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Chelated Copper, Zinc and Manganese Improved Performance, Haematological and Serum Biochemical Indices of Layers (late-lay)

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ABSTRACT
The growth performance, haematological and serum biochemical indices of laying hens (late lay) fed diets supplemented with inorganic or chelated blends of copper, zinc and manganese were evaluated. A 77-day feeding trial was carried out using 540 (58 weeks old) Nera Black Hens. Birds were randomly allocated to five dietary treatment groups with 12 replicates of 9 birds each. The diets consisted of control (0, 0 and 0 mg kg^{-1} of Cu, Zn and Mn respectively), inorganic trace minerals (ITM) supplementation at 16, 64 and 64 mg kg^{-1} of Cu, Zn and Mn, chelated trace mineral (CTM) supplementation at 16, 64 and 64 mg kg^{-1} of Cu, Zn and Mn (100 % CTM), CTM supplementation at 8, 32 and 32 mg kg^{-1} of Cu, Zn and Mn (50 % CTM) and CTM supplementation at 4, 16 and 16 mg kg^{-1} of Cu, Zn and Mn (25 % CTM) in that order. On day 77 of the experiment, 2.5 mL of blood was collected individually from 4 birds per replicate via brachial vein puncture and transferred to sample tubes containing EDTA for hematological analyses. Another 2.5 mL of blood was collected into sample tubes for serum biochemical analyses. Data collected were subjected to One-Way Analysis of Variance with 5 % significance in a Completely Randomized Design. Feed intake was increased (p<0.05) with ITM supplementation while 50 % CTM and 100 % CTM reduced feed intake (117.29 and 116.18 g/b/d respectively). Kg feed/kg egg was better for diets supplemented with CTM. CTM supplementation at 50 and 100% increased (p<0.05) packed cell volume (PCV) while white blood cell count was similar (p>0.05) across all treatment groups. Supplementation of CTM led to a higher (p<0.05) total serum protein and albumin. Globulin, creatinine and ALT were not affected (p>0.05) while 100% CTM increased AST (153.45 IU/L). ITM supplementation resulted in increased (p<0.05) cholesterol (192.35 mg/dL) and LDL (140.47 mg/dL) levels in the sera while CTM supplementation increased (p>0.05) HDL. This study revealed that CTM supplementation improved kg feed/kg egg, PCV, total serum protein and albumin of layers in late lay.
Keywords: Chelated trace minerals, haematology, inorganic salts, performance, serum biochemistry

Introduction
Copper, zinc and manganese are involved in several metabolic and physiological processes required for normal functioning of the animal. Chelates may improve performance and blood parameters of animals because they move to the site of absorption without any chemical interference due to their electrically neutral nature (Liu *et al*., 2016). This enhances nutrient

absorption and utilization by the animals. The physiological response of animals to nutrition is depicted by their haematological and serum biochemical indices (Madubuike and Ekenyem, 2006). Hence, this study evaluated the performance, haematological and serum biochemical indices of laying hens (late-lay) fed diets supplemented with inorganic or chelated blends of copper, zinc and manganese

Material and Methods

This study was carried out according to the research ethics and guidelines of the Animal Care and Review Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria. A 77-day feeding trial was carried out using 540 (58 weeks old) Nera Black Hens. Birds were housed in battery cages and randomly allocated to five dietary groups with 12 replicates of 9 birds each. The treatments are displayed in Table 1. The cages were equipped with nipple drinkers and trough feeders. Feed conversion ratio was calculated using data obtained from egg production (collected daily) and feed consumption (determined every 2 weeks with the exception of initial 7 days of adaptation period). On day 77 of the experiment, 2.5 mL of blood was collected individually from four (4) birds per replicate via brachial vein puncture and transferred to sample tubes containing EDTA for hematological analyses. Another 2.5 mL of blood was collected into sample tubes for serum biochemical analyses. Data collected were subjected to One-Way Analysis of Variance with 5 % significance in a Completely Randomized Design.

Table 1: Treatments with level of trace mineral supplementation

Treatment	Composition
Control	Basal Diet (**BD**) – 0 mg kg^{-1} of supplemental Cu, Zn and Mn
100% Inorganic Trace Mineral (ITM) supplementation	BD + 16, 6 4 and 6 4 mg kg^{-1} of inorganic Cu, Zn and Mn respectively
100% Chelated Trace Mineral (CTM) supplementation	BD + 16, 6 4 and 6 4 mg kg^{-1} of chelated Cu, Zn and Mn respectively
50% Chelated Trace Mineral (CTM) supplementation	BD + 8, 3 2 and 3 2 mg kg^{-1} of chelated Cu, Zn and Mn respectively
25% Chelated Trace Mineral (CTM) supplementation	BD + 4, 1 6 and 1 6 mg kg^{-1} of chelated Cu, Zn and Mn respectively

Results and Discussion

Performance characteristics of laying chickens fed supplemental trace minerals is presented in Table 2. Feed intake was increased ($p<0.05$) with ITM supplementation while 50 % CTM and 100 % CTM reduced feed intake and the Kg feed/kg egg (FCR) was better for diets supplemented with CTM. The reduced feed intake and better FCR observed in groups fed diet supplemented with CTM in this study may be due to a more balanced amino acid profile as a result of the organic ligands to which the trace minerals were chelated. This implies that the diet was able to meet up with the nutrient requirements of the birds faster than the inorganic sources and control groups, which might have elicited the better FCR. This agrees with the work of Salami *et al*. (2016) who reported an improved FCR in turkeys fed diets supplemented with chelated trace mineral blend even with reduced feed intake.

