

BLOOD BIOCHEMICAL CONSTITUENTS IN CALVES FOLLOWING SUBCLINICAL LEVELS OF FLUORIDE TOXICOSIS

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SUMMARY: To investigate the effect of dietary fluoride (F) on some blood biochemical constituents, twenty male cross-bred calves (aged 6-8 months) were divided into equal groups and fed for 20 weeks on diets of a concentrate mixture and green maize (50:50 for the first 3 months and 40:60 during the later phase). The four dietary treatments differed only with respect to the F content of the mineral mixture. In treatments 1 and 2, the mineral mixture contained dicalcium phosphate, which was replaced with rock phosphate in treatments 3 and 4. However, treatments 2 and 4 were also supplemented with NaF so as to provide an additional 80 mg F/kg diet. The resultant dietary F levels were 7, 79, 132 and 191 ppm in groups 1 to 4, respectively. Blood serum analysis at biweekly intervals (carried out for 18 weeks) indicated that while serum P content did not change significantly, the serum alkaline phosphatase activity increased ($p < 0.01$) at higher levels of F intake. A decline in serum thyroxine (T4) level upon F addition was observed, which, however, failed to alter significantly the serum triiodothyronine (T3) levels.

Keywords: Alkaline phosphatase, Blood biochemistry, Calves, Fluoride supplement, Subclinical toxicosis, Thyroxine, Triiodothyronine.

INTRODUCTION

Mineral supplements that include phosphate rock and other fertilizer grade phosphate supplements are considered as potential dangers in animal feeding since they contain high levels (3-4%) of F.¹ A maximum limit of F (0.04% or 400 ppm) in mineral mixture has been suggested,² and defluorinating procedures are recommended,³ but they are not routinely followed due to their high cost and other technical problems. Moreover, the extent of defluorination is not usually ascertained. High F content has always been a caution in the use of rock phosphate for animal feeding as a phosphate supplement, and adequate information is available on clinical aspects of fluorosis. However, information is scarce on subclinical levels of excess dietary F and consequent metabolic changes. The present study was therefore undertaken to investigate the effect of subclinical levels of excess dietary F upon various blood biochemical constituents.

MATERIALS AND METHODS

Twenty Karan Fries (Tharparkar x Holstein Friesian) male calves aged 6-8 months age were divided into four equal groups in a randomised block de-

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sign. They were kept for 20 weeks on a diet of a concentrate mixture and green maize (50:50 for first 3 months and 40:60 during the later phase) as per standard recommendations.⁴ The concentrate mixture consisted of barley 40, groundnut cake 40, wheat bran 17 and mineral mixture 3 parts (crude protein 22% and total digestible nutrients 75%). The mineral mixture (per 3 kg) of groups 1 and 2 contained 1.65 kg dicalcium phosphate (0.018% F) as phosphorus supplements, whereas in groups 3 and 4, dicalcium phosphate was replaced with rock phosphate (1.5% F). Fluorine (as NaF) was added to the mineral mixture of groups 2 and 4 to achieve additional 80 ppm F in the diet. Moreover, the mineral mixture of the four groups was supplemented with required amounts of other major minerals (CaCO₃ 0.3312 kg, MgCO₃ 0.09 kg, and NaCl 0.9 kg) together with required amount of trace elements (0.0288 kg supplying Fe, Zn, Cu, Mn, Co, and I). The dietary F levels were 7, 79, 132, and 191 ppm in the four treatments groups, respectively. Blood samples were collected from the jugular vein in the beginning and subsequently at fortnightly intervals for 18 weeks. These samples were analysed for alkaline phosphatase activity,⁵ phosphorus concentration,⁶ and thyroxin (T4) and triiodothyronine (T3) (radio immuno assay).

Fluorine content in samples was determined by a fluoride selective ion electrode following procedures described by Villa⁷ and Tusl.⁸ The data were analysed statistically.⁹

RESULTS

Data obtained at fortnightly intervals on serum biochemical constituents (Table 1) indicated that alkaline phosphatase activity increased at higher dietary F levels. The activity was found to be highest in group 2, followed by groups 3 and 4, which were similar statistically. Serum phosphorus values, however, remained unaffected by the various treatments. There was some influence of dietary F levels on T4 but not on T3. With the increase in F dose, there was decline in T4 values, but the decline was not reflected appreciably in the final body weights of the calves (Table 2). Since differences due to replicates, time periods, and treatments were significant ($p < 0.05$) for T4 and T3, the effect of treatments alone was examined by regression equations between T4 and T3 level (Y) and weeks (X) after feeding for each treatment group. Regression lines (Figures 1 and 2) were developed with each regression equation. The respective regression equations for the four treatments groups for T4 were:

$$Y = 55.25 - 0.345X, Y = 59.60 - 0.844X, Y = 50.56 - 0.663X, \text{ and} \\ Y = 57.47 - 1.165X; \text{ for T3 these were } Y = 1.06 - 0.022X, \\ Y = 1.06 + 0.023X, Y = 0.84 + 0.026X, \text{ and } Y = 0.98 + 0.027 X.$$

These equations indicated that the serum T4 level in groups 2, 3 and 4 showed a significant decline compared to group 1. However, no such trend was observed for T3.

