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# Effects of Dietary Inclusion of Non-protein Nitrogenous Compounds on Performance and Serum Biochemistry of Broiler Chickens

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**ABSTRACT**

This study aimed to evaluate partial substitution of soybean meal (SBM) with certain non-protein nitrogenous compounds (especially ammonium sulphate (Amm. sulphate) and ammonium chloride (Amm. chloride) in diets of broilers. A total of 201 one-day old broiler chicks were fed starter (0-3 weeks) and finisher (4-7 weeks) diets. Amm. chloride was added at levels of 1.50% in the starter diet and then 1.50, 2.80 and 4.0% in the finisher diet, whereas, Amm. sulphate was added at levels of 1.80% in the starter diet, and then 1.80, 3.50 and 5.0% in the finisher diet. Performance data were monitored and blood samples were collected at the third and seventh weeks. All performance data (feed intake, body weight gain and feed conversion ratio) were negatively altered in experimental treatments, when compared to control, except for 1.50% Amm chloride (during the finisher period only) and 1.80% Amm. sulphate (throughout the experiment) groups. Serum analyses of glucose, liver and kidney function indices, and lipid profile were unaffected by the dietary treatments except ALT, total protein and albumin, which were influenced by high levels of ammonium sulphate at the finisher period. Therefore, Amm. sulphate can be used at a level of 1.80% in broiler diets substituting a portion of SBM, but Amm. chloride (1.50%) could be used only in the finisher period.

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## Introduction

Normal development and growth performance of poultry mainly depend on protein. Protein represents about 25 to 40% of the diet of animals and birds, it is responsible for half of the economic cost of the ration. Recently, because of the big expansion of population in all over the world and the shortage in feed resources mainly protein, it is become urgent to find out new replacements for protein in order to face this problem (Xing *et al*., 2018).

Non-protein nitrogenous (NPN) substances are essential sources to supply ruminant animals with protein where the rumen bacteria are able to convert the nitrogen to protein. However, little information is available about the possibilities of using NPN sources as a partial protein substitute for broilers. Non-protein nitrogenous compounds are still unconventional item in poultry feeding unlike ruminant animals (Chowdhury *et al*., 1996).

There are conflicting results concerning utilization of urea and other NPN compounds in poultry. Some studies reported that nitrogen from urea and diammonium citrate (DAC) could be utilized by chicks (Shannon *et al*., 1970; Karasawa, 1989a,b; Karasawa and Maeda, 1994). However, other researchers found no beneficial effects of urea nitrogen as well as DAC and dibasic ammonium (Amm.) phosphate in chicks (Isikwenu, 2012; Fallah *et al*., 2016) or fattening chickens (Prieto *et al*., 1978).

Baker and Molitoris (1974) observed that supplementation of diet with urea stimulated the growth of chicks. Moreover, Sibbald and Hamilton (1975) found that growing chicks can tolerate high levels of dietary urea (3-15 %) for several weeks without observing any evident signs of toxicity. Reduction in weight gain and decreases in feed efficiency may be explained on the basis of dietary dilution with urea. The authors claimed that there are no reasons to believe that the urea had any direct effect on the performance of the birds. Therefore, it is not clear from these findings whether the effect on performance of birds was due to urea or due to dilution of the diets.

The information about using ammonium salts in nutrition of poultry is rare. In this concern, Oyedeji *et al*. (2003) clarified that inclusion of Amm. sulphate in the ration of broiler with 1.0 to 3.0% resulted in a decrease in the feed intake but did not induce any alteration in the feed/gain ratio. The authors added that addition of Amm. sulphate was effective in the

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starter diet than in the finisher diet. On the other hand, Han *et al.* (2004) observed that Amm. chloride up to 1.0% did not influence the feed intake (FI) and feed efficiency in broilers.

There is a lack of information about using non-protein nitrogenous compounds especially ammonium salts in poultry diets. Therefore, the current study was complemented to assess the impact of inclusion of certain NPN substances (especially Amm. sulphate and Amm. chloride) in the diets of broilers on the performance and also on the liver and kidney functions. These compounds were used as sources of dietary nitrogen with a partial substitution of commonly used protein source (soybean meal) in poultry diets.

## Materials and methods

*Birds, management and nutrition*

The present experiment was carried out at Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

A total of 201, day-old, broiler chicks (Hubbard, avian 43) were weighed and sorted to achieve equal starting weights (about 46 g) across all treatments. The chicks were divided into 3 groups; 51 chicks for control group and 75 chicks for each group of ammonium salts (Amm. chloride and Amm. sulphate (Sigma-Aldrich, USA) groups). In every group, the birds were allocated into three replicates, each with 17 birds in control and 25 in other groups. All chicks were housed in pens and were kept under similar management conditions. The birds were fed on control (containing no NPN source), Amm. chloride diet and Amm. sulphate diet. Amm. chloride (25.20% N) and Amm. sulphate (20.20% N) were used as a source of non-protein nitrogen, substituting portions of soybean meal in the diets. The protein equivalent (N X 6.25) of these compounds was 157.50% and 126.25% for Amm. chloride and ammonium sulphate, respectively. The experiment lasted for 7 weeks. During the starter period (0-3 weeks), the NPN substances were added to the diets at the rate of 1.50% Amm. chloride and 1.80% Amm. sulphate for Amm. chloride and Amm. sulphate diets, respectively. These compounds supplied the diets with about 2.30% crude protein (CP) of the total dietary protein. The levels of ammonium salts were used according to the results of Oyedeji *et al.* (2003) and Han *et al.* (2004).

