Nigerian J. Anim. Sci. 2019, 21 (3): 117-127

Dietary salt potentiates folliculogenesis and modulates the functionality of the oviduct in late production layers

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Target audience: *Poultry farmers, Reproductive Physiologists, Endocrinologists and Researchers with a bias in the Biological Sciences.*

Abstract

This experiment was conducted to investigate the effects of varied levels of inclusion of dietary salt on follicular development, gonadal morphometry and hormonal profile of laying chickens in their last phase of production. Four experimental diets were formulated with varied levels of dietary salt. The control diet had 0.25% salt, whilst the remaining diets had 0.50%, 0.75% and 1.00% levels of salt respectively. One hundred and twenty layers were randomly assigned to the four experimental diets each containing 30 birds replicated thrice with 10 birds per replicate. At the end of the eight weeks feeding trial, thirty-six birds were slaughtered and dissected. Their reproductive tract and ovaries were weighed, counted or measured accordingly while blood samples were collected to determine circulating estradiol, luteinizing (LH) and follicle stimulating (FSH) hormones. The results showed significant dietary effects ($P<0.05$) on the weight of ovary, the length of the infundibulum and number of follicles. Birds fed 1.00% dietary salt had the highest ($P<0.05$) weight of entire reproductive tract and ovary and also produced the highest number of pre-vitellogenic follicles. Layers in their late phase of production produced very high level of circulating estradiol that seemed to exert a negative feedback mechanism on FSH and LH especially at 0.75% inclusion of dietary salt. Conclusively, maintaining the dietary salt allowance of late production layers at 0.75% could be used as a management strategy to sustain them in production until it is economically favourable to dispose them off as spent layers.It was also discovered that high levels of circulating LH and FSH would not always translate to maximum egg production without optimum level of dietary NaCl.

Keywords: *Dietary salt, follicles, gonadal morphometry, hormones, layers.*

Description of the Problems

Sodium (Na) and chlorine (Cl) are two of body's main electrolytes and are usually found chemically combined together as sodium chloride (NaCl, or common salt). The functions of NaCl are many and varied among which are the maintenance of acidity levels in body fluids and proper osmotic pressure in body cells. Excessive levels of salt are however toxic to poultry, hence caution must be exercised in the inclusion of salt in poultry's diets. Poultry on high-salt diets increase their consumption of water almost in proportion to the excess salt (1) hence, NaCl toxicity is rare if livestock have unhindered access to drinking water. Previous research studies have been limited to the effect of salt (deficiency or excess) on the general performance of birds as regards feed intake, egg production and egg shell quality. Sodium chloride deficiencies adversely affect utilization of dietary protein and energy, and interfere with reproductive performance (2).

Little or no work has been done to determine

the effect of NaCl on reproductive parameters like folliculogenesis and gonadal morphometry of laying birds. Folliculogenesis is a complex process during which oocytes increase in size and develop into a mature form, accompanied by proliferation and differentiation of the surrounding granulosa cells (3, 4). Also, reproductive hormones like oestrogen, luteinizing hormone (LH) and follicle stimulating hormone (FSH) play pivotal roles in the formation, growth, maturation, and eventual ovulation of the egg cells. All these are some of the underlying factors that determine the egg producing capacity of the laying chicken.

The principal determinant for culling off laying chickens especially in their late phase of production is reduced hen-day production below the economic threshold needed to keep them in production. At this point, it is ill-advised to further keep them in production and they are usually sold off as spent layers. This culling oftentimes may not fall within the festive seasons when these spent layers could be sold off at a premium price to the farmers. The rational farmer may therefore be constrained to hold on to the birds till it is economically favourable for him to dispose them off as spent layers. However, within this interim, the old layers which have now become deficient in terms of egg production are at the same time immunologically weak and prone to high mortality. Since dietary sodium chloride (NaCl) has been reported to enhance egg production (5, 2, 6) and boost the immuno-competence of farm animals (7, 6), it seems a worthwhile intervention strategy to experiment with these attributes of NaCl to sustain egg production and improve the livability in the old layers until it becomes economically favourable to dispose them off. The effects of NaCl on follicullogenesis, reproductive tract morphometry and concentration of some of the hormones that potentiate follicular development have not been exhaustively investigated in layers in their late phase of production. This study was therefore designed to investigate the probable effects of feeding graded levels of salt on folliculogenesis, gonadal morphometry and hormonal profiles of laying chickens in their late phase of production.

Materials and Methods

The research work was carried out at the Teaching and Research Farm (poultry section) of the Federal University of Technology Akure, Ondo State, Nigeria. Akure is situated at an altitude of 350.52m above mean sea level and on latitude 7° 15′N and 5° 12′E. The average annual temperature is 26.2°C and the relative humidity is 78% (8). The vegetation of the area is that of the rainforest characterized by hot and humid climate and a bimodal rainfall distribution chart. One hundred and twenty late production layers of Nera Black breed were used for this experiment. Water and feed were given to the birds *ad-libitum* while medication and vaccination were given according to the standard practices in layers' management. The layers were randomly assigned to four dietary treatments comprising 0.25%, 0.50%, 0.75% and 1.00% of salt. The treatment with 0.25% salt (T1) served as the control treatment while the remaining three diets were labeled as T2, T3 and T4 respectively. Each treatment contained 30 birds replicated thrice with 10 birds per replicate. The feeding trial lasted for eight weeks. Table 1 shows the gross composition of the experimental diet.

Thirty-six birds were slaughtered at the end of the eight week trial. The birds were dissected and the various parts of the reproductive organs such as the ovary, infundibulum, magnum, isthmus, uterus, vagina and the entire reproductive tract were weighed and measured accordingly. The follicles in each of the birds were counted, measured with digital calipers and recorded.

The follicles were divided into matured/Graafian follicles; stage3 pre-ovulatory or maturing follicles; stage2 pre-ovulatory follicles; stage1 pre-ovulatory follicles and pre-vitellogenic follicles according to (9).

For the hormonal assay, blood samples were collected from the birds upon slaughter into test tubes which were held in a slanted form for about 12 hours for clotting to take place. From the clotted blood, sera were separated following centrifugation at 3000rpm for about 10 minutes. The sera of 12 birds (3 birds per treatment group) were then taken to the Hormonal Assay Department of the Federal Medical Centre (FMC), Owo in Ondo State, Nigeria where an enzyme based immunoassay procedure was employed to determine oestradiol (E2), follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in the serum samples. The protocol used for the analyses was as described by the kit producers. The ELISA kit used for the analyses of oestradiol was produced by Inteco Diagnostic UK Ltd. and the kit had its code number as EST: LOT; 115052002 QC: 15-449.

Before the commencement of the assay, all the reagents, serum references and controls were brought to room temperature. Thereafter, the micro-plate's wells for each sample to be assayed were formatted in duplicate and the unused micro-well strips were replaced back into the aluminium bag, sealed and stored at 2 – 8°C. Subsequently, 25µl of the appropriate serum specimen was drawn with a pipette into the assigned wells and then, 50µl of oestradiol biotin reagent was added to the wells. After this, the micro-plate was swirled gently for 30 seconds so as to enhance its mixture. This was followed by the covering and incubation for 30 minutes at room temperature. Thereafter, 50µl of oestradiol enzyme reagent was added directly on top and the reagent dispensed in the well. Again, the micro-plate was swirled gently for 30 seconds to achieve proper mixture. Thereafter, it was covered and incubated for 90 minutes at room temperature. The contents of the micro-plate were then decanted. After decanting, the plate was blotted dry with absorbent paper.

Table 1: Gross composition (g/100g) of the experimental diets

Ingredients	T1	T2	T3	T4
Maize	55.00	55.00	54.80	54.70
GNC	10.00	10.00	10.00	10.15
PKC	20	19.75	19.65	19.40
Fishmeal	2.50	2.50	2.50	2.50
Bone meal	4.00	4.00	4.00	4.00
Limestone	7.95	7.95	7.95	7.95
Layers premix	0.30	0.30	0.30	0.30
Salt	0.25	0.50	0.75	1.00
Total	**100.00**	**100.0**	**100.00**	**100.00**
Calculated Analysis				
M.E. (Kcal/kg)	2650.00	2644.76	2635.68	2630.81
CrudeProtein (%)	13.80	13.77	13.74	13.77
Crude Fibre (%)	4.00	3.99	3.98	3.97
Calcium (%)	4.49	4.48	4.48	4.48
Phosphorus (%)	0.78	0.77	0.77	0.77

GNC = Groundnut cake; PKC = Palm kernel cake; T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; M.E. = Metabolizable energy.

