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PATHOGENIC, ENTEROTOXIN-PRODUCING STAPHYLOCOCCI IN MINK FEED AND MINK FEED RAW MATERIALS

By

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JUOKSLAHTI, T., S. LINDROTH and A. NISKANEN: *Pathogenic, enterotoxin-producing staphylococci in mink feed and mink feed raw materials.* Acta vet. scand. 1980, *21*, 516—522. — Samples of 51 mink feeds and 101 raw material batches were analysed for pathogenic staphylococci. Of the feed samples 37 % were contaminated with pathogenic staphylococci, the mean count being 3.02 log CFU(colony forming units)/g and the highest count being 4.48 log CFU/g of feed. Of the raw materials investigated, slaughter-house offals were most frequently contaminated and contained highest counts of S. aureus, 60 % of the samples being positive with a mean log count of 3.86/g and a maximum of 5.70/g. Forty-eight staphylococcal isolates were tested for enterotoxin production. Toxin A, B or C were produced by eight isolates, A being the most common. None of the isolates produced B or E toxins.

mink feed; staphylococci; enterotoxins.

In the previous studies it was shown that ready-mixed mink feed and its raw materials are invariably contaminated with various bacteria (*Juokslahti* 1978, 1979). Of the feed samples investigated 95.4 % contained haemolytic bacteria, and especially raw materials derived from slaughter-houses had high counts of haemolytic bacteria. In the investigations bacteria colonies suggesting pathogenic staphylococci were frequently observed on primary blood agar plates. Because of the practical feeding management — feed is kept on cage nettings on farms at outdoor temperatures for a feeding period of 24 h — suitable conditions for staphylococcal outgrowth exist during warm seasons. Moreover, staphylococci have been shown to multiply rapidly in mink feed at 30°C, a possible outdoor temperature on warm summer

days (*Chou & Marth* 1969). According to *Niskanen* (1977) staphylococci enterotoxin production is closely associated with cell growth.

Staphylococcus aureus is one of the most prevalent food poisoning bacteria in man, and the presence of coagulase-positive staphylococci in foods represents a serious food-poisoning risk. Staphylococcal infections are known to occur in minks (*Head* 1959, *Budd et al.* 1966, *Trautwein & Helmboldt* 1966, *Löliger* 1970, *Crandell et al.* 1971), and the authors have shown that mink is susceptible to staphylococcal enterotoxin per os but the amounts are higher than needed to cause intoxication in man (*Juokslahti et al.* 1980). Disease outbreaks which resemble staphylococcal enterotoxin intoxications commonly occur in mink farming.

The purpose of the present study was to investigate the extent to which ready-mixed mink feed and its raw materials are contaminated with staphylococci, the number of staphylococci enterotoxin producers and the most common toxin types produced. The data should reveal the enterotoxicosis potentiality of mink feed as well as the raw materials that bear the highest risk in this respect.

MATERIALS AND METHODS

Samples of 51 ready-mixed mink feeds and 101 raw material batches were obtained through the regular feed quality control of the Finnish Fur Breeders' Association. The samples were collected and forwarded to the Feed Laboratory, Vaasa, in the manner described in earlier papers (*Juokslahti* 1978, 1979).

Total bacterial count (TBC) and staphylococcal enumeration. A 2 g portion of the sample was homogenized for 2 min in 20 ml of a sterile saline solution with an Ultra Turrax homogenizer (Janke & Kunkel, Stauffen, Germany). Serial dilutions of the homogenate were made and 0.1 ml aliquots were plated on Nutrient Agar (Orion Diagnostica, Espoo) for total bacterial count and on Baird Parker agar (BP; Bacto Baird Parker Agar Base, Difco, with added Bacto Egg Yolk Tellurite Enrichment, Difco) for staphylococcal enumeration. Black, shiny colonies 1.0 to 1.5 mm in diameter surrounded by a clear zone 2 to 5 mm in width were counted as staphylococci. The Nutrient Agar plates were incubated for 3 d at 30°C and the BP plates for 2 d at 37°C.

Coagulase and thermonuclease production. Typical staphylococcal colonies were tested for coagulase and heat-stable deoxyribonuclease production. Coagulase was tested with Coagulase Plasma (Difco) by the tube method. The tubes were incubated at 37°C and examined after 2 and 4 h. Completely or partially coagulated plasma was interpreted as a positive result. Thermonuclease was tested by the metachromatic well-agar diffusion technique described by *Lachica et al.* (1972).

Enterotoxin production. Forty-eight coagulase and thermonuclease positive staphylococcal isolates were analysed in the Food Research Laboratory of the Technical Research Centre of Finland, Espoo. Toxins were produced by the sac-culture method of *Donnelly et al.* (1967), and the culture supernatant fluids were tested for enterotoxin A, B, C, D and E by the microslide technique as described by *Casman & Bennett* (1965). Control toxins A and C as well as the corresponding antisera were obtained from Prof. H. Fey, the Veterinary-Bacteriological Institute, University of Bern, Bern, Switzerland, and those of B, D and E from Prof. M. S. Bergdoll, the Food Research Institute, University of Wisconsin, Madison, Wisconsin, USA.

RESULTS

Table 1 shows that 37 % of the ready-mixed mink feed batches were contaminated with pathogenic staphylococci, the mean count being 3.02 log CFU per g and the highest count 4.48 log CFU per g. Of the raw materials investigated, the highest staphylococcal contamination was found in slaughter-house offals. The contamination frequency was 60 %, the mean count 3.86 and maximum 5.70 log CFU/g. Four of 21 slaughter blood samples, one of five chicken offal and one of eight other miscellaneous raw material samples contained staphylococci. The lowest mean count, 2.69 log CFU/g, was recorded in slaughter blood samples. All BP colonies tested were coagulase and thermonuclease positive.

