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THE EFFECT OF THE BACTERIOLOGICAL STATUS OF FEED ON SOME HAEMATOLOGICAL AND BLOOD CHEMICAL DATA ON MINK

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JUOKSLAHTI, T.: *The effect of the bacteriological status of feed on some haematological and blood chemical data on mink.* Acta vet. scand. 1980, 21, 504—515. — Blood samples from 100 minks from a research farm using bacteriologically high quality feed and from 55 minks from another farm supplying bacteriologically inferior feed, as well as from nine minks from Denmark from two farms providing still better quality feed than both Finnish farms — all minks apparently clinically healthy — were analyzed for some haematological and chemical data: total leucocyte count, haemoglobin, ornithine carbamoyltransferase (OCT), alkaline phosphatase (AP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), γ -glutamyltransferase (γ -GT), total bilirubin and creatinine. The Finnish minks supplied with high quality feed had more optimal values of total leucocytes, haemoglobin, OCT, AP and creatinine than minks receiving feed with higher bacterial contamination. The Danish minks had better blood values in the investigated parameters except for lower haemoglobin and total bilirubin, which showed no significant difference.

blood parameters in mink; feed quality.

Feeds consumed by ranch mink differ greatly in both rheological form and chemical composition from conventional livestock rations, and the ingredients are more diversified. The feeds have high levels of protein and fat (40—50 % and 18—20 %, respectively, on a dry matter basis) and are relatively low in carbohydrates. The moisture content is about 70 %. The type and origin of the major ingredients of mink feeds render them vulnerable to bacteriological spoilage. Previous studies have shown that ready-mixed mink feed and its raw materials in Finland have relatively high bacterial counts (*Juokslahti 1978*,

1979). Enteric diseases of bacterial aetiology are invariably observed on mink farms. Feed-borne diseases account for 50—90 % of the diseases encountered in mink farming in Norway (*Loftsgård* 1968). German investigations (*Wenzell & Keil* 1980) have shown that about 60 % of all diseases registered during the rearing season are disturbances and damages arising from faulty feeding. Feed-borne bacterial infections have also been observed to be the main cause of mink diseases in the USA (*Turner & Howell* 1969), as confirmed by symptoms, necropsies, bacteriological studies and response to antibacterial drugs.

Aside from causing various diseases among mink herds, stress from high levels of bacteria in feed can be assumed to cause subclinical disturbances in the health of the animals. For evaluating subclinical diseases and metabolic disturbances among mink herds, the usefulness of clinical-chemical and haematological surveys of blood samples from different mink herds has merited attention (*Poulsen* 1977, 1980, *Wenzell & Keil*). This research was conducted in order to study the effects of bacteriologically low quality feed on the health of minks, monitored with some physiologically important blood parameters.

MATERIAL AND METHODS

The experimental minks were from two research farms owned by the Finnish Fur Breeders' Association. Farm A is located in southern Finland (geo. lat. 60°), its breeding female stock consists of 1200 standard and dawn minks. Farm B is situated in central Finland (lat. 63°) with a breeding stock of 2000 standard and dawn female minks. Animals from weaning age (early July) to pelting (late November) from both research farms were used in various feeding trials in the present experiments. The composition of the basic diets used on the farms during July-November are presented in Table 1.

Feeding trials on Farm A were performed by substituting 15—30 % of the whole fish and filleting offals indicated in Table 1 with frozen fish material of the following species: sandeel (*Ammodytes tobianus*), capelin (*Mallotus villosus*), sprat (*Clupea sprattus*), blue whiting (*Micromesistius poutassou*), Norway pout (*Gadus esmarchii*) and roach (*Leuciscus rutilus*) and acid-preserved sprat and roach (neutralized with calcium hydroxide).

Table 1. Percentage composition of basic diets used on two research farms during July-November.