This was followed by the addition of 350µl of wash buffer which was decanted. A manual plate washer was then used to wash it three times. This was followed by the addition of 100µl working substrate solution to all wells. It was then incubated at room temperature for twenty minutes. Penultimately, 50µl of stop solution was added to each well and gently mixed for 20 seconds. Lastly, the absorbance was read on the ELISA micro-plate reader, specifically the AD TOUCH model and the results were read within thirty (30) minutes of adding the stop solution.

The ELISA kits used for the analyses of Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH) were also produced by Inteco Diagnostic UK Ltd. and the kits had their code numbers as FSH: LOT; 115051303 QC: 15-422 and LH: LOT; 115051801 QC: 15-169 respectively. Just as it was done prior to the analyses of oestradiol (E2) all the reagents, serum references and controls were brought to room temperature. Thereafter, the micro-plate's wells for each sample specimen to be assayed were formatted in duplicate and the unused micro-well strips were replaced back into the aluminium bag, sealed and then stored at 2 – 8^{o}C. Subsequently every other step was followed according to manufacturer's protocols.

Data analysis

The data collected were subjected to one way analysis of variance (ANOVA) in a completely randomized design using the IBM SPSS (10) version 22 statistical package. Significant differences where necessary were separated using Duncan Multiple Range Test of the same statistical package. Significance was accepted at $P<0.05$.

Results and Discussion

Performance of late production layers fed varying levels of dietary salt

The performance of late production layers fed varying levels of dietary salt is shown in Table 2. The hen-day production, hen-housed production, feed conversion ratio (calculated as kilogram of feed/dozen eggs), total feed intake, total eggs laid and mortality were significantly different ($P<0.05$) among the dietary treatments while average egg weight was statistically similar. Hen-day production was observed to be highest in T4 (51.40%) followed sequentially by T3 (50.75%), T1 (50.25%) and T2 being the least (44.65%). Birds in T3 recorded the highest hen-housed production (50.75%), a slight decrease was recorded in T4 (50.65%) which was followed by T1 (48.25%) and T2 (42.70%). No mortality was recorded in T3, while T1 and T2 recorded the same percentage mortality (6.67%) while T4 recorded 3.34% mortality.

Table 2: Performance of late production layers fed varying levels of dietary salt

Parameters	T1	T2	T3	T4
Total Egg Laid	400.50±3.50^{b}	354.50±22.50^{c}	422.50±0.50^{a}	393.50±34.50^{b}
Average Egg Weight (g)	56.80±1.30	55.19±2.74	57.98±0.86	57.29±0.79
Hen-day Production (%)	50.25±1.65^{a}	44.65±0.65^{b}	50.75±0.25^{a}	51.40±0.20^{a}
Hen-housed Production (%)	48.25±0.35^{a}	42.70±2.60^{b}	50.75±0.25^{a}	50.65±0.95^{a}
Total Feed Intake (kg)	93.90±0.20^{c}	94.10±0.15^{b}	93.95±0.03^{b}	94.55±0.05^{a}
FCR (Kg of feed/dozen egg)	2.81±0.03^{b}	3.20±0.20^{a}	2.67±0.01^{b}	2.91 ± 0.26^{b}
Mortality (%)	6.67±0.25^{a}	6.67±0.25^{a}	0.00±0.00^{b}	3.34 ± 0.18^{b}

$^{a, b, c}$ = Means on the same row but with different superscripts are statistically ($P<0.05$) significant.

T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; ±SEM= ±Standard Error of the mean.

The result obtained from this research showed that birds in T3 (0.75% salt inclusion) produced the highest number of eggs and they also had the best feed conversion ratio. It was also reported (5) that pullets fed diets containing 0.25% to 0.50% salt were significantly more efficient in their utilization of feed for egg production than those fed low salt diet. The higher feed intake in the high salt diets relative to the control is in consonance with reports (11, 12) that sodium chloride (NaCl) in the diet is an appetite enhancer. It showed that even at 1.00% salt as used in the trial, the tolerable limit of dietary salt for these birds has not been exceeded. The hen-housed production is a function of mortality (13). This explains why T3 that recorded zero mortality had the highest hen-housed production (50.75%). Also, T4 that recorded 3.33% mortality had a better hen-housed production (50.65%) than those recorded for both T1 and T2 with hen-housed production of 48.25% and 42.90% respectively. The lowest mortality was recorded in the high salt diets (T3 and T4). This supports earlier reports in the literature that dietary salt confers a high level of immunity on animals (7) hence its essentiality in the diets of both man and his stock (14). It could be observed from this trial that the best immunity could be conferred on late production layers with 0.75% dietary salt in their diets.

