EFFICACY OF SEVERAL ORGANIC ACIDS AGAINST MOLDS

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Primary Audience: Researchers, Feed Manufacturers, Quality Assurance Personnel

SUMMARY

The efficacy of several organic acids against growth of molds typically found in animal feed was determined. A plate assay was used in which paper disks soaked in a spore solution were placed on the surface of agar plates containing increasing concentrations of the respective organic acid. Mold growth radiated from the disks, and the radius was considered indicative of the degree of inhibition as compared to a control. The efficacy of each of the organic acids against the various molds varied substantially. Valeric acid, propionic acid, and butyric acid displayed the highest efficacy against all molds with the effective concentrations ranging from 0.05 to 0.25%. Other acids, including acetic acid, lactic acid, and benzoic acid, required concentrations of 10 lb/ton or more for effective mold inhibition. Susceptibility of molds to inhibition by organic acids was in the order Fusarium spp. > Aspergillus spp. > Penicillium spp., with Fusarium spp. being the most susceptible mold.

Key words: Efficacy, grain quality, mold inhibition, organic acids, propionic acid

1999 J. Applied Poultry Res. 8:480-487

DESCRIPTION OF PROBLEM

Molds are ubiquitous and thus, unavoidable contaminants in all animal feeds. Virtually all animal feeds contain molds and viable mold spores, and due to changing practices in grain production, e.g., less tilling, molds continue to pose a threat to grain quality. Conditions favoring molds include moisture levels higher than 12%, warm temperatures, the presence of oxygen, and prolonged storage time [1, 2, 3]. High contamination levels are also frequently associated with preharvest physiological stress such as drought or insect damage. Many molds are toxigenic and produce mycotoxins. The growth of molds and production of mycotoxins by these molds in feed ingredients can cause significant economic losses [2, 4, 5]. Mold growth depletes the nutrient density and affects feed palatability; mycotoxins cause serious health problems that include growth retardation, decreased resistance to infections, impaired immunity, and decreased egg and milk production [6].

Several management tools such as the use of mold inhibitors and drying of grain are available to help control mold contamination. Organic acids are known in the feed industry as an effective and affordable tool to control mold growth. A variety of organic acids such

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were then placed on the PDA. The plates were
when necessary. These suspensions were
0.05%, 0.35%, and 0.4%, 0.45%, and 0.5% (vol/vol) of
each acid to test the efficacy of the acid for
mold inhibition. Organic acids were added
after sterilization and prior to solidification of
the PDA. The paper disks carrying the spores
were then placed on the PDA. The plates were
allowed to incubate for 48 hr at 25°C. Mold
growth spread radially from the paper disks
onto the agar. For each treatment and each
acid tested, five plates were prepared with

as acetic acid, lactic acid, propionic acid, or
blends of acids are used to help control mold
contamination. However, there is little re-
search available on the relative efficacy of
these acids at various concentrations and,
therefore, the industry continues to rely almost
solely on research conducted in 1973 by Pelhate [7]. The purpose of this study was to
determine the relative efficacy of eight organic
acids against six molds commonly found in
animal feeds.

**Materials and Methods**

An *in vitro* plate assay was used to measure inhibition of mold growth by various
organic acids. For the *in vitro* assay, paper
disks soaked in a spore solution were placed
on the surface of agar plates containing in-
creasing concentrations of the respective or-
ganic acid. Mold growth radiated from the
disks and the radius was considered indicative
of the degree of inhibition compared to a con-
 troll. Molds were isolated from poultry feed
ingredients. In order to obtain pure mold
cultures, isolated molds were plated repeated-
edly on potato dextrose agar (PDA) when
necessary. For the preparation of the spore
solutions, mold cultures were grown on PDA
until confluence. Ten (10) mL of sterile phos-
phate buffer was added to the surface of each
of the sporulating and overgrown plates and
the spores were scrubbed off with a sterile
loop to prepare a concentrated spore suspen-
sion. Spore suspensions were adjusted to
5 × 10^5 CFU/mL by adding additional spores
when necessary. These suspensions were
aseptically transferred to a sterile tube. Sterile
paper disks were soaked with these spore
suspensions and placed in petri dishes. The
petri dishes were partially covered with a lid
and dried by placing them separately in a
sterile hood.

PDA was prepared and treated with 0,
0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%,
0.35%, 0.4%, 0.45%, and 0.5% (vol/vol) of
each acid to test the efficacy of the acid for
mold inhibition. Organic acids were added
after sterilization and prior to solidification of
the PDA. The paper disks carrying the spores
were then placed on the PDA. The plates were
allowed to incubate for 48 hr at 25°C. Mold
growth spread radially from the paper disks
onto the agar. For each treatment and each
acid tested, five plates were prepared with

four disks each for a total of 20 disks. The
zones of mold growth were then determined
by measuring the radius from the center of the
paper disks to the edge of the mold circle for
a total of 20 measurements for each data point.
The average of the 20 measurements was
calculated. The average of the measurements
for the 20 control plates was considered 100%
mold growth. When necessary, measurements
for control plates were taken where mold
growth from two adjacent disks did not over-
lap.

The molds used in this study were isolated
from field samples of contaminated feeds.
These molds were *Aspergillus* spp.,
*Geotrichum* spp., *Mucor* spp., *Fusarium* spp.,
*Penicillium* spp., and *Scopulariopsis* spp. The
acids and PDA were purchased from Fisher
Scientific (Pittsburgh, PA). The acids used
were propionic, acetic, lactic, undecylenic, bu-
tyric, valeric, benzoic, and sorbic. Sterile disks
were purchased from DIFCO (Detroit, MI).

