Original Research Article

Effect of sulphur-based amino acids with or without formic acid on apparent nutrient digestibility and gut morphology of broiler birds

ABSTRACT

Aim: Nutrient digestibility and gut morphological response of broiler birds fed diets supplemented with sulphur-based amino acids with or without formic acid were investigated in a 56-day feeding trial.
Methodology: One hundred and ninety-two one-day old unsexed Arbor Acre broilers were used. The birds were brooded for 7 days after which they were randomly allotted to 4 dietary treatments with 4 replicates of 12 birds each. The experimental treatments were: diet 1: basal diet + DL-methionine without formic acid, diet 2: basal diet + DL-methionine with 0.8% formic acid, diet 3: basal diet + methionine hydroxyl analogue without formic acid, diet 4: basal diet + methionine hydroxyl analogue with formic acid.
Experimental design: The design of the experiment was a completely randomised design in a 2X2 factorial arrangement.
Results: Formic acid supplementation had a significant ($P<0.05$) influence on apparent nutrient digestibility of all the nutrients assessed. Apparent nutrient digestibility was significantly ($p<0.05$) improved in birds fed with diet 2 relative to birds fed other diets. There were significant ($P<0.05$) differences observed in the wall thickness, villus height, villus width and crypt depth of the birds. Formic acid supplementation significantly ($P<0.05$) reduced gut wall thickness and increased villus height, villus width and crypt depth in birds fed with diet 4. The interaction between formic acid and thetype of sulphur amino acid sources was significant for wall thickness, crypt depth, villus height and villus width ($P<0.0069$ to 0.0488) of the jejunum.

Conclusion: The gut parameters were better for birds fed with diet 2. Likewise birds fed with diet 4 showed better gut morphology. Formic acid supplementation improved apparent nutrient digestibility and gut morphology of broiler chickens used in the study.

Keywords: DL-methionine, gut morphology, formic acid, methionine hydroxyl analogue, microbial load

1. INTRODUCTION

All animals need to be well fed and healthy if they are to grow to their potential. The nutrition of an animal is therefore of great importance if this is to be achieved in practice. Feed additives provide mechanism by which dietary deficiencies can be addressed, this benefit not only the nutrition and thus the growth rate of the animal concerned, but also its health and welfare. In the modern day farming, the nutritional requirements of farm animals are well understood and all the requirements can be met through direct dietary supplementation of the limiting nutrient in concentrated form. Organic acids are considered to be any organic carboxylic acid including fatty acids and amino acids, of the general structure R-COOH. Not all of these acids have effects on gut microflora *(Canibe et al 2005)*. In fact, the organic acids associated with specific antimicrobial activity are short chain acids (C1 – C7). They are either simple or monocarboxylic acids such as formic, acetic, propionic and butyric acids, or carboxylic acids bearing an hydroxyl group (usually on the alpha carbon) such as lactic, malic, tartaric and citric acids *(Rickel 2003)*. The inclusion of organic acid in poultry diet was considered due to its ability to render unfavourable microflora such as salmonella inactive by decreasing pH in the gastrointestinal tract (GIT). In contrast it was to promote favourable environment in the GIT for growth of the microflora resistant to pH<7 (such as Lactobacillus). Thus organic acids create an ideal flora in the GIT, improve digestion and nutrient absorption, stimulate growth and increase efficiency *(Choct 2004)*.

Methionine is required in avian species for it feather growth and protein synthesis. It is however classified as a first limiting amino acid in avian species because it is limited in plant protein sources. It is therefore necessary to supply it in diets deficient in the required amount of methionine *(Chaiyapoom 2009)*. Methionine sources include DL-methionine, liquid methionie hydroxyl analogue (HMTBA), calcium salt of methionine hydroxyl analogue, DL-methionine sodium salt etc. The two methionine sources are absorbed in the animals GIT, converted to L-methionine and used in protein synthesis and other metabolic functions *(Buttin 1999)*. The study was conducted to investigate the effect of sulphur-based amino acids with or without formic acid on apparent nutrient digestibility and gut morphology of broiler chickens.

2. MATERIAL AND METHODS

This study was carried out at the Teaching and Research Farm, University of \ibadan, Nigeria. One hundred and ninety-two Arbor Acre broiler chicks were used for the study. The birds were reared in a well-ventilated poultry house with natural lightening. After 7 days brooding, the birds were randomly allotted to 4 dietary treatments. Each dietary treatment had 4 replicates of 12 birds each. Experimental diets and water were given *ad libitum*. Composition of the experimental diet is shown in Tables 1 and 2. The experimental design was a 2x2 factorial arrangement in a completely randomised design.

