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Effect of glucosamine supplementation on performance and egg quality of laying chicken

TRIPATHI M K[1]✉, KAMAL R K[2], KUMARI RAJNI[1], RAMAN R K[1], RAY P K[1], CHANDRAN P C[1], DAYAL S[1], DEY A[1] and SARMA K[1]

ICAR-Research Complex for Eastern Region Patna, Bihar, India, 800014

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ABSTRACT

The experiment was conducted to explore the effect of supplemental glucosamine on egg production and quality performance of Vanaraja Chickens. The chickens of same age (8 week) were divided into three equal groups i.e. Control (C), Group 10, and Group 20. Birds in group G10 and G20 were supplemented with glucosamine hydrochloride @ doses of 10 mg/kg and 20 mg/kg of body weight/day respectively, via drinking water individually in a plastic container kept in cages. The amount of the supplemented glucosamine was based on their body weight taken fortnightly. The effect of glucosamine on laying performance of the treated groups was compared with control group. For hormonal estimation, blood samples were collected at 15 weeks and 22 weeks of age. Data were analysed using ANOVA (Analysis of Variance) and paired t-test. It was found that the age at first egg laying (AFE) was lesser in G10 and G20 as compared to control group. Egg production was highest in the G10 group based on initial six month records. There was no significant difference of glucosamine supplementation in the important quality parameters including egg weight, shell weight, shell thickness, yolk weight and albumin weight. There was significant increase in the plasma estrogen and leptin level between 15 weeks and 22 weeks of age in all the three groups indicating their role in attaining puberty. Our results indicate that glucosamine hydrochloride may have role in egg laying performance in poultry.

Keywords: Age at first egg, Egg quality, Estrogen, Glucosamine hydrochloride, Leptin

Glucosamine is the basic structural unit of chitin and chitosans. Glucosamine supplementation has been reported to have several beneficial effects in human, animals and poultry. Supplementation of glucosamine sulfate in the diet had resulted in enhanced weight gain in broilers (Martins *et al*. 2020). Chitosans when used as feed additive for poultry and farm animals, have shown antibacterial, anti-inflammatory, antitumor, antioxidative, immunostimulatory hypocholesterolemic and growth promoting properties (Kamal *et al.* 2022). Chitosan oligosaccharide is a potent preventative and therapeutic agent for premature ovarian failure which acts by enhancing the ovarian immune response and inhibiting germ cell senescence (Li *et al*. 2023).

Ryczko *et al.* (2016) had reported that orally administered glucosamine leads to enhanced leptin hormone production in the circulation in mice. The orally administered GlcNAc is rapidly absorbed, enters systemic circulation, and then the glucosamine enters into the cell via glucose transporter proteins (GLUTs) and get metabolized through the hexosamine biosynthetic pathway to increase intracellular UDP-GlcNAc pool (Ryczko *et al.* 2016., Pizzolatti *et al.* 2018). The increased tissue concentration of UDP-N-acetylglucosamine (UDP-GlcNAc) results in rapid and marked increase in leptin in skeletal muscle and adipose tissues (Wang *et al.* 1998, Considine *et al.* 2000). Glucosamine rapidly activates *ob* gene expression (that encodes leptin hormone) in skeletal muscle (Wang *et al.* 1998). Considine *et al.* (2000) also reported that hexosamine biosynthesis regulates leptin production in human adipose tissue and there is significant positive relationship between adipose tissue UDP-N-acetylglucosamine and serum leptin.

