

Full Length Research Paper

Direct-fed microbial on the health status, productive performance and internal egg characteristics of layer chickens under hot humid environmental conditions

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Dietary direct-fed microbial (DFM) and antibiotics supplementation on health status, egg laying performance and internal egg characteristics under hot humid environmental conditions were studied. Three hundred (300) layer birds at point of lay (22 weeks old) were used for the study in a completely randomized design. There were four dietary treatments which are; Basal diet (BD), BD + DFM at the rate of 1.5 ml/kg, BD + antibiotics at the rate of 10 mg/kg feed and BD + DFM+ antibiotics at the same rate as above. Feed intake was significantly ($p<0.05$) lower for birds fed the DFM diet as compared with the control. However, body weight gain of DFM fed birds was not negatively affected and was relatively more efficient in converting feed into body weight and eggs. Hen-day and hen-housed production and egg shell thickness were not significantly ($P>0.05$) influenced by dietary treatments. Eggs laid by birds fed DFM diet were significantly ($P<0.05$) heavier and consistent throughout the experimental period. Serum and egg cholesterol concentration were significantly reduced by DFM supplemented diet (64 and 409 mg) as compared with the control (75 and 483 mg), antibiotics (74 and 481 mg) and DFM+ antibiotics combined diet (70 and 430 mg). Intensity of yolk colour was higher for eggs laid by birds fed DFM supplemented diet. It was concluded that DFM could be a suitable alternative to antibiotics supplementation for improved health status and productive performance of layer chickens under hot and humid environmental conditions.

Key words: Direct-fed microbial, layer chickens, egg cholesterol, sub-therapeutic antibiotics.

INTRODUCTION

Eggs from poultry industry have been one major source by which protein malnutrition can be eradicated in most developing countries. This is as a result of nutritious but relatively cheaper eggs that are obtained from the poultry production. However, there are a number of factors militating against the total realization of this objective as a result of high feed cost and disease challenges due mainly to stress responses (Osei, 2010). Chickens suffer

depressed immune responsiveness when exposed to higher environmental conditions and managerial stressors. The result is weakening of the immune system with subsequent increase in colonization by pathogenic microbes and reduced productive performance (Lee et al., 2010; Yang et al., 2011). As side these production challenges, the egg cholesterol concentration has raised a lot of consumer health concerns regarding vascular

Table 1. Composition of basal diet (BD) and calculated analysis.

Feed ingredients (kg)	Inclusion rate
Yellow maize	550
Fishmeal (anchovy)	80
Soya bean meal	85
Wheat bran	200
Oyster shells	70
Dicalcium phosphate	10
*Premix	2.5
Sodium chloride	2.5
Calculated analysis (g/kg)	
Crude protein	174
Crude fibre	46
Ether extract	44.6
Calcium	34
P (Available)	6.2
Lysine	7.4
Methionine	3.8
Metabolisable energy MJkg ⁻¹	11.8

*Vitamin mineral premix provided the following per kg of diet: vitamin A, 10,000 IU; D, 400,000 IU; E, 3,000 IU; K, 2,000 IU; B1 200 mg B2, 900 mg; B12, 2,400 mg; niacin, 5,000 mg; Fe, 900 mg; Cu, 500 mg; Mn, 12,000 mg; Co, 1000 mg; Zn, 10,000 mg; Se, 4.

