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Research Article
Effect of Dietary Protein Sources and Amino Acid Balances on Performance, Intestinal Permeability and Morphology in Broiler Chickens

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Abstract

Objective: This study was conducted to evaluate the effects of dietary protein soybean meal (SBM) and cottonseed meal (CSM) sources and Amino Acid (AA) balances on performance, intestinal permeability and morphology in broiler chickens. **Methodology:** Five hundred and twenty broiler chickens were fed an experimental diet from 21-42 days of age. A completely randomized block design was used with four treatments of SBM+CSM with AA Balance (B) and Imbalance (I) (SBM_B and SBM_I, SBM+CSM_B and SBM+CSM_I) were replicated 10 times with 13 broiler chickens per replicate. All respective treatments were kept in the same condition provide the diet twice a day and access to water *ad libitum*. **Results:** The results showed that Daily Weight Gain (DWG), Daily Feed Intake (DFI), feed efficiency, Feed Conversion Ratio (FCR), carcass yield and dressing percentage were improved with SBM_B in comparison with all treatments. Furthermore, SBM_B significantly increased most of morphological findings in the intestinal parts studied. Intestinal permeability was ($p<0.05$) increased in SBM_I and SBM+CSM_I treatments. **Conclusion:** It is concluded that the best performance indices could be obtained by using SBM_B carcasses and morphological findings in comparison with other treatments.

Key words: Protein sources, performance, intestinal permeability, morphology, chickens

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry feed is composed based on the digestibility of ingredients and absorption of nutrients in diet, as the digestible amino acids (AAs) are crucial in evaluating appropriate protein sources and dietary supplements. Digestible AAs highlight the importance of ingredients, for their nutritive values as well as growth performance in poultry. Various dietary protein sources need to be changed in the diets of mono-gastric animals to improve production[1-3]. The diet, dietary protein sources, supplementation of amino acid (AA) and methodology can all affect growth performance in broilers[4,5]. Soybean meal (SBM) is a rich source of high-quality vegetable protein and is most widely used in poultry diets because of its digestible amino acids and metabolic energy[6,7]. The SBM increased daily weight gain and daily feed intake in the growing phase of chicks[8]. Compared with more conventional diets, SBM is better for poultry[1]. Soy products are easy to produce, cheap and a rich source of dietary protein for poultry. The metabolic energy, digestible AA concentration and true digestibility AAs in SBM make it better than other protein meals fed to poultry[9]. Previous studies have been conducted on soy products but there have not been any to investigate the AA balance and imbalance in poultry.

Cottonseed meal (CSM) is a byproduct of cottonseeds with 41-44% of CP from the extraction process and is a high-protein feed for poultry[10]. The CSM contains naturally occurring anti-nutrient factors (ANFs) but if used with other dietary protein sources, the effects of ANFs can be reduced and CSM can become a digestible dietary protein source for poultry[11]. The CSM, corn and wheat are major feed ingredients used with SBM for cheap feed formulations for poultry[12,13]. Besides, lysine supplemented to CSM frequently binds and inactivates gossypol[14].

It has been observed that digestible AAs decrease the output of nitrogen (N) in excreta and improve digestibility of dietary protein[7,15]. There are various dietary protein sources and feed ingredients that need to be analyzed for their digestible AAs and as inexpensive sources of feed formulated for poultry diets. However, SBM and CSM in diets affect broiler growth performance due to their imbalance in digestible AA. It is hypothesized that the CP levels and two dietary protein sources are enough to provide a balanced diet for broilers in the growing phase and balanced diets would increase the growth performance. Thus, the aim of the study was to evaluate dietary protein sources (SBM and CSM, including AA balance and imbalance) and their effects on growth performance and intestinal integrity and morphology.

MATERIALS AND METHODS

Experiment design: The experiment was conducted to evaluate broiler growth performance and intestinal permeability. All treatments were designed 20% CP levels in finisher ration, SBM_B and $SBM+CSM_B$ with AA balanced, whereas SBM_I and $SBM+CSM_I$ were imbalanced digestible AAs in methionine, lysine and threonine, according to nutritional requirements of NRC[9]. The broilers remained on the treatment diets from the 3rd-6th week, fed twice daily and given access to water *ad libitum* (Table 1).

Experimental procedure and management: This experimental study was approved by the Animal Care and Use Committee of Northwest A and F University, Shaanxi province,

Table 1: Ingredient specification and composition of experimental diets (as-fed)

	Diets*			
Items	SBM_B	$SBM+CSM_B$	SBM_I	$SBM+CSM_I$
Ingredients (%)				
Corn	57.60	55.66	55.97	54.22
SBM	33.00	27.50	34.50	29.00
CSM	-	6.00	-	6.00
Oil	5.60	7.00	6.20	7.50
Limestone	1.40	1.40	1.40	1.40
$CaHPO_4$	1.50	1.45	1.40	1.45
Salt	0.30	0.30	0.30	0.30
Trace minerals#	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.10
DL-Met	0.24	0.25	-	-
Lys	0.15	0.20	-	-
Thr	0.08	0.11	-	-
Vitamin premixΦ	0.03	0.03	0.03	0.03
Estimated composition (%)				
AMEn (kcal kg^{-1})	3005.00	3009.00	3007.00	3008.00
Crude protein	20.00	20.00	20.00	20.00
Calcium	0.90	0.90	0.90	0.90
Available phosphorus	0.36	0.36	0.36	0.36
Linoleic acid	4.60	5.31	4.91	5.56
Fiber	2.60	2.90	2.70	3.00
Crude fat	8.20	9.50	8.80	10.00
Digestible Met	0.49	0.50	0.26	0.26
Digestible Lys	0.98	0.98	0.90	0.85
Digestible Cys	0.27	0.27	0.28	0.28
Digestible Met+Cys	0.77	0.77	0.54	0.55
Digestible Thr	0.70	0.70	0.64	0.61
Digestible Trp	0.18	0.18	0.19	0.19

