Effects of alphamune g on the performance, serum and haematological parameters of *Escherichia coli* challenged turkey poults

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Abstract

A study was conducted to determine the response of turkey poults to graded levels of Alphamune G (0.00+, 0.03, 0.04, 0.05, 0.06 and 0.00 %) when challenged with *Escherichia coli* orally for 7 days. The graded levels were the treatments viz 0.00%+ (positive control), Alphamune G at 0.03, 0.04, 0.05, 0.06% and 0.00% (negative control; infected without Alphamune G supplementation). Each treatment was allotted 3 replicates of 6 poults. The experiment which was conducted for 36 days employed a completely randomized design. E. coli was isolated from the intestinal digesta of a colisepticaemic chicken. 108 turkey poults were used in this study. Poults were infected with *E.coli* for 7 days through the drinking water and given the treatment. The performance parameters of Alphamune G supplementation were significantly affected. The cumulative weight, feed intake and weight gain were highest for turkey poults fed 0.06% Alphamune G supplementation. These values were also directly proportional to the supplementation levels of Alphamune G. The birds given the negative treatment (0.00 %) had relatively poor performance compared to the other treatments. The specific enzymes studied were significantly affected (p<0.05) by the treatments. ALT and AST were significantly highest for turkey poults fed the negative control. Enzyme values became optimum at 0.05% Alphamune G supplementation. At 0.06% of Alphamune G supplementation, cellular mitigations of the effects of *E. coli* was measurable. Urea and creatinine were not significantly (p>0.05) influenced by the treatments. Haematological indices such as WBC and specific differential counts (lymphocytes and neutrophils) were affected significantly (P<0.05) by supplemental levels of Alphamune G. The inclusion of Alphamune G at 0.06% in the diets improved performance of turkey poults when challenged with *Escherichia coli*.

Keywords: alphamune g, performance, blood parameters, *Escherichia coli*, turkey poults

Introduction


There are a number of potential immune-nitribiotics are used mainly to protect poultry from pathogenic organisms, enhance their growth and health. However, the emergence of antibiotic resistance by pathogenic bacteria has led to international reconsideration of the use of antibiotics in livestock feeds (Thwaites and Frost, 1999; Bywater, 2005). Early concerns about the development of antibiotic resistance in human pathogens and recommendations to ban subtherapeutic use in animal feeds have been documented (Castanon, 2007; Dibner and Richards, 2005).

It has been reported that antibiotic resistance of *E. coli* of poultry has remained at a relatively high level since the 1950s (Gustafson and Bowen, 1997). Antibiotic Growth Promotion (AGP) has been practiced for about 50 years in many countries. In addition, subtherapeutic antibiotics have been used to enhance gastrointestinal maturity (Dibner and Richards, 2005). Antibiotic resistance has been displayed by field *Escherichia coli* isolates from commercial turkey farms, including resistance to Enrofloxacin, one of the most recently approved antibiotics for
use in poultry (Fairmodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in turkey production. The β-glucans are polymers of glucose that can be derived from the cell walls of yeast, bacteria, fungi, and cereals such as oats, barley, and rye. They have been found to increase the functional activity of macrophages and neutrophils (Reynolds et al., 1980; Yun et al., 2003). Mannan-oligosaccharides are polysaccharide-protein complexes derived from yeast that are indigestible to non-ruminant animals can function as prebiotics, providing favourable conditions for beneficial intestinal Lactobacillus spp. (Flickinger and Fahey, 2002). They also provide competitive binding sites for pathogens with mannose-specific fimbriae such as Salmonella, causing them to pass through the intestine, thus decreasing attachment and colonization (Sims et al., 2004; Zdunczyk et al., 2002) and improve feed conversion efficiency in turkeys grown to 20 week (Fritts and Waldroup, 2003).

