



Iron and Calcium Source Interaction for Egg Production

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Does Dietary Calcium Source and Iron Interaction Affects Egg Laying Performance, Quality and Yolk Iron Enrichment ?

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ABSTRACT

Present study was conducted to assess the interaction between dietary powdered and particulate form of limestone as calcium source *vis a vis* graded levels of inorganic iron supplementation on egg production, quality and yolk iron enrichment. In 3x2 factorial experimental design, a total of 72 BV-300-layer hens between 20-47 weeks of age were subjected to total 6 treatments (with 3 replicates having 4 birds each) comprised of three dietary levels (50, 75 and 100 mg per Kg feed) of iron and two levels of substitution (50 and 75 %) of fine limestone powder (LSP) with limestone grit (LSG). The source of calcium and iron interaction had significant effect on egg production and substitution of 75 % of LSP with LSG along with increased iron supplementation significantly improved percent hen housed and hen day egg production and feed conversion ratio for egg mass production but increasing LSG inclusion, only significantly (Pd^{0.05}) improved egg shell thickness. Although, increasing LSG or iron supplementation did not improve egg yolk iron but dietary substitution of LSP with LSG upto 75% at supplementation of 100 mg iron/ kg feed improved egg production performance of layers, especially during late laying phase.

KEYWORDS: Egg production, Egg quality, Limestone grit, Limestone powder, Yolk iron enrichment.

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INTRODUCTION

Iron deficiency anemia is worldwide problem and in India around 50% population suffers from iron deficiency anemia. While in vulnerable age group of children, adolescent girls, pregnant and breastfed women of reproductive age the prevalence ranges much higher between 80-90% (Kaur, 2014). Iron fortified egg contains 8-15 mg iron per 100 g of yolk (United State Patent no. 5464637) (Horikowa et al., 1995) has gained popularity across the world as an important food item with highly bio available iron along with cheaper source of high quality protein. Earlier a number of workers attempted to increase egg yolk iron through increased dietary supplementation of inorganic and organic source of iron (Qui, 2003; Skrivan et al., 2005 and Revell and Hughes, 2009). But a number of other dietary factors like anti-nutrient and minerals interaction also influences iron metabolism in poultry (Hurrel

et al., 1997; Ramadan et al., 2010; Abbasi et al., 2015; Farina et al., 2020) and corresponding production and iron enrichment of eggs. Among minerals, calcium is most abundantly required for laying hens and reported to have inhibitory effect on iron absorption in human, if fed in same diet (Whiting, 1995; Lynch, 2000; Beck et al., 2014) but to the best of our knowledge no study has been conducted on dietary calcium and iron interaction in poultry. If so, dietary calcium manipulation can be alternative economical approach to produce iron enriched eggs without increasing rate of dietary iron supplementation. Large particle calcium has prolonged retention time in gizzard with slow dissolution (Rao et al., 1992; Guinote et al., 1995) ensuring calcium absorption, during off fed hours in the night at the time of egg shell formation (Pavlovski et al., 2003; Lichovnikova, 2007), therefore fine powdered calcium can be substituted

with large particle calcium to minimize gut iron absorption after feeding during day hours. Keeping in view, present experiment was conducted to study the interaction between dietary inclusion levels of limestone powder (LSP), limestone grit (LSG) and ferrous sulphate on egg production, quality and yolk iron enrichment of laying hens.

MATERIALS AND METHODS

Present study was conducted at the Poultry Farm of Department of Livestock Production Management, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana with permission of Institution Animal Ethics Committee

with No. GADVASU/2018/IAEC/47/12. The experiment was conducted on total of 72 BV300 layer birds from 20 to 48 weeks of age. Experimental birds were housed in 2 tier M type shallow California cage with linear channel feeder and drinker. The experimental design was fully randomized 3 X 2 factorial design consisted of three different dietary iron at the rate of 50, 75 and 100 mg per Kg supplemented through ferrous sulphate and two different ratios of limestone powder (LSP) and (LSG) grit as calcium supplement. In which commonly used LSP and LSG ratio form 50: 50 was increased to 25:75. Dietary treatments comprised of following six different experimental diets.

