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Brief Communication

From the National Veterinary Institute, Oslo, Norway.

ENDOTOXINS IN ANIMAL FEEDSTUFFS*

Microbial heat stable endotoxins comprise part of most animal feedstuffs. These components have become the subject of considerable interest in later years because of the demonstration of acute toxicity, intravascular coagulation and generalized Shwartzman's reaction in connection with intravenous injections of endotoxins (*Nordstoga* 1976). Endotoxins are normally present in the intestine of man and animals, but seem only to be absorbed under special conditions. Of particular interest is the "sudden infant death syndrome" which has been described for infants receiving cow milk (*Di Luzio & Friedmann* 1973). Absorption of endotoxins may also occur in adults e.g. in connection with γ-irradiation, immunosuppression, transplantations, severe traumatic lesions and burns (*Nordstoga*). It is supposed that endotoxins play an important role in the development of shock under these conditions (*Nordstoga*). *Fine* (1972) reported that exogenously administered endotoxins may break down the defence mechanisms and lead to continuous absorption of endotoxins from the intestine.

Until recently, no convenient method was available for the detection of endotoxins in complex organic materials. A method is now described, however, the Limulus Amebocyte Lysate** test, which is primarily a method for the qualitative detection of endotoxins (*Levin et al.* 1972).

Work has been done at the National Veterinary Institute in order to modify this method for the semiquantitative analyses of endotoxins in feedstuffs.

Several organic solvents are used for the extraction of endotoxins. Among these ethanol, phenol and diethyleneglycol were tested in the present investigation for endotoxin analyses. It was found, however, that all the tested standard endotoxin samples derived from Escherichia coli became negative in the presence of even low concentrations of each of the 3 organic solvents mentioned when using the Limulus Amebocyte Lysate test.

Extraction with 1 M-NaOH and 1 M-H_2SO_4 with subsequent neutralization was also tested. It was found, however, that the most sensitive detection of endotoxins using standard endotoxin samples was obtained with distilled water using the procedure described below:

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** Mallinkrodt Chemical Work, St. Louis, Mo., USA.

One gram of a representative finely ground sample of the feed is added to 20 ml of pyrogen free water (as tested by the Limulus test). The suspended material is frequently shaken during 1 hr. at room temperature and then centrifuged at low speed (about 50 × g) for about 5 min. One ml of the supernatant is diluted 1:50 in pyrogen free water. From the diluted sample (A), 0.1 ml is mixed with 0.1 ml of the Limulus extract. A positive endotoxin control and a negative pyrogen free water control are included in each analysis. Serial 10-fold dilutions of the 1:50 diluted sample (A) are prepared and tested with Limulus extract until a negative reaction is obtained.

A number of samples from various types of feedstuff have been analysed for endotoxins according to the procedure described (Table 1). Some of the products examined were suspected as being the cause of feed intoxication. Because of the uncertainty in determining the endpoint dilution for a positive endotoxin test, only serial 10-fold dilutions were carried out. Thus, the method described must be considered as semiquan-

Table 1. Analyses for the counts of total viable bacteria, viable coliforms, viable sulphite reducing clostridiae, viable moulds and endotoxin titre in samples of "normal" feed and in some samples connected with supposed feed intoxication. The endotoxin titres refer to samples containing 1 mg feed material per ml of water.

Samples from	Viable total counts per g	Viable coliforms per g	Viable sulphite reducing clostridiae per g	Viable moulds per g	Endotoxin titre
Guar meal	700000	3000	100	2100	1: 100
Cotton expellers	600000	100	—	4000	1:10000
Raps meal	23000	—	—	100	1: 10
Corn meal	3000	—	—	300	1: 10
Guinea corn meal	400000	—	—	600	1: 100
Oats, Norwegian product	2.900000	—	—	3900	1: 1000
Oats, Norwegian product	12000	—	—	—	1: 100
Barley, Norwegian product	84000	—	—	—	1: 100
Barley, Norwegian product	600000	—	—	7000	1: 100
Fish meal	7000	200	300	300	1: 1
Fish meal	200000	170	1000	600	1: 10
Meat-bone meal	0	—	—	—	1: 10
Meat-bone meal	10000	—	30	—	1: 1
Milk replacer for calves	60000	—	—	—	1: 100
Feed mixture for hens	1.000000	2000	50	1000	1: 1000
Feed mixture for piglets	15000	—	10	100	1: 100
Feed mixture for chinchilla	15000	—	—	—	1: 100
Feed mixture for rabbits and guinea pigs	12000	—	10	—	1: 100
Feed mixture for mice and rats	100000	2000	1000	—	1: 1000
Feed mixture for swine	300000	100	300	—	1: 1000
Feed mixture for horses*	100.000000	1.000000	1000	1.000000	1:10000
Feed mixture for cows*	100.000000	—	10	50000	1:10000
Feed mixture for swine*	3.000000	10000	40	25000	1: 1000
Feed mixture for cows*	55000	2000	—	500	1:10000
Milk replacer for calves*	20000	—	—	7000	1: 1000

* Feed samples from cases connected with a supposed feed intoxication.

titative where the concentration is calculated on the basis of the last positive dilution, the next 10-fold dilution being negative. The figures given in the table must be regarded as examples of the endotoxin content in various "normal" feedstuffs and in some samples connected with a probable feedstuff intoxication.

The content of endotoxins in the types of feedstuff examined, varies considerably, the highest titre being 1:10000. It can be noted that a titre of 1:10000 was found in 3 out of 5 samples suspected of being the cause of feed intoxication. A titre of 1:10000 was, however, also found in a few "normal" feed samples.

In order to evaluate the reproducibility of the endotoxin analyses, 10 different samples from each of 2 feedstuff lots were analysed. The mean endotoxin titre and standard deviation were found to be 82 ± 38 and 9100 ± 2850 for the 2 lots, respectively.

There is no obvious correlation between the endotoxin titre and the viable total counts, viable coliforms or viable moulds. This lack of correlation may be due to the fact that the products were heat treated. During this treatment viable cells are killed while the heat stable endotoxins may remain active. The types of microorganisms present in the feed are probably also of importance for the presence of endotoxins.

Thus the Limulus test should, in addition to giving data for the present endotoxin content also give indications of the hygienic quality of the feedstuff before heat treatment.

According to the literature cited, there are reasons to believe that a high concentration of endotoxins in feedstuffs may be harmful to animals under special conditions, particularly for newborn and young animals. However, the significance of endotoxins and the health of animals of various species remain to be investigated, and tolerance limits to be given.

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