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Full Length Research Paper

Effect of direct-fed microbial and antibiotics supplementation on the health status and growth performance of broiler chickens under hot humid environmental conditions

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Abstract

The effect of dietary direct-fed microbial (DFM) and antibiotics supplementation on health status and growth performance of broiler chickens under hot humid environmental conditions was studied. A total of 600 one-day-old Cobb hybrid broilers were randomly allotted to three dietary treatments. Three hundred and twenty (320) birds were selected out of the 600 after phase 1 and allotted to four dietary treatments: (i) Basal diet (BD) control, ii) BD plus DFM at the rate of 1.5 ml/kg, (iii) BD plus antibiotics at the rate of 100 mg/kg and (iv) BD plus DFM and antibiotics at the same rate as earlier described. In phase 1, birds fed DFM diet had lower feed intake (P<0.05) which did not affect weight gain but were more efficient in converting feed to gain. In phase 2, increased feed intake, improved weight gain and feed efficiency was observed for birds fed DFM diet. Growth performance of birds fed DFM diet was similar to that of antibiotic diet. Mortality reduced in both phases in favour of DFM and was least susceptible to disease occurrence. Increased intestinal acidity from a pH of 5.79 in control to 5.05 was recorded for birds fed DFM diet. A reduction in serum cholesterol was recorded from 110.25 mg/dl in control to 91.25 mg/dl in DFM fed group. It was more economical rearing broilers on DFM diet. It was concluded that DFM can be a suitable alternative natural feed additive to conventional antibiotics as growth and health promoter in a hot humid environment.

Key words: Direct-fed microbial, antibiotics, cholesterol, broiler.

INTRODUCTION

Intensive production causes considerable stress on poultry with adverse effect on their performance (Osei, 1984). This leads to a marked reduction in feed efficiency and utilization, productive performance and consequently increasing further the already high cost of production, especially cost of feed which accounts for over 70% of production cost (Ademosum, 1976; Begum, 2005).

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Feed additives have been the major intervention used to improve performance and profitability of commercial poultry enterprise (Mandal et al., 2000). The well known additives are the antibiotics used at sub-therapeutic levels to improve growth, health, feed efficiency and subsequent economic improvement benefit (Brorsen et al., 2002). However, concerns about the routine use of antibiotics and anti-microbial resistance development and transference gene from animal to human microbiota make

Table 1. Composition and calculated analysis of broiler starter and finisher experimental basal diets.

Ingredient (g/kg)	Starter	Finisher
Maize	550	550
Wheat bran	135	200
Fish meal	120	110
Soybean meal	170	110
Oyster shell	15	20
Dicalcium phosphate	5	5
*Premix	2.5	2.5
Sodium chloride	2.5	2.5
Calculated analysis (gkg^{-1})		
Crude protein	224.7	210
Fibre	34	45.1
Ether extract	45	30.1
Ca	11.9	11
P(available)	7	8.2
Lysine	12.7	11.4
Methionine	4.6	5.0
Metabolizable energy ($MJkg^{-1}$)	11.8	12

*Vitamin mineral premix per kg of diet: Fe 100 mg, Mn 110 mg, Cu 20 mg, Zn 100 mg, I 2 mg, Se 0·2 mg, Co 0·6 mg, sanoquin 0·6 mg, retinal 2000 mg, cholecalciferol 25 mg, α-tocopherol 23000 mg, menadione 1·33 mg, cobalamin 0·03 mg, thiamin 0·83 mg, riboflavin 2 mg, folic acid 0·33 mg, biotin 0·03 mg, pantothenic acid 3·75 mg, niacin 23·3 mg and pyridoxine 1·33 mg.

it unsafe for use (Castanon, 2007). The seriousness of the problem has led to withdrawal of approval for antibiotics as growth promoters in the European Union (EU) since January 1, 2006 due to negative human health issues of antibiotic resistance (Immersoel et al., 2004; Castanon, 2007). The antibiotic resistance development and the subsequent ban have resulted to an increased interest in finding alternatives for poultry and livestock production. The use of direct-fed microbial (DFM) have been reported to have the potential as alternative to the sub-therapeutic antibiotics as a result of the DFM's ability to prevent internal colonization of enteropathogenic micro-organisms, produce antimicrobial substances (organic acid and bacteriocins) and enzymes (Sieo et al., 2005; Liu et al., 2007), stimulate intestinal immunity of birds (Nurmi and Rantala, 1973; Fuller, 2000) and also reduce stress in animals (Line et al., 1997).

Most of the DFM researches have been conducted in Europe, America and Asian countries where the set of environmental conditions are different from Africa of which Ghana is included, where ambient temperatures during the year generally exceeds 32°C and coincide with declining performance of both broiler and layer chickens (Osei, 1984).

