Research Note: Effect of dietary cottonseed meal and soybean oil concentration on digesta passage time and amino acids digestibility in roosters

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ABSTRACT Two experiments were conducted to study the effect of dietary cottonseed meal (**CM**) and soybean oil (**SO**) on passage time of digesta and standardized ileal digestibility (**SID**) of amino acids (**AA**). The experimental design was a 2 × 2 factorial arrangement evaluating the levels of CM (20 or 40 %) and SO (0 or 10 %). Experiment 1 estimated the effect of CM and SO on the passage time of digesta. Twenty-five Chinese yellow-feathered roosters (BW = 2.61 ± 0.08 kg; 26-wk-old) were individually weighed and allocated to 5 diets in 5 randomized complete blocks by initial BW. Experimental diet 1 contained 20% CM and 0 SO, diet 2 contained 20% CM and 10% SO, diet 3 contained 40% CM and 0% SO, diet 4 contained 40% CM and 10% SO, and a nitrogen-free diet was also fed. Passage time through the total digestive tract was determined by time-relative cumulation of dry excreta. Experiment 2 estimated the effect of CM and SO on SID of AA in CM. Thirty Chinese yellow-feathered roosters (BW = 2.91 ± 0.05 kg; 26-wk-old) were allocated to the 5 experimental diets in 6 randomized complete blocks by initial BW to determine the SID of AA. Increasing CM concentration significantly reduced the time for 50% relative cumulation of dry excreta ($P < 0.05$). Adding 10% SO tended to increase the time for 50% relative cumulation of dry excreta ($0.05 < P < 0.10$) relative to diets without SO. Dietary CM and SO did not affect the SID of indispensable AA or dispensable AA of CM significantly, but increasing dietary CM tended to reduce the SID of Lys ($0.05 < P < 0.10$). Increasing SO tended to reduce the SID of Met ($0.05 < P < 0.10$). There were no significant interactive effects of SO and CM ($P > 0.10$). These results suggest passage time is increased with dietary SO, and reduced with dietary CM, but digestibility of AA in CM was not significantly affected by dietary CM and SO.

Key words: amino acids, passage time, standardized ileal digestibility

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INTRODUCTION

Accurate evaluation on amino acids (**AA**) digestibility of feed ingredients is essential for formulation of poultry diets. In the determination of standardized ileal digestibility (**SID**) of AA for feed ingredients, experimental diets are generally formulated to contain similar dietary protein to that of practical poultry diets. However, miscellaneous meals are limited to low inclusion in practical commercial diets due to the presence of antinutritional factors (Olukomaiya et al., 2019). In previous studies, the inclusion of miscellaneous meals varies widely among different studies (Ravindran et al., 2005; Adedokun et al., 2009). Little information can conclude whether the AA digestibility is identical in miscellaneous meals contained at different levels in the experimental diets. High concentration of miscellaneous meals in experimental diets usually leads to high fiber contents which can accelerate digesta passage consequently reduce nutrient digestibility. Because faster passage rate generally contributes to less time for dietary substrates to interact with digestive enzymes and are negatively correlated with digestibility of gross energy (**GE**) (Hughes, 2004) and AA (Toghyani et al., 2020). Mateos et al. (1982) and Goff et al. (2002) reported that nutrient digestibility of feed improved with lower fiber or higher fat concentration, which might due to that lower fiber or higher fat concentration prolong the digesta passage time in the gut of poultry. However, the result of Hakim et al. (2020) was contradictory with these studies. Therefore, this study aims to change the passage time of digesta by inclusion level of cottonseed meal (**CM**) and soybean

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oil (**SO**), then evaluate the effect of digesta passage time on AA digestibility of CM.

MATERIALS AND METHODS

Experimental procedures related to the use of live roosters were approved by the animal care and welfare committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). The code of ethical inspection was IAS 2020-28.

Birds Management and Experimental Diets

Fifty-five Chinese yellow-feathered roosters (Wen's Yellow 2; 26-wk-old) were obtained from the broiler experimental station of Wen's Food Group Co. Ltd. (Guangdong, China). All roosters were raised in individual cages (0.45 × 0.45 × 0.55 m) and provided with free access to water via a suspended nipple drinker line in an environmentally controlled room (23°C) with 16 h of light per day.

Diets were formulated in a 2 × 2 factorial arrangement to contain CM at 20% or 40% and SO at 0% or 10 %. Experimental diet 1 contained 20% CM and 0% SO, diet 2 contained 20% CM and 10% SO, diet 3 contained 40% CM and 0% SO, diet 4 contained 40% CM and 10% SO, and a nitrogen-free diet was formulated as recommended by Adedokun et al. (2011). Dietary levels of vitamins and minerals met or exceeded the estimated requirements for yellow-feathered chicken (China Agricultural Industry Standard, 2004). Titanium dioxide (**TiO_2**) was included as an indigestible marker at 0.5% of the diets (Table 1). The CM was ground through a 2 mm screen and then mixed with other ingredients. Each diet was pelleted using a laboratory non-steam press pellet mill (Model SKJ 150, Funong machine Co., Zhengzhou, Henan, China) to maintain sample uniformity.

Experimental Design

Experiment 1 The objective of this experiment was to estimate the effect of CM and SO concentrations on passage time of digesta. Twenty-five Chinese yellow-feathered roosters (Wen's Yellow 2; BW = 2.61 ± 0.08 kg; 26-wk-old) were individually weighed, assigned to one of 5 blocks by initial BW, and allocated to 5 diets (diets 1 to 4 and a nitrogen-free diet) randomly. Passage time through the total digestive tract was determined according to the method of time-relative cumulation of dry excreta described by Sibbald (1979). Roosters were allowed free access to diets for 5 d, diets were removed at 08:00 am on d 6, and 60 g of each diet except for nitrogen diet was provided at 08:00 am on d 7. The roosters that fed nitrogen free diet during the 5 d adaption period were kept fasting to determine the endogenous excretion. Excreta of each treatment was collected quantitatively using a plastic bag fixed around cloaca of roosters at 2, 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, and 32 h after provision of feed. Excreta were transferred to a glass dish then dried at 105°C for 5 h and weighed.

Experiment 2 The objective of this experiment was to estimate the effect of CM and SO concentrations on SID of AA in CM. Thirty Chinese yellow-feathered roosters

Table 1. Composition of the experimental diets (g/kg as-fed basis).

Item	Diet 1	Diet 2	Diet 3	Diet 4	Nitrogen-free diet
Ingredients					
Maize starch	179.50	155.70	131.80	107.90	200.50
Dextrose	573.00	496.80	420.70	344.60	640.00
Soybean oil	0.00	100.00	0.00	100.00	50.00
Cottonseed meal	200.00	200.00	400.00	400.00	-
Sodium chloride	3.00	3.00	3.00	3.00	-
Limestone	13.00	13.00	13.00	13.00	13.00
Dicalcium phosphate	19.00	19.00	19.00	19.00	19.00
Choline chloride	2.50	2.50	2.50	2.50	2.50
Titanium dioxide	5.00	5.00	5.00	5.00	5.00
Vitamin-mineral premix[1]	5.00	5.00	5.00	5.00	5.00
Solka Floc[2]	-	-	-	-	50.00
Potassium carbonate	-	-	-	-	2.60
Magnesium dioxide	-	-	-	-	2.00
Sodium bicarbonate	-	-	-	-	7.50
Potassium chloride	-	-	-	-	2.90
Total	1,000.00	1,000.00	1,000.00	1,000.00	1000.00
Nutrient content[3,4]					
DM, %					
GE, kcal/kg	3,386	4,087	3,590	4,215	3546
CP, %	10.11	10.06	19.42	20.02	0.19
Ether extract, %	0.15	10.05	0.37	10.45	3.70
Crude ash, %	5.01	5.16	6.32	6.49	4.57
Crude fiber, %	2.42	2.44	4.30	4.10	4.32

[1]Supplied per kilogram of diets: vitamin A, 5,000 IU; vitamin D3, 1,000 IU; vitamin E, 10.0 IU; vitamin K3, 0.50 mg; thiamine 1.8 mg; riboflavin, 3.0 mg; vitamin B6 3.0 mg; vitamin B12 10.0 μg; pantothenic acid 10.0 mg; nicotinic acid 25.0 mg; folic acid 0.55 mg; biotin 0.15 mg; Cu (as copper sulfate) 8.0 mg; Fe (as ferrous sulfate) 80 mg; Mn (as manganese sulfate) 80 mg; Zn (as zinc sulfate) 60 mg; I (as calcium iodate) 0.35 mg; Se (as sodium selenite) 0.15 mg.

