Original Research Article

Effect of vitamin D_3enrichment by ultra violet blue (UVB) radiation and dietary zinc supplementation onegg production and feed consumption in layer birds

ABSTRACT

The present study to evaluate the influence ofvitamin D_3 enrichment by UVB radiation and dietary zinc supplementation on egg production and feed consumption in layer birds was conducted at Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, for a period of 12 weeks from 01 February 2020 to 01 May 2020.

A total of sixty four laying hens, belonging to crossbred (Whie Leghorn N strain and Desi) housed in animal house attached to the department were used in the experiment. The treatments of this study included a control diet (according to BIS 2007) fed group (group I), group with zinc supplementation alone (group II), group with UVB exposure without zinc supplementation (group III) and group with both UVB exposure and zinc supplementation (group IV). The four treatments were tested in 28week old crossbred hens in a completely randomised experimental design. Each of the experimental treatment was having four replicates with four birds with all standard managemental conditions.

In the current study egg production in birds were not significantly affected by UVB light exposure and dietary zinc supplementation. However, the effect of UVB exposure and zinc supplementation via feed was highly significant ($p<0.01$) for feed consumption. Highest overall mean feed intake (113.18±0.14 g/day) was recorded with *those birds exposed to UVB and fed with inorganic zinc. While the lowest value (107.17±0.24 g/day) was recorded in group of birds fed with zinc supplementation in diet without UVB exposure.*

Key words: Egg production, Feed consumption, UltaViolet Blue, Vitamin D, Zinc

INTRODUCTION

Eggs, irrespective of table or fertilized, are considered as natural life supporting chemical storehouse which provides highly nutritious food with biological packaging (egg shell) without any chance of adulteration. Table eggs of chicken are vital part of the diet which has been used as a food by human beings since ancient times. The poultry eggs are regarded as inexpensive, convenient and low calorie sources of high quality protein with several other essential nutrients. Over the last four decades, poultry production in India had made impressive progress. Nutritionists have recently been involved in enriching or altering the nutritional profile of poultry products, such as meat and eggs, in relation to increased consumer interest in the nutritional value of foods.

The Indian industry is presently focused in the production of eggs that have higher or enriched levels of certain nutrients. These eggs are capable of safeguarding the health of consumers. Designer eggs have high market demand because of the consumers' willingness to purchase designer eggs due to its' nutritional qualities in addition to regular eggs. Considering these facts, the current study was planned to produce vitamin

D_3enriched eggs by UVB exposure and to produce zinc enriched eggs by supplementing laying hens' diet with required levels of zinc. The dual enrichment with these two provide healthcare benefits over other food sources.

MATERIALS AND METHODS

The study was conducted using sixty four numbers of 28 weeks old crossbred (White Leghorn N strain X Desi) layer birds procured fromAll India Coordinated Research Projects (AICRP) on Poultry for Eggs, Mannuthy, KVASU. The birds were randomly distributed in a completely randomized experimental design and they were placed into four treatment groups, each with four replicates having four birds in each replicate (Table 1).

All birds were fed with a standard layer diet and raised under standard managemental conditions up to 28 weeks of age at AICRP on Poultry for Eggs Mannuthy, KVASU. The birds were immunized against diseases as per standard protocol followed in the farm. On completion of 28 weeks of age, the birds (average body weight of 1.28±0.06 kg) were brought and housed in well ventilated cagesat 24.5 ± 0.5˚C ambient temperature and at relative humidity ranging from 60-80 per cent (Megha and Ramnath, 2021). A photoperiod of 16 h per day was ensured throughout the period.

Birds of treatment groups III and IV were exposed to UVB (280-315 nm) light (M/s Philips India Ltd., Hyderabad) for 3h daily @ 8.00-8.30 a.m., 11.00 a.m.-12.00 p.m., 2.00-3.00 p.m. and 4.30-5.00 p.m. The UVB radiation dosage at a distance of 20 cm was 76 µW/cm^{2}as claimed by the manufacturer. A 60cm long, 240 V, 50 Hz and 36 W UVB tube equipped with heat protection reflector was placed in the lower front part of the cages to ensure optimal UVB light exposure on the featherless skin of feet and legs of birds,especially during the feeding time (Megha and Ramnath, 2023). The zinc level in control diet was formulated according to BIS 2007 standards (Table 2). Treatment groups II and IV were provided with a diet incorporating inorganic zinc sulphate at the level of 75 mg/kg mash diet (Megha *et al*., 2021).