Alphamune is an alternative to Antibiotics Growth Promoter (AGP) (Alpharma Animal Health, 2004; Alpharma, 2011). Alphamune is an extract of Saccharomyces cerevisae that has been spray dried to a tan powder and granulated (Bolu et al., 2011). It is a feed supplement that improves performance and immuno-competence system of animals. It enables the animal withstand occurring pressure within its own physiological competence (Huff et al, 2006). Alphamune G is a combination of 1-3, 1-6 β glucans and mannans oligosaccharides. β-Glucan has been found to possess immunomodulatory function and mannans, a prebiotic effect within the biological systems (Bent and Jesen, 2000). It has been reported that Alphamune G supplementation in pig diet improved performance when compared to salinomycin (an AGP). Optimal performance of Alphamune has been recorded at 500 g/tonnes of feed for broiler chicks (Alpharma Animal Health, 2004).

This study was conducted to determine the performance serum and haematological parameters of Escherichia coli-challenged turkey poults fed Alphamune G based diets.

Materials and Methods
Study Area
The experiment was carried out in the Animal Pavilion of the Department of Animal Production, University of Ilorin (80°28’0N, 4°41’0E), Ilorin, Nigeria.

Experimental Birds
One hundred and eight, four (4) week old poults were obtained from a commercial hatchery. The poults were weighed and randomly allotted to 6 treatments. Each treatment was replicated three times consisting of 6 poults per replicate raised in metabolic battery cages. A basal diet was formulated to contained 2,900 kcal of ME/kg and 26.0% CP to meet the nutrient requirement of young poults (NRC, 1994). The dietary treatments (Table 1) consisted of 6 supplemental levels of Alphamune G (0.00%+, 0.03%, 0.04%, 0.05%, 0.06%, and 0.00%- per 100kg of feed). The graded levels were the treatments viz: 0.00%+ (positive control), Alphamune G at 0.03, 0.04, 0.05, 0.06% and 0.00%- (negative control; infected without Alphamune G supplementation).

Source of E. coli
Escherichia coli were isolated from chickens with colisepticemia. Samples of the intestinal digesta were collected with sterile cotton swab. The inoculum was prepared by adding 2 inoculating loops of the sample on blood agar to 100 ml of Tryptose Phosphate Broth (TPB) and incubating for 2.5 h in a 37°C sonicator.
water bath. *Escherichia coli* colony was identified by a distinctive dark with metallic green sheen colour. A sterile needle was used to retreak an overnight culture *Escherichia coli* new culture plate to obtain pure cultures. The culture was incubated overnight at 4°C while a standard plate count was made. Ten-fold dilutions were then made in TPB based on the standard plate count and the challenge dilution titre equivalent to $1.23 \times 10^7$ cfu/poult (Sarkozy et al, 2000) was verified with another plate count. The poults were challenged via the dilution for a period of 7 days in drinking water. Turkey poults in treatments 2, 3, 4, 5 and 6 were challenged with *Escherichia coli*.

Routine management and vaccination were followed. Feed and water were given *ad libitum* for the 56 days of feeding trial. The basal diet contained 2,900 kcal of ME/kg and 28.0% CP which were in accordance with NRC recommended allowances (NRC, 1994).

Data were collected daily on feed intake and weight gain. Feed:gain was computed from the data of daily feed intake as a ratio of weight gain. At the end of the third week of study, a nitrogen retention study was conducted. Feed was weighed and given to birds and fecal samples collected over a period of 72 hours employing total collection method. Fecal samples collected were oven-dried, ground and analyzed for proximate composition. Proximate compositions of feed and fecal samples were carried out using the methods of AOAC (1990).

At four weeks of the study, blood samples were randomly taken from the wing veins of four (4) birds from treatment across replicates into bijou bottles containing EDTA (anticoagulant). Packed cell volume (PCV), haemoglobin concentration, total RBC, WBC and differential counts were evaluated according to Dacie and Lewis (1997). Serological samples were taken from collected blood (without anticoagulant), centrifuged at 4000 rpm for 3 min. and the supernatant sera harvested in bijou bottles for the determination of specific serum biochemical indices. Enzyme assay for serum aspartate amino transferase (AST, EC 2.6.1.1) and alanine amino transferase (ALT, EC 2.6.1.2) were determined by the colorimetric method of Reitman and Frankel (1957) while alkaline phosphatase (AP, EC 3.1.1.3) was determined by the kinetic method of Frajola et al. (1965). Response criteria were subjected to analysis of variance (ANOVA) using the SAS statistical package (SAS, 1985). Differences between treatment means were separated using Duncan multiple range test (Duncan, 1955).