The starter and finisher diets formulated were offered to the birds from day 8 to 28 and day 29 to 56 respectively. Diet 1 was the control which had the inclusion of DL-methionine without formic acid in the basal diet; diet 2 was basal diet with DL-methionine and 0.8% liquid formic acid; diet 3 contained basal diet with methionine hydroxyl analogue (MHA) without formic acid while diet 4 contained basal diet with MHA and 0.8% liquid formic acid.

Metabolic study

At week 7, 8 birds were randomly selected from each treatment ad placed in metabolic cages (i.e. 2 birds/replicate/cage) for collection of faeces for apparent nutrient retention determination. The birds were left in the cages for four days to acclimatize. Fresh faeces were collected in the morning, the faecal samples were wrapped in foil, weighed (the weights were recorded) and oven dried at 60^0C until constant weights were obtained. The oven-dried faeces were milled, analysed and consequently used for digestibility calculation as nutrient in diet consumed – nutrient in faeces /nutrient in diet consumed.

Intestinal morphology

Approximately 5cm length each of the jejunum from birds from each replicate selected at random were removed to carry out a histological morphometric analysis of the jejuna mid-epithelium. Histological examinations were carried out according to the method of Iji et al (2001). Intestinal samples from each section were immersed in 10% formaldehyde, before fixation in Bouin's solution and paraffin embedding. The samples were transferred into 70% ethanol after 24 hours. Paraffin sections at 6µm thickness made from each sample were stained haematoxylin and eosin, and examined under microscope. Villus height (from the tip of the villus to the villus crypt junction), crypt depth (depth of invagination between adjacent villi), whole wall thickness and smooth muscle width were analysed from each preparation. These values were examined to predict the absorption ability of the experimental animal in retrospect to the test ingredients.

Proximate analysis

The proximate composition of the diets and faecal sampes were carried out according to the method of A.O.A.C (2000).

Statistical analysis

Data obtained were analysed by means of the General Linear Model using SAS statistical software (SAS 2004). Differences among means were separated using Duncan Multiple Range Test significant at P<0.05 (Steel *et al* 1997).

3. RESULTS AND DISCUSSION

Nutrient digestibility

Apparent nutrient digestibility of broiler chickens fed with experimental diets is shown in Table 3. There were improvements in the nutrient digestibility of birds fed 0.8% formic acid (diets 2 and 4). This is in agreement with the findings of Ghazalah *et al.* (2011) who observed improved nutrient digestibility compared to the control with the best result obtained from 0.5% formic acid inclusion. Similar trend was found by Hernandez *et al.* (2006), Garcia *et al. (2007)* and Helen and Christian (2010) on apparent ileal digestibility. This improvement may be due to the ability of organic acids to create an ideal flora in the GIT, improve digestion and nutrient absorption, stimulate growth and increase efficiency *(Choct 2004)*. On the other hand, neither the different methionine sources nor the interaction of the different methionine sources and formic acid significantly influenced the nutrient digestibility of the birds.

Gut morphology

Table 4 shows gut morphology of birds fed experimental diets. The whole wall thickness was highest in the jejuna segment of broilers fed the control diet (diet 1). Broilers fed other experimental diets responded significantly similar. This result is harmony with the findings of Gunal *et al. (2006)* who reported a reduction in muscularis thickness of birds fed acidified feed. The results of the present study also revealed a reduction in the cell wall thickness of birds on diets 2, 3 and 4. This may be attributed to the effect of acidification which had antibacterial effect (i.e. its ability to reduce negative bacteria count). During a pathogenic bacteria infection, lymphocytes accumulate to kill the pathogens and cause inflammation which in turn increases the wall thickness. Organic acid reduces microbial population numbers and their production of toxin and by-products in the lumen, thereby reducing lymphocyte accumulation and subsequently inflammation and whole wall thickness. Reduced whole wall thickness is helpful in improving the digestion and absorption of nutrients.

An increased villus height is parallel by increased digestive ad absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzyme an nutrient transport system (Caspary 1992). The results of the present study showed that the villus height and villus width were significantly (P<0.05) higher in the jejuna section of broiler fed diet 4. These results are consistent with the previous findings by Sakata (1987) who reported increased villus height in the jejunum by most organic acidifiers. Also, dietary inclusion of organic acid being short fatty acid decreases the production of ammonium and also stimulates the proliferation of the epithelial cells of the GIT (Sakata 1987; Ichikawa *et al* 1999.

