Pakistan J. Zool., vol. 51(6), pp 2323-2328, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2323.2328

Effects of Graded Levels of Betaine Supplementation on Growth Performance and Intestinal Morphology in Indigenous Young Yellow Feather Broilers

Chen-Yu Sun, Wen-Chao Liu*, Mei Xiao, Zhi-Hui Zhao and Li-Long An*

Department of Animal Science, College of Agriculture, Guangdong Ocean University, Zhanjiang, Guangdong 524088, P. R. China

Chen-Yu Sun and Wen-Chao Liu contributed equally to this work as co-first authors.

Article Information
Received 22 February 2019
Revised 27 March 2019
Accepted 20 April 2019
Available online 12 September 2019

Authors' Contribution
WCL and LLA conceived and designed the study. CYS performed experimental work and laboratory analysis. MX and ZHZ helped in preparation of the manuscript. WCL and CYS analyzed the data and wrote the article.

Key words
Betaine, Growth promoter, Huaixiang chickens, Indigenous young broilers, Intestinal morphology.

ABSTRACT

This 28-d feeding trial was conducted to evaluate the effects of dietary supplementation with different levels of betaine on growth performance and intestinal morphology in Chinese indigenous young yellow feather broilers. A total of 360 male, one-d-old Huaixiang chickens with an average initial BW of 44.52 ± 1.07 g were randomly allotted to 4 treatments. Each dietary treatment consisted of 6 replicate cages, with 15 birds per replicate. The 4 dietary treatments were corn-soybean meal-based diets and supplemented with 0, 500, 1000 and 2000 mg/kg betaine, respectively. During d 1-14, with increasing the dietary betaine levels from 0 to 2000 mg/kg, the BWG was improved (quadratic, $P<0.05$), whereas the FCR was decreased (linear, $P<0.01$). During d 14-28, dietary supplementation of graded level of betaine had no significant effects on BWG, feed intake and FCR ($P>0.05$). Overall (d 1-28), there was a quadratic increase in BWG ($P<0.05$) and a linear decrease in FCR ($P<0.01$) with increasing betaine supplementation. In addition, dietary supplementation with different levels of betaine increased the villus width (linear, $P<0.05$), serosal layer thickness, muscular thickness, and wall thickness of duodenum (quadratic, $P<0.05$), and the serosal layer thickness and wall thickness of jejunum were also improved by inclusion of different level of betaine (cubic, $P<0.05$). Moreover, dietary supplementation of betaine increased the length of ileum (quadratic, $P<0.05$). In conclusion, the betaine could be used as growth promoter for Chinese indigenous young yellow feather broilers (Huaixiang chickens), and the betaine also exerted positive effects on intestinal morphology. The suitable recommended supplemental amount is 500 mg/kg.

INTRODUCTION

Due to the prohibition of subtherapeutic antibiotic usage in animal feed, the interest in searching alternatives to antibiotics in feed has increased. It is well known that the feed additives, including probiotics, prebiotics, enzymes and plant extracts, which could be used as potential alternatives to antibiotics, might improve growth performance and gut health (Liu and Kim, 2017; Liu *et al.,* 2018). As a trimethyl derivative of glycine, betaine is evidenced to produce positive effects on animal performances (Rao *et al.,* 2011). Betaine is known to have two major roles in the body, as a methyl group donor and an organic osmolyte. It is found naturally in a variety sources including sugar beets, wheat bran, spinach, shrimp and many others (Choe *et al.,* 2010). On the other hand, betaine has been shown to protect cells from osmotic stress and allow them to continue regular metabolic activities in conditions that would normally inactivate the cell (Hamidi *et al.,* 2010). Previous studies indicated that betaine supplementation as feed additives plays important roles such as improving growth performance, fat distribution (Wang *et al.,* 2004; Sun *et al,* 2008; Attia *et al.,* 2009; Liu *et al*., 2019), immune response and act as a coccidiostat enhancer (Kettunen *et al.,* 2001; Attia *et al.,* 2009; Chand *et al.,* 2017) in broilers. It is reported that dietary supplementation of betaine also improves the digestibility of specific nutrients, such as methionine, protein and crude fat (Eklund *et al.,* 2005). Suggesting that betaine has positive influence on intestinal health, but few studies focused on the effects of betaine on intestinal morphology and development of broiler chickens.

Yellow feather broilers are increasingly favored by consumers because of their good meat quality, and its proportion in the Chinese broiler market is also growing.

