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Influence of Level of Dietary Inorganic and Organic Copper and Energy Level on the Performance and Nutrient Utilization of Broiler Chickens

T. K. Das, M. K. Mondal, P. Biswas*, B. Bairagi and C. C. Samanta

Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, 37 Kshudiram Bose Sarani, Kolkata- 700 037, India

ABSTRACT : An experiment was conducted to determine the influence of dietary inorganic (copper sulfate) and organic (copper proteinate) forms of copper and energy level on performance and nutrient utilization of broiler chickens. Two hundred day-old commercial Vencobb broiler chicks were purchased and randomly distributed to 20 cages of 10 birds each. These replicates were randomly assigned to one of five treatments in a ((2×2)+1) factorial arrangement. These two factors were sources of Cu ($CuSO_4$ vs. Cu-proteinate) and dose of Cu supplements (200 mg and 400 mg/kg dietary dry matter) and the control (no supplemental Cu). After the starter period (up to 3 weeks), from d 22 onwards another factor i.e. energy at two levels (2,900 vs. 2,920 kcal/kg diet) was introduced with the previous factorial arrangements by subdividing each replicate into two equal parts, for two energy levels, without disturbing the dose and source of Cu supplement. Cu-salt supplementation linearly increased ($p<0.01$) live weight (LW), live weight gain (LWG) and feed conversion ratio (FCR) at 3 weeks, whereas cumulative feed intake (CFI) was unaffected ($p>0.05$). LWG and FCR were higher ($p<0.01$) in Cu-proteinate supplemented birds compared to $CuSO_4$ supplementation. A linear dose response ($p<0.01$) of Cu was found for the performance of broiler chickens. Birds having a higher energy level in the finisher stage increased ($p<0.01$) LWG and FCR. Cumulative feed intake was similar ($p>0.05$) across the groups up to the 5^{th} week. Cu-proteinate increased performance of broiler chickens compared to $CuSO_4$ Dose of supplemental Cu-salt irrespective of source showed a linear response ($p<0.01$) for performance. Supplementation of Cu-proteinate increased metabolizability of DM ($p<0.01$), NFE ($p<0.05$), total carbohydrate ($p<0.01$) and OM ($p<0.01$) at the starter period. Increased dose of Cu-salt linearly increased ($p<0.01$) metabolizability of DM, CP, CF, NFE and OM. Higher energy level in the diet improved DM ($p<0.05$), EE ($p<0.01$), NFE ($p = 0.01$), total carbohydrate ($p<0.01$) and OM ($p<0.01$) metabolizability. Cu-proteinate supplementation showed better nutrient utilization compared to $CuSO_4$. Dose of Cu linearly increased DM, CP, EE, NFE, total carbohydrate and OM metabolizability. CF metabolizability was unaffected ($p>0.05$) among the treatments. In conclusion, dietary supplementation of Cu-salt more than the requirement may improve performance and nutrient utilization in broiler chickens even with a high energy finisher diet. Cu-proteinate showed better performance and nutrient utilization compared to $CuSO_4$.
(**Key Words** : Copper Proteinate, Energy, Performance, Nutrient Utilization, Broiler Chicken)

INTRODUCTION

The essentiality of copper for poultry and livestock is well documented (Davis and Mertz, 1987). Cu is an essential mineral which serves as co-factor in many enzyme systems in the body. Cu-sulfate ($CuSO_4 5H_2O$) is the most commonly used dietary Cu supplement. Copper in the form of Cu-sulfate improves growth rate and feed efficiency in broilers (Choi and Paik, 1989; Baker et al., 1991) and in pigs (Roof and Mahan, 1982; Edmonds et al., 1985; Cromwell et al., 1989) at supernormal level (125 to 250 mg/kg). Growth promoting effect of dietary Cu has been attributed to its antimicrobial action (Fuller at el., 1960; Bunch et al., 1961; Burnell et al., 1988). Improved availability of Cu from organic Cu complexes compared with the commonly used Cu salts recently has been suggested. Chelates, complexes or proteinates are the organic form of Cu and are usually considered for use in animal diet as alternatives to inorganic Cu source. More bioavailability of Cu is probably due to better absorption, which enhances its efficiency (Downs et al., 2000; Yu et al., 2000; Guo et al., 2001). Baker and Ammerman (1995) reported that relative bioavailability estimate of organic Cu sources ranged from 88% to 147% of the response to cupric sulfate in poultry, Swine, sheep and cattle. Improvements in the digestibility of proteins (Braude, 1965; Castell and Bowland, 1968) and retention of nitrogen (Braude, 1965)

* Corresponding Author: P. Biswas. Tel: +91-33-2550-2229, Fax: +91-33-2557-1986, E-mail: maicrp@yahoo.com
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Table 1. Composition of basal diets

