



Enhancing the immunity and egg production of stressed laying birds by supplementing organic chromium

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ABSTRACT

An experiment was conducted to improve the immunity and egg production of stressed laying birds by organic chromium supplementation for 6 weeks. Birds (5,000 White Leghorn chicken, BV 300 strain) were divided into 5 groups (T1 to T5) having 10 replicates in each group and each replicate having 100 birds with an initial age of 33 weeks. The experimental groups are as follows: T1 is control unaffected by Newcastle disease (ND); all other groups (T2 to T5) were affected by ND and recovering from the disease. T2 is not supplemented with chromium source. T3, T4 and T5 were supplemented with chromium chloride, chromium yeast and chromium picolinate @ 300 ppb, respectively. The results revealed that weekly average egg production was significantly high in T1 followed by T4 during first week of supplementation and increased further. The feed intake was similar in all groups except control group. The mean HI titre for NDV was significantly higher in T4 followed by T5 and least in T2 and T3 during first week of vaccination. The titre was reached at the level of unaffected highly immune bird by second week in T4. Organic chromium supplementation (T4 and T5) reduced the mortality significantly in laying birds. The return on investment was high in chromium yeast (8.28) than the chromium picolinate (5.43). Supplementation of organic chromium to disease stressed laying birds increases egg production and HI titre and it could be useful feeding strategies during biotic stress condition.

Key words: Disease stress, Egg production, Immunity, Layer, Organic chromium

Chromium (Cr) is an essential mineral was first demonstrated by Schwarz and Mertz (1959) in rats and the essentiality of Cr was demonstrated in humans in 1977 (Jeebhoy *et al.* 1977). The beneficial effects of chromium can be observed more efficiently under environmental, dietary and hormonal stresses. Supplemental dietary chromium is recommended by NRC (1997) for animals undergoing environmental stress. Currently there are no NRC recommendations for chromium in poultry diets (NRC 1994). Dietary chromium supplementation positively affect the egg production and performance in laying hens (Sahin *et al.* 2001, Sahin *et al.* 2002a, Southern and Page 1994) and broiler (Jackson *et al.* 2008). Immunological function was enhanced by Cr and its effects were more pronounced during times of stress (Borgs and Mallard 1998). Organic source of chromium is over 10 times more bio-available than inorganic sources (Lyons 1994). However, organic

chromium has not been explored much for immune response during infective stress. Objective of the present experiment is to study the effect of inorganic and organic chromium on production and immune response in stressed layer.

MATERIALS AND METHODS

Location: The experiment was carried out at commercial layer farm at Namakkal (Poultry Hub of India), Tamil Nadu, India. Namakkal is known for poultry industry in India having 400 million layer birds with production of approximately 10,000 million eggs per year. This location is known for world highest density of laying birds population per sq. km (i.e) about 40 million per sq. km. Hence spread of disease from one farm to other farms is more prone and farmers are adopting high level of bio-security measures. However, economic loss for the farmers is mainly due to disease related production loss. Hence, production loss to the farmers has to be reduced by means of improving immunity by vaccination and other immune booster. Borgs and Mallard (1998) reported that immunity was improved when birds under stress condition by Cr supplementation.

Birds and treatments: In a 42-day experiment, 5,000 birds (BV 300 strain) were divided into 5 groups (T1 to T5) having 10 replicates in each group and each replicates

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Table 1. Physical and nutrient composition of the feed used during trial period

Ingredient	Quantity	Nutrient composition	%
<i>Physical composition</i>			
Maize (%)	44.61	Linoleic acid	1.04
De-oiled rice bran (%)	25.96	Lysine	0.94
Soyabean meal (solv.) (%)	15.59	Methionine	0.34
Fish meal (%)	6.0	Cystine	0.30
Calcite powder (%)	4.0	MET+CYS	0.64
Shell grit (%)	3.28	Arginine	1.20
Dicalcium phosphate (%)	0.30	Histidine	0.50
Salt (%)	0.15	Leucine	1.57
Trace mineral mixture (%)	0.11	Isoleucine	0.74
Total	100.00	P. Alanine + tyrosine	1.56
<i>Supplements</i>			
D.L.methionine g	20	Threonine	0.72
L.Lysine g	100	Tryptophan	0.19
NSP enzyme g	50	Valine	0.85
Phytase 2500 g	20	Serine	0.84
Ultrasil (toxin binder) g	100	Glutamic acid	2.77
Hepatocare (liver tonic) g	50	Calcium	3.25
Nutrient composition	Units	T.phosphorus	0.82
ME (Mcal/kg)	2.58	A.phosphorus	0.46
CP (%)	17.67	Na+K-Cl	23.93
Calorie : crude protein	146.16	Na:Cl	1.51
C.Fibre %	6.83	Na:K	5.82
Fat %	2.56	Chloride	2.41
		Sodium	1.60
		Potassium	9.30