Leptin plays a central role in the metabolic control of puberty and causes early onset of reproductive function. The circulating leptin level and leptin gene expression increase markedly and linearly in cows and girls progressing into puberty (Garcia *et al.* 2002, Zhu *et al.* 2016). Age at first egg laying is commonly used in defining female sexual maturity in hen and is significantly associated with reproduction performance (Tan *et al.* 2021). In female chickens exogenous leptin significantly advances the onset of puberty (Paczoska- Eliasiewicz *et al.* 2006). This early onset of puberty is accomplished by attenuation of ovarian apoptosis, enhancement of folliculogenesis and a rise in

Present address: [1]ICAR-Research Complex for Eastern Region Patna, Bihar, 800014 India, [2]ICAR-Research Complex Eastern Region, System Research Centre for Hill and Plateau Region, Ranchi, Jharkhand, 834010, India. ✉Corresponding author email:tripathimanoj123@rediffmail.com

luteinizing hormone, estradiol, and progesterone hormones (Paczoska- Eliasiewicz *et al.* 2006). Similar reports are available in case of Japanese quail wherein administration of leptin during embryonic development induced faster development and evoked earlier onset of sexual maturity. Females treated with leptin reached the 100% egg laying earlier and their production of total egg mass per quail and the egg laying rate was higher (Ma´cˇajova´ *et al*. 2002). Leptin had a dose-dependent impact that accelerated pubertal development in quails (Gündüz 2023)

Considering the previous reports that glucosamine rapidly activates leptin production, we hypothesize that the supplementation of glucosamine will increase its intracellular pool and thus push leptin level to a threshold value to trigger earlier sexual maturity. Reduced age of onset of sexual maturation may result in greater egg production in poultry and overall reproductive efficiency. In addition the use of chitosan to improve egg production has been documented (Li *et al.* 2022). Keeping these in view, we undertook this experiment with the objective of supplementing glucosamine on egg laying performance of the chicken.

MATERIALS AND METHODS

*Ethical approval statement:*The study was conducted in accordance with the ethical standards and guidelines of the Institutional Animal Ethics Committee (IAEC) of ICAR-Research Complex for Eastern Region, Patna, India.

Production of Vanaraja chickens and experimental design: The study was conducted on Vanraja chickens maintained in the Experimental Livestock Farm Complex of ICAR-Research Complex for Eastern Region, Patna, Bihar. The parent stock was maintained in deep litter system. Eggs were collected, cleaned and transferred to automated incubator at optimum temperature and humidity. The hatched chicks were grown as per standard protocol. Total eighteen female birds at 8 weeks of age were divided into three equal groups i.e. control, Group 10, and Group 20 and kept in cages. The body weights (g) of the selected birds at 8 week of age for control, Group 10 and Group 20 were similar (605.38±8.15, 610.076±8.39 and 613.46±6.95 respectively). Group 10 and Group 20 were supplemented with glucosamine hydrochloride @ 10 and 20 mg/kg body weight/day, respectively, in drinking individually in a plastic container kept in cages. All the birds were kept under good nutritional and management condition. Egg production was recorded for six months and egg quality parameters were evaluated of first egg produced (E1) and egg laid after 3 months of first egg (E2) from all the groups. The blood was collected at 15 weeks of age and 22 weeks of age (approaching laying) for hormonal estimation.

Hormonal estimation: Serum hormonal profiles (leptin, estrogen) were estimated using chicken leptin and estrogen ELISA (Enzyme linked immune sorbent assay) kit (catalogue no-ITECH 0095 and ITECH 0079, G-biosciences respectively). The assay was performed as per manufacturer's protocol.

Egg production and quality assessment: Age of first egg laying was recorded and the external and internal egg quality parameters of the first egg (E_1) produced was analysed for all the birds. This was followed by assessment of egg quality parameters of the egg produced after 3 months (E_2). The total egg production for first six months was recorded. The external egg quality traits *viz*. gross egg weight (EW) in g, egg length (L) in cm, egg width (W) in cm, shell weight (SW) in g, and the internal egg quality traits such as yolk weight (YW) in g, albumin weight (AW) in g, yolk height (YH) in mm, albumin height (AH) in mm, yolk diameter (YD) in cm and albumin diameter (AD) in cm, were estimated. The eggs were weighed on an electronic balance to determine their weights. Subsequently, L and W of the eggs were measured by slide callipers. Each egg was broken on a table and its contents were poured into a plate. Then the yolk was separated from the albumen with the help of a spoon and weighed. The AW was calculated by subtracting YW and SW from the gross EW (*i.e.* AW = EW-(YW + SW)]. Screw gauge was used to determine the shell thickness from the broad end, narrow end and the middle of the shell, and the average of the three measurements was taken as shell thickness in millimetre. Albumen and yolk heights were measured using a spherometer.

