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EFFECTS OF DIRECT-FED MICROBIAL (DFM) ON THE PRODUCTIVE AND REPRODUCTIVE PERFORMANCE AND HAEMATOLOGIC TRAITS OF FEMALE INDIGENOUS GUINEA FOWLS (NUMIDA MELEAGRIS)

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ABSTRACT

A 2-phase (grower and layer) study was undertaken to investigate the effects of direct-fed microbial (DFM) on the growth, production and reproductive performance of indigenous guinea fowls (Numida meleagris) raised on-station. During the grower phase, two hundred and sixteen (216) nine-week-old pearl guinea fowl growers of mean weight of 510 grams were allotted to four dietary treatment groups, each with three replicates containing 18 birds (15 pullets and 3 cockerels) in a completely randomized design (CRD). At 20 weeks of age, the birds were transferred to four-layer dietary treatments. The control diet contained no DFM while DFM was added to the various treatment diets at the respective rates of 1.0ml/kg, 1.5 ml/kg and 2 ml/kg. Feed and water were provided to the birds ad libitum in both the grower and layer phases. Parameters studied included feed consumption, growth rate, feed conversion ratio, egg production, egg fertility and hatchability and blood traits. The dietary incorporation of DFM had no significant effect ($p > 0.05$) on feed intake, egg weight and quality, weight of first egg and body weight at first egg as well as blood cholesterol levels but significantly improved final liveweight, feed conversion ratio (FCR), age at first egg, egg weight, egg fertility and hatchability ($p < 0.05$) compared with the control. It is concluded that the addition of 1.5 to 2ml of DFM/kg diet for indigenous guinea fowls improves both productive and reproductive performance.

Keywords: DFM, guinea fowls, haematology, production, reproduction

INTRODUCTION

Ghana's guinea fowl industry has the potential to create thousands of jobs, generate revenue by selling to the local and international markets and help alleviate poverty (World Bank, 2022). In Ghana, indigenous guinea fowls are found all over the country, but mostly in the northern savannah regions (84.54%) (Dei and Karbo, 2004) where the number of birds in each household ranges from five to 200 birds with an average of about 20 (Apiiga, 2004) and are largely raised free-range. Guinea fowls concurrently serve multiple purposes as parent stock, and source of eggs and meat. However, guinea fowl production in Ghana faces a myriad of constraints including slow growth rate, poor egg hatchability, high rates of male infertility, high keet mortality and lack of knowledge and skills for proper management (Oke *et al*., 2004). Research strategies to improve productivity have included the use of growth promoters, predominantly antibiotics. However, the development of antibiotic resistance has led to a search for alternatives that give similar or enhanced production as the antibiotics currently provided to food animals (Bostoglou and Fletouris, 2001; Hume, 2011). Probiotics (direct-fed microbial, DFM) have been studied as alternatives to antibiotics in livestock diets including layers, and broilers and have been reported to reduce the incidence of bacterial diseases and consequently increase the profitability of layers and broilers (Yoruk *et al*. 2004; Pan *et al*. 2011; Ezema, 2012; Bonsu *et al*. 2014). Neveling and Dicks (2021) have listed some of the benefits of direct-fed microbial in broiler chickens as improved feed conversion, improved weight gain, improved immune response and enhanced feed digestion. There is little data however, on the utilization of direct-fed microbial in indigenous guinea fowl production in Ghana. Therefore, this study aimed to evaluate the beneficial effects of DFM on indigenous guinea fowls raised on-station.

MATERIALS AND METHODS

Study Location

The experiment was carried out at the Poultry Section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology, Kumasi. The area is located within the semi-deciduous humid forest zone of Ghana. The zone is characterized by a bimodal rainfall pattern with an annual rainfall averaging around 1400mm (unpublished meteorological data, Department of Animal Science, KNUST). Daily temperatures range from 20^0C to 35^0C with a mean of 26^0C. The relative humidity varies from 97% during the early morning in the wet season to as low as 20% during the late afternoon in the dry season.

Ethical Approval

The procedures reported in this study were approved by the Animal Ethics Committee of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Direct Fed Microbial (DFM)

The DFM (commercial name: RE3) used in this study is a product of Basic Environmental Systems and Technology Inc. (Canada) and contains lactobacilli ($1x10^8$ cfu/g), bacillus ($1x10^{12}$ cfu/g) and *Saccharomyces cerevisiae* (yeast, $1x10^5$ cfu/g).

Experimental Design and Duration of the Experiment

Two hundred and sixteen (216) nine-week-old indigenous guinea fowls were randomly allotted to four dietary treatments with three (3) replications each in a completely randomised design. Each replicate was made up of 18 keets consisting of 15 females and 3 males with a mean weight of 510g. The control diet (T1) contained no DFM while Diets 2, 3

and 4 contained DFM at the respective rates of 1ml per 1kg, 1.5 ml/kg and 2.0 ml/kg diet.

The trial consisted of two phases (grower and layer). The grower phase began when the keets were 9 weeks old and ended at 20 weeks while the layer phase was between 20 and 40 weeks. Feed and water were also provided *ad libitum*. The composition and nutrient contents of the diets are shown in Table 1.

Table 1: Composition and calculated nutrient contents of experimental diets

Ingredients	Quantity (g/100g)	
	Grower diet	Layer diet
Maize	63	65
Wheatbran	22.5	26
Soybean meal	6	3
Fishmeal	7	3
Oyster shell	0.3	1.6
Dicalcium Phosphate	0.4	0.6
*Vitamin-mineral premix	0.4	0.6
Table salt	0.4	0.2
Total	100	100
Calculated nutrient content (%) except for ME (MJ /kg)		
Crude protein	18	15.2
Crude fibre	4.3	4.7
Ether extract	3.46	3.5
Calcium	0.5	0.7
Available phosphorus	0.6	0.8
Lysine	0.8	0.6
Methionine	0.3	0.3
Cystine	0.19	0.18
Metabolisable energy (MJ/Kg)	12.3	12.3

*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; B_1 200 mg B_2, 900 mg; B_{12}, 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, 4mg

Parameters studied

During the grower phase, the data collected included feed intake, growth rate, and feed conversion ratio; during the reproductive phase, however, data were collected on age and weight at first egg and live weight at end of the trial, egg production rate, egg weight and internal egg quality, fertility and hatchability of eggs and blood profile.

Blood profile studies

A total of 24 guinea fowl females, two per replicate, were randomly selected and 5ml blood samples were collected from the jugular vein of each bird into anticoagulant heparin bottles. The samples were analysed for total red blood cells (RBC), haemoglobin (HB), packed cell volume (PCV) and white blood cells (WBC) using Sysmex- 2IN Auto Analyser (Sysmex Corporation, Kobe, Japan) and the biochemical factors with Flexor Junior auto-analyser (Vital Scientific, Spankeren, Netherlands).

Egg Production, Weight, and internal egg quality

Eggs were collected twice daily: in the morning (9 h local time) and afternoon (15 h) to determine hen-day and hen-housed production. Eggs were weighed individually using an Ohaus electronic balance (Ohaus Scout STX123 120gx 0.001g, Parsippany, NJ.). Internal quality traits of the eggs were determined at the start, during and at the end of the laying period. Three eggs from each replicate were randomly selected and numbered. The width and the shell length of the eggs were measured using a tripod micrometre (Focus Technology, Boston, USA). Each egg was broken along the narrow end of the shell and the albumen and yolk separated into pre-weighed glass plates and subsequently weighed by means of an Ohaus electronic balance. The albumen and yolk heights were determined using a spherometer (Osaw Industrial Products, Haryana, India). The yolk colour was determined by comparison with the Roche colour fan (Hoffmann-La Roche, Switzerland). Egg Shape Index was determined according to the procedure outlined by Reddy *et al*. (1979). Egg shell thickness (mm) was calculated as the average of measurements at the broad, narrow and mid sections of the shell after any sticking albumen was removed by hand after the shells were washed under gently flowing water. The washed shells were left to dry in the open air for 24 hours.