*SBM_B: Amino acid balanced diet with SBM as sole protein source, $SBM+CSM_B$: Amino acid balanced diet with SBM+CSM as sole protein source, SBM_I: Amino acid imbalanced diet with SMB as sole protein source, $SBM+CSM_I$: Amino acid imbalanced diet with SBM+CSM as sole protein source, #Trace mineral premix provides per kilogram of diet: Iron 60 mg, manganese 80 mg, copper 10 mg, zinc 80 mg, iodine 0.4 mg, selenium 0.3 mg, ΦVitamin premix provides per kilogram of diet: vitamin A 8000 IU, vitamin D3 1500 IU, vitamin E 30 IU, vitamin K3 3 mg, vitamin B1 4 mg, vitamin B2 6 mg, B6 4 mg, vitamin B12 30 μg, folic acid 2 mg, niacin acid 35 mg, calcium pantothenate 15 mg, biotin 0.3 mg

Yangling, People's Republic of China. Day-old broiler chickens (n = 520) were purchased from Shaanxi Zhengda Co., Ltd., China. All birds were fed normally from day 1-21 days (3 weeks), for acclimation and experimental design. Birds were allowed access to water *ad libitum* and offered commercial diet. The diet was feed formulated according to NRC[9] that was purchased from a local market. When the birds reached 21 days of age, birds were divided in to 4 treatments, with each treatment having 10 replicates of 13 birds both male and female broilers. All birds were fed experimental diets continually for 3 weeks (from days 21-42), during this period, birds were measured and observed daily for growth performance, feed intake, body weight gain, physical appearance, motility, morbidity, behavior and production. On day 42, randomly one bird was selected from each replication and killed for further analysis.

Performance parameters: The birds were kept in cages from days 21-42. Daily were monitored of DWG, DFI and FCR, mortality and morbidity along with a physical examination and observation of behavior. On the completion of the experimental period on day 42, the broilers from each replication were weighed before and after sacrifice. After dressing the bird, the carcass weight was recorded and the dressing percentage was calculated.

Intestinal morphology: The intestinal morphological study was conducted on the duodenum, jejunum and ileum of the broiler. An approximately 1 cm piece was cut from the middle of each section and then immediately placed in 10% formalin for histological study. The intestinal morphology was analyzed by the procedure described previously[16-18]. The tissues were cut with a Leica microtome (Leica RM 2235, Bio-system Nussloch GmbH D-69226, Heideberger str. 17-19, Germany) into approximately 5 μm thick slices and further stained with hematoxylin and eosin. After the staining process, a tissue morphological study was conducted using a Nokia 80i microscope with NIE-elements documentation software according to Arias and Koutsos[19]. The intestinal villus height was measured top to base and villus width was measured three times from the right to left of the villus under 100× magnifications. The crypt depth and mucosal thickness were measured from the base of the villus to the basolateral membrane under 100× magnifications.

Intestinal transepithelial permeability: Intestinal transepithelial electrical permeability was measured by the short circuit current method (ISC) and analyzed as described previously[20]. The birds were killed on day 42 and the ileum was immediately removed and mounted in a chamber system (World Precision Instruments, Inc., FL, USA). Both sides of the ileum were washed with 5 mL of Ringer's solution. The medium was warmed to 37 by circulating water jacket and smoothly oxygenated with 95% O_2 and 5% CO_2. The short circuit current (ISC) was measured through the agar bridge of KCl (3% agar in 3 mol L^{-1} of KCl), which was connected to an automatic voltage clamp using the chamber system (present clamp EVC-4000 voltage, World Precision Instruments, Inc., FL, USA). The resistance of the solution was measured before the ileum was added; therefore, it was easy to account for the background voltage readings caused by the clamp current, the electrolyte path between the voltage electrodes and the surface of the membrane. The transepithelial electrical resistance (TEER) measurement of the cell monolayer was completed by using a epithelial voltohmmeter (World Precision Instruments, Inc., FL, USA) using a chamber system with TEER above 300 Ω cm^{-2}, the output was digitized by a data acquisition board (Lab-Trax-4/16) and recorded by Lab-Trax software (World Precision Instruments, USA).

Statistical analysis: All data were analyzed in IBM SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Two main factor of data analysis, significant was declared at ($p<0.05$). When a significant effect of treatment was detected, difference among mean was tested using Tukey's multiple comparison test. The results were expressed as the treatment mean with pooled SEM and their Interaction. A probability value of $p\leq0.05$ was considered statistically significant.

RESULTS

Performance parameters: The effects of all treatments on broiler growth performance, including DWG, DFI and FCR were significant ($p<0.05$) among groups shown in Table 2. The SBM_B and $SBM+CSM_B$ with the balanced AA had higher growth performance compared to the SBM_I and $SBM+CSM_I$ AA imbalanced groups. Throughout the experimental feeding period 3 weeks growth performance was consistently significantly different among the groups ($p<0.05$).

