

## EFFECTS OF FLUORIDE ON GROWTH AND THYROID FUNCTION IN YOUNG PIGS

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**SUMMARY:** This study was undertaken to investigate the effects of fluoride on growth and thyroid function in young pigs. Three groups of eight crossbred barrows were exposed to 100, 250, and 400 mg F<sup>-</sup>/kg (from NaF) in their diets for 50 days. Compared to a control group of eight pigs, the average daily gain in weight was significantly reduced, and serum thyroxine (T<sub>4</sub>) and free thyroxine (FT<sub>4</sub>) levels also decreased significantly. On the other hand, the level of serum thyroid-stimulating hormone (TSH) was significantly increased, but no significant differences were observed in serum triiodothyronine (T<sub>3</sub>) and free triiodothyronine (FT<sub>3</sub>). The activity of Na/K-ATPase in the thyroid was significantly inhibited as well as thyroid peroxidase (TPO). The results suggest that excessive fluoride in the diet can cause growth depression and hypothyroxinemia in pigs. Accompanying thyroid lesions were attributed to fluoride acting as a TSH analogue in concert with elevated TSH levels.

Keywords: Fluoride and growth; Growth depression; Hypothyroxinemia in pigs; Thyroid enzymes; Young pigs.

### INTRODUCTION

Prevalent in many parts of the world, chronic fluorosis, which is caused by prolonged ingestion of excessive amounts of fluoride, endangers the health of animals as well as humans. In addition to its well-known deleterious dental and skeletal effects, excess fluoride can exert toxic effects on many other tissues and organs, giving rise to a broad array of symptoms and pathological changes.<sup>1</sup>

Thyroid hormones play crucial roles in regulating development, differentiation, and metabolism of almost every tissue in the body of mammals, including bone and brain.<sup>2</sup> The thyroid gland is one of the most sensitive organs in its histopathological and functional responses to excessive amounts of fluoride.<sup>3</sup> In a 1976 review, McLaren noted that fluoride can accumulate in the thyroid and cause structural and functional changes.<sup>4</sup> However, in an earlier review published by the World Health Organization, Demole stated that fluoride does not accumulate in the thyroid gland, does not decrease the uptake of iodine by the thyroid, and has no effect on the synthesis of thyroxine.<sup>5</sup> More recently, Desai et al. found evidence that fluoride-induced goitres were associated with anatomical or structural changes rather than functional changes.<sup>6</sup>

In view of these conflicting reports, the present study was undertaken to assess and highlight the effects of fluoride on growth and thyroid function in young pigs.

### MATERIALS AND METHODS

Thirty-two 50-day-old barrows (Duroc × Landrace × Yorkshire) with an average body weight of about 17 kg were acclimatized for one week under uniform housing conditions and then allotted randomly to four groups of eight. These

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animals were the same pigs we used earlier,<sup>7</sup> which were fed the same basal diet containing 6.2 mg F<sup>-</sup>/kg in the control group 1, supplemented by 100, 250, and 400 mg F<sup>-</sup>/kg diet from NaF in experimental groups 2, 3, and 4, respectively. The fluoride content in the diet was determined according to the method (ID number 7.115) of AOAC (1984).<sup>8</sup> On the 50th day of the feeding trial, all pigs were deprived of food for 12 hr and then slaughtered. Blood samples were collected. The thyroid gland was carefully dissected out, immediately quick-frozen in liquid nitrogen and stored at -70°C prior to analysis.

Serum triiodothyronine (T<sub>3</sub>), free triiodothyronine (FT<sub>3</sub>), thyroxine (T<sub>4</sub>), free thyroxine (FT<sub>4</sub>), and thyroid stimulating hormone (TSH) levels were measured using radioimmunoassay (RIA) kits. The thyroid gland from the slaughtered pigs was homogenized and centrifuged. The supernatant was saved for determining the activities of Na/K-ATPase and thyroid peroxidase (TPO). The hydrolytic activity of Na/K-ATPase was calculated as ouabain-sensitive ATP hydrolysis by measuring the release of inorganic phosphate (P<sub>i</sub>) from ATP.<sup>9,10</sup> Na/K-ATPase specific activity was taken as the difference in P<sub>i</sub> concentration per milligram of protein per hr in the absence and presence of ouabain. The activity of TPO was assayed according to Hosoya et al.<sup>11</sup> The amount of enzyme, which produced a change of 1.0 absorbance unit per min, was taken as 1 unit and expressed as guaiacol units (GU). Protein contents in all homogenates were determined by the method of Bradford<sup>12</sup> with bovine serum albumin as standard.