Reproductive tract morphometry of the laying chickens fed varying levels of dietary salt.

The reproductive tract morphometry of the laying chickens fed varying levels of dietary salt is presented in Table 3. The length of isthmus was not significantly different ($p>0.05$) among the different dietary salt levels while the weight of ovary, weight of the entire reproductive tract, the length of magnum, uterus, vagina and infundibulum were significantly ($P<0.05$) affected by the dietary salt levels. Birds in T4 recorded the highest weight for the entire reproductive tract (95.45g) and the least weight was observed in T2 (83.88g). The same trend was also noticed in ovary weight. It can also be observed that T4 had the longest magnum (37.73cm) which was closely followed by T1, T2 and T3 respectively. T2 recorded the longest infundibulum (13.65cm) and the shortest was in T4 (10.12cm). There was a decrease in uterus length in the high salt diets relative to the control. T4 had the longest vagina among the dietary treatments with the least from T3.The weight of the entire reproductive tract was highest in T4 which was also reflected in the weight of its ovary.

Table 3: Reproductive tract morphometry of laying chickens fed varying levels of dietary salt

Parameters	T1	T2	T3	T4	±SEM
Weight of entire tract (g)	94.78^{a}	83.88^{b}	93.50^{a}	95.45^{a}	±6.55
Weight of ovary (g)	28.97^{ab}	23.12^{b}	28.84^{ab}	34.56^{a}	±3.48
Length of infundibulum (cm)	12.11^{ab}	13.65^{a}	10.94^{b}	10.12^{b}	±1.08
Length of magnum (cm)	37.39^{a}	36.43^{ab}	34.95^{b}	37.73^{a}	±1.59
Length of isthmus (cm)	11.07	10.91	10.84	11.97	±1.21
Length of uterus (cm)	11.65^{a}	9.56^{b}	10.44^{b}	10.68^{a}	±1.06
Length of vagina (cm)	5.80^{a}	5.25^{a}	4.52^{b}	6.00^{a}	±1.11

$^{a, b, ab}$ = Means on the same row but with different superscripts are statistically significant.

T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; ±SEM = ± Standard error of the mean

This increase in the ovarian weight had been reported by (15,16) that FSH and LH usually synergistically promote the secretion of oestrogen (oestradiol) in the ovary which results in increase in the ovarian weight. The observed decrease in the length of uterus in birds fed the high salt diets did not follow a well defined trend as values recorded for T2 and T3 diets were lower than that obtained with the T4 diet which was statistically similar to the T1 (control) diet. The development of the ovary and the entire reproductive tract of the female farm animals is under the control of the gonadotropins notably the follicle stimulating hormone (FSH) and the luteinizing hormone (LH). The FSH initiates the process of follicular development or folliculogenesis in the ovary (17) and the mobilization of yolk proteins and lipid into the pre-vitellogenic follicles thus increasing their number, mass and size. Folliculogenesis in the present study seems to be enhanced by dietary salt as the increase in number (Table 4), mass (Table 3) and size (Table 5) of the follicles were favoured in the high salt diets (T3 and T4) which resulted in the heaviest weight of the ovary and the entire reproductive tract observed in these high salt diets. Further developments of the pre-vitellogenic follicles to pre-ovulatory and then to Graafian follicles lead to the production of estradiol (oestrogen) which maintains the functionality of the avian oviduct. This further increased the ovarian mass (15, 16) and the oviduct as evidenced in the current study,

Number of matured, pre-ovulatory and pre-vitellogenic follicles in laying chickens fed varying levels of dietary salt.

