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Mycotoxins and Swine Performance

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Fungi

Plants and animals may serve as excellent hosts for many fungi. Spores from fungi (molds) are primarily spread by water and air and come into contact with plants in the field or with grain in storage facilities.

Factors that influence the degree of fungal infestation in grain are moisture, temperature and availability of oxygen. Other factors such as insect population, physical condition of grain or susceptibility of certain grain hybrids will also influence whether fungal proliferation will occur under a given set of environmental conditions.

In general, the livestock consumption of feedstuffs containing fungi is not toxic. Most fungal-infected grain is not toxic because toxin-producing species of fungi must compete with nontoxic species to grow; only a small portion of the fungal species produces toxins; and suitable environmental conditions for fungal growth may be different from the conditions suitable for toxin production. Quality of the grain can be reduced by fungal infestations, but most problems with livestock consuming fungal-infested grain result from consumption of mycotoxins produced by fungi.

Feedstuffs	Fungi Genera/Species	Mycotoxins Family/Toxins
Corn, Wheat Rice, Barley Oats, Rye	Aspergillus fiavus parasiticus	Aflatoxins B ₁ , B ₂ G ₁ , G ₂ M ₁ , M ₂
Milk Blood Meal	nomius ochraceus	Ochratoxin Ochratoxin A
Stored Corn, Wheat, Barley	Penicillium viridicatum	Ochratoxin Citrinin
Rye, Wheat Barley	Claviceps purpurea	Ergot
Corn Wheat, Barley Mixed Feed	Fusarium graminearum moniliforme	Trchothecenes Deoxynivalenol Diacetoxyscirpenol Diacetylivalenol Nivalenol T-2 Toxin Resorcylic acid lactones Zearalenone Fumomisin B ₁ , B ₂

Table 1. Feedstuffs that support growth of various fungi, the genera and species of fungi commonly found, and the family of mycotoxins and toxins that are known to impair performance of swine.

Mycotoxins

Mycotoxins are toxins produced by fungi on or in grain or feedstuffs when conditions are favorable for their development. Fungi that produce mycotoxins of economic importance to pork producers are *Aspergillus*, *Penicillium*, *Claviceps* and *Fusarium*. These fungi produce the following mycotoxins: aflatoxins, ochratoxins, ergots, trichothecenes and resorcylic acid lactones (Table 1).

Aflatoxins. Aflatoxins are produced by *Aspergillus flavus*. This fungus can germinate at lower moisture levels of 15-17%, but infection and growth require higher moistures. Aflatoxin production appears to be higher at grain moisture levels of 22-26% and temperatures of 82°-90°F. Conditions for growth are ideal when temperatures remain high both day and night, but growth decreases dramatically at temperatures above 95°F. Although *Aspergillus flavus* is abundant in the southeastern United States, drought-stressed corn in Indiana and Illinois in 1978, 1983, 1988 and 1991 contained aflatoxin in scattered fields.

The risk from aflatoxin-contaminated grain depends on the age and health of the pig as well as the concentration of the toxin in the feed. Symptoms occur with concentrations in the parts per billion (ppb) range. Small amounts can depress performance and general well-being. Aflatoxins suppress the immune system and thus make pigs more susceptible to bacterial, viral or parasitic diseases. These more subtle effects are insidious because often they are unnoticed. Overtime, profits are reduced due to lost efficiency, slower growth and increased medical costs. If levels are high enough, death may result. The direct effects of aflatoxin on reproduction have not been determined.

Aflatoxin B₁ has been the most extensively studied mycotoxin. Young swine are extremely sensitive to aflatoxins but susceptibility decreases with age. At low concentrations (20-200ppb), aflatoxin decreases feed intake, which in turn depresses growth rate and immunity. The detrimental effects of aflatoxins may be lessened by altering key nutrients in the diet. For example, a reduction in average daily gain was observed when an 18% crude protein diet was spiked with aflatoxin (182ppb). If pigs were fed a 20% crude protein diet with the aflatoxin (182ppb), no reduction in average daily gain was noted. Similar results were obtained with the addition of .25% L-lysine HCL. Adding 5% fat to diets prevented a depression in feed intake, but no improvement in growth was observed.