Egg production

Daily egg production was recorded as follows and expressed in hen housed and hen day egg production per cent.

$$\text{Hen Housed egg production} = \frac{\text{Average daily number of eggs produced}}{\text{Total number of birds housed at the beginning}} \times 100$$

$$\text{Hen day egg production} = \frac{\text{Number of eggs produced on that day}}{\text{Total number of live hens on that day}} \times 100$$

Feed consumption

Feed intake was recorded in each replicate at the end of every 28 day period as the difference between total feed offered and the cumulative feed left over. From the above data feed intake per bird/day was calculated.

RESULTS AND DISCUSSIONS

Hen housed egg production

The results of the influence of UVB light treatment and zinc feeding on hen housed egg production are presented in table 3 (a) and (b).

The effect of influence of UVB light treatment and zinc feeding was not significant for mean hen housed egg production. During the entire period, egg production did not vary between treatment groups.Egg production parameters showed no significant changes due to UVB treatment and zinc feeding. The overall mean percent hen housed egg production was also not significantly differed among treatment groups.

Hen day egg production

Data presented in table 4 (a) and (b) illustrate the effect of UVB light treatment and zinc feeding on mean hen day egg production.

The effect of UVB treatment and zinc supplementation was not significant for the mean hen day egg production during the experimental period. In the present study, the UVB light treatment and zinc supplementation did not cause any significant change in both hen housed egg number as well as hen day egg number over the entire experimental period of 29 to 40 weeks of age, as shown in Table 3 (a) and 4 (a), respectively. Similarly the mean per cent hen housed and hen day egg production also did not significantly get affected by the treatments employed in the study [Table 3(b) and 4(b)].

Similar to the present findings, Supplee *et al.* (1958) found no change with respect to egg production when zinc was supplemented in the feed for layers maintained on the floor or cages. Stahl *et al.* (1986) recorded that 28 ppm of zinc in the control diet was appropriate for egg production and at no time layers consuming this basal diet had lower egg production than Zn supplemented group of birds. Hence 28 ppm of zinc which was naturally supplied was sufficient for egg production, hatchability and growth of progeny.

However, Durmus *et al.* (2004) recorded a graded response on egg production, when the level of inclusion of zinc increased two times (1.21% more eggs), three times (1.64% more eggs) and 3.5 times (1.76% more eggs) than the control group though it was statistically non significant.On contrary, Kim and Patterson (2005) remarked that hens fed with diet containing 3000 ppm Zn brought about a significant decrease in egg production, egg weight and shell thickness.

Extra zinc supplementation in the diet was practised as a mode for inducing moulting in birds, in order to boost the production in subsequent cycle. Zinc can also cause follicular atresia and interrupt the laying of eggs (Scott and Creger, 1976; Berry and Brake, 1985). Shippee *et al*. (1979) also found that the addition to the layer ration with 10,000 ppm (1 per cent) zinc, such as zinc oxide or zinc acetate for 14 days, caused decline in hen day egg production from 60 to 0% in 6 days, which was marked with induction of moulting.

The trend of egg production recorded in this study fully agreed with reports of Plaimast*et al.* (2008) and Bahakaim*et al.* (2014) who supplemented both inorganic (zinc sulfate) and organic (amino acid chelate) zinc at levels of up to 600 ppm in the diet and it did not have a detrimental impact on the production of eggs and on quality of production.

On contrary to above reports so far discussed, Aghai*et al.* (2017) concluded that dietary supplementation with Zn beyond 40 mg/kg of diet improved egg production and egg shell strength in laying Japanese quail.

In the present study as discussed, zinc supplementation and UVB treatment did not bring any significant changes on egg production.Nowadays, the usage of insect traps lighted with UV bulbs is more common in poultry houses. Since the hypothalamic photosexual responses to direct UV reception, is very weak because of shorter wavelength and poor penetration power of UV rays. Hence it did not involve in hypothalamic control of reproduction in controlled environment of poultry houses (Lewis and Gous, 2009).

In the present study, the UVB exposure for a long period of 3h/day did not make any change in egg production. These findings were in accordance with the reports of Kuhn *et al*. (2015) and Hogsette *et al.* (1997) who demonstrated that UVB light treatment could cause any significance in laying performance.