* Corresponding author: wenchao2011@hotmail.com; anlilong_gdou@163.com
0030-9923/2019/0006-2323$ 9.00/0

Huaixiang chicken is a famous and excellent indigenous yellow feather broiler breed in China. Because of the young broilers' intestinal development is imperfect and low resistance, subtherapeutic antibiotics are widely used in young broilers feed. From the foregoing, it hypothesized that dietary betaine can improve the growth performance and intestinal development. However, no research has been reported to study the effect of supplementing betaine as alternatives to antibiotics in indigenous young yellow feather broilers. Therefore, the present experiment was conducted to evaluate the effects of graded levels of betaine supplementation on growth performance and intestinal morphology in Chinese indigenous young yellow feather broilers (Huaixiang chickens).

MATERIALS AND METHODS

All experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Guangdong Ocean University, Zhanjiang, Guangdong, P. R. China.

Birds, diets and experimental procedures

A total of 288 male, one-d-old Huaixiang chickens (Chinese indigenous yellow feather broilers) with an average initial BW of 44.52 ± 1.07 g were obtained from a local hatchery, and randomly distributed to 4 treatments. Each dietary treatment consisted of 6 replicate cages, with 12 birds per replicate. All birds were raised in stainless steel pens with concrete floors covered with clean rice bran in an environmentally controlled house. The ingredient composition and nutrient content of basal diets for both experimental phases are presented in Table 1. Basal diets were formulated to meet or exceed Chinese indigenous yellow feather broilers recommendations based on Chinese Chicken Feeding Standard (NY /T33-2004). The 4 dietary treatments were corn-soybean meal-based diets and supplemented with 0, 500, 1000 and 2000 mg/kg betaine, respectively. The betaine was obtained from a Chinese commercial company (Anhydrous betaine, the purity is 99%, Shandong Jianchuan Biotechnology Co., Ltd., Shandong, China). All experimental diets were given to the birds in mash form. Birds had free access to feed and water. Lighting was continuous, and room temperature was maintained at 33 ± 1 °C for the first 3 d, and then gradually reduced by 2 °C a week until reaching 24 °C.

Sampling and measurements

Broilers were weighed on a cage basis on d 0, 14 and 28, and feed consumption was recorded throughout the experiment. The body weight gains (BWG), feed intake and feed conversion ratio (FCR) were then calculated using these information for each phase.

Table I. Basal diet composition (as-fed basis).

Item	Contents (%)
Ingredients	
Corn	60.99
Soybean meal	29.00
Soybean oil	2.00
Limestone	1.28
Wheat bran	2.16
Fish meal	3.00
CaHPO4	1.26
Premix[1]	0.31
Total	100.00
Nutrient levels[2]	
ME (MJ/kg)	12.30
Crude protein (%)	20.03
Ca (%)	1.00
Met (%)	0.45
Lys (%)	1.09
Phosphorus	0.68

[1]Premix Provided per kilogram of diet: 5,000 IU of vitamin A, 1000 IU of vitamin D_3, 10 IU of vitamin E, 0.5 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 10 µg of vitamin B_{12}, 25 mg of niacin, 0.55 mg of folic acid, 0.2 mg of biotin, 500 mg of choline, and 10.5 mg of pantothenic acid. 60 mg of Zn, 80 mg of Mn, 80 mg of Fe, 3.75 mg of Cu, and 0.35 mg of I.
[2]Except for ME, others are measured values.

At the end of the experiment (d 28), all birds were fasting 24 h, and then 6 birds (1 bird per cage) were randomly selected from each treatment were weighted, and killed by cervical dislocation for the examination of intestinal morphology. The small intestine were separated, the length and relative weight (intestinal weight/ live weight before slaughter, 100%) of duodenum, jejunum and ileum were determined. Subsequently, approximately 2 cm segments of the duodenum, jejunum and ileum at consistent locations were collected immediately. The intestinal samples from each section were fixed in 10 % buffered formalin until analyzed. Each intestinal segment was embedded in paraffin. A 7-µm section of each sample was placed onto a glass slide and stained with alcian blue/ haematoxylin and eosin for examination with a light microscope. Villus height and crypt depth of the small intestine were measured at 100 × magnification using computer software (Sigma Scan, Jandel Scientific, San Rafael, CA. USA), then villus height to crypt depth ratio were calculated. The villus width, serosal layer thickness, muscular thickness, wall thickness and mucosal thickness were measured according to Yamauchi *et al.* (2006).

