though at 10 and 21 days of age, the V/C ratio in the 3.93% and 4.82% groups was significantly greater than in the 5.71% group ( $P < 0.05$ ).

In the jejunum, at 4 days of age, the V/C ratio in the 3.93% and 4.82% groups was significantly greater than in the 3.04% and 5.71% groups ( $P < 0.05$ ). At 10, 14, and 21 days of age, no significant differences were observed between the 4.82% and 3.04% groups ( $P > 0.05$ ), but the V/C ratio in the 5.71% group was significantly lower than in the 3.93% group ( $P < 0.05$ ). At 14 days of age, the V/C ratio in the 3.93% group was significantly greater than in the 3.04% group ( $P < 0.05$ ), and the 4.82% group was significantly greater than the 5.71% group ( $P < 0.05$ ). At 21 days of age, the V/C ratio in the 3.93% group was significantly greater than in all other groups ( $P < 0.05$ ).

In the ileum, at 7 days of age, the V/C ratio in the 4.82% and 5.71% groups was significantly lower than in the 3.04% group ( $P < 0.05$ ). At 10, 14, and 21 days of age, the V/C ratio showed a consistent trend, with the 3.93% and 4.82% groups having greater values than the 3.04% and 5.71% groups. At 14 and 21 days of age, the V/C ratio in the 3.93% and 4.82% groups was significantly greater than in the 5.71% group ( $P < 0.05$ ).

### 3.1 Effects of BCAAs on Broiler Growth Performance

Farran et al. reported that feeding any single BCAA alone to 3-week-old male broilers did not improve body weight gain, whereas simultaneous supplementation of all three BCAAs improved weight gain and feed conversion efficiency. Waldroup et al. found that as BCAAs level increased from 3.93% to 6.86%, broiler body weight remained unchanged while feed intake decreased and feed/gain ratio improved, but body weight decreased significantly when BCAAs reached 7.71%. In this experiment, as dietary BCAAs increased from 3.04% to 4.82%, no significant differences were observed in body weight or average daily gain among the 3.04%, 3.93%, and 4.82% groups, while average daily feed intake and feed/gain ratio decreased gradually, consistent with previous findings. This may be attributed to BCAAs supplementation reducing the blood tryptophan (Trp)/BCAAs ratio, thereby decreasing feed intake, with the improved feed/gain ratio primarily resulting from reduced feed intake.

Furthermore, when BCAAs level reached 5.71%, average daily feed intake decreased significantly and average daily gain was also reduced compared to the 4.82% group, impairing growth performance. Tang et al. reported that excessive BCAAs intake affects animal growth, particularly when one BCAA is excessive, as antagonism among BCAAs can severely hinder absorption and conversion of

other amino acids, reducing feed intake and growth rate and affecting normal physiological functions. Other researchers have reported that excessive Leu intake reduces animal feed intake and daily gain. Harper et al. and Fernstrom noted that excessive BCAAs intake decreases brain serotonin levels, thereby reducing feed intake. These results consistently show that excessive BCAAs reduce feed intake, aligning with our findings. Thus, appropriate dietary BCAAs levels improve broiler growth performance, while excessive levels are detrimental.

### **3.2 Effects of BCAAs on Small Intestinal Length, Relative Weight, and Unit Length Weight at Different Ages**

As the primary site for nutrient digestion and absorption, intestinal development is closely related to poultry growth performance. Changes in small intestinal length and weight can alter nutrient absorption, thereby affecting animal growth. Our results showed that as dietary BCAAs increased from 3.04% to 4.82%, duodenal unit length weight, jejunal relative weight, and jejunal unit length weight increased accordingly. Since rapid animal growth is often accompanied by rapid protein synthesis, this effect may occur because BCAAs promote intestinal protein synthesis, thereby stimulating intestinal development. Yin et al. demonstrated that BCAAs deficiency in diets reduces intestinal protein synthesis and mucosal mass in piglets. Conversely, dietary supplementation with 0.27% Leu (1.61% of diet) significantly increased protein synthesis in the proximal small intestine of piglets, promoting digestive tract growth. Similarly, Torrazza et al. reported that adding Leu at 4% of diet to low-protein diets stimulated jejunal protein synthesis in animals. Additionally, BCAAs affected different intestinal segments differently, possibly due to morphological and structural differences among small intestinal sections. When dietary BCAAs increased from 4.82% to 5.71% in this experiment, duodenal relative weight and jejunal length and relative weight decreased, impairing healthy intestinal growth and development. This may be caused by reduced average daily feed intake due to excessive BCAAs levels. Research indicates that during early animal development, feed intake is closely related to intestinal development and health, with higher feed intake promoting intestinal development and improving structural and functional integrity, while lower intake impairs intestinal development. Therefore, within a certain range, increasing dietary BCAAs level improves small intestinal development, whereas excessive BCAAs levels may impair healthy intestinal development by reducing feed intake.