diseases especially arteriosclerosis and has slightly reduced patronage. Therefore, any move to reducing feed cost and diseases of layers as well as decrease in egg cholesterol concentration would increase egg production and consumption while reducing protein malnutrition ultimately. The use of antibiotics in feed at sub-therapeutic level to birds as production enhancers has been adopted by many producers. However, the challenge of antimicrobial resistance and their possible transference to human microbiota (Castanon, 2007) has made antibiotics use not an ideal solution to production challenges. The search for a suitable alternative to antibiotics that would promote health status and productive performance of the layer chickens other than giving more feed is necessary. The use of Direct-fed microbial (DFM) in poultry has been suggested as a viable alternative (Bonsu et al., 2012; Chapman, 1989). DFM has the potential to prevent entero-pathogenic colonization and proliferation, promote better feed utilization from improved intestinal environment, improved immune responses and reduced impact of stress (Liu et al., 2007; Fuller, 2000; Line et al., 1997). However, microbial performance is temperature sensitive. This research was conducted to determine the effect of DFM on health status, productive performance and internal eggs characteristics of layers reared under hot and humid environmental conditions.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the animal house of the Department of Animal Science, 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. The relative humidity is 93% in the morning (06.00 h) and 75% in the afternoon (15.00 h).

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 (RE3) was composed by Lactobacilli (1×10^8 cfu/g), Bacillus (1×10^{12} cfu/g) and *Saccharomyces cerevisiae* (yeast, 1×10^9 cfu/g). The DFM was stored at room temperature (30°C).

Experimental birds and procedure

A total of three hundred (300) Isa brown layer birds at point of lay (22 weeks old) were used for the study in a completely randomized design. There were four dietary treatments. Each treatment consisting of seventy five (75) birds was replicated three times. The four dietary treatments were: a control (basal diet, BD) (Table 1), which contained none of DFM or antibiotic and three others, designated as DFM, Antibiotic (ANT), and DFM+ANT combined diet which contained 1.5 ml DFM, 10 mg antibiotic, and 1.5 ml + 100 mg per kilogram of BD, respectively. Feed and water were provided *ad libitum*.

Data collection

Parameters measured included feed intake, body weight and weight gain, egg production and egg characteristics, and haematological parameters.

Haematological studies and egg cholesterol concentration

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 (EDTA) bottles and analyzed for total red blood cells (RBC), haemoglobin (HB), packed cell volume (PCV) and white blood cells (WBC) using a Haematological Auto Analyzer. Three eggs from each replicate were opened into beakers, stirred and analysed for egg cholesterol concentration. Serum cholesterol and egg cholesterol were analyzed according to the procedure outlined by Cheesbrough (1984).

Egg production

The number of eggs laid by the hens per replicate was recorded daily and from that, Hen-day production, (HDP) and Hen-housed production (HHP) were calculated. Eggs were collected 3 times daily (9.00 am, 1.00 pm and 5.00 pm). Egg weights from each replicate were measured daily using the electronic scale and then manually graded based on weight into: extra large (≥ 60 g), medium (≥ 50 g < 60 g) and small (≥ 45 g ≤ 50 g). A random sample of eggs

Table 2. Effect of dietary treatments on the laying performance (11months) of layer chickens.

Parameter	Treatment				COV
	BD	DFM	ANT	DFM+ANT	
Initial body weight (g/bird)	1436.0	1422.5	1485.0	1392.3	3.67
Feed intake (g/bird)	29645.5 ^a	29259.1 ^c	29514.9 ^{ab}	29486.7 ^b	0.27
Final body weight (g/bird)	1841.9	1871.5	1860.7	1910.3	3.09
Body weight gain (g/bird)	404.9	445.7	381.0	505.0	13.99
FCR	2.44 ^{ab}	2.35 ^b	2.51 ^a	2.44 ^{ab}	2.81
HDP (%)	70.5	71.8	69.1	71.0	3.53
HHP (%)	70.5	69.5	65.0	69.3	5.31
Mean egg weight (g)	60.9	62.7	60.4	61.2	1.79
Age at 50% production (days)	161	166	161	161	1.49
Egg grades (%)					
1 st Grade (> 60 g)	13.5	15.9	11.0	9.1	26.26
2 nd Grade (50-60 g)	43.7	47.6	38.4	46.0	15.53
3 rd Grade (< 50 g)	42.8	36.0	50.7	44.9	15.58
Egg volume (ml)	55.6	57.2	55.1	55.9	1.79
Egg yolk colour	8.0 ^c	9.8 ^a	8.5 ^{bc}	9.0 ^b	4.72
Shell thickness (mm)	0.33	0.36	0.36	0.37	6.54
Haugh unit (%)	87.1	87.6	85.2	86.6	8.45
Egg cholesterol (mg dl ⁻¹)	483 ^a	409 ^c	480 ^a	430 ^b	2.43
Livability (%)	100	96	89	96	

