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# Effect of Sulphur-Based Amino Acids with or without Formic Acid on Performance and Microbial Load of Broiler Chickens

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors ADO, OAA and OOA designed the study. Author OAA carried out the feeding trial supervised by Authors ADO, OOA and IOA performed the statistical analyses, wrote the protocol, and wrote the first draft of the manuscript. Authors OAA and IOA managed literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of the study was to investigate the effect of sulphur-based amino acids with or without formic acid on performance and microbial load of broiler chickens in a 56-day feeding trial. 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. The design of the experiment was a completely randomised design in a 2X2 factorial arrangement. Dietary treatments had no significant influence on the feed intake (FI) pattern of the birds. However, the inclusion of formic acid and the sulphur amino acid sources significantly affected body weight gain (WG) and feed conversion (FCR). Birds fed with diet 2 had significantly ($P<0.05$) improved WG (3.16kg/bird) and FCR (1.42) compared with birds fed with the other diets with values ranging from 2.28 to 2.64kg/bird. The microbial load of the digesta from

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selected segments of the gastrointestinal tract (i.e. duodenum and ileum) were also significantly ($P<0.05$) affected by the dietary treatments. The total bacteria count and coliform count were significantly ($P<0.05$) reduced with formic acid supplementation (1.60 to 6.26 logCFU/ml digesta) relative to the total bacteria and coliform count observed in the digesta of birds fed diets without formic acid supplementation (12.60 to 33.20 logCFU/ml digesta). Formic acid supplementation had positive effect on body weight gain and microbial population of the experimental birds.

*Keywords: Broiler feed; DL-methionine; formic acid; methionine hydroxyl analogue; microbial load.*

## 1. INTRODUCTION

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. Type and composition of rations are to be considered as the most important factors affecting the economic performance of poultry. With restrictions on the use of antibiotics, other additives have been included in the diet to improve the performance of birds. Among those used on a wider basis, are organic acids, probiotics, prebiotics and phytogenics [1].

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 [2]. 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 [3]. Salts of some of these acids have also been shown to have performance benefits. Other acids such as sorbic and fumaric acids which are short chain carboxylic acids containing double bonds, have also been observed to possess antifungal activity [4]. 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 [5].

There is a strong requirement for methionine in avian species to support feather growth and protein synthesis. It is however limited in plant protein sources and it is therefore necessary to supply it in diets deficient in the required amount. [6]. There are a few methionine sources namely: DL-methionine, liquid methionie hydroxyl analogue (HMTBA), calcium salt of methionine hydroxyl analogue, DL-methionine sodium slat etc. Both Dl-methionine (powder feed supplement) and methionine hydroxyl analogue (dry granulated feed supplement) can provide methionine activity for animals. The two methionine sources are absorbed in the animals GIT, converted to L-methionine and used in protein synthesis and other metabolic functions [7]. Methionine hydroxyl analogue is an organic acid owning to the fact that it possesses an hydroxyl group on its α carbon instead of having an amine group [8]. The study was conducted to investigate the effect of sulphur-based amino acids with or without formic acid on performance and microbial load of broiler chickens.

## 2. MATERIALS 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.

**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*

The variables studied include weight gain (WG), feed intake (FI), feed conversion ratio (FCR) and mortality, which were assessed weekly during this trial and recorded as they occurred. On day 56, two birds from each replicate were selected and weighed. The birds were slaughtered and the digestive tract was carefully excised. The digestive tract between the pyloric junction to the distal most point of insertion of the duodenal mesentry (as the duodenum) and digestive tract between Meckel's diverticulum and ileo-cecal junction (as ileum) were severed. The duodenal and ileal digesta were gently stripped into sterile sampling tubes and immediately transferred on ice to Microbial Laboratory for the microbial study.

### Statistical Analysis

Data obtained were analysed by means of the General Linear Model using SAS statistical software [9]. Differences among means were separated using Duncan Multiple Range Test significant at $P<0.05$ [10].

## 3. RESULTS AND DISCUSSION

### 3.1 Performance Characteristics

Performance characteristics of broiler chickens fed with experimental diets are shown in Table 3. The FI showed no significant differences across the treatments. The result of the present study is in agreement with the finding of Hernandez et al [11] who reported no significant improvement in the FI of birds fed diets supplemented with formic acid and other organic acids. However, Prigozlievet et al [12] found reduction in the feed consumption of birds fed diets supplemented with organic acid. The reduction was attributed to the strong taste of organic acids which would decrease the palatability of the diet as well as reduced the FI. The result of the present study also agrees with the findings of Bertram et al [13], who reported similar FI trend in birds fed diets containing DL-methionine and MHA.

The WG pattern obtained for birds fed diet 2 was significantly improved. It was observed that the different methionine sources as well as formic acid influenced this result. This observation is in agreement with results reported by Van Weerdenet et al. [14] who found that broilers fed diets containing DL-methionine had improved growth rate compared to those fed MHA. As opposed to the report of Hernandez et al. [11], who found no significant differences in the weight gain of birds fed diets with different levels of formic acid. The improvement in body WG may be due to direct antimicrobial effect of organic acids as postulated by Ricke [3] that organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism causing bactericidal effect.

Organic acids supplementation has pH-reducing properties in various gastrointestinal segments of broiler chickens as observed by Abdel-Fattah et al. [15]. This lowered pH is conducive for growth of favourable bacteria simultaneously hampering the growth of pathogenic bacteria which grow relatively at higher pH. Together the direct antimicrobial and pH reducing properties of organic acid might have resulted in inhibition of intestinal bacteria. This in turn can reduce bacteria competition with host for available nutrients and diminution in the level of toxic bacteria metabolites. As a result of lessened bacteria fermentation, protein and energy digestibility was improved, thereby ameliorating the WG and performance of broiler chickens.