Statistical Analysis: The impact of glucosamine on Vanaraja chicken among the three groups (Group 10, Group 20 and control) was compared using Analysis of Variance (ANOVA) following Random Block Design (RBD). The treatments means were compared using Tukey's test. Chickens of two different ages at 15 weeks and 22 weeks are compared for selected parameters using paired T-test and their significant difference was measured. The ANOVA was performed using SAS 9.3 software.

RESULTS AND DISCUSSION

To the best of our knowledge, the potential of glucosamine to induce early sexual maturity (or age at first egg production) has not yet been evaluated in any domestic species including poultry and the literature available is scanty.

Table 1. Hormonal profile of chicken at 15 and 22 weeks of age among control and treatments (G10 & G20) groups

Parameters	15 weeks age			22 weeks age		
	Control	G10	G20	Control	G10	G20
Leptin (pg/ml)	231.61±66.00[a]	211.42±80.59[a]	286.40±80.68[b]	1298.21±1096.17[a]	965.22±556.62[a]	1569.49±790.14[a]
Estrogen (pg/ml)	166.21±80.19[a]	247.58±144.95[a]	340.56±123.41[a]	392.89±53.43[a]	450.04±94.45[a]	430.77±58.10[a]

Values being different superscripts (a, b) differ significantly (p > 0.05) within a row for 15 week and 22 weeks of age.

Table 2. Comparison of chicken hormonal profile in control and treatments (G10 & G20) groups at 15 and 22 weeks of age

Parameters	Groups	15 week age	22 week age	p value
Leptin	Control	231.60	1298.21	<0.05
	(treatment1)	211.42	965.22	<0.01
	(treatment2)	286.40	1569.49	<0.01
Estrogen	Control	166.21	392.89	<0.01
	(treatment1)	247.58	450.04	<0.05
	(treatment2)	340.56	430.77	<0.05

Age at first egg laying and hormonal profile: The age at first egg laying in control, G10 and G20 groups was 164.16±5.41, 159.16±3.31 and 162.33±7.42 days respectively. The data revealed that the age of first egg is lesser in G10 and G20 as compared to control.

Leptin: The levels of serum leptin in control, G10 and G20 at 15 weeks and 22 weeks of age have been given in table 1. The serum leptin levels were higher in G20 group followed by control and G10 group in both 15 weeks and 22 weeks of age. There was significant increase ($p<0.05$) in serum leptin in all the three groups at 22 weeks of age as compared to 15 weeks of age (table-2). In addition there was significant effect of supplementation of glucosamine in G20 groups on serum leptin level at 15 weeks of age; however, there was no significant difference of supplementation of glucosamine at 22 weeks of age (time approaching puberty) among the three groups.

Leptin hormone level in circulation in Vanaraja chickens in our study is comparable to the findings of Panigrahy *et al.* (2016). Our finding that there was significant increase in serum leptin in the birds approaching sexual maturity (22 weeks) as compared to at 15 week of age in all the groups, indicates that leptin hormone may have a role in sexual maturity in birds also. Our finding also supports the other previously published reports wherein it has been reported that serum leptin increases linearly as puberty approaches in rodents and heifers (Ahima *et al.* 1997, Garcia *et al.* 2002). It has been reported that exogenous leptin significantly advances the onset of puberty in female chickens and Japanese quail (Paczoska- Eliasiewicz *et al.* 2006, Ma´cˇajova´*et al.* 2002, Gündüz 2023). On contrary, Ni *et al.* (2013) reported that sexual maturation in hens was not associated with increase in the serum leptin. Munoz *et al.* (2002) also did not found any increase in circulating concentrations of leptin after five days of glucosamine infusion in ewes however; its infusion had effects on folliculogenesis and aromatase expression.