[2]Fiber Sales and Development Corp. (Urbana, OH).

[3]Values were determined values (as-fed basis).

[4]Abbreviation: GE, gross energy.

(Wen's Yellow 2; BW = 2.91 ± 0.05kg; 26-wk-old) were assigned to 6 blocks by initial BW and randomly allocated to 5 diets within each block. Diets were available ad libitum for 5 d, and then roosters were euthanized with xylazine hydrochloride on d 6. The section between Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction was removed. The digesta of the posterior two-thirds of this section was gently flushed with distilled water, and immediately stored at −20°C. Subsequently, samples were freeze-dried for chemical analysis.

Chemical Analysis

Diets were ground finely in a laboratory mill fitted with a 0.3-mm mesh screen prior to chemical analysis. Digesta samples were ground with a mortar and pestle prior to chemical analysis. The DM content (method 934.01; AOAC, 1990) was determined by oven drying at 105°C for 5 h. Diets were analyzed for GE by a Parr 6400 automatic adiabatic calorimeter (Parr Instrument Co., Moline, IL) with benzoic acid as the calibration standard. Diets were analyzed for CP (Kjeldahl N; method 954.01; AOAC, 1990), ether extract (method 920.39; AOAC, 1990), crude fiber (method 962.09; AOAC, 1990), and ash (method 942.05; AOAC, 1990). The Concentrations of TiO_2 in diets and digesta were determined according to the method described by Myers et al. (2004) and Wang and Adeola (2018).

AA were determined in an AA analyzer (Biochrom, Version 30, Biochrom Ltd., Cambridge, UK) according to AOAC.994.12. In brief, samples were reacted with 27% (vol/vol) hydrogen peroxide with phenolic formic acid solution for 16 h at 0°C to oxidate cystine and methionine, then neutralized with a small amount of sodium disulphite (approximately 0.8 g). Finally, the samples were hydrolyzed with 6 N HCl (containing phenol) for 23 h at 110°C. The AA was detected on an ion-exchange column and the chromatograms were integrated using the OPENLAB software with AA simultaneously detected at 570 nm and 440 nm. Cystine and methionine were determined as cysteic acid and methionine sulphone, respectively. Tryptophan was determined in an HPLC instrument (Shimadzu HPLC system, type 20A with LC-Solution software, Kyoto, Japan). The samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110°C for 20 h in an autoclave. After adjusting the pH of the hydrolysate to 3.0, tryptophan was separated by reversed-phase chromatography RP-18 on a HPLC column, and the chromatograms were integrated using the Lab solution software with a fluorescence detector.

Calculation and Statistical Analysis

Relative cumulation of dry excreta for each rooster was calculated as cumulative fractions of the total amount using the following equation:

Relative cumulation of dry excreta (%)

$$= \frac{\sum_{i=0}^{t} \text{Dry excreta}}{\sum_{i=0}^{32h} \text{Dry excreta}} \times 100$$

where t is time (h).

The time-relative cumulation of dry excreta curve was expressed as described by Ferrando et al. (1987) using the following mathematical model:

$$y(\%) = \frac{t^n}{t^n + k} \times 100$$

where t is time (h), y is relative cumulation of dry excreta.

Apparent ileal digestibility (**AID**) of AA for diets was calculated as described by Kadim et al. (2002) using the following equation:

$$\text{AID}(\%) = \left[1 - \frac{\text{AA}_{\text{digesta}}}{\text{AA}_{\text{diet}}} \times \frac{\text{M}_{\text{diet}}}{\text{M}_{\text{digesta}}}\right] \times 100$$

Endogenous amino acid (**EAA**) flow was calculated using the following equation:

$$\text{EAA flow} = \text{AA}_{\text{digesta}} \times \frac{\text{M}_{\text{diet}}}{\text{M}_{\text{digesta}}}$$

SID of AA for diets was calculated as described by Kadim et al. (2002) using the following equation:

$$\text{SID}(\%) = \text{AID} + \frac{\text{EAA flow}}{\text{AA}_{\text{diet}}} \times 100$$

where $AA_{digesta}$ is the AA concentration in digesta, AA_{diet} is the AA concentration in diet, $M_{digesta}$ is the TiO_2 concentration in digesta, M_{diet} is the TiO_2 concentration in diet.

