

Limiting Mold Growth in Dog Food Through Natural Compounds

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ABSTRACT

Mold contaminated dog food has become a growing concern as many mold poisoning epidemics in the past have killed hundreds of dogs and left others severely sick. With current methods of reducing mold growth in dog food failing, it's important to explore new methods of limiting mold growth. Recognizing natural compounds like cumin, astragalus, ginger, and apple cider vinegar's high antimicrobial properties, it's important to explore their effectiveness as mold inhibitors in dog food. Thus, this experiment explores the present question: Can natural compounds, added in safe amounts, effectively act as mold inhibitors and limit mold growth in dog food? To answer this question, an experiment was developed in which each natural compound (turmeric powder, astragalus root powder, ginger powder, cumin powder, and apple cider vinegar) was added into dog food along with mold samples. After a four week period, mold growth was measured and compared to a control batch which had no natural compound additives. Results indicate that ginger had a significant effect on limiting mold growth. Similarly, apple cider vinegar noticeably limited mold growth when compared to the control. It's thus important to further explore these compounds' efficacies as mold inhibitors in dog food.

Introduction

Mold is defined as any fungi that form mycelium, a root-like structure, on the surface of organic matter ("Mold | Fungus | Britannica," 2023). Mold can be found almost anywhere that moisture is present. Mold also plays a critical role in a variety of processes including cheese production, bread making, and liquor fermentation. While mold has a variety of beneficial applications, it can also be dangerous if it contaminates food products. *Penicillