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Assessment of Manganese and Phyto-Additives (Turmeric) Supplementation in Low Input Technology (LIT) Layer Bird Diets on Egg Quality Parameters and Hatchability

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ABSTRACT

A study was conducted to evaluate the effect of supplementation of manganese and turmeric in a class of Low Input Technology (LIT) layer birds' diet namely Him-Smridhi (HS) under a complete randomized design. For this purpose, 44-week-old HS birds were divided into 4 treatment groups viz. T0 (BF), T1 (TP0.5%), T2 (Mn50mg/kg diet), T3 (TP 0.5% & Mn 50mg/kg diet) . Each treatment group had three replicates, with ten birds (9 female, 1 male) per replicate (T0)BF served as control group and was given standard feed, T1 was supplemented with 0.5 per cent turmeric powder w/w, treatment T2 offered with feed additional manganese 50mg/Kg and treatment T3 was supplemented with additional manganese (50mg/Kg) + (0.5%)) turmeric powder w/w. Fertility per cent, per cent total egg set hatched and per cent fertile egg set hatched were calculated during trial period (44th to 53rd week). Significant ($p<0.05$) effect of turmeric supplementation was observed on average albumen height and average haugh unit and reduction in egg yolk cholesterol level. Significant ($p<0.05$) higher albumen manganese, eggshell calcium and eggshell manganese was recorded with additional Mn supplementation affecting improve eggshell strength. Mn and turmeric powder supplementation showed no effect on hatchability in LIT bird Him-samridhi, but numerically 2.98 per cent higher hatchability with supplementation of turmeric powder alone was exhibited.

KEYWORDS: Egg yolk Cholesterol, Hatchability, Manganese, Turmeric.
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INTRODUCTION

Poultry is one of the fastest growing sectors in the Indian agricultural industry according to Indian Chamber of Food and Agriculture. Egg production increased from 78.48 billion in 2014-15 to 129.60 billion Nos. in 2021-22. Egg production in the country is growing at the rate (CAGR) of 7.4 per cent per annum (PIB,2024). The prevalence of protein deficiency in rural areas, especially among pregnant and lactating mothers and growing children, is very high. This problem can be addressed through the introduction of Low Input Technology Birds (LIT Birds) in rural backyard poultry initiatives. Him-samridhi is crossbreed of native chicken from the North Indian state (Himachal Pradesh) with a popular Dahlem Red (DR) poultry breed (Sharma et al., 2021). Poor eggshell quality is a major problem in the poultry industry, responsible for decreased hatchability, increased egg mortality, decreased protection against penetrating pathogens such as *Salmonella* spp. Eggs with shell defects contribute around 6-10 per cent of total egg produced, which negatively impacts the profitability of egg production (Nys, 2001). In past studies, dietary effect of macro minerals and vitamins on eggshell quality were under focus, however recent experiments showed positive results of using micro-minerals in layer feed.

Manganese (Mn) and Zinc (Zn) along with feed additive shows positive affect on eggshell quality. Manganese and Zinc act as co-factors of certain metallo-enzymes (Mn- Glycosyltransferase, Zn-Carbonic anhydrase) which helps in carbonate and mucopolysaccharides synthesis. Mn involved in the biosynthesis of proteoglycans keratan and dermatan sulphate which are responsible for egg shell structure and strength. (Arias et al., 1993; Nys et al., 2001). Thus, Mn may affect the mechanical strength of eggshell.

Phyto-additives are substances of plant origin added to animal diets at recommended levels with the aim of improving animal performance. Essential oils, herbs, and spices, all serve as sources for bioactive ingredients, e.g., phenols and flavonoids. Besides enhancing performance, phyto-additives also have antioxidant property, the effects of which are associated with essential oils (EOs) and their components. Turmeric (*Curcuma longa*) is a perennial herb belonging to the family of *Zingiberaceae*, distributed throughout tropical and subtropical regions of the world (Beevers C.S et al., 2011). The active ingredients are tetrahydrocurcuminoids, curcumin, demethoxycurcumin and bisdemethoxycutcumin (Osawa et al., 1995; AL-Sultan, 2003). Curcuminoid is primary active principle present in turmeric and is responsible for various biological actions *i.e.* hypocholesteremic, hepatoprotective, hypotensive, antiviral, antifungal, antibacterial anti-inflammatory, antifertility, and antioxidant etc.