During the finisher period (4-7 weeks), the birds in group 2 and 3 were subdivided into 3 subgroups, each with 21 birds (three replicates; 7 birds per replicate), whereas the control group (42 birds; 14 birds in each replicate) was kept as in the starter period without any NPN- supplement. Three graded levels of Amm. chloride and Amm. sulphate were added to group 2 and 3, respectively. In group 2, the birds were fed on a diet containing 1.50, 2.80 and 4.0% Amm. chloride, whereas in group 3, Amm. sulphate was added to the diet at 1.80, 3.50 or 5.0%.

All experimental diets (Tables 1 and 2) were formulated to have nearly identical nutrient contents and to meet the nutrient requirements of broiler chicks according to NRC (1994). The diets were formulated to be isocaloric and isonitrogenous. Lysine and methionine were added to ensure that all diets provided a similar level of amino acids. Fat (soybean oil) was added at different levels to adjust energy density of the diets. The used NPN compounds provided about 2.30, 4.40 and 6.30% crude protein of the total dietary protein for the corresponding graded levels of NPN, respectively. The nutrient con-

Table 1. Composition of diets fed to control and experimental birds during starter period (0-3 weeks; as fed)

| Ingredients | Control | Amm. Chloride (1.50%) | Amm. Sulphate (1.80%) |
|---|---|---|---|
| Yellow corn (%) | 58.13 | 61.92 | 60.58 |
| Soybean meal (44% CP, %) | 25.91 | 20.44 | 21.53 |
| Broiler concentrates (52% CP, %) | 12.73 | 12.73 | 12.73 |
| Soybean oil (%) | 2.5 | 2.5 | 2.5 |
| Bone meal (%) | 0.08 | 0.08 | 0.08 |
| Salt (%) | 0.23 | 0.23 | 0.23 |
| DL- Methionine (%) | 0.12 | 0.18 | 0.17 |
| L- Lysine (%) | - | 0.12 | 0.08 |
| Minerals and vitamins premix[a] (%) | 0.3 | 0.3 | 0.3 |
| Amm. chloride (%) | - | 1.5 | - |
| Amm. sulphate (%) | - | - | 1.8 |
| Chemical composition | | | |
| Metabolizable energy (kcal/kg) | 3084 | 3089 | 3079 |
| Crude protein (%) | 22.99 | 23.27 | 23.19 |
| Crude fiber (%) | 3.27 | 2.97 | 3.03 |
| Methionine (%) | 0.5 | 0.5 | 0.5 |
| Lysine (%) | 1.2 | 1.2 | 1.2 |
| Calcium (%) | 1.1 | 1.1 | 1.1 |
| Available phosphorus (%) | 0.51 | 0.5 | 0.5 |
| Sodium (%) | 0.28 | 0.28 | 0.28 |

[a]Minerals and vitamins premix: each 3kg contains Vit. A, 12,000,000 IU; Vit.D3, 2,000,000 IU; Vit. E, 10,000mg; Vit.K.3, 2000 mg; Vit.B1, 1000 mg, Vit.B2, 5000 mg; Vit.B6, 1500 mg; Vit. B12, 10 mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid,1000 mg, choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000mg; Fe, 30000mg; Cu, 10000mg; I, 1000mg; Se, 100mg; Co, 100mg; and calcium carbonate up to 3kg.

Table 2. Composition of diets fed to control and experimental birds during finisher period (4-7 weeks; as fed)

| Ingredients | Control | Amm. Chloride | | | Amm. Sulphate | | |
|---|---|---|---|---|---|---|---|
| | | 1.5 % | 2.8 % | 4.0 % | 1.8 % | 3.5% | 5.0 % |
| Yellow corn (%) | 62.81 | 66.59 | 67.8 | 69.57 | 65.2 | 68.14 | 72.24 |
| Soybean meal (44% CP, %) | 21.0 | 15.53 | 11.88 | 8.23 | 16.62 | 10.78 | 4.94 |
| Broiler concentrates (52% CP, %) | 12.04 | 12.04 | 12.04 | 12.04 | 12.04 | 12.04 | 11.5 |
| Soybean oil (%) | 3.5 | 3.5 | 4.5 | 5.0 | 3.5 | 4.5 | 5.0 |
| Bone meal (%) | - | - | - | - | - | - | 0.03 |
| Salt (%) | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| DL- Methionine (%) | 0.09 | 0.16 | 0.18 | 0.24 | 0.16 | 0.21 | 0.28 |
| L- Lysine (%) | - | 0.12 | 0.24 | 0.36 | 0.12 | 0.27 | 0.45 |
| Minerals and vitamins premix[a] (%) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Amm. chloride (%) | - | 1.5 | 2.8 | 4.0 | - | - | - |
| Amm. sulphate (%) | - | - | - | - | 1.8 | 3.5 | 5.0 |
| Chemical composition | | | | | | | |
| Metabolizable energy (kcal/kg) | 3201 | 3206 | 3262 | 3273 | 3193 | 3239 | 3259 |
| Crude protein (%) | 20.87 | 21.14 | 21.24 | 21.38 | 21.06 | 21.24 | 21.22 |
| Crude fiber (%) | 3.02 | 2.72 | 2.5 | 2.29 | 2.77 | 2.42 | 2.09 |
| Methionine (%) | 0.41 | 0.45 | 0.45 | 0.49 | 0.45 | 0.47 | 0.51 |
| Lysine (%) | 1.08 | 1.03 | 1.03 | 1.03 | 1.06 | 1.03 | 1.0 |
| Calcium (%) | 1.04 | 1.02 | 1.01 | 1.0 | 1.02 | 1.01 | 0.96 |
| Available phosphorus (%) | 0.47 | 0.46 | 0.45 | 0.44 | 0.46 | 0.45 | 0.42 |
| Sodium (%) | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.27 |