**Results and Discussion**

The performance parameters of Alphamune G supplementation were significantly affected (Table 2). The cumulative weight, feed intake and weight gain were highest for turkey poults fed 0.06% Alphamune G supplementation. As the levels of Alphamune G increased, the body weights also increased. The values for all performance parameters measured for birds given the negative treatment (0.00 %) were comparatively low. Nitrogen retained was significantly influenced by Alphamune G supplementation of *E. coli* challenged poults. Poults fed the negative control diet recorded the lowest value for nitrogen retained. Results of the performance suggest that Alphamune G may have mitigated the adverse effects of *E.coli* (toxaemia) on the poults. These mitigations are voluntary feed intake and subsequently rapid muscle development cumulating to 5.20kg for poults fed 0.06% Alphamune G supplementation. Saif,
observed that the major clinical signs of colibacilosis include ill-thrift, ruffled feathers, enlarged and swollen navel, decreased appetite (anorexia), depression, diarrhea and pasting of feathers around vent. The increased voluntary feed intake, weight gain and nitrogen retained in proportion of supplemental levels of Alphamune G suggests that dietary Alphamune-G may have aided nutrient digestion, especially energy. NRC (1994) observed that feed intake in poultry is inversely related to the energy content of the diet. Through a feedback mechanism, energy satiety can reduce voluntary feed intake. When compared to the negative control pouls, Alphamune G proportionally enhanced the efficiency of feed conversion culminating in relatively higher weight gains. Bolu et al., (2009) reported that dietary Alphamune at 0.04% and 0.05% improved the performance of broilers. These findings also corroborates the reports of Miles and Bootwalla, (1991); Madrigal et al., (1993); Bradley et al., (1994); Santin et al., (2001). This improvement may be related with the balanced microbial population in the gastrointestinal tract (prebiotic effect) which has an important role in the health and performance of the broilers (Santin et al, 2001). Cumulative weight gain is a function of nutrition; Alphamune-G and other yeast cell complex have been shown to reduce feed intake and improve weight gain. 

Table 1. Composition of Experimental Treatments

<table>
<thead>
<tr>
<th>Diet</th>
<th>Infected with E. coli</th>
<th>Supplemented with Alphamune G (%)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>Positive control</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>0.03</td>
<td>0.03%</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>0.04</td>
<td>0.04%</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>0.05</td>
<td>0.05%</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>0.06</td>
<td>0.06%</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
<td>Negative control</td>
</tr>
</tbody>
</table>

Basal diet contained(%); Corn, 34.74; SBM, 9.15; GNC, 18.29; Fish meal, 12.85; Wheat offal, 13.41; Bone meal, 2.00; Lysine, 0.30; Methionine, 0.30; Salt, 0.30 and Vitamin/Mineral Premix** 0.30. Analysed Nutrient Content (%): DM, 90.98; CP, 26.00; EE, 6.65; CF, 3.60; Ash, 5.40.

**Premix supplied per kg of diets; Vitamin A: 8x10^6 IU, Vitamin D3: 1500IU, Vitamin E: 10IU, Vitamin D3: 1.5mg, Vitamin B1: 1.6mg, Vitamin B2: 4mg, Vitamin B6: 1.5mg, Vitamin B12: 0.0mg, Niacin: 20mg, Pantothenic acid: 5mg, Folic acid: 0.05mg, Biotin: 0.75mg, Choline Chloride: 1.75X10^6 mg, Cobalt: 0.2mg, Copper: 0.2mg, Iodine: 1mg, Iron: 20mg, Manganese: 40mg, Selenium: 0.2mg, Zinc: 80mg, Antioxidant: 1.25mg.
to improve feed conversion efficiency, nitrogen retention and increased body weight in chickens (Bolu et al., 2009; Zhang et al., 2005). Broilers chicks fed 0.04% dietary inclusion of Alphamune G gave the best performance (Bolu, et al., 2009).