Statistical analysis

All data were analyzed by using GLM procedure of SAS (SAS Institute Inc., Cary, NC). Data on growth performance were analyzed using cage as the experimental unit, whereas intestinal morphology were analyzed using individual broiler as the experimental unit. Orthogonal polynomial contrasts were used to test the linear, quadratic and cubic effects of the increasing levels of dietary betaine. Differences among treatment means were determined using the Tukey's multiple range test. Variability in the data was expressed as standard error of means (SEM) and P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Growth performance

As described in Table II, during d1-14, with increasing dietary betaine levels from 0 to 2000 mg/kg, the BWG was improved (quadratic, P<0.05). Addition of 500 and 1000 mg/kg betaine significantly increased the BWG (P<0.05). Whereas the FCR was decreased (linear, P<0.01), and supplementation of betaine at 500, 1000 and 2000 mg/kg significantly reduced the FCR. During d14-28, dietary supplementation of graded level of betaine had no significant effects on BWG, feed intake and FCR (P>0.05). Overall (d 1-28), there was a quadratic increase in BWG (P<0.05) and a linear decrease in FCR (P<0.01) with increasing betaine supplementation. Dietary 500 mg/kg betaine supplementation significantly improved BWG (P<0.05) and inclusion level at 1000 and 2000 mg/kg betaine significantly decreased the FCR (P<0.05). In summary, the suitable amount of betaine supplementation for growth promotion at 500 or 1000 mg/kg.

Young broilers growth faces many challenges because the low resistance and digestive physiology of young chicks is nt yet fully developed. The present study demonstrated for the first time that betaine can act as a growth promoter for Chinese indigenous young yellow feather broilers. The results from the present study are in agreement with the findings of the reports from Zhan *et al.* (2006), who revealed that supplementation of betaine (0.5 g/kg of diet) to the diet increased weight gain and decreased the FCR of Arbor Acres male broilers. Similarly, Sun *et al.* (2008) suggested that 400 mg/kg betaine supplementation improved the BW at d 21 and ADG of d 1-21 in Arbor Acres (mixed sex) broiler chickens. Alirezaei *et al*. (2012) demonstrated that inclusion of betaine at the dosage of 1 g/kg showed positive influence on body weight gain of Cobb broiler chickens. He *et al*. (2015) also reported that betaine-supplemented Arbor Acres broilers groups showed higher feed consumption, body weight gain, and lower feed: gain ratio compared with the heat stressed control group.

Table II. Effects of dietary betaine supplementation on growth performance in Chinese indigenous young yellow-broilers.

Items[1]	Dietary betaine levels (mg/kg)				SEM[2]	P-value			
	0	500	1000	2000		ANOVA	Linear	Quadratic	Cubic
Initial									
BW	45.44	44.55	44.00	44.06	1.07	0.7623	0.3417	0.6606	0.9544
1-14d									
BWG	31.28b	40.68a	38.95a	36.24ab	2.20	0.0424	0.2005	0.0147	0.3170
Feed intake	129.24ab	141.09a	110.36b	111.44b	6.29	0.0091	0.0092	0.4054	0.0184
FCR	4.36a	3.48b	2.87b	3.10b	0.282	0.0101	0.0033	0.0678	0.6486
15-28d									
BWG	155.23	164.78	163.78	160.22	4.86	0.5188	0.5296	0.1977	0.7185
Feed intake	333.21	344.72	349.37	326.23	8.52	0.2451	0.6753	0.0600	0.5911
FCR	2.15	2.10	2.13	2.05	0.04	0.3331	0.1557	0.6888	0.2735
Overall (1-28d)									
BWG	186.50b	205.45a	202.73ab	196.47ab	5.30	0.0979	0.2703	0.0313	0.4563
Feed intake	460.44ab	485.80a	459.72ab	437.67b	10.88	0.0506	0.0569	0.0544	0.2891
FCR	2.48a	2.37ab	2.27bc	2.24c	0.042	0.0029	0.0004	0.3422	0.3422

[1]BW, body weight; BWG, body weight gain; FCR, feed conversion ratio (feed/gain).
[2]SEM, Standard error of means.

Table III. Effects of dietary betaine supplementation on intestinal morphology in Chinese indigenous young yellow-broilers.