In our experiment, oral supplementation of glucosamine hydrochloride caused decrease in AFE in glucosamine supplemented groups as compared to control. There was significant effect of supplementation of glucosamine in G20 groups on serum leptin level at 15 weeks of age. This indicates that glucosamine supplementation @ 20 mg/kg body weight may induce leptin before puberty, however; at 22 weeks of age at time closer to puberty there was no significant difference of supplementation of glucosamine among the three groups. The effect of glucosamine on leptin production was highest in the G20 group however; the AFE waslesser in G10 group indicating that the reduction in AFE caused by glucosamine might have occurred through other mechanism and not through leptin production.

Estrogen: The levels of serum estrogen (E2) at 15 weeks and 22 weeks of age have been given in table 1. There was significant increase ($p<0.05$) in serum estrogen in all the three groups in 22 week of age as compared to 15 week of age (table-2). At 15 weeks and 22 weeks of age, serum estrogen level was higher in both G10 and G20 groups as compared to control however; the same does not differ significantly among the groups (table1).

In poultry, estradiol-17β (E2) is the major hormone involved in reproduction and the process of sexual maturation (Van der Klein *et al.* 2019). Higher estrogen level may be associated with the earlier AFE as supported by the findings of Van der Klein *et al.* (2019) who reported that the *ad libitum* feeding during the rearing period resulted in higher E2 levels and an earlier AFE compared to restricted feeding. Mehlhorn *et al.* (2022) reported that the concentration of estradiol -17ß is strongly linked with the laying performance in poultry. Our results showed that the serum oestrogen levels were higher in glucosamine treated groups as compared to control in the birds both at 15 weeks and 22 weeks of age. The higher estrogen levels in the birds approaching sexual maturity may be associated with earlier AFE in treatment groups as compared to control. The glucosamine supplementation appears to be more effective in G10 group wherein the estrogen level at 22 week of age (about one week prior to average AFE) was the highest. Our results gain support from the finding of Hanlon *et al.* (2022) who reported that regardless of strain, the first egg was laid one week after the appearance of functional peak in estradiol. Our presumptions that glucosamine hydrochoride supplementation may be involved in estrogen production, gain support from the finding of Feng *et al.* (2020) who reported that glucosamine (GlcN) supplementation before mating improves reproductive outcomes and causes significant increase in maternal estradiol concentrations estimated on day 19.5 of pregnancy in rats. Very little literature is available on the effect of glucosamine on leptin and estrogen hormone production and its subsequent effect on AFE, however recently it has been reported that dietary glucosamine sulphate increased serum, estrogen (Wang *et al.* 2024) in chicken.

Laying performance: Total egg production per bird in control, G10 and G20 from start of egg laying to six month were 78.77, 85.07, 73.85 eggs respectively showing the highest egg production for first six month in G10 group. The high egg production in G10 may be due to earlier start of egg production in the group as well as the higher concentration of estrogen which has been reported to be strongly linked with the laying performance in poultry (Mehlhorn *et al.* 2022). Our finding is alignment with the finding of Li *et al.* (2022) who reported that addition of

Table 3. Egg quality parameters of control, G10 and G20 groups at first egg (E1) and egg laid after 3 months (E2)