Intestinal weight was different ($p<0.05$) whereas the intestinal length was ($p>0.05$) similar among the treatments (Table 3). The carcass weight and dressing percentage were highly ($p<0.05$) significantly different among the treatment groups ($p<0.05$). The carcass weight and dressing percentage

Table 2: Effects of dietary protein sources and amino acid balance on growth performance of broiler chickens (n = 10)

	*AAs balance		**AAs imbalance			p-value		
Items	SBM_B	$SBM+CSM_B$	SBM_I	$SBM+CSM_I$	SEM	Balance	Protein sources	B×P
DWG (g/d)								
21-28	58.75[a]	54.52[b]	47.80[c]	45.35[d]	0.898	0.001	0.001	0.148
28-35	61.51[a]	56.98[b]	54.72[c]	50.70[d]	0.702	0.001	0.001	0.698
35-42	76.03[a]	67.96[b]	59.24[c]	53.37[d]	0.396	0.001	0.011	0.051
21-42	65.43[a]	59.82[b]	53.59[c]	49.81[d]	0.227	0.001	0.001	0.207
DFI (g/d)								
21-28	96.59[a]	94.95[b]	88.38[d]	92.11[c]	0.642	0.001	0.220	0.003
28-35	110.45[a]	108.32[b]	109.23[b]	106.56[c]	0.451	0.075	0.006	0.741
35-42	139.51[a]	136.57[b]	130.25[c]	126.47[d]	0.920	0.001	0.001	0.630
21-42	115.52[a]	113.28[b]	109.29[c]	108.38[c]	0.521	0.001	0.003	0.179
FCR (g feed/g gain)								
21-28	1.64[a]	1.74[b]	1.85[c]	2.03[d]	0.026	0.001	0.001	0.120
28-35	1.80[a]	1.90[b]	2.00[c]	2.10[d]	0.023	0.001	0.001	0.987
35-42	1.83[a]	2.01[b]	2.20[c]	2.37[d]	0.035	0.001	0.001	0.469
21-42	1.77[a]	1.89[b]	2.03c	2.18[d]	0.026	0.001	0.001	0.806

[a-d]Different letters with in a row differ significantly (p<0.05), *AAs balance: Amino acids balance diet as a sole protein source of SBM_B and $SBM+CSM_B$, **AAs imbalance: Amino acids imbalanced diet as a sole protein source of SBM_I and $SBM+CSM_I$, SEM: Standard error of mean, DWG: Daily weight gain, DFI: Daily feed intake, FCR: Feed conversion ratio

Table 3: Effects of dietary protein sources and amino acid balance on digestive organ morphometry and carcass yield of broiler chickens (n = 10)

	*AAs balance		**AAs imbalance			p-value		
Items	SBM_B	$SBM+CSM_B$	SBM_I	$SBM+CSM_I$	SEM	Balance	Protein sources	B×P
Intestine weight (g)	146.58[a]	139.52[c]	143.92[b]	138.95[c]	0.876	0.286	0.487	0.001
Intestine length (cm)	160.10	154.36	159.60	155.62	1.511	0.904	0.771	0.117
Carcass weight (g)	937.43[a]	884.97[b]	809.51[c]	740.68[d]	12.411	0.001	0.254	0.001
Carcass yield (%)	61.36[a]	59.18[b]	57.45[c]	55.51[d]	0.413	0.001	0.793	0.001

[a-d]Different letters with in a row differ significantly (p<0.05), *AAs balance: Amino acids balance diet as a sole protein source of SBM_B and $SBM+CSM_B$, **AAs imbalance: Amino acids imbalanced diet as a sole protein source of SBM_I and $SBM+CSM_I$, SEM: Standard error of mean

Table 4: Effects of dietary protein sources and amino acid balance on intestinal duodenum morphology of broiler chickens (n = 10)

	*AAs balance		**AAs imbalance			p-value		
Items	SBM_B	$SBM+CSM_B$	SBMI	$SBM+CSM_I$	SEM	Balance	Protein sources	B×P
Villus height (μm)	865.50[a]	786.50[b]	734.80[c]	669.50[d]	14.890	0.001	0.730	0.001
Villus width (μm)	121.45	117.80	109.70	101.65	3.587	0.055	0.756	0.410
Crypt depth (μm)	204.20[a]	181.50[b]	166.70[c]	153.60[d]	6.001	0.005	0.660	0.106
VH:CD (ratio)	4.24	4.33	4.41	4.36	0.156	0.434	0.406	0.883
Mucosal thickness (μm)	404.30	379.40	355.20	339.70	13.192	0.099	0.859	0.446

[a-d]Different letters with in a row differ significantly (p<0.05), *AAs balance: Amino acids balance diet as a sole protein source of SBM_B and $SBM+CSM_B$, **AAs imbalance: Amino acids imbalanced diet as a sole protein source of SBM_I and $SBM+CSM_I$, SEM: Standard error of mean, VH:CD: Villus height: Crypt depth

in treatments with digestible balanced AA were significantly higher compared to the other treatments.

Duodenum morphology: Duodenum villus height (p<0.05) was found to be significantly different among treatments (Table 4). However, the villus width, crypt depth, VH:CD and mucosal thickness were similar among treatments (p>0.05, Fig. 1 shows the histological appearance).

Jejunum morphology: Jejunum villus height (p<0.05) and villus width (p<0.05) were significantly different among treatments but crypt depth, VH:CD and mucosal thickness were not significantly different (p>0.05) as shown in Table 5. The differences in histological findings among treatments are shown in Fig. 2.

Ileum morphology: Ileum villus height (p<0.05) and villus width (p<0.05) were to be found statistically significantly different among all treatments, whereas the crypt depth, VH:CD and mucosal thickness similar found as shown in Table 6. Figure 3 clarifies the significant histological differences among the groups.