[a]Minerals and vitamins premix: each 3kg contains Vit. A, 12,000,000 IU; Vit.D3, 2,000,000 IU; Vit. E, 10,000mg; Vit.K.3, 2000 mg; Vit.B1, 1000 mg, Vit.B2, 5000 mg; Vit.B6, 1500 mg; Vit. B12, 10 mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid,1000 mg, choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000mg; Fe, 30000mg; Cu, 10000mg; I, 1000mg; Se, 100mg; Co, 100mg; and calcium carbonate up to 3kg.

tents of different experimental diets were calculated according to NRC (1994) and the chemical composition is presented also in Tables 1 and 2. Feed and water were available ad libitum throughout the experiment.

All experimental procedures and the study protocol were approved by the Animal Ethics Committee at Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

*Performance traits*

Broilers were weighed at the start (day 1) as well as at weekly intervals throughout the experiment. Weighed amounts of feed were offered daily and refusals were weighed back every week to calculate weekly feed intake (FI). Accordingly, the feed conversion ratio (FCR) of the birds was calculated. The calculations of feed intake and FCR were done according to Youssef *et al.* (2008). Mortality rates were recorded daily throughout the treatments.

*Collection of blood samples*

Blood samples were collected through sacrificing the birds, by stunning (through a blow to the head) followed by cutting the neck vessels (Youssef *et al*., 2012). The samples were collected from 9 birds per group/subgroup at the end of the 3rd and 7th week throughout the experiment, via taking the blood from three birds per replicate. During sacrificing the birds, samples of blood were collected in plain tubes, and then centrifuged at 3.000 rpm for 15 minutes for separating serum. Afterwards, the serum samples were frozen at -20 °C until analysis.

*Biochemical analysis*

The samples were analyzed for different blood parameters including serum glucose levels, liver indices (total serum bilirubin, alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as well as total serum protein, albumin and globulins), kidneys function indicators (Creatinine, urea and uric acid) and some of lipid profile (Serum triglycerides and total serum cholesterol). These parameters were determined by spectrophotometer (CE 1021 UV/ visible light, Cecil instruments limited company, Cambridge, England), using their corresponding colorimetric kits (Sigma-Aldrich, USA) according to the manufacture protocol.

*Statistical analysis*

The results were analysed using the statistical SAS software (2002). The data were analysed using the General Linear Models (GLM) procedure for analysis of variance. The results of the starter period were subjected to one-way ANOVA, whereas that of the finisher period were analysed by two-way ANOVA (2 x 3 factorial analyses). These tests accompanied with Duncan's multiple range test to detect the differences among the treatments. The effects of level and source, and their interaction, of ammonium salts were statistically evaluated. Differences were considered to be significant when $p < 0.05$. Significant differences are denoted with different superscripts. Values are presented as arithmetical means with standard deviation (Mean ±SD).

## Results

*Effect of ammonium salts inclusion in broiler diets on performance during the starter period*

The performance data of birds during the starter period (0-3 weeks) is shown in Table 3 and Figs. 1-3. The body weight gain (BWG) of broiler chicks fed diet containing Amm. chloride was significantly ($P < 0.05$) lower than those fed control diets (Fig. 1). However, the BWG of broiler chicks fed Amm. sulphate diet did not differ ($P > 0.05$) than that of the control especially during the second and third weeks of age. Moreover, it was observed that the feed intake was slightly lower ($P < 0.05$) all over the starter period in the broiler chicks fed either Amm. chloride or Amm. sulphate salts in comparison to the control group (Fig. 2). Nevertheless, the FCR was higher ($P < 0.05$) only in birds fed Amm. chloride diet compared to the control, whereas it was similar ($P > 0.05$) in chicks fed Amm. sulphate