Total bacterial count in mink feed and mink feed raw materials was recorded since it is regarded as a general indicator of the bacteriological quality of the product. The highest mean count, 7.3 log CFU/g, was in chicken offals, followed by slaughter offals with 6.36 log CFU/g. The lowest mean count of total bacteria, 4.59 log CFU/g, was in the group of other miscellaneous

Table 1. Total bacterial and staphylococcal counts in mink feed and mink feed raw materials.

Product	Number of samples examined	Total bacterial count (log CFU/g)		Staphylococci[a]		
		mean	maximum	positive samples	(log CFU/g) mean	maximum
Mink feed	51	5.93	7.20	19 (37 %)	3.02	4.48
Raw materials:						
Slaughter-house offals	67	6.36	7.38	40 (60 %)	3.86	5.70
Slaughter blood	21	6.09	7.17	4 (19 %)	2.69	3.30
Chicken offals	5	7.13	7.25	1 (20 %)	3.70	3.70
Other raw materials	8	4.59	6.65	1 (12 %)	2.78	2.78
In total	152	6.06	7.38	65 (43 %)	3.54	5.70

[a] Based on the ability to form colonies on Baird Parker agar.

raw materials. Ready-mixed mink feeds had a mean TBC of 5.93 log CFU/g.

Table 2 shows that eight of 48 staphylococcal isolates produced some enterotoxin. Six of the enterotoxigenic strains were isolated from slaughter-house offals. The most common toxin type was enterotoxin A, produced by four strains. C and D toxins were produced by two strains. None of the investigated strains produced B or E toxin. All enterotoxigenic strains (17 %) produced only one type of toxin.

Table 2. Enterotoxin production by staphylococcal strains isolated from mink feed and mink feed raw materials.

Product	Isolates tested	Enterotoxin production				
		A	B	C	D	E
Mink feed	10	—	—	—	1	—
Raw materials:						
Slaughter-house offals	30	4	—	2	—	—
Slaughter blood	5	—	—	—	—	—
Chicken offals	2	—	—	—	1	—
Other raw materials	1	—	—	—	—	—
In total	48	4	—	2	2	—

DISCUSSION

Pathogenic staphylococci contaminated 37 % of the ready-mixed mink feed batches. The highest incidence rate of staphylococci, 60 %, was found in slaughter-house offals, which are common raw materials for mink feed production. Slaughter-house offals (*Morehouse & Wedman* 1961) and frozen meat by-products from slaughter-houses (*Chou & Marth* 1969) have in previous studies been found to contain staphylococci with reported frequencies of 57 and 40 %, respectively. Pathogenic staphylococci present in mink feed raw materials are easily transmitted to the final feed since heat treatment which would kill bacteria is not used in mink feed production.

The mean staphylococcal counts in the present study were from 4.9×10^2 to 7.2×10^3 CFU/g. Since staphylococci can readily multiply in feed products (*Chou & Marth*) and mink feed is kept on cage netting at outdoor temperatures for up to 24 h, the warm season provides a real possibility for the presence of staphylococci in mink feed in excess of 10^6 CFU/g, the level considered minimum for enterotoxin production in foods (*Bergdoll* 1972). Enterotoxin was produced by 17 % of all staphylococcal isolates and 20 % of slaughter-house offal isolates. The most common type of toxin produced was enterotoxin A. About 40 % of S. aureus strains isolated from livers of commercially slaughtered poultry (*Genigeorgis & Sadler* 1966) and 62 % of staphylococcal strains isolated from broiler and hen carcasses at processing plants (*Gibbs et al.* 1978) were found enterotoxigenic. Enterotoxin was reported in 69 % of S. aureus strains isolated from foods (*Niskanen & Koiranen* 1977) and in 41 % of S. aureus strains isolated from mastitic milk samples (*Niskanen et al.* 1978). The toxin most often encountered was also enterotoxin A.

High total bacterial counts were also found in slaughter-house offals in connection with high staphylococcal counts, indicating that staphylococci can also readily grow in feeds in spite of competition from other bacteria. Furthermore, the increasing use of antibacterial feed additives in mink feed may facilitate the outgrowth of resistant staphylococci. The results of the present study indicate that mink feed is a potential causative agent to enterotoxicoses in minks and that the raw materials for feed originating from slaughter-houses are the most risky raw materials in this respect.

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SAMMANFATTNING

Förekomst av patogena, enterotoxinproducerande stafylokocker i minkfoder och -råvaror.

Foder- och råvaruprov, 152 ialt, blev analyserade för förekomst av patogena stafylokocker; 37 % av foderproven var kontaminerade med patogena stafylokocker, i medeltal var bakteriehalten 3.02 log per gram foder, högsta halten var 4.48 log bakterier per gram foder. Av undersökta råvaror var slaktavfallsprodukterna mest kontaminerade, 60 % av proven innehöll stafylokocker, i medeltal 3.86 log bakterier per gram, högsta halten var 5.70 log bakterier per gram. Fyrtioåtta isolerade bakteriestammar testades med avseende på enterotoxinbildning, 17 % av stammarna producerade A, C eller D toxin, ingen av stammarna producerade B eller E toxin.

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