having 100 birds with an initial age of 33 weeks. Birds were housed in raised cages. Automatic nipple system used for watering and semi automatic feeding system was followed. They were fed with uniform feed except chromium supplementation. Physical and chemical composition of feed offered during experimental period is presented (Table 1). The experimental groups are as follows: T1 is control unaffected by very virulent Newcastle disease (ND); all other groups (T2 to T5) were affected by ND. T2 is not supplemented with chromium source and act as negative control. T3, T4 and T5 were supplemented with chromium chloride, chromium yeast and chromium picolinate @ 300 ppb. All birds were vaccinated at the start of experiment with RDV Lasota live vaccine. The average

maximum and minimum ambient temperature was 38° C and 25.5° C. All the birds were provided with uniform gage and feeder space and were reared under standard managerial conditions throughout the experimental period of 42 days.

Parameters studied

Performance parameters: The performance variables studied include egg production, feed intake and mortality. Egg production for each group was recorded on a daily basis and expressed in percentage over the number of birds in each group. From this the weekly average percentage of egg production was calculated for each group. Cumulative feed intake for each group was measured for every 15 days (i.e. thrice during the study period) and total feed consumption was calculated. It was expressed as the amount of feed (g)/bird/day.

Immune parameter

HI test: The test was carried out as per Alexander (1988) using 4HA units of LaSota virus as the source of antigen and 1% fresh chicken erythrocytes (CRBC) as the indicator system. The highest dilution of serum that showed complete button formation was considered to be the HI titre of that serum.

Mortality: Mortality of the birds in each group was recorded on daily basis and expressed as a percentage over the total number of birds in each group at the start of the experiment.

Statistical analysis: Data were analyzed for analysis of variance using SPSS for windows. Statements of statistical significance are based on $P < 0.05$.

RESULTS AND DISCUSSION

Performance parameters: The performance parameter, viz. mean weekly egg production and mean cumulative feed consumption of layer as influenced by dietary inclusion of chromium are presented in Table 2. The weekly average egg production during first, second and fourth week of the trial period was significantly ($P < 0.01$) increased in T4 followed by T5 compared to un-supplemented T2 and chromium chloride supplemented group (T3). In third and fifth week chromium yeast supplemented group performed significantly ($P < 0.01$) better than the chromium picolinate and chromium chloride supplemented groups. At the end of sixth week chromium yeast and chromium picolinate

Table 2. Effect of chromium supplementation on production performance of layer bird

Groups	Weekly egg production % hen day						Feed intake g/bird/day **
	First**	Second**	Third**	Fourth**	Fifth**	Sixth**	
T ₁	97.79 ^a ±0.3	98.04 ^a ±0.2	97.63 ^a ±0.4	98.31 ^a ±0.2	97.89 ^a ±0.4	97.87 ^a ±0.4	109.40 ^a ±0.3
T ₂	74.09 ^d ±0.8	75.50 ^d ±0.9	83.88 ^{bc} ±0.8	83.81 ^c ±0.9	85.20 ^c ±0.9	85.23 ^b ±1.0	105.10 ^b ±0.8
T ₃	73.18 ^d ±0.7	73.60 ^d ±0.8	82.45 ^c ±0.9	83.36 ^c ±1.8	85.53 ^c ±0.8	82.71 ^b ±2.4	105.70 ^b ±0.9
T ₄	81.07 ^b ±1.1	83.18 ^b ±0.9	85.79 ^b ±0.8	88.21 ^b ±1.0	92.77 ^b ±1.1	95.37 ^a ±0.8	105.30 ^b ±0.8
T ₅	77.34 ^c ±1.1	80.13 ^c ±1.6	82.75 ^c ±0.4	85.16 ^{bc} ±1.3	87.20 ^c ±1.3	93.77 ^a ±1.5	106.70 ^b ±0.6

^{abcd}Mean bearing different superscript differ significantly (** $P < 0.01$)

supplemented groups improved its performance up to the level of healthy birds. The average increase in egg production during the trial period was 7.98% for chromium yeast and 3.86% for chromium picolinate. The percentage improvement was much higher in the last week of trial in chromium yeast supplemented groups and approaches to control group. However, trend was not encouraging in case of chromium picolinate and it was as low as -1.13 in third weeks as compared to un-supplemented group (T2). Similarly, chromium chloride supplemented group (T3) did not increase egg production at any point of time when compare to other organic chromium supplemented group. The higher egg production performance of organic chromium might be due to its better availability. Similarly, Underwood and Suttle (1999) suggested that organic Cr-compounds like Cr-nicotinic acid, Cr-picolinate or Cr-yeast complex may elicit better responses from livestock owing to the better absorbability of organic Cr though contradictory reports are also available (Nam *et al.* 1995).