Values with different superscripts in the same row differ significantly ($P < 0.05$); COV, coefficient of variation; FCR, feed conversion ratio; HDP, hen– day production, HHP, hen- housed production; BD, basal diet; DFM, direct fed microbials; ANT, antibiotics; DFM+ANT, direct fed microbials and antibiotics.

collected from each replicate was measured at monthly intervals for shell thickness using a micrometer screw gauge, with measurements being taken at the equatorial plane of the egg after the shell membranes had been carefully removed. Broken, misshaped and cracked eggs were recorded.

Feed conversion ratio

The monthly feed conversion ratio was calculated as the amount of feed consumed in relation to the number and weight (per kg) of eggs produced during that month.

Internal egg quality

Samples of eggs (5 eggs) from each replicate were measured monthly for egg weight (W), height (H) and width using a tripod micrometer from which egg volume and haugh unit were calculated. The haugh unit (HU) was calculated using the equation

$$HU = 100 \times \text{Log} [H - 1.7^{W^{0.37}}] + 7.6$$

Yolk colour scores were also assessed by visual comparison of the fresh yolk with a Roche yolk colour fan.

Economics of production

The economics of production was calculated for each treatment. The assessments were done based on the cost of birds, feed, medication and returns on sale of eggs.

Statistical analysis

Data recorded were subjected to analysis of variance using Costat 6.204 (2003) (Bartlett's Test). Data on egg production and egg grades were log transformed prior to statistical analysis. However the mean percent values were presented for easy comprehension. Differences among treatment means were isolated at 5% significance level.

RESULTS AND DISCUSSION

The effect of dietary DFM on egg production parameters are presented in Table 2. Dietary treatments had significant influence ($P < 0.05$) on feed consumption. Chickens fed the DFM diet and the combined DFM and antibiotics diet had lower feed intakes, however, birds on the basal diet consume more feed, while that of the antibiotics diet was intermediate. The reduced feed intake of birds fed the DFM diet could be attributed to improved GIT environment. Final body weight and weight gain was not significantly influenced. Efficiency of feed utilisation was significantly ($P < 0.05$) different among dietary treatments. Chickens fed the DFM- supplemented diet were most efficient (Bozkurt et al., 2011) whereas those fed the antibiotics diet were least efficient. It has been hypothesized that improvement in feed efficiency in laying hens may partially be attributed to the establishment of an