Various factors affecting hatchability are genetics, age, nutrition, egg selection and handling of fertile eggs, position of egg during incubation, temperature and humidity during incubation and metabolism of chick embryo. Both quality and quantity of feed is important for optimum reproductive performance *i.e.* production of good quality ova in females and sperm in males. Fertile egg contains all essential nutrients for development of embryo till hatching. Owing to the role of manganese in eggshell formation and synthesis of steroid hormones, the present study was thus undertaken to evaluate the effect of additional supplementation of manganese and turmeric on hatchability, physical and chemical parameters of eggs laid in LIT bird (Him-Smridhi).

MATERIALS AND METHODS

The experiment was conducted in University Poultry Farm, Department of Animal Genetics and Breeding, DGCN COVAS, CSKHPKV, Palampur, Himachal Pradesh, India. The experiment encompassed 120 old LIT layer birds (Him-samridhi) of age ranging from 44th to 53rd week. At the beginning of the experiment, all birds were dewormed and randomly distributed into four experimental groups: T0 (BF), T1 (TP0.5%), T2 (Mn50mg/kg diet), T3 (TP 0.5% & Mn 50mg/kg diet). The birds were kept under deep litter system during experimental period for 10 weeks. Each treatment group had three replicates, with ten birds (9 female, 1 male) per replicate. T0 served as control group and was given standard feed as per ICAR 2013 standards (basal diet), T1 supplemented with (0.5 %) turmeric powder w/w, T2 offered feed with additional calculated Mn (50mg/Kg) and T3 with additional calculated Mn (50 mg/Kg) + 0.5 % turmeric powder w/w. The experimental diets (Table 1) were formulated to meet nutritional requirements for energy, protein, minerals, and vitamins in accordance with the ICAR 2013 standards. Inorganic manganese sulphate and turmeric powder were purchased locally in Palampur, Himachal Pradesh, India. Routine activities involved feeding of treatment feed (120gm/bird/day), watering (*ad libitum*), collection of eggs (4 times a day) and shifting to hatchery (A.P. Poultry Equipment). The eggs were collected for 8-10 day and stored in egg storage room at temperature ranging between 13-15°C and relative humidity ranging from 75 - 80 per cent. After every 8 days, collected eggs were set in the egg setter after its fumigation. Eggs were evaluated for fertility after 18 days and unfertile eggs were removed, and fertile eggs were transferred to hatcher room for 3 days.

The estimation of proximate principles of feed ingredients (Table 1) utilized in formulating experimental diets was done by employing standard AOAC (2005) techniques. Metabolizable energy was calculated using the equation suggested by Lodhi et al. (1976).

Table 1. Ingredients and Chemical composition of experimental diets (% D.M.B)

Ingredients (%)	T0	T1	T2	T3
Soy flake	20.1	20.1	20.1	20.1
Maize	60.25	60.25	60.25	60.25
DORB	5	5	5	5
FM	5	5	5	5
MnSO4	0	0	0.0167	0.0167
Limestone	6.65	6.65	6.65	6.65
DCP	3	3	3	3
Salt	0.2	0.2	0.2	0.2
Premix	0.5	0.5	0.5	0.5
Turmeric powder	0	0.5	0	0.5
Chemical Composition (%)				
Crude Protein	16.59	16.59	16.59	16.59
Crude Fibre	3.91	3.91	3.91	3.91
Ether extract	0.98	0.98	0.98	0.98
LA	1.34	1.34	1.34	1.34
Ca (%)	3.39	3.39	3.39	3.39
P (%)	1.33	1.33	1.33	1.33
Manganese (mg/kg)	64	64	114	114
ME (KCal/Kg)	2569	2569	2569	2569
E/P	154.85	154.85	154.85	154.85

