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Effect of Supplemental Drinking Boron on Morphology of African Ostrich Cerebrum

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Abstract: The aim of this study was to determine the effects of supplemental drinking boron on morphology of ostrich chicks' cerebrum. Twenty four hatched ostrich chicks were divided into six group (I-VI) and supplemented by the water with 0, 40, 80, 160, 320 and 640 mg L^{-1} boron, respectively for 45 days. Cerebrums were obtained and weighed after dissection then measured the transverse diameter, longitudinal diameter and height of cerebral hemisphere immediately. Paraffin embedded sections of cerebral tissues (4 μm thick) were stained with HE, Nissl's and argentaffin and then micro photographed. It showed significant increase ($p<0.05$ or $p<0.01$) in each anatomy index of group IV in comparison with the other groups while group VI showed significant decrease. Histology study showed that neurons of the cerebrum of group I and II were alike, nerve fibers passed horizontally within the cortex. Neurons of group III were varied in size and shape and with abundance of nerve fibers passed horizontally within the cortex tighter. Whereas neurons of group IV had more types than the rest of groups and were well arranged from the edge to inside by size, the nerve fibers were rich and interweaved. In contrast, neurons of group V and VI were monomorphic with less neurite and nerve fibers were tenuous and sparse. Findings showed that 80-160 mg L^{-1} supplemental drinking boron promotes cerebrum development, neurons differentiation, neurite formation and nerve fibers elongation of the cerebrum of 0-45 days old ostrich chicks.

Key words: Ostrich chicks, boron, cerebrum, morphology, neorons

INTRODUCTION

African ostrich, *Struthio camelus* is the largest living bird in the modern world. Wild ostriches had been embossed out from their origin and now raised in the North East of Africa and the desert regions of the Arabian Peninsula in Asia. It is a primitive animal that plays an important role in the process of evolution. In addition, it has high economic value. Ostrich had been list on the Convention on International Trade in Endangered Species (CITES) Appendix ((include all species threatened with extinction which are or may be affected by trade) (Goldsmith, 1978). Nonetheless, the decline in wild ostrich population continues (BirdLife International, 2013). Boron is an essential trace element in maintaining normal biological functions of humans and animals (Hunt, 2012; Nielsen, 2008). Low-dose boron ingestion has contributions to promote the metabolism of bone (Cheng *et al*., 2011), energy substrate (Nielsen, 2009) and other trace elements (Ross *et al*., 2011) to promote the proliferation and differentiation of blood cells (Bozkurt *et al*., 2012), T and B lymphocytes and germ cells (Pizent *et al*., 2012) to improve growth performance (Guelu *et al*., 2006) functions of brain (Penland, 1994) and immune system (Fry *et al*., 2010) and embryo development (Rowe and Eckhert, 1999) to alter blood biochemical index (Bozkurt *et al*., 2012) and various hormone (Kucukkurt *et al*., 2013). Conversely, high-dose boron ingestion, however will cause undesirable impact or even toxic effect (Devirian and Volpe, 2003; Cortes *et al*., 2011). Effect of boron to the cerebrum of African ostrich has not been reported so far. In the present study, researchers

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launch the research from the morphological changes of cerebrum of African ostrich chicks take in different levels of boron.

MATERIALS AND METHODS

Animals, grouping, feed and management: Twenty four newly hatched African ostrich chicks (Henan Ostrich Farm, China) weighing (0.80±0.05) kg were acclimated for 1 week and divided into control group (group I) and test group (group II, III, IV, V and VI) randomly. Four chickens in each group were marked on the outer leg for group mark identification. Boron added water with different levels were given to these groups (group I, 0 mg L^{-1}; group II, 40 mg L^{-1}; group III, 80 mg L^{-1}; group IV, 160 mg L^{-1}; group V, 320 mg L^{-1} and group VI, 640 mg L^{-1}), until the birds were 45 days old. Apart from the additional boron in the water, the ostrich chicks were fed with a feeding and management condition of the same.

Tissue and processing: Ostrich chicks were weighted and anesthetized with 15% urethane (1 g kg^{-1} body weight), bleed to death, dissected and removed the brain. Cerebrum were weighted the by electronic analytical balance. The transverse diameter, the longitudinal diameter and the height of cerebral hemisphere were measured by the caliper. Then put the cerebral tissue into 4% paraformaldehyde solution immediately. Samples of cerebrum of ostrich chicks were fixed, paraffin embedded, sectioned (4 μm thick) and stained with HE, Nissl's and argentaffin staining. Finally, the slides were observed under bright field optical microscope, photomicrography were taken by OLYMPUS DP2_BSW Imaging System.

