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Effect of Germination on Vomitoxin Level in Grain

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Kallela, K., I. Saastamoinen and H. Saloniemi: Effect of germination on vomitoxin level in grain. Acta vet. scand. 1991, 32, 483–489. – The present investigation clarified the effect of enzymes or other substances formed during the germination process on the vomitoxin level of contaminated oats. The studies found that oats containing vomitoxin germinated very poorly; the decrease in toxins was also slight. The amount of pure vomitoxin added to toxin-free grain decreased (barley 53 %, oats 40 %, wheat 20 %) during germination (5 d). In homogenized mixture of germinated grain (2.4 and 7 d) and toxic grain no decrease in toxin amount occurred during a 1–7 day period. In contrast, when germinating toxin free grains and toxic oats in a grain mixture the toxin level decreased at first, but later rose considerably. On the basis of these results, the utilization of germination processes for the improvement of grain containing vomitoxin is of questionable value.

mycotoxins; trichothecenes inactivation.

Introduction

During warm and moist years abundant fungal growth may form on cereal, sometimes during the growth stage (*Abbas et al.* 1988) but usually during the harvesting and storage periods. A subsequent change to conditions unfavourable to the vegetative growth of fungi may cause several fungal strains to form mycotoxins, which are harmful to humans and animals (*Smith & Moss* 1985). Under Finland's environmental conditions, the most common toxins, zearalenone and trichotechenes, are produced by the Fusarium fungi.

The most prevalent trichotechene encountered in Finland is deoxynivalenol (DON) or vomitoxin, which has been shown to depress feed palatability and cause vomiting and diarrhea in test animals (*Ueno* 1985). Usually a number of toxins are simultaneously present in feeds, in which case the symptoms in animals are more virulent than those encountered under experimental conditions. Often the clinical pathological picture is confusing (*Bauer* 1988, *Kubena et al.* 1989). Preventative measures can considerably inhibit fungal growth and toxin formation. However, pre-existing toxins are difficult to destroy. Thus far, the most effective agents have been shown to be formaldehyde for zearalenone (*Bennett et al.* 1980, *Kallela & Saastamoinen* 1981a, 1981b, 1982) and sodium bisulphite for trichotechenes (*Kallela et al.* 1987, *Young et al.* 1987). In animals, the destruction of toxins mainly occurs enzymatically. Plants also appear to possess enzymes whose effect, like that of some other plant constituents, may reduce the level of toxins in feedstuffs (*Ueno & Matsumato* 1975, *Miller et al.* 1983). During the germination of grain enzymatic neutralization of toxins occurs, on account of which malt products do not usually contain toxins (*Krogh et al.* 1974, *Chu et al.* 1975).

Because of the last mentioned findings, we considered it appropriate to carry out a study to determine if the germination process could be utilized to improve the utility of toxin-containing grain.

Materials and methods

A batch of oats relatively abundant in vomitoxin (3–18 mg/kg) was made available for the studies. In addition, the material contained trace amounts of other trichotechenes (3-AcDON, nivalenol, diacetoxyscirpenol) and some zearalenone (0.8–0.9 mg/kg). The present study was concerned only with vomitoxin. The following subexperiments were performed to determine: 1. The decrease in concentration of vomitoxin (DON) during germination of the toxic oats. 2. The degradation of pure vomitoxin added to toxin-free grain (oats, barley, wheat) during the germination period. 3. The effect of enzymes in germinated grain on the vomitoxin level of toxic oats. 4. The degradation of vomitoxin in toxic oats when germinating toxic oats and toxin free grain in a grain mixture.

Before the actual experiments were started, the germinability of the toxin-free grains employed in the study was determined. In the same manner the effects on the germinability of toxin-free grains by the addition DON, by the methanol used as the DON solvent, or by the germination of a mixture of toxin-free grain/toxic oats were investigated. The grains were soaked in water for about 24 h at room temperature (approximately 22°C), thus forming the following moisture percentages: oats 39.8, barley 42.9, wheat 41.9. Germination was allowed to proceed in petri dishes between 2 layers of moist filter paper (distilled water 25 cm^3/ petri dish was used for moistening). For each grain type there were 2 petri dishes each containing 100 grains of oats. The petri dishes were first kept for a few h at +5°C (to break the dormancy phase), thereafter up to 24 h at +10°C, and later at room temperature. Germination was checked at 24 h periods. The average germination percentage varied from 86 (barley) to 90 (wheat); wheat was the fastest to germinate, oats the slowest. The DON addition decreased germinability in all grain species. The toxic oats mixture also decreased the germinability of toxin-free oats and barley to some extent. The methanol used as the DON solvent did not have a clear effect on germinability (Table 1).

In the actual experiments, the germination process was carried out as described above. In particular, an attempt was made to ensure the uniformity of the toxin level in the samples. For that purpose, a sufficient amount of carefully mixed oats was reserved for all the subexperiments. To assure the results of the present research, two replications were performed on subexperiments 1, 2, and 4 under uniform conditions.