The mean for relative cumulation of dry excreta at each time and SID of AA were calculated with the MEANS procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC). Treatment effects were assessed as a 2 × 2 factorial arrangement using the GLM procedure of SAS 9.0 (SAS Inst. Inc.), and the dependent variables were relative cumulation of dry excreta at each time and SID of AA. Duncan's multiple comparison test was conducted to separate the difference of treatment means. Significance level was set at $P < 0.05$, with trends being noted at $0.05 < P < 0.10$. The time-relative cumulation of dry excreta curves were established using the NLIN procedure of SAS 9.0 (SAS Inst. Inc.).

RESULTS AND DISCUSSION

As shown in Table 2, increasing dietary CM increased the relative cumulation of dry excreta at all points measured from 2 to12 h after feeding ($P < 0.05$) and tended to increase the relative cumulation of dry excreta at 14 and 26 h after feeding ($0.05 < P < 0.10$). Dietary SO significantly reduced the relative cumulation of dry excreta at 10 h after feeding ($P < 0.05$) and tended to reduce

Table 2. Percentage of relative accumulative dry excreta (%).

Item	Diet 1	Diet 2	Diet 3	Diet 4	CM		SO		SEM	*P*-value		
					20%	40%	0%	10%		CM	SO	CM × SO
Time after feeding, h												
2	0.90	2.83	5.62	4.23	1.87	4.93	3.26	3.53	1.20	0.022	0.825	0.186
4	5.75	8.16	17.02	14.85	6.96	15.93	11.38	11.51	1.91	<0.001	0.950	0.247
6	24.82	20.81	34.67	31.88	22.82	33.28	29.75	26.35	2.27	<0.001	0.155	0.790
8	39.64	37.56	52.68	46.27	38.60	49.48	46.16	41.91	3.50	0.007	0.242	0.545
10	58.59	47.09	67.65	60.29	52.84	63.97	63.12	53.69	3.96	0.013	0.030	0.608
12	66.86	60.89	79.38	68.73	63.87	74.05	73.12	64.81	4.13	0.025	0.061	0.579
14	72.96	67.61	83.29	73.23	70.28	78.26	78.12	70.42	4.20	0.076	0.085	0.583
24	92.09	93.03	95.71	92.60	92.56	94.15	93.90	92.81	2.24	0.487	0.634	0.380
26	92.37	93.48	96.63	97.14	92.93	96.89	94.50	95.31	1.96	0.060	0.686	0.880
28	95.09	99.09	97.77	97.81	97.09	97.79	96.43	98.45	1.27	0.591	0.131	0.138
30	98.39	99.77	99.65	99.65	99.08	99.65	99.02	99.71	0.82	0.496	0.411	0.407
32	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	-	-	-	-
50% relative cumulative of dry excreta, h	9.23	10.16	7.59	8.63	9.70	8.11	8.41	9.40	0.51	0.007	0.071	0.911

Abbreviation: CM, cottonseed meal; SO, soybean oil.
Diet 1 contained 20% of CM and 0% of SO.
Diet 2 contained 20% of CM and 10% of SO.
Diet 3 contained 40% of CM and 0% of SO.
Diet 4 contained 40% of CM and 10% of SO.

relative cumulation of excreta at 12 and 14 h after feeding ($0.05 < P < 0.10$). Dietary CM and SO concentrations had no interaction effect on the relative cumulation of dry excreta. As shown in Figure 1, the fit models of relative cumulation of dry excreta against time after feeding obviously produced 4 separate curve lines for 4 diets. The relative cumulation of dry excreta curve lines in diets 3, 4, 1, and 2 were decreased sequentially. According to the fit models, the time for 50% relative cumulation of dry excreta were 9.23 h, 10.16 h, 7.59 h, and 8.63 h for diets 1 to 4 respectively (Table 2). In previous studies, the time for 50% relative cumulation of inert marker varied from 5.1 to 8.5 h in 8- to 10-wk-old broilers fed commercial diets (Ferrando et al., 1987) or from 5.5 to 8.9 h in broilers fed barley diets for 2 wk (Almirall and Esteve-Garcia, 1994). The higher time for 50% relative cumulation of dry excreta in the current study may relate to different experimental diets and long fasting after a quick feed intake. Increasing dietary CM concentration significantly reduced the time for 50% relative cumulation of dry excreta ($P < 0.05$). Dietary fiber in CM is 77.8% insoluble and 22.2% soluble (Mou et al., 2020). CM was the sole source of dietary fiber in this experiment, the concentration of insoluble