Ingredients	Composition		
	Broiler starter	Broiler finisher	
		High energy	Low energy
Ingredients (% air dry basis)			
Ground maize	53.0	62.5	48.0
Soybean meal	40.0	31.5	32.0
Deoiled rice bran	3.0	2.0	11.96
Di-calcium phosphate	1.8	1.8	1.8
Limestone powder	1.2	1.2	1.2
Soybean oil	-	-	4.0
Trace mineral mixture[a]	0.10	0.10	0.10
Iodized Salt	0.30	0.29	0.29
Ventrimix[b]	0.02	0.03	0.03
Ventribee plus[c]	0.04	0.04	0.04
Vitamin E (50%)	0.01	0.01	0.01
Choline chloride (60%)	0.05	0.10	0.10
Furazolidone	0.02	0.02	0.02
Livoliv	0.05	0.05	0.05
Maduramycin	0.05	0.05	0.05
Natuzyme[d]	0.03	0.03	0.03
$NaHCO_3$	0.10	0.10	0.10
L-lysine	0.05	0.03	0.03
DL-methionine	0.18	0.15	0.15

[a] Provided per kg of diet: Zn, 60 mg; Mn, 90 mg; Fe, 110 mg; KI, 2.5 mg.
[b] Each gm contains: vitamin A, 82,500 IU; vitamin B_2, 50 mg; vitamin D_3, 12,000 IU; vitamin K, 10 mg.
[c] Each gm contains: vitamin B_1, 4 mg; vitamin B_6, 8 mg; vitamin B_{12}, 40 mg; vitamin E, 40 mg; calcium-D-pantothenate, 40 mg; niacin, 60 mg.
[d] Each kg contains: cellulase, 6,000,000 U; Xylanase, 10,000,000 U; β-Glucanase, 700,000 U; α-Amylase, 700,000 U; protease, 3,000,000 U; pectinase, 70,000 U; phytase, 40,000,000 U.

Table 2. Chemical composition, calculated

Chemical composition (DM basis)[1]	Broiler starter	Broiler finisher	
		High energy	Low energy
ME (kcal/kg)[2]	2,803	2,898	2,920
Dry matter (%)	89.45	90.54	90.74
Crude protein (%)	21.72	19.18	19.21
Crude fat (%)	2.88	2.74	6.79
Crude fibre (%)	4.11	4.16	4.62
Ash (%)	8.42	8.21	8.44
Nitrogen free extract (%)	62.87	65.71	60.94
Organic matter (%)	91.58	91.79	91.56
Calcium (%)	0.98	0.96	1.02
Total phosphorus (%)	0.61	0.62	0.65
Mg (%)	1.91	1.95	1.98
Cu (ppm)	10.57	10.94	10.79
Zn (ppm)	81.72	82.01	81.81
Mn (ppm)	110	117	121
Fe (ppm)	296	312	316
Methionine (%)[2]	0.54	0.48	0.48
Methionine+cystine (%)[2]	0.68	0.60	0.61
Lysine (%)[2]	1.22	1.01	1.02
Available phosphorus (%)[2]	0.512	0.493	0.528

[1] Assayed values.
[2] Calculated on the basis of standard values applicable under Indian Condition (Singh and Panda, 1996).

have been reported in young pigs fed diet containing added Cu. Studies by Dove and Haydon (1992) and Dove (1995) have indicated that addition of 250 mg Cu/kg improved digestibility and utilization of the fat of weaned pigs, but results have limited and inconclusive for chickens when Cu added to the feed fortified with oil.

To increase the energy level in the finisher diet and to obtain higher weight of a broiler chicken, oil and fat are used because of its high energy content. Soybean oil which stimulates growth rate, when included in poultry diet (Nitsan et al., 1997) is most commonly used oil source in Indian condition. Unsaturated vegetable fats (like soybean oil) are more energetic than saturated animal fat. Therefore, the main objective of this study was to investigate the influence of level of dietary inorganic and organic forms of Cu and energy level on performance and nutrient utilization of broiler chicken.

MATERIALS AND METHODS

Birds and experimental design

A total of 200 d old Vencobb broiler chicks purchased from a commercial supplier was used in this experiment. The chicks were individually weighed and allocated to 20 cages of 10 birds each, so that each cage had birds with similar (p>0.5) average weight. These cages were randomly allotted to five treatments of four replicate in a ((2×2)+1) factorial arrangement. These two factors were source of Cu-salt (inorganic $CuSO_4$ vs. organic Cu-proteinate) and dose of Cu supplements (200 and 400 mg/kg dietary DM) and one control (no supplemental Cu). After starter period (0 to 3 weeks), from d 22 onwards each treatment were again subdivided into two, resulting 40 replicate with 4 birds each (2 birds slaughtered at d 21 from each replicate) for addition of soybean oil (SBO) at the rate of 4% of diet. Here the experimental design was converted to ((2×2×2)+2)) factorial arrangement, where the new factor was energy level (2,900 vs. 2,900 kcal/kg) with two controls having two different energy level.