Birds fed diet 2 had the best FCR. Both the methionine sources ad formic acid influenced the FCR of the birds. This improvement is due to better utilisation of nutrients which consequently resulted in increased WG in birds fed organic acid. The results of this study were in agreement with reports of earlier researchers [16-18] who reported that supplementation of organic acid improved FCR of broiler chicks.

## 3.2 Microbial Load of the Digesta

Tables 4 and 5 show the microbial load of the duodenal and ileal digesta of birds fed experimental diets respectively. Dietary treatments modified the microbial population in selected segments of the intestine (duodenum and ileum). Biochemical conditions in the digesta, as a result of varying feed composition have the tendency to affect substrate availability as well as modify microbial population. The total bacteria and coliform count in the digesta of birds fed diets without formic acid supplementation were significantly higher than those fed diets with formic acid supplementation. It could thus be inferred that formic acid at 0.8% was able to reduce the total bacteria and coliform count in the digesta of birds. This is in agreement with the results of the study by Gunal et al. [19], who reported that the use of organic acid mixture reduced the total bacteria count and the gram negative bacteria count. Improved lactobacilli population was observed in birds fed diets containing formic acid (Diets 2 and 4). This observation is similar to what was reported by Hinton et al. [20] who recorded high lactobacilli population and low pH in the GIT of birds. This was said to result in decreased salmonella occurrence. Alp et al. [21] reported reduced *Enterobacteriaceae* count in the ileum of broilers in response to the separate or combined inclusion of organic acid blend containing lactic acid, fumaric, propionic, citric and formic acid. All the digesta samples from the birds in the present study were free from *Salmonella spp*. It is noteworthy that birds fed diets supplemented with MHA and 0.8% formic acid had the lowest value of total bacteria and coliform count in the duodenal digesta. Although the difference in the data obtained were not significantly affected by the different methionine sources. This may be due to the organic acid nature of MHA in synergy with the organic nature of formic acid. Birds fed diet supplemented with DL-methionine and 0.8% formic acid had the least total bacteria and coliform count in the ileal digesta. The differences in the pattern of results from the duodenal and ileal digesta may be due to the thinning antimicrobial effect of organic acids as it moves along the GIT due to strong buffering action of the poultry GIT [11]. Moreover, the magnitude of the antimicrobial effects of organic acids varied from one acid to another and also dependent on concentration and pH of the organic acid [22].

**Table 3. Performance characteristics of broiler birds on experimental diets**

| Parameters | DL-methionine | | Methionine hydroxy analogue | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | Without formic acid (diet 1) | With formic acid (diet 2) | Without formic acid (diet 3) | With formic acid (diet 4) | | Effect of formic acid | Effect of the sulphur amino acid sources | Interaction formic acid* sulphur amino acid sources |
| Feed Intake (kg) | 4.54 | 4.42 | 4.57 | 4.44 | 0.05 | N.S. | N.S. | N.S. |
| Body weight gain (kg/bird) | 2.61b | 3.16a | 2.28b | 2.64b | 0.06 | ** | ** | N.S. |
| Feed conversion ratio | 1.73b | 1.42c | 2.02a | 1.69b | 0.04 | ** | ** | 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. Microbial load of the duodenal digesta of birds fed experimental diets**

| Parameters (logCFU/ml digesta) | DL-methionine | | Methionine hydroxy analogue | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | Without formic acid (diet 1) | With formic acid (diet 2) | Without formic acid (diet 3) | With formic acid (diet 4) | | Effect of formic acid | Effect of the sulphur amino acid sources | Interaction formic acid* sulphur amino acid sources |
| Coliform | 33.20a | 2.93b | 12.60ab | 1.60b | 3.35 | ** | N.S. | N.S. |
| Total bacteria | 29.88a | 6.26b | 19.15ab | 2.64b | 4.00 | ** | N.S. | N.S. |
| Lactic acid bacteria | 0.90a | 25.45a | 3.83b | 26.06a | 2.79 | ** | 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 5. Microbial load of the ileal digesta of birds fed experimental diets**

| Parameters (logCFU/ml digesta) | DL-methionine | | Methionine hydroxy analogue | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | Without formic acid (diet 1) | With formic acid (diet 2) | Without formic acid (diet 3) | With formic acid (diet 4) | | Effect of formic acid | Effect of the sulphur amino acid sources | Interaction formic acid* sulphur amino acid sources |
| Coliform | 37.82a | 1.83c | 18.35b | 2.15c | 1.97 | *** | * | * |
| Total bacteria | 31.57a | 1.68b | 17.04ab | 3.37b | 2.98 | * | N.S. | N.S. |
| Lactic acid bacteria | 1.06a | 26.78a | 1.78b | 3.37b | 2.98 | * | 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) differen*

## 4. CONCLUSION

Diet 2 recorded significantly improved body weight gain as well as the best feed conversion ratio, which implies that birds on diet 2 seemed to better utilise the nutrients in the diet. The total bacteria and coliform count in the digesta of birds fed diets without formic acid supplementation were significantly higher than those fed diets with formic acid supplementation. Formic acid supplementation had positive effect on body weight gain and microbial population of the experimental birds.

## ETHICAL APPROVAL

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.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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