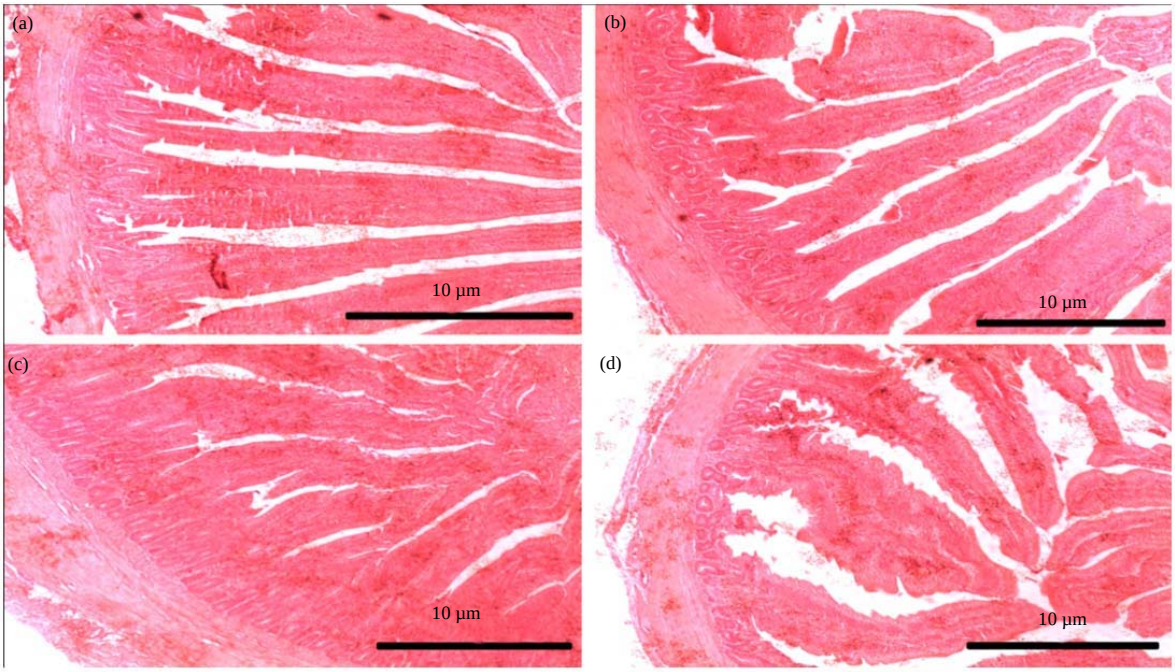

Fig. 1(a-d): Morphology of broiler intestinal duodenum epithelial tissues villus height, (a) SBM_B, (b) $SBM+CSM_B$, (c) SBM_I and (d) $SBM+CSM_I$. Scale bar = 10 µm

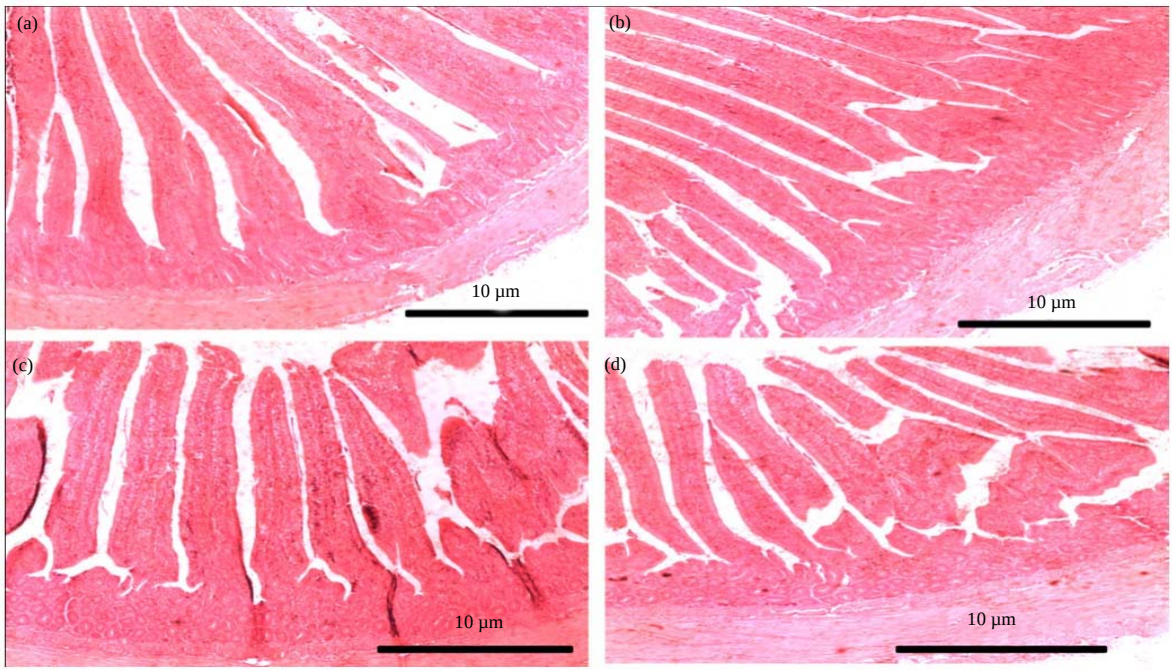

Fig. 2(a-d): Morphology of broiler intestinal jejunum epithelial tissues villus height, (a) SBM_B, (b) $SBM+CSM_B$, (c) SBM_I and (d) $SBM+CSM_I$. Scale bar = 10 µm

Intestinal transepithelial permeability: Figure 4 shows that the intestinal transepithelial permeability was increased in the AA Imbalanced (I) treatment as an increase in statistically significant ISC levels was measured ($p<0.05$), whereas in AA Balanced (B) treatments, constant or reduced ISC levels were measured ($p<0.05$).