Treatment	Rate of iron supplementation	LSP: LSG ratio
T1	50 mg/kg	
T2	75 mg/kg	50: 50 (30 g+ 30 g per kg feed)
T3	100 mg/kg	
T4	50 mg/kg	
T5	75 mg/kg	25: 75 (15 g+ 45 g per kg feed)
T6	100 mg/kg	

A total of 72 birds were distributed to 6 treatments with 12 birds per treatment. Further, each treatment had 3 replicates with 4 birds per replicate.

Daily a weighed amount of experimental diet

(Table 2) was offered to each replicate of different treatments twice in the morning and evening. Each bird had access to *ad libitum* water through channel water drinkers

Table 1. Ingredient and nutrient composition of experimental layer diet (per Kilogram)

Ingredient	T1	T2	T3	T4	T5	T6
Maize (g)	480	480	480	480	480	480
Soybean meal (g)	200	200	200	200	200	200
Rice Polish (g)	120	120	120	120	120	120
De-oiled Rice bran (g)	120	120	120	120	120	120
Inorganic Ferrous sulphate (20%) (g)	0.25	0.375	0.5	0.25	0.375	0.5
Dicalcium phosphate (g)	15	15	15	15	15	15
Limestone powder (g)	30	30	30	15	15	15
Limestone grit (g)	30	30	30	45	45	45
Common salt (g)	5	5	5	5	5	5
Metabolizable energy (Kcal)	2602	2602	2602	2602	2602	2602
Crude protein (%)	16.3	16.3	16.3	16.3	16.3	16.3
Calcium (g/kg)	30	30	30	30	30	30
Phosphorous (g/kg)	9	9	9	9	9	9
Iron (g/kg)	0.05	0.075	0.1	0.05	0.075	0.1
Lysine (g/kg)	7	7	7	7	7	7
Methionine+ Cysteine (g/kg)	4.4	4.4	4.4	4.4	4.4	4.4

Feed additives: Indomix 0.2, Indobee 0.25, Neftin 0.25, Coccidiostat 0.5, Choline chloride 0.05, Merivit (B12) 0.2, Vitamin C 0.2, Trace minerals 36 and Toxin binder 0.5 g/ Kg feed.

The data on daily feed offered, egg production, egg mass weight and mortality, if any was recorded. Egg production performance was assessed for the persistency of egg production, as average daily eggs production of hens housed in the beginning of period, expressed as percent then housed egg production (HHEP %) and intensity of egg production was determined as percent eggs produced by actual live birds and expressed as percent hen day egg production (HDEP%). However, feeding efficiency was assessed for number and mass of eggs production as feed consumed in kilogram per dozens of egg production and per Kg egg mass production. All production parameters were determined for early, mid and late laying period corresponding to 20-28, 29-37 and 38-48 weeks of age.

A total of 15 eggs per treatment were randomly collected during early, mid and late laying periods. These eggs were utilized to assess the haugh unit, albumen height, yolk colour, egg grade using automatic egg analyzer (Orka® Food Technology Ltd.). While to assess the egg shell quality, shell thickness was measured using screw gauge at broad and narrow end after removing shell membrane and average of these were taken average egg shell thickness.

For separation of iron, egg yolk was dried overnight in hot air oven at around 75°C. Subsequently, 1 gm of dried yolk sample was mixed with 10 ml. diacid (HNO₃: HCl, 3:1) in conical flask for 24 hours. After that, conical flask was heated on heating plate till yellow colour fumes evaporated and white precipitates appeared. After that samples were filtered through Whatman filter paper number 1 and washed with deionized water to make final volume of 25 ml filtrate. The egg yolk iron content was analysed by Atomic Absorption Spectrometer (ECIL Model 414) with standards Fe flame operating at 248.3 nm wavelength, air acetylene fuel, 5 milliampere, lamp current 50%, 3 milliseconds integration time.