Table 4 shows the number of matured, pre-ovulatory and pre-vitellogenic follicles in laying chickens fed varying levels of dietary salt. There were no significant differences ($P>0.05$) observed in the matured and pre-ovulatory (stages 2 and 3) follicles. The pre-vitellogenic follicles and stage1 pre-ovulatory follicles were however significantly different ($P<0.05$) with T4 producing the pre-vitellogenic follicles in the highest number (22.67), followed successively by T3 (22.00), T1 (20.33) and T2 (17.00). The T3 also produced the highest number (14.50) of stage1 pre-ovulatory follicles.

Table 4: Number of follicles in laying chickens fed varying levels of dietary salt

Parameters	T1	T2	T3	T4	±SEM
Matured/Graafian follicles	1.00	1.00	1.00	1.00	0
Pre-ovulatory follicles 3	1.00	1.00	1.00	1.00	0
Pre-ovulatory follicles 2	1.00	1.00	1.00	1.00	0
Pre-ovulatory follicle1	1.00^{b}	1.00^{b}	14.50^{a}	1.00^{b}	±7.21
Pre-vitellogenic follicles	20.33^{ab}	17.00^{b}	22.00^{ab}	22.67^{a}	±1.97

$^{a, b, ab}$ = Means on the same row but with different superscripts are statistically significant.
T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; ±SEM = ± Standard error of the mean

The initial proliferation in the number of pre-vitellogenic follicles as a result of the increase in dietary salt levels support the report of (18, 19) that dietary NaCl aids the absorption of amino acids and carbohydrates needed for oocyte production, development and maturation. The progressive decrease in the number of follicles up the growth ladder is suggestive of the normal apoptosis synonymous with the growth process (20) but

more apoptosis of maturing follicles at high salt concentration of the diets as observed in this trial is indicative of sub-normal salt toxicity beyond 0.75% inclusion level.

Dimension of follicles of laying chickens fed varied levels of dietary salt.

Table 5 reveals the dimension of follicles of laying chickens fed varied levels of dietary salt. There were significant differences ($P<0.05$) in the dimension of the three stages of growth of the pre-ovulatory follicles in all the dietary treatments. T4 had the largest (11.70mm) stage1 and stage2 (17.40mm) pre-ovulatory follicles. The largest stage3 pre-ovulatory (23.00mm) and matured (28.50mm) follicles were recorded in T1 and T3 respectively. The increased rate of apoptosis and atresia of the follicles as a result of increase in the dietary salt was also observed here. The selection of follicles based on dimension determines those that will become atretic and those that will undergo further development to final ovulation (9). This finding is similar to the report of (20) in their study on folliculogenesis in ducks following the administration of a gonadotropin-releasing hormone 1 (GnRH) analogue.

Table 5: Dimensions of follicles in laying chickens fed varying levels of dietary salt

Parameters	T 1	T 2	T 3	T 4	±SEM
Matured follicles (25mm and above)	27.90	27.30	28.50	27.30	±1.50
Pre-ovulatory 3 (20-24mm)	23.00^{a}	20.80^{a}	22.00^{a}	19.90^{b}	±2.70
Pre-ovulatory 2 (15-19mm)	16.30^{a}	15.00^{a}	14.80^{b}	17.40^{a}	±2.40
Pre-ovulatory 1 (10-14mm)	11.20^{b}	12.30^{a}	11.30^{b}	11.70^{a}	±0.90

$^{a, b,}$ = Means on the same row but with different superscripts are statistically significant.
T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; ±SEM = ± Standard error of the mean

Hormonal profile of late production layers fed graded levels of dietary salt

Table 6 shows the hormonal profile of the experimental birds fed graded levels of dietary salt. It could be observed that all the hormones: oestradiol (E2), follicle stimulating hormone (FSH) and luteinizing hormone (LH) were significantly ($P < 0.05$) influenced by the dietary salt levels. The level of E2 was highest in T3 and lowest in T2 while the level of FSH was highest in T2 but lowest in T3. A somewhat inverse relationship was thus established between these two hormones under the interplay of varied levels of dietary NaCl. The level of circulating LH decreased in the high NaCl diets relative to the control but the lowest value was recorded with the T3 diet.