Premix was prepared by mixing following in 500gm maize flour. Traymix =20gm (Vitamin A-82,500 IU, B2-50mg, D3-12,000 IU, K-10mg/gm). VENTRIBEEPLUS 20g (vitamin B1-25 mg, B6-35 mg, B12-250 µg, E-225 mg, Pantothenate -225 mg, Niacinamide -300 mg, Folic acid-20 mg/5 g). E-care Se fort 10 gm (Vitamin E- 0.20g, Se-0.04 mg/g). Toxin binder-10 gm. Trace minerals – 100gm (Manganese sulphate=20gms, Ferrous sulphate =30gms, Zinc oxide=13gms, Copper sulphate=3.7gms, Potassium iodide=2.5gms, Dicalcium phosphate=30.8gms), Choline chloride=10gms, Methionine=14gms, Lysine=15gms.

* DORB: De-oiled rice bran, FM: Fish meal, DCP: Dicalcium phosphate

Digestion of the samples for mineral estimation was done using QLAB microwave digestion system: Questron Technologies Corp. (Canada). Mineral estimation of eggshell (Ca, Mn) was done using microwave assisted digestion method. The eggshells from respective treatment eggs were collected and grinded into homogenized powder form. Eggshell powder kept in hot air oven at 98°C for 12 hours. Digestion of eggshell powder, egg yolk and whole egg (Mn) samples was done by microwave assisted acid digestion in which 0.2 gm of homogenized sample was taken in digestion tube (eVHP TFM liner). The egg yolk and albumen were separated after boiling the eggs in water for 10-15minutes. Egg yolk samples and albumin were kept in hot air oven for 48-72hrs at 60°C and thereafter homogenized with a mechanical mixer. Acids used were nitric acid: perchloric acid: hydrogen peroxide in ratio 7: 1.5: 1.5 respectively. With an atomic absorption spectrophotometer, the final filtrate was used for analysis to determine the presence of Ca and Mn. Estimation of calcium and manganese was done by Atomic Absorption Spectrometer (AAS) (LABINDIA AA8000) and estimation of phosphorous was done spectrophotometrically (Spectronic 200 spectrophotometer) by method proposed by Parks and Dunn (1963).

External egg parameters such as egg weight, egg length, egg width, shape index, shell thickness and percentage shell were recorded. Eggs were weighed using electronic balance to an accuracy of 0.01 g. (Danwer scales India**.** The length of the eggs was measured using digital vernier calipers (Aerospace Inc.). The width of the eggs was measured using digital vernier calipers (Aerospace Inc.). Shape index was calculated as the ratio of egg width to egg length × 100. Shell thickness was measured using a micrometer. Percentage shell was measured by taking the weight of eggshell to egg weight × 100. Shell weight was measured after washing the interior egg membrane and after its drying at 60°C for 48 h Venglovska et al. (2014).

Internal egg parameters such as yolk width, yolk height, yolk weight, albumen width, albumen height, albumen weight, Yolk: Albumen ratio and haugh unit were recorded during trial period (44th to 53rd week). Yolk width was measured using a digital vernier caliper (Aerospace Inc.). Yolk height was recorded using the vernier caliper (Aerospace Inc.). Yolk weight is measured by separating the yolk from albumen of egg. Albumen width was recorded using the digital vernier caliper (Aerospace Inc.). Albumin height was measured using spherometer in millimeters. Albumen weight was calculated by subtracting the shell and yolk weights from the egg weight. Yolk: Albumen ratio was calculated using weight of yolk/ weight of albumen. Haugh unit= 100 log ($H + 5.57 - 1.37\ W^{0.37}$), Where H is albumen height (mm), and W is egg weight (g).

Egg hatchability records such as fertility per cent, per cent total egg set hatched (TESH), per cent fertile egg set hatched (FESH) and per cent infertile egg (IE) on total egg set were recorded during trial period (44th to 53rd week).

Fertility per cent = Number of fertile eggs / Total eggs set ×100

TESH = Number of eggs hatched / Total eggs set × 100

FESH = Number of eggs hatched / Fertile egg set × 100

IE (Total egg set) = Total infertile number/ Total egg set × 100

Total cholesterol in egg yolk was measured according to Zaltkis et al. (1953) with minor modifications as reported by Rajkumar et al. (2004) using a cholesterol test kit (Agappe).

