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Calcium intake on Gramasree male bird's semen quality

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ABSTRACT

A study was assigned in 18 Gramasree male birds of 32nd weeks of age, randomly distributed to 3 experimental groups (G1, G2 and G3) with 6 birds in each group and housed separately in male cages for 91 days. During this experimental period, layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to all experimental male birds. Egg shell powder (ES) and shell grit (SG) having more than 2 mm particle size was given as free choice after feeding to G2 and G3 birds, respectively to meet their extra calcium requirement. The feed intake (FI) and body weight (BWT) were significantly different (P<0.01) between the treatment groups. ES and SG intake was 0.67 and 0.72 g/ rooster/day, respectively. Total calcium (Ca) intake was 3.20, 3.44 and 3.55 g/rooster/day in G1, G2 and G3, respectively. Semen was collected by abdominal massage at weekly intervals from 33rd to 44th weeks of age and analysed for semen volume, colour, sperm concentration and sperm motility and significantly no difference was found between the groups. The serum Ca and P content, tibial characteristic and tibial-ash were similar in all treatment groups but the tibial Ca content was significantly (P<0.01) higher in SG group. It can be concluded that, Ca intake (3.20 g/rooster/day) through feed is sufficient for male birds to meet their Ca requirements and extra ES, SG given as free choice feeding had not influenced the semen qualities without any detrimental effects.

Key words: Calcium, ES, Chemical composition, Gramasree Male birds, Semen quality

INTRODUCTION

In India, poultry rearing was primarily a backyard system until the early 1960s, and indigenous desi birds were mainly utilised for egg and meat production, despite their low productivity. The scenario of poultry production has transformed over the last six decades, with indigenous desi birds gradually being replaced by highly specialized improved varieties. Gramasree was one of the important germplasms developed by KAU to exploitation their productivity under field conditions. Their progenies were synthetic crosses of different breeds such as Barred Plymoth rock, New Hampshire, Rhode Island Red and desi breeds.
According to Kanyinji *et al.* (2010) feeding dietary Ca at 3.1 per cent level had shown enhanced seminal Ca level, sperm motility, thermo-tolerance and cryo-survivability due to increase in Ca seminal plasma in Barred Plymouth Rock roosters. Tyler *et al.* (2021) had mentioned high Ca level (3.05%) showed significantly poorer sperm concentration at 42 and 57 weeks of age of broiler breeder male birds.

In backyard poultry farms, usually layer breeder ration was fed to male birds, which contains more Ca content and effect of excess Ca feeding and data on Ca requirements for improved varieties of native chicken male birds are scanty. In this scenario, the aim of the present study was ascertained to find out the maximum level of Ca intake and effect on semen quality of male Gramasree birds by providing free-choice feeding of ES and SG.

MATERIALS AND METHODS

Experimental procedures were approved (approval number, IAEC/COVAS/PKD/8/2021) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

The chicken ES used in this study was collected

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from bakeries, washed in running tap water and soaked in water for 5 h. Then, shell membrane was manually removed and sundried for 48 h and dried again in hot air oven at 120°C for 24 h, powdered in laboratory mill then packed in bags for further processing. The SG was purchased from the local market.

The particle size of ES and SG was studied by taking 100 g of representative samples of egg shell and SG were manually sieved in BSS 8 (0.75 mm) and BSS 22 (2 mm) sieves for five minutes at one minute interval and quantities were weighed in electronic balance. The solubility of Ca sources was determined by in-vitro method proposed by Zhang and Coon (1997).

The experiment was conducted at Poultry Farm, ILFC, College of Veterinary and animal sciences, Pookode, during May to July'21. Eighteen Gramasree male birds of 32nd weeks of age was randomly distributed to 3 experimental groups (G1, G2 and G3) with 6 birds in each group and housed separately in male cages for 91 days. During this experimental period, layer breeder ration (control) was prepared as per ICAR, (2013) recommendations and fed to all experimental male birds. The ingredient composition was shown in the table-1.