Ingredient (%)	Farm A	Farm B
Slaughter-house offals	20.0	13.0
Slaughter blood	5.0	1.5
Cod filletting offals	25.0	15.0
Whole fish	20.0	25.0
Fish silage, neutralized	—	5.0
Fish meal	4.0	5.0
Meat meal	—	2.0
Soybean meal	—	1.0
Wheat meal, boiled	—	4.0
Wheat meal, extruder boiled	10.0	4.0
Potatoes, boiled	—	6.0
Brewer's yeast	0.5	0.5
Vegetable oil	—	0.5
Potato fiber	—	0.7
Vitamin mixture ^a	1.5	1.5
Water	14.0	15.3
Total	100.0	100.0

^a Contained (per kg) vitamin A, 500,000 i.u.; vitamin D₃, 150,000 i.u.; ascorbic acid, 5,000 mg; vitamin E, 2,000 mg; vitamin K, 10 mg; thiamine hydrochloride, 400 mg; riboflavin, 300 mg; pyridoxine hydrochloride, 120 mg; vitamin B₁₂, 1 mg; cholin, 1,700 mg; pantothenic acid, 500 mg; niacin, 1,400 mg; folic acid, 50 mg; biotin, 3 mg.

The feeding trials on Farm B were carried out by adding separately the above-mentioned fish silage, potato protein and meat meal to the basic feed. Samples of ready-mixed experimental feeds were taken during the rearing season and forwarded to the Feed Laboratory, Vaasa, where analyses of nutrient contents were made using conventional feed analysis methods. The results are presented in Table 2. The nutrient contents are within recommended levels (*Ahman* 1961).

The basic and experimental diets on Farm A were prepared in the farm kitchen. On Farm B the basic diet was supplied by the local central mink feed kitchen, and the experimental additions to the basic diet were made on the research farm.

The bacteriological quality of the feeds differed between the farms. In Farm A feed only high quality slaughter-house offals were used, as opposed to the Farm B basic feed in which also bacteriologically low quality ingredients were included. The

Table 2. Chemical analysis of nutrient contents of feed mixtures used on two research farms during July-November (mean \pm s).

	Farm A	Farm B
Dry matter (%)	31.2 \pm 3.6	33.4 \pm 2.7
In dry matter (%):		
Ash	7.6 \pm 2.6	12.4 \pm 2.1
Crude protein	45.2 \pm 4.0	43.7 \pm 14.6
Crude fat	21.4 \pm 6.7	18.8 \pm 2.3
Crude carbohydrates	25.8 \pm 4.5	25.1 \pm 7.5
Number of samples	31	11

central feed kitchen specifically uses Product D containing slaughter-house offals, which in a previous study have been shown to be highly contaminated with bacteria (*Juokslahti* 1979). During the rearing season (July-November) 16 samples of Farm A feed and six samples of Farm B feed were analyzed for total bacterial count, faecal streptococci, coliform bacteria, haemolytic bacteria, sulphite-reducing bacteria, peroxide value and free fatty acids. The bacteriological analyses were conducted in the manner described earlier (*Juokslahti* 1978), while peroxide value was determined iodometrically with sodium thiosulfate, and free fatty acids were titrated with sodium hydroxide (*Horwitz* 1975). The results of the analysis are presented in Table 3.

The table indicates that the mean bacterial counts for faecal streptococci, coliform bacteria, haemolytic bacteria and sulphite-reducing bacteria were significantly higher in samples from Farm B than the means for samples from Farm A ($P < 0.001$ and $P < 0.01$). Several cases of bacterial enteritis were observed on Farm B during the experimental period in July-November, requiring five antibiotic treatment periods of five—seven days' duration during the rearing season to combat the enteritis. The minks had been vaccinated against Mink Virus Enteritis (MVE) in June. Thus MVE as an aetiological factor in the enteric diseases could be excluded. On Farm A the health of the animals was good throughout the experimental period. The bacteriological quality of Danish mink feeds has been shown to be better than Finnish feeds (*Juokslahti* 1978, 1980). The feed used for the Danish minks in this experiment was checked systematically by the Mink Feed Control of the Danish Fur Breeders' Association, and was shown to be of average quality (*Poulsen, H.*, personal communication).

Table 3. Bacterial counts, peroxide value and free fatty acids in samples from Farm A feed and Farm B feed during July-November (mean \pm s).