STATISTICAL ANALYSIS

The data obtained were subjected to two ways ANOVA using GLM of SPSS 20 as per following statistical model:

$$Y_{ij} = \mu + Fe_i + Ca_j + Fex\ Ca_{ij} + E_{ijk}$$

Where,

μ : Overall mean

Fe_i : Effect of dietary iron supplementation

Ca_j ratio: Effect of dietary LSP and LSG ratio

$Fex\ Ca_{ij}$: Effect of interaction between dietary iron and LSP and LSG ratio

E_{ijk} : Error

Further, interaction means were compared with Post-hoc test for statistical difference at 5% level of significance.

RESULTS AND DISCUSSION

The data on effect of rate of inorganic iron supplementation in relation to limestone grit and limestone powder ratio, showed that during early laying period between 20-28 weeks of age (Table. 2) rate of iron and limestone grit supplementation did not have any significant effect on HHEP, HDEP, FCR per dozen egg production and FCR per kg egg mass production. Similarly, during mid-laying period between 29-37 weeks also, egg production, egg weight and feed intake among different treatment groups did not vary but FCR per Kg egg mass tends to improve with increasing rate of iron supplementation and limestone grit inclusion and was significantly ($P < 0.05$) better in group subjected to dietary iron supplementation of 100 mg/kg with 75% LSG in feed. However, iron supplementation rate and limestone source interaction were more apparent during late laying period between 38-47 weeks and found that HDEP and FCR for egg mass basis was significantly better in groups subjected to 75% of dietary LSG and 100 mg iron /kg feed treatment.

Table 2. Effect of dietary calcium source and inorganic iron interaction on egg production Performance during different period

Parameter	LSP: LSG ratio	Early laying period (20-28 weeks age)						Mid laying period (29-37 weeks age)						Late laying period (38-47 weeks age)					
		50		75		100		50		75		100		50		75		100	
		Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM
Hen house egg production (%)	50:50	81.78	1.74	82.92	1.74	82.62	1.74	76.31	2.88	78.75	2.88	79.34	2.88	73.20 ^{ax}	2.88	80.30 ^y	2.88	76.26 ^{xy}	1.75
	25:75	84.07	1.74	84.83	1.74	83.97	1.74	81.09	2.88	83.13	2.88	84.17	2.88	81.03 ^b	2.88	78.33	2.88	78.31	1.75
Hen day egg production (%)	50:50	81.78	1.78	82.92	1.78	82.62	1.78	79.04	2.95	81.80	2.95	79.34	2.95	79.79 ^{xy}	2.95	83.40 ^y	2.95	76.97 ^{ax}	1.63
	50:75	84.07	1.78	84.95	1.78	85.55	1.78	81.09	2.95	85.88	2.95	87.91	2.95	81.03	2.95	80.62	2.95	82.30 ^b	1.63
Average egg weight (g)	50:50	49.80	0.99	49.00	0.99	50.00	0.99	57.58	1.03	58.60	1.03	59.82	1.03	57.68	1.03	57.54	1.03	57.27	1.13
	25:75	51.00	0.99	50.40	0.99	50.80	0.99	61.04	1.03	58.28	1.03	59.64	1.03	58.99	1.03	58.30	1.03	59.39	1.13
FCR /dozen egg	50:50	1.48	0.03	1.43	0.03	1.48	0.03	1.72	0.05	1.72	0.05	1.79	0.05	1.80	0.05	1.81	0.05	1.89	0.08
	25:75	1.43	0.03	1.41	0.03	1.40	0.03	1.64	0.05	1.64	0.05	1.56	0.05	1.79	0.05	1.74	0.05	1.77	0.08
Feed intake per bird (kg)	50:50	6.21	0.03	6.11	0.03	6.23	0.03	8.98	0.09	9.07	0.09	8.98	0.09	8.85	0.09	9.19	0.09	9.13	0.04
	25:75	6.21	0.03	6.16	0.03	6.13	0.03	9.02	0.09	8.94	0.09	8.89	0.09	9.12	0.09	9.06	0.09	9.21	0.04
FCR (Kg)	50:50	2.18	0.07	2.02	0.07	2.13	0.07	2.85 ^y	0.12	2.40 ^x	0.12	2.66 ^{xy}	0.12	2.60 ^{xy}	0.12	2.49 ^x	0.12	2.69 ^{xy}	0.06
	25:75	2.03	0.07	1.90	0.07	1.98	0.07	2.58 ^{xy}	0.12	2.71 ^y	0.12	2.28 ^{ax}	0.12	2.52	0.12	2.63	0.12	2.52 ^a	0.06