Table 1. Ingredient composition of Layer breeder ration (per cent)

Raw material	Composition
Maize	54.0
De-oiled Rice Bran	5.0
Wheat Bran	4.0
Soya bean meal	25.4
Limestone powder	4.0
Shell grit	5.0
Di Calcium Phosphate	1.4
Dl-Methionine (98.5 per cent)	0.2
Salt	0.5
Trace Mineral Mix	0.125
Vitamin Premix	0.05
Choline Cholride	0.2
Toxin Binder	0.1
Liver extract powder	0.025
Analytical values	
Crude Protein (%)	17.65 ± 0.24
Crude Fibre (%)	9.26 ± 0.15
Total Ash (%)	14.99 ± 0.35
Calcium (%)	4.85 ± 0.27
Total Phosphorus (%)	1.90 ± 0.19

All the birds were maintained under standard management conditions prevailing in the farm during the entire period of experiment. The birds were provided with 2 times feed and ad-libitum water. They were exposed to a 16 h photoperiod (16 Light: 8 Dark) daily and lights were turned off manually. The feed, ES and SG intake were calculated from offered quantity and left over on daily basis and Ca intake from feed and different Ca sources were calculated at weekly interval. The body weight was taken before and after the end of the experiment.

The semen was collected in a sterile glass funnel, between 10.00 - 10.30 am by abdominal massage method (Burrows and Quinn, 1937) from four roosters housed in cages from each group at weekly interval from 33nd to 44th weeks of age. Semen

volume was measured use of a collection tube with graduation to measure 0.01 ml. The semen colour was scored as 1–5 by visual examination (Score 1-Watery or clear semen; Score 2 -Watery semen with white streaks; Score 3-Medium white semen; Score 4-Thick white semen; Score 5-Very viscous chalky white semen) by McDaniel and Craig (1959) procedure.

Immediately after semen collection, the semen was four-fold diluted with semen diluent as per Chaudhuri and Lake (1988) and stored for further semen quality analysis. Sperm motility (per cent) was examined by under the low power of light microscope (40 x) field and scored as per Wheeler and Andrews (1943). Sperm concentration was estimated as per the procedure suggested by Taneja and Gowe (1960).

The serum Ca and P (Farrell E C, 1984) were measured using biochemical kits in a semi-automated clinical chemistry analyser (Merck, Microlab 300). At end of experimental period, four roosters from each group were randomly selected to estimate tibial ash and Ca content (Gongruttananun, 2011). The chemical composition of the layer breeder feed, ES and SG were analysed as per AOAC (2016).

Data of the study were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The chemical composition of egg shell and SG is presented in table 2.

Table 2. Chemical composition of Egg shell powder and Shell grit fed to experimental birds during 33rd to 44th weeks of age (%), on dry matter basis

Attributes	Eggshell	Shell grit
Moisture	0.56 ± 0.24	0.70 ± 0.24
Crude Protein	8.30 ± 0.41	7.47 ± 0.41
Crude Fibre	2.90 ± 0.26	1.58 ± 0.26
Ether Extract	0.70 ± 0.06	0.48 ± 0.06
Total Ash	93.45 ± 0.60	94.86 ± 0.60
Acid insoluble ash	0.34 ± 0.11	0.97 ± 0.11
Calcium	42.16 ± 0.48	39.11 ± 0.48
Phosphorous	1.70 ± 0.32	3.46 ± 0.32

In this present study, egg shell without shell membrane contains 8.30 % CP, 42.16 % Ca and 1.70 % P which is higher than reported values reported by Islam (2021) in kitchen-extruded egg shell and hatchery-extruded egg shell. Islam (2021) reported kitchen-extruded egg shell contains, 4.24 % CP, 29.75 % Ca and 14.82 % available P and hatchery–extruded egg shell contains 13.80 % CP, 25.53 % Ca and 13.87 % available P. The estimated CP in the present study was higher than kitchen-extruded egg shell and lesser than hatchery-extruded egg shell reported by Islam (2021), however lesser value of 2.14 % CP was reported by Lertchunhakiat *et al.* (2016).