Table 6: Hormonal profile of laying hens fed graded levels of dietary salt

Parameters	T1	T2	T3	T4
E2 (pg/ml)	1700.00 ±115.00^{b}	434.07 ± 3.51^{d}	1876.70 ± 122.50^{a}	461.00 ± 2.00^{c}
FSH (IU/ml)	8.89 ± 0.88^{b}	34.53 ± 4.65^{a}	3.34 ± 0.11^{d}	4.29 ± 0.55^{c}
LH (IU/ml)	15.80 ± 0.80^{a}	9.53 ± 1.55^{b}	0.30 ± 0.20^{d}	4.63 ± 2.25^{c}

^{a,b,c,d}= means on the same row but with different superscripts are statistically ($P<0.05$) significant. Each value represents the mean for 3 birds selected at random per treatment; E2 = Oestradiol; FSH = Follicle Stimulating Hormone; LH= Luteinizing Hormone; T1 = Diet with 0.25% salt; T2 = Diet with 0.50% salt; T3 = Diet with 0.75% salt; T4 = Diet with 1.00% salt; ±SEM = ± Standard error of the mean

The mean values for E2 in these experimental birds were higher than the normal range of 26 – 364pg/ml for female chickens reported by (21). Similarly, (22) reported lower values for E2 than those observed in these hens. Oestradiol seemed to have a negative feedback mechanism on follicle stimulating hormone (FSH) and luteinizing hormone (LH) since it was highest in T3 when both FSH and LH were at their lowest (Figures 1, 2 and 3) in the birds fed 0.75% dietary salt. This observation supports the report of (23) that oestradiol otherwise known as oestrogen could have negative feedback mechanism on the gonadotropins (FSH and LH). The egg production performance (Table 2) revealed that T3 with the highest level of E2 had the highest number of eggs produced among the four treatment diets while T2 had the least. According to (21), the normal range of Luteinizing hormone for the female chicken is 1.75 – 3.38IU/ml. However in this current study, none of the values for this hormone fell within this range. While T1, T2 and T4 were well above this range, T3 with the highest egg production fell below this range. The normal assay range of follicle stimulating hormone (FSH) of chickens as reported by (24) is 0.2 – 60IU/ml. Observably, all the values obtained for this hormone in all the birds fell within this reported range. Drawing from the fact that FSH concentration in birds fed T3 fell within this normal range, it could be opined that high circulating oestradiol and normal concentration of FSH in T3 kept the circulating LH in abeyance through the negative feedback mechanism of E2 in circulation. Thus E2 which is an ovarian steroid hormone was able to lower, through negative feedback mechanism, the secretions of FSH and LH which are both gonadotropins when it (oestradiol) was being maximally produced through steroidogenesis in the ovary under the probable optimum NaCl concentration achieved in the present study with 0.75% inclusion of NaCl. (15) reported that under experimental conditions, FSH and LH usually synergistically promote the secretion of oestrogen (oestradiol) from the ovary with a concomitant increase the ovarian weight. It was also reported by (25) that small amounts of LH can do as much as larger amounts of FSH and this could be observed particularly in those that were on 0.75% salt level (T3). It was also discovered that high LH and FSH in the blood as observed in T1 and T2 would not always translate to maximum egg production without optimum level of dietary NaCl.

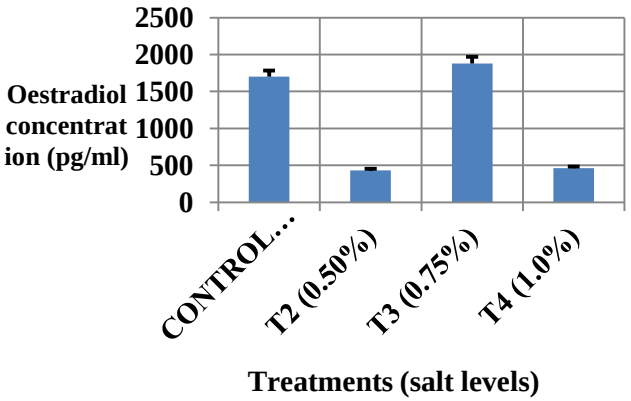

Figure 1: Oestradiol concentration in laying hens fed graded levels of dietary salt.