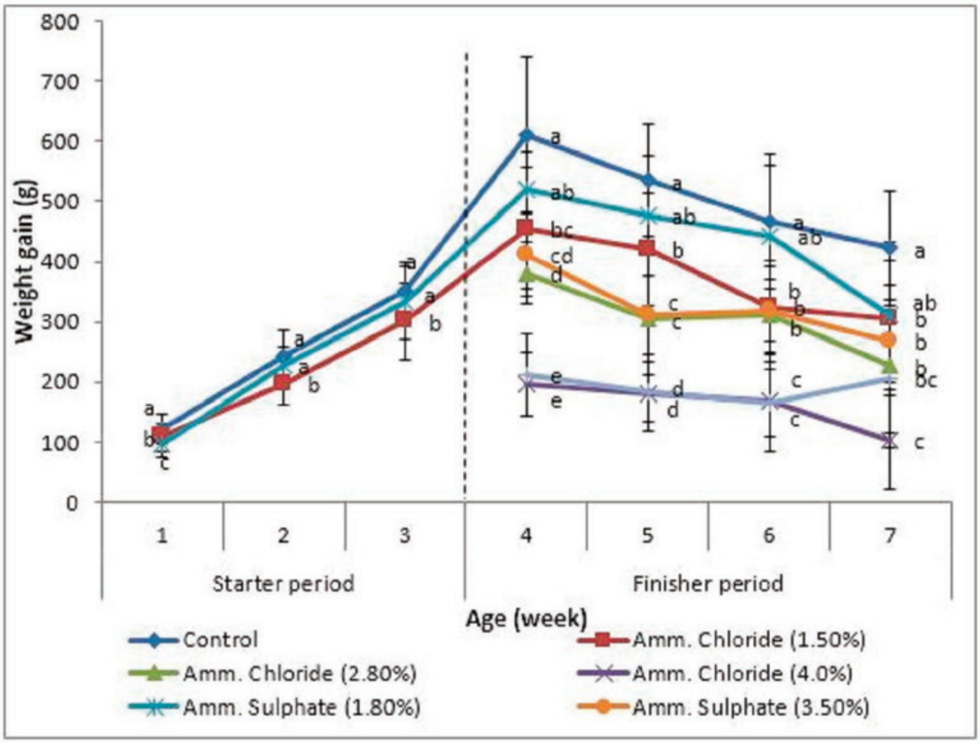


Fig.1. Weekly weight gain (g) of birds, fed different experimental diets, during starter and finisher periods. In the same week, different letters (a,b,c,d,e) mean significant.

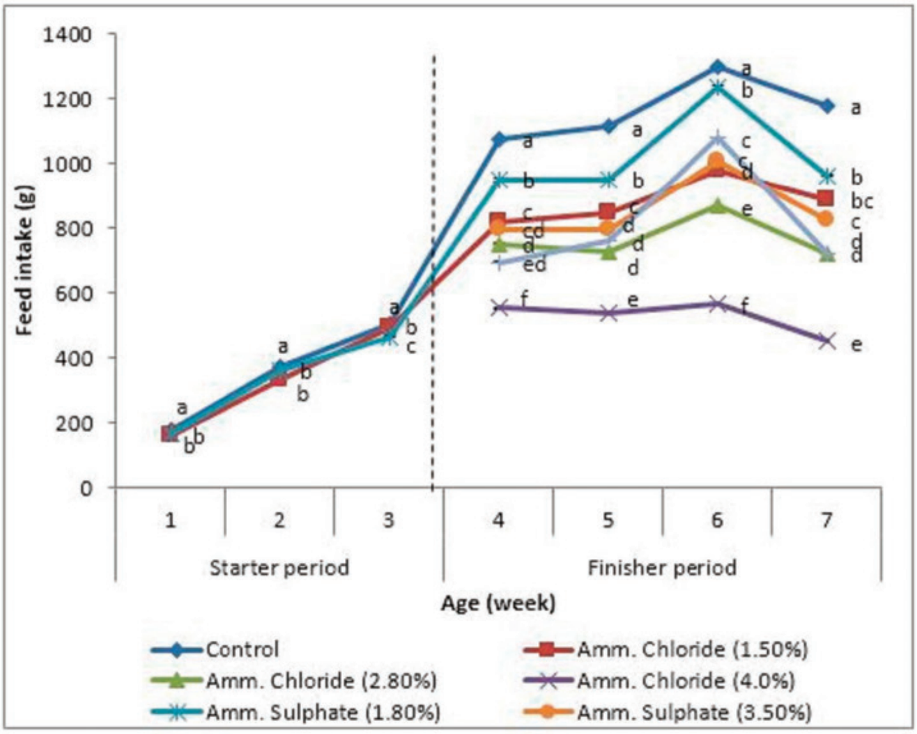


Fig. 2. Weekly feed intake (g) of birds, fed different experimental diets, during starter and finisher periods. In the same week, different letters (a,b,c,d,e) mean significant.

Table 3. Weight gain (g/bird/period), feed intake (g/bird/period), feed conversion ratio, and mortality rate (%) of birds during the starter period (0 - 3 weeks)

| Parameter | Control | Amm. Chloride (1.50%) | Amm. Sulphate (1.80%) | p- value |
|---|---|---|---|---|
| Weight gain | 238.4±103.8[a] | 201.2±90.7[b] | 217.3 ±102.0[a] | <0.001 |
| Feed intake | 351.4 ±54.7[a] | 328.7 ±55.1[b] | 329.4 ±50.5[b] | 0.033 |
| FCR* | 1.48 ±0.02[b] | 1.61±0.03[a] | 1.58 ±0.06[b] | 0.04 |
| Mortality rate | - | 1.3 | 4 | |

*FCR: feed conversion ratio (kg/kg). [a,b] Means in the same row with different superscripts are significantly different (p < 0.05).

and control diets especially during the second and third weeks of life (Fig. 3). The percentage of mortality rate was monitored throughout the experiment, and it was about 0, 1.3 and 4.0% in control, Amm. chloride and Amm. sulphate, respectively.