High concentrations of aflatoxin (1,000-5,000ppb) result in acute effects, including death. Aflatoxin M₁, a metabolite of aflatoxin, has been found in milk of sows fed diets containing aflatoxin. Piglets nursing sows consuming feed with 500-750ppb of aflatoxin had increased mortality and slower growth. Piglets were permanently stunted and performance was reduced to market weight even through they were not exposed to aflatoxin after weaning.

Ochratoxin. Ochratoxin A is the best characterized of several structural-related mycotoxins produced by *Aspergillus ochraceus* and *Penicillium viridicatum*. Ochratoxin A is found on a variety of feedstuffs grown on the southeastern coast of the United States. Ochratoxin at concentrations greater than 5-10ppm in feed results in a number of pathological conditions. These include impairment of kidney function, blood in the urine, enteritis, necrosis of lymph nodes and fatty liver changes. Ochratoxins have been found in wheat, barley, oats, corn, dry beans and peanuts. The economic impact of ochratoxins has not been determined.

Ergot. *Claviceps purpurea* invades rye, wheat and barley plants and produces alkaloid toxins termed ergot. Ergot reduces weight gain, lowers reproductive efficiency and promotes agalactia (lack of milk flow) in several livestock species. Signs of ergotism include staggers, convulsions, temporary posterior paralysis and loss of blood flow to limbs, ears and the tail. This loss of blood flow sometimes leads to gangrene and eventual loss of extremities.

Sows fed a diet containing .5% to 1.0% ergot develop agalactia and farrow fewer and smaller live pigs as compared to sows fed uncontaminated feed. Diets containing 1% ergot reduce the growth of pigs. Higher concentrations cause feed wastage and slow growth.

Ergot appears on the heads of rye, barley and wheat as hard, black, elongated structures that replace kernels (sclerotia) at harvest time. The black sclerotia can readily be seen at harvest. Grain with ergot should be stored separately and not fed to young pigs and breeding animals. Growing-finishing swine should not be fed diets with more than 10% to 20% of grain contaminated with ergot.

Trichothecenes. Deoxynivalenol, frequently referred to as DON, feed refusal factor or vomitoxin, is a mycotoxin produced by *Fusarium graminearum* (*Gibberella zeae*) that occurs often on corn (Gibberella ear rot), but also on wheat and barley (Head scab). The fungus develops on corn after silking, during cool, damp weather. Visual signs of Fusarium infection of corn include a white to pink to reddish fungus starting at the tip of the ear and developing towards the base. However, there is not necessarily a direct relationship between the extent of visual signs and the amount of toxin produced. Visual examination of corn ears growing in the field for white to pink to reddish fungus may give an indication of potential problems.

Vomitoxin is most prevalent in the upper midwestern United States and the Canadian provinces of Ontario and Quebec. These areas tend to have shorter growing seasons and have cool, damp weather during the first month after silking. Since *Fusarium graminearum* (*Gibberella zeae*) produces both deoxynivalenol and zearalenone, contaminated feedstuffs may contain both of these mycotoxins.

In pigs, vomitoxin at levels above 1ppm may cause a reduction of feed intake and consequently, rate of gain. As the dietary concentrations increase above 5 ppm, depression of feed intake may become severe and at 10ppm, there will be a severe feed refusal resulting in weight loss. The marginal reduction in feed intake and weight gain caused by low levels of vomitoxin may contribute to a substantial economic loss and may be more important than vomiting.

Vomiting, as the common name of the toxin implies, is one of the signs. Vomiting, however, does not usually occur unless the dietary concentration of the toxin approaches 10ppm or more. At that level, the pig will initially consume a sufficient amount of the diet to induce vomiting but, thereafter, the pig voluntarily reduces intake so that vomiting ceases. Thus, one must be present to observe the initial vomiting symptom. At concentrations approaching 20ppm, vomiting may be observed in pigs within approximately 15 minutes of initial consumption. Feed consumption resumes almost immediately after highly contaminated feed is replaced with uncontaminated feed. No other visual signs or gross pathology are apparent with vomitoxin.

Resorcylic acid lactones. Of all mycotoxins produced in feedstuffs, zearalenone affects reproduction most seriously since it mimics the reproductive steroids of the estrogen family. Estrogenic compounds naturally produced by plants are commonly referred to as phytoestrogens. Zearalenone is produced by *Fusarium graminearum* (*Gibberella zeae*). It may occur with deoxynivalenol in scabby wheat and in many cases with Gibberella ear rot of corn. Zearalenone contamination is more likely to occur in storage than in the field.