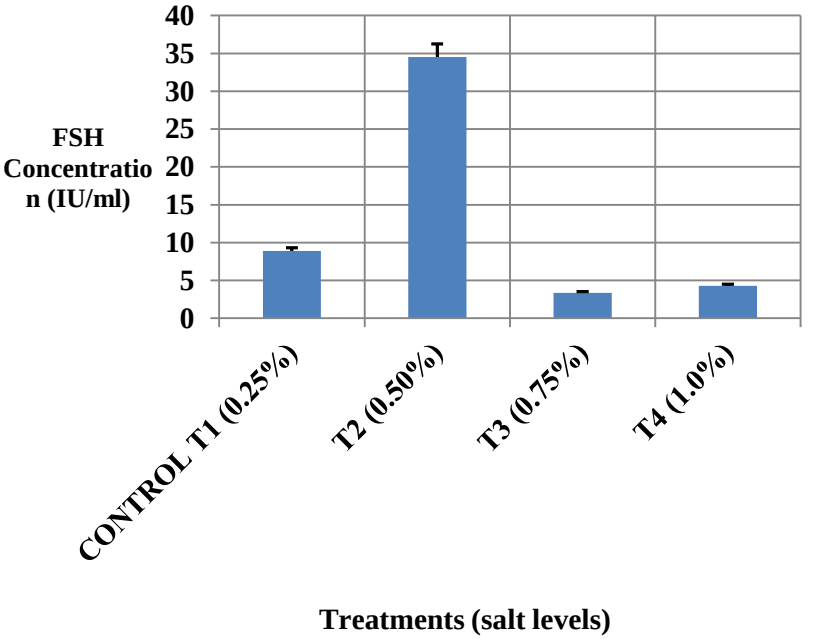

Figure 2: Follicle stimulating hormone (FSH) concentration in laying hens fed graded levels of dietary salt.

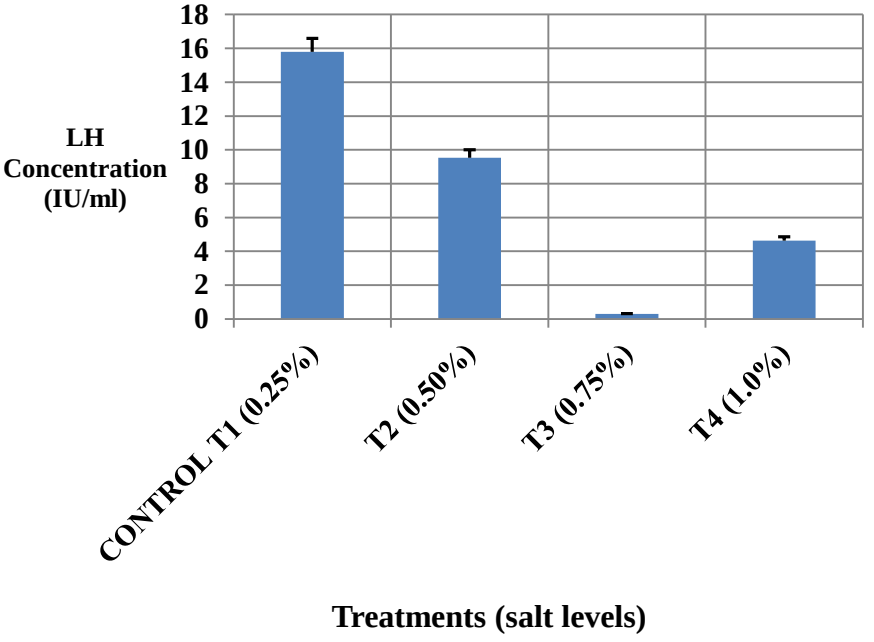

Figure 3: Luteinizing hormone (LH) concentration in laying hens fed graded levels of dietary salt.

Conclusions and Application

1. Dietary salt significantly affected the morphometry of the ovary and the oviduct. Birds fed with 1.00% dietary salt had the heaviest ovaries, highest number of pre-vitellogenic follicles and the largest stage1 pre-ovulatory follicles.
2. Inclusion of dietary salt beyond 0.75% is ill-advised in order to forestall increased rate of follicular apoptosis and atresia that could result in subsequent developmental stages.
3. The 0.75% salt promotes better livability through reduction in mortality rate and maximal production of estradiol to sustain the ovary and the oviduct for increased egg production.
4. Layers in their latter stage of production produce high levels of estradiol with probable negative feedback mechanism on LH and FSH production.
5. The 0.75% NaCl seemed the optimum level for enhancement of egg production and livability in laying chickens at their late phase of egg production. Also high levels of circulating LH and FSH would not always translate to maximum egg production without optimum level of dietary NaCl.

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