Items	Dietary betaine levels (mg/kg)				SEM[1]	P-value			
	0	500	1000	2000		ANOVA	Linear	Quadratic	Cubic
Duodenum									
Villus height	494.46	500.38	475.62	473.72	21.06	0.7527	0.3704	0.8551	0.5782
Villus width	65.75b	76.99a	77.21a	84.81a	3.30	0.0085	0.0358	0.4106	0.3968
Crypt depth	77.55	74.79	93.27	80.18	5.96	0.1760	0.3384	0.3999	0.0664
Villus height: crypt depth	6.61	6.98	5.19	6.12	0.63	0.2589	0.2673	0.6654	0.1074
Serosal layer thickness	26.83b	31.65ab	37.39a	23.99b	2.77	0.0195	0.8260	0.0050	0.1265
Muscular thickness	55.61b	75.01a	65.68ab	59.29b	4.65	0.0478	0.9353	0.0142	0.1486
Wall thickness	82.44b	106.65a	103.07a	83.28b	6.41	0.0287	0.9711	0.0037	0.6921
Mucosal thickness	561.37	568.15	560.03	541.77	21.14	0.8356	0.4899	0.5624	0.9603
Jejunum									
Villus height	428.30	392.54	434.41	416.09	18.61	0.4245	0.9506	0.6460	0.1184
Villus width	78.91	69.66	89.84	86.52	6.22	0.0863	0.0953	0.5789	0.0685
Crypt depth	65.68	70.08	78.89	75.05	5.07	0.3132	0.1243	0.4289	0.4634
Villus height: crypt depth	6.65	5.71	5.53	5.71	0.45	0.3262	0.1598	0.2358	0.8404
Serosal layer thickness	17.04b	24.65a	19.85ab	21.61ab	1.87	0.0586	0.3020	0.1385	0.0384
Muscular thickness	50.73b	61.98a	54.36ab	56.35ab	3.40	0.1717	0.5538	0.1935	0.0809
Wall thickness	67.77b	86.63a	74.2ab	77.96ab	4.41	0.0439	0.3717	0.1069	0.0293
Mucosal thickness	487.41	452.89	511.26	478.88	21.63	0.3298	0.7393	0.9611	0.0770
Ileum									
Villus height	261.10b	340.19a	296.02ab	290.28ab	24.05	0.1824	0.2395	0.0981	0.3518
Villus width	75.42	75.18	76.91	82.06	8.10	0.9238	0.6227	0.7439	0.7486
Crypt depth	53.09	61.76	55.46	57.71	5.82	0.7547	0.4513	0.5901	0.5918
Villus height: crypt depth	5.03	5.63	5.50	5.34	0.57	0.8892	0.6764	0.5163	0.9743
Serosal layer thickness	26.59	27.11	25.11	23.94	2.93	0.8658	0.6566	0.7756	0.5194
Muscular thickness	63.67	75.09	65.72	64.09	7.07	0.6388	0.7414	0.3705	0.3951
Wall thickness	90.25	102.20	90.83	88.02	9.68	0.7325	0.9153	0.4575	0.4141
Mucosal thickness	303.61b	391.89a	342.00ab	336.84ab	25.15	0.1436	0.2035	0.0830	0.3170

[1]SEM, Standard error of means.

Our observation and the reports from previous studies for weight gain in the betaine-treated broilers supports the idea that betaine is associated with antioxidant and methyl donor properties through its involvement in cell membrane stabilisation and homocysteine remethylation (Alirezaei *et al.,* 2012). However, contrary to the results above, reports from Schutte *et al.* (1997) and Esteve-Garcia and Mack (2000) indicated that betaine had a small and non-significant effect on growth performance of broilers. These inconsistent findings may be due to the inclusion level, feed composition or chick breed.

Intestinal morphology, length and relative weight

As shown in Table III, dietary supplementation with different levels of betaine increased the villus width (linear, $P<0.05$), serosal layer thickness, muscular thickness, and wall thickness (quadratic, $P<0.05$) of duodenum. Also, the serosal layer thickness and wall thickness of jejunum were improved by inclusion of different level of betaine (cubic, $P<0.05$). In addition, supplementation of betaine at 500

Table IV. Effects of dietary betaine supplementation on intestinal length and relative weight in Chinese indigenous young yellow-broilers.