The significance of the difference between means was determined by analysis of variance (ANOVA) with p<0.05 being considered significant.

## RESULTS

With increasing fluoride in the diet, the average daily gain (ADG) in weight of the pigs decreased significantly in groups 2, 3, and 4 (Table 1).

**Table 1.** Effect of fluoride on 50-day growth performance in young cross-bred barrow pigs<sup>a,b</sup>

|                      | Group  |         |         |         | SEM <sup>c</sup> |
|----------------------|--------|---------|---------|---------|------------------|
|                      | 1      | 2       | 3       | 4       |                  |
| Initial weight (kg)  | 17.16  | 17.25   | 17.18   | 17.31   | 0.18             |
| Final weight (kg)    | 42.85  | 40.93   | 39.08   | 38.41   | 0.73             |
| ADG <sup>d</sup> (g) | 513.75 | 473.50* | 438.00* | 421.75* | 13.41            |

<sup>a</sup>Values are means; n = 8 per group; <sup>b</sup>Groups 1, 2, 3, and 4 received the basal diet supplemented with 0, 100, 250, or 400 mg F<sup>-</sup>/kg diet, respectively; <sup>c</sup>Standard error of the mean; <sup>d</sup>ADG = average daily gain; \*p<0.05.

Compared to the control group 1, serum total and free T<sub>4</sub> levels were significantly lower in groups 3 and 4, whereas the serum TSH level was correspondingly increased; serum total and free T<sub>3</sub>, however, showed no significant change (Table 2).

**Table 2.** Effect of fluoride on serum T<sub>3</sub>, FT<sub>3</sub>, T<sub>4</sub>, FT<sub>4</sub>, and TSH concentrations after 50 days in young cross-bred barrow pigs<sup>a-c</sup>

|                          | Group |       |        |        | SEM <sup>d</sup> |
|--------------------------|-------|-------|--------|--------|------------------|
|                          | 1     | 2     | 3      | 4      |                  |
| T <sub>3</sub> (ng/mL)   | 0.66  | 0.60  | 0.61   | 0.57   | 0.04             |
| FT <sub>3</sub> (pmol/L) | 3.22  | 3.12  | 2.97   | 2.73   | 0.24             |
| T <sub>4</sub> (ng/mL)   | 35.93 | 29.44 | 28.30* | 27.88* | 2.60             |
| FT <sub>4</sub> (pmol/L) | 6.38  | 5.22  | 4.48*  | 4.15*  | 0.49             |
| TSH (μIU/mL)             | 0.98  | 0.91  | 1.31*  | 1.32*  | 0.11             |

<sup>a</sup>Values are means; n = 8 per group; <sup>b</sup>Groups 1, 2, 3, and 4 received the basal diet supplemented with 0, 100, 250, or 400 mg F<sup>-</sup>/kg diet, respectively; <sup>c</sup>T<sub>3</sub> = triiodothyronine; FT<sub>3</sub> = free triiodothyronine; T<sub>4</sub> = thyroxine; FT<sub>4</sub> = free thyroxine; TSH = thyroid-stimulating hormone; <sup>d</sup>Standard error of the mean; \*p<0.05.

Fluoride supplementation also significantly inhibited the activity of thyroid Na/K-ATPase in all fluoride-treated groups and TPO (thyroid peroxidase) in groups 3 and 4 (Table 3).

**Table 3.** Effect of fluoride on the activities of Na/K-ATPase and TPO after 50 days in thyroid tissue of young cross-bred barrow pigs<sup>a,b</sup>

|                                           | Group |       |       |       | SEM <sup>c</sup> |
|-------------------------------------------|-------|-------|-------|-------|------------------|
|                                           | 1     | 2     | 3     | 4     |                  |
| Na/K-ATPase (μmoles P <sub>i</sub> /mg/h) | 0.92  | 0.76* | 0.74* | 0.72* | 0.05             |
| TPO <sup>d</sup> (GU/mg)                  | 0.52  | 0.55  | 0.39* | 0.34* | 0.04             |

<sup>a</sup>Values are presented as means; <sup>b</sup>Groups 1, 2, 3, and 4 received the basal diet supplemented with 0, 100, 250, or 400 mg F<sup>-</sup>/kg diet, respectively; <sup>c</sup>Standard error of the mean; <sup>d</sup>TPO = thyroid peroxidase; n = 8 per group; \*p<0.05.