In this study, Ca content estimated as 42.16 % on DMB which was higher than the previously mentioned values of 33.5– 34.8 % Ca in eggshell by Muir *et al.* (1976) and Olgun *et al.* (2015) reported value of 32.3 %. Further to that, as explained by Islam (2021), egg shells from hatchery had lower Ca and P content because of Ca and P utilization by embryo during incubation. The variation in nutritive value of egg shell might be due to source of collection, processing involved and study materials used in this study was collected from bakeries which are infertile eggs.

The dietary Ca requirement for birds was highly influenced by particle size, solubility and source of Ca. The relative proportion particle size, in-vitro solubility of egg shell and SG was studied and presented in table 3.

Table 3. Particle size and in-vitro solubility of shell grit and egg shell

Particle size (mm)	Egg shell	Shell grit	SEM	P value
Quantity (per cent, by weight)				
Coarse (> 2.00)	84.13[a]	69.11[b]	0.46	0.001*
Medium (< 2.00)	15.36 [b]	22.56 [a]	0.43	0.001*
Fine (< 0.75)	0.39 [b]	7.01 [a]	0.06	0.001*
In-vitro solubility (per cent)				
Coarse (> 2.00)	40.61 [b]	56.21 [a]	0.54	0.001*
Medium (< 2.00)	57.33 [b]	76.51 [a]	1.35	0.001*
Fine (< 0.75)	55.39 [b]	92.44 [a]	0.74	0.001*

[ab] Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 [ns] non-significant

It was found that coarse particle (>2.00 mm) in ES (84.13 %) was comparatively more than SG (69.11 %) and fed to the male birds in this study. The in-vitro solubility of coarse egg shell (more than 2 mm) used in this study was comparatively lesser than SG. Like that, Pizzolante *et al.* (2011) mentioned that, limestone powder with 0.44 mm particle size had 31 % solubility, 2.40 mm coarse limestone powder with 28 % and 46 % in-vitro solubility was recorded for oyster shell which similar to SG used in this study.

The mean feed intake (FI) of the roosters in this experiment was 87.58, 86.86 and 88.28 g/rooster/day, in control, egg shell and shell group, respectively and illustrated in the table 4.

Table 4. Mean values of Calcium Intake, Semen quality and BWT of Gramasree roosters from 33 to 44 weeks of age

Parameter	Group			SEM	p-value
	Control	Egg shell	Shell grit		
Feed intake (g)	87.58[b]	86.86[c]	88.28[a]	0.22	0.001*
Egg shell powder and shell grit intake (g)	-	0.67[b]	0.72[a]	0.00	0.002*
Calcium intake from feed (%)	3.20[b]	3.18$_c$	3.25[a]	0.01	0.001*
Calcium intake from egg shell and shell grit (%)	-	0.26	0.30	0.00	0.06[ns]
Total calcium intake (%)	3.20	3.44	3.55	0.01	0.04[ns]
Semen characteristics					
Semen volume (mL)	0.13	0.16	0.14	0.04	0.54[ns]
Semen colour (score)	2.35	2.81	2.83	0.25	0.32[ns]
Sperm motility (%)	47.92	61.04	61.46	8.19	0.28[ns]
Sperm concentration (x109 cells /mL)	4.25	5.49	5.31	0.88	0.45[ns]
Serum parameters					
Calcium (mg/dL)	13.66	17.38	17.43	2.34	0.40[ns]
Phosphorous (mg/dL)	6.19	6.32	6.18	0.85	0.27[ns]
BWT (kg/male bird)					
32 weeks	2.307	2.177	2.400	0.09	0.25
44 weeks	2.603[a]	2.311[b]	2.029[c]	0.108	0.01*

[a, b, c] Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 [ns] non-significant

As age increased, control group birds FI was significantly (P<0.01) increased and not evidenced in other treatment group birds. Gongruttananun (2011) reported FI was more in egg shell group (118.56 g/ rooster/ day) and Lertchunhakiat *et al.* (2016) reported that the ES inclusion at 10.8 % level did not influence the FI and BWT. Meanwhile, the BWT was reduced in SG group compared to control and egg shell group birds in this experiment. The present study reports revealed that, male birds consumed 3.46, 3.49 and 3.25 g of Ca/rooster/day in control, egg shell and shell group, respectively during the experimental period and observed male birds consumed only 0.67 g/bird/day of eggshell and 0.72 g/bird/day of SG, which contributed 0.26 and 0.3 g of Ca/bird/day, which fed as free choice source of Ca. However, ICAR (2013) recommends minimum Ca requirements of 0.8 % for Gramapriya male breeder birds.