Fertility and hatchability of eggs

Thirty eggs were selected per treatment (10 per replicate) for hatchability studies. The eggs were collected during the 25th week of the trial. The selected eggs had mean weight of 29.31g. Parameters measured were percent fertility and percent hatchability of fertile and total eggs set.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using Genstat (2012). Where there was a statistical ($P<0.05$) difference between means, the least significant (LSD) difference method was used to separate them.

RESULTS AND DISCUSSION

Grower Phase

Feed intake was not influenced significantly ($P>0.05$) by the addition of DFM (Table 2). The initial body weights of pullets did not differ for the treatment groups (mean of 510g/bird) (Table 2). However, birds on DFM diets weighed significantly ($P< 0.05$) heavier than their counterparts on the control diet (mean of 1.45 kg versus 1.36 kg respectively). The highest daily weight gain ($P<0.05$) was registered by pullets on the 1.5 ml DFM /kg diet. These results compare with those of Torres- Rodriguez *et al*., (2007) who observed increased average daily gain and market body weight of turkeys fed DFM (FM-B11). In a similar trial using exotic guinea keets imported from the Netherlands, Yeboah *et al* (2020) reported that only the highest inclusion rate of RE3, 2 ml/kg, had a significant positive effect on growth. The addition of DFM did not have any significant ($P > 0.05$) impact on feed conversion ratio (FCR). Aghaii *et al. (*2010) working with laying Hyline W-36 strains

reported significantly improved feed utilization efficiency with probiotic supplementation (2.03g/g, against 2.263g/g for the control). Aghaii *et al* (2010) however, used a commercial probiotic, Bioplus 2B, containing *Bacillus subtilis* and *Bacillus licheniformis* with a minimum of $3.2x10^9$ cfu g^{-1} and it was added at 0, 1000 and 2000 g ton^{-1} of feed.

Table 2: The effects of DFM on growth performance of indigenous Guinea fowl pullets

Parameters	Treatments				SEM	P-value
	Control	1ml DFM	1.5ml DFM	2.0ml DFM		
Initial body weight (g)	510	510	510	510	0.07	0.89
Body weight @ 19 weeks(kg)	1.26	1.28	1.33	1.31	0.01	0.001
Average daily weight gain (g)	10.11	10.33	11.04	10.73	0.13	0.004
Average daily feed intake (g)	69.9	69.5	69.9	69.0	0.02	0.276
Feed to gain ratio	6.91	6.73	6.33	6.42	0.10	0.168

a-c Values within the same row followed by different subscripts differ significantly ($P < 0.05$); SEM = Standard Error of Means. P= Probability values

Table 3 presents blood parameters of pullets as influenced by DFM. DFM had no significant ($P>0.05$) influence on serum total protein, albumin and globulin contents of growing indigenous guinea fowls although birds on the 1ml DFM/kg diet recorded the highest values.

Alkhalf *et al*. (2010) previously showed that a commercial DFM (Bactocell) did not affect ($P>0.05$) total protein, lipids and albumin concentrations. At all levels of DFM, albumin content was higher than the corresponding globulin content. Feng *et al*. (2002) have stated that albumin-to-globulin ratios greater than unity are normally associated with better antibody production. Packed cell volume did not significantly vary ($P>0.05$) although birds on the control diet tended to have numerically higher values. Mohan *et al*. (1996) observed that packed cell volume did not show any variation as a result of DFM supplementation. Total serum cholesterol values showed no significant influence due to DFM ($P>0.05$). Droualt *et al*. (2002), however, observed a significant reduction in cholesterol and triglycerides concentrations after feeding a probiotic containing *Lactobacillus spp. Si*milarly, haemoglobin concentration, red and white blood cell counts did not appear to be affected by DFM ($P> 0.05$).