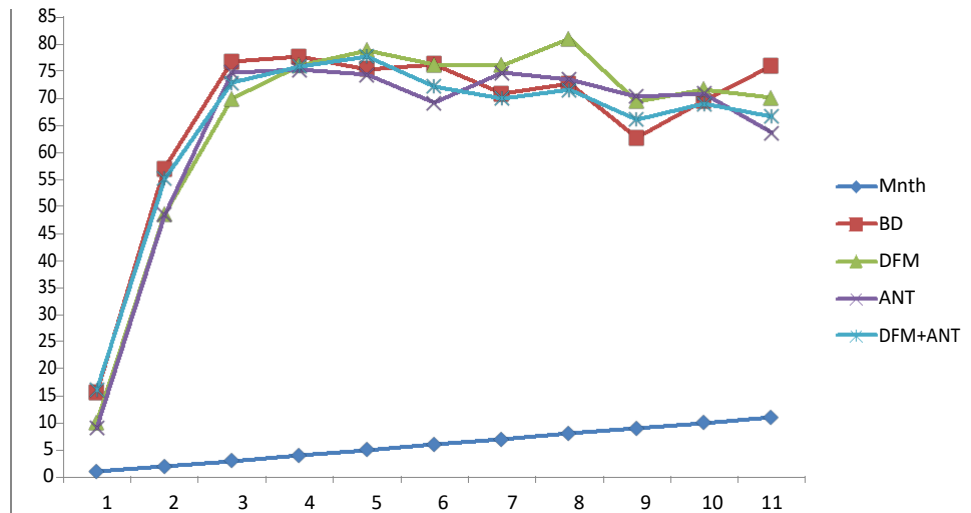


Figure 1. Effect of dietary treatments on egg production. Mnth, month; BD, basal diet; DFM, direct fed microbials; ANT, antibiotics; DFM+ANT, direct fed microbials and antibiotics.

intestinal bacterial population that favoured improved nutrient retention (Liu et al., 2002). Beneficial microbes stimulate numerous intestinal brush border enzymes to maintain normal digestive functions, secrete many factors including enzymes that may reduce dietary protein allergies following gastroenteritis and polyamines that stimulate brush border hydrolases, proteases, and transport carriers. It stimulates D-glucose and sodium absorption and produces short-chain fatty acids. These improve nutrient digestion, retention and utilization.

Egg production parameters (HDP and HHP) did not indicate significant ($P>0.05$) difference among dietary treatments. However, the production line was consistent with that of Etches (1996) who observed that during the typical 12-month laying period, the rate of hen-day production (HDP) rises quickly after laying begins, reaches its maximum level after 7 to 8 weeks and then declines gradually until the flock is disposed off when the revenue from eggs no longer covers the cost of production (Figure 1). Although HDP was relatively higher for birds fed the DFM diet (71.82%), since the denominator for HDP is altered daily depending on the stocking density at the time, the statistic is not influenced by mortality. It is therefore possible to have excellent performance indicator from HDP which is not economically viable because mortality may be excessive. However, HHP which uses the number of hen present in the facility at the start of egg production as a constant denominator can be used to derive a combined estimate of mortality and egg production.

In that way, a more accurate reflection of economic viability of flock performance can be deduced. HHP was over 1% better for chickens fed the basal diet than that of the other treatments due partly to mortalities that occurred for the respective dietary treatments.

Layers fed the DFM diet produced the heaviest number of eggs and resulted in the highest number of extra large eggs (Grade 1) and medium size eggs than the other dietary treatments (Figure 2). The improvement was 2.5% (extra large) and 4% (medium) than the control.

Birds fed the antibiotics supplemented diet laid the highest number of small eggs (Grade 3). This observation indicated that the use of DFM in layer diet therefore resulted in a shift from smaller to larger eggs. Improved mean egg weight and size could be attributed to improved intestinal condition, nutrient retention and utilization (Nahashon et al., 1994).

The values obtained for egg volume were not significantly ($P>0.05$) different among dietary treatments, it was numerically higher for eggs laid by birds fed the DFM-supplemented diet. The pattern of effect of dietary treatments tended to follow that of the egg weight. The increased volume of eggs laid by birds fed on DFM diet further suggested that the eggs were not only heavier by mass but were also larger by volume.

Mean egg shell thickness was not significantly ($P>0.05$) affected by dietary treatments. However, improvement of 9% of the shell thickness was observed for dietary treatments other than the control. Nahashon et al. (1994, 1996) in a work done with laying hens observed that the addition of Lactobacillus-based DFM to the diet improved N, Ca and P retention. This probably accounted for the improvement observed in shell thickness. This is in agreement with results of studies conducted by Yalcin et al. (2008).