This study was therefore carried out to determine the effect of DFM on the health and productive performance of broiler chickens in a hot humid environment, while assessing the value of DFM on the economics of production in practical poultry enterprise.

MATERIALS AND METHODS

Experimental site

The experiment and the laboratory studies were conducted at the Kwame Nkrumah University of Science and Technology, Kumasi. The climate of the experimental site is generally described as hot and humid and is located within the semi-deciduous forest zone of Ghana. Geographically, it is located within latitude 0°43'N and longitude 01°36'W and experiences maximum and minimum temperature of 33 and 22°C, respectively. Relative humidity varies from 75 to 90%.

Source of DFM and storage

The DFM (multi-strain) used in this study was obtained from the Basic Environmental Systems and Technology (BEST), Canada. The DFM was composed by Lactobacilli (1×10^{8} cfu/g), Bacillus (1×10^{12} cfu/g) and *Saccharomyces cerevisiae* (yeast, 1×10^{5} cfu/g). The DFM was stored in a cold room at 18°C.

Experimental birds and procedure

Phase 1

A total of 600 one-day-old unsexed Cobb commercial broiler chicks were used. The chicks were balanced by weight and randomly allotted to 3 treatment groups in a completely randomized design. Each treatment, consisting of 200 birds, was replicated thrice. Three experimental treatments were formulated: a control (basal starter diet, BD) (Table 1). The BD contained none of DFM or antibiotic and two others, designated as DFM and antibiotic (ANT),

Table 2. Effect of diets on growth performance of broiler chicks from 0 to 3 weeks.

Parameter	Treatment			CV
	BD	DFM	ANT	
Initial body weight (g)	45.1	44.98	45.1	1.21
Final body weight (g)	555	555.63	419.6	26.12
Feed intake (g)	910.85^{b}	896.85^{c}	923.43^{a}	0.18
Weight gain (g)	480.08^{b}	489.77^{b}	510.42^{a}	1.36
FCR	1.9^{a}	1.83^{b}	1.81^{b}	1.48
Livability (%)	96.6	98.0	99	

Values with different superscripts in row differ significantly (P<0.05); CV, coefficient of variation; BD, basal diet; DFM, direct-fed microbial; ANT, antibiotics; FCR, feed conversion ratio.

contained 1.5 ml DFM and 100 mg antibiotic kg^{-1} BD, respectively. Feed and water were provided *ad libitum*.

Phase 2

A total of 320 birds were selected out of the initial 600 birds based on weight, condition and absence of physical deformities. Twenty birds with 1 male: 1 female ratio were balanced by weight and randomly allotted to each replicate in a completely randomized design. There were four treatments and four replicates, each replicate had birds selected from their respective treatment from the previous phase which ensured treatment continuity. An additional treatment which involved supplementation of the basal diet with DFM and antibiotics at rate of 1.5 and 100 mg/kg, respectively was introduced. This treatment was from birds reared on antibiotics or DFM supplemented birds. A finisher basal diet (Table 1) was supplemented with DFM or antibiotics or their combination at the same rate as in phase 1. Feed and water were supplied *ad libitum*.

Data collection and statistical analysis

Parameters measured include feed intake, body weight and weight gain, and carcass characteristics. The data obtained were analyzed using Costat 6.204 (2003) at 5% significant level.

Total microbial count and intestinal pH

Total microbial counts were determined on the small intestine digesta by method outlined by Bainbridge et al. (1996), while identification of microbes which involved the examination of colonial characteristics, morphology, motility, staining and biochemical properties was carried out using the guidelines of the International Commission Specification for Food (1978). The intestinal pH was measured on the small intestine digesta with a digital pH meter.

Haematological studies

Blood samples were collected randomly from two birds from each replicate. Blood samples were collected (after feed withdrawal for 12 h) from jugular vein into anticoagulant (heparin) bottles and analyzed for total red blood cells (RBC), haemoglobin (HB), packed cell volume (PCV), white blood cells (WBC) and serum cholesterol using a Haematological Auto Analyzer.

RESULTS AND DISCUSSION

Feed intake was significantly (P<0.05) affected by dietary treatments, whereas birds fed diet containing DFM had a lower feed intake. Birds fed diet containing antibiotics had the highest feed intake, while birds fed the BD had intermediate feed intake. Supplementation of DFM resulted in a reduction or no increase in feed intake relative to the basal diet. The reduction has been attributed to improved nutrient retention and utilization arising from a better gastrointestinal tract (GIT) environment enabled by the beneficial microorganisms (Table 2).

Weight gain of chickens fed on diet containing DFM did not differ markedly from those fed on the BD. Birds fed antibiotic supplemented diet had a significantly (P<0.05) higher weight gain and were also the most efficient, although not significantly different from those on the DFM supplemented diet (Table 2).