Of all domestic species and stages of maturity, the prepuberal gilt is the most sensitive to zearalenone. The genital system of immature gilts exhibits gross and histologic changes after ingestion of zearalenone. Gross changes include reddening of the vulva, increased size and weight of the uterus and mammary enlargement. In extreme cases, rectal and vaginal prolapses may occur.

Although the gross and histologic changes that are induced by zearalenone are well characterized in prepuberal gilts, it is unclear what effect this hyperestrogenism has on puberty or subsequent reproduction. Ingestion of diets containing 10 ppm zearalenone has had variable effects on the onset of puberty in gilts. However, results from several studies indicate that the estrogenic properties of zearalenone are not permanent and that gilts can successfully enter the breeding herd without a reduction in fertility after a two week withdrawal from zearalenone ingestion.

In cycling gilts or sows, zearalenone causes multiple reproductive dysfunctions. Diets containing 25-100ppm zearalenone that were fed continuously from weaning to rebreeding produce constant estrus, pseudopregnancy and ultimately infertility. When cycling gilts are administered either 20mg zearalenone or 2mg estradiol benzoate in the feed on days 6-10 or days 11-5 of the estrous cycle, the interval between estrus is extended. Usually these gilts will return to estrus within 30 days after zearalenone is removed from the diet and can be rebred and produce normal litters.

Numerous observations of Fusarium-contaminated feedstuffs causing stillbirths, neonatal mortality, fetal mummification, splay-leg of piglets, abortion, abnormal return to estrus and other abnormalities have been reported. However, the specific action of zearalenone in each of these situations is not well characterized. In many cases, fungalinfected feedstuffs were not assayed for zearalenone and conclusions are made from field observations rather than from controlled experiments. Therefore, it is possible that other mycotoxins in conjunction with zearalenone are interacting to produce the effects.

When pregnant gilts are fed diets containing low concentrations zearalenone (3.6-4.3ppm) from mating to day 80 of gestation, embryonic development is not affected. Higher doses of zearalenone (60-90ppm) consumed by gilts from day 2 to 15 postmating completely arrest development of embryos. It appears that the critical period for zearalenone to exert its detrimental actions on embryonic development is days 7 to 10 after mating. Not only is reproductive efficiency reduced when bred gilts consume zearalenone during this early period of gestation because embryos are lost, but it may be several months before these females will return to estrus and can be bred successfully.

The lactating sow also is susceptible to zearalenone at high concentrations. Sows fed 50-100ppm zearalenone for 2 weeks before weaning and for 63 days after weaning exhibit constant estrus. Sows fed a diet containing 10ppm zearalenone during the last 14 days of lactation exhibit an extended interval from weaning to estrus. However, fertility at the first postweaning estrus will not be adversely affected. Low concentrations of zearalenone (2.1-4.8ppm) fed throughout pregnancy and lactation will not affect postweaning rebreeding. The effect of zearalenone toxicoses on sexual development of boars has been evaluated in a few studies. Consumption of diets containing 60ppm zearalenone for 8 weeks does not alter libido or semen quality characteristics of mature boars. Similarly, mature boars consuming feed with 200ppm zearalenone have normal libido scores and normal sperm concentrations when compared with boars consuming a normal ration. When prepuberal boars consume 40ppm of zearalenone from 14-18 weeks of age, their libido scores are lower than the untreated boars. This reduction in sex drive is associated with a reduced concentration of blood testosterone, the male sex hormone responsible for sex drive. Feeding diets containing lower concentrations of zearalenone (9ppm) does not influence sexual behavior of boars. Further experimentation is needed to determine if prepuberal and postpuberal boars react differently to diets containing zearalenone.

Fumonisin. Fumonisin is a more recently recognized family of mycotoxins of concern to the swine industry. Fumonisin is produced by *Fusarium moniliforme*. Recently, acute pulmonary edema (filling of the lungs with fluid) has been reported as a symptom of fumonisin toxicity. All ages of pigs have been reported to be affected. Mortality rates have been recorded in the range of 10-40%. Only limited information is available on fumonisin. More information will be generated as the incidence of problems with this mycotoxin is identified.

Control of Fungal Growth

In order to have mycotoxins, there must be a feedstuff on which a fungus can grow, a fungus capable of producing mycotoxins, and environmental conditions favorable for fungal growth and mycotoxin production. To prevent the production of mycotoxins in feedstuffs, each of these areas must be addressed. Since fungi are commonly found in nature, keeping feed from being exposed to fungi is impractical. Controlling factors that promote the growth of fungi is a more practical approach.

Damaged feedstuffs are readily available food sources for fungal growth. Anytime the kernel is cracked and the endosperm is exposed, there is high probability of fungal growth. Drought-stressed corn, kernels cracked during harvesting and screenings are three examples. Even healthy corn in the field is at some risk. Drought-stressed corn is less resistant to fungi and should be considered to be of high risk. Proper operation of harvesters will help to reduce the incidence of cracked kernels. Corn screenings are excellent media for fungal growth and have been incriminated in Fumonisin toxicity.

The two major environmental factors associated with fungal growth are temperature and humidity. Anytime humidity exceeds 62%, temperature exceeds 80°F and grain moisture levels exceed 14% to 15%, there is a greater chance that fungi will grow. The exception is zearalenone which is produced under cool temperatures (less than 70°F) and moist conditions. Regardless of all other factors, the critical point for controlling fungal growth in storage is grain moisture levels. Grain that is dry when placed in storage and kept dry (less than 14% moisture) will be unlikely to support growth of fungi that produce mycotoxins.

Ground feed is an ideal source of food for fungal growth. Therefore, it should be utilized rapidly. This is especially true during periods of high humidity and heat. Feed storage bins should be cleaned at frequent intervals to prevent bridging of feedstuffs and creation of "hotspots."

Fungal inhibitors, such as propionic acid, may be effective in preventing fungal growth on stored grains.

However, producers are cautioned that fungal inhibitors have no effect on mycotoxins already present in the corn at the time of application. They only prevent future growth of fungi. There are a number of companies manufacturing products to curb fungal growth. Storage of grain in oxygen-tight silos reduces growth of fungi on the grain but has no effect on mycotoxins already present.

Detection of Fungi and Mycotoxins

There are four methods of detecting either the fungi that produce mycotoxins or mycotoxins themselves: 1) visual inspection, 2) blacklight, 3) immunoassays, and 4) chromatography.

To detect *Gibberella*-damaged corn (*Fusarium graminearum*), the ear or individual kernels can be visually evaluated. A red to pink fungus, usually beginning at the tip of the ear, is a sign of *Gibberella*-infected corn. Husks frequently are tightly adhered to the ear in fungal-infested corn. Individual kernels infected by *Gibberella* are usually shrunken, discolored and often display a watermark. If more than 2% to 3% of kernels display these signs, the *Gibberella* fungus may be present and producing sufficient levels of DON or zearalenone to adversely affect performance.

Test Name/Manufacturer	Mycotoxin Tests	Test Type
Agri Screen Neogen Corp. 620 Leshar Place Lansing, MI 48912 517/372-9200	aflatoxin vomitoxin T-2 toxin zearalenone	rapid radio-immunoassay
EZ-Screen Environmental Diag. PO Box 908 Burlington, NC 27215 1-800-334-1116	aflatoxin ochratoxin T-2 toxin zearalenone	color test compared to standards
Alfa Test-10 Cambridge-Naremco Springfield, MO 65801 1-800-641-7515	aflatoxin	measures fluorescence
Signal Accucup Int. Diagnostics PO Box 799 Saint Joseph, MI 40085 616/983-3122	aflatoxin	color test
SAM-A-SAM Papillion Ag. Prod. PO Box 1161 Easton, MD 21601 1-800-888-5688	aflatoxin zearalenone	measures fluorescence

Table 2. Partial list of commercially available test kits for mycotoxins.

A black light will cause a bright greenish-yellow fluorescence to appear if *Aspergillus flavus* is present in the grain. The black light is commonly used, especially at grain buying stations, because it is a very rapid procedure. The major drawback is that it is only an indicator of the presence of *Aspergillus* and not aflatoxin. The fungus may have been present, disappeared, and left the mycotoxin to affect swine performance. This is commonly referred to as a "false negative reading." "False positive readings" also are possible as foreign material also may cause fluorescence. To perform the black light test, all kernels in the sample should be cracked and viewed by an operator who is not affected by color blindness. The black light test detects no other mycotoxin producing fungi.

