Effect of Boron Administration on the Morphology of Ostrich Chick Kidney Tissue

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ARTICLE HISTORY (15-059)

Received: February 02, 2015
Revised: April 03, 2015
Accepted: June 15, 2015

Key words:
Boron
Kidney tissue
Morphology
Ostrich chicks

ABSTRACT

This study was to determine the effect of boron supplementation on the morphology of kidney tissue in ostrich chicks. A total of 48 newly hatched ostrich chicks were used and randomly assigned to six groups and exposed to various doses of boric (0, 40, 80, 160, 320, 640 mg/L) in drinking water for 90 days, respectively. The kidneys were removed and tissue sections were stained with HE for morphological examination and semi-quantitative evaluation. Microscopic observation showed that compared with the control group (0 mg/L), the structure of renal corpuscle and renal tubular was clearer in ostrich chicks fed with 40 and 80 mg/L boron. Furthermore, the brush borders were more abundant. On the other hand, in groups fed with 320 and 640 mg/L boron, histopathological degeneration occurred with the characters of cellular swelling and dissociation, loose cytoplasm, nuclear pyknosis, and basement membrane detachment in some tubular cells. Debris deposit was accumulated in the tubular lumen, especially in the proximal tubule. And the percentage of injured tubules was more than 50% in these groups. Moreover, the extent of kidney injury in group receiving 640 mg/L boron was more obvious with increasing tubular dilatation, tubular cell granular degeneration, interstitial inflammation and associated capsular retraction. Taken together, these findings showed that 40 and 80 mg/L boron supplementation may promote the kidney development; while 320 and 640 mg/L boron supplementation have obviously adverse effect, and the toxic effect was dose-dependent.

INTRODUCTION

Growing evidence has demonstrated that boron plays a central role in the development of animals (Hunt et al., 1994; Hunt and Stoecker, 1996; Nielsen et al., 2000; Hunt, 2012), and appropriate supplementation of boron has a protective effect on immune function (Hunt, 2003), growth performance (Rossi et al., 1993; Armstrong and Spears, 2001; Eren et al., 2006), and the development and microstructure of the immune organs (Jin et al., 2014) in broilers. Ostrich chicks are the largest living birds in the world, with the characteristics of high economic value in meat, eggs, fur, feather and egg shell. They also have a particular feature of rapid growth, particularly in the first third month of their lives (Bunter and Cloete, 2004), which is critical to the life circle. Kidney, as the most important excretion organ, is correlated to the whole body growth. However, no study has reported the effect of boron on kidney tissue morphology in ostrich chicks so far. In this study, we used the ostrich chicks as the research object, and supplemented different doses of boric acid into the drinking water to investigate the role of boron played in kidney tissue. Through this research, we are able to advance the knowledge of boron effects in other species from another perspective, so as to provide theoretical basis of science in the correct use of boron in breeding industry.

MATERIALS AND METHODS

Animals and experimental design: A total of 48 newly hatched ostrich chicks weighing (0.80±0.05) kg were obtained from Henan Ostrich Farm, China. Ostrich chicks were randomly divided into a control group and five boron fed groups. These groups were fed with boric acid in drinking water with 40, 80, 160, 320, 640 mg/L until the birds were 90 days, respectively. Except the boron supplemental doses in drinking water, the other condition
were all the same. All experiments were conducted in accordance with the Institutional Animal Ethical Committee.

Sample collection and assessment of renal tubular injury: Eight ostrich chicks from each group were anesthetized with 15% urethane (1 g/kg BW) and the kidney tissue were removed immediately. For morphological examination, the kidney tissues were fixed in 4% paraformaldehyde, dehydrated by graded ethanol treatment, cleared in xylene, paraffin embedded, sectioned at 4 µm thickness and stained with HE. The kidney structures were observed under the bright field optical microscope, and photomicrography were taken by OLYMPUS DP2-BSW imaging system (Fig. 1 & 2).

In addition, the slides were reviewed and scored using a semi-quantitative scale method according to the severity and range of tubular damages. The magnitude of renal tubular damage including nuclear pyknosis, tubular dilatation and tubular cell granular degeneration was scored into five levels (0: none; 1: less than 25%; 2: 25 to 50%; 3: 50% to 75%; and 4: more than 75%), which was based on the percentage of injured tubules. Ten high-power fields but non-overlapping cortical areas (magnification ×400) of each variable were randomly selected, and each slide was reviewed by two professional researchers and tubular damage scores were recorded (Table 1).