Egg yolk colour was significantly ($P<0.05$) affected by dietary treatments. The overall high score of the yolk colour was due to the yellow maize used in the basal diet. However, the intensity of the yolk colour was significantly higher for eggs of birds fed the DFM diet followed by the

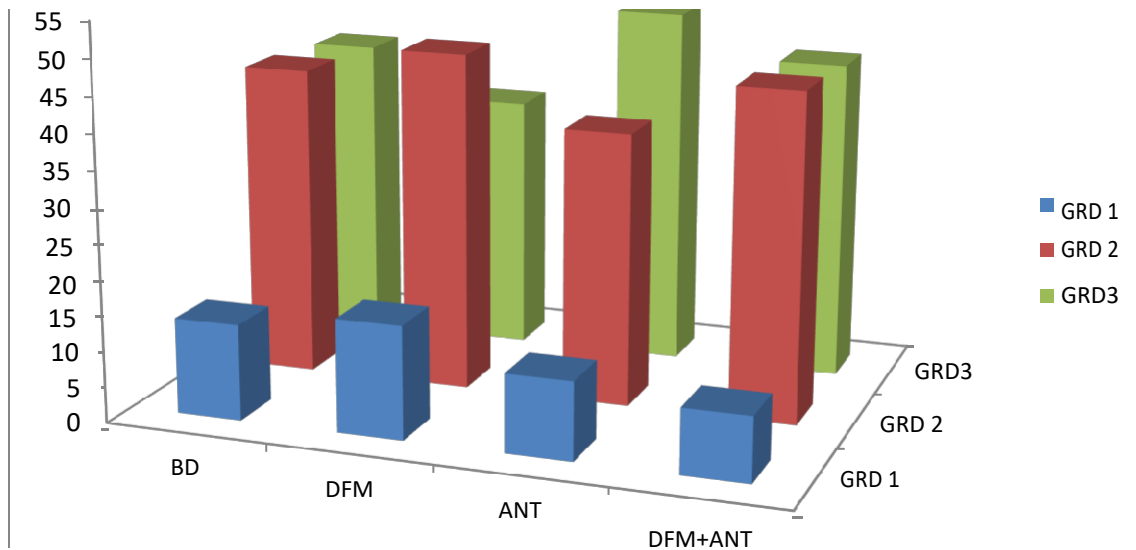


Figure 2. Effect of dietary treatments on distribution of eggs into grades. BD, basal diet; DFM, direct fed microbials; ANT, antibiotics; DFM+ANT, direct fed microbials and antibiotics; GRD, grade.

Table 3. Effect of dietary treatments on blood parameters of layers.

Parameter	Treatment				COV
	BD	DFM	ANT	DFM+ANT	
WBC $\times 10^9$ L	118.8 ^d	134.83 ^a	133.43 ^a	135.5 ^a	3.92
RBC $\times 10^{12}$ L	2.27	2.49	2.38	2.3	5.74
PCV %	31	32.78	34.18	40.03	13.90
HB g dl ⁻¹	12.3	12.5	12.88	12.96	3.21
Serum cholesterol (mg/dl)	75 ^a	64.25 ^d	73.75 ^a	70.33 ^{ab}	5.31

Values with different superscripts in the same row differ significantly ($P < 0.05$); COV, coefficient of variation; BD, basal diet; DFM, direct fed microbials; ANT, antibiotics; DFM+ANT, direct fed microbials and antibiotics.

combined diet and antibiotics diet. The increased yolk colour of DFM eggs also affirms their nutrient retention ability by being able to retain more of the carotene in the yellow maize. This is also in agreement with the results obtained by Li et al. (2006) and Salma et al. (2007).

Total egg cholesterol concentration was significantly ($P < 0.05$) reduced from 483 mg/dl in the control to 409 mg/dl in the DFM diet and accounted for 15.3% reduction. Layers fed on the combined diet also had a reduced egg cholesterol levels. However, the reduction was less than was achieved with that of the DFM diet. This reduction in egg cholesterol might be explained by the reduced absorption, synthesis, or both, of cholesterol in the gastrointestinal tract (Mohan et al., 1995). Antibiotics supplementation had no effect on egg cholesterol concentration and could be attributed to lack of ability to deconjugated bile salt and consequently reduced fat emulsification and absorption (Yalcin et al., 2008).