*Effect of ammonium salts inclusion in broiler diets on performance during the finisher period*

The performance results of broilers during the finisher period (4-7 weeks) are presented in Table 4 and also in Figs. 1-3. There was a significant (P 0.05) reduction in the BWG of birds fed diets containing 1.50, 2.80 and 4.0% Amm. chloride in comparison to the control group (Fig. 1). The same results were reported in groups fed diets comprising 3.50 and 5.0% Amm. sulphate. It was noticed that, the more the level of ammonium salts was included in the diets the more reduction in the weight gain was obtained. However, all over the finisher period, only the weight gain of birds fed 1.80% Amm. sulphate showed no significant change (P > 0.05) compared to the control. In addition, the feed intake of broilers fed diets including 1.50% Amm. chloride and 1.80% Amm. sulphate was slightly lower (P < 0.05) than that of the control, whereas the reduction in feed intake was found to increase linearly with increasing the inclusion level of ammonium salts in the diet (Fig. 2). Even though, the FCR of the birds fed diets containing 1.50% Amm. chloride and 1.80% Amm. sulphate did not differ (P > 0.05) than that of the control (Fig. 3). Moreover, the feed conversion ratio was higher (P < 0.05) in other groups when compared to the control group, and it was mostly in ascending trend with increasing the level of the used ammonium salts. In addition, the mortality rate was nearly nil in all groups during the finisher period except for birds fed diet containing 3.50% Amm. sulphate where the mortality percentage was about 4.50%.

Generally, the birds fed diets containing high levels of ammonium salts exhibited clinical signs of diarrhea and shrinkage in their body size.

*Effect of ammonium salts supplementation to broiler diets on biochemical parameters*

Unexpectedly, as shown in Table 5, regarding all studied biochemical indices including serum glucose, liver function indicators (serum levels of total bilirubin, ALT, AST, ALP, total protein, albumin and globulin), kidney function markers (serum levels of creatinine, urea and uric acid) and lipid profile (serum levels of triglycerides and total cholesterol) during the starter period, it was found that all of these parameters were not al-

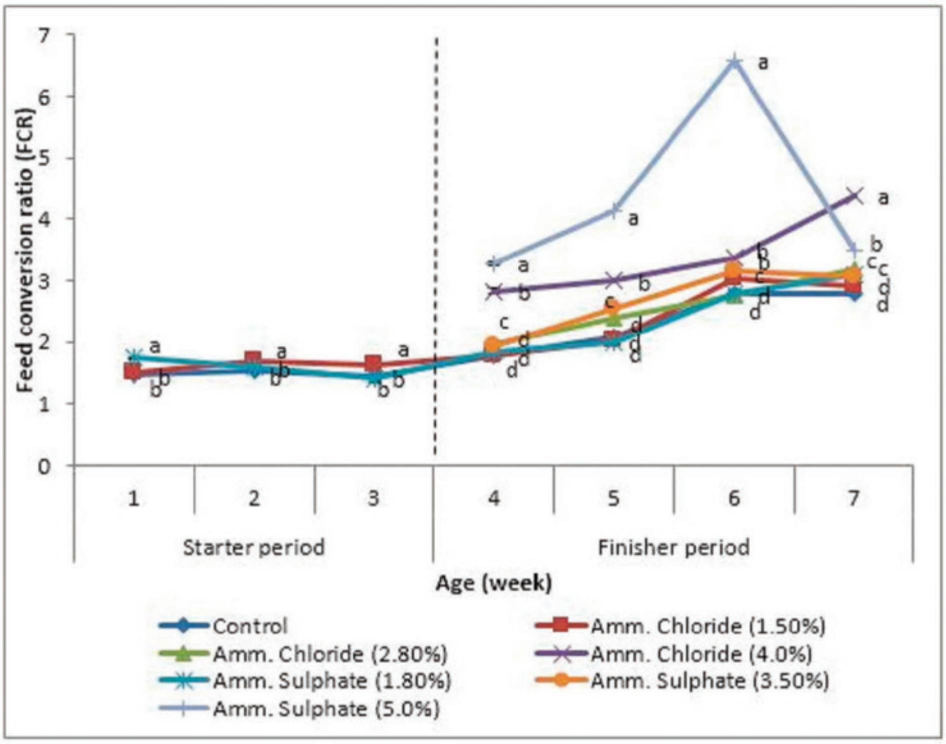


Fig. 3. Weekly feed conversion ratio (FCR) of birds, fed different experimental diets, during starter and finisher periods. In the same week, different letters (a,b,c,d) mean significant.

Table 4. Weight gain (g/bird/period), feed intake (g/bird/period), feed conversion ratio, and mortality rate of birds during the finisher period (4 – 7 weeks)

| Parameter | Control | Amm. Chloride | | | Amm. Sulphate | | | Level effect | Source effect | Level X source |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5 % | 2.8 % | 4 .0% | 1.8 % | 3.5 % | 5 .0% | | | |
| Weight gain | 518.8±129.5[a] | 376.0±138.3[b] | 302.5±124.4[b] | 161.7±79.2[c] | 434.3±146.5[ab] | 324.0±116.2[b] | 184.9±75.6[c] | <0.001 | 0.01 | 0.022 |
| Feed intake | 1168.4±32.8[a] | 882.6±23.9[c] | 767.8 ±22.9[d] | 527.3±17.5[e] | 1025.9 ±46.6[b] | 855.8±34.2[c] | 813.7±59.7[d] | <0.001 | 0.01 | 0.042 |
| FCR* | 2.36 ±0.17[d] | 2.44 ±0.21[d] | 2.58±0.17[c] | 3.40 ±0.23[b] | 2.43±0.20[d] | 2.68±0.19[c] | 4.38±0.51[a] | <0.001 | 0.01 | 0.036 |
| Mortality rate | - | - | - | - | - | 4.5 | - | | | |

*FCR: feed conversion ratio (kg/kg).