Table 3. Mean blood values of growing indigenous female guinea fowls

Blood Parameters	Treatments				SEM	P-value
	Control	1ml DFM	1.5ml DFM	2.0ml DFM		
Total protein (g/l)	54.1	62.2	60.1	52.8	3.51	0.245
Globulin (g/l)	22.56	25.86	24.22	21.44	1.02	0.090
Albumin (g/l)	29.2	37.2	35.9	29.1	2.45	0.064
Total cholesterol (mmol/l)	4.66	4.54	4.80	3.84	0.54	0.591
Haemoglobin (g/dl)	14.43	14.48	14.56	14.98	0.52	0.880
Haematocrit (PVC) %	47.17	38.31	38.31	41.56	2.56	0.880
Red blood cells ($x10^{12}$/1)	2.33	2.38	2.18	2.33	0.06	0.189
White blood cells($x10^{9}$/l)	314.6	315.7	311.1	317.4	3.82	0.7

*(P < 0.05) SEM- Standard Error of Means. P- Probability values

Semen quality traits of experimental birds are presented in Table 4. DFM had no significant (P > 0.05) effects on semen colour, sperm concentration, sperm motility, viable sperm count and dead sperm. However, there was a significant (P < 0.05) improvement in semen volume of birds fed diets containing DFM. El-Deep *et al.* (2012) reported that semen volume was significantly increased by 29.1 and 21.9% in cocks of a local Egyptian chicken strain fed DFM diets compared with the control.

Table: 4. Semen quality parameters of guinea fowl cockerels as influenced by DFM

Parameters	Treatments				SEM	P-value
	Control	1ml DFM	1.5ml DFM	2.0ml DFM		
Semen volume (µl)	17^{a}	21^{bc}	19b	22^{bc}	0.76	0.007
Motility (%)	80	82	79	80	5.20	0.98
Viable count (%)	76	78	70	78	5.77	0.737
Dead sperm (%)	24	22	30	22	5.77	0.737
Sperm concentration (10^{9}cell/ml)	1.41	1.41	1.39	1.39	0.03	0.938

a-b Values within the same row followed by different subscripts differ significantly (P < 0.05) SEM- Standard Error of Means. P- Probability values.

Layer phase

Table 5 presents a summary of laying performance. As with the pullets, the feeding of DFM significantly (P < 0.05) enhanced growth with DFM birds recording mean final live weights between 1.45 and 1.46 kg as against 1.28 kg for the control birds. Similar results were reported in turkeys when they were fed diets containing the DFM called FM=B11 (Torres-Rodriguez *et al*., 2007). DFM had no significant effect on (P>0.05) either total or daily feed intake which agrees with

the report of Goh and Hwang (1999) although their work was with broilers.

The feeding of DFM increased the efficiency of feed conversion to eggs (P<0.05) with fowls on the 2.5 ml DFM per kg diet birds being almost twice as efficient as the control birds. Similar observations were made by Aghaii *et al*. (2010) in laying White Leghorn and Hy-Line hens.

Kalsum *et al.* (2012) reported that DFM supplementation improved egg production by up to 9.5% in Japanese quails. DFM at 1.5ml and 2.0ml/kg feed significantly improved egg weights (31.4g and 33,0 g) respectively compared to eggs produced by fowls on either control or 1.0 ml DFM (P < 0.05). Bonsu *et al.* (2014) recorded similar results. DFM exerted no significant (P>0.05) influence on egg shell thickness, egg yolk height, egg yolk colour, egg yolk weight and egg albumin weight. This observation agrees with Kalsum *et al*. (2012) and Bonsu *et al*. (2014) who reported that DFM had no effect on egg quality.