On contrary to the present findings, Barrot *et al.* (1951), Carson and Junnila (1953) and Wei *et al.* (2020) recorded an increase in egg production, when hens were exposed to UV lamp which could be attributed to the favourable effects brought about by germicidal UV light of 253.7 nm wavelength. The beneficial effects of UVB exposure in older birds to maintain egg production was reported by England and Ruhunke (2020).

Even though UV light did not play any role in photosexual responses related to egg production, it possessed the ability to synthesise vitamin D_3 which would lead to improved egg production in birds receiving vitamin D_3 deficient diet.

The current study was in accordance with reports of Carson and Beall (1955) and Schutkowski*et al.* (2013), as they found neither UVB light exposure nor vitamin D_3 supplementation brought any significant change in egg production. Since, daily supplementation of white incandescent light with 8h to 12h of UV radiation did not cause a shift in timing of the release of pre-ovulatory luteinising hormone which determined the time of oviposition (Lewis *et al.,* 2000b).

Feed consumption

Results of the effect of UVB light treatment and zinc feeding on mean feedintake (g)/bird/day between 29 and 40 weeks of age are presented in table 5.

The effect of UVB light treatment and inclusion of inorganic zinc sulphate in diet was highly significant ($p<0.01$) for mean daily feed consumption during the entire three periods of experiment. Throughout the period I, significantly lower feed consumption was observed in group of birds fed with inorganic zinc sulphate without UVB treatment (106.16 g) and in UVB light treated group without zinc supplementation (108.04 g) compared to control group I (110.00 g). However highest feed intake was observed in group which received UVB light treatment along with zinc supplementation (115.80) during period I. In periods II and III, the group with zinc supplementation alone (group II) displayed a similar pattern, showing lower feed intake relative to other groups, while the group with both UVB light therapy and zinc supplementation (group IV) showed the highest feed consumption. Feed intake was significantly different for all the groups during period II and period III. The overall feed consumption was found to be significantly different ($p<0.01$) and mean overall feed intake was lower in group II (107.17 g) while no significant differences were seen in control group I (109.94 g) and UVB light treated group III (109.70) however, the highest mean feed intake was noticed in group IV (113.18). On the other hand, feed intake in groups with both UVB treatment and zinc supplementation were greater. Hens in group II did not consume feed as generally expected. Overall mean of feed intake was significantly different among treatments and the lowest value was recorded in layers fed diet with zinc supplementation alone.

The mean daily feed consumption during the entire three periods of experiment and the overall feed consumption during the experimental period was significantly ($p<0.01$) affected by UVB light treatment as well as inorganic zinc sulphate in diet, as summarized in Table 5.

During the present research study, significantly lower feed consumption was observed in the group of birds fed with inorganic zinc sulphate without UVB treatment (group II) compared to other groups. Such a finding was in full agreement with reports of Shippee *et al*. (1979) and Tabatabaie *et al*. (2007) who reported reduced feed intake in zinc fed group.

On contrary to the present study findings, Idowu *et al.* (2011) and Naz *et al*. (2016) reported that additional supplementation of Zn enhanced feed intake and improved growth in poultry birds. However, Sunder *et al.* (2008) found that there was no major effect on body weight, feed consumption and feed conversion efficiency by supplementing Zn to the basal diet at graded levels.

Addition of high level of zinc decreases plalatability (Fox, 1989) and depress appetite (Brink *et al*., 1959) which could contribute to reduced feed intake and subsequent production performance. This hypothesis was well supported by findings of Kim and Patterson (2005) who recorded that supplementation of zinc (3,000 to 20,000 µg Zn/g diet) could reduce hens' feed intake due to the anorectic effect at high levels.

In current study feed intake was higher in group of birds which received UVB light treatment along with zinc supplementation than the birds with zinc supplementation alone. These results were in line with previous studies of Kuhn *et al.* (2015) who exposed birds to UVB light (76 mW/cm^2) for varying periods ranging from 0 to 6h per day, where they found significant increase ($p < 0.01$) in food intake. However, Schutkowski*et al.* (2013) reported that UVB radiation and dietary vitamin D_3 did not influence food intake. Similarly, Lewis *et al.* (2000a) reported that feed intake, growth andfeed conversion efficiency were similar for all groups, irrespective of UV supplementation (60-160 mW/m^2 intensity at bird-head height) when turkeys were given nutritionally complete diets. Therefore feed consumption and feed conversion efficiency were not significantly affected by UV supplementation in turkeys.