[a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$).

tered ($P > 0.05$) by addition of the different levels of either Amm. chloride or Amm. sulphate when compared to the control group. Moreover, serum ALT concentration was higher ($P < 0.05$) in 1.80% Amm. sulphate than that of 1.50% Amm. chloride (20.9 vs. 14.9 IU/ml), but the ALT levels in both groups did not differ significantly than that of the control (17.8 IU/ml). However, at the finisher period, the data in Table 6, displayed that all values of the studied biochemical parameters in both Amm. chloride and Amm. sulphate groups were similar to the corresponding values of the control except for the values of ALT, AST, total protein, albumin and albumin/globulin ratio. It was found that serum ALT concentration was increased ($P < 0.05$) in birds fed high level (5.0%) of Amm. sulphate, in comparison to the control, while it was not affected in the other treatments. On the other hand, AST level showed no significant change compared to the control group in 1.50% Amm. chloride and 1.80% Amm. sulphate groups only, whereas it was decreased ($P < 0.05$) in other groups. The total serum protein level was lower in 5.0% Amm. sulphate group, but it was not affected by other treatments. Also, the albumin and its ratio with globulin were lower ($P < 0.05$) in 3.50 and 5.0% Amm. sulphate only, but it had no significant changes in the other groups in comparison to the control group. However, the globulin serum concentration did not differ significantly among the dietary treatments.

## Discussion

All over the world, there is a common problem in using protein sources for poultry especially soybean meal due to increasing up of their prices. Therefore, all the researchers try to find out alternative protein sources. Therefore, this study aimed to investigate the effects of inclusion of NPN compounds, especially ammonium salts, in diets to substitute some amounts of the expensive SBM on the growth performance of broiler chicks and also to assess if they have any adverse effects on the hepato-renal functions and lipid profile as well as serum glucose level. The ammonium compounds, especially Amm. chloride and Amm. sulphate, were used to supply the diet with about 2.0, 4.0 or 6.0% CP of the total dietary protein. These salts were used in the diet to replace about 4.50 to 16.0 % of dietary soybean meal. Furthermore, the ammonium compounds were used in the starter diet at low levels (1.50% to 1.80%) as the birds cannot tolerate the high levels during the early days of life. However, the graded high levels (1.50 to 5.0%) of these salts were used during the finisher period as the birds became older. The levels of the ammonium salts were chosen to replace the dietary soybean meal as well as to provide the diets with the same levels of nitrogen or protein equivalent (2.0 to 6.0%).

Regarding the effects of ammonium salts on performance of the broiler chicks, the current results revealed that except for 1.80 % Amm. sulphate (during the starter and finisher periods) and 1.50 % Amm. chloride (during the finisher period only), all other inclusion levels of both ammonium salts significantly altered the BWG, feed intake and FCR in comparison to control group. These data come in agreement with Fallah *et al*. (2016), who reported that addition of urea to the diet of broilers resulted in a significant decrease in the BWG and also, the authors found that increasing the level of urea in the diet induced a significant decrease in feed intake when compared to the control group. In addition, the authors observed that the FCR increased significantly in comparison to the control, especially when the levels of total volatile nitrogen in the diet increased above 1.5 % urea. In a study made by Sahraei *et al*. (2012), it was demonstrated that feeding poultry with diet containing total volatile nitrogen (TVN) at a concentration of 209 mg/100g reduced feed intake, BWG and altered the FCR

Table 5. Levels of different blood parameters in serum of birds fed different experimental diets at the end of starter period (at the end of 3rdweek)

| Parameter | Control | Amm. Chloride (1.50%) | Amm. Sulphate (1.80%) | p- value |
|---|---|---|---|---|
| Glucose (mg /dl) | 233.7±24.6[a] | 212.9 ±33.0[a] | 197.7 ±12.9[a] | 0.114 |
| Total bilirubin (mg/dl) | 0.06±0.03[a] | 0.06 ±0.03[a] | 0.11 ±0.08[a] | 0.156 |
| ALT (IU/l) | 17.8±2.96[ab] | 14.9 ±3.50[b] | 20.9±8.19[a] | 0.035 |
| AST (IU/l) | 331.2±68.2[a] | 332.5±13.1[a] | 363.8±111.5[a] | 0.739 |
| ALP (IU/l) | 99.4±0.97[a] | 99.0±1.04[a] | 97.0±5.66[a] | 0.488 |
| Total protein (g/dl) | 2.76±0.32[a] | 2.54±1.19[a] | 3.21±1.19[a] | 0.336 |
| Albumin (g/dl) | 1.49±0.18[a] | 1.51±0.19[a] | 1.94±0.68[a] | 0.178 |
| Globulin (g/dl) | 1.28±0.15[a] | 1.03±0.13[a] | 1.27±0.63[a] | 0.473 |
| A/G ratio | 1.17±0.09[a] | 1.49±0.28[a] | 1.71±0.54[a] | 0.058 |
| Creatinine (mg/dl) | 0.61±0.47[a] | 0.66±0.19[a] | 0.97±0.67[a] | 0.437 |
| Urea (mg/dl) | 6.00±0.89[a] | 6.37± 1.00[a] | 8.06±2.63 [a] | 0.057 |
| Uric acid (mg/dl) | 4.22±1.35[a] | 4.12±1.76[a] | 2.90±0.93[a] | 0.265 |
| Triglycerides (mg/dl) | 43.04±9.61[b] | 50.88±16.33[ab] | 48.12±6.56[ab] | 0.528 |
| Total cholesterol (mg/dl) | 156.1±26.2[a] | 175.8±3.40[a] | 165.4±40.4[a] | 0.056 |

a,b Means in the same row with different superscripts are significantly different ($p < 0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, A/G ratio: albumin /globulin ratio.