The serum cholesterol level was reduced ($P < 0.05$) from

75 mg/dl in chickens fed the basal diet to 64.25 mg/dl in the DFM-supplemented diet (Table 3). Birds fed the combined diet managed a marginal reduction whereas no effect was observed for those fed the antibiotics-supplemented diet. Dietary treatment had no effect on blood haemoglobin (HB) and red blood cells (RBC). Long term use of DFM supplementation has been implicated to reduce HB level due to competition for Fe and folic acid with the host (Coates, 1962). However, this was not the case in this layer studies unlike the broiler experiment where the HB level of birds fed DFM diet was reduced (Bonsu et al., 2012). White Blood cell (WBC) count were higher ($P < 0.05$) for birds on all dietary treatments other than those fed the basal diet. The higher WBC of birds fed the DFM diet and the combined diet affirmed earlier observation of the ability of Lactobacillus to stimulate the immune system resulting in higher number of white blood cells. Packed cell volume (PCV) was highest in favour of birds fed the combined diet and lowest in respect of birds fed the basal diet. However, blood parameters are all

within the range specified by Bone (1988).

Mortality of birds in various dietary treatments varied. Whereas 100% livability was observed in birds fed the basal diet, 4, 11 and 4% mortality were recorded for birds fed the DFM diet, antibiotics diet and the combined diet respectively. Cause of deaths was not attributed to dietary treatments. In the case of deaths which occurred in the DFM and the combined diet, it was associated with reproductive disorder especially impacted oviduct accompanied by inflammation of the peritoneum (peritonitis). However, this condition was goaded by increased egg size of these dietary treatments. Birds then pecked affected ones to death especially in the absence of caretakers. Post-mortem examination of dead birds fed the antibiotics-supplemented diet indicated higher deposition of abdominal fat and about half of the deaths were as a result of peritonitis. Interestingly, birds fed the antibiotic-supplemented diet were not laying larger eggs to have triggered this condition. However, Mandal et al. (2000) indicated that antibiotics supplementation reduced intestinal or gut thickness which increased nutrient absorption. It is thought that the gut thickness reduction might have affected the strength of the peritoneum to withstand constant passage of eggs which could also predispose the birds to this condition.

Economics of layer production

Monthly cost of production for dietary treatment was relatively higher for chickens fed the combined diet (DFM+ANT) GH¢370 (US\$195) followed by the antibiotics diet GH¢361 (\$190), the basal diet GH¢302 (US\$168) and the DFM diet GH¢317 (US\$167). Birds fed the DFM diet incurred the least cost as a result of reduced feed intake. Profit on returns per month for dietary treatment was relatively higher for chickens fed the DFM diet GH¢48 (US\$48) followed by the Basal diet GH¢86 (US\$45), the combined diet GH¢67 (US\$37) and the antibiotics diet GH¢63 (US\$35). The improved profit margin for birds fed the DFM diet was attributed to higher feed conversion efficiency which resulted from efficient feed utilization and also to the laying of heavier and larger eggs which increased the sale of more larger and medium sized eggs. Birds fed the antibiotics diet were expected to improve egg production above that of the basal diet. However, fewer number and small sized eggs characterized their production. It was therefore more economical to rear layers on DFM-supplemented diet.

Conclusion

The results of the present study show that DFM can be used in layer diets without any negative effects on their growth and productive performance. DFM improves the general health status of layers and reduces egg and

blood cholesterol concentration significantly. Further, DFM results in a shift from small to large size eggs while making the production more efficient and economical through savings from feed and sale of extra large eggs. DFM can be used in hot humid environments as a valuable alternative to the use of conventional sub-therapeutic antibiotics.