Statistical analysis

ANOVA (analysis of variance) utilizing the complete randomized design (CRD) was performed on all recorded and computed data as explained by Snedecor and Cochran (1968). A 5 per cent level of significance was used to evaluate the outcomes.

RESULTS AND DISCUSSION

The egg production was not affected by the supplementation of Mn and turmeric powder. Average value of feed conversion ratio (FCR) was found to be 1.65, 1.74, 1.73 and 1.65 in control T0 and treatment groups T1, T2 and T3 respectively. The result obtained revealed that the average value of average feed conversion ratio (FCR) exhibited non- significant differences among control and treatment groups (Table 2). The effect of supplementing additional Mn^{50mg} along with $TP^{0.5\%}$ on the egg quality parameters is presented in Table 3. Results obtained implied that by the end of the trial, egg weight, egg width, egg length, shape index, yolk width, albumen width, yolk: albumen ratio, shell thickness, per cent shell exhibited non-significant ($p>0.05$) difference in treatment groups compared to BF on weekly basis. However, Haugh Unit (HU) and albumin height exhibited significant ($P<0.05$) difference in treatment groups compared to control, BF.

Table 2. Overall Egg production & FCR

Parameters	T0	T1	T2	T3
Total Egg Produced	937±2.27	884±3.50	897±4.60	927±3.10
Avg. Egg Wt (gm)	52.13±1.21	51.41±0.45	51.10±1.12	52.80±1.05
Total Egg Mass Produced (gm)	48845.81±0.045	45446.44±0.042	45836.70±0.035	48945.60±0.036
Total Feed Intake (gm)	80750.0±0.053	79200.00±0.036	79410.00±0.065	79850.00±0.043
FCR	1.65±0.03	1.74±0.01	1.73±0.02	1.63±0.03

FCR = Total feed consumed (kg) ÷ Total egg mass produced (kg)
Total egg mass produced (kg) = Numbers of eggs produced * Avg. Egg Wt
*Mean bearing different subscript within row differ significantly ($P<0.05$) from each other.

HU is a measure of the quality of eggs and higher value of HU enhances the grade of the egg. Interestingly HU value was significantly ($p<0.01$) higher in $TP^{0.5\%}$ whereas albumin height was recorded to be higher in Mn^{50mg} and in combination with $TP^{0.5\%}$. In our study, the results obtained reinforced the earlier findings implying that turmeric and Mn help to maintain freshness of egg components such as albumen and yolk. These findings are consistent with those of park et al. (2012b), where supplementation of layer diet with turmeric (Zadeh et al., 2022, Park et al., 2012, & Devi et al., 2023) improved the HU significantly. Improvement in HU due to turmeric powder consumption need further study for examination of variations in mineral and moisture levels in the albumen. It is well documented with previous studies that turmeric contains curcumin which prevents lipid peroxidation and Mn as a cofactor of superoxide dismutase prevents oxidation activity and maintains freshness of egg. Increased HU may be attributed to the antioxidant properties of curcumin present in turmeric powder. In our study, no effect on HU was recorded with Mn ^{50mg} supplementation but many of the authors have reported higher HU with Mn ^{50mg} supplementation. (Kim et al., 2022, Gumus et al., 2017).

The average albumen height in BF, $TP^{0.5\%}$ Mn ^{50mg}, $Mn^{50}TP^{0.5}$ during trial period (44th to 53rd week) was 6.76, 6.8, 7.23 and 6.98 mm respectively and significant (P<0.05) results were obtained in Mn ^{50mg}, $Mn^{50}TP^{0.5}$ compared to BF. Supplementing turmeric alone did not influence the albumin height, where as additional Mn^{50mg} supplementation alone and in combination with $TP^{0.5\%}$ influenced albumin height. Increase in albumin height was also observed by other workers owing to Mn supplementation. (Rubio Zapata NK 2016, Attia et al., 2018, Zadeh et al., 2022).Contrary to our findings, Park et al. (2012) found improvement in albumen height due to turmeric powder consumption and attributed it to the antioxidant properties of curcumin present in turmeric powder. Gumus et al. (2017) and Laganá et al. (2011) also reported similar results in turmeric supplemented groups.