in comparison to control group. These findings are in a close agreement with that presented by Botta *et al.* (1984), who observed that, addition of poultry by-product meal comprising 209 mg/100g TVN in the diet of broiler chicken was found to alter their growth performance. The adverse effects of high level of ammonium salts on the growth performance could be attributed to the diarrhea that caused by inclusion of both ammonium salts, as reported in the present study, which explains why BWG was reduced and consequently the FCR was elevated. In this regard, the report of Takagi *et al.* (1999), who found that feeding of rats with diets containing different concentrations of Amm. sulphate (0%, 0.38%, 0.75%, 1.5%, and 3.0%) showed diarrhea only in the 3% group. Also, the authors demonstrated that inclusion of Amm. sulphate didn't cause any alteration in organ weights, serum biochemical indices and histological examinations. These findings indicate that Amm. sulphate is less toxic substance and doesn't cause any toxic symptoms on growth performance unless being in high level and also its high level is not too toxic to induce alteration on organs function, which met an accordance with the findings of the present study.

However, addition of 1.80% Amm. sulphate in the starter and finisher diets of broilers and addition of 1.50% Amm. chloride in the finisher diet didn't have any detrimental effects on growth performance. The obtained results are in harmony with that detected by Shahzad *et al.* (2012), who demonstrated that the addition of low level of urea (1 %) in the diet had no detrimental effects on the performance of chickens. Also, Isikwenu (2012) reported that the replacement of groundnut cake in the diet of cockerel chicks with different levels of urea (25, 50, 75 and 100 %) induced significant decrease in the final BWG and feed intake in comparison to control. Also, the author observed that FCR of the cockerel chicks is not influenced with low inclusion level of urea (25%) ($P > 0.05$) but significantly ($P < 0.05$) affected with higher inclusion levels (50, 75 and 100%). Also, Isikwenu *et al.* (2008) reported similar results in broiler chicks. In the same line, Trakulchang and Balloun (1975) showed that supplementation of the diet with low levels of urea (0.43%) didn't induce any negative effects on the feed intake and the BWG of chicken. Thus, from the obtained results, it could be suggested that the effect of both ammonium salts on the broiler performance is dose dependent as the performance of broiler was maintained within normal line with the low levels inclusion of ammonium salts (1.80% Amm. sulphate and 1.50% Amm. chloride) in the diets, while it was adversely affected with high levels inclusion.

Although the high inclusion levels of ammonium salts decreased the performance of broiler chicks, it didn't affect the mortality rate especially during the finisher period in comparison to the control, which indicated that the high inclusion levels of ammonium salt in the diets are not severely toxic. This come in accordance with Isikwenu (2013), who found similar mortality rates in both the control and urea- treated groups.

At the starter period, the serum levels of glucose, liver enzymes (ALT, AST and ALP), bilirubin, total serum protein, albumin, creatinine, urea, uric acid, triglycerides and total serum cholesterol in broilers of both Amm. sulphate and Amm. chloride groups showed insignificant ($P > 0.05$) changes compared to the control group. These results were consistent with the results of Isikwenu (2013), who found that the serological and biochemical indices for rabbits fed urea included diet showed similar pattern with those fed control diet. Also, previous studies of Isikwenu and Omeje (2007); Ojebiyi *et al.* (2009) and Isikwenu (2012) reported that inclusion of urea in the diet of broiler chicks didn't cause any detrimental effects on the haematological parameters including red blood cell count, packed cell volume and haemoglobin concentration. These results indicated that urea inclusion in the diet didn't give rise to anaemic conditions, which give obvious indication about presence of normal and healthy liver and kidney (McHutchison *et al.*, 2006). On the other side, during the finisher period, the significant increase of serum ALT level in 5.0% Amm. sulphate or the significant decrease of serum AST in high levels of Amm. chloride or sulphate indicates that the liver could be influenced by these levels of dietary treatments. Also, the decrease of total serum protein level in 5.0% Amm. sulphate may be attributed to the lower albumin concentration in this group. The same reason can be used to explain the decrease of albumin/globulin ratio in this group. It is possible that the liver is affected by high level of Amm. sulphate (5.0%), which resulted in a lower protein synthesis. Thus, these abnormal findings may point to hepatocellular damage (Ghany and