Methods of analysis: The body and cerebrum weight, transverse and longitudinal diameter and the height of cerebral hemisphere of every ostrich chicks were recorded. The cerebral organ index was calculated. All data were analyzed with SPSS Version 19.0 to assess the effects of different levels of boron supplemented in drinking water on cerebrum tissue and were expressed as M±SD (Mean±Standard Deviation). Duncan's multiple range tests were performed to detect differences between groups.

RESULTS

Anatomical data of ostrich chick's cerebrum: The longitudinal diameter, the transverse diameter and the height of cerebral hemisphere were measured by a caliper which anatomical data were as the Fig. 1a-c. The results of the significant test of difference among groups of anatomical data of ostrich chicks cerebrum were as follows (Table 1).

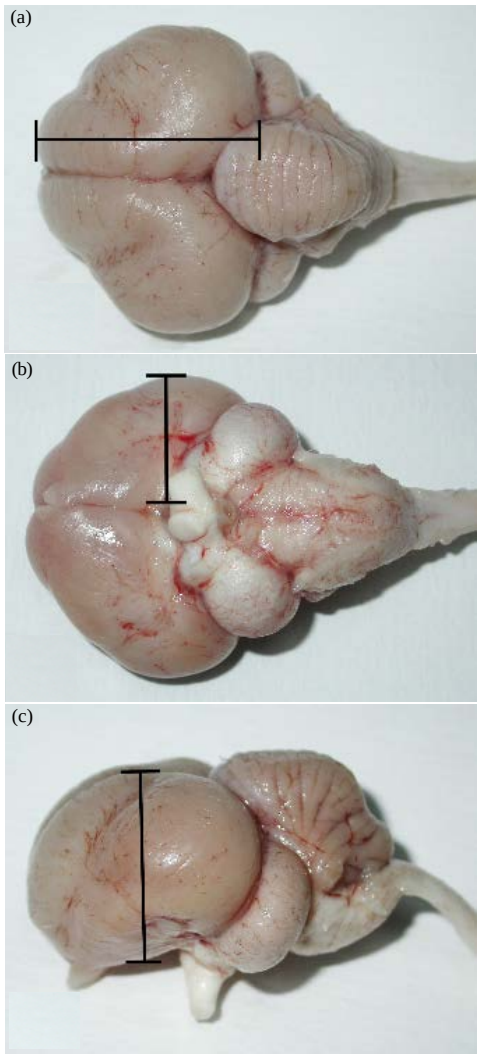

Fig. 1: Brain of African ostrich; a) dorsal surface of ostrich brain, the line represents the longitudinal diameter of the cerebral hemispheres; b) ventral surface of ostrich brain, the line represents the transverse diameter of the cerebral hemispheres; c) left surface of the ostrich brain, the line represents the height of the cerebral hemispheres

Table 1: Effects of boron supplemented in drinking on the Organ Index of cerebrum (OI) as well as the Longitudinal Diameter (LD) the Transverse Diameter (TD) and the Height (H) of cerebral hemisphere in ostrich chicks

Groups	OI (0/100)	LD (mm)	TD (mm)	H (mm)
I	$7.37±0.21^{Bb}$	$15.04±0.42^{Bb}$	$12.72±0.41^{Bbc}$	$11.16±0.42^{ABb}$
II	$7.41±0.15^{Bb}$	$15.12±0.30^{Bb}$	$12.34±0.58^{BCbc}$	$11.64±0.30^{ABab}$
III	$7.51±1.07^{Bb}$	$15.32±2.19^{Bb}$	$12.42±0.66^{BCbc}$	$11.67±0.70^{ABab}$
IV	$8.36±0.19^{Aa}$	$17.05±0.39^{Aa}$	$13.98±0.51^{Aa}$	$12.33±0.32^{Aa}$
V	$7.70±0.28^{ABb}$	$15.71±0.58^{ABb}$	$13.04±0.38^{Bab}$	$11.50±0.50^{ABab}$
VI	$6.54±0.62^{Cc}$	$13.33±1.27^{Cc}$	$11.61±1.52^{Cc}$	$10.79±1.85^{Bb}$

All the data were expressed as means±SD according to Duncan's multiple range. Capital letters in the same item indicated significant differences among groups ($p<0.05$) lowercase letters indicated highly significant difference among groups ($p<0.01$), n = 4

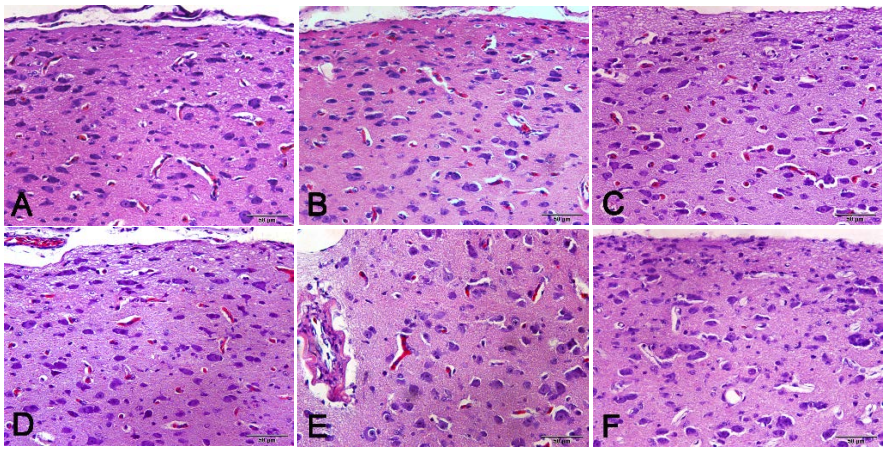

Fig. 2: Effects of supplemental boron on histology of the cerebrum of 45 days ostrich chicks from group I-VI (HE staining); A) the cerebral cortex of group I; B) the cerebral cortex of group II; C) the cerebral cortex of group III; D) the cerebral cortex of group IV; E) the cerebral cortex of group V and F) the cerebral cortex of group VI

The statistical analysis showed significant difference among groups of the cerebrum index, the longitudinal and transverse diameter and the height of cerebral hemisphere. All parameters in group IV were significantly increased while in group VI were significantly decreased. There was no significant difference in anatomical indicators in the cerebrum of ostrich chickens among group II, III, V as compared to group I. The organ data of cerebrum of ostrich chicks of group II-V were increased 0.54, 1.90, 13.43 and 4.48% than group I respectively while group VI were decreased 11.26% than group I. The longitudinal diameter of cerebral hemisphere in ostrich chicks of group II-V were increased 0.53, 1.86, 13.36 and 4.45% than group I, respectively while group VI were decreased 11.37% than group I. The transverse diameter of cerebral hemisphere in ostrich chicks of group V and VI were increased 9.91%, 2.52% than group I, respectively while group II, III, VI were decreased 2.99, 2.36 and 8.73% than group I, respectively. The height of cerebral hemisphere in ostrich chicks of group II-V were increased 4.30, 4.57, 10.48 and 3.05% than group I, respectively while group VI were decreased 3.32% than group I. Among the data there were no significant differences between group II, III, V and group I as well as the height of cerebral hemisphere in ostrich chicks of group IV, VI and group I ($p>0.05$). In addition there were highly significant differences between group IV and VI, the organ index of cerebrum, longitudinal diameter, transverse diameter and height of cerebral hemisphere in ostrich chicks of group IV were increased 27.83, 27.91, 20.41 and 14.27% than group VI, respectively.

Morphological changes of ostrich chick's cerebrum: The morphological changes of 45 days old ostrich chicks of each group were as follows: HE staining of the cerebrum of 45 days old ostrich chicks showed: the cerebral cortex of ostrich chicks of group I (control group; Fig. 2A) had many neurons that were irregularly arranged, similarly formed and single-typed, most of the neurons were granule cells with deeply stained cell body. The arrangement of the neurons of the cerebral cortex of ostrich chicks of group II (Fig. 2B) and group III (Fig. 2C) were similar to those of group I whereas the neurons of group III were in different forms with different types, most of them were stellate cells with few neurites. Moreover, the neurons were larger and larger from the edge of cerebral cortex to inside of the cerebrum. The neurons of group IV (Fig. 2D) were most stellate cells and some pyramidal cells with more and clearer neurites, the neurons were regularly arranged and were larger and larger from the edge of cerebral cortex to inside of the cerebrum. The neurons of group V (Fig. 2E) and group VI (Fig. 2F) were fewer than that of the other groups they were irregularly arranged with lightly stained cell body.