Mortality was reduced from 3.4% in the BD to 2 and 1% in birds fed the DFM and the antibiotics supplemented diets, respectively. This observation shows the benefits derived from early DFM supplementation in the diet of the chicks. The reduction in mortality could be attributed to elimination of entero-pathogenic micro-organisms through ‘competitive exclusion’ aided by the production of antimicrobial substance (lactic acid) (Nurmi and Rantala, 1973; Stern et al., 2001).

Phase II

The feed intake by birds fed DFM diet increased from the 5th week, but this was not significantly (P>0.05) different from feed intake of birds fed the antibiotic diet and the combined DFM and antibiotic diet. Birds fed BD had a significantly (P<0.05) lower feed intake when compared with the other dietary treatments. Weight gain and feed conversion ratio (FCR) of chickens fed the diet containing DFM, antibiotics and the combined DFM+ANT were significantly (P<0.05) better than those fed BD (Table 3). This improvement might be attributed to the increased

Table 3. Effect of diets on growth performance of broilers from 4 to 8 weeks.

Parameter	Treatment				CV
	BD	DFM	ANT	DFM+ANT	
Initial body weight (g)	555	555.63	419.6	555	26.12
Final body weight (g)	2302.38^b	2571.25^a	2636.75^a	2654.08^a	3.02
Feed intake (g)	4839.31^b	4987.41^a	4995.74^a	5069.74^a	1.69
Weight gain (g)	1762.87^b	2002.9^a	2045.23^a	2004.95^a	2.16
FCR	2.74^a	2.49^b	2.44^b	2.53^b	2.84
Livability (%)	93.75	100	97.5	98.75	

CV, Coefficient of variation. Values with different superscripts in a row differ significantly (P<0.05).

Table 4. Effect of diets on health status of broilers.

Parameter	Treatment				CV
	BD	DFM	ANT	DFM+ANT	
pH	5.79^a	5.05^b	5.79^a	5.46^{ab}	5.05
Microbial population × 10^6	16.8	1.43	17.8	15.8	
Disease occurrence (rank)	1	4	2	3	
Mortality (0 to 3 weeks) %	3.4	2.0	1		
Mortality (4 to 8) %	6.25	0.0	2.5	1.25	

CV-Coefficient of variation. Values with different superscripts in row differ significantly (P<0.05).

feed retention and nutrient utilization arising from a better GIT environment devoid of entero-pathogenic micro-organisms. Although, higher numerical feed intake was recorded for birds fed the combined diet which could be due to synergetic effect of the DFM and antibiotics, however, weight gain was marginally increased which made them less efficient as compared to DFM and antibiotics supplemented sole diets.

Health status of birds

The intestinal pH of chickens fed DFM supplemented diet was significantly lower (P<0.05) than the other dietary treatments. However, those on basal diet and antibiotics supplemented diet had the same pH value which was higher than the DFM supplemented diet, but lower than the pH of chickens on the combined (DFM+ANT) diet. The intestinal pH of chickens fed on DFM diet (5.05) and the combined diet (5.46) were outside the range (acidic) as given above (5.7 to 6.5) and this could be attributed to the antimicrobial substance (lactic acid) produced by the DFMs intestinally. This further, evidenced the viability of the administered DFM. This is because viable administered lactobacillus will produce lactic acid which will increase intestinal acidity. There is a delicate balance between beneficial and pathogenic bacteria and this relationship is sensitive to pH. A low pH value (4 to 5) favors beneficial microbes (DFMs) and a high pH value (6 to 7) is optimal for *Escherichia coli* and other pathogenic microbes. The lower pH (acidic) of DFM supplemented birds reduced substantially the pathogenic microbial population through “competitive exclusion” (Nurmi and Rantala, 1973; Chapman, 1989). A lower microbial population count (14.3 × 10^5) was obtained from the intestines of chickens fed DFM diet and could be as a result of non-motile nature of lactobacilli (Cheesbrough, 1984). On the contrary, chickens fed antibiotics supplemented diet had the largest microbial count (17.8 ×10^6). This could be attributed to the antibiotics used in sub-therapeutic levels, which resulted in entero-pathogenic organisms not completely excluded, probably due to development of antibiotic resistance.

Chickens fed the DFM-supplemented diet were the least susceptible to disease occurrence and as a result had reduced mortality (ranked 4) than those of the basal diet, which were susceptible to disease occurrence, especially coccidiosis and resulted in relatively higher mortality (Table 4). Guillot (2000) showed that probiotics increased the resistance of the bird to colonization by pathogenic species of bacteria and furthermore, stimulated the immune response of the birds through the mechanism of action that has not been clarified (Lu et al., 2008).