Overall, results for average egg weight over a period exhibited non-significant (p>0.05) difference, although numerically highest value of egg weight was exhibited by $Mn^{50}TP^{0.5}$ (52.82 gm) and lowest value of egg weight was exhibited by Mn ^{50mg} (51.11 gm). Our findings are in agreement to those of Xiao et al. (2014) and Zarghi et al. (2023), that supplementing layer diet with Mn didn't affect egg weight. As Sazzad et al. (1994) concluded that layers fed diet enriched with Mn conferring to the minimal manganese requirements do not impact egg weight & egg production. Further, Kujero et al. (2021) and Attia et al. (2018) concluded that layers fed diet enriched with turmeric didn't exhibit significant egg weight. This might be linked to the relatively low levels or concentrations of essential compounds such as protein and fats, along with the volatility of secondary compounds like volatile oils in turmeric powder. These factors could potentially be necessary to improve egg weight. However, results of the experiment though revealed no impact of Mn and TP fed alone, but their combination led to 3.23 per cent increase in average egg weight. The higher egg weight in Mn and TP containing diet may be due to the fact that Mn is required for growth and development and also for normal functioning of enzymes, hormones, and carbohydrate metabolism. (McDowell LR 1992). Similarly in one of the studies, compared with the control diet, the levels of turmeric powder supplementation significantly improved nutrient digestibility (p < 0.001). (Mohamed A Fawaz et al., 2023). It is thus apparent that the combination of Mn^{50mg} and $TP^{0.5\%}$ might be having a synergistic action by increasing the availability of nutrients at the time of digestion and metabolism leading to average increase in egg weight.

Cited literature in previous studies on commercial egg layers attributed the supplementation of turmeric powder to enhance the fertile egg per cent by improving the semen quality because of the antioxidant action of turmeric preventing the lipid membrane from oxidation. Whereas in our study, the results obtained (Table 4) revealed non-significant (p<0.05) difference in treatment groups compared to control on fertile eggs at 18 days of candling. Numerically the highest fertile egg per cent at 18 days was recorded in treatment group $TP^{0.5\%}$ (92.55) compared to BF (91). Results of per cent TESH and FESH (Table 3) and per cent IE (total egg set) revealed non-significant (p>0.05) difference in treatment groups compared to control.

Table 3. Egg hatchability

		T0	T1	T2	T3
0-18th day	Total egg set (no.)	413	287	345	344
	Infertile eggs (no.)	37	22	36	47
	Infertile eggs %	8.96	7.66	10.43	13.66
	Fertility (%)	91±3.39	92.55±1.56	89.23±3.65	86.21±6.48
18th -21st day	Egg hatched (no.)	339	245	281	272
	TESH (%)	82.13±3.28	85.11±2.26	80.68±4.86	78.96±5.12
	FESH (%)	90.33±4.25	92±3.94	90.38±2.36	91.64±1.55
	Total infertile eggs (no.)	72	41	64	72
	IE (%)	17.43±3.28	14.28±2.26	18.55±4.86	21±5.17

*Mean bearing different subscript within row differ significantly (P<0.05) from each other

Cholesterol in egg yolk mg/100g (Table 3) exhibited significant ($p<0.05$) difference in $TP^{0.5\%}$ and $Mn^{50}TP^{0.5}$ compared to BF. Dalal et al. (2018) explained that turmeric powder has the potential to lower cholesterol levels through mechanisms such as enhancing the activity of cholesterol-7-α hydrolase or inhibiting HMG Co-A reductase. Curcumin, a component of turmeric, promotes the conversion of cholesterol into bile acid, facilitating its elimination from the body by inhibiting reabsorption of dietary cholesterol. In our study, the results obtained reinforced the earlier findings implying that turmeric powder reduces the egg yolk cholesterol. (Amir Mosayyeb Zadeh et al ., 2022, Xu et al., 2016 and Li et al., 2023). The anti-atherogenic properties of curcumin contribute to decreased blood cholesterol levels, leading to a reduction in the transfer of cholesterol into eggs. Hence, egg yolk cholesterol level was reduced in turmeric supplemented treatments.

