Effect of Dietary Supplementation of Active Chromium-Insulin Compound on Egg Quality, Performance and Serum Biochemical Parameters of Laying Hens.

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Abstract

An experiment was conducted to investigate the effect of chromium-insulin mixture on egg quality indices, performance and some serum parameters of laying hens. 120 Isa brown pullets at point-of-lay were acquired and randomly divided into four experimental groups, each group was replicated thrice with ten birds per replicate. The birds were fed for 16 weeks with diets supplemented with varying levels of chromium-insulin mixture at 0ppm, 250ppm, 500ppm and 750ppm for treatments 1(control), 2, 3 and 4 respectively. Parameters such as egg weight; albumen weight, width; yolk index and colour; haugh unit; shell thickness and weight; weight gain, feed intake(FI), feed conversion ratio(FCR), protein efficiency ratio(PER), hen-day egg production, average egg production, total protein(TP), albumin, globulin, albumin–globulin ratio and cholesterol were evaluated. Egg weight, albumen weight and width of birds on treatments 3 and 4 were statistically superior ($p<0.05$) to those on treatments 1 and 2. Other egg quality indices were not significantly affected by the treatments. FI of birds on treatments 1 and 2 was significantly ($p<0.05$) higher than those on treatments 3 and 4. However, lowest mean value (2.04±0.34) for FCR was obtained in treatment 4 suggesting that the birds required less feed for egg production. Hen-day egg production and average egg production for birds on treatment 4 were statistically different ($p<0.05$) compared with those on control diet and have higher mean values than treatments 2 and 3 respectively. Glucose concentration decreased significantly as the chromium–insulin levels increased, while other serum parameters were not significantly influenced by the dietary treatments.

Keywords: Chromium, Egg Quality, Insulin, Layers, Performance, Serum.

Introduction

Poultry production has become one of the largest agricultural enterprises and its improvement is one of the major objectives of the industry for the past few decades. Also consumers' preference for high quality poultry products over these decades has prompted the poultry nutritionists to fashion out ways of dealing not only with how to improve production efficiency, but also how to improve poultry products.

Poultry meat is indisputably the leanest when compared to beef and pork, but due to the dynamics of consumers demand the industry cannot be satisfied with this advantage. Recently, the cut in the consumption of animal fat and cholesterol has led to a substantial erosion of the previously accepted dietary status of egg as nutritionally near perfect food. The present concerns and views on animal fats and cholesterol consumption are so ingrained that to reinstate the egg with the consumers will require great effort on the part of the egg industry and the animal scientists (Paik and Blair, 1996).

Also, peak of performance is the ultimate goal of animal scientists and that of farmers and the adequate nutrition of laying hens is imperative to achieve this optimum performance. In view of this, countless researches are been carried out on nutritional improvement of laying birds so as to improve their production to the best of their genetic potential which will definitely increase the availability of egg and meat to the ever booming population.

One of the most important means of achieving high quality poultry products is through the inclusion of one or more essential mineral elements in poultry diets, which in recent years there has been considerable research interest in utilization of chromium in animal feed. Chromium is an essential micro element for man and animals (Anderson, 1987). Since the first report on the essentiality in rat (Schwarzt and Mertz, 1950), the metabolic rate of chromium has been widely studied in human and animals. Its essentiality has been proved in the normal metabolism of carbohydrate, protein and lipid in man and animals (McDowell, 1992). Also its benefits in human health are well documented and include a role in maintenance of normal blood sugar and cholesterol level (Press *et al.*, 1990; Rabinovitz *et al.*, 2004). Chromium as an integral part of glucose tolerance factor (GTF), helps to promote or potentate the action of insulin, one of the anabolic hormones, helps to control appetites, hypoglycemia and protein uptake and also plays protective role against heart diseases (Merzt, 1993).

Chromium as a co-factor of insulin can also reduce blood levels of lipid total cholesterol, low-density lipoprotein (LDL) and increase high density lipoprotein (Press *et al*, 1990). Chromium is also considered as anti-stress factor and increase immune response with chromium forms varying in their bioavailability within the animal body system (Mowat, 1997). Chromium exists in two major oxidation states which are trivalent and hexavalent and only trivalent state is found to be physiologically and biologically active in living organisms and important in human animal glucose metabolism and the absorption scores range from 0.4 to 3% (Davis *et al.*, 1997).

On the other hand, insulin, an anabolic hormone secreted by the pancreas helps in building muscle tissue, fat metabolism and cholesterol utilization. If glucose cannot be utilized by the body cells due to insulin activity, it is converted into fat cells and also if adequate amino acids cannot enter the cells, muscle cannot be built (Hossan *et al.*, 1998).