The specific enzymes studied were significantly affected (p<0.05) by the treatments (Table 3). ALT and AST were significantly highest for turkey poults fed the negative control. The trend for these enzymes in relation to Alphamune G supplementation suggests that there was a proportionate rise in the values obtained for the enzymes with increasing levels of supplementation. However, the rise in the enzyme values became optimum at 0.05% Alphamune G supplementation. At 0.06% of Alphamune G supplementation, cellular mitigations of the effects of *E. coli* was measurable; the values observed for these enzymes were less. ALP was significantly influenced by the treatments, although most of the values are within the range for turkey poults (MVM,1986). Pouls fed the positive control had low value for ALP. Increasing levels of Alphamune G tended to increase ALP value similar to the other enzymes. However, the level of this enzyme reported for pouls fed 0.06%, suggest mitigated effects of *E. coli*. According to Otsele et al., (1991); Kecceci et al., 1998 serum biochemistry is a generalized medium of assessing the health status of animals. Blood parameters are potent indices of physiological, pathological and nutritional status of an organism (Babatunde and Olusanya, (1992). Changes in blood constituent are indirect indices to assess the metabolic stage of an animal as well as quality of feed. Prescott and Baggot (1993) reported that growth promoters perform best when the animal is in poor health and unhygienic living condition; thus, challenging the poults with *E. coli* enhanced the immunomodulatory effects of

### Table 2 Effects of Graded Levels of Alphamune G on Performance of Turkey Poults

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DIETS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. Initial weight (kg)</td>
<td></td>
<td>0.96</td>
<td>0.95</td>
<td>0.97</td>
<td>0.95</td>
<td>0.94</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Av. Final weight (kg)</td>
<td></td>
<td>5.81b</td>
<td>4.75a</td>
<td>5.17ab</td>
<td>5.55b</td>
<td>6.14c</td>
<td>4.57a</td>
<td>0.42</td>
</tr>
<tr>
<td>Cumulative weight (kg)</td>
<td></td>
<td>4.85bc</td>
<td>3.80a</td>
<td>4.20ab</td>
<td>4.60b</td>
<td>5.20c</td>
<td>3.60a</td>
<td>0.48</td>
</tr>
<tr>
<td>Weight g/bird/day</td>
<td></td>
<td>86.61b</td>
<td>67.86a</td>
<td>75.00b</td>
<td>73.21b</td>
<td>92.85d</td>
<td>64.28a</td>
<td>4.53</td>
</tr>
<tr>
<td>Feed intake g/bird/day</td>
<td></td>
<td>167.4b</td>
<td>151.24a</td>
<td>184.95c</td>
<td>189.49c</td>
<td>200.14d</td>
<td>172.58b</td>
<td>9.40</td>
</tr>
<tr>
<td>Feed: gain</td>
<td></td>
<td>1.94bc</td>
<td>2.18b</td>
<td>2.46c</td>
<td>2.59d</td>
<td>2.16b</td>
<td>2.68c</td>
<td>0.08</td>
</tr>
<tr>
<td>Nitrogen retained (%)</td>
<td></td>
<td>68.12c</td>
<td>60.14b</td>
<td>64.56c</td>
<td>65.55c</td>
<td>67.62b</td>
<td>52.34a</td>
<td>4.35</td>
</tr>
</tbody>
</table>