Table: 5. Effects of DFM on laying performance of indigenous guinea fowls

Parameters	Treatments				SEM	P-value
	Control	1ml DFM	1.5ml DFM	2.0ml DFM		
Initial body weight (kg)	1.26^{a}	1.28^{b}	1.33^{d}	1.31^{c}	0.01	0.001
Final body weight (kg)	1.36^{a}	1.46^{b}	1.45^{b}	1.45^{b}	0.58	0.003
Average daily feed intake (g)	77.81	75.95	75.56	75.46	0.68	2.23
Average total feed intake (kg)	10.89	10.63	10.58	10.56	0.1	0.126
Kg feed per kg egg	2.26^{c}	1.81^{b}	1.9^{bc}	1.28^{a}	0.1	0.004
Hen-day production (%)	16.3	20.2	17.8	28.1	1.93	0.183
Hen-housed production (%)	16.0	20.2	17.7	27.9	1.96	0.168
Total egg production by treatment (Numbers)	303	381	335	527	67.1	0.168
Shell thickness (mm)	0.7	1	0.8	0.62	0.13	0.356
Egg yolk weight (g)	10.33a	10.31	11.28	10.28	1.21	0.187
Egg yolk height (mm)	9	9.55	9.84	8.99	0.297	0.187
Egg albumin weight (g)	15.10	14.51	16.90	16.2	4.46	0.793
Egg yolk colour	8.5	8.67	8.57	8.6	0.12	0.793

*a-c Values within the same row followed by different subscripts differ significantly (P < 0.05). FCR-feed conversion ratio; SEM- Standard Error of Means. P- Probability values

DFM had varying effects on blood values of guinea fowls (Table 6). The administration of DFM significantly (P < 0.05) increased both total protein and globulin content but not albumin although the latter tended to increase as DFM levels increased. While DFM apparently did not affect the levels of haemoglobin, WBC, RBC and PCV contents, all tended to increase with increases in dietary DFM. In contrast, Priya and Babu (2012) found that supplementing broiler diets with *Saccharomyces cerevisiae* increased total protein, albumin, globulin, haemoglobin, red blood cells and pack cell volume.

Serum cholesterol of laying guinea fowls was not significantly affected by DFM levels. Blood or serum cholesterol levels have been shown to be significantly reduced by supplementation of diets with DFM (Bonsu *et al*. 2014; Priya and Babu, 2012).

Table 6. DFM effects on blood values of laying indigenous guinea fowls

Blood Parameters	Treatments				SEM	P-value
	Control	1ml DFM	1.5ml DFM	2.0ml DFM		
Total protein (g/l)	54.3^{a}	63.8^{b}	68.8^{bc}	68.8^{b}c	2.75	0.018
Globulin (g/l)	23.33^{a}	28.83^{b}	31.83^{b}	31^{b}	1.06	0.002
Albumin (g/l)	31	35.67	37	37.83	1.64	0.071
Total cholesterol (mmol/l)	4.43	4.83	4.50	4.98	0.19	0.217
Haemoglobin (g/dl)	13.90	14.3	15.13	14.08	0.32	0.080
Haematocrit (PVC) %	43.43	46.13	43.03	43.52	0.32	0.184
Red blood cells (x10^{12}/1)	1.87	2.02	2.02	2.15	0.11	0.380
White blood cells(x10^{9}/l)	265.5	273.8	274.5	274.3	3.02	0.548

a-b Values within the same row followed by different subscripts differ significantly (P < 0.05) SEM-Standard Error of Means. P- Probability values

The summary of the reproductive performance is shown in Table 7. DFM significantly hastened (P<0.05) the age at first egg but had little effect (P > 0.05) on weight of first egg and live weight at first egg. Ezema *et al*. (2012) fed chicken pullets palm kernel cake-based diets supplemented with DFM and observed a reduction in the age of onset of egg laying from 22 weeks in control birds to 18 weeks in pullets fed DFM without affecting egg weight and body weight at first egg. Fertility and hatchability of eggs set were significantly enhanced by both 1ml and 2ml of DFM/kg diet but not DFM at 1.5 ml per kg diet (P>0.05). Percent hatchability of fertile eggs was increased at all levels of DFM (P<0.05), a result that is similar to that of El-Deep *et al*. (2012) that DFM improved the fertility and hatchability percentages of chicks fed diet with different levels of DFM. The hatchability and fertility values from this study compared favourably with those reported by Atawalna *et al.,* (2020) for 50.7% and 30.5% for fertility and hatchability respectively. Adu-Aboagye *et al*., (2020) in a related study also reported a fertility rate of 56.9% which affirms the fertility values reported in this study.