In subexperiment 1, the toxin level was determined prior to germination (0 d) and 3 and 6 days into the germination process. Twenty grams of grain were weighed into each of the petri dishes.

In subexperiment 2, pure vomitoxin in methanol was added to healthy grain (oats, barley, wheat). The germination periods were 0, 3 and 5 days. When determining toxin level both the toxin present in the grain and the toxin adhering to the germination media were taken into consideration.

In subexperiment 3, toxin-free oats were germinated in petri dishes (9 dishes á 10 g) for 2, 4, and 7 days afterwhich 10 g of the "water treated" toxic oats were added to each dish of germinated oats. The samples were then ground and kept at room temperature 1–2, 3–4, and 4–6 days, afterwhich the vomitoxin level of the samples was determined.

Table 1. Germinability of toxin-free grains (oats, barley, wheat) and the effect on germinability of DON (10 mg/kg in methanol), methanol (10 ml/kg) and toxic grain (in a mixture of toxin-free grain/toxic oats ana partes).

Grain species	Germination time (d)	Germinability %			
		Untreated grain	DON treated	Methanol treated	In toxic oats mixture
Oats	3	52	41	61	44
	4	63	52	75	59
	5	65	54	78	60
	6	70	58	82	61
	7	77	64	86	71
	10	86	71	92	80
	12	88			
Barley	3	61	50	52	58
	4	76	60	69	64
	5	79	60	74	64
	6	81	61	76	65
	7	83	64	77	66
	10	86	65	80	68
	12	86			
Wheat	3	83	62	83	82
	4	87	75	86	85
	5	89	76	86	89
	6	89	78	86	90
	7	89	82	86	91
	10	90	82	86	91
	12	90			

In subexperiment 4, 10 g of toxin-free grain (oats, barley, wheat) and 10 g of toxic oats were weighed into petri dishes. Toxin level was determined after the germination period (0, 3 and 6 d).

The toxin levels of the samples were determined by gas chromatography according to *Karppanen et al.* (1985) employing a Romer column (*Romer* 1986) for the purification step.

Results

Subexperiment 1 showed that the toxin damaged oats germinated poorly. A slight decrease occurred in the vomitoxin level of oats. The results are the means of 2 replicates (Table 2).

Subexperiment 2. Table 3 shows the change in the amount of pure vomitoxin added into toxin-free grain (oats, barley, wheat) and the vomitoxin amounts measured from the filter paper of the germination vessel (mean of 2

Table 2. Changes in the DON level of toxic oats during germination.

Germination time(d)	%	DON amount mg/kg	%
0	–	9.1	100
3	3.1	8.8	97
5	4.1	8.0	88

Table 3. Changes in the level of DON added into toxin-free grain (oats, barley, wheat) during the germination period.

Grain species	Germination days	DON amount: In grain mg/kg	In growth vessel mg/kg	Total mg/kg	Total %
Oats	0	5.5		5.5	100
	3	3.9	0.7	4.6	84
	5	2.8	0.5	3.3	60
Barley	0	5.5	–	5.5	100
	3	4.6	1.0	5.6	102
	5	1.6	1.0	2.6	47
Wheat	0	5.5	–	5.5	100
	3	2.1	1.8	3.9	71
	5	3.1	1.3	4.4	80

experimental series). The level of toxin decreased quite clearly. According to the germinability studies done on the grains, the DON addition had to some extent lowered normal germinability (Table 1).

Subexperiment 3. The DON concentrations in toxic oat samples which were stored 1–6 days in a ground mixture with 2, 4, 7-day germinated oat samples are presented in Table 4. No consistent changes occurred in DON concentrations.

Table 4. Effect of enzymes in germinated oats on the DON level in toxic oats in a mixture of germinated toxin-free oats/toxic oats (ana partes).

Germination of toxin-free oats days	Storage of grain mixture at room temp. days	Amount of DON in grain mixture mg/kg	%
0	0	5.0	100
2	1	4.2	84
2	3	5.0	100
2	5	4.5	90
4	2	6.0	120
4	4	4.2	84
4	6	5.6	112
7	1	6.7	134
7	2	6.6	132
7	4	4.8	96

Subexperiment 4. Table 5 shows the changes that occurred in DON level during germination in the mixture of toxic oats and toxin-free grain (oats, barley, wheat). The 2 replicates are presented separately due to the dissimilarity of the results. In both experimental series the DON levels decreased initially. As germination progressed the DON level strongly increased in experimental series 2. According to the germinability experiments it appears likely that the germinability of toxin-free oats and barley in the toxic oats mixture had decreased.

Discussion

When evaluating the results it should be noted that the variation of toxin levels in the samples will be large as the toxin level in grain is largely on a per-grain basis (*Smith & Moss* 1985, *Tubbs & Dekich* 1989). Also in the present investigations, the toxin level of grain clearly varied in spite of careful mixing; in subexperiment 1 the greatest difference measured in toxin level among untreated control samples (4) was 2.8 mg/kg. The respective differences for subexperiments 3 and 4 (3 and 6 samples) were 3.6 and 2.6 mg/kg. After 2 completely separate experi-

Table 5. Changes in DON level in a mixture of toxic oats and toxin-free grain (oats, barley, wheat) (ana partes) during germination.

Grain mixture	Germination time days	DON amount experiment 1 mg/kg	%	experiment 2 mg/kg	%
Oats/oats	0	3.6	100	5.8	100
	3	2.6	72	5.0	86
	6	1.7	47	5.3	91
Oats/barley	0	3.6	100	5.8	100
	3	3.1	86	3.7	64
	6	3.2	89	11.5	198
Oats/wheat	0	3.6	100	5.8	100
	3	1.9	59	3.7	64
	6	2.4	67	10.1	174

mental series had been performed on sub-experiments 1, 2, and 4 in order to verify the results, comparatively reliable conclusions could then be drawn.

Based on practical experience, it appears that toxin in grain is degraded during germination. The malting process completely degrades ochratoxin A in moderately contaminated barley lots (*Krogh et al.* 1974). On the other hand, the use of germination of detoxify citrinin in barley has not been successful because contaminated grain does not germinate (*Krogh et al.* 1974). A similar result was obtained in the present experiments in which toxic oats germinated very poorly and the decrease in vomitoxin level was very slight, though according to the rule.

In contrast, the pure DON added to the toxin free grain decreased quite clearly during the 5 days germination period: in barley the decrease was 53 % (in experimental series 1.75 %, in experimental series 2.31 %), in oats 40 % (49 and 31 %) and in wheat 20 % (33 and 7 %). The result is consistent with that presented by *El-Bannan* (1987) on the degradation of pure toxins (DON, citrinin) during the germination of barley. However in those studies the degradation of toxins was greater than in the present results. Differences may be due to the fact that the present investigations also measured the level of toxin transferred to the moist germination media. Also experimental conditions in the present study were not completely identica.

A difference among the grain species was also found in the study. Considering the divergence of the results between the experimental series and the different germination rates of the grains, the differences in effect among the grain species studied are inconclusive without further study.

In the study where grain was germinated (oats, barley, wheat) 2, 4, and 7 days before being combined with toxic grain, no consistent decrease in the toxin level of the grain mixtures could be shown after a 1–7 day storage period. Thus, neither enzymes nor other substances were formed which would have been able to neutralize the vomitoxin in the contaminated grain. A similar result has been found earlier on the effect of germinated barley on exogenous DON (*El-Banna* 1987). Apparently, toxins must

be present during the germination process in order to be inactivated.

When germinating toxin-free grain (oats, barley, wheat) together with contaminated oats (ana partes) a decrease was found initially in the vomitoxin levels of all samples. Later, divergent results were obtained in the experimental series. Particularly in experimental series 2, a strong increase of toxins occurred in the barley and wheat mixture as germination progressed. A possible explanation for the phenomenon is that existing toxin-forming strains elaborated toxins after the "water treatment" made conditions more favourable. Toxins form more readily in grains which have been remoistened after a faulty drying (*Smith & Moss* 1985). Also according to this research, toxins appear to form more easily in a barley and wheat mixture that in oats. A similar increase in vomitoxin has been shown also in experiments with pure enzymes (Uridine 5 diphosphoglucuronyltransferase) where the DON level in toxic oats at first decreased after addition of the enzymes, but later increased simultaneously with the formation of abundant mould growth in the grain (unpublished result).

Based on these investigation, the vomitoxin level of grain decreases during the germination process. Practical application of the methods seems questionable; contaminated grain germinates poorly and the degradation of toxin free grains is relatively slight. In addition there is a danger that the toxin level might, without preventative agents against fungal growth, rise at a certain stage. Apparently, when using pure enzymes for the destruction of toxins it is also necessary to simultaneously employ agents to prevent fungal growth.

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Sammandrag

Groningens effekt på vomitoxinhalten i säd.

I detta arbete undersöktes effekten av enzym och andra substanser, som bildats under groningsprocessen, på vomitoxinhalten i kontaminerad havre. Resultaten visade att havre innehållande vomitoxin grodde mycket dåligt; minskningen av toxinmängden var också låg. Mängden av rent vomitoxin tillsatt till toxinfri säd minskade (korn 53 %, havre 40 %, vete 20 %) under groning (5 d). I en homogeniserad blandning av grodd säd (2.4 och 7 d) och toxisk säd förekom ingen minskning av toxinmängden under en 1–7 dagars period. Vid groning av toxinfri säd och toxisk havre i blandning minskade däremot toxinhalten först, men ökade senare betydligt. På basen av dessa resultat är användandet av groningsprocessen för att förbättra säd innehållande vomitoxin av ifrågasatt betydelse.

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