Change in egg production pattern (%) is depicted in Fig.1. It indicated that T4 expressed 9.4, 10.2 and 11.9 % increased in egg production when compared to T2 group during first, second and fourth week respectively. Similarly, T5 group showed 4.4, 6.1 – 1.3 and 10.0 % increase/decrease in egg production during first, second, third and fourth week, respectively, when compared to T2. Inorganic chromium did not increase in egg production at any point of time except fifth (0.4 % increase). The result is in agreement with previous studies indicating an increase in

egg production with chromium supplementation (Sahin *et al.* 2002a). Southern and Page (1994) observed an increase in egg production of laying hens with chromium picolinate supplementation at 100 and 200 ppb, whereas at 400 ppb and above there was a reduction in egg production. The authors recorded an increase of 5.3% in egg production with 100 ppb dosage similarly in the current study the increase in production was 3.86 % with chromium picolinate at higher dosage of 300 ppb. The 2 experiments differed in the stress condition of the birds and the chromium source. Average daily feed intake was higher in uninfected group than infected group. Increased egg production at a lower feed consumption in T4 and T5 demonstrates better feed efficiency. Dietary Cr-supplementation positively affected the growth rate and feed efficiency in growing poultry (Lien *et al.* 1999, Kheiri and Toghyani 2009).

Immune parameter

HI test: HI titre (log 2) against Newcastle disease virus are presented in Table 3. All the birds were vaccinated against NDV on first day of experiment and titre was evaluated every week and result is presented. The mean HI titres (log 2) ranged from 4.80 ± 0.13 to 8.7 ± 0.15 and found that uninfected control group has gained titre as early as first week.

Change in HI titre was depicted in Fig. 2. HI titre was 2.1, 27.1 and 18.8 % was higher in T3, T4 and T5 group respectively compared to T2 group in first week and further improved in T4 and T5 in second week. This showed that

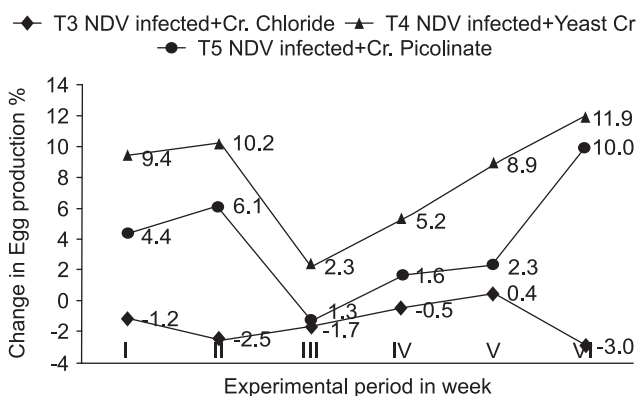


Fig. 1. Change in egg production (%) pattern in layer infected with NDV and with supplementation of chromium chloride (T3), chromium yeast (T4) and chromium picolinate (T5).

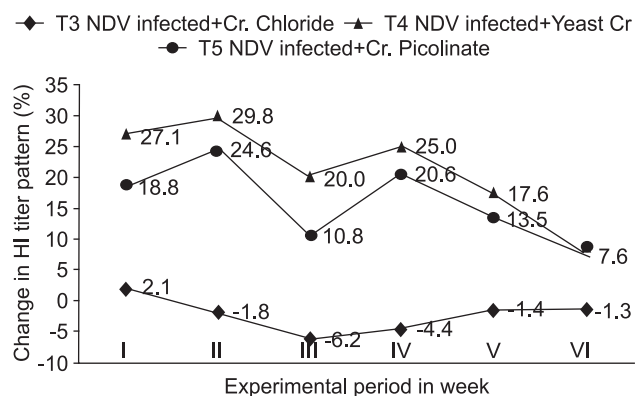


Fig. 2. Change in HI titer (%) pattern in layer vaccinated with NDV live vaccine and with supplementation of chromium chloride (T3), chromium yeast (T4) and chromium picolinate (T5).