Parameters	Control	G10	G20	Control	G10	G20
		E1 (first egg)			E2 (Egg after 3 month)	
Egg weight(g)	41.11±2.31^{a}	42.37±2.42^{a}	42.30±2.44^{a}	53.99±1.25^{a}	52.74±0.61^{a}	52.25±0.83^{a}
Egg length(cm)	5.13±0.10^{b}	5.05±0.16^{b}	5.39±0.23^{a}	5.66±0.16^{a}	5.60±0.10^{a}	5.65±0.05^{a}
Egg width(cm)	3.79±0.10^{a}	3.85±0.06^{a}	3.79±0.06^{a}	4.10±0.08^{a}	4.03±0.08^{a}	4.05±0.05^{a}
Shell weight (g)	4.54±0.39^{a}	4.50±0.48^{a}	4.68±0.40^{a}	5.05±0.40^{a}	5.47±0.22^{a}	5.46±0.33^{a}
Shell thickness (mm)	0.30±0.05^{a}	0.28±0.05^{a}	0.33±0.06^{a}	0.28±.02^{a}	0.30±0.01^{a}	0.31±0.02^{a}
Yolk diameter (cm)	3.55±0.23^{a}	3.70±0.32^{a}	3.60±0.13^{a}	4.22±0.08^{a}	4.33±0.07^{a}	4.28±0.11^{a}
Albumin height (mm)	6.54±1.17^{a}	6.97±1.37^{a}	6.39±0.99^{a}	6.85±0.13^{a}	6.81±0.33^{a}	6.33±1.00^{a}
Yolk height(mm)	12.40±0.35^{b}	13.78±0.72^{a}	12.61±0.77^{b}	13.73±1.14^{a}	12.56±0.41^{b}	12.40±0.40^{b}
Yolk weight (g)	12.14±3.02^{a}	14.52±2.97^{a}	12.31±0.45^{a}	19.01±3.48^{a}	20.44±1.33^{a}	19.81±2.33^{a}
Albumin weight (g)	24.38±2.81^{a}	23.34±2.36^{a}	25.14±1.86^{a}	28.30±2.55^{a}	25.27±0.90^{a}	25.57±2.49^{a}
Albumin diameter (cm)	6.09±0.39^{a}	6.19±0.67^{a}	5.95±0.43^{a}	7.18±0.34^{a}	7.13±0.11^{a}	7.33±0.28^{a}

* Values being different superscripts (a, b) differ significantly (p > 0.05) within a row for E1 and E2 eggs.

chitosan to layer hen diet could increase egg production.

External quality: The effect of glucosamine on egg quality parameters is presented in table 3. There was significant increase in egg weight from E1 to E2 eggs among all the groups. However; the egg weight differed non-significantly in treatment groups as compared to control in both E1 and E2. Our finding match with the finding of Wang *et al.* (2024) who recorded no significant difference in the egg weight of chickens from 21 to 29 week of age in glucosamine treated groups as compared to control. We found that the egg length was significantly ($p<0.05$) higher in G20 as compared to other two groups in E1 eggs while there were no significant differences in egg width, and egg length among the control and treatment groups in E2 eggs.

The egg shell thickness was found to be higher in G20 as compared to control and G10 in E1 eggs. There was dose dependant non-significant increase in the shell thickness in E2 eggs also. There were no significant differences among the control and treatment groups in egg shell weight of first egg (E1), however; it was highest in G20. In the E2 eggs, egg shell weight was higher in treatment groups. Egg shell strength is highly associated with thickness (Ha *et al*. 2007). Chicken egg shell contains D-glucosamine in calcified shell (Liu *et al*. 2016). In our result the higher shell thickness and shell weight in G20 group as compared to control in both E1 and E2 eggs might be due to the enhanced utilisation of supplemental glucosamine in egg shell formation. Our results are in agreement with the findings of Wang *et al.* (2024) who reported that dietary glucosamine sulphate exerted a positive effect on the eggshell weight, eggshell thickness and eggshell strength in chicken.

Internal quality: There were no significant differences in the weight, height, and diameter of albumin among the control and treatment groups in first egg (E1). The albumin height, albumin weight and albumin diameter were significantly ($p<0.05$) higher in E2 eggs as compared to E1 in control group. Ovalbumin constitutes more than half of

Table 4. Comparison of egg quality parameters between control, G10 and G20 groups of first egg (E1) and egg laid after 3 months (E2)