Most poultry diets are mainly composed of ingredients of plant origin which are generally low in chromium (Giri *et al.*, 1990). Recent findings on the effects of chromium supplementation for pigs on carcass leanness and on reproductive parameters have been quite impressive. It has been reported that chromium supplementation increase the rate of glucose utilization by 16% which result in an improvement in egg production, egg weight and egg quality parameters (Sahin *et al.*, 2001). Some studies also reveal that increasing dietary chromium supplementation linearly increase albumen and yolk weight (Yildiz *et al.*, 2004).

Dietary chromium supplementation has also been shown to positively affect growth rate and feed conversion efficiency of growing birds (Cupo and Donaldson, 1987).

However, published research on the effects of chromium supplementation on poultry performance, egg quality indices and serum biochemical profile is limited.

Therefore, this experiment was designed to investigate the effects of graded levels of active chromium and insulin mixtures on performance, egg quality indices and some serum parameters of laying hens.

Materials and methods

Experimental site and materials

This study was carried out at the poultry unit of the Teaching and Research farm, Ekiti State University, Ado Ekiti, Nigeria. 120 Isa brown strain at point-of-lay were acquired from a reputable farm in Oyo State, Nigeria. After two weeks of acclimatization, the hens were randomly divided into four experimental groups of thirty birds per treatment and each treatment was replicated three times such that each replicate consisted of 10 birds. The birds were managed in a deep litter housing system. The birds were fed grower's and layer's mashes at growing and laying phases respectively which were supplemented with active chromium and insulin mixture at the rate of 0ppm for treatment 1(control), 250ppm for treatment 2, 500ppm for treatment 3 and 750 ppm for treatment 4. The birds were unrestrictedly fed for 16 weeks with their respective experimental diets. Tables 1 and 2 show the gross composition of the experimental diets. The diets were prepared to meet the nutritional requirements to support both maintenance and production functions of the birds throughout the period of the experiment.

Source of active chromium and insulin mixture

Synthetic form of active chromium-insulin mixture sold commercially as Lay more(Trade name) was procured from Metrovet Nigeria Limited, Ado-Ekiti, Ekiti State.

Table 1: Gross composition of the growers mash (kg/100kg)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Maize	40	40	40	40
Soybeans meal	11	11	11	11
Palm kernel cake	15	15	15	15
Rice bran	11	11	11	11
Brewer's dried grain	15.25	15.25	15.25	15.25
Fish meal (72%)	1	1	1	1
Limestone	2	2	2	2
Bone meal	4	4	4	4
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Grower's premix	0.2	0.2	0.2	0.2
Salt	0.25	0.25	0.25	0.25
Silicon forte	0.15	0.15	0.15	0.15
Active chromium and Insulin mixture (ppm)	---	250	500	750

Calculated composition
Crude protein= 16%
Metabolizable Energy= 2645 Cal/kg
Ppm= parts per million

Table 2: Gross composition of the growers mash (kg/100kg)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Maize	48	48	48	48
Soybeans meal	18	18	18	18
Palm kernel cake	10	10	10	10
Wheat offal	11	11	11	11
Fish meal (72%)	0.8	0.8	0.8	0.8
Limestone	9	9	9	9
Bone meal	2.5	2.5	2.5	2.5
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Layer's premix	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Active chromium and Insulin mixture (ppm)	---	250	500	750

Calculated composition
Crude protein=16%
Metabolizable Energy= 2580 Cal/kg
Ppm= parts per million

Data collection and analysis.

Egg collection and analysis
Egg samples were collected when production reached its peak at about 27 weeks of age by random sampling. Twelve eggs were collected from each treatment per week for egg quality indices analysis. This was done for 4 consecutive weeks.
The external and internal qualities of these eggs were determined as described by Ogunlade *et. al.* (2013).

Blood collection and analysis.
Blood samples for biochemical assay were collected from the jugular vein of sample birds using needle and syringe into vacutainer tubes devoid of anticoagulants. The tubes were kept in slanting position in a plastic rack while the blood samples were allowed to clot and the serum were separated by decanting after appropriate spinning using centrifuge machine. The samples were kept deep frozen prior to analysis. Serum total protein, albumin, globulin and albumin-globulin ratio were determined as described by Ogunlade and Egbunike (2013).

Determination of Cholesterol.
Cholesterol was determined by enzymatic hydrolysis and oxidation using CHOD-POD method (cholesterol liquicolour). This is an enzymatic calorimetric test for cholesterol with lipid clearing factor (LCF). The indicator quinoeinine is formed from hydrogen peroxide and 4-amino-phenazone in the presence of phenol and peroxide.

Determination of glucose
Fresh blood samples were collected in anticoagulant bottles containing flouride oxalate. The samples were spun in centrifuge at 3,000 rpm for 10 minutes to retract the plasma. The plasma was incubated for 10 minutes, 1000µl of distilled water was added to halt the reaction and reading was done in spectrophotometer at 500nm wavelength.

Data collection on performance
Data on weight gain were collected on weekly basis using weighing scale and subtracting the initial body weight from the final body weight. It was calculated and recorded averagely on g/bird/day basis. Data on feed intake was collected on weekly basis using the weighing scale to measure the quantity of feed offered and leftover. Differences between the feed offered and the feed leftover were calculated

and recorded averagely on g/bird/day basis.
The feed conversion ratio was calculated as ratio of feed consumed in kg to number eggs laid

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Hen- day egg production was calculated using the formula:

$$\frac{\text{No of eggs produced}}{\text{No of hen in flock}} \times 100$$

The protein efficiency ratio was calculated using the formula:

$$\frac{\text{Gain body mass (g)}}{\text{Protein intake (g)}} \times 100$$

The average egg produced was calculated using the formula:

$$\frac{\text{No of egg produced}}{\text{No of days}}$$

Experimental design and statistical analysis

The experiment was a completely randomized design. Data obtained were subjected to analysis of variance and differences in treatment means were separated using Duncan's Multiple Range Test. Statistical Analysis Software (SAS, 2003) package was used for the analysis.

Results

Table 3 shows the egg quality parameters of laying birds fed diets fortified with graded levels of active chromium and insulin mixture.

The results revealed that egg and albumen weights were significantly ($p<0.05$) influenced by graded levels of active chromium and insulin mixture with the weights of birds on treatment 4 (750ppm) being significantly superior to those on control diet and treatment 2 but statistically similar to treatment 3. The albumen width was significantly affected across the treatment by the dietary treatments. Albumen width of birds on treatment 4 was significantly superior to those on treatments 2 and 1but statistically similar to treatment 3. Other egg quality indices investigated were not significantly affected by the dietary treatments.

Some serum biochemical parameters of laying hens fed diet supplemented with graded levels of active chromium and insulin are shown in table 4. The results indicate that there were no significant differences ($p<0.05$) in the albumin, globulin, total protein, cholesterol and albumin/globulin ratio across the treatment. However, active chromium –insulin mixture in diets significantly ($p<0.05$) influenced serum glucose concentration of laying hens as it decreased with increasing chromium –insulin mixture across the treatments. Treatment 4 with 750ppm in diet was significantly lower than treatment 1 (control) while it was similar to treatments 2 and 3 with 250ppm and 500ppm respectively.

Table 5 shows the performance of laying hens fed different levels of active chromium –insulin mixture. It indicated that weight gain and protein efficiency ratio were not significantly ($p<0.05$) influenced by the dietary treatments. Feed intake was however influenced by the treatments ($p<0.05$). Feed intake for experimental birds on treatments 1 and 2 were higher than those on treatments3 and 4 respectively. Also, feed conversion ratio were significantly ($p<0.05$) affected by the treatments with treatment 2 having the highest mean value (2.21 ± 0.31) which is statistically similar to control (2.16 ± 0.36) and both are superior to treatments 4 with lowest mean value 2.04 ± 0.34. Hen- day egg production (HDEP) was significantly ($p<0.05$) influenced by varying levels of chromium –insulin mixture in diets of laying hen. The highest mean value was obtained in treatment 4 which was statistically similar to those of treatments 2 and 3 but superior to the control group. Averagely daily egg production was also significantly ($p<0.05$) influenced by the dietary treatments. The pattern of influence of dietary treatments on average daily egg production follow similar trend with hen- day egg production.

Table 3: Egg quality parameters of laying birds fed graded levels of active chromium and insulin mixture.