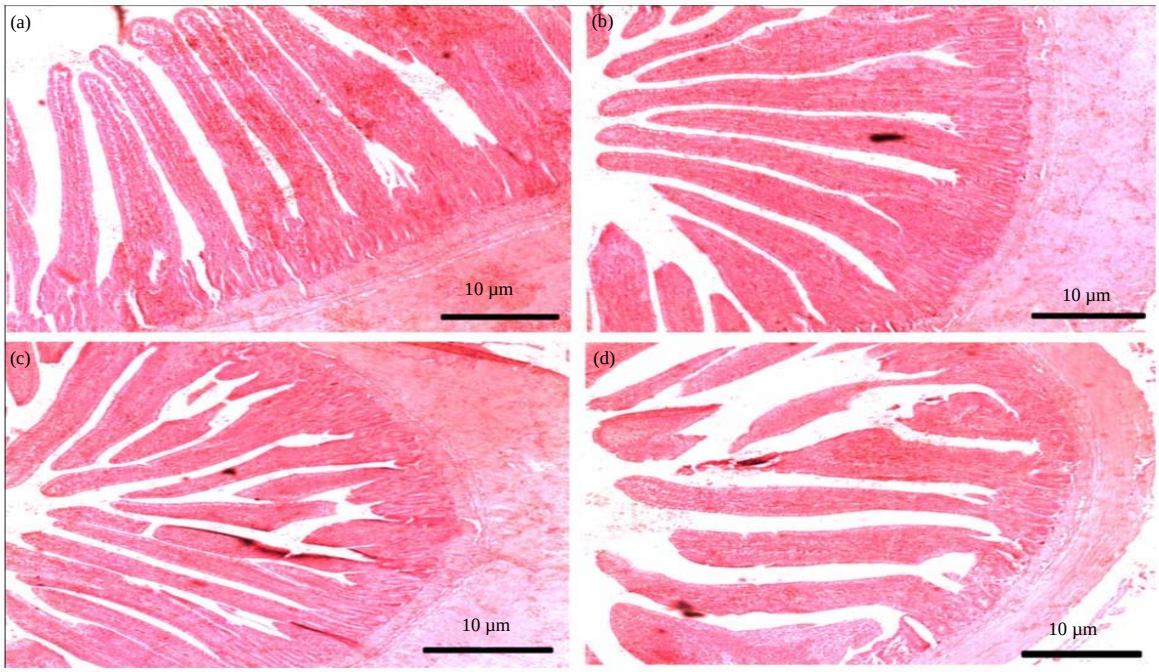

Fig. 3(a-d): Morphology of broiler intestinal ileum epithelial tissues villus height, (a) SBM_B, (b) $SBM+CSM_B$, (c) SBM_I and (d) $SBM+CSM_I$. Scale bar = 10 µm

Table 5: Effects of dietary protein sources and amino acid balance on intestinal jejunum morphology of broiler chickens (n = 10)

	*AAs balance		**AAs imbalance			p-value		
Items	SBM_B	$SBM+CSM_B$	SBM_I	$SBM+CSM_I$	SEM	Balance	Protein sources	B×P
Villus height (µm)	937.10^a	849.70^b	812.30^c	765.20^d	16.605	0.001	0.471	0.019
Villus width (µm)	139.55^a	127.35^b	111.29^c	104.45^c	3.863	0.001	0.688	0.159
Crypt depth (µm)	153.20	155.30	151.20	133.90	4.602	0.209	0.296	0.412
VH/CD (ratio)	6.12	5.47	5.37	5.71	0.242	0.878	0.872	0.155
Mucosal thickness (µm)	376.60	340.30	321.50	336.70	14.007	0.308	0.370	0.712

$^{a-d}$Different letters within a row differ significantly ($p<0.05$), *AAs balance: Amino acids balance diet as a sole protein source of SBM_B and $SBM+CSM_B$, **AAs imbalance: Amino acids imbalanced diet as a sole protein source of SBM_I and $SBM+CSM_I$, SEM: Standard error of mean, VH/CD: Villus height:Crypt depth

Table 6: Effects of dietary protein sources and amino acid balance on intestinal ileum morphology of broiler chickens (n = 10)

	*AAs balance		**AAs imbalance			p-value		
Items	SBM_B	$SBM+CSM_B$	SBM_I	$SBM+CSM_I$	SEM	Balance	Protein sources	B×P
Villus height (µm)	802.60^a	732.10^b	712.10^{bc}	659.40^c	12.891	0.001	0.668	0.005
Villus width (µm)	139.95^a	118.20^b	109.95^c	96.80$_c$	5.263	0.011	0.658	0.078
Crypt depth (µm)	150.90^a	136.50^{ab}	123.60^c	115.80^c	4.491	0.006	0.691	0.186
VH/CD (ratio)	5.32	5.36	5.76	5.69	0.221	0.784	0.518	0.500
Mucosal thickness (µm)	418.10^a	373.80^b	321.10^c	304.50^c	15.312	0.006	0.627	0.288

$^{a-c}$Different letters within a row differ significantly ($p<0.05$), *AAs balance: Amino acids balance diet as a sole protein source of SBM_B and $SBM+CSM_B$, **AAs imbalance: Amino acids imbalanced diet as a sole protein source of SBM_I and $SBM+CSM_I$, SEM: Standard error of mean, VH/CD: Villus height:Crypt depth

DISCUSSION

Crude protein and dietary protein sources with balanced and imbalanced AAs affected the growing phase in broilers when exposed from days 21-2. Highly significant differences were observed among the treatment groups in the present study. The SMB_B diets had increased digestibility, due to the presence of digestible AAs and a balanced ratio of AAs, resulting in increased DWG and DFI measurements up to day 42. The age of broilers affects the ileal AA digestibility and absorption of nutrients[5,21]. Low-CP diets decreased growth performance and carcass traits in broiler chickens[22]. Balanced