Figures bearing different superscripts across the rows and columns differ significantly (P d” 0.05)

The data on overall production performance (Table. 3) indicates that cumulative HDEP, FCR for dozen eggs were also significantly (P≤ 0.05) better in birds supplemented with dietary iron at the rate of 100 mg /kg feed and higher inclusion rate of LSG. Earlier workers reported that increasing dietary supplementation of iron from 0 to 30 mg/Kg (El Husseiny et al., 2009) and up to 100 mg /kg (Ramadan et al., 2010) significantly improved (P≤ 0.05) egg production and FCR for egg mass basis, but iron supplementation beyond 64 mg/kg feed did not result any significant improvement in egg laying performance (Buckiuniene et al., 2016) and further, Abbasi et al., (2015) suggested 65-68 mg iron supplementation /kg feed to optimize performance of broiler breeder hens during later stage of production. However, Araujo et al. (2011) and Swaiatkiewicz et al. (2015) also found no significant effect on egg production with substitution of LSP with LSG. In present study, either increasing iron supplementation from 50-100 mg/kg or LSG from 50 to 75 % of feed did not improve egg production performance during any phase of experiment. But these findings suggest that increasing iron supplementation along with coarse calcium (LSG) had synergistic effect on egg production of laying hens especially during late laying period. As iron is an important trace mineral, actively involved in energy and protein metabolism (Drygalski and Adamson, 2013; Abbaspour et al., 2014; Ma et al., 2016; Zhang et al., 2016), so iron indispensably affect availability of nutrients for egg production. Possible explanation of synergistic interaction can be that partial replacement of LSP with LSG improved iron absorption due to prolonged retention time of LSG in gizzard (Rao et al., 1992) thus not interfering with iron absorption in duodenum after feeding during day hours. Further, slow dissolution of LSG led to sustained availability of calcium during the time of egg shell formation in the night hours. Thus, LSG and iron together, possibly facilitated energy, protein and calcium metabolism during the process of egg formation and improved laying performance.

Table 3. Overall egg production performance during (20-47 weeks age) entire experiment period

Parameter	LSP: LSG ratio	Fe (mg/kg)			SEM
		50	75	100	
Hen house egg production (%)	50: 50	77.12 ^{ax}	82.72 ^y	79.17 ^{xy}	1.33
	25:75	81.92 ^b	79.81	82.02	
Hen day egg production (%)	50: 50	80.24 ^x	84.97 ^y	79.44 ^{ax}	1.26
	25:75	81.92 ^{xy}	81.69 ^x	85.23 ^{by}	
Average egg weight (g)	50: 50	55.21	55.21	55.89	1.26
	25:75	57.34	55.76	56.47	
FCR /dozen egg	50: 50	1.68	1.60	1.73 ^b	0.119
	25:75	1.67	1.67	1.59 ^a	
FI per bird (kg)	50: 50	24.05	24.37	24.35	0.04
	25:75	24.35	24.17	24.24	
FCR (Kg)	50: 50	2.54 ^y	2.28 ^{ax}	2.51 ^{by}	0.06
	25:75	2.40 ^{xy}	2.48 ^{by}	2.28 ^{ax}	