Ultimately, organic acids function by decreasing the inflammatory reactions at the intestinal mucosa. This in turn increases the villus height and functions of secretion, digestion and absorption of nutrients by the mucosa. The crypt depth is considered as the villus factory and deeper crypt indicates fast tissue turn over to permit the renewal of the villus as needed in sloughing or inflammation from pathogens or their toxins and high demands for tissue (Yasaon 1987). Saki *et al.* (2011) and Garcia *et al.* (2007) reported increased crypt depth with increasing inclusion rate of dietary organic acid. Awad *et al.* (2008) reported that increased villus height is an indication of an increased surface area for greater absorption of available nutrients while deeper crypt depth is implicated in a greater production of enterokinase which is the precursor for the production of trypsin. Trypsin is needed for the digestion of protein which culminates in increased availability of amino acids which is vital for improved bird performance. Crypt depth of broilers fed diet 4 in this study was significantly (P<0.05) higher than broilers fed other experimental diets. The findings of Abdel-Fattah *et al.* (2008) showed that chicks whose diets were provided by organic acids had longer and thicker villi than the control. Organic acids have trophic effects on the mucosa of the GIT *(Dibner and Buttin 2002)*. Once MHA is in an acidic environment it completely dissociates into HTMBA. It had reported that HTMBA has a significant antibacterial effect on the intestine of monogastric animals *(Dibner and Buttin 2002)*.

4. CONCLUSION

The gut morphology parameters measured in this study showed that birds fed 0.8% formic acid were better than those on diet without formic acid supplementation. Likewise, birds fed diet supplemented with MHA with 0.8% formic acid showed the better gut morphology results when compared with birds fed other experimental diets. Formic acid supplementation improved apparent nutrient digestibility and gut morphology of broiler chickens used in the study.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

Abdel-Fattah SA, El-Sanhoury MH, El-Medonary NM, Abdel-Azeem F. Thyroid activity, some blood constituents, organic morphology and performance of broiler chicks fed supplemental organic acids. International Journal of Poultry Science, 2008,7(3):215-222

Awad WA, K Ghareeb, J Bohm. Intestinal structure and function of broiler chickens on diet supplemented with a symbiotic containing *Enterococcus faecium* and oligosaccharides. Int. J. Mol. Sci., 2008,9:2205-2216

Canibe N, Hojberg O, Hojsgaard S, Jensen BB. Feed physical form and formic acid addition to the feed affect gastrointestinal ecology and growth performance of growing pigs. J. Anim. Sci., 2005,83(6):1287-1302

Caspary WF. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr., 1992,55:299s-308s

Chaiyapoom B. Role of methionine in poultry production. Poultry Science, 2009,46(3):169-179

Choct N. Effect of organic acids, prebiotics and enzyme on control of necrotic enteritis and performance of broiler chicken. School of Rural Science and Agriculture, University of New England. Available at http://www.jcu.edu.au/school/bms/avpa/avpaconfapr_2002/abstracts/choct.pd

Dibner JJ, Buttin P. Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research, 2002,11:453-463

Garcia V, Catala-Gregori P, Hernandez F, Megias MD, Madrid J. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology and meat yield of broilers. Journal of Applied Poultry Research, 2007,16:555-562

Ghazalah AA, Atta AM, Kout E, Moustafa MEL, Riry FHS. Effect of dietary supplementation of organic acids on performance, nutrient digestibility and health of broiler chicks. International Journal of Poultry Science, 2011,10(3):176-184

Gunal M, Yayli G, Kaya O, Karahan N, Sulak O. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Inetrnational Journal of Poultry Science, 2006,5:149-155

Helen B, Christian L. Performance enhancement through the use of diformates in broiler. ADDCON company in Germany, Porsgrunnpublication, date 01/02/2010

Hernadez,F., V. Garcia, J. Madrid, J. Orengo, P. Catala and M.D . Megias, 2006. Effect of formic acid on performance, digestibility, Intestinal histo-morphology and plasma metabolite levels of broiler chickens. Br. Sci., 47: 50-56

Ichikawa H, T Kuroiwa, A Inagaki, R Shineha, T Nishihira, S Satomi, T Sakata. Probiotic bacteria stimulate gut epithelial cell proliferation in rat. Digestive Diseases and Sciences, 1999,44:2119-2123

Iji PA, Saki AA, Tivey DR. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Animal Feed Science and Technology, 2001,89:175-188