### **3.3 Effects of BCAAs on Small Intestinal Mucosal Development at Different Ages**

The small intestine is the main site for nutrient digestion, absorption, and transport, and good small intestinal mucosal structure is particularly important for complete digestive physiological function and promoting animal growth and development. In this experiment, as BCAAs level increased, villus height in all

small intestinal segments of the 3.04%, 3.93%, and 4.82% groups increased gradually, indicating that appropriate BCAAs supplementation can promote villus development and improve nutrient absorption capacity. However, villus height in the 5.71% group decreased as BCAAs level increased, corresponding to the effect of high BCAAs level on average daily feed intake. The V/C ratio comprehensively reflects small intestinal functional status, with increased V/C indicating improved intestinal development and decreased V/C indicating intestinal damage and impaired growth. In this experiment, as dietary BCAAs increased from 4.82% to 5.71%, the small intestinal V/C ratio decreased significantly, further demonstrating that appropriate BCAAs supplementation promotes small intestinal development, while excessive BCAAs impairs development, affecting nutrient absorption and slowing broiler growth.

Sun et al. reported that increasing Leu level to twice that in sow milk significantly increased duodenal villus height and ileal V/C ratio in suckling piglets. This aligns with our finding that duodenal villus height increased as BCAAs level rose from 3.04% to 4.82%, though ileal V/C ratio did not increase significantly, possibly because ileal crypt depth also increased with BCAAs level while villus height increased. The mechanism by which small intestinal morphology changes with BCAAs level may involve: 1) BCAAs affecting intestinal amino acid transporter expression, thereby influencing intestinal development. Studies have shown that feeding high amino acid diets increases amino acid transport, primarily due to increased expression of amino acid transporters. Zhang et al. found that supplementing 0.63%-3.19% BCAAs to low-protein diets significantly increased amino acid transporters in the jejunum of weaned piglets, playing an important role in maintaining normal small intestinal development. 2) BCAAs affecting the mammalian target of rapamycin (mTOR) signaling pathway, thereby influencing intestinal development. The mTOR signaling pathway comprehensively regulates cell growth, proliferation, apoptosis, and autophagy and is sensitive to amino acid signals. Apelo et al. demonstrated that Ile promotes mTOR phosphorylation, linearly increases ribosomal protein S6 phosphorylation, regulates small intestinal protein synthesis, and promotes villus development. Wang et al. found that Leu significantly increases mTOR gene expression in broiler intestinal epithelial cells, promoting cell division and proliferation. Therefore, appropriate BCAAs levels may promote small intestinal morphological development by increasing amino acid transporters or promoting intestinal epithelial cell proliferation.

However, excessive BCAAs are detrimental to small intestinal morphological development. Our results showed that as dietary BCAAs increased from 4.82% to 5.71%, small intestinal villus height and V/C ratio decreased significantly, impairing nutrient absorption and utilization and negatively affecting intestinal development. Apelo et al. found that the effects of Leu and Ile on mTOR depend on their antagonism with each other and with other amino acids. Amino acid antagonism reduces effective amino acid utilization, impairs expression of mTOR and related genes, slows intestinal epithelial cell proliferation, and hinders intestinal mucosal growth. The specific mechanisms underlying the adverse

effects of excessive BCAAs on the small intestine remain unclear and require further investigation.

## Conclusions

1. Under the conditions of this experiment, increasing dietary BCAAs level can reduce feed/gain ratio in broilers, but high BCAAs levels significantly decrease average daily feed intake.
2. As dietary BCAAs level increased from 3.04% to 4.82%, duodenal unit length weight, jejunal relative weight, and small intestinal villus height increased, thereby promoting small intestinal growth and development. However, when BCAAs level reached 5.71%, intestinal villus height and V/C ratio decreased significantly, slowing small intestinal growth and development.

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