	Treatments			
Parameters	0PPM	250PPM	500PPM	750PPM
Egg weight (gm)	$62.09^{c}\pm1.55$	$63.75^{b}\pm1.26$	$64.33^{ab}\pm1.99$	$65.92^{a}\pm1.79$
Yolk weight (gm)	15.50±0.5	15.54±0.54	15.58±0.62	15.99±1.14
Yolk width (gm)	4.09±0.34	4.10±0.42	4.14±0.51	4.15±0.48
Yolk height (gm)	1.30±0.02	1.34±0.60	1.35±0.37	1.37±0.41
Yolk colour (gm)	3.96±1.80	13.92±1.77	3.92±1.77	4.00±1.94
Yolk index (%)	30.80±4.63	31.50±3.50	33.18±3.11	32.67±3.60
Yolk ratio (%)	24.33±1.13	25.05±1.31	24.68±1.39	24.13±1.77
Albumen weight (gm)	$40.92^{c}\pm1.81$	$41.87^{bc}\pm1.64$	$43.08^{ab}\pm2.82$	$44.00^{a}\pm3.19$
Albumen height (cm)	8.94±0.74	9.34±0.47	9.35±0.33	9.47±0.66
Albumen width (cm)	$6.05^{b}\pm0.21$	$6.27^{ab}\pm0.26$	$6.32^{ab}\pm0.18$	$6.66^{a}\pm0.43$
Albumen ratio (%)	65.67±1.44	65.65±2.37	65.82±2.17	66.93±2.49
Haugh unit (%)	93.36±3.45	94.73±3.86	95.30±1.34	95.51±2.92
Shell thickness (cm)	0.15±0.02	0.16±0.02	0.15±0.03	0.15±0.02
Shell weight (cm)	6.25±0.62	6.21±0.44	6.42±0.40	6.33±0.24
Egg diameter (cm)	4.60±0.05	4.62±0.11	4.58±0.11	4.59±0.03
Egg height (cm)	5.83±0.06	5.77±0.11	5.82±0.12	5.94±0.05
Shape index (%)	78.89±1.68	80.10±2.97	78.85±2.93	77.31±0.36
Shell ratio (%)	9.80±0.98	9.99±0.56	9.97±0.31	9.61±0.58

abc: Means with different superscript across the row are significantly different ($p<0.05$). ppm: parts per million

Table 4: Some serum biochemical parameters of laying birds fed graded levels of active chromium and insulin mixture.

Treatments

Parameters	0PPM	250PPM	500PPM	750PPM
Glucose (g/dL)	$14.73^{a}\pm1.79$	$11.41^{ab}\pm1.90$	$11.03^{ab}\pm0.36$	$10.37^{b}\pm3.65$
Albumin (g/dL)	28.31±6.69	30.01±8.23	27.20±6.67	32.22±11.63
Total protein (g/dL)	47.71±4.56	48.32±8.56	48.92±4.56	53.15±6.54
Cholesterol (g/dL)	3.47±0.89	2.80±0.40	3.40±0.17	2.55±0.61
Globulin (g/dL)	19.39±5.79	18.31±0.93	25.94±11.25	20.45±2.77
Albumin/Globulin ratio (g/dL)	1.55±0.46	1.63±0.43	1.04±3.79	2.48±2.14

ab: Means with different superscript across the row are significantly different ($p<0.05$). ppm: parts per million

Table 5: Performance of laying birds fed graded levels of active chromium and insulin mixture.

	Treatments			
Parameters	0PPM	250PPM	500PPM	750PPM
Weight gain	5.64±8.38	5.53±8.04	5.43±9.24	5.93±10.75
Feed intake	$133.60^{a}\pm12.7$	$131.90^{a}\pm11.4$	$129.20^{b}\pm13.9$	$128.90^{b}\pm11.1$
FCR	$2.16^{ab}\pm0.36$	$2.21^{a}\pm0.31$	$2.08^{bc}\pm0.40$	$2.04^{c}\pm0.34$
PER	0.35±0.53	0.35±0.50	0.38±0.51	0.37±0.67
HDEP(%)	$58.10^{b}\pm11.2$	$60.00^{ab}\pm9.06$	$60.30^{ab}\pm9.56$	$65.50^{a}\pm11.11$
AEP (%)	$61.60^{b}\pm2.84$	$64.21^{ab}\pm2.62$	$64.21^{ab}\pm2.62$	$67.95^{a}\pm2.71$

abc: Means with different superscript across the row are significantly different ($p<0.05$). ppm: parts per million
FCR: Feed conversion ratio, PER: Protein efficiency ratio, HDEP: Hen day egg production, AEP: Average egg production.

Discussion

In this study, the egg weight was significantly influenced by the treatments. There were improvements in eggs weight with increasing levels of chromium –insulin mixture. Similar result was reported by Kim *et al* (1997), that feeding 800 ppb chromium picolinate resulted in improvement of egg production and weight when compared with control group in laying hens. Improvement in egg weight may be due to ability of chromium and insulin to improve protein uptake and utilization of lipid (Mertz, 1993; Hossan *et al.*, 1998). Protein and lipid are two important materials involved in egg formation.