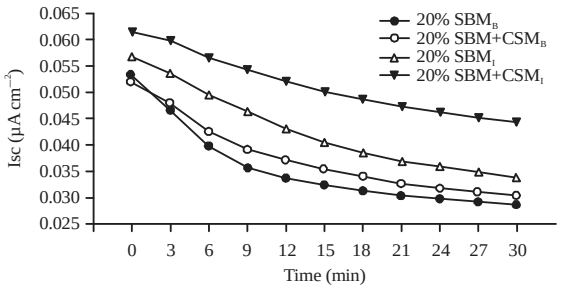

Fig. 4: Effects of dietary protein sources and amino acid balance on integrity and permeability of ileum in broiler intestine by short circuit current (Isc)

diets have been observed to significantly increase growth during the grower-finisher period in mono-gastric animals[1]. The results of the present study are in agreement with previous studies[1,23] that found that SBM in balanced diets affect growth performance in growing phase and the performance is enhanced with supplementation of other sources or treated with enzymes. Furthermore SBM+CSM diet improves growth performance and increased digestibility in the production phase[24]. A diet of SBM with balanced AAs had higher growth performance, including carcass yield and carcass weight in broilers than the SBM+CSM with balanced AAs. The other treatments, SBM with an AA imbalance and SBM+CSM with an AA imbalance had reduced performance. Previous studies of Feng *et al.*[8] Clarke and Wiseman[25] and Valencia *et al.*[26] showed that the SBM diet increased ileal AA digestibility in broilers. Therefore, SBM and CSM with balanced AAs are better for growth performance and dietary feed supplementation[27]. The supplementation of CSM with other dietary protein sources frequently inactivates gossypol[16]. Dietary supplementation of SBM as a control diet provided better performance results compared to other diets in previous experimental diet analysis[12]. Growth performance is improved by intestinal integrity and the absorption of nutrients from diets[28]. In present study, the balanced AA diets improved growth performance, including digestibility of ingredients with crude protein levels. However, the treatments that were imbalanced for AA, despite the presence of the two rich protein sources, SBM and CSM did not improve performance as a result of imbalanced AAs. For broilers, dietary AAs are crucial for growth performance and absorption of nutrients.

To improve "Performance" in poultry, specifically in broilers, means to increase carcass yield and dressing percentage, it has been suggested that better growth performance increases muscle and reduces abdominal fat[29,30]. It is reported that dietary crude protein and threonine supplementation increased growth performance, carcass weight, breast meat weight and relevant carcass in body weight compared to the same parameters in broilers fed basal diets[31]. In the present study, performance and carcass weight increased in broilers fed the balanced diets. Intestinal weight and length was indicated the performance and digestibility of diet with better physiological mechanism[32].

Morphological studies showed that nutrient absorption was affected by the balance and imbalance of AAs in diets. The various protein diet supplementations also affected intestinal morphology and barrier integrity in broilers[33,34]. The gastrointestinal tract of broiler chickens was observed to be associated with growth performance and production[35]. Dietary sources influenced villus height, villus width, crypt depth and mucosal thickness, these were observed in the gross histology of the intestine[16,34,36]. Protein sources increased changes in gut morphology and had significant effects on villus height and crypt depth in broiler chicken[37]. The SBM diets increased the absorption of nutrients as observed in the statistically significant histological findings. In all treatments, villus height was increased extensively as a result of absorption and transportation of nutrients. Our results are in agreement with Yi *et al.*[38] who found that changing dietary glutamine supplementation affects the intestinal morphology in broilers and increases villus height due to the absorption of nutrients. Reduction of protein in broiler diets resulted in significant decrease in crypt depth ($p<0.01$) and villus height ($p<0.05$) in jejunal epithelial cells but the use of low-protein diets with threonine (110 and 120%) of the recommended levels allowed overcoming these alterations[22]. If the villus height is increases, this indicates the performance and health of the intestine has improved[37]. Another study observed that dietary leucine supplementation improved intestinal development by enhancing jejunum and ileum villus height in broilers[39]. Nutrient digestibility affects broiler intestinal morphology[40]. Significant effects on intestinal histology, most frequently a change in villus height were observed in all treatments, this may be a result of low and high secretion, absorption of nutrients and ions in the small intestine.

The intestinal transepithelial permeability of ileum in broiler significantly different among the groups fed with balanced and imbalanced AAs diets as a result of transportation and absorption of ions. Changes in ISC values reflect the integrity of intestinal epithelial cells and transportation of ions[20]. The short circuit current of the intestine rises when the digestible AAs absorption and transportation process increases during dietary Aas

supplementation. The intestine is responsible for electrolyte balance and water absorption[41,42]. High absorption rates of ions provided by dietary nutrient supplementation were measured in the small and large intestines of birds. Intestinal integrity and permeability were affected by the transportation of ions[43]. The TEER is a straight forward quality indicator, which differentiates between epithelial cells with transportation and absorption of nutrients[20].

CONCLUSION

The present results show that performance, carcass trait, intestinal morphology and permeability were affected by feeding the broilers on SBM_B and $SBM+CSM_B$ diets with balanced or imbalanced AAs. The dietary protein sources with balanced AA significantly increased the performance of broilers. Carcass yield and dressing percentage increased due to the presence of digestible AA with metabolize energy in the SBM diets. Alternatively, the imbalanced AA diets caused reduced performance due to insufficient AAs present in the diets. Therefore, all treatments affected the intestinal morphology and permeability of broilers. The villus height, villus width, crypt depth and mucosal thickness increased due to improved nutrient absorption and transportation in intestine by the balanced AAs diets. Moreover, intestinal transepithelial permeability was increased due to improved ion secretion and absorption. The intestinal permeability and integrity increased because of the availability of digestible AA and protein sources in broiler diets.