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REFERENCES

- Bone JF (1988). Animal Anatomy and Physiology. 3rd Edition, Prentice-Hall, Inc. New Jersey, USA, pp. 234- 248.
- Bonsu FRK, Donkoh A, Osei SA, Okai DB, Baah J (2012). Effect of direct-fed microbial and antibiotics supplementation on the health status and growth performance of broiler chickens under hot humid environmental conditions. *Int. J. Livestock Prod.* 3(5):66-71.
- Castanon JRI (2007). History on the use of antibiotics as growth promoters in European poultry feeds. *Poult. Sci.* 86:2466-2471.
- Chapman JD (1989). Probiotics, acidifiers and yeast culture: A place for natural additive in pigs and poultry production, *Biotechnology in Feed Industry*. Alltech Inc. Technical publications, Nicholasville, Kentucky, pp. 63-77.
- Cheesbrough M (1984). Medical Laboratory Manual for Tropical Countries- Microbiology. Volume 2, 2nd edition, Butterworth-Heinemann Ltd. Halley Court, Jordan Hill, Oxford OX2 8EJ.
- Costat 6.204, copyright© (2003). CoHort software. 798 Light House Ave. PMB 320, Monterey, CA 93940USA. <http://www.cohort.com>.
- Etches RJ (1996). Reproduction in poultry. CAB International, Wallingford Oxon OX10 8DE UK.
- Fuller R (2000). The chicken gut microflora and probiotic supplements. *Poult. Sci.* 38:189-196.
- Lee KW, Lee SH, Lillehoj HS, Li GX, Jang SI, Babu US, Park MS, Kim DK, Lillehoj EP, Neumann AP, Rehberger TG, Siragusa GR (2010). Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poult. Sci.* 89:203-216.
- Li C, Xu CL, Ji C, Ma Q, Hao K, Jin ZY, Li K (2006). Effect of a dried *Bacillus subtilis* culture on egg quality. *Poult. Sci.* 85(2):364-368.
- Line JE, Bailey JS, Cox NA, Stern (1997). Yeast treatment to reduce salmonella and campylobacter population associated with broiler chickens subjected to transport stress. *Poult. Sci.* 76:1227-1231.
- Liu JR, Lai SF, Yu B (2007). Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanases as a probiotic for broiler chickens fed on a wheat-based diet. *Brit. Poult. Sci.* 48(4):507-514.
- Liu Z, Qi G, Yoon I (2002). Effect of yeast culture on production parameters and intestinal microflora in laying hens. *Poultry Science Association 91st Annual Meeting Abstracts*. August 11-14, 2002. Newark, DE. Abstract No: 381, P. 89.
- Mandal L, Mandal SK, Baidua N, Sarkar SK (2000). Pro and antibiotic in sequence perform well in broiler diet. *Feed Mix* 8(1):18-20.
- Mohan B, Kadirvel M, Bhaskaran M, Natarajan A (1995). Effect of probiotic supplementation on serum / yolk cholesterol and on egg shell thickness in layers. *Brit. Poult. Sci.* 36:799 - 803.
- Nahashon SN, Nakave HS, Mirosh LW (1996). Nutrient retention and production parameters of Single Comb White Leghorn layers fed with

- varying crude protein levels and supplemented with Direct- Fed Microbial. Anim. Feed Sci. Tech. 61:17- 26.
- Nahashon SN, Nakave HS, Mirosh LW (1994). Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented with Direct- Fed Microbial. Poul. Sci., 73:1699-1711.
- Osei SA (2010).The influx of imported animal product onto the Ghanaian market and the impact on animal production, processing and marketing: The case of poultry meat. Ghana J. Anim. Sci. 5 (1):1-9.
- Salma U, Miah AG, Tareq KMA, Maki T, Tsuji H (2007). Effect of dietary *Rhodobacter capsulatus* on egg yolk cholesterol and laying hens performance. Poul. Sci. 86:714-719.
- Yalçin S, Ozsoy B, Erol H, Yalçin S (2008). Yeast culture supplementation to laying hen diet containing soybean meal or sunflower feed meal and its effects on performance, egg quality trait and blood chemistry. J. Appl. Poul. Res. 17:229-236.
- Yang XY, LI WL, Feng Y, Yao JH (2011). Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. Poul. Sci. 90 (12):2740-2746.