	Farm A	Farm B	Significance ^a
Total bacterial count (log/g)	5.59 \pm 0.33	5.77 \pm 0.16	ns
Faecal streptococci (log/g)	2.43 \pm 1.20	4.06 \pm 0.80	***
Coliform bacteria (log/g)	1.38 \pm 1.49	4.01 \pm 0.81	***
Haemolytic bacteria (log/g)	2.85 \pm 0.35	3.75 \pm 0.57	**
Sulphite-reducing bacteria (log/g)	1.87 \pm 1.56	4.18 \pm 0.47	**
Peroxide value (meq/kg)	78.4 \pm 61.5	61.0 \pm 43.0	ns
Free fatty acids (%)	14.7 \pm 3.5	14.3 \pm 4.1	ns
Number of samples	16	6	

^a Significance of differences between Farm A and Farm B:

ns P > 0.05

** P < 0.01

*** P < 0.001

During the pelting time from November eight—ten male kits aged seven months were randomly taken from each experimental feeding group for blood sampling. The sampling was performed by the author on November 21—22 with a total of 100 minks from Farm A, and on November 29 with 55 animals from Farm B. The minks were withheld feed from 4.00 p.m. the day before sampling, which was carried out from 8.00—12.00 a.m. on the following day. The minks were captured in portable cages and anaesthetized in an ether chamber. Under anaesthesia a 10 ml blood sample was drawn by cardiac puncture with a heparinized needle and syringe. Haemoglobin and total leucocyte determinations were made immediately. Haemoglobin was determined with a Spencer haemoglobinometer (American Optical Co., Buffalo, USA), according to *Schalm et al.* (1975) and total leucocyte count was made under microscope with a Bürker counting chamber (*Schalm et al.*). Blood was centrifuged and plasma removed and frozen in CO₂ ice. The frozen plasma samples were

forwarded for analysis to the laboratory at the Department of Biochemistry, College of Veterinary Medicine. Ornithine carbamoyltransferase (OCT; EC 2.1.3.3.) was measured according to the manual method of *Ohshita et al.* (1976), except that the incubation time at 37°C was increased from 20 min to 45 min to obtain better sensitivity. Alkaline phosphatase (AP; EC 3.1.3.1.), alanine aminotransferase (ALAT; EC 2.6.1.2.), aspartate aminotransferase (ASAT; EC 2.6.1.1.) and γ -glutamyltransferase (γ -GT; EC 2.3.2.2.) were analyzed using a Gilford System 3500 Computer directed Analyzer (Gilford Instrument Company, Oberlin, Ohio, USA) according to the standard methods of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974, 1976).

Total bilirubin was analyzed by diazotization in the presence of caffeine sodium benzoate and quantitation of the blue alkaline form of azobilirubin, and creatinine was analyzed according to the kinetic Jaffe reaction, both these with a Gilford Analyzer.

For comparison with the blood values of the Finnish minks, nine minks aged five months from two Danish farms were sampled by cardiac puncture under Althesin® anaesthesia with Combelen® premedation. The frozen plasma specimens were forwarded by air to our laboratory, and the samples were analyzed in the same manner as the Finnish samples. The health of the minks from both Danish farms had been normal throughout the rearing season (*Brandt*, personal communication).

After blood sampling the Finnish minks were killed by cervical dislocation, and liver specimens were taken for total lipid analysis with a Foss-Let Analyzer (A/S N. Foss Electric, Kolding, Denmark) according to the method of *Pettinati & Swift* (1975). Statistical analysis was performed using the Student's t-test.

RESULTS AND DISCUSSION

The results of the haematological and blood chemical analyses are presented in Table 4. The mean total leucocyte count of the Farm A minks was significantly lower than the corresponding mean for Farm B animals ($P < 0.01$). The minks on both Finnish farms had higher mean leucocyte counts than the minks from the Danish farms ($P < 0.001$ and $P < 0.05$). *Rotenberg & Jørgensen* (1971) report a mean value of $5.6 \pm 1.4 \times 10^6$ leucocytes/l in blood of six month-old male minks in Denmark,

Table 4. Haematological and blood chemical data (mean \pm s) of minks on two research farms (Farm A and Farm B) and of minks on two Danish farms.

	Farm A	Farm B	Danish farms	Significance of differences between groups*
Number of minks	100	55	9	
Total leucocytes ($10^6/l$)	6.4 \pm 3.1	8.1 \pm 2.7	4.5 \pm 2.6	c***a**b*
Haemoglobin (mmol/l)	11.5 \pm 1.3	10.9 \pm 0.8	8.4 \pm 2.1	bc***a**
Ornithine carbamoyl-transferase (nkat/l)	77.5 \pm 39.0	110.2 \pm 53.8	26.7 \pm 14.2	abc***
Alkaline phosphatase (μ kat/l)	1.64 \pm 0.43	2.23 \pm 1.78	1.35 \pm 0.29	bc**a*
Alanine aminotransferase (μ kat/l)	3.22 \pm 2.19	3.96 \pm 2.34	1.17 \pm 0.50	bc***a ^{ns}
Aspartate aminotransferase (μ kat/l)	2.56 \pm 1.03	2.89 \pm 1.51	1.66 \pm 0.41	bc***a ^{ns}
γ -glutamyl transferase (μ kat/l)	0.34 \pm 0.21	0.38 \pm 0.12	0.07 \pm 0.03	bc***a ^{ns}
Total bilirubin (μ mol/l)	2.07 \pm 2.14	2.21 \pm 1.80	2.38 \pm 2.80	abc ^{ns}
Creatinine (μ mol/l)	64.79 \pm 12.85	77.07 \pm 17.76	50.51 \pm 17.98	ac***b**

* a = between Farm A and Farm B

b = between Farm A and Danish farms

c = between Farm B and Danish farms

ns P > 0.05

* P < 0.05

** P < 0.01

*** P < 0.001

and in the USA *Wehr et al.* (1980) report a mean value of $6.2 \pm 2.4 \times 10^6$ leucocytes/l in seven and a half month-old male standard minks. *Fletch & Karstad* (1972) report $4.4 \pm 0.3 \times 10^6$ leucocytes/l in seven month-old standard minks. Their investigation revealed no difference between various colour mutants. A report from the Soviet Union gives the mean leucocyte count of six month-old minks to be $4.2 \times 10^6/l$ and seven—eight month-old minks to be $4.8 \times 10^6/l$ (*Berestov* 1974). The samples of *Rotenberg & Jørgensen* were obtained from arteria caudalis on unanaesthetized minks, by cardiac puncture under ketamine anaesthesia in *Wehr et al.*'s investigation, and by cardiac puncture under ether anaesthesia in *Fletch & Karstad*'s investigation.

Jepsen et al. (1979) have shown that the leucocyte count in mink is unaffected by the anaesthesia methods mentioned above. The mean leucocyte counts for minks from both Finnish farms were higher than those reviewed. Leucocytosis is almost always associated with infectious diseases in furred animals but can be observed with some non-infectious diseases as well (*Berestov*). For instance tocopherol deficiency has been shown to cause elevated leucocyte counts in mink (*Stowe & Whitehair* 1964). The tocopherol supplementation and fat quality of the feeds were the same on both experimental farms. The elevated leucocyte levels in this experiment could be indicative of poor bacteriological quality of the feed.

The minks on Farm A had significantly higher mean haemoglobin values than the mean for minks on Farm B. The haemoglobin value in growing minks is age-dependent and steadily increases until the pelting season (*Berestov*), so the lower mean haemoglobin values for Danish minks are not directly comparable to the corresponding Finnish values of older animals. Fish-induced anaemia is well known in minks when feed is inadequately supplemented with iron. Both research farms did not differ in this respect and in the amounts of fish fed, thus excluding the possibility of fish-induced anaemia in Farm B minks. In the development of the red cell and its constituents a number of nutritionally important substances play a part, so that deficiencies in the absorption of any of these factors may interfere with the development of the normal haemoglobin content in blood (*Hoffman* 1970, *Schalm et al.* 1975). The haemoglobin content of blood is also affected by nutrition (*Greig & Boyne* 1956). The lower mean haemoglobin content of the blood of the Farm B minks could be due to the enteric outbreaks occurring during the rearing season.

The mean activities of plasma enzymes in minks of Farm A were lower than the respective means for Farm B minks, the difference for OCT being significant at the $P < 0.001$ level and for AP at the $P < 0.05$ level. The Danish minks had lower mean values for all enzymes investigated when compared to the mean values for both Finnish farms (OCT, ALAT, ASAT, γ -GT significant at the $P < 0.001$ level and AP at the $P < 0.01$ level). These enzymes are elevated in the serum when cellular damage or increased cell membrane permeability occurs in the organs where these enzymes are located (*Freedland & Kramer* 1970).

OCT in mink is strictly liver specific (Juokslahti *et al.* 1980), and the elevation of its activity in blood is an indication of liver damage. AP is a membrane-bound enzyme distributed in the organism in the epithelial cells of intestines, kidney tubuli cells, endothelial cells of capillaries, especially in liver, biliary tubuli cells and osteoblasts (Thorén-Tolling 1979): in mink the highest activities were observed in intestines and kidneys (Juokslahti *et al.*). The blood AP activity in mature animals is mainly from the liver, but activity of intestinal origin may be found in some animal species (Thorén-Tolling). As the age of the sample minks on both Finnish farms was the same, the osteoblastic AP is presumably equal in the specimens, and therefore the elevation of AP activity observed in minks from Farm B may be attributed to cell damage in the tissues listed above. Microbes and microbial toxins have shown to have a deleterious effect on the liver. In experimental salmonella infection in rats, liver alkaline phosphatase activity has been demonstrated to increase (Wachstein *et al.* 1962). Experimental aflatoxicosis causes a marked increase in plasma alkaline phosphatase indicating liver damage in minks (Chou *et al.* 1976). The difference in the ages of the Finnish and Danish minks does not permit a direct comparison of AP activities, but the significantly lower activities in Danish minks are, however, presumably indicative of lesser cell damage in the organs mentioned. ALAT is found in mink liver and heart muscle in higher activities, ASAT is found especially in musculature and also in many other organs, and γ -GT is mainly found in kidneys and pancreas (Juokslahti *et al.*). The lower activities of these enzymes in the plasma may indicate that there is a lesser degree of cell damage in the Danish minks. Mean ALAT, ASAT and γ -GT activities were lower in minks from Farm A than Farm B minks, although the differences were not significant. The mean total bilirubin concentrations did not differ between the farms. The minks on Farm B had significantly higher mean creatinine concentrations than Farm A minks and the Danish minks ($P < 0.001$), and the Farm A minks had significantly higher concentrations than the Danish minks ($P < 0.01$). Elevated creatinine blood levels indicate renal insufficiency (Hoffman 1970). The mean total lipid content of the liver specimens of the minks from Farm A was 5.90 ± 1.90 % and the minks from Farm B 6.11 ± 2.08 , the difference being insignificant.

The analytical data of this investigation indicates that despite the apparent clinical health of the animals, significant differences exist in the clinical blood parameters of mink from different farms. The minks from Farm A thus had more optimal clinical blood parameters than the minks from Farm B, and the Danish minks had even more optimal clinical blood parameters than the Finnish minks. These discrepancies may be attributed to differences in the bacteriological qualities of the mink feeds used.

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SAMMANFATTNING

Betydelsen av fodrets bakteriella kvalitet på några blodvärden hos kliniskt friska minkar.

Blod från 100 minkar från en försöksfarm med foder av bakteriologiskt god kvalitet, från 55 minkar från en annan farm med foder av bakteriologiskt dålig kvalitet och från nio minkar från Danmark med ett foder av bättre bakteriologisk kvalitet än de båda finländska gruppernas — samtliga djur kliniskt friska — undersöktes på några hematologiska och kemiska värden: totalt leukocyttal, hemoglobin, ornitinkarbamyltransferas (OCT), alkalisk fosfatas (AP), alanin-aminotransferas (ALAT), aspartat-aminotransferas (ASAT), γ -glutamyltransferas (γ -GT), totalt bilirubin och kreatinin. De finska minkarna, som erhöll kvalitativt gott foder, hade signifikant bättre värden vad gäller totala leukocyttalet, hemoglobin, OCT, AP och kreatinin jämfört med minkarna som fick starkare infekterat foder. Blodvärdena hos de danska minkarna var bäst så när som på hemoglobin, vars värde var signifikant lägre, och bilirubin, som inte visade någon signifikant skillnad.

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