Results for whole egg Mn (ppm) and yolk Mn (ppm) (Table-3) in treatment diets exhibited non-significant ($p>0.05$) difference compared to control. Whereas results for albumen Mn (ppm) and eggshell Ca (Table 3) exhibited significant ($p<0.05$) difference Mn^{50mg} and $Mn^{50}TP^{0.5}$ compared to BF. Results of the experiment revealed enhanced deposition of Mn and Ca in egg albumin and eggshell in treatment groups by additional Mn supplementation. It is well documented with previous studies that manganese plays a role in the activity of glycosyl transferases involved in cartilage muco-polysaccharide synthesis. These mucopolysaccharide act as store house of Ca^{++} ion during eggshell formation in their mammillary lobes. In our study, the results obtained reinforced the earlier findings implying that Mn supplementation improves the Ca^{++} deposition in eggshell.

Table 4. Egg quality parameters

Parameters	T0	T1	T2	T3
Egg weight (gm)	52.12±2.11	51.42±3.32	51.11±1.61	52.82±3.55
Egg breadth (mm)	40.94±0.24	40.86±1.26	40.45±3.45	41.32±1.12
Egg length (mm)	55.11 ±0.51	54.66±0.46	54.22±0.92	55.22±1.52
Shape Index (SI)	74.37±1.47	74.66±1.96	74.77±7.17	74.93±1.43
Albumin height (mm)	6.76a±0.26	6.8a±0.38	7.23b±0.74	6.98ab±1.53
Yolk width (mm)	42.52±0.68	42.43±2.07	43.83±4.47	43.13±4.27
Albumen width (mm)	101.65±4.45	97.35±6.25	97.17±6.77	97.02±4.62
Yolk height (mm)	17.57±0.67	17.5±0.6	17.16±0.76	17.65±0.65
Yolk weight (gm)	16.63±0.73	16.6±1.41	6.78±0.68	17.18±0.48
Albumen weight (gm)	27.68±0.98	27.33±2.13	27.4±1.3	27.61±1.61
A:Y ratio	0.60±0.03	0.61±0.03	0.61±0.02	0.63±0.05
Shell thickness (mm)	36.6±1.00	36.06±1.66	34.93±1.83	35.57±2.77
Per cent shell (%)	9.03±0.2	9.09±0.41	8.99±0.35	8.99±0.36
Haugh unit	84.37a±2.53	89.43b±1.53	84.16a±1.96	84.76a±1.36
Whole egg Mn(ppm)	0.02±0.01	0.03±0.01	0.03±0.01	0.05± 0.03
Yolk Mn(ppm)	0.04±0.00	0.03±0.02	0.03±0.02	0.05±0.03
Egg Albumen Mn(ppm)	0.036a±0.000	0.049a±0.007	0.135ab±0.005	0.083b±0.003
Eggshell Ca (ppm)	5.37a±0.3	5.31a±0.9	6.32ab±0.6	8.28b±0.4
Eggshell Mn(ppm)	0.0080a±0.000	20.0086ab±0.000	20.0080a±0.0004	0.0160b±0.0008
Egg Yolk cholesterol (mg/100 gm)	199.77ab ±28.38	164.93b ±14.94	191.52a±23.22	160.88b ±17.98

*Mean bearing different subscript within row differ significantly ($P<0.05$) from each other.

CONCLUSION

It is thus concluded that additional supplementation of Mn @ 50 mg/ Kg feed enhanced the HU significantly whereas turmeric powder supplementation increased the albumin height and lowered the egg yolk cholesterol significantly ($p<0.05$). Higher content of albumen Mn, eggshell Ca & eggshell Mn was recorded with supplementation of additional Mn and turmeric powder improving the eggshell strength. No significant ($p>0.05$) effect of Mn and turmeric powder on hatchability in LIT bird Him-samridhi was recorded, but numerically 2.98 per cent higher hatchability with supplementation of turmeric powder alone was exhibited.

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