Figures bearing different superscripts across the row and column differ significantly ($p \leq 0.05$)

Table 4. Egg quality and yolk iron content during different production phase

Parameter	LSP: LSG ratio	Early laying period (20-28 weeks age)						Mid laying period (29-37 weeks age)						Late laying period (38-47 weeks age)					
		50		75		100		50		75		100		50		75		100	
		Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM	Fe (mg/kg)	SEM
Haugh unit	50:50	52.40	5.97	55.60	5.97	60.68 ^b	64.48	6.91	64.48	6.91	40.54 ^a	51.84	6.91	53.65	57.49	53.65	57.49	57.49	4.45
	25:75	65.60	58.20	58.00	58.20	37.36 ^{ax}	73.72 ^y	63.18 ^{by}	63.18 ^{by}	63.18 ^{by}	63.18 ^{by}	57.19	0.52	51.72	54.19	51.72	54.19	54.19	4.45
Albumen height (mm)	50:50	3.20	0.45	3.20	0.45	4.46 ^b	4.78	0.52	4.78	0.52	2.90 ^a	3.87	0.52	4.02	3.92	4.02	3.92	3.92	0.35
	25:75	4.20	0.45	3.80	0.45	2.60 ^{ax}	5.56 ^y	4.42 ^{bx}	4.42 ^{bx}	4.42 ^{bx}	4.42 ^{bx}	4.00	0.52	3.52	3.66	3.52	3.66	3.66	0.35
Yolk colour grade	50:50	2.60 ^a	0.31	2.60	0.31	5.80 ^a	4.80	0.43	4.80	0.43	4.60	4.171	0.43	5.542	4.833	5.542	4.833	4.833	0.42
	25:75	3.00 ^b	0.48	3.60	0.48	4.00 ^b	4.80	0.55	4.80	0.55	4.80	5.333	0.55	6.083	5.083	6.083	5.083	5.083	0.42
Egg grade	50:50	2.80	0.48	2.80	0.48	3.80	3.60	0.55	3.60	0.55	2.20	2.42	0.55	2.42	2.17	2.42	2.17	2.17	0.18
	25:75	3.60	0.48	3.40	0.48	1.80	4.60	0.55	4.60	0.55	3.60	2.25	0.55	2.00	2.08	2.25	2.08	2.08	0.18
Average egg shell thickness (mm)	50:50	0.414 ^a	0.027	0.425 ^a	0.027	0.390 ^a	0.390 ^a	0.012	0.390 ^a	0.012	0.402 ^a	0.385 ^a	0.012	0.371 ^a	0.386 ^a	0.371 ^a	0.386 ^a	0.386 ^a	0.01
	25:75	0.524 ^b	0.027	0.518 ^b	0.027	0.404 ^b	0.430 ^b	0.012	0.430 ^b	0.012	0.424 ^b	0.398 ^b	0.012	0.391 ^b	0.396 ^b	0.391 ^b	0.396 ^b	0.396 ^b	0.01
Yolk iron (mg/100g)	50:50	14.32	1.75	14.54	1.75	10.54	10.42	2.01	10.42	2.01	13.26	13.67	2.01	16.48	14.38	16.48	14.38	14.38	2.44
	25:75	13.29	1.75	17.20	1.75	13.83	11.51	2.01	11.51	2.01	13.17	11.79	2.01	17.45	13.05	17.45	13.05	13.05	2.44

Figures bearing different superscripts across the row and column differ significantly ($p \leq 0.05$)