Haematological and biochemical indices

The results of haematological studies indicated no health-related problems during the experimental period that

Table 5. Effect of dietary treatments on blood parameters of broiler chickens.

Parameter	Treatment				CV
	BD	DFM	ANT	DFM+ANT	
WBC $\times 10^9$/L	127.57[c]	131.23[a]	127.13[c]	129.35[b]	0.73
RBC $\times 10^{12}$/L	2.25	2.18	2.27	2.2	2.73
PCV (%)	27.13	26.69	27.09	26.92	1.42
HB g/dl	11.11[a]	10.55[b]	11.18[a]	10.66[b]	1.10
SCC mg/dl	110.25[a]	91.25[b]	108.98[a]	97.55[b]	1.19

[a-c]Values with different superscripts in the same row differ significantly (P<0.05). WBC, white blood cell; RBC, red blood cell; PCV, packed cell volume; HB, haemoglobin; SCC, serum cholesterol concentration.

Table 6. Effect of dietary treatments on carcass characteristics

Parameter	Treatment				COV
	BD	DFM	ANT	DFM+ANT	
Dressing percent	76.65	76.35	76.50	77..03	3.34
Abdominal fat (g)	59.50	59.30	60.00	63.75	20.13
Gizzard (g)	56.25	57.50	68.13	63.13	8.16
Liver (g)	69.38	70.00	71.25	65.63	9.17
Heart (g)	13.00	13.10	14.2	13.95	2.10
Intestine (g)	148.13	140.63	148.10	146.25	10.23

could be attributed to the various dietary treatments. However, the haemoglobin (Hb) concentration of chickens fed DFM diet and the combined DFM and antibiotics diet were significantly lower than those fed on the basal diet and the antibiotics supplemented diet. Coates (1962) indicated that probiotics might compete with the host for supply of iron which in the long run would affect the Hb concentration. This could probably be the reason for the lower Hb level. The number of white blood cells (WBC) was highest (P<0.05) for chickens fed on DFM supplemented diet followed by the combined DFM and antibiotics diet, however, it did not differ for birds fed the basal diet and the antibiotics supplemented diet. The increased WBC count could be attributed to improvement in the immune system of the chickens brought about by improved stimulation of different subset of Th 2 cytokines induced by lactobacilli (Christensen et al., 2002) in response to potential infection or disease. However, values obtained from the haematological analysis are within the normal physiological ranges reported by Bone (1988).

Serum cholesterol concentration was markedly reduced in chickens fed the diet containing DFM and the combined DFM and antibiotics diet (Table 5). Mohan et al. (1996) and Jin et al. (1998) obtained similar results, and attributed the reduction to cholesterol assimilation by lactobacillus cells (Gilliland et al., 1985) and to the co-precipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993).

Carcass characteristics

The overall slaughter data (Table 6) indicated that dietary DFM and antibiotics supplementation may not have a significant influence on the dressing percentage and weight of internal organs. This is in agreement with the observation made by the study of Mandal et al. (2000) and Mohan et al. (1996). This showed that the use of DFM had no adverse effect on the performance and internal organs of broilers.

Production cost

Cost of production per bird was relatively highest for chickens fed the combined diet (DFM + ANT), GH¢2.84 (US$2.18) followed by the antibiotics diet, GH¢2.76 ($2.12), the DFM diet, GH¢2.67 (US$2.05) and the basal diet, GH¢2.61 (US$2.01). Although, the basal diet fed chickens incurred the least cost per bird, it is worth noting that apart from vaccines administered, drugs such as antibiotics, coccidiostats and others used normally in poultry production were not included in the production cost. These drugs were not used in the production in order for the full health improvement potential of DFM supplementation to be assessed. Profit on returns per bird was relatively higher and same for chickens fed the combined DFM and antibiotics diet and the antibiotics supplemented diet, GH¢3.29 (US$2.53) followed by the

DFM diet, GH¢3.22 (US$2.48). In spite of the lower production cost, the least profit per bird of GH¢2.69 (US$2.07) was realized from chickens fed the basal diet. As a result of the reduced mortality of chickens fed diet containing DFM, a better overall profit, GH¢197.6 (US$198.4) on returns was accrued from their production. It was therefore more economical to rear broilers on DFM-supplemented diet.

Conclusions

The results of the present study show that DFM can be used in broiler diets without any negative effects on their growth performance and carcass traits. In fact, DFM improves the general health status of broilers and reduces blood cholesterol concentration. Further, it makes the production more efficient and economical through savings from feed and medications. DFM can be used in hot humid environments (Ghana) as a valuable alternative to the use of conventional sub-therapeutic antibiotics.

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