Findings from this study revealed that increase in levels of tests ingredients led to increase in albumen weight. This was in concordance with the report of Yildiz *et al* (2004) who observed increase in albumen weight of eggs from Japanese quails fed chromium picolinate. Although the yolk weight among the treatments were not significantly influenced but the data obtained showed that increasing dietary chromium and insulin mixture linearly increased yolk weight. Similar observation was made and reported by Sahin *et al* (2001).

Previous study by Uyanik *et al* (2001) reported no significant difference in albumen index in two out of their 3 samplings when chromium was supplemented in layer's diet. This report is in agreement with the result obtained in this present study where chromium –insulin mixture have no significant influence on albumen index. Though there were no documented reports on the effect of chromium-insulin mixture on yolk colour, the non-significant result obtained in this study among treatment may be due to absence of carotenoids in the test ingredient. Carotene is the pigment responsible for yellow colouration of egg yolk. Shell weight and thickness were not significantly affected by chromium –insulin mixture. Similar observations were made by Uyanik *et al* (2001) and Sahin *et al* (2001) when dietary chromium and chromium–zinc mixture were respectively supplemented in the diets of laying hens. The non-significant effect may be due to inability of the test ingredient to mobilize calcium and phosphorous as it does for glucose (Sahin *et al.*, 2001). Calcium and phosphorus are the two main minerals used in shell formation.

The dietary treatments had no significant effects on the weight gain of the experimental birds across board. Similar result was obtained by Uyanik *et al* (2001) who reported that graded levels of chromium in layer's diet did not significantly influence weight gain.

The feed intake was significantly higher in the treatments with 0ppm and 250ppm particularly compared with treatments having 500 and 750ppm chromium-insulin mixture. This is contrary to the result reported by Hossain *et al* (1998) that supplemental chromium in diet of laying hens has no significant effect on feed intake. Chromium –insulin mixture as used in this study has no significant effect on protein efficiency ratio. This result is in line with the report of Steele *et al* (1979) that supplemental chromium does not have any significant effect on protein efficiency ratio.

With respect to layers, feed conversion ratio is interpreted as the quantity of feed required per unit weight of egg laid and the lower the value of feed conversion ratio the better the conversion efficiency. The lowest mean value for feed conversion ratio was obtained at 750ppm supplementation indicating the treatment with the best utilization of feed. Feed intake at this level was also the lowest compared to other treatment groups indicating that birds in this treatment were feeding less per unit weight of egg laid and this is similar to findings of Steele and Rosebrough (1979) that chromium supplementation improves efficiency of conversion of laying hens.

Hen-day egg production HDEP or rate of lay is a percentage which reflects the number of egg produced by flock in a given period of time. The HDEP for birds on 750ppm for a period of sixteen weeks was found to be 65.5% which is superior to 62.05% of HDEP estimated by

North (1978). The average egg production for 16 weeks of laying was also calculated and was found to be highest at inclusion level of 750ppm. It was estimated to be 68 which is lower to average egg production of 86 reported by North M.O (1978) for the same period. This may be attributed to environmental differences as this current research was carried out in the tropical region while North's report was made for temperate region. Moreover, it may also be due to the effect of differences in housing system adopted. Deep litter housing system was used for this present research. Better performance of layers reared in conventional cage system have been reported in previous studies (Hulzebosch, 2006; Valkonen *et al.*, 2010).

None of the serum biochemical parameters except for glucose were significantly influenced by chromium–insulin mixture. Serum glucose decreased as chromium –insulin mixture inclusion level increased. This observation is similar to those of Lien *et al* (1999), Sahin *et al* (2001), Uyanik *et al* (2002) and Yildiz *et al* (2004) who reported decrease in serum glucose level in broiler chicken, laying hens and Japanese quail respectively by dietary supplementation of chromium picolinate.

Chromium is widely accepted as the active component of glucose tolerance factor which works synergistically with insulin by increasing the sensitivity of tissue receptor to insulin, resulting in increased glucose uptake by cells. Thus, the decrease in serum glucose concentration in this study may be as a result of enhanced sensitivity of tissue receptors to the insulin by chromium.

Conclusion

Supplementation of active chromium–insulin mixture at all graded levels yielded better results in egg quality indices, utilization of serum glucose and performance of the laying hens when compared to the control group. Data on birds fed 500ppm and 750ppm are comparable and are superior to 250ppm treatment and control groups. Therefore, in practical 500 ppm (500 g/tonne) inclusion rate may be adopted by animal nutritionists and farmers in the diet of laying hens. However, further studies may focus on investigating the possibility of chromium toxicity in laying hens.

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