The data on egg quality parameters (Table 4 & 5) indicates that increasing iron or LSG grit supplementation only, did not improve albumen quality in any egg laying phase but during peak laying period between 29-37 weeks of age iron dose and calcium source interaction was observed in term of significantly ($P \leq 0.05$) better haugh unit and albumen height in birds supplemented with iron at the rate of 100 mg/kg feed and 75% LSG. As egg quality in negatively correlated with egg production rate (Anene et al., 2020) so probably effect of iron and LSG on albumen quality was more apparent during peak production in mid laying period. El Husseiny et al. (2009) with supplementation of iron at the rate of 30 mg/kg feed and Xie et al. (2019) up to 60 mg iron /kg feed through iron sulphate and iron glycine complex reported significant effect on haugh score. Improvement in egg albumen could be attributed to critical role of iron in promoting energy metabolism (Ma et al., 2016, Zhang et al., 2016). However, irrespective of iron supplementation, substitution of 75% of LSP with LSG significantly ($p \leq 0.01$) increased egg shell thickness during entire laying period. Earlier, Lichovnikova (2007), Araujo et al. (2011) and Swiatkiewicz et al. (2015) also recommended inclusion of coarse particle calcium source up to 75% to increase calcium retention and egg shell formation. This improvement in egg shell thickness could be attributed to increased calcium retention due to lower solubility of large particle calcium source (Rao and Roland, 1990 and Zhang and Coon, 1997).

Table 4. Overall egg quality and yolk iron content experimental (20-47 weeks) laying period

Parameter	LSP: LSG ratio	Fe (mg/kg)			SEM
		50	75	100	
Haugh Unit	50: 50	54.91	54.74	50.59	4.03
	25:75	53.99	60.17	57.47	
Albumen height (mm)	50: 50	3.94	3.98	3.30	0.31
	25:75	3.67	4.14	3.78	
Yolk Colour	50: 50	4.250	4.375	3.813	0.364
	25:75	3.938	4.844	4.563	
Egg grade	50: 50	3.063	2.813	2.188	0.287
	25:75	2.500	3.250	2.875	
Average egg shell thickness (mm)	50: 50	0.392 ^a	0.383 ^a	0.428	0.016
	25:75	0.434 ^b	0.439 ^b	0.436	
Yolk iron (mg/100g)	50: 50	13.55	14.21	13.98	1.17
	25:75	13.00	14.81	13.48	

Figures bearing different superscripts across the row and column differ significantly ($p \leq 0.05$)

Egg yolk iron concentration was not affected by either rate of iron concentration or substitution of LSP with LSG during any laying period. Egg yolk iron among different treatments groups during different laying period ranged from minimum 10.42 mg during mid-laying period to maximum 17.45 mg/100 g yolk during late laying period. Egg yolk iron concentration 8-15 mg/100 g yolk among all treatments during different laying periods was within the range of enriched egg yolk (Horikawa et al., 1995). Earlier, Paik et al. (2009), Ramdan et al. (2010) and Bess et al. (2012) reported significant increase in yolk iron content of eggs of birds supplemented with iron up to 100 mg iron/ kg feed than birds fed control diets without iron. However, Cornescu et al. (2014) and Buckiuniene et al. (2016) reported non-significant improvement in egg yolk iron concentration with increment in inorganic and organic source of iron supplementation of from 68 to 145 mg/Kg feed. Yolk iron deposition does not linearly increase with increasing rate of dietary iron supplementation but also limited by gut iron absorption due to minerals interaction, iron binding protein phosvitin and iron transportation protein transferring (Skrivan et al., 2005; El Husseiny et al., 2009). Abbasi et al. (2015) analyzed that 68 mg iron /kg dietary iron concentration is optimum to achieve maximum egg yolk iron level in broiler breeder hen.

CONCLUSION

It is concluded that dietary substitution of fine LSP with coarse LSG and increasing iron supplementation did not improve rate of egg yolk iron deposition, but dietary substitution of LSP with LSG up to 75% at 100 mg iron/ kg feed improved egg production performance of layers, especially during late laying phase. As iron and limestone grit interaction was more apparent during late laying phase, further experiment for prolonged period up to 80 weeks of commercial production cycle can be conducted to assess impact on laying performance, egg quality and economics of production.

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