Supplementation of SBO and graded dose of Cu in the diet

The starter and finisher diets of the experiment were formulated to meet or exceed the nutrient requirement as per Bureau of Indian Standard (1992). Ingredient and chemical composition of all the basal diets were presented in Table 1 and 2. Starter feed was given to the birds up to 3 weeks and finisher feed from 4 to 6 weeks. The starter and finisher diet were supplemented with 200 and 400 mg elemental Cu/kg dietary DM either as $CuSO_4$ ($CuSO_4 \cdot 5H_2O$; Merck Limited, Mumbai, India; MW: 249.68; Minimum assay 99%) or as Cu-proteinate (as Bioplex Copper supplied by Alltech Inc. Nicholasville, USA, MW:

<1,500 Da, minimum assay 15%). To prepare the graded dose of Cu both in starter and finisher diet, 0.785 g and 1.571 g of $CuSO_4$/kg feed and 1.333 g and 2.667 g of Cu-proteinate/kg feed was mixed with premix (required major and trace mineral without Cu) and subsequently prepared the complete diet. In the finisher diet SBO was added with the diet at 4% level to prepare diet with two energy levels (2,898 vs. 2,920 kcal/kg).

Housing and management

Chicks were housed in a battery type California cages which were cleaned thoroughly with formaldehyde and potassium permanganate solution three days prior to arrival of birds. The d old chicks were offered electrolyte solution upon arrival. Birds were maintained on a 24 h constant light schedule. The brooding temperature was maintained close to their requirement, first by heating device for 3 days following arrival of chicks. Then no additional heating was required as the summer room temperature was found appropriate up to 3 weeks and finally by turning cooler fan during day time for the last 3 weeks of rearing period. The birds were vaccinated against Ranikhet disease and Infectious Bursal Disease on d 7, 14 and 21 and provided antibiotic for the first 5 days as per recommendation.

Details of the feeding regimens

The chicks were offered maize soybean meal based diet (broiler starter and broiler finisher in mash form). These diets were formulated to meet or exceed the BIS (1992) nutritional requirement of broiler chicken. The diets were fortified with mineral and vitamin premix as per the standard stipulated by the Bureau of Indian Standard for broiler chickens (1992). Total amount of feed offered during 24 h to a replicate under a specific treatment groups was divided into 3 equal proportions. The amount and timing of feed was adjusted in such a way that the birds consume the whole of the diet offered at any one time. As a result hardly any residue can be obtained from the replicate after a days feeding. These ensured the consumption of the mineral elements that might have been precipitated in the dust portion of the feed.

Record keeping

Body weight was recorded at the initial day followed by at weekly interval upto 6 weeks before offering feed. Mortality was recorded as it occurred. Body weight gain and feed intake were obtained by calculation. Feed conversion ratio (FCR), feed intake to live weight of birds was recorded at weekly intervals. Separate records of feed intake of birds during metabolic trial were kept for last 3 days of starter (19-21 d) and finisher (40-42 d) period. Excreta were collected and recorded its weight.

Metabolism trial

Two metabolic trial of 3 days duration were conducted at the end of 3rd and 6th week of feeding trial. During the metabolic trial total amount of feed consumed and total amount of excreta voided from each replicate of the individual experimental group was quantified. The excreta from each replicate was collected in a previous weighed clean and dry petridish and was oven dried at 100±2°C for subsequent estimation of DM, organic nutrients. A part of weighed excreta from each replicate was preserved in concentrated H_2SO_4 (Minimum assay 98%) for estimation of nitrogen fraction.

Chemical analysis of feed and faeces samples

The dietary ingredients and faeces were dried at 70°C for 12 h in a hot air oven and ground to pass through a 1 mm sieve, were analyzed for DM, total Ash and Organic Matter (OM), Crude Fibre (CF), Ether Extract (EE), Nitrogen Free Extract (NFE) and total carbohydrate (AOAC, 1995). Calcium, Mg, Cu, Zn, Mn and Fe concentration of feed samples were determined by Flame Atomic Absorption Spectrophotometer (A Analyst 100, Perkin-Elmer Inc., USA). Phosphorous (P) in the feeds were determined colorimetrically (AOAC, 1995).

Statistical analysis

The experiment was divided into two parts i.e. starter and finisher. There were two factors up to 3 weeks i.e. salt and dose of Cu but another factor i.e. level of energy was introduced after 3 weeks. So up to 3 weeks data were collected and were analyzed by General Linear Model of SPSS (1997) with replicates as experiment units. Data obtained during this period were analyzed separately to determine the main effects of Cu-salt and dose of supplemental Cu at 21 day. Salt and dose interaction was also determined. For this, Cu salt and dose were used as fixed factor in this model. Polynomial contrast (linear and quadratic) was applied to determine the effects for different dose levels (0 vs. 200 and 400 mg/kg) of supplemental Cu. Data of finisher phase were analysis by the same model to determine the main effect of energy, Cu salt and dose of supplemental Cu, where salt, dose and SBO was taken as fixed factor. Salt×dose and salt×dose×energy interaction were estimated. A probability of $p<0.05$ was considered to be statistically significant.

RESULTS

Performance

Performance of broiler chicken supplemented with two sources of Cu-salt ($CuSO_4$ and Cu-proteinate) during starter and finisher period were presented in Table 3 and 4 respectively.

Table 3. Performance of broiler chicken supplemented with two sources (Cu-sulfate and Cu-proteinate) of Cu salt upto 3rd weeks (starter period) of Cu supplementation (each group having 4 replicates)

Attributes	Control	Source and dose of supplemental copper mg/kg diet DM				SE	Significance of treatment effects (p>F)			
		Copper sulfate		Copper proteinate			Salt	Dose		S×D
	0	200	400	200	400			Linear	Quadratic	
Live weight (g/bird)										
1 wk	140.96	145.81	148.75	146.96	151.33	1.255	0.321	0.001	0.600	0.700
2 wk	322.21	339.25	346.50	342.50	345.31	1.100	0.511	0.000	0.000	0.168
3 wk	551.57	576.44	583.83	580.77	595.75	1.877	0.000	0.000	0.005	0.034
Live weight gain (g/bird)										
1 wk	96.27	100.96	103.54	102.01	106.36	1.290	0.316	0.001	0.613	0.642
2 wk	277.51	294.40	301.29	297.55	300.34	0.997	0.436	0.000	0.000	0.157
3 wk	506.87	531.59	538.63	535.82	550.78	1.886	0.000	0.000	0.006	0.025
Cumulative feed intake (g/bird)										
1 wk	128.14	132.00	135.00	132.75	135.75	1.380	0.722	0.008	0.739	1.000
2 wk	377.00	390.25	390.50	391.50	389.00	1.754	0.963	0.001	0.005	0.607
3 wk	813.75	835.25	831.00	829.00	831.75	2.859	0.517	0.002	0.022	0.412
Feed conversion ratio										
1 wk	1.33	1.31	1.30	1.30	1.28	0.006	0.058	0.002	0.445	0.205
2 wk	1.36	1.33	1.30	1.32	1.30	0.004	0.409	0.000	0.294	0.490
3 wk	1.61	1.57	1.54	1.55	1.51	0.006	0.000	0.000	0.400	0.397

Table 4. Performance of broiler chicken supplemented with two sources (Cu-sulfate and Cu-proteinate) of Cu salt with different energy level from 4 to 6 weeks (finisher period) of Cu supplementation (each group having 4 replicates)

	Diet with 2,900 kcal/kg					Diet with 2,920 kcal/kg					SE	Significance of treatment effects (p>F)			
	Control	Source and dose of supplemental copper mg/kg diet DM				Control	Source and dose of supplemental copper mg/kg diet DM								
		Copper sulfate		Copper proteinate			Copper sulfate		Copper proteinate			Energy	Salt	Dose	
	0	200	400	200	400	0	200	400	200	400				Linear	Quadratic
Live weight (g per bird)															
4 wk	894.08	920.35	948.45	933.25	962.45	925.45	932.29	958.18	939.44	973.17	3.374	0.000	0.000	0.000	0.398
5 wk[f]	1,236.79	1,336.32	1,383.52	1,347.18	1,401.15	1,311.39	1,366.44	1,392.21	1,361.15	1,412.59	3.825	0.000	0.001	0.000	0.046
6 wk[a]	1,745.36	1856.45	1,933.45	1,896.18	1,975.51	1,830.48	1,886.71	1,955.49	1,916.69	1,982.54	3.846	0.000	0.000	0.000	0.202
Live weight gain (g/bird)															
4 wk	849.38	875.50	903.24	888.30	917.48	880.75	887.44	912.97	894.49	928.20	3.374	0.000	0.000	0.000	0.398
5 wk[cf]	1,192.10	1,291.47	1,338.32	1,302.23	1,356.18	1,266.70	1,321.59	1,347.00	1,316.20	1,367.62	3.825	0.000	0.001	0.000	0.046
6 wk[a]	1,700.66	1,811.60	1,888.24	1,851.23	1,930.54	1,785.78	1,841.86	1,910.28	1,871.74	1,937.57	3.816	0.000	0.000	0.000	0.203
Cumulative feed intake (g per bird)															
4 wk	1,508.50	1,530.00	1,543.50	1,532.50	1,543.50	1,522.33	1,530.83	1,537.11	1,521.83	1,534.33	7.334	0.932	0.660	0.000	0.783
5 wk	2,163.17	2,287.67	2,312.50	2,278.17	2,304.50	2,204.33	2,264.50	2,287.78	2,259.50	2,274.33	9.526	0.432	0.197	0.000	0.000
6 wk[dh]	3,318.17	3,300.00	3,315.83	3,331.50	3,379.17	3,324.67	3,213.50	3,338.11	3,265.95	3,312.67	12.693	0.001	0.003	0.448	0.002
Feed conversion ratio															
4 wk	1.78	1.75	1.71	1.73	1.68	1.73	1.73	1.68	1.70	1.65	0.003	0.000	0.000	0.000	0.345
5 wk[fg]	1.81	1.77	1.73	1.75	1.70	1.74	1.71	1.70	1.72	1.66	0.005	0.000	0.000	0.000	0.965
6 wk[d]	1.95	1.82	1.76	1.80	1.75	1.86	1.74	1.75	1.74	1.71	0.004	0.000	0.000	0.000	0.001