Ricke S.C., 2003. Perspective on the use of organic acids and short chain fatty acids as antimicrobials Poult. Sci., 82: 632-639

Sakata T. Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanantion for tropic effects of fermentable fibre, gut microbes and luminal tropic factors.
British Journal of Nutrition, 1987,58:95-103

Saki AA, Eftekhari SM, Zamani P, Aliarabi H, Abbasinezhad M. Effects of organic acid mixture and methionine supplements on intestinal morphology, protein and nucleic acid content, microbial population and performance of broiler chickens. Animal Production Science, 2011,51:1025-1033

SAS Institute. SAS Users' Guide. Statistics. Version 9.2. SAS Institute Inc. Cary, NC, 2004

Steel RGD, Torrie JH, Dickey DA. Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw-Hill Publishing, New York, 1997

Yason CV, Summers BA, Schat KA. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. American Journal of Vererinary Research, 1987,6:927-93

Table 1. Composition of experimental broiler starter diets (g/100gDM)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Maize	59.00	59.00	59.00	59.00
Soyabean meal	35.00	35.00	35.00	35.00
Fish meal	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Common salt	0.25	0.25	0.25	0.25
Broiler premix	0.25	0.25	0.25	0.25
DL-methionine	0.12	0.12	0.00	0.00
MHA	0.00	0.00	0.12	0.12
Formic acid (%)	0.00	0.80	0.00	0.80
Metabolisable enenergy (Kcla/kg)	2992.10	2992.10	2992.10	2992.10
Crude protein (%)	22.75	22.75	22.75	22.75

MHA = methionine hydroxy analogue

Table 2. Composition of experimental broiler finisher diets (g/100gDM)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Maize	50.00	50.00	50.00	50.00
Soyabean meal	30.50	30.50	30.50	30.50
Brewer's dried grain	15.00	15.00	15.00	15.00
Fish meal	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00

Common salt	0.25	0.25	0.25	0.25
Broiler premix	0.25	0.25	0.25	0.25
DL-methionine	0.08	0.08	0.00	0.00
MHA	0.00	0.00	0.08	0.08
Formic acid (%)	0.00	0.80	0.00	0.80
Metabolisable enenergy (Kcla/kg)	2808.30	2808.30	2808.30	2808.30
Crude protein (%)	21.90	21.90	21.90	21.90

MHA = methionine hydroxy analogue

Table 3. Apparent nutrient digestibility of broiler birds in experimental diets

Parameters (%)	DL-methionine		Methionine hydroxy analogue			P-value		
	Without formic acid (diet 1)	With formic acid (diet 2)	Without formic acid (diet 3)	With formic acid (diet 4)	SEM	Effect of formic acid	Effect of the sulphur amino acid sources	Interaction formic acid* sulphur amino acid sources
Crude fiber	62.28b	68.63a	62.74b	67.05a	0.55	***	N.S.	N.S.
Crudeprotein	54.24b	63.28a	54.73b	59.33ab	1.01	**	N.S.	N.S.
Ether extract	56.09b	66.41a	59.45ab	64.30ab	1.52	*		
Ash	60.27b	71.16a	57.99b	62.26ab	1.55	*		
Dry matter	58.92b	67.44a	59.52b	61.20ab	1.1	*	N.S.	N.S.

N.S.=not significant at P>0.05, *0.05>P>0.01, **0.01>P>0.001, ***P<0.001, SEM=pooled standard error of mean. *Means on the same row with different superscripts are significantly (P<0.05) different.

Table 4. Gut morphology of birds fed experimental diets

Parameters (logCFU/ml digesta)	DL-methionine	Methionine hydroxy analogue	P-value

	Without formic acid (diet 1)	**With formic acid (diet 2)**	**Without formic acid (diet 3)**	**With formic acid (diet 4)**	**SEM**	**Effect of formic acid**	**Effect of the sulphur amino acid sources**	**Interaction formic acid* sulphur amino acid sources**
Wall thickness	3038.60a	2472.80b	2562.2b	2250.10b	55.77	**	N.S.	**
Crypt depth	195.01c	220.42bc	232.93b	285.24a	5.55	**	***	N.S.
Villus height	843.26c	872.04c	1226.50b	1394.86a	15.72	***	**	*
Villus width	371.19d	535.21c	980.48b	1051.31a	8.04	***	***	*

N.S.=not significant at P>0.05, *0.05>P>0.01, **0.01>P>0.001, ***P<0.001, SEM=pooled standard error of mean.
*Means on the same row with different superscripts are significantly (P<0.05) different.