On contrary to the findings discussed so far, Lewis *et al.*, (2000b) reported thatthe supplementation of continuous incandescent illumination with 12 h of UVA from a blacklight-blue lamp inexplicably reduced feed intake in laying hens during the period of exposure, eventhough the total daily feed intake was not significantly different from that of non UVA supplemented controls.

In the present study, it was recorded that during period II (33 to 36 weeks) the egg production performance was at its peak and the feed consumption was increased in groups with UVB treatment along with zinc supplementation. This may be due to the enhanced calcium-binding protein formation as well as calcium absorption from the duodenal segment which were channelized for egg laying as reported by Williams *et al.* (1982) and Tian *et al.* (1994). Higher feed intake and egg production were recorded in period II as per the present study.

CONCLUSION

There were no significant differences were observed in egg production of birds due to UVB light exposure and zinc supplementation, since the present study, neither caused any toxicity nor imparted any negative effect on the production performance. However, highly significant effect was observed on feed consumption between the treatment groups.

REFERENCES

Aghai, A., Khosravinia, H., Mamuoei, M., Azarfar, A. and Shahriari, A. (2017). Effects of dietary supplementation of zinc and α-tocopheryl acetate on performance and zinc concentrations in egg and tissues of Japanese quails. Poult. Sci. J. 5(1): 57-64.

Bahakaim, A., Abdel Magied, H., Osman, S., Omar, A., Abdel Malak, N.Y. and Ramadan, N. (2014). Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment.Egypt. Poult. Sci. J. 34(1): 39-56.

Barott, H.G., Schoenleber, L.G. and Campbell, L.E. (1951). The effect of ultraviolet radiation on egg production of hens. Poult. Sci. 30(3): 409-416.

Berry, W.D. and J. Brake. (1985). Comparison of parameters associated with molt induced by fasting, zinc and low dietary sodium in caged layers. Poult. Sci. 64(11): 2027-2036.

Brink, M.F., Becker, D.E., Terrill, S.W. and Jensen, A.H. (1959). Zinc toxicity in the weanling pig. J. Anim. Sci. 18(2): 836-842.

Carson, J.R. and Beall, G. (1955). Absence of response by breeder hens to ultraviolet energy. Poult. Sci. 34(2): 256-262.

Carson, J.R. and Junnila, W.A. (1953). Ultraviolet irradiation of the turkey hen. Poult. Sci. 32(5): 871-873.

Durmus, I.S., Atasoglu, C., Mizrak, C.E.N.G.I.Z.H.A.N., Ertas, S. and Kaya (2004). Effect of increasing zinc concentration in the diets of brown parent stock layers on various production and hatchability traits. Archiv. Anim. Breed. 47(5): 483-489.

England, A. and Ruhnke, I. (2020). The influence of light of different wavelengths on laying hen production and egg quality. World's Poult. Sci. J. 76(3): 443-458.

Fox, M.R.S. (1989). Zinc in human biology. Springer, London, 365-370.

Hogsette, J.A., Wilson, H.R. and Semple rowland, S.L. (1997). Effects on White Leghorn hens of constant exposure to ultraviolet light from insect traps. Poult. Sci. 76(8): 1134-1137.

Idowu, O.M.O., Ajuwon, R.O., Oso, A.O. and Akinloye, O.A. (2011). Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. Int. J. Poult. Sci. 10(3): 225-230.

Kim, W.K. and Patterson, P.H. (2005). Effects of dietary zinc supplementation on hen performance, ammonia volatilization, and nitrogen retention in manure. J. Environ. Sci. Health B. 40(4): 675-686.

Kuhn, J., Schutkowski, A., Kluge, H., Hirche, F. and Stangl, G.I. (2014). Free-range farming: A natural alternative to produce vitamin D-enriched eggs. Nutr. 30(4): 481-484.

Lewis, P.D. and Gous, R.M. (2009). Responses of poultry to ultraviolet radiation. World's poult. Sci. J. 65(3): 499-510.