Table 2: Effect of supplemental inorganic and organic trace minerals on the Performance characteristics of laying chickens.

Parameters	Control	100% ITM	100 % CTM	50 % CTM	25 % CTM	SEM	P value
Initial Weight (g/bird)	1668.96	1735.39	1698.00	1696.43	1735.40	12.69	0.4700
Feed intake (g/bird/day)	118.63^{b}	120.04^{a}	116.18^{d}	117.29^{c}	118.81^{b}	0.18	0.0001
FCR (kg feed/kg egg)	2.65^{c}	2.47^{bc}	2.06^{a}	2.17^{a}	2.44^{b}	0.04	0.0001

^{a,b,c,d} Means with different superscripts in a row are significantly (P<0.05) different

The effect of supplemental inorganic and organic trace minerals on the haematological and serum biochemical indices of laying chickens is displayed in Table 3 and 4 respectively. Chelated trace mineral supplementation at 50 and 100 % increased (p<0.05) packed cell volume (PCV) while white blood cell count was similar (p>0.05) across all treatment groups. The higher PCV value in groups fed diet supplemented with 100% CTM showed that red blood cell occupies a higher proportion of the blood volume in this groups which would have aided oxygenation of cells responsible for laying. This is in accordance with the report of Wikihow (2013).

Supplementation of CTM led to a higher (p<0.05) total serum protein and albumin. Globulin, creatinine and ALT were not affected (p>0.05) while 100% CTM increased AST (153.45 IU/L). The increased serum total protein in groups fed diet supplemented with CTM could be attributed to the positive influence of the amino acid (Methionine Hydroxyl Analogue; a precursor of methionine) ligand in which the chelated trace minerals (Cu, Zn and Mn) were bound to. As a result, higher concentrations of free amino acids must have been present in the blood which would have stimulated protein synthesis in the blood. This would have ensured a better nutrient utilization for groups fed chelated (amino acid) trace minerals as compared to groups without chelates supplementation. This is in consonance with the work of Zhai *et al*. (2016) who reported a higher free amino acid in the blood of chicken when additional lysine and methionine were included in the diets of chicken. Improved serum albumin in groups fed 100% CTM as compared to the control group could be as a result of increased protein synthesis in the liver resulting from a more balanced amino acid profile. Albumin is known to be the major protein secreted by the liver and it helps in maintaining intravascular colloid osmotic pressure, neutralizes toxins and transport therapeutic agents (Call, 2015). This suggests that supplementation of CTM at 100% may have capability to improve the health status of the birds which is subject to confirmation in subsequent studies.

Inorganic trace minerals supplementation resulted in increased (p<0.05) cholesterol (192.35 mg/dL) and LDL (140.47 mg/dL) levels in the sera while CTM supplementation increased (p>0.05) HDL. The improvement observed in the serum AST of birds fed chelated trace minerals as compared to other treatment groups can be as a result of the complimentary amino acids obtained from the which increased the serum protein and hence, stimulated the serum enzymes.

Table 3: Effect of supplemental inorganic and organic trace minerals on the Haematological parameters of laying chickens

Parameters	Control	100% ITM	100 % CTM	50 % CTM	25 % CTM	SEM	P value
PCV (%)	27.67^{b}	29.33^{ab}	32.00^{a}	31.33^{a}	29.33^{ab}	0.50	0.023
HB (g/dL)	8.93	11.30	10.70	10.53	9.77	0.35	0.056
RBC (10^{6}/μL)	2.50	2.88	2.89	2.86	2.70	0.07	0.055
WBC (x10^{6}/μL)	13.27	12.80	10.43	10.77	10.97	0.42	0.071

^{a,b} Means with different superscripts in a row are significantly (P<0.05) different

Table 4: Effect of supplemental inorganic and organic trace minerals on the Serum biochemistry of laying chickens

Parameters	Control	100% ITM	100 % CTM	50 % CTM	25 % CTM	SEM	P value
Protein (g/L)	61.17^{c}	61.77^{c}	73.47^{a}	71.13^{ab}	66.40^{bc}	1.41	0.001
Albumin (g/L)	33.13^{b}	36.87^{ab}	40.93^{a}	39.33^{ab}	35.70^{ab}	0.89	0.001
Globulin (g/L)	28.03	24.90	32.53	31.80	30.70	1.00	0.113
Creatinine (mg/dL)	1.17	1.33	1.53	1.53	1.47	0.06	0.211
AST (IU/L)	134.17^{b}	132.97^{b}	153.45^{a}	152.27^{a}	149.97^{ab}	2.80	0.001
ALT (IU/L)	14.33	15.67	15.47	15.53	15.47	0.64	0.172
Cholesterol	138.07^{b}	192.35^{a}	151.17^{ab}	179.40 ^{ab}	172.63 ^{ab}	6.62	0.001
Triglycerides	104.33	106.57	105.33	108.17	103.83	2.73	0.060
HDL	23.91^{c}	30.57^{bc}	31.93^{bc}	40.47^{ab}	35.93^{ab}	1.62	0.001
LDL	93.30^{b}	140.47^{a}	98.17^{b}	117.30^{ab}	115.93^{b}	5.52	0.001

^{a,b,c,d,e} Means with different superscript in a row are significantly ($P<0.05$) different

Conclusions and Outlook

Chelated Trace Mineral supplementation improved FCR (kg feed/kg egg) of layers in late lay. Chelated Trace Mineral supplementation at 100% and 50% increased PCV and total serum protein of layers in late lay while albumin was enhanced with 100% CTM supplementation.

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