Parameters	Groups	E1	E2	p value
Egg weight (EW)	Control	41.11	53.99	<0.01
	(treatment1)	42.37	52.75	<0.01
	(treatment2)	42.30	52.25	<0.01
Egg length (L)	Control	5.13	5.67	<0.01
	(treatment1)	5.05	5.60	<0.01
	(treatment2)	5.39	5.65	0.015
Egg width (W)	Control	3.79	4.10	<0.01
	(treatment1)	3.85	4.03	<0.01
	(treatment2)	3.79	4.05	<0.01
Shell weight (SW)	Control	4.54	5.05	0.06
	(treatment1)	4.50	5.47	<0.01
	(treatment2)	4.68	5.46	<0.01
Shell thickness (ST)	Control	0.30	0.28	<0.01
	(treatment1)	0.28	0.30	0.32
	(treatment2)	0.33	0.31	0.23
Yolk diameter (YD)	Control	3.55	4.22	0.21
	(treatment1)	3.70	4.33	<0.01
	(treatment2)	3.60	4.28	<0.01
Albumin height (AH)	Control	6.54	6.85	<0.05
	(treatment1)	6.97	6.81	0.39
	(treatment2)	6.39	6.33	0.46
Yolk height (YH)	Control	12.4	13.73	<0.01
	(treatment1)	13.7	12.56	<0.01
	(treatment2)	12.61	12.40	0.28
Yolk weight (YW)	Control	12.14	19.01	<0.01
	(treatment1)	14.52	20.44	<0.01
	(treatment2)	12.31	19.81	<0.01
Albumin weight (AW)	Control	24.38	28.31	<0.05
	(treatment1)	23.34	25.27	<0.05
	(treatment2)	25.14	25.57	0.33
Albumin diameter (AD)	Control	6.09	7.18	<0.01
	(treatment1)	6.19	7.13	<0.01
	(treatment2)	5.95	7.33	<0.01

the egg white and is the important ingredient of egg white. The content of ovalbumin affects albumin height and the glucosamine sulfate is a component of ovalbumin (Wang *et al.* 2024). We did not find any effect of glucosamine on albumin height in both E1 and E2 eggs which in contrast to the finding of Wang *et al.* (2024) who reported that glucosamine sulphate increased albumin height. There were no significant differences in yolk weight among the control and treatment groups in both E1 and E2 eggs. The yolk height were significantly lesser ($p<0.05$) in the treatment groups of E2 egg as compared to control and did not agree with the findings of Wang *et al*. (2024). The yolk weight and height were significantly higher in E2 eggs as compared to E1 in control group. Xu *et al*. (2020) reported that supplementation of chito-oligosaccharide improved egg production, egg mass and exerted positive effects on eggshell strength and eggshell thickness and feed conversion.

Table 5. Percentage (%) increase in egg albumin and egg yolk weight in E2 eggs (with respect to E1 eggs) in control, G10 and G20 groups

Percentage (%) increase in egg albumin weight and egg yolk weight in E2 eggs (with respect to E1 eggs)			
Parameters	control	G10	G20
Albumin weight	16.08	8.27	1.71
Yolk weight	56.59	40.78	60.92

The significantly higher egg weight, length, width, shell weight, yolk weight; and albumin diameter in E2 eggs as compared to E1 in all the groups (table 4) indicates that the reproductive maturation is not completed at first egg production in hen and continued thereafter. Our results show that the change in the egg weight between E1 and E2 is largely contributed by changes in yolk weight whereas the proportional increase in egg albumin is relatively lesser (table 5). In hen the yolk formation and deposition is regulated by the liver-blood-ovarian axis involving many hormones especially oestrogen (Liu *et al.* 2018). Our results that yolk formation increased by about 40-60% after 3 month from first egg production (159 to164 days) gain support from the finding of Liu *et al.* (2018) who reported that liver lipid synthesis increased in hens from D90 (before laying) to D150 (early laying) and remained at higher levels at D280 (peak laying) and the serum 17β-estradiol level increased from D90 to D280. This indicates that the lipid synthesis is positively related with estrogen level peak. In our results also, the estrogen level as well as yolk weights were higher in G10 group.

In conclusion, our results indicated that glucosamine hydrochloride supplementation may have variable effects on different egg quality parameters and egg laying performance in poultry.

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