Lewis, P.D., Perry, G.C. and Morris, T.R. (2000a). Ultraviolet radiation and laying pullets. Br. Poult. Sci. 41(2): 131-135.

Lewis, P.D., Perry, G.C., Sherwin, C.M. and Moinard, C. (2000b). Effect of ultraviolet radiation on the performance of intact male turkeys. Poult. Sci. 79(6): 850-855.

Megha, P.S. and Ramnath, V.(2021). Production of Vitamin D3 Enriched Designer Chicken Eggs by Direct Ultra Violet Blue (UVB) Light Exposure.J. Anim. Res. 11(6): 977-982.

Megha, P.S. and Ramnath, V. (2023). Influence of direct ultra violet blue (UVB) light exposure on serum levels of vitamin D3, calcium and phosphorus in layer chickens. J. Pharm. Innov. 12(1): 16-19.

Megha, P.S., Ramnath, V., Raji, K., Babitha, V. and Binoj, C. (2021). Production of zinc enriched designer eggs through dietary supplementation. J. Vet. Anim. Sci. 52(1): 77-80.

Naz, S., Idris, M., Khalique, M.A., Alhidary, I.A., Abdelrahman, M.M., Khan, R.U., Chand, N., Farooq, U. and Ahmad, S. (2016). The activity and use of zinc in poultry diets. World's Poult. Sci. J. 72(1): 159-167.

Plaimast, H., Sirichakwal, P., Puwastien, P. and Kijparkorn, S. (2008). Effect of supplementary zinc from organic and inorganic sources on laying performance and zinc deposition in eggs. Thai. J. Vet. Med. 38(3): 47-53.

Schutkowski, A., Kramer, J., Kluge, H., Hirche, F., Krombholz, A., Theumer, T. and Stangl, G.I. (2013). UVB exposure of farm animals: study on a food-based strategy to bridge the gap between current vitamin D intakes and dietary targets. PloS One. 8(7): e69418.

Scott, J.T. and C.R. Creger. (1976). The use of dietary zinc as an effective molting agent in laying hens. Poult. Sci. 55(5): 2089.

Shippee, R.L., Stake, P.E., Koehn, U., Lambert, J.L. and Simmons Iii, R.W. (1979). High dietary zinc or magnesium as forced-resting agents for laying hens. Poult. Sci. 58(4): 949-954.

Stahl, J.L., Cook, M.E. and Sunde, M.L. (1986). Zinc supplementation: its effect on egg production, feed conversion, fertility, and hatchability. Poult. Sci. 65(11): 2104-2109.

Sunder, G.S., Panda, A.K., Gopinath, N.C.S., Rao, S.R., Raju, M.V.L.N., Reddy, M.R. and Kumar, C.V. (2008). Effects of higher levels of zinc supplementation on performance, mineral availability, and immune competence in broiler chickens. J. Appl. Poult. Res. 17(1): 79-86.

Supplee, W.C,Blamberg, D.L. Keene, O.D. Combs G.F. and. Romoser, G.L (1958).Observations on zinc supplementation of poultry rations. Poult. Sci. 37(5): 1245-1246

Tabatabaie, M.M., Aliarabi, H., Saki, A.A., Ahmadi, A. and Siyar, S.A. (2007). Effect of different sources and levels of zinc on egg quality and laying hen performance. Pak. J. Biol. Sci. 10(19): 3476-3478.

Tian, X.Q., Chen, T.C., Lu, Z.H.I.R.E.N., Shao, Q.I.N.G. and Holick, M.F. (1994). Characterization of the translocation process of vitamin D_3 from the skin into the circulation. Endocrinol. 135(2): 655-661.

Wei, Y., Zheng, W., Li, B., Tong, Q., Shi, H. and Li, X. (2020). Effects of b-wave ultraviolet supplementation using light-emitting diodes on caged laying hens during the later phase of the laying cycle. Anim. 10(1): 15.

Williams, S.N., Miles, R.D., Ouart, M.D. and Campbell, D.R. (1989). Short-term high level zinc feeding and tissue zinc concentration in mature laying hens. Poult. Sci. 68(4): 539-545.