*a,b,c,d. Means within a row with different superscripts are significantly different (p < 0.05)*
Aphamune G supplementation. Enzymes are protein catalysts present mostly in living cells that are constantly and rapidly degraded although, renewed by new synthesis (Coles, 1986). According to Zilva and Pannall (1984), normal enzyme level in serum is a reflection of a balance between synthesis and their release as a result of the different physiological processes in the body. These enzymes measured in this study are found predominantly in the hepatocytes and renalocytes. However, during condition that may predispose liver and kidney damage, these enzymes are found abundantly in the blood (Bolu et al, 2008). In the same vein, Keele and Neil (1971) reported that serum AST is significantly high under disease and morbid conditions (as we have in the negative controlled poults) involving injuries to large numbers of metabolically active cells. Shipman et al (2013) reported that ALP are predominantly found in the liver, bone, kidney, intestine and placenta, however, circulating ALP are hepatic and the bone. Liver damage can be indirectly detected with ALP values at interval. Urea and creatinine were not significantly (p>0.05) influenced by the treatments (Table 3). Creatinine is a waste product of muscle metabolism and a good measure of kidney function (Siamak, 2011). The values of the serum creatinine and urea are indicative of kidney condition.

Haematological indices such as WBC and specific differential counts (lymphocytes and neutrophils) were affected significantly (P<0.05) by supplemental levels of Aphamune G (Table 3). There was significant rise in these values for turkey pouls fed the negative control diets in response to *E. coli* challenge. The unchallenged bird had the least values. Aphamune G, a prebiotic have relatively reduced the immunologic response to *E. coli* challenge, resulting in the significantly low values of these cell mediated products comparable to the unchallenged pouls. White blood cells in the avian species perform phagocytic functions similar to their mammalian counterparts (Campbell and Coles, 1986) and are used as indicators of stress response and sensitive biomarkers crucial to immune function (Shaniko, 2003). Leucocytes values of indigenous chickens have been reported to be higher than those of exotic breeds, lending credence to their higher susceptibility to avian pathogenic agents (Uko and Ataja, 1996; Talebi et al., 2005). Heterophils are the most abundant leukocyte in the peripheral blood of most species of birds in most studies, whereas some avian species are lymphocytic (have lymphocytes as the predominant cell type in the differential count) (Fudge, 2000; Latimer et al, 1988). The turkeys in this study had lymphocyte as the most abundant leukocyte in the peripheral blood further corroborating earlier works (Bolu, et al 2009, 2011 and 2012). Hematological studies of wild turkeys showed a similar condition (Bounous et al., 2000). Bounous et al (2000) reported that the lymphocytes are the predominant leukocyte in the peripheral blood of chickens and turkeys. That the Lymphocyte values were higher in birds fed Aphamune G inclusion than in control with the least value (62%) further attested to the immunomodulatory function of Aphamune G by conferring high immunity in the pouls (Bolu et al, 2012; Adeyemo and Longe, 2007).

Conclusion
The results of this study suggest that Aphamune G supplementation at 0.06% enhanced the performance and blood parameters of *E. coli* challenged pouls and could effectively substitute for antibiotic
Table 3 Effects of graded levels of Alphamune-G on Serum and Haematological Parameters of Escherichia Coli Challenged Pouls.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>DIETS</th>
<th>SEM ±</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Alanine Aminotransferase (iu/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.40</td>
<td>24.20</td>
</tr>
<tr>
<td>Aspartate Amino Transferase (iu/l)</td>
<td>104.60</td>
<td>113.30</td>
</tr>
<tr>
<td>Alkaline Phosphatase (iu/l)</td>
<td>40.40</td>
<td>36.90</td>
</tr>
<tr>
<td>Urea (Mmol/l)</td>
<td>6.30</td>
<td>9.20</td>
</tr>
<tr>
<td>Creatinine (Mmol/l)</td>
<td>0.70</td>
<td>1.00</td>
</tr>
<tr>
<td>Packed Cell Volume (%)</td>
<td>26.90</td>
<td>24.00</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>7.60</td>
<td>6.50</td>
</tr>
<tr>
<td>White Blood Cell (x10⁹/l)</td>
<td>7.00</td>
<td>8.40</td>
</tr>
<tr>
<td>Red Blood Cell (x10⁹/l)</td>
<td>5.80</td>
<td>5.00</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>62.00</td>
<td>63.00</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>30.00</td>
<td>37.00</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

a, b, c, d. Means within a row with different superscripts are significantly different (p < 0.05) ns = not significantly different (p>0.05)

growth promoter.

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