a = Energy×salt interaction ($p<0.05$), c = Energy×dose interaction ($p<0.05$), d = Energy×dose interaction ($p<0.01$).
f = Source×dose interaction ($p<0.01$), g = Energy×source×dose interaction ($p<0.05$), h = Energy×source×dose interaction ($p<0.01$).

Starter period

Main effect of supplemental Cu salt ($p<0.01$) was found at 3 week in respect to live weight (LW), live weight gain (LWG) and feed conversion ratio (FCR) whereas, cumulative feed intake (CFI) were not affected ($p>0.05$) by the Cu-salt. Live weight gain and FCR was higher ($p<0.01$) in Cu-proteinate supplemented birds compared to $CuSO_4$ supplementation. A linear dose response ($p<0.01$) of Cu was found for the performance of broiler chickens. Four hundred mg Cu/kg feed was given better performance than 200 mg Cu/kg irrespective of source. The dose response was also quadratic for LWG ($p<0.01$) and CFI ($p<0.05$). Salt×dose interaction was found for LW ($p<0.05$) and LWG ($p<0.05$) at 3 weeks.

Finisher period

Live weight gain and FCR was increased ($p<0.01$) when energy level was increased in the finisher diet. Cumulative feed intake was similar ($p>0.05$) across the groups up to 5th week. The main effect of energy on FCR was found from 4th week onwards which was evidenced from the compare of two controls (2,900 vs. 2,920 Kcal/kg) where FCR was 1.95 and 1.86 respectively. Copper proteinate increased performance of broiler chickens compared to $CuSO_4$. Increased dietary energy level decreased CFI as expected. Dose of supplemental Cu-salt irrespective of source showed

Table 5. Nutrient intake (g/d), fecal out go (g/d) and metabolizability (coefficient) of broiler chicken supplemented with two sources (Cu-sulfate and Cu-proteinate) of Cu salt at 3rd weeks of Cu supplementation (each group having 4 replicates)

Attributes	Control	Source and dose of supplemental copper mg/kg diet DM				SE	Significance of treatment effects (p>F)			
		Copper sulfate		Copper proteinate			Salt	Dose		S×D
	0	200	400	200	400			Linear	Quadratic	
Dry matter										
Intake	62.39	63.57	62.93	62.50	63.25	0.532	0.492	0.303	0.564	0.211
Fecal	20.57	18.46	16.97	17.48	16.42	0.268	0.012	0.000	0.040	0.428
Metabolizability	0.67	0.71	0.73	0.72	0.74	0.003	0.009	0.000	0.006	0.902
Crude protein										
Intake	13.55	13.81	13.67	13.58	13.74	0.116	0.492	0.303	0.564	0.211
Fecal	3.26	3.03	2.71	2.91	2.63	0.112	0.387	0.000	0.926	0.857
Metabolizability	0.76	0.78	0.80	0.79	0.81	0.007	0.426	0.000	0.935	0.921
Crude fat										
Intake	1.80	1.83	1.81	1.80	1.82	0.015	0.492	0.303	0.564	0.211
Fecal	0.23	0.19	0.17	0.18	0.16	0.005	0.071	0.000	0.002	0.952
Metabolizability	0.87	0.90	0.91	0.90	0.91	0.003	0.105	0.000	0.001	0.641
Crude fiber										
Intake	2.56	2.61	2.59	2.57	2.60	0.022	0.492	0.303	0.564	0.211
Fecal	1.31	1.36	1.31	1.32	1.33	0.102	0.949	0.971	0.796	0.767
Metabolizability	0.49	0.48	0.50	0.49	0.49	0.042	0.983	0.924	0.859	0.895
Nitrogen free extract										
Intake	39.23	39.97	39.56	39.29	39.77	0.335	0.492	0.303	0.564	0.211
Fecal	13.27	11.47	10.45	10.76	10.06	0.223	0.026	0.000	0.014	0.482
Metabolizability	0.66	0.71	0.74	0.73	0.75	0.005	0.020	0.000	0.002	0.813
Total carbohydrate										
Intake	41.79	42.58	42.15	41.86	42.37	0.372	0.492	0.303	0.588	0.211
Fecal	14.58	12.83	11.76	12.08	11.39	0.242	0.046	0.000	0.026	0.481
Metabolizability	0.65	0.70	0.72	0.71	0.73	0.008	0.018	0.000	0.004	0.846
Organic matter										
Intake	57.14	58.22	57.63	57.24	57.92	0.488	0.492	0.303	0.564	0.211
Fecal	18.07	16.05	14.64	15.17	14.18	0.223	0.009	0.000	0.023	0.357
Metabolizability	0.68	0.72	0.75	0.74	0.76	0.003	0.005	0.000	0.002	0.795