Table 1: Experimental layout

Treatment Groups	**Treatment**	**No of birds**

I	Control with standard layer diet (BIS 2007)	4X4
II	Zn enriched diet	4X4
III	UVB light exposed	4X4
IV	Zn enriched diet + UVB light exposed	4X4

Table 2: Percent ingredient composition of experimental diet

Sl. No.	**Feed ingredients**	**Percentage**
1	Yellow maize	56.50
2	De-oiled rice bran	6.50
3	Soya bean meal	27.50
4	Calcite powder	7.50
5	Dicalcium phosphate	1.50
6	Salt	0.50
Total		100.00
Feed Supplements (g/100 kg feed)		
1	L-Lysine	100
2	DL-Methionine	100
3	Vitamin premix	50
4	Toxin binder	100
5	Choline chloride	100
6	Trace mineral mixture	100

7	Liver tonic powder	25
Total		575

Table 3 (a): Mean ±S.E. hen housed egg number in four treatment groups during 29-40 weeks of age

Treatment Groups	**Period/age in weeks**			
	Period I 29-32	**Period Π 33-36**	**Period Ш 37-40**	**Overall mean 29-40**

I	21.13±0.16	21.88±0.16	19.63±0.13	20.88±0.12
II	20.75±0.10	21.75±0.10	19.69±0.12	20.73±0.09
III	21.00±0.10	22.13±0.07	19.56±0.06	20.90±0.06
IV	20.63±0.16	22.13±0.07	20.00±0.10	20.92±0.08
p-value	0.082	0.073	0.053	0.476

Table 3 (b): Mean ±S.E. hen housed egg percent in four treatment groups during 29-40 weeks of age

Treatment Groups	**Period/age in weeks**			
	Period I 29-32	**Period Π 33-36**	**Period Ш 37-40**	**Overall mean 29-40**
I	75.45±0.58	78.13±0.58	70.09±0.45	74.55±0.45
II	74.11±0.36	77.68±0.36	70.31±0.43	74.03±0.31
III	75.00±0.36	79.02±0.26	69.87±0.22	74.63±0.22
IV	73.66±0.58	79.02±0.26	71.43±0.36	74.70±0.27
p-value	0.082	0.073	0.053	0.476

Table 4 (a): Period-wise mean ±S.E. hen day egg number in four treatment groups during 29-40 weeks of age

Treatment Groups	**Period/age in weeks**			
	Period I 29-32	**Period Π 33-36**	**Period Ш 37-40**	**Overall mean 29-40**
I	21.13±0.16	21.88±0.16	19.63±0.13	20.88±0.12

II	20.75±0.10	21.75±0.10	20.40±0.82	20.96±0.30
III	21.00±0.10	22.13±0.07	19.56±0.06	20.90±0.06
IV	20.63±0.16	22.13±0.07	20.00±0.10	20.92±0.08
p-value	0.082	0.073	0.490	0.989

Table 4 (b): Period-wise mean ±S.E. hen day egg percent in four treatment groups during 29-40 weeks of age

Treatment groups	**Period/age in weeks**			
	Period I 29-32	**Period Π 33-36**	**Period Ш 37-40**	**Overall mean 29-40**
I	75.45±0.58	78.13±0.58	70.09±0.45	74.55±0.45
II	74.11±0.36	77.68±0.36	72.86±2.93	74.84±1.06
III	75.00±0.36	79.02±0.26	69.87±0.22	74.63±0.22
IV	73.66±0.58	79.02±0.26	71.43±0.36	74.70±0.27
p-value	0.082	0.073	0.490	0.988

Table 5: Mean ±S.E.daily feed consumption (g/bird) under UVB light treatment and zinc feeding

Treatment Groups	**Period/age in weeks**			
	Period I 29-32	**Period Π 33-36**	**Period Ш 37-40**	**Overall mean 29-40**
I	110.00^{b}±1.27	115.54^{b}±0.83	104.29^{b}±0.26	109.94^{b}±0.72
II	106.16^{a}±0.40	112.68^{a}±0.26	102.68^{a}±0.07	107.17^{a}±0.24

III	108.04^{ab}±0.16	114.82^{b}±0.22	106.25^{c}±0.07	109.70^{b}±0.13
IV	115.80^{c}±0.15	116.96^{c}±0.13	106.79^{d}±0.16	113.18^{c}±0.14
p-value	0.00**	0.00**	0.00**	0.00**

Means bearing different superscript within a column differ significantly

** Highly significant ($p<0.01$)