Nissl's staining of the cerebral cortex of 45 days old ostrich chicks of each group showed: the neurons of ostrich chicks of group I (control group; Fig. 3a) were deeply stained with 1 or 2 neurites that were clearly observed; the neurons of group II (Fig. 3b) and group III (Fig. 3c) were stained deeper than group I, 1 or 2 and sometimes 3 neurites were found stained in most of the neurons; molecular layer of the cerebral cortex were found in the cerebral cortex of group IV (Fig. 3d), most of the neurons of group IV had 2-4 neurites some of them had more neurites could be found whereas the neurons of group V (Fig. 2e) and group VI (Fig. 2f) were fewer than other groups, neurites of the neurons were hard to be observed or even many neurons had fewer or no neurites could be found.

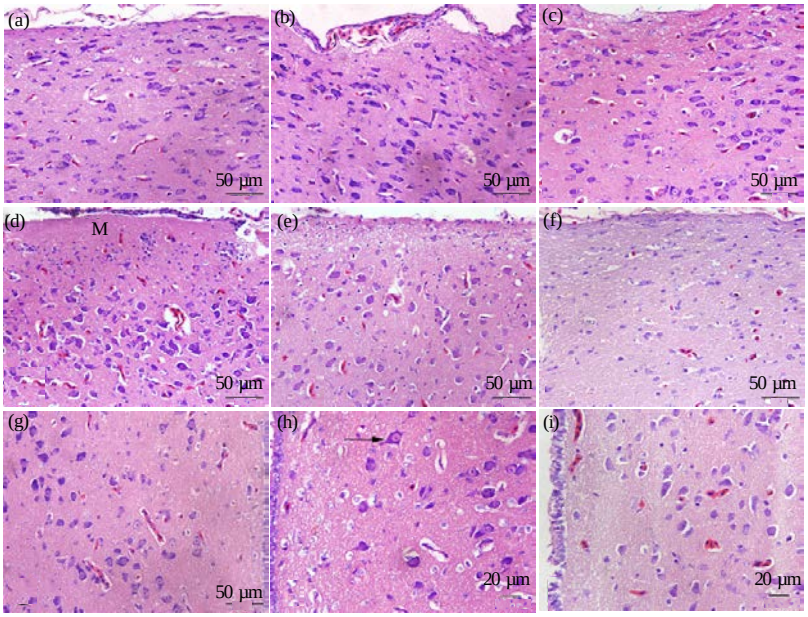

Fig. 3: Effects of supplemental boron on histology of the cerebrum of 45 days ostrich chicks from group I-VI (Nissl's staining); a) the cerebral cortex of group I; b) the cerebral cortex of group II; c) the cerebral cortex of group III; d) the cerebral cortex of group IVl; e) the cerebral cortex of group V; f) the cerebral cortex of group VI; g) the hippocampus of group I; h) the hippocampus of group IV; i) the hippocampus of group VI of 45 days ostrich; M: The molecular layer of group IV. Arrow for stellate cells with more neurites than other neurons

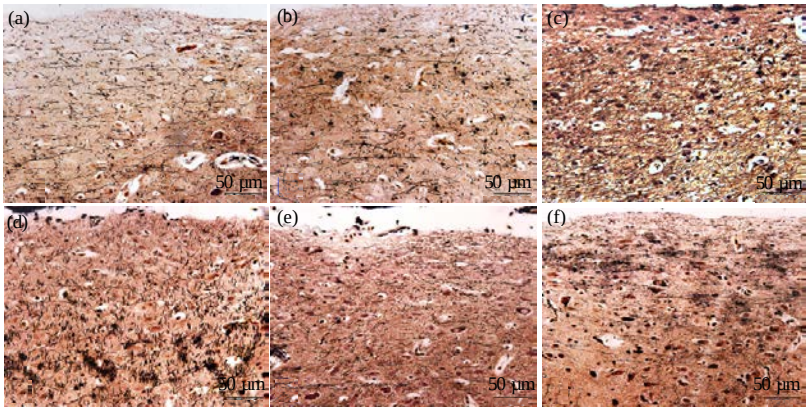

Fig. 4: Effects of supplemental boron on histology of the cerebrum of 45 days ostrich chicks from group I-VI (argentaffin staining); a) the cerebral cortex of group I; b) the cerebral cortex of group II; c) the cerebral cortex of group III; d) the cerebral cortex of group IV; e) the cerebral cortex of group V; f) the cerebral cortex of group VI