Statistical analysis: Data were analyzed using the Statistical Package for the Social Sciences (SPSS, v.17.0; SPSS, Chicago, IL, USA) to evaluate the effect of boron supplementation on the morphology of kidney tissue in ostrich chicks, and were expressed as mean±SEM. Duncan’s multiple range tests were performed to assess differences between six groups. The level of statistical significance was assigned at P<0.05.

RESULTS

Effects of boron on microstructure of kidney tissue in ostrich chicks: In the kidney of the control group, the renal corpuscle was composed normally by vascular and renal capsule with the apparent Bowman’s capsules. The proximal tubular cells were composed of simple cuboidal epithelium cells with the characteristic of big cell body deeply stained, but unclear boundary between cells. The distal tubular cell body was smaller and lightly stained with the nucleus located in the central. Moreover, the tubular lumen of distal tubule was bigger and more regular than that in the proximal tubule (Fig. 1A).

Compared with the control group, the structure of renal corpuscle and renal tubules were clearer and the epithelial cells were in good arrangement in the boron supplemented groups of 40 and 80mg/L. Furthermore, brush border in the free surface and basal striation were clearly observed (Fig. 1B & 1C).

Surprisingly, the group with the 160 mg/L boron supplementation did not show obvious improvement of kidney tissue shown in Fig. 2A. However, no obvious morphological changes were observed, except slight expansion of glomerular capillary and steatosis in few renal tubular epithelial cells compared with control group (Fig. 2A).

While the microstructures in the 320 mg/L boron group showed different degrees of pathological changes in kidney. The cells of renal glomeruli were normal, but Bowman’s space was slightly dilated. There was a uniformly morphological injury in the renal tubule, except for some distal tubule, encompassing partial renal tubular epithelial cells with granular degeneration, cellular swelling and dissociation, nuclear pyknosis and loose cytoplasm (Fig. 2B).

Meanwhile, in the group fed with 640 mg/L boron, damage of kidney tissue was more obvious than 320 mg/L boron group, which had a remarkable severe tubular damage mainly in some kidney cortex. The number of renal tubules with granular degeneration was increased. In addition, some cells obviously detached from the basement membrane with the characteristics of swelling and dissociation. Tubular lumen dilation and proteinaceous cast formation were notably seen in the lumen. Those histopathological degenerative changes were observed especially in proximal tubular cells. Furthermore, renal corpuscle was also directly damaged including Bowman’s space with scattered blood cells and the glomeruli swelling apparently (Fig. 2C).

Kidney tubular damage scores: Quantification of tubular damage in the each group showed that morphology of renal tubular in the control group was approximate normal, showing no damage. The severity of tubular damages fed with 40 mg/L and 80 mg/L boron in drinking water were not different from controls. Besides, the supplementation of 160 mg/L boron showed, to some degree, a significant slight tubular damage. However, compared with the control group, the damage of tubular in ostrich chicks receiving high doses of boron (320 and 640 mg/L in drinking water) caused obvious extensive damage with the significantly increasing percent of injured tubules reaching about 56.65% and 70.85%, respectively (Table 1). In addition, the difference between 320 and 640 mg/L boron groups was also significant (P<0.05) (Table 1).

<table>
<thead>
<tr>
<th>Dosage (mg/L)</th>
<th>Number in each group</th>
<th>Severity of Renal tubular damages</th>
<th>Renal tubular damage scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>-</td>
<td>0d</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>-</td>
<td>0d</td>
</tr>
<tr>
<td>80</td>
<td>8</td>
<td>-</td>
<td>0d</td>
</tr>
<tr>
<td>160</td>
<td>8</td>
<td>++</td>
<td>9.25±0.07c</td>
</tr>
<tr>
<td>320</td>
<td>8</td>
<td>++++</td>
<td>56.65±0.63b</td>
</tr>
<tr>
<td>640</td>
<td>8</td>
<td>++++</td>
<td>70.85±0.92a</td>
</tr>
</tbody>
</table>

* represents normal; + represents mild; ++ represents moderate; +++ represents severe; ++++ represents very severe; +++++ represents extensive damage. Different letters (a-d) in the same column indicate significant differences (P<0.05), and the same letters indicate no significant difference (P>0.05).