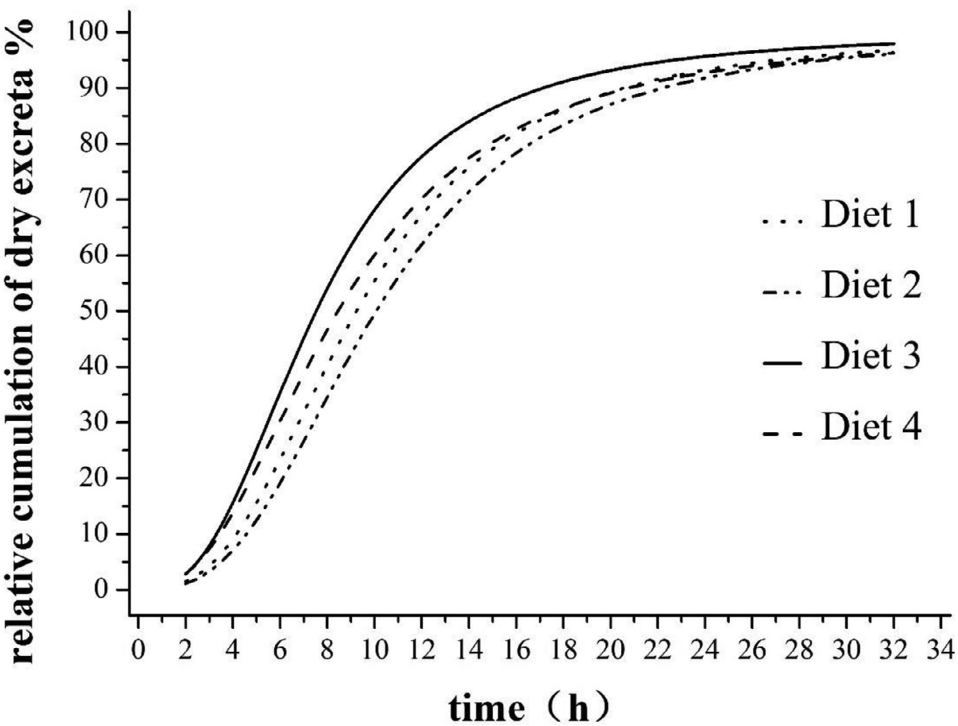

Figure 1. Relative cumulative dry excretion curves of diet 1 contained 20% of cottonseed meal and 0% of soybean oil, diet 2 contained 20% of cottonseed meal and 10% of soybean oil, diet 3 contained 40% of cottonseed meal and 0% of soybean oil and diet 4 contained 40% of cottonseed meal and 10% of soybean oil fed to yellow-feathered rooster of 26-wk-old. Passage time was determined with 5 replicates of 1 rooster.

Table 3. Standardized ileal digestibility of AA in CM of experiment 2 (%).

Item	Diet 1	Diet 2	Diet 3	Diet 4	CM		SO		SEM	*P*-value		
					20%	40%	0%	10%		CM	SO	CM × SO
Indispensable AA												
Arg	90.4	90.9	89.8	89.4	90.6	89.6	90.1	90.1	0.9	0.221	0.947	0.444
His	80.3	81.4	82.0	79.5	80.9	80.7	81.1	80.5	1.6	0.934	0.731	0.238
Ile	74.8	73.1	79.4	70.3	74.0	74.8	77.1	71.7	2.6	0.721	0.124	0.162
Leu	77.6	75.9	81.3	73.6	76.7	77.4	79.4	74.7	2.3	0.736	0.123	0.200
Lys	69.0	68.6	63.8	63.2	68.8	63.5	66.4	65.9	3.1	0.068	0.908	0.969
Met	74.4	71.2	79.9	70.0	72.8	75.0	77.2	70.6	2.8	0.420	0.093	0.228
Phe	84.0	83.8	86.6	82.4	83.9	84.5	85.3	83.1	1.5	0.660	0.226	0.193
Thr	71.6	69.8	76.3	66.2	70.7	71.2	74.0	68.0	2.8	0.826	0.130	0.124
Trp	73.6	77.2	78.8	75.2	75.4	77.0	76.2	76.2	2.0	0.438	0.991	0.106
Val	77.7	76.4	80.8	73.8	77.1	77.3	79.2	75.1	2.2	0.906	0.151	0.196
Dispensable AA												
Ala	76.0	74.5	78.9	71.7	75.2	75.3	77.5	73.1	2.4	0.969	0.162	0.223
Asp	78.9	78.4	80.5	76.0	78.6	78.3	79.7	77.2	1.9	0.843	0.287	0.279
Cys	78.1	78.2	75.7	74.1	78.2	75.0	76.9	76.2	2.0	0.129	0.744	0.650
Glu	86.2	86.3	87.3	84.4	86.2	85.9	86.8	85.4	1.3	0.770	0.375	0.272
Gly	75.4	75.3	76.4	71.7	75.3	74.1	75.9	73.5	2.1	0.515	0.358	0.274
Pro	78.7	77.7	81.5	78.1	78.2	79.8	80.1	77.9	1.9	0.411	0.342	0.538
Ser	77.2	75.7	79.4	73.0	76.4	76.2	78.3	74.4	2.3	0.908	0.182	0.282
Total AA	80.7	80.3	82.4	78.0	80.4	80.2	81.5	79.2	1.8	0.855	0.288	0.266