The mean semen volume of G1, G2 and G3 group roosters are 0.13, 0.16 and 0.14 ml/rooster, respectively during the experimental period and Chauhan *et al.* (2009) reported in Gramapriya cocks average ejaculate volume of 0.39 ± 0.02 ml/rooster with sperm concentration and total sperm count of 2.49 ± 0.03 x 109/ ml and 0.98 ± 0.04 x 109/ ejaculate. Whereas, weekly mean semen concentration recorded in present study was 4.25, 5.49 and 5.31 x 109/ ml in control, egg shell and SG groups, respectively and higher than Chauhan *et al.* (2009) reported value. However, no significant differences were found in semen concentration between the groups and the results were similar to Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

Like that, Gongruttananun (2011) and Lertchunhakiat *et al.* (2016) also reported 0.37 ml/ ejaculate and 0.39 ml/ejaculate, respectively and reported that source of Ca not affected the semen volume, corroborates with our findings. Meanwhile, semen colour average score evaluated during this study are 2.35, 2.80 and 2.83 and results were similar to findings of Shanmugam *et al*. (2012) and Lertchunhakiat *et al.* (2016). The average semen motility reported in this study was 47.92, 61.04 and 61.46 % in control, egg shell and SG groups, respectively and no significant difference in semen motility between the groups was observed and same findings were reported by Shanmugam *et al.* (2012) and Lertchunhakiat *et al.* (2016).

In agreement to the present findings, Namntu, (2011) reported that the effect of feeding female ration to male broiler breeders and found that it had no influence on male fertility but it increased the male BWT. Like that, Khalil *et al.* (2012) reported that cocks fed with 2 and 3 % Ca levels had superior forward sperm motility, live sperm and fertility percentage compared with those fed with 1 % Ca.

Table 5. Tibia Characteristics of experimental Gramasree male birds

Treatment	Control	Egg shell	Shell grit	SEM	p-value
Tibial weight (g)	6.11^{b}	6.80^{a}	5.58^{c}	0.001	0.001*
Tibial length (mm)	126.27^{a}	126.4^{a}	120.90^{c}	0.269	0.001*
Tibial thickness (mm)	6.10^{b}	6.53^{a}	5.97^{c}	0.072	0.001*
Tibial width (mm)	13.33	13.33	13.07	0.39	0.86^{ns}
Tibial Ash (%)	53.42	56.52	55.82	2.16	0.59^{ns}
Tibial calcium (%)	19.66^{c}	20.96^{b}	26.58^{a}	0.61	0.001*

^{abc} Mean values with different superscripts within a row differ significantly

* Significant at p<0.01 ^{ns} non-significant

It was understood that, extra Ca intake through feed had not influenced the serum Ca, P content and similar in all the treatment groups. The tibial bone weight, length, thickness and tibial Ca content were significantly differed between the groups. Higher tibial Ca content (P<0.01) and lesser tibial bone weight was observed in SG group compared to other groups and similar findings were reported by Gongruttananun (2011). The increased tibial bone Ca content in SG fed group might be due to increase

in bio-availability of Ca as evidenced by in-vitro solubility studies of SG. The fecal Ca excretion studies may clarify further, which not studied in this experiment.

CONCLUSION

It can be concluded that, breeder layer ration with 3% Ca was sufficient to maintain the semen quality without any detrimental effects and Ca intake up to 3.49 g/male bird/day had not influenced the semen qualities and ES can be used as extra Ca source for Gramasree male birds.

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