Table 3. Effect of chromium supplementation on HI titre (log₂) against Newcastle disease virus and mortality of layer bird

Groups	Weekly HI titre (log ₂)						% mortality**
	First**	Second**	Third**	Fourth**	Fifth**	Sixth**	
T ₁	7.60 ^a ±0.2	7.70 ^a ±0.2	8.10 ^a ±0.2	8.40 ^a ±0.2	8.60 ^a ±0.2	8.10±0.3	0.80 ^c ±0.2
T ₂	4.80 ^c ±0.1	5.70 ^c ±0.2	6.50 ^{bc} ±0.2	6.80 ^b ±0.1	7.40 ^b ±0.2	7.90±0.2	8.30 ^a ±1.3
T ₃	4.90 ^c ±0.4	5.60 ^c ±0.3	6.10 ^c ±0.3	6.50 ^b ±0.3	7.30 ^b ±0.4	7.80±0.3	8.10 ^a ±0.9
T ₄	6.10 ^b ±0.1	7.40 ^a ±0.2	7.80 ^a ±0.1	8.50 ^a ±0.2	8.70 ^a ±0.2	8.50±0.2	3.10 ^b ±0.5
T ₅	5.70 ^b ±0.2	6.50 ^b ±0.2	6.80 ^b ±0.1	7.90 ^a ±0.2	8.10 ^a ±0.28	8.40±0.2	3.40 ^b ±0.5

^{abcd} Mean bearing different superscripts differ significantly (* P<0.05). NS, nonsignificant (P>0.05).

Table 4. Calculation of return on investment (ROI) of supplementation of chromium yeast and chromium picolinate in stressed laying bird

Source	Flock size	Average increase in egg production	Extra egg per day	Egg production for 6 weeks	Income (₹)	Source expense (₹)	Net income	Return on investment
Chromium yeast	50000	7.98	3990	167580	418950	45150	373800	8.28
Chromium picolinate	50000	3.86	1930	81060	202650	31500	171150	5.43

chromium yeast supplemented group gained titre value as early as second week. However, chromium picolinate supplemented group gained titre value on fourth week only. Chromium chloride supplemented group gained titre on sixth week as par with T1. Amir *et al.* (2010) reported that broilers received 1,200 and 600 $\mu\text{g kg}^{-1}$ Cr-nicotinate had the highest antibody titer against Newcastle and influenza viruses at 20 day, respectively.

Elevating in antibodies titre may be related to Cr role on insulin and cortisol hormones. The immune function may be affected in association with insulin and cortisol activity, because of cortisol acting as a suppressor for antibodies (Borgs and Mallard 1998). Lee *et al.* (2003) observed an improving in antibody titre against infectious bronchitis in broiler were consumed 400 $\mu\text{g kg}^{-1}$ Cr-picolinate. In other research, supplementation with different levels of Cr-picolinate specially at 1,500 $\mu\text{g kg}^{-1}$ level in diet of heat-stressed broiler chicks tend to increase antibody titre against Newcastle and influenza viruses (Toghyani *et al.* 2007).

The present study was also supported by Lien *et al.* (2005) who studied the immune response in weanling pigs with a supplementation of chromium propionate (0.2 mg/kg). Pigs supplemented with Cr had higher antibody titres specific for sheep red blood cells and total serum globulin.

Mortality: The percentage of mortality in each group during the experiment period is presented (Table 3). It is clear that the treatments with organic chromium reduced the mortality significantly ($P < 0.01$) in birds. The mortality was observed to be more during the initial days of the trial due to various reasons including stress due to heat and disease. The reduction in the mortality compared to untreated T2 is about 5.2 % in T4 and 4.9% in T5. However, chromium chloride supplemented group did not show any improvement in the livability of the bird.

Reduced mortality may be related to the beneficial effect of Cr on immune response. Reduced morbidity in response to Cr supplementation is well documented with calves (Burton *et al.* 1993, Chang *et al.* 1995) but no such data are available with layer chicks. The NRC (1994) did not specify any recommendation for Cr in poultry diets, which are basically composed of ingredients of plant origin containing only small amounts of Cr (Giri *et al.* 1990). Broilers reared in high environmental temperatures on corn-soybean meal diets probably have a moderate Cr deficiency. In addition, the reduction in the mortality is also due to stress relieving effect of organic chromium as discussed earlier and it was

not observed in inorganic chromium supplemented groups (T3).

Return on investment: The return on investment was high (Table 4) in chromium yeast (8.28) than the chromium picolinate (5.43). Supplemental chromium as chromium picolinate improved the feed efficiency in laying Japanese quails under heat stress (Sahin *et al.* 2002b). Uyanik *et al.* (2002) observed 1.88% reduction in feed consumption and 4.28% improvement in the efficiency of feed utilization of laying hens with the supplementation of chromium as chromium chloride. However, this experiment showed negative effect on chromium chloride supplementation. Perhaps during disease condition, chromium chloride could not be handled by the birds.

It is concluded that supplementation of organic chromium in disease affected birds could relieve the stress quickly and improve egg production and immunity. Increase in egg production in ND affected birds and enhancing the immunity by means of supplementation of chromium yeast to layer feeds could be useful feeding strategies. The return on investment was high in chromium yeast (8.28) than the chromium picolinate (5.43) during stress condition. The improvement in egg production and return on investment on supplementation of chromium ensures economical benefits to the farmer.

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