Table 6. Levels of different blood parameters in serum of birds fed different experimental diets at the end of finisher period (at the end of 7thweek)

| Parameter | Control | | Amm. Chloride | | Amm. Sulphate | | | Level effect | Source effect | Level X source |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5 % | 2.8 % | 4.0 % | 1.8 % | 3.5 % | 5.0 % | | | |
| Glucose (mg /dl) | 219 ±17.7[a] | 215±16.6[a] | 226±20.2[a] | 217±16.6[a] | 204 ±21.8[a] | 227±28.1[a] | 215±18.8[a] | 0.318 | 0.568 | 0.231 |
| Total bilirubin (mg/dl) | 0.11 ±0.04[a] | 0.15±0.05[a] | 0.12±0.04[a] | 0.12±0.01[a] | 0.13±0.08[a] | 0.13±0.06[a] | 0.17±0.08[a] | 0.635 | 0.234 | 0.453 |
| ALT (IU/l) | 20.7±2.65[bcd] | 19.0±3.31[d] | 20.1±4.35[cd] | 19.8±3.46[cd] | 24.7±4.01[abc] | 25.4±3.80[ab] | 25.9±2.10[a] | 0.031 | 0.046 | 0.029 |
| AST (IU/l) | 507.0±121[a] | 418.0±48.2[ab] | 371.0±95.6[b] | 394.0 ±38.8[b] | 463.0±109[ab] | 383.0±113[b] | 372.0±77.7[b] | 0.016 | 0.132 | 0.112 |
| ALP (IU/l) | 76.0 ±16.0[a] | 80.0±17.4[a] | 64.0±31.5[a] | 72.0 ±33.5[a] | 62.9±14.5[a] | 56.9±32.2[a] | 79.3±29.2[a] | 0.435 | 0.413 | 0.513 |
| Total protein (g/dl) | 3.84 ±0.43[a] | 3.85±0.54[a] | 3.47±0.67[a] | 3.94 ±1.31[a] | 3.51±0.26[a] | 3.52±0.44[a] | 2.68±0.76[b] | 0.011 | 0.048 | 0.044 |
| Albumin (g/dl) | 2.05 ±0.32[a] | 2.07±0.22[a] | 1.77±0.29[abc] | 1.89±0.31[ab] | 1.48±0.27[ab] | 1.43±0.25[b] | 1.00±0.22[d] | 0.021 | 0.039 | 0.032 |
| Globulin (g/dl) | 1.80 ±0.40[a] | 1.78±0.34[a] | 1.70±0.44[a] | 2.05±1.07[a] | 2.04±0.49[a] | 2.07±0.20[a] | 1.68±0.64[a] | 0.616 | 0.312 | 0.445 |
| A/G ratio | 1.22 ±0.48[a] | 1.19±0.13[a] | 1.07 ±0.19[ab] | 1.04±0.31[ab] | 0.80±0.37[ab] | 0.70±0.16[b] | 0.68±0.31[b] | 0.022 | 0.04 | 0.048 |
| Creatinine (mg/dl) | 0.39±0.23[a] | 0.48±0.21[a] | 0.54 ±0.29[a] | 0.62±0.17[a] | 0.39±0.13[a] | 0.49±0.15[a] | 0.48±0.15[a] | 0.247 | 0.113 | 0.221 |
| Urea (mg/dl) | 6.55± 1.29[a] | 6.44±1.31[a] | 7.47±1.69[a] | 8.48±1.83[a] | 6.86±1.18[a] | 6.91±0.72[a] | 6.79±2.17[a] | 0.124 | 0.241 | 0.312 |
| Uric acid (mg/dl) | 3.54±0.95[a] | 3.31±1.25[a] | 2.72±0.85[a] | 2.89±1.25[a] | 3.29±0.94[a] | 3.09±1.23[a] | 2.45±0.64[a] | 0.327 | 0.311 | 0.461 |
| Triglycerides (mg/dl) | 46.3±15.3[a] | 34.2±11.6[a] | 38.5±15.7[a] | 39.4±7.48[a] | 48.5±13.5[a] | 54.1±9.86[a] | 38.1±14.0[a] | 0.104 | 0.212 | 0.298 |
| Total cholesterol (mg/dl) | 119.0±21.1[a] | 119.0±35.2[a] | 121.0±32.9[a] | 106.0±20.3[a] | 192.0 ±39.7[a] | 200.0±35.9[a] | 124.0±54.7[a] | 0.278 | 0.412 | 0.378 |

a,b Means in the same row with different superscripts are significantly different ($p < 0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, A/G ratio: albumin /globulin ratio.

Hoofnagle, 2005). Therefore, based on the current biochemical results, inclusion of low levels of ammonium salts in the diets of broilers is usually accompanied with healthy and functioning liver and kidney as well as normal lipid profile. However, high levels of Amm. sulphate (3.50 and 5.0%) when fed for long period (7 weeks) could induce hepatocellular damage.

This study draw attention about the probability of adulteration of poultry diets with high levels of ammonium salts in a trial to decrease the diet cost, especially this adulteration may be accompanied with normal liver and kidney functions. However, this can negatively affect the growth performance and accordingly decrease the body mass revenue.

## Conclusion

The present study showed that the high levels of ammonium salts adversely affect the performance of broiler chickens, and also have negative effects on some serum biochemical indices, especially ALT, AST, total protein and albumin. The obtained results indicate that 1.80% Amm. sulphate can be used in the starter and grower diets of broilers, substituting portions of expensive SBM, without any detrimental effects on performance and serum biochemical indices. In addition, Amm. chloride can be safely used at a level not more than 1.50 % in the finisher diets only, but not in the starter diets.

## Conflict of interest

The authors declare that there is no conflict of interest.

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