Items	Dietary betaine levels (mg/kg)				SEM[1]	P-value			
	0	500	1000	2000		ANOVA	Linear	Quadratic	Cubic
Duodenum									
Length	19.75	21.2	20.13	19.68	0.96	0.6645	0.7713	0.3366	0.4754
Relative weight (%)	1.353	1.19	1.483	1.155	0.122	0.0902	0.4747	0.3840	0.0493
Jejunum									
Length	35.78	40.83	36.88	35.53	1.87	0.2059	0.5828	0.1080	0.1861
Relative weight (%)	1.88	1.697	2.028	1.745	0.109	0.1754	0.8819	0.6516	0.0343
Ileum									
Length	31.07b	38.45a	34.30ab	31.23b	1.44	0.0079	0.5794	0.0025	0.0690
Relative weight (%)	1.165	1.263	1.238	1.098	0.074	0.4159	0.5088	0.1298	0.9803

[1]SEM, Standard error of means.

mg/kg increased the villus height and mucosal thickness of ileum (P<0.05). The effects of betaine on intestinal length and relative weight were presented in Table IV. Dietary betaine supplementation at the level of 500 mg/kg increased the ileum length (P<0.05), but no differences were observed on the length and relative weight of duodenum and jejunum among the groups in the current study (P>0.05). Taken together, the supplementation level at 500 mg/kg is most beneficial to intestinal morphology and development of Huaixiang chickens.

In our experiment, when supplemented with betaine to Chinese indigenous young yellow feather broilers (Huaixiang chickens), the intestinal development and histomorphology of gut microstructure was improved. Similarly, Kettunen *et al.* (2001) found that dietary betaine supplementation increased the epithelial villus–crypt ratio in bird gut and they thought the improved mucosal structure was due to both the methyl group donor nature and the osmotic nature of betaine. However, few other researchers investigated the effects of betaine on intestinal morphology and development of broilers, so no more comparisons can be made. A study in rats carried by Wang *et al*. (2018) who demonstrated that betaine supplementation improved villus heights of the duodenum, jejunum and ileum in high-salt stressed rats, and similar results were observed in the ratio of villus height to crypt depth. Moreover, it was assumed that gut microbiota also contributed to the intestinal development (Liu and Kim, 2017). Therefore, betaine might be involved in the gut microbiota and then promoted the intestinal development and microstructure morphology. However, the underlying mechanism is not quite clear yet and need to be verified by further studies.

CONCLUSION

On the whole, dietary supplementation with graded levels of betaine in Chinese indigenous young yellow feather broilers diets improved the growth performance, and showed positive effects on intestinal morphology, the recommended supplemental amount is 500 mg/kg. However, further research is necessary to confirm the conclusions and to illustrate the underlying mechanism.

ACKNOWLEDGMENTS

This research was funded by the Talent Research Start-up Project of Guangdong Ocean University (R18007); Innovative Strong School Engineering Youth Talent Project (2017KQNCX090) and Key Platform Projects: Innovative Platform for Efficient Use of Animal Genetic Resources in the Tropics and Healthy Farming (2018302) by Department of Education in Guangdong Province; and Natural Science Foundation of Guangdong Province (2018A030307023).

Statement of conflicts of interest

The authors declare they have no conflict of interest.

REFERENCES

Alirezaei, M., Reza Gheisari, H., Reza Ranjbar, V. and Hajibemani, A., 2012. Betaine: a promising antioxidant agent for enhancement of broiler meat quality. *Br. Poult. Sci.* **53**: 699-707. https://doi.org/10.1080/00071668.2012.728283

Attia, Y.A., Hassan, R.A. and Qota, E.M.A., 2009. Recovery from adverse effects of heat stress on slow-growing chicks in the tropics 1: Effect of ascorbic acid and different levels of betaine. *Trop. Anim. Hlth. Prod.,* **41:** 807-818. https://doi.org/10.1007/s11250-008-9256-9

Chand, N., Naz, S., Maris, H., Khan, R.U., Khan, S. and Qureshi, M.S., 2017. Effect of betaine supplementation on the performance and immune response of heat stressed broilers. *Pakistan J. Zool.,* **49:** 1857-1862. https://doi.org/10.17582/journal.pjz/2017.49.5.1857.1862

Chicken Feeding Standard, NY /T33-2004 [S]. Ministry of Agriculture of the People's Republic. Beijing: Agriculture Press, China 2004.