Table 7. Reproductive performance of female indigenous guinea fowls as affected by different levels of DFM.

Parameters	Treatments				SEM	P-value
	Control	1ml/ DFM	1.5ml/ DFM	2.0ml/ DFM		
Age at first egg (days)	140	126^{b}	126^{b}	126^{b}	4.20	0.001
Mean liveweight at first egg (kg)	1.26	1.27	1.26	1.27	0.01	0.482
Weight of first egg (g)	25.96	26.2	27.65	25.63	1.71	0.884
Fertility of eggs (%)	51.44^{b}	51.63^{b}	48.03^{a}	52.71^{c}	0.91	0.001
Hatchability of eggs set (%)	44.9^{b}	46.32^{c}	42.51^{a}	47.38^{cd}	1.3	0.001
Hatchability of fertile eggs (%)	84.69^{a}	87.11^{c}	85.71^{b}	85.71^{b}	0.92	0.001

*a-c Values within the same row followed by different subscripts differ significantly ($P < 0.05$), SEM- Standard Error of Means. P- Probability values

Table 8 is a summary of the carcass parameters. Bled weight, de-feathered weight, shank weight, neck weight, liver weight, heart weight, full gizzard weight, empty gizzard and full intestine weight expressed as a percent of pre-slaughter body weight were all higher ($P < 0.05$) in birds fed the control diet. The abdominal fat content significantly declined progressively ($P< 0.05$) as DFM amounts increased; however, at the highest inclusion rate of DFM (2ml/kg), abdominal fat decreased almost 40% compared to the control. Paryad and Mahmoudi (2008) have also reported a decline in abdominal fat resulting from the consumption of diets containing DFM.

Table: 8. Carcass characteristics of indigenous guinea fowl

Parameters	Treatments				SEM	P-value
	Control	1ml/ DFM	1.5ml/ DFM	2.0ml/ DFM		
Live weight (g)	1350^{a}	1520^{d}	1440^{b}	1470^{c}	0.58	0.018
Bled weight (g)	99.16^{d}	96.71^{b}	97.22^{c}	95.92^{a}	0.82	0.001
Defeathered weight (g)	97.0^{4}	94.08^{c}	91.67^{b}	88.44^{a}	0.58	0.001
Dressed weight (g)	82.22^{c}	80.92^{a}	83.33^{d}	81.63^{b}	0.58	0.001
Shank weight (g)	2.47^{b}	2.27^{a}	2.28^{a}	2.31^{ab}	0.29	0.001
Neck weight (g)	5.76^{b}	5.36^{a}	5.82^{b}	5.39^{a}	0.58	0.001
Liver weight (g)	1.68^{b}	1.63^{b}	1.43^{a}	1.39^{a}	0.05	0.001
Head weight (g)	3.24^{d}	2.94^{a}	3.18^{c}	3.03^{b}	0.05	0.001
Heart weight (%)	0.61^{c}	0.51^{a}	0.52^{b}	0.52^{b}	0.06	0.001
Full gizzard weight (g)	3.01^{b}	2.94^{ab}	2.72^{a}	2.90^{a}	0.04	0.001
Empty gizzard (g)	3.22^{c}	2.32^{a}	2.34^{a}	2.54^{b}	0.03	0.001

Full intestine (g)	4.01[c]	4.36[d]	3.16[a]	3.39[b]	0.06	0.001
Empty intestine (g)	2.65[a]	3.08[b]	2.64[a]	2.86[ab]	1.16	0.014
Abdominal fat (g)	1.58[b]	1.54[b]	1.50[b]	0.99[a]	0.87	0.001

a-d Values within the same row followed by different subscripts differ significantly, SEM-Standard Error of Means, P- Probability values.

CONCLUSION AND RECOMMENDATION

The addition of 1.5ml/kg DFM to the diet of indigenous guinea fowls improved productive performance and 2ml improved both productive and reproductive performances. It is therefore recommended that guinea fowl farmers intending to use DFM (RE3) should include it at the rate of between 1.5 and 2.0 ml per kilogram of diet.

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