Nissl's staining of the hippocampus of 45 days old ostrich chicks of each group showed: the neurons of hippocampus of group I and VI were larger and larger from the inner side of hippocampusto edge of the cerebral cortex, however, the neurons of group VI were irregularly arranged. The neurons of group I were stained deeply, the neurons had 2 or more neurites; the neurons of group IV were stained deeper than group I and had at least 3 neurites some neurons had 5 neurites. Furthermore, the root of neurites were thicker than those of group I; the neurons of group VI were lightly stained, the cell body were oblate, un-sharp and smaller than group I, the neurites of the neurons of hippocampus of group VI were hard to be observed as only 1 neurite could be found.

Argentaffin staining of the cerebrum of 45 days old ostrich chicks of each group showed: the nerve fibers of

group I (control group; Fig. 4a) were clear and slender they were regularly arranged that were parallel to superficial edge of cerebrum however, the cell bodies were lightly stained; the nerve fibers of group II (Fig. 4b) were stained deeper than those of group I and were thicker they were regularly arranged in general but with some parts irregularly arranged and were also parallel to superficial edge of cerebrum whereas cell bodies were also lightly stained. The nerve fibers of group III (Fig. 4c) were stained the deepest, arranged the closest and paralleled to superficial edge of cerebrum, some tiny nerve fibers that were perpendicular to superficial edge of cerebrum and the cell bodies were stained the deepest among each group; the nerve fibers of group IV (Fig. 4d) were deeply stained but lighter than those of group III they were irregularly arranged, some of them were parallel to superficial edge of cerebrum that were long and thin and numbers of them were perpendicular to superficial edge of cerebrum that were short and thick these two kinds of nerve fibers interweaved a net. In addition, the reticular formation were more and more apparent from the edge of cerebrum to the inside, the cell bodies were deeply stained but lighter than those of group III; the nerve fibers of group V (Fig. 4e) and group VI (Fig. 4f) were stained lightly, short and tiny and they were arranged irregularly, the cell bodies were stained deeply with uneven size.

DISCUSSION

Boron is a necessary element for brain development: The development of cerebrum was not only the weight gain of brain tissue but also morphological changes of cerebrum such as the proliferation and differentiation of the neurons (Qiu *et al*., 2007) the formation and growth of the nerve fibers (Borrell and Reillo, 2012) the formation and increase of synapses (Chen and Liu, 2011) and so on. The development was suffered by the effects of hereditary, environment and their interactions these factors may affect to the histological structure and function or even cause long time or permanent brain injury (Gao *et al*., 2000; Money and Stanwood, 2013). In fact, research has shown that lack of boron would give rise to the changes of the frequency of brain waves of sexual mature rats; the increase of low frequency activities and the decrease of high frequency activities proved the brain activity was in decline (Penland and Eberhardt, 1993). Likewise, lack of boron would experience symptoms as the changes of constitution of mineral element in brain (Nielsen, 1997) but not that violent (Penland, 1994). Hence, it was necessary to supply a dose of boron according to the requirement for animals to maintain brain activities as normal.