linear response ($p<0.01$) for performance. Two hundred mg/kg supplemental Cu with high energy diet showed better ($p<0.01$) FCR compared to birds not supplemented with Cu. Source×dose interaction ($p<0.01$) was found at 5th week for all parameters studied under performance except CFI. Energy×salt interaction ($p<0.05$) was noted for LW (6 week) and LWG (6 week). Energy×dose interaction ($p<0.05$) was found only in LWG at 5th week. Energy×dose interactions ($p<0.01$) was observed for CFI and FCR at 6 week. Energy×source×dose interaction was found for 5th week FCR ($p<0.05$). Energy×source×dose interaction ($p<0.01$) was also present for CFI ($p<0.01$) at 6th week.

Effect on nutrient metabolizability

Starter period : Nutrient intake and metabolizability coefficient of broiler chicken supplemented with two sources of Cu and different energy level were presented in Table 5 and 6. Supplementation of Cu-proteinate increased metabolizability of DM ($p<0.01$), NFE ($p<0.05$), total carbohydrate ($p<0.01$) and OM ($p<0.01$) at d 21. Increased dose of Cu-salt in the feed showed a linear increased ($p<0.01$) in metabolizability of DM, CP, CF, NFE and OM. Quadratic effect were also found ($p<0.01$) for DM, EE and OM. No significant salt×dose interaction was found for the metabolizability of the nutrients at d 21. Supplementation of Cu-proteinate at 400 mg/kg showed highest DM (0.74), OM (0.76) and total carbohydrate (0.75) metabolizability.

Finisher period : Increased level of energy in the diet improved DM ($p<0.05$), EE ($p<0.01$), NFE ($p = 0.01$), total carbohydrate ($p<0.01$) and OM ($p<0.01$) metabolizability. Cu-priteinate supplementation showed better nutrient utilization compared to $CuSO_4$. Highest DM metabolizability (0.80) was found in birds supplemented with 400 mg Cu-proteinate/kg with higher energy. EE metabolizability also improved ($p<0.01$) in Cu-proteinate supplemented birds compared to $CuSO_4$. Linear dose response ($p<0.01$) was found for DM, CP, EE, NFE, total carbohydrate and OM metabolizability. Intakes of all the nutrients were varied ($p<0.01$) among the treatments as was expected. Metabolizability of CF was unaffected ($p>0.05$) among the treatments. Quadratic dose response ($p<0.01$) was noted for DM, OM, total carbohydrate and NFE

Table 6. Nutrient intake (g/d), fecal out go (g/d) and metabolizability (co-efficient) of broiler chicken supplemented with two sources (Cu-sulfate and Cu-proteinate) of Cu salt and different energy level at 6 weeks of Cu supplementation (each group having 4 replicates)