An immunoassay is sometimes referred to as a serologic assay or ELISA (enzyme linked immunosorbent assay) test. Commercial kits are available for detecting aflatoxin, DON and zearalenone. They are easy to run and relatively inexpensive. They serve also as relative indicators of the amount of mycotoxin within a test sample. A partial list of commercial kits available from companies is presented in Table 2.

Chromatographic tests, such as the minicolumn, the HPLC (high performance liquid chromatograph) and TLC (thin-layer chromatography) are used mainly in laboratory settings or in situations where a more accurate indication of the mycotoxin concentration is needed. Chromatographic tests require sophisticated techniques and equipment and are expensive to perform.

Test Sample Collection. Samples collected for testing should be randomly taken from several locations within the batch. It is not uncommon for there to be "hotspots" within a storage compartment. While these "hotspots" have a relatively high concentration of mycotoxin, other areas may be very low. Using a grain probe at several evenly distributed locations within a storage compartment is an effective way to collect samples. Samples collected at periodic intervals from grain being augured also is an effective sampling technique. A random sample from multiple (10-30) locations of a large quantity is the most useful. The sources of error in determining the aflatoxin content of corn can be classified as sampling, subsampling or analysis error. Sampling error accounts for 88% while subsampling and analysis error account for only

12%. Obviously sampling is critical. Collect at least 10 one-pound samples from each lot of feed or ingredients and thoroughly mix and grind the entire sample before subsampling. To decrease the chance of fungal growth while the samples are in transit to the laboratory, use paper instead of plastic bags. Plastic bags retain moisture which promotes fungal growth.

Utilization of Mycotoxin Contaminated Feedstuffs Decontamination

Producers often are confronted with finding a way to utilize a contaminated feedstuff. Research has focused on the decontamination of corn containing toxins via extraction, acid or base treatment, physical separation or heat treatment. Roasting to 300°F has been shown to reduce the level of aflatoxin present by 50% to 60%, but some destruction of amino acids in the grain also occurred. Ammoniation appears to be the most reliable method to detoxify grain of aflatoxins. Procedures have been established for on-farm processing of small batches of grain, but ammonia is hazardous to handle, toxic and extremely corrosive. Treatment of feedstuffs with anhydrous ammonia has not been approved by the Food and Drug Administration (FDA). Although the technology exists, there are no practical methods to economically decontaminate large volumes of mycotoxin-contaminated grain.

Pig	Dietary Concentration		
	Deoxynivalenol ppm	Zearalenone ppm	Aflatoxin ppb
Breeding Herd	1.0	2.0	100
Young	1.0	1.0	20
Growing	1.0	1.0	--*
Finishing	1.0	3.0	200
Young Males	1.0	3.0	--*
Old Males	1.0	3.0	--*

Table 3. Recommended maximum concentrations of toxin in swine diets (modified from Michigan State University) *Concentration not determined.

Blending

Feeding mycotoxin-contaminated products carries risk. Producers must consider the consequences and work to minimize detrimental effects. Remember that young animals are most susceptible. If possible, segregate the contaminated grain and avoid feeding it to nursery pigs, breeding animals or replacement gilts. If all the grain is heavily contaminated, “clean” grain should be purchased for the more susceptible animals in the herd. Often, contaminated products are damaged and are of generally lower quality. Knowing the concentration of mycotoxins in the feed is important to allow proper utilization.

Increased awareness and monitoring have led to fewer market outlets for grains containing mycotoxins. There are no official FDA tolerances for any mycotoxins. This means a zero tolerance. However, FDA has established an action level which permits grains or feedstuffs to be marketed in interstate commerce with up to 20ppb aflatoxin. At the present time, the tolerance for feed destined for market hogs is 200ppb and 100ppb for the breeding herd. Even though a tolerance level has been established, no “safe” level has been established for any mycotoxin in any diet.