**Results and Discussion**

Figure 1 demonstrates the principle of the
assay using *Aspergillus* spp. and propionic acid
as an example. The photograph displays four
agar plates treated with 0, 0.05%, 0.15%, and
0.2% propionic acid, respectively (from left to
right). The zones of mold growth decrease in
size with higher propionic acid inclusion rates.
No growth is observed at the 0.2% inclusion
level.

Figures 2–9 display the results of the plate
assays. Valeric acid displays the highest mold-
inhibiting activity, followed by propionic acid
and butyric acid, which show similar efficacy.
With all three acids, a complete inhibition of
growth was achieved at concentrations not
higher than 0.35%. With valeric acid, a 50%
reduction of mold growth is observed in a
concentration range between 0.04% and
0.13%. Valeric acid is effective against all the
molds tested. For both propionic acid and
butyric acid, a concentration of 0.20% or less
was able to reduce the growth rate of almost
all molds tested to 50%. The other acids tested
(acetic acid, sorbic acid, benzoic acid, un-
decylenic acid, and lactic acid) showed lower
efficacy as mold inhibitors. Lactic acid was the
least effective compound. Even at concentra-
tions as high as 0.5%, inhibition of growth
with lactic acid never surpassed 60%. A
comparison of the organic acids’ effect on
FIGURE 1. Photograph of Aspergillus spp. grown on potato dextrose agar plates treated with 0, 0.05, 0.15, or 0.2% propionic acid, respectively.

FIGURE 2. Efficacy of valeric acid to inhibit growth of molds.
specific molds shows that among all molds tested, *Fusarium* was the most susceptible to organic acids. With effective inclusion rates of 0.1% and 0.2%, propionic acid and butyric acid were equal in their efficacy to inhibit *Aspergillus* spp. or *Fusarium* spp., respectively, the two most common molds found in grains.

This study confirms the efficacy of propionic acid and butyric acid to inhibit the growth of various molds commonly found in feed ingredients. These two acids inhibited mold growth effectively in a concentration range of 0.1 to 0.2%, which agrees well with commonly used application levels in the field. However, in this study, a laboratory test involving artificial media was used and conclusions for application rates under field conditions must be drawn with caution. Although valeric acid
displayed the highest efficacy in controlling mold growth, it is not used as a mold inhibitor due to its potent smell and high cost. Similarly, the use of butyric acid is limited to mold inhibitor blends due to its low palatability. All other organic acids tested required a concentration of up to 0.5% to achieve good efficacy. The findings of this study allow the conclusion that under the experimental conditions used, efficacy reflects the level of propionic acid and/or butyric acid contained in a mold inhibitor product. It is reasonable to assume that similar results would be obtained also in field situations.

The mechanism of inhibition of growth of molds by organic acids is generally not considered a pH phenomenon. However, it is well known that growth and morphology of
molds is influenced by the pH of media [8]. The pH values in the PDA media used in these experiments were determined before solidification and before and after addition of the organic acids to be tested. The pH values revealed that except for undecylenic acid and sorbic acid, addition of 0.5% of each of the organic acids decreased the pH from 5.7 to between 3.35 and 4.82. Lesser acidification was seen for undecylenic acid and sorbic acid at 0.5% and for all acids at 0.2%. However, little correlation was observed between the pH of an organic acid and its relative efficacy. For example, while the pH values for lactic acid and acetic acid were 3.35 and 3.81 respectively, these two acids displayed little efficacy in controlling mold growth. The pH data allow the conclusion that in this in vitro assay pH effects were likely secondary effects. Furthermore, comparison of the pH values and the
pKa values of the acids suggest that the organic acids operated well below their pKa values. The plate assay, therefore, may be indicative of the relative efficacy of organic acids in practical applications where grains and animal feeds are treated at a low moisture content of 12–15%.

Similar results were found by Pelhate in a 1973 study for propionic acid. Pelhate [7] examined the relative efficacy of propionic, sorbic, acetic, and formic acid and found propionic acid to be the most effective of these four organic acids. With 0.2 to 0.4% required for maximum efficacy, inclusion levels were comparable in both studies. A few studies have confirmed the efficacy of propionic acid as a mold inhibitor using corn rather than a laboratory assay. Vandegraft et al. and Stewart et al. found that propionic acid prevents aflatoxin and ochratoxin formation in high-moisture corn [4, 9]. Sauer and Burroughs determined that propionic acid and acetic acid are more effective than calcium or sodium propionate, sorbic acid, and potassium sorbate at preserving high-moisture corn in a mold-free state [10].

The microbiological quality of feed has been a comparatively unexplored area but is receiving more attention due to the recognition of mycotoxins as a widespread economic threat [2, 4, 5]. Contamination by molds and contamination by mycotoxins can be greatly reduced by using organic acids as inhibitors.

**CONCLUSIONS AND APPLICATIONS**

1. Propionic acid and butyric acid were equally effective in inhibiting mold growth in a laboratory assay. They are superior to several other organic acids tested.
2. Due to its high efficacy and its relatively good palatability at lower inclusion rates, as well as its relatively low cost, propionic acid may reasonably be considered one of the most economical organic acids for field applications of those tested here. Only 0.1 to 0.2% is needed to effect almost total inhibition of molds in the laboratory assay used.
REFERENCES AND NOTES


