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Brief communication

STUDIES ON THE ANEMIOGENIC PROPERTIES OF TRIMETHYLAMINE OXIDE, AN ETIOLOGICAL FACTOR IN FISH-INDUCED ANEMIA IN MINK

A hypochromic microcytic iron deficiency anemia associated with light underfur and increased mortality in mink kits is caused by the feeding of various species of raw marine fish, especially coalfish (Gadus virens) and whiting (Gadus merlangus) as first detected in Norway by *Helgebostad* (1957). The same disorder caused by fish-feeding was later described from other countries (*Stout et al.* 1960, *Jørgensen & Christensen* 1966, *Kangas et al.* 1967). Nutritional anemia has also been induced in rats by feeding a raw coalfish diet as stated by *Gjønnes & Helgebostad* (1965).

Though the percentage of kits which develop anemia varies from year to year, the disorder is practically prevented by feeding *boiled* instead of *raw* fish (*Helgebostad*). Studies on the nature of the observed anti-anemic effect of *boiled* fish are in progress.

The etiology of this peculiar disorder has attracted much attention from fur breeders in the last decade. Causal factors have, up to now, been obscure, even though the anemic condition can be more or less reduced by supplementary feeding of meat products (*Helgebostad & Ender* 1961, *Helgebostad et al.* 1961).

Preliminary studies with radioactive iron (Fe^{59}) by *Havre et al.* (1967) revealed that iron absorption in the anemic mink was lowered, probably due to one or more substances in the raw fish which make iron less available to the animal.

Further studies led *Ender* to the idea that of the many possible factors present in raw fish which might be responsible for this disorder, there was reason to associate the anemic condition with the presence of one or more of the various methylamines, i.e. trimethylamine oxide, di-, and/or trimethylamine, all of which occur naturally in most raw marine fish (*Shewan* 1951), but not in noticeable amounts in meat products. The following research program was therefore initiated based on this assumption.

Preliminary studies revealed that severe anemia could be induced in adult mink after intraperitoneal injections of trimethylamine oxide (triox) in doses of 4 g weekly. Hemoglobin (Hb) and hematocrit (Ht) values in 10 mink dropped from an average level of 18.4 and 58 %, down to 12.4 and 49 %, respectively, after a test period of 7 weeks.

In experiments carried out with 10 mink kits, anemia could be induced after a feeding period of 5 months, if triox was administered at a level of 0.6—0.8 g daily, which was added to the boiled coalfish. No anemia was observed in the control group without triox (20 animals). A more pronounced anemiogenic effect of triox could also be demonstrated very clearly in a three-month experiment with 9 adult mink in which triox was added to a *raw* coalfish diet, compared with animals fed the same diet without triox supplement. On an average, a reduction of hemoglobin values from 17.1 to 10.6 % resulted when triox were fed in doses up to 3 g daily.

The degree of anemia seems to vary in relation to the level of triox in the raw fish, which showed a variance from 0.09 to 0.40 %. Raw fish of inferior quality is distinguished by a low triox content and elevated levels of trimethylamine. In feeding trials with raw fish possessing low levels of triox, the anemic condition increased when the dose of triox was augmented, which thus is considered to be an anemiogenic agent. In 10 adult mink which did not present any anemic signs, hemoglobin values dropped from a level of 17.5 % to a level of 14.3 % after a period of 2½ months during which increasing doses of triox (1—4 g) were added to the raw fish diet. No hemoglobin reduction was observed in the control group. Increased mortality and reduced fur quality to the same extent as generally observed in commercial mink-raising were observed in the experiments with kits. The other methylamines mentioned above had no anemiogenic effect.

The results of our experiments reveal that anemia can be induced in practically all mink, both young and adult, as demonstrated by the low Hb and Ht levels observed. Our experiments thus give substantial proof of the anemiogenic properties of triox. Evaluation of all hemoglobin and hematocrit values showed statistical significance.

The amount of triox in raw fish is generally reduced by about 35 % during boiling. Total elimination requires boiling for about 20 hrs. The anemiogenic effect of triox is, no doubt, caused

by the fact that triox, as shown in in vitro experiments, reacts with ferrous sulphate to produce a practically insoluble ferric hydroxide-oxide, the chemical nature of which will be described in a following paper. Great quantities of triox $(CH_3)_3NO.2H_2O$, with melting point 96°C, were synthesized.

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