## DISCUSSION AND CONCLUSIONS

Significant variations in individual thyroid hormone levels were observed in all groups, including the control group. However, the overall trend pointed to hypothyroxinemia, and the serum hormone levels were similar to those normally observed in iodine deficiency (ID), such as high TSH, low T<sub>4</sub> and normal T<sub>3</sub>.<sup>13,14</sup>

Normal serum T<sub>3</sub> levels in the presence of high TSH and low T<sub>4</sub> indicate a compensatory mechanism to maintain euthyroidism. In this process, the type 2 (target-organ) iodothyronine deiodinase (D2) is the key enzyme responsible for modifying systemic T<sub>3</sub> levels, and its activity increases as T<sub>4</sub> levels decline. In fact, D2-generated T<sub>3</sub> production accounts for approximately 71% of the peripheral T<sub>3</sub> production in hypothyroidism.<sup>15</sup> Moreover, D2 is under external TSH control.<sup>16,17</sup> Recent studies have shown that, although serum T<sub>3</sub> levels might be normal in ID, numerous tissues which depend on local T<sub>4</sub> to T<sub>3</sub> conversion, such as the brain, cerebellum, and pituitary, might be hypothyroid.<sup>18</sup>

That fluoride might cause thyroid disturbances as are normally observed in ID, in spite of adequate iodine intake, has been documented for many years,<sup>4,6,19</sup> most recently in children in India.<sup>20</sup> Other recent investigations have shown that fluoride causes DNA damage in thyroid cells similar to that observed in ID, effects which were augmented when fluoride was given together with a low iodine diet.<sup>21</sup>

Abnormal thyroid pathology and the process of goitrogenesis in ID are thought to be the consequence of increased TSH stimulation.<sup>22</sup> Fluoride is a well-

established TSH analogue, which explains the similarities in abnormal thyroid pathology with ID. Not only may fluoride act like TSH in its absence, but it can also enhance TSH effects and alter the expression of G proteins,<sup>23</sup> thereby influencing all aspects of iodine uptake, transport, and T<sub>4</sub> to T<sub>3</sub> conversion.

In the present study, lassitude, anorexia, and sluggishness were observed in the fluoride-treated pigs during the experimental period. Growth depression affected by excessive fluoride ingestion has been reported in other animals.<sup>24,25</sup> Fluoride-induced growth depression might be ascribed to the bio-chemically-induced hypothyroidism which, in turn, causes decreased serum concentrations of growth hormone (GH) and reduced GH-binding activity.<sup>26,27</sup> Stunted growth as a result of ID is well documented.

In this study, we found that fluoride inhibited Na/K-ATPase and TPO (thyroid peroxidase) in the thyroid gland. The iodine required for hormone synthesis is known to accumulate in the thyroid gland through the combined actions of the Na/K-ATPase and the Na/I symporter,<sup>28</sup> a process modulated by TSH and thyroid hormones.<sup>29</sup> Decreased activity of Na/K-ATPase could adversely affect accumulation of iodide in the thyroid, which is opposite to stimulation of Na/K-ATPase induced by hypothyroidism.<sup>30</sup> This effect might be due to accumulation of fluoride in the thyroid, directly inhibiting the Na/K-ATPase,<sup>31</sup> or the combined activity of fluoride and high TSH on activation of the protein kinase C, which decreases the activity of Na/K-ATPase.<sup>32</sup>

TPO is also an integral membrane protein that is present in the apical (colloid-facing) plasma membrane of thyroid epithelial cells, and is also under external TSH control through adenylate cyclase (AC)/cAMP activation.<sup>33</sup> Similar to TSH, fluoride at low levels stimulates AC and at higher doses inhibits AC.<sup>34</sup>

Our previous reports have shown that the concentration of malondialdehyde was significantly increased and the activities of superoxide dismutase and glutathione peroxidase were significantly decreased in the thyroid, liver, and kidney of fluorotic pigs.<sup>7</sup> Thyroid hormone also modulates superoxide dismutase, malondialdehyde, and glutathione peroxidase activity in virtually all tissues.<sup>35-38</sup> In some organs, such as the liver, superoxide dismutase activity correlates closely with TSH levels.<sup>39</sup> Oxidative stress can damage the structure and function of thyroid cells as well as certain biomacromolecules such as proteins and nucleic acids.

In summary, this study demonstrated that excessive fluoride in the diet of young pigs caused growth depression, hypothyroxinemia, and altered thyroid hormone levels like those observed in iodine deficiency.

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