Effect of boron in different tissue development is dose related: Previous studies have shown that low level dietary or drinking boron for animals could make contributions to growth performance (Guclu *et al*., 2006) bone development (Cheng *et al*., 2011) and strength (Naghii *et al*., 2012) blood biochemical index balance (Hunt, 2012) immune cells proliferation and differentiation, immune organs development (Fry *et al*., 2010) and so forth at the same time, high level had toxicity effect obviously. Previous studies in rats have shown, a 40 mg L^{-1} supplement of boron in drinking water promoted the development of adrenal gland (Li *et al*., 2012) tongue mucosa and taste buds, gastric mucosa and glands (Li *et al*., 2008c) the genesis and proliferation of blood cells (Li *et al*., 2009c) however, a 80-640 mg L^{-1} hinder those organs and cells development or even had toxicity effect; as to the histological development of thymus (Li *et al*., 2009e) and pancreas (Li *et al*., 2011) 40-80 mg L^{-1} supplement of boron in drinking water promoted well, 160-640 mg L^{-1} were harmful. Previous studies in Gushi chick have shown, a 100 mg L^{-1} supplement of boron in drinking water had effect to the proliferation and differentiation of blood cells (Li *et al*., 2008b) T and B lymph cells and had benefits to the histological development of myocardium (Li *et al*., 2009d) hepar and pancreas (Cai *et al*., 2010), kidney (Li *et al*., 2009b), spleen (Li *et al*., 2008a), thymus (Gu *et al*., 2007) and bursa of Fabricius (Li *et al*., 2009a), it stunted the development slightly in early phase then promoted obviously but 200-400 mg L^{-1} had detrimental effect to those organs and cell earlier. And studies in broilers (Kurtuglu *et al*., 2005; Rossi *et al*., 1993) and laying hens (Olgun *et al*., 2013) have shown, 5 mg B/kg diet made contribution to bone development (Kurtoglu *et al*., 2005) growth performance (Rossi *et al*., 1993) and eggs quality (Olgun *et al*., 2013) 10-25 mg B/kg diet had adverse impact. Previous studies in barrows (Armstrong and Spears, 2001) have shown that 5-15 mg B/kg diet were good for the growth performance and bone strength, 5 mg B/kg diet was better. Previous studies in ostrich have shown that 100-200 mg L^{-1} supplement of boron in drinking water were beneficial to tibia development, 200 mg L^{-1} was better but 400 mg L^{-1} was harmful (Cheng *et al*., 2011). In the present study, 40-80 mg L^{-1} supplement of boron in drinking water did something well for cerebrum development mildly; 160 mg L^{-1} promoted the development of the ostrich chicks brain the best greatly; 320-640 mg L^{-1} stunted the development of ostrich chicks brain but no abnormality of the histological structure has been seen so, that the inhibition of 320-640 mg L^{-1} were explicit but there was no toxicity.

Requirements of boron are diverse in different organ of different species: The difference of promotion, inhibition or toxicity of boron to organs of species were different. Thus, the requirement and the tolerance dose were

different among organs of species. By summarizing prior research, it showed that larger animals such as swine and ostriches or higher boron content organs for instance bone, brain, kidney, spleen, liver and heart (Devirian and Volpe, 2003) the requirement and the tolerance dose were higher meanwhile smaller animals like rats and chicks or lower boron content organs for example digestive glands and adrenal gland, the requirement and the tolerance dose were lower. Studies have pointed out that >82% of the absorbed boric acid or borate excreted as original form with urine (Devirian and Volpe, 2003). At the same low dose of drinking or dietary boron, larger animals had to drink or ingest multiples of the size of smaller animals to satisfy the quantity of the development of their organs in effect, the larger animal needed to drink or ingest much more than earlier mentioned because the large amount of excretion. Therefore, only a higher dose of drinking or dietary boron would satisfy their organ development for they could not drink or ingest that much in low dose. As African ostrich is the largest avian and whose brain is one of the high boron content organs, 40-160 mg L^{-1} supplement of boric acid in drinking water were good to ostrich chick's brain development and the optimal dosage (160 mg L^{-1}) was higher than rats or chicks; 320-640 mg L^{-1} stunted the development but no toxicity effects it means ostrich had more tolerance than smaller animals. Furthermore, the structure and function of cerebrum and bone tissue were different, the optimal dosage for cerebrum tissue (160 mg L^{-1}) and bone tissue (200 mg L^{-1}) were close (Cheng *et al*., 2011) however, toxic dose to cerebrum tissue were <640 mg L^{-1} and that to bone tissue was 400 mg L^{-1} (Cheng *et al*., 2011). The dose of toxicity differed more depended on the difference of organ.

CONCLUSION

The ostrich chicks under 45 days old experience their critical period of development of brood stage. The results of this research indicated that supplemental boron in drinking water had positive effect on cerebrum development of ostrich chicks but high level might stunt their growth and development. Researchers found that the dosage of boron was influenced by different size of individual and different boron content of various organ. According to the data of this research, a 160 mg L^{-1} supplement of boron in drinking water was the optimal dosage for cerebrum development of 0-45 days old ostrich chicks.

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