DISCUSSION

It is well known that the organ structure determines its relevant functions. More and more studies demonstrated that the boron supplementation with appropriate doses is beneficial to the early embryonic development and reproductive system (Fort et al., 1998; Rowe et al., 1998), immune organs development (Fry et al., 2010), the mitogen-activated protein kinase (MAPK) pathway (Park et al., 2004), as well as regulation of inflammation and immune function (Armstrong et al., 2001) in domestic animals.
In addition, appropriate addition of boron produced positive effects on immune function of broilers and increased the serum antibody in broilers and rats (Hunt and Herbel, 1994). Moreover, Cheng et al. (2011) indicated that boron supplementation was helpful for ostrich chick bone development, and that 200 mg/L concentration appeared to be the most beneficial. In the current study, we observed the renal corpuscle and renal tubular structure in the groups supplemented with 40 and 80 mg/L boron, with the good arrangement of epithelial cells, were clearer than that of control group in ostrich chick. Furthermore, the brush border was more abundant. The brush border contributes to the reabsorption function of proximal tubules. These results suggested that low-dose boron may have a promoting effect on the kidney tissue development, so as to the kidney function, which was consistent with the previous studies on other organs mentioned above. However, it is noted that the optimal dosage of boron supplementation depends on the animal species and ages.

On the other hand, high-dose boron has an obviously adverse or even toxic effect on kidney in domestic animals (Pah et al., 2001; Hunt et al., 1994). More and more experiments have shown that high-dose boron (boron concentration more than 200 mg/L) have obvious inhibitive effect on the functions of brain (Penland, 1994), development of central immune organs (Jin et al., 2014), and immune system (Fry et al., 2010). In addition, high-dose boron can also decrease the body weight (Wilson and Ruszler, 1998), and damage the structure of thymus (Gu et al., 2007), and spleen (Hu et al., 2014). In our present study, morphological injury of the renal tubules was detected in both 320 and 640 mg/L boron groups when compared with the control group, including the loose cytoplasm, nuclear pyknosis, and basement membrane.
detachment in some cells. These cells demonstrated the characteristics of swelling, dissociation, and structure indistinct. Besides, cast formation has been seen in most of the lumens, especially in renal proximal tubules. Moreover, the extent of kidney injury was more obvious in 640 mg/L boron group than 320 mg/L boron group. These observations were consistent with the previous studies by Tavil Sabuncuoglu et al. (2006), indicating that high-dose boron resulted in histopathological changes in kidney tissue and the toxic effect was dose-dependent.

The renal tubule, considered as the important functional structure on the regulation of urine volume and composition, is one of the main targets damaged by high-dose boron supplementation. When renal tubule was injured, on the one hand, then the injured tubular cells underwent the apoptosis, and produced cast formation in the lumen of renal tubules observed in both 320 and 640 mg/L boron groups. But the mechanisms underlying the high-dose boron induced damage were not clear by far. We speculated the reason may be numerous. First, when absorbed in the blood, most of boron absorbed is ultimately eliminated as

\[ \text{blood, most of boron absorbed} \]

ultimately eliminated as

\[ \text{induced damage were not clear by far. We speculated the} \]

lumen of renal tubules observed in both 320 and 640 mg/L boron group.

In the present study, we evaluated the effect of boron supplementation on ostrich chicks’ kidney tissue, and to provide a comprehensive evaluation for the effect of boron on kidney tissue at different dosage and scientific basis for the ostrich chick breeding.

**Conclusion:** In the present study, we evaluated the effect of boron supplementation on ostrich chicks’ kidney tissue with different doses in drinking water. Results from this study indicated that 40 and 80 mg/L boron supplementation may promote kidney tissue growth; whereas, 320 and 640 mg/L of boron caused histopathological changes in kidney tissue and the damage effect was dose-dependent. These results suggested that adding a certain dose of boron in the drinking water may promote the kidney development of ostrich chick. More study is needed to confirm the appropriate dosage and the timing of supplementation during the ostrich chick breeding.

**Acknowledgement:** This study was supported by the National Natural Science Foundation of China No.31272517 and No.30972152.

**Author’s contribution:** KMP and JW conceived the experimental protocols. JW, PPS and KX executed the experiment. JW, JT and WW analyzed the data. All authors critically reviewed the manuscript and approved the final version.

**REFERENCES**


