

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 56 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

Ahild *et al.*, 2001).

There are a number of potential immun-
antibiotics 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 ~~N~~old *Escherichia coli*
isolates from commercial turkey farms,
including resistance to Enro~~N~~oxacin, 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 cerevisiae* 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 mannan 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 poult 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 (8° 28'0N, 4° 41'0E), Ilorin, Nigeria.

Experimental Birds

One hundred and eight, four (4) week old poult were obtained from a commercial hatchery. The poult were weighed and randomly allotted to 6 treatments. Each treatment was replicated three times consisting of 6 poult 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 poult (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 re-streak 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 1.23×10^8 cfu/poult (Sarkozy et al, 2000) was verified with another plate count. The poult was challenged via the dilution for a period of 7 days in drinking water. Turkey poult 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 faecal samples collected over a period of 72 hours employing total collection method. Faecal samples collected were oven-dried, ground and analyzed for proximate composition. Proximate compositions of feed and faecal 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 bijoux 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 bijoux 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 poult 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 poult. Poult 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 poult. These mitigations are voluntary feed intake and subsequently rapid muscle development cumulating to 5.20kg for poult fed 0.06% Alphamune G supplementation. Saif,

Table 1. Composition of Experimental Treatments

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

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: 8×10^6 IU, Vitamin D3: 1500IU, Vitamin E: 10IU, Vitamin ₃: 1.5mg, Vitamin B1: 1.6mg, Vitamin B₂: 4mg, VitaminB6: 1.5mg, Vitamin B12:0.0mg, Niacin: 20mg,Pantothenic acid: 5mg, Folic acid: 0.05mg, Biotin:0.75mg, Choline Chloride: 1.75×10^4 mg, Cobalt:0.2mg, Copper: 0.2mg, Iodine: 1mg, Iron: 20mg,Manganese: 40mg, Selenium: 0.2mg, Zinc: 80mg,Antioxidant: 1.25mg.

(2003); Austic and Nesheim, (1990) observed that the major clinical signs of colibacillosis 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 poults, 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 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 poult 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 poult (MVM, 1986). Poults 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 poults fed 0.06%, suggest mitigated effects of *E. coli*. According to Otesile *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

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

a, b, c, d, Means within a row with different superscripts are significantly different (p < 0.05)

Alphamune 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 Alphamune G (Table 3). There was significant rise in these values for turkey poults fed the negative control diets in response to *E. coli* challenge. The unchallenged bird had the least values. Alphamune 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 poults. 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 Alphamune G inclusion than in control with the least value (62%) further attested to the immunomodulatory function of Alphamune G by conferring high immunity in the poults (Bolu et al, 2012; Adeyemo and Longe, 2007).

Conclusion

The results of this study suggest that Alphamune G supplementation at 0.06% enhanced the performance and blood parameters of *E. coli* challenged poults and could effectively substitute for antibiotic

Table 3 Effects of graded levels of Alphamune–G on Serum and Haematological Parameters of *Escherichia Coli* Challenged Poults.

PARAMETERS	DIETS						SEM ±
	1	2	3	4	5	6	
Alanine Aminotransferase (iu/l)	19.40 ^a	24.20 ^a	26.50 ^a	28.40 ^a	25.90 ^a	33.50 ^b	9.23
Aspartate Amino Transferase(iu/l)	104.60 ^a	113.30 ^a	98.50 ^a	124.20 ^b	120.70 ^{ab}	126.0 ^b	10.06
Alkaline Phosphatase (iu/l)	40.40 ^a	36.90 ^a	48.30 ^b	46.00 ^b	40.60 ^a	43.00 ^b	6.22
Urea(Mmol/l)	6.30	9.20	9.20	10.10	9.80	9.60	4.38
Creatinine(Mmol/l)	0.70	1.00	1.30	1.20	1.90	2.50	2.11
Packed Cell Volume (%)	26.90	24.00	27.00	28.00	28.00	28.00	4.68
Haemoglobin(g/dl)	7.60	6.50	6.80	6.30	6.60	6.80	2.21
White Blood Cell(x10 ⁹ /l)	7.00 ^a	8.40 ^a	7.60 ^a	7.80 ^a	8.30 ^a	10.50 ^b	1.35
Red Blood Cell(x10 ⁹ /l)	5.80	5.00	5.90	4.80	5.60	5.90	1.21
Lymphocyte (%)	62.00 ^a	63.00 ^a	70.00 ^b	65.00 ^{ab}	69.00 ^b	64.93 ^{ab}	4.91
Neutrophil (%)	30.00 ^a	37.00 ^c	30.00 ^a	35.00 ^a	30.00 ^a	45.00 ^b	7.82
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	1.00	3.17
Monocytes (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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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