SIGNIFICANCE STATEMENTS

- The present study was planned to evaluate the effects of dietary protein sources and amino acid balances in broiler
- Weight gain, feed intake, feed efficiency, feed conversion ratio, carcass yield and dressing percentage were improved with SBM_B in comparison with all treatments
- The SBM_B increased the most of morphological findings in the intestinal parts studied
- Intestinal permeability was increased in SBM_I and $SBM+CSM_I$ treatments

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REFERENCES

1. White, G.A., L.A. Smith, J.G.M. Houdijk, D. Homer, I. Kyriazakis and J. Wiseman, 2015. Replacement of soya bean meal with peas and faba beans in growing/finishing pig diets: Effect on performance, carcass composition and nutrient excretion. Anim. Feed Sci. Technol., 209: 202-210.
2. Jeong, J.S., J.W. Park, S.I. Lee and I.H. Kim, 2015. Apparent ileal digestibility of nutrients and amino acids in soybean meal, fish meal, spray-dried plasma protein and fermented soybean meal to weaned pigs. Anim. Sci. J., 87: 697-702.
3. Upadhaya, S.D., J.W. Park and I.H. Kim, 2017. Effect of replacement of de-hulled barley with water-soaked barley in corn-soybean meal-based diet on growth performance, blood characteristics and meat quality in finishing pigs. J. Applied Anim. Res., 45: 164-169.
4. Adedokun, S.A., O. Adeola, C.M. Parsons, M.S. Lilburn and T.J. Applegate, 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci., 90: 1737-1748.
5. Adedokun, S.A., A. Helmbrecht and T.J. Applegate, 2016. Investigation of the effect of coccidial vaccine challenge on apparent and standardized ileal amino acid digestibility in grower and finisher broilers and its evaluation in 21-day-old broilers. Poult. Sci., 95: 1825-1835.
6. Kaczmarek, S.A., M. Hejdysz, M. Kubis and A. Rutkowski, 2016. Influence of graded inclusion of white lupin (*Lupinus albus*) meal on performance, nutrient digestibility and intestinal morphology of broiler chickens. Br. Poult. Sci., 57: 364-374.
7. Jezierny, D., R. Mosenthin and E. Bauer, 2010. The use of grain legumes as a protein source in pig nutrition: A review. Anim. Feed Sci. Technol., 157: 111-128.
8. Feng, J., X. Liu, Z.R. Xu, Y.Y. Liu and Y.P. Lu, 2007. Effects of *Aspergillus oryzae* 3.042 fermented soybean meal on growth performance and plasma biochemical parameters in broilers. Anim. Feed Sci. Technol., 134: 235-242.
9. NRC., 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC., USA., ISBN-13: 978-0309048927, Pages: 176.
10. Henry, M.H., G.M. Pesti, R. Bakalli, J. Lee, R.T. Toledo, R.R. Eitenmiller and R.D. Phillips, 2001. The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine. Poult. Sci., 80: 762-768.

11. Batonon-Alavo, D.I., D. Bastianelli, P. Lescoat, G.M. Weber and M.U. Faruk, 2016. Simultaneous inclusion of sorghum and cottonseed meal or millet in broiler diets: Effects on performance and nutrient digestibility. Animal, 10: 1118-1128.
12. De Keyser, K., L. Kuterna, S. Kaczmarek, A. Rutkowski and E. Vanderbeke, 2016. High dosing NSP enzymes for total protein and digestible amino acid reformulation in a wheat/corn/soybean meal diet in broilers. J. Applied Poul. Res., 25: 239-246.
13. De Sousa Lima, V.B., L.R.B. Dourado, L.P. Machado, D. Biagiotti and S.B.P. de Lima *et al*., 2016. Cottonseed oil in diets for broilers in the pre-starter and starter phases. PloS One, Vol. 11. 10.1371/journal.pone.0147695
14. Begum, I., A. Azim, S. Akhter, M.I. Anjum and M. Afzal, 2010. Mineral dynamics of blood and milk in dairy buffaloes fed on calcium and phosphorus supplementation. Pak. Vet. J., 30: 105-109.
15. Huang, K.H., V. Ravindran, X. Li, G. Ravindran and W.L. Bryden, 2007. Apparent ileal digestibility of amino acids in feed ingredients determined with broilers and layers. J. Sci. Food Agric., 87: 47-53.
16. Hu, C.H., Z.C. Qian, J. Song, Z.S. Luan and A.Y. Zuo, 2013. Effects of zinc oxide-montmorillonite hybrid on growth performance, intestinal structure and function of broiler chicken. Poult. Sci., 92: 143-150.
17. Yoon, J.H., S.L. Ingale, J.S. Kim, K.H. Kim and J. Lohakare *et al*., 2013. Effects of dietary supplementation with antimicrobial peptide-P5 on growth performance, apparent total tract digestibility, faecal and intestinal microflora and intestinal morphology of weanling pigs. J. Sci. Food Agric., 93: 587-592.
18. Choi, S.C., S. Ingale, J.S. Kim, Y.K. Park, I.K. Kwon and B.J. Chae, 2013. Effects of dietary supplementation with an antimicrobial peptide-P5 on growth performance, nutrient retention, excreta and intestinal microflora and intestinal morphology of broilers. Anim. Feed Sci. Technol., 185: 78-84.
19. Arias, V.J. and E.A. Koutsos, 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult. Sci., 85: 999-1007.
20. Wang, X., Y. Li, X. Yang and J. Yao, 2013. Astragalus polysaccharide reduces inflammatory response by decreasing permeability of LPS-infected Caco2 cells. Int. J. Biol. Macromol., 61: 347-352.
21. Adedokun, S.A., C.M. Parsons, M.S. Lilburn, O. Adeola and T.J. Applegate, 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci., 86: 2590-2597.
22. Abbasi, M.A., A.H. Mahdavi, A.H. Samie and R. Jahanian, 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. Braz. J. Poult. Sci., 16: 35-44.
23. Stefanello, C., S.L. Vieira, G.O. Santiago, L. Kindlein, J.O.B. Sorbara and A.J. Cowieson, 2015. Starch digestibility, energy utilization, and growth performance of broilers fed corn-soybean basal diets supplemented with enzymes. Poult. Sci., 94: 2472-2479.
24. De Almeida, G.F., T.A. Del Valle, P.G. De Paiva, E.F. De Jesus and R.V. Barletta *et al*., 2016. Effects of whole raw soybean or whole cottonseed on milk yield and composition, digestibility, ruminal fermentation and blood metabolites of lactating dairy cows. Anim. Prod. Sci., Vol. 57. 10.1071/AN15266
25. Clarke, E.J. and J. Wiseman, 2005. Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. Anim. Feed Sci. Technol., 121: 125-138.
26. Valencia, D.G., Serrano, M.P., R. Lazaro, E. Jimenez-Moreno and G.G. Mateos, 2009. Influence of micronization (fine grinding) of soya bean meal and full-fat soya bean on the ileal digestibility of amino acids for broilers. Anim. Feed Sci. Technol., 150: 238-248.
27. Ortega, R.M., M.F. Paulino, E. Detmann, L.N. Renno and L. de Vieira Barros *et al*., 2016. Substitution of soybean meal for cottonseed meal in multiple supplements for grazing beef heifers in the dry season. Semina: Ciencias Agrarias, 37: 495-506.
28. Emami, N.K., S.Z. Naeini and C.A. Ruiz-Feria, 2013. Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids. Livest. Sci., 157: 506-513.
29. Kwon, H.J., Y.K. Choo, Y.I. Choi, E.J. Kim and H.K. Kim *et al*., 2014. Carcass characteristics and meat quality of Korean native ducks and commercial meat-type ducks raised under same feeding and rearing conditions. Asian-Aust. J. Anim. Sci., 27: 1638-1643.
30. Aghwan, Z.A., A.Q. Sazili, K.K. Kadhim, A.R. Alimon, Y.M. Goh and K.D. Adeyemi, 2015. Effects of dietary supplementation of selenium and iodine on growth performance, carcass characteristics and histology of thyroid gland in goats. Anim. Sci. J., 87: 690-696.
31. Ciftci, I. and N. Ceylan, 2004. Effects of dietary threonine and crude protein on growth performance, carcase and meat composition of broiler chickens. Br. Poult. Sci., 45: 280-289.
32. Wang, J.P., Z.F. Zhang, L. Yan and I.H. Kim, 2016. Effects of dietary supplementation of emulsifier and carbohydrase on the growth performance, serum cholesterol and breast meat fatty acids profile of broiler chickens. Anim. Sci. J., 87: 250-256.
33. Song, J., L.F. Jiao, K. Xiao, Z.S. Luan, C.H. Hu, B. Shi and X.A. Zhan, 2013. Cello-oligosaccharide ameliorates heat stress-induced impairment of intestinal microflora, morphology and barrier integrity in broilers. Anim. Feed Sci. Technol., 185: 175-181.