Attributes	Diet with 2,900 kcal/kg: Control	Source and dose of supplemental copper mg/kg diet DM: Copper sulfate		Copper proteinate		Diet with 2,920 kcal/kg: Control	Source and dose of supplemental copper mg/kg diet DM: Copper sulfate		Copper proteinate		SE	Significance of treatment effects (p>F): Energy	Salt	Dose: Linear	Quadratic
	0	200	400	200	400	0	200	400	200	400					
Dry matter															
Intake[adh]	156.75	137.39	136.17	142.95	145.85	152.05	128.79	142.55	136.59	140.92	0.870	0.000	0.000	0.000	0.000
Fecal	46.33	33.00	32.33	31.67	30.33	41.00	31.00	32.00	29.33	28.67	1.111	0.001	0.015	0.000	0.000
Metabolizability	0.70	0.76	0.76	0.78	0.79	0.73	0.76	0.78	0.79	0.80	0.007	0.024	0.000	0.000	0.003
Crude protein															
Intake[adh]	30.06	26.35	26.12	27.42	27.97	29.21	24.74	27.38	26.24	27.07	0.167	0.000	0.000	0.000	0.000
Fecal	8.28	6.67	5.96	6.48	6.14	7.68	6.63	6.12	6.13	4.94	0.235	0.011	0.019	0.000	0.052
Metabolizability[e]	0.72	0.75	0.77	0.76	0.78	0.74	0.73	0.78	0.77	0.82	0.009	0.139	0.001	0.000	0.405
Crude fat															
Intake[beh]	4.30	3.76	3.73	3.92	4.00	10.32	8.75	9.68	9.27	9.57	0.038	0.000	0.000	0.704	0.598
Fecal	1.30	1.04	0.89	0.99	0.87	2.69	1.64	1.62	1.54	1.37	0.051	0.000	0.008	0.001	0.098
Metabolizability	0.70	0.72	0.76	0.75	0.78	0.74	0.81	0.83	0.83	0.86	0.008	0.000	0.000	0.000	0.308
Crude fiber															
Intake	6.52	5.72	5.66	5.95	6.07	7.02	5.95	6.59	6.31	6.51	0.037	0.000	0.000	0.001	0.000
Fecal	3.19	2.80	2.71	2.88	2.99	3.36	2.82	3.19	3.02	3.18	0.049	0.000	0.001	0.004	0.000
Metabolizability	0.51	0.51	0.52	0.52	0.51	0.52	0.53	0.52	0.52	0.51	0.007	0.157	0.463	0.669	0.450
Nitrogen free extract															
Intake[bdh]	103	90.28	89.48	93.93	95.84	92.66	78.49	86.87	83.24	85.87	0.559	0.000	0.000	0.005	0.002
Fecal	27.82	17.72	18.21	16.34	15.9	21.21	15.02	15.89	13.69	14.2	0.897	0.000	0.016	0.000	0.000
Metabolizability	0.73	0.80	0.80	0.83	0.83	0.77	0.81	0.82	0.84	0.83	0.010	0.008	0.001	0.000	0.001
Total carbohydrate															
Intake	109.52	96.00	95.14	99.88	101.91	99.68	84.44	93.46	89.55	92.38	0.581	0.000	0.000	0.008	0.005
Fecal	31.01	20.52	20.92	19.22	18.89	24.57	17.84	19.08	16.71	17.38	0.723	0.000	0.011	0.000	0.000
Metabolizability	0.72	0.79	0.78	0.81	0.81	0.75	0.79	0.80	0.81	0.81	0.009	0.006	0.002	0.000	0.002
Organic matter															
Intake[aeh]	143.88	126.11	124.99	131.22	133.87	139.21	117.92	130.51	125.06	129.02	0.798	0.000	0.000	0.000	0.000
Fecal	40.59	28.22	27.77	26.68	25.89	34.94	26.11	26.83	24.38	23.69	1.045	0.000	0.011	0.000	0.000
Metabolizability	0.72	0.78	0.78	0.80	0.81	0.75	0.78	0.79	0.81	0.82	0.008	0.005	0.000	0.000	0.003

a = Energy×salt interaction ($p<0.05$), b = Energy×salt interaction ($p<0.01$), d = Energy×dose interaction ($p<0.01$).
e = Source×dose interaction ($p<0.05$), f = Source×dose interaction ($p<0.01$), h = Energy×source×dose interaction ($p<0.01$).

metabolizability. Energy×salt interaction ($p<0.05$) was found in DM intake, CP intake and OM intake. Energy×salt interaction ($p<0.01$) was found for NFE, total carbohydrate and EE intake. Energy×dose interaction ($p<0.05$) was found in CP metabolizability. Energy×dose interaction ($p<0.01$) was noted in NFE and total carbohydrate metabolizability. Salt×dose interaction ($p<0.05$) was found for EE intake and OM intake. Intake of OM, NFE, EE and CP was affected by energy×salt×dose interaction ($p<0.01$).