Choe, H.S., Li, H.L., Park, J.H., Kang, C.W. and Ryu, K.S., 2010. Effects of dietary betaine on the secretion of insulin-like growth factor-I and insulin-like growth factor binding protein-1 and -3 in laying hens. *Asian-Austral. J. Anim. Sci.,* **23:** 379–384. https://doi.org/10.5713/ajas.2010.70653

Eklund, M., Bauer, E., Wamatu, J. and Mosenthin, R., 2005. Potential nutritional and physiological functions of betaine in livestock. *Nutr. Res. Rev.,* **18:** 31–48. https://doi.org/10.1079/NRR200493

Esteve-Garcia, E. and Mack, S., 2000. The effect of DL-methionine and betaine on growth performance and carcass characteristics in broilers. *Anim. Feed Sci. Technol.,* **87:** 85–93. https://doi.org/10.1016/S0377-8401(00)00174-7

Hamidi, H., Jahanian, R., Pourreza, J., 2010. Effect of dietary betaine on performance, immunocompetence and gut contents osmolarity of broilers challenged with a mixed coccidial infection. *Asian J. Anim. Vet. Adv.,* **5:** 193–201. https://doi.org/10.3923/ajava.2010.193.201

He, S., Zhao, S., Dai, S.F., Liu, D. and Bokhari, S.G., 2015. Effects of dietary betaine on growth performance, fat deposition and serum lipids in broilers subjected to chronic heat stress. *Anim. Sci. J.,* **86:** 897-903. https://doi.org/10.1111/asj.12372

Kettunen, H., Tiihonen, K., Peuranen, S., Saarinen, M.T. and Remus, J.C., 2001. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. *Comp. Biochem. Physiol. A.,* **130:** 759-769. https://doi.org/10.1016/S1095-6433(01)00410-X

Liu, W.C. and Kim, I.H., 2017. Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. *Poult. Sci.,* **96:** 566-573. https://doi.org/10.3382/ps/pew258

Liu, W.C., Ye, M., Liao, J.H., Zhao, Z.H., Kim, I.H. and An, L.L., 2018. Application of complex probiotics in swine nutrition– A review. *Annls. Anim. Sci.,* **18:** 335-350. https://doi.org/10.2478/aoas-2018-0005

Liu, W.C., Yuan, Y.L., Sun, C.Y., Balasubramanian, B., Zhao, Z.H. and An, L.L., 2019. Effects of betaine on growth performance, digestive function, carcass traits, and meat quality in indigenous yellow-feathered broilers under long-term heat stress. *Animals,* **9**: 506. https://dx.doi.org/10.3390/ani9080506

Rao, S., Raju, M., Panda, A., Saharia, P. and Sunder, G.S., 2011. Effect of supplementing betaine on performance, carcass traits and immune responses in broiler chicken fed diets containing different concentrations of methionine. *Asian Austral. J. Anim. Sci.,* **24:** 662–669. https://doi.org/10.5713/ajas.2011.10286

Schutte, J.B., Jong, J.D., Smink, W. and Pack, M., 1997. Replacement value of betaine for DL-methionine in male broiler chicks. *Poult. Sci.,* **76:** 321–325. https://doi.org/10.1093/ps/76.2.321

Sun, H., Yang, W.R., Yang, Z.B., Wang, Y., Jiang, S.Z. and Zhang, G.G., 2008. Effects of betaine supplementation to methionine deficient diet on growth performance and carcass characteristics of broilers. *Am. J. Anim. Vet. Sci.,* **3:** 78-84. https://doi.org/10.3844/ajavsp.2008.78.84

Wang, Y.Z., Xu, Z.R. and Feng, J., 2004. The effect of betaine and methionine on growth performance and carcass characteristics in meat ducks. *Anim. Feed Sci. Technol.,* **116:** 151-159. https://doi.org/10.1016/j.anifeedsci.2004.05.003

Wang, H.C., Li, S.S., Fang, S.L., Yang, X.J. and Feng, J., 2018. Betaine improves intestinal functions by enhancing digestive enzymes, ameliorating intestinal morphology, and enriching intestinal microbiota in high-salt stressed rats. *Nutrients,* **10:** 907. https://doi.org/10.3390/nu10070907

Yamauchi, K., Buwjoom, T., Koge, K. and Ebashi, T., 2006. Histological intestinal recovery in chickens refed dietary sugar cane extract. *Poult. Sci.,* **85:** 645–651. https://doi.org/10.1093/ps/85.4.645

Zhan, X.A., Li, J.X., Xu, Z.R. and Zhao, R.Q., 2006. Effects of methionine and betaine supplementation on growth performance, carcase composition and metabolism of lipids in male broilers. *Br. Poult. Sci.,* **47:** 576-580. https://doi.org/10.1080/00071660600963438