34. Long, Y., S. Lin, J. Zhu, X. Pang and Z. Fang *et al*., 2016. Effects of dietary lysozyme levels on growth performance, intestinal morphology, non-specific immunity and mRNA expression in weanling piglets. Anim. Sci. J., 87: 411-418.
35. Husveth, F., L. Pal, E. Galamb, K.C. Acs and L. Bustyahazai *et al*., 2015. Effects of whole wheat incorporated into pelleted diets on the growth performance and intestinal function of broiler chickens. Anim. Feed Sci. Technol., 210: 144-151.
36. Khonyoung, D., K. Yamauchi and K. Suzuki, 2015. Influence of dietary fat sources and lysolecithin on growth performance, visceral organ size and histological intestinal alteration in broiler chickens. Livestock Sci., 176: 111-120.
37. Qaisrani, S.N., P.C.A. Moquet, M.M. Van Krimpen, R.P. Kwakkel, M.W.A. Verstegen and W.H. Hendriks, 2014. Protein source and dietary structure influence growth performance, gut morphology and hindgut fermentation characteristics in broilers. Poult. Sci., 93: 3053-3064.
38. Yi, G.F., G.L. Allee, C.D. Knight and J.J. Dibner, 2005. Impact of glutamine and Oasis hatchling supplement on growth performance, small intestinal morphology and immune response of broilers vaccinated and challenged with *Eimeria maxima*. Poult. Sci., 84: 283-293.
39. Chang, Y., H. Cai, G. Liu, W. Chang and A. Zheng *et al*., 2015. Effects of dietary leucine supplementation on the gene expression of mammalian target of rapamycin signaling pathway and intestinal development of broilers. Anim. Nutr., 1: 313-319.
40. Alshelmani, M.I., T.C. Loh, H.L. Foo, A.Q. Sazili and W.H. Lau, 2016. Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, intestinal morphology and gut microflora in broiler chickens. Anim. Feed Sci. Technol., 216: 216-224.
41. Chen, S., R. Einspanier and J. Schoen, 2015. Transepithelial electrical resistance (TEER): A functional parameter to monitor the quality of oviduct epithelial cells cultured on filter supports. Histochem. Cell Biol., 144: 509-515.
42. Torras-Llort, M., J.F. Soriano-Garcia, R. Ferrer and M. Moreto, 1998. Effect of a lysine-enriched diet onl-lysine transport by the brush-border membrane of the chicken jejunum. Am. J. Physiol. Regul. Integr. Comp. Physiol., 274: R69-R75.
43. Amat, C., J.A. Piqueras, J.M. Planas and M. Moret, 1999. Electrical properties of the intestinal mucosa of the chicken and the effects of luminal glucose. Poult. Sci., 78: 1126-1131.