DISCUSSION

In some of the previous studies Cu from cupric citrate (organic source) increased BWG in broiler at 125 mg/kg (Pesti and Bakalli, 1996) or lower levels 100 mg/kg Cu-methionine (Paik, 2001). Average body weight was increased at 35 days onwards in Cu supplemented groups compared to control (Ewing et al., 1998). The increased in CFI of birds fed dietary Cu-Met chelate during 1-5 week resulted in FCR closed to control up to 150 mg but an improvement at the higher level 200 mg/kg (Chowdhury et al., 2004). These findings are consistent with the present findings. In previous studies birds showed improved FCR in broilers when Cu was supplemented (Paik et al., 1999; Paik et al., 2000; Paik, 2001) where weight gain was the determining factor. In other study, Fox et al. (1987) reported no effect on weight gain and feed conversion even with 500 mg/kg level of $CuSO_4$ in broilers. On the other hand Wang et al. (1987) observed growth depression with little effect on feed conversion at the same dietary level. Cu functions biochemically as a component of several Cu dependent enzymes and as a cofactor for numerous other enzymes (Zapsalis and Beck, 1985; Sorenson, 1987). It is possible that high dietary Cu concentration enhance growth of broiler chicken by stimulating activities of the enzymes involved in nutrient utilization. Lim and Paik (2006) found dietary supplementation of 100 ppm Cu-methionine chelate increased the performance of layer compared to control. In the present investigation, Cu supplementation up to 400 mg/kg from Cu-proteinate showed better performance compared to $CuSO_4$ which supports the previous findings. This finding indicates Cu-proteinate has the ability to improve performance in broiler chicken over $CuSO_4$ supplementation due to its better absorption than $CuSO_4$.

Supplemental SBO at the rate of 3% in the diet improved LWG (6.9%, $p<0.05$) than the diet containing 0% SBO (Nitsan et al., 1997). They also observed higher LWG (3.4%) in group of broilers fed 6% SBO than 3% SBO added diet. Franco et al. (1996) found that inclusion of SBO (3%) in the diet improved LWG significantly ($p<0.05$) of broilers during 22-49 days of age. Campos et al. (1987) found that although there were no significant difference among the groups of broiler but weight gain increased with the increasing amount of fat (1.25, 2.50 and 3.75%) in the diet. Above 6% SBO containing diet increased the LWG of broilers, might be due to the higher ratio of protein and energy (Ali et al., 2001). Feed conversion efficiency was increased with increased level of SBO up to 6% but with higher level (e.g. 8 and 10%) the FCR were decreased. Franco et al. (1996) and Nitsan et al. (1997) stated that feed conversion efficiency improved ($p<0.05$) with addition of 3% SBO in the diet. In addition to its direct energy contribution, fat is also reported to enhance feed efficiency via an "extra caloric" effect. This effect has been observed in both chicks (Vermeersch and Vanschoubroek, 1968) and poults (Jensen et al., 1970). In the present investigation SBO was used 4% of the diet, which increased its energy content resulting, higher FCR. Ewing et al. (1998) suggest that under commercial condition average BWG is a better indicator of effectiveness of Cu supplementation than FCR because calculation of adjusted FCR is not routinely practiced in the industry but in the present study FCR was calculated considering the mortality.

Addition of Cu improved nitrogen retention which can be compared with results from Dove (1995), Braude (1965), Castell and Bowland (1968). Zhou et al. (1994ab) demonstrated that Cu given by either intravenous or oral intake increase plasma mitogenic activity (mitogenic peptides, an indicator of blood growth factor activity) in weanling pigs and numerically increased pituitary growth hormone mRNA concentration. Cu also had been shown to stimulate growth hormone secretion from bovine pituitary explants *in vitro* (LaBella et al., 1973). Therefore it is possible that Cu enhance protein retention and protein synthesis by stimulating hormone and growth factors in broiler chickens. The addition of Cu increase metabolizability of oil used in this trial, indicating that Cu played a role in the utilization of vegetable oil. This may indicated that birds needed additional Cu in the diet to efficiently digest supplemental vegetable fat. The improved fat metabolizability resulting from Cu addition would lead to the increased absorption of fatty acid and fat soluble vitamins and affects other aspect of nutrient metabolism in the body and therefore stimulate growth of broiler chickens. Improvement of fat metabolizability due to Cu addition may be partially due to increase lipase and phospholipase activity in the small intestine.

Addition of oil increased energy content leading to improved DM metabolizability which contradicts the previous findings (Dove, 1995; Cera et al., 1988; Li et al., 1990) who reported decreased DM metabolizability in pigs. In the present experiment, SBO was used as a fat source and its supplementation significantly increased fat metabolizability in broiler chicken.

From the result it is apparent that Cu may be supplemented in the feed of broiler chicken more than the requirement to improve performance and nutrient utilization as evident from the FCR, LWG and nutrient metabolizability. As supplementation of oil is commonly practiced by the industry to increase the energy content in finisher stage, so it can be said that Cu can be supplemented to improve the performance of broiler chicken. Cu-proteinate showed better performance and nutrient utilization compared to $CuSO_4$. However, further trials are warranted to consolidate the present findings and cost effectiveness of using Cu-proteinate.

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