Table 1. Serum biochemical constituents in calves under different dietary treatments after 18 weeks

Serum measurement*	Treatment group			
	1	2	3	4
Alkaline Phosphatase (10 ¹ EU/mL)	4.74 ^a ± 0.20	6.00 ^c ± 0.29	5.38 ^b ± 0.37	5.14 ^b ± 0.34
Phosphorus (mg/100mL)	8.25 ± 0.15	8.15 ± 0.16	8.59 ± 0.16	8.31 ± 0.12
Thyroxine (ng/mL)	52.30 ^a ± 2.04	50.42 ^a ± 2.02	43.84 ^b ± 1.81	45.61 ^b ± 2.84
Triiodothyronine (ng/mL)	1.23 ^a ± 0.10	1.24 ^a ± 0.08	1.06 ^b ± 0.08	1.20 ^a ± 0.08

*Means bearing different superscripts in a row differ significantly (p<0.05).

Table 2. Fluorine balances, retention, and body weight of calves under different dietary treatments after 20 weeks

Attribute*	Treatment group			
	1	2	3	4
<i>Fluorine retention</i>				
Intake (mg/d)	31.79 ^a ± 3.41	302.00 ^b ± 18.47	448.11 ^b ± 58.37	786.63 ^c ± 112.34
Voided (mg/d)				
Faeces	9.16 ^a ± 1.32	17.42 ^a ± 2.38	55.31 ^b ± 0 8.10	092.17 ^b ± 22.73
Urine	19.74 ^a ± 2.35	83.02 ^b ± 2.26	96.34 ^b ± 15.81	156.26 ^c ± 12.72
Balance	2.90 ^a ± 1.09	196.00 ^b ± 15.71	296.47 ^b ± 44.36	538.20 ^c ± 80.12
Balance % of intake	9.12 ^a ± 3.08	64.53 ^b ± 1.56	65.58 ^b ± 02.87	068.22 ^c ± 0.94
Body wt (kg) [†]	131.99 ± 7.93	125.70 ± 6.86	119.09 ± 7.38	130.50 ± 7.41

*Means bearing different superscripts in a row differ significantly (p<0.05).

†Average of body weight recorded from 0 to 18 weeks.

DISCUSSION

The four dietary treatments tested in the present study were similar with respect to energy, protein, and mineral supply. The group differences were due only to two factors the amount of dietary F supply and the source of phosphorus supplement. Whereas dicalcium phosphate is an accepted phosphorus supplement,¹⁰ rock phosphate, due to its high F content and expensive defluorination procedures, is not widely used. In the present study, an attempt was made, therefore, to investigate the effect on certain blood biochemical constituents of higher levels of F in dicalcium phosphate and rock phosphate by adding NaF to them.

Data on serum alkaline phosphatase activity indicated that adding F to dicalcium phosphate (group 2) increased its activity. However, higher F levels in groups 3 and 4 (132 and 191 ppm F, respectively) did not cause further increase in activity. Probably at levels around group 3, body F retention as percent of intake attained saturation, and with further increase in dietary F level, rapid excretion of F through urine in amounts in excess of saturation occurred (Table 2). No significant differences in this enzyme activity in groups 2, 3, and 4 also support this interpretation. An increased activity of serum alkaline phosphatase at higher levels of F intake in different species has also been reported earlier.¹¹⁻¹⁴

It is also clear from Table 2 that there was some influence of dietary F levels in lowering the T4 values (Figure 1). But the degree of change was probably too little to be reflected in depressed growth as found earlier.¹⁵ Direct evidence of the effects of F on thyroid function in dairy cattle is contradictory.¹⁶⁻¹⁸ It has been suggested that F may interfere with I, due to competitive inhibition, when thyroxine is synthesised, but this effect may be dependant upon the level of both F and I intake.¹⁹ This proposal has been supported by an *in vitro* study with intact thyroid gland of mice.²⁰ In the present investigation, however, this competitive influence was not sufficient to manifest any significant effect on growth (Table 2). Therefore, F is probably not able to influence thyroid hormones in blood at lower levels of intake, but at higher levels, when actual clinical fluorosis occurs, there may be a decline in both T4 and T3 levels.

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Figure 1. Changes in serum thyroxine (T4) in calves at different levels of fluoride intake.

Finally, some workers²¹ have reported raised serum P level at high F intake (up to 3125 ppm) indicating nephritic dysfunction, whereas low serum P in field cases was observed at a feed F level of 45-96 ppm.²² Supplemental P sources in the present investigation probably maintained a normal serum P level and masked any influence, if present, of differences in dietary F levels. Our *in vitro* studies²³ also suggest no influence of F levels on ³²P uptake and absorption by intestinal segments and thus support the above finding.

Figure 2. Changes in serum triiodothyronine (T3) in calves at different levels of fluoride intake.

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