Abbreviation: CM, cottonseed meal; SO, soybean oil.
Diet 1 contained 20% of CM and 0% of SO.
Diet 2 contained 20% of CM and 10% of SO.
Diet 3 contained 40% of CM and 0% of SO.
Diet 4 contained 40% of CM and 10% of SO.

dietary fiber increased with increasing CM in diets. Therefore, the diets containing 40% CM were excreted more quickly than these containing 20% CM. This result is consistent with that reported by Cao et al. (2003) who observed increasing insoluble dietary fiber generally reduced passage time of digesta in poultry. Adding 10% SO tended to increase the time for 50% relative cumulation of dry excreta ($0.05 < P < 0.10$) relative to diets without SO. Mateos et al. (1982) also observed that yellow grease slowed excretion of digesta in white leghorn hens. The possible mechanism is that fat in the distal gut might induce the release of peptide YY, which inhabits transit through the proximal small intestine (Lin et al., 1996). However, others found that including 5 to 20% poultry fat did not affect the passage time of chyme for broilers (Golian and Maurice, 1992). These variable findings indicate that different types of fat may affect passage time of digesta differently. The interaction of CM and SO did not significantly affect time for 50% relative cumulation of dry excreta, which indicates passage time of digesta is influenced by fiber and fat concentration independently.

As shown in Table 3, the concentration of CM and SO did not affect the SID of dispensable AA or indispensable AA of CM significantly. No interaction was observed between CM and SO on the SID of AA. These results were similar to the conclusion that the dietary concentration of toasted soybean or corn gluten meal increased from 15 to 30% had no effect on AA digestibility of these 2 ingredients (Rezvani et al., 2008). Although the passage time of digesta was changed by the CM and SO concentration in the diets, the inconsistent change between passage time of digesta and AA digestibility could partly due to the high variation of AA digestibility within replicates, which is commonly found by the collection of distal ileal digesta method (Rezvani et al., 2008). However, increasing concentration of CM tended to reduce the SID of Lys ($0.05 < P < 0.10$). Lys is the first limiting AA in cottonseed meal for poultry, the Lys content in diets containing 20% cottonseed meal was much lower than rooster requirement relative to diets containing 40% cottonseed meal, therefore roosters may improve the digestion and absorption of Lys in diets containing 20% cottonseed meal. Increasing dietary SO concentration tended to reduce the SID of Met ($0.05 < P < 0.10$). Generally, high fat concentration increases the passage time thus might improve the digestibility of non-fat components in basal diet (Wiseman and Lessire, 1987). However, opposite result can be concluded from the data presented by Aardsma and Parsons (2016) who observed the digestibility of corn oil was identical but its ME determined using difference method declined with increasing rate from 5 to 10% in the diets, which means the digestibility of basal diet is decreased. In this study, 10% of SO numerically reduced the digestibility not only for Met but also for other 14 AA. It means the presence high fat might reduce the digestibility of AA.

In conclusion, our findings indicate that fiber content and fat content of diets affected the passage time of digesta, but it has little impact on AA digestibility.

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DISCLOSURES

The authors declare no financial or personal conflicts of interest.

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