Blending contaminated and uncontaminated feeds can be difficult from both an economic and logistic point of view. FDA oversees blending of grains that are moved through market channels. On-farm blending is only an option for those who desire to feed mycotoxin-contaminated grain to their pigs. However, mixing contaminated grain with uncontaminated grain contaminates all of the grain. Because of their susceptibility, 4- to 5-month old prepubertal gilts make excellent models to test suspect grain for zearalenone. Swollen vulvas would indicate that zearalenone or vomitoxin is present in the feed. Blending should only occur shortly before the feed will be consumed. Using freshly mixed feed will reduce the chance of growth of mycotoxin-producing fungi and minimize contamination of the clean grains. For this reason, separate storage is required for the contaminated and uncontaminated products.

The producer must have sufficient uncontaminated grain in order to blend quantities of highly contaminated products to acceptable concentrations. For example, if 1,000 bushels of corn are contaminated with 1,000ppb aflatoxin B₁, it would require 49,000 bushels of uncontaminated corn in order to dilute the aflatoxin to 20ppb. It may be difficult to purchase, store and routinely blend sufficient quantities to dilute the concentration to acceptable levels.

Ration Formulations

Interactions of aflatoxins with riboflavin, vitamin D, vitamin A and thiamin have been reported. Fungi can destroy vitamins in feeds. The destruction of vitamins in ingredients is of little consequence since synthetic

vitamins are added to diets. However, after the vitamins are combined with other ingredients, reduced potency can occur. Because of this, always keep feed fresh. If vitamins are supplied by a base mix or premix, the inventory should be rotated to assure vitamin potency. Adequate vitamin supplementation is particularly important when feeds contain mycotoxins.

Binding Agents

Addition of non-nutritive binding agents such as sodium bentonite and certain zeolites to contaminated feed have alleviated growth depression in pigs. Research has shown that adding 10lb/ton sodium bentonite almost completely prevented the growth depression caused by feeding corn containing 750ppb aflatoxin. Similar benefits have been reported from the addition of anti-caking agents (hydrated sodium calcium aluminosilicate) to diets containing aflatoxin. However, addition of aluminosilicates did not alter the effects of DON on performance of starter pigs. Recent research has shown that these compounds are only partially effective at binding toxins in the digestive tract and reducing their absorption. The cost of these products varies, but many are relatively inexpensive and appear to offer promise. They have not been cleared for use by FDA as mycotoxin binding agents.

Summary

1. Fungi (molds) that are capable of producing mycotoxins invade grains and feedstuffs during plant growth, maturity, harvesting, storage, and processing.
2. Mycotoxin is a term used to specifically refer to toxins produced by fungi on feedstuffs when environmental conditions support their growth.
3. *Aspergillus*, *Claviceps*, *Fusarium* and *Penicillium* are four genera of fungi of economic concern to the swine industry. These fungi produce five families of mycotoxins, namely aflatoxins, ochratoxins, ergots, trichothecenes and zearalenone.
4. Specific testing for the presence and quantities of mycotoxins is essential to determine toxicity. The presence of fungi only determines the potential for toxins to be produced. Mycotoxins may be present after fungi have lost their viability.
5. Recommended maximum allowable concentrations of toxins in swine diets are listed in Table 3.
6. The potential for mycotoxins is reduced by timely grain harvest, drying to 1% to 2-1/2% below maximum moisture for storage (grain 14% to 15%), removal of all foreign material, cracked kernels, routine aeration of stored grains to prevent moisture accumulation, as well as weevil and temperature control in the grain (less than 80°F). The use of fungal inhibitors, such as propionic/acetic acid (1 to 2%) will help prevent fungal growth in grain and finished feed.
7. A number of alternative methods can be used for detection of fungi. These include visual analysis, black light, immunoassay and chromatography. Quantitative tests for specific mycotoxins are essential to determine the value of infected grains.
8. There are no practical methods of economically decontaminating large volumes of mycotoxin-contaminated grain. Dilution with clean corn may be helpful when mycotoxin levels are near the lower threshold where contamination begins to show slight animal effects. The use of absorbing clays or binding agents such as sodium bentonite or hydrated sodium calcium aluminosilicate has been reported to be beneficial at levels of 5 to 20 lb/ton of feed when aflatoxins are near the lower threshold of toxicity.
9. Performance testing and pig reaction to grains suspected to be infected are useful methods of detecting potential problems. Close observations of animal behavior for feed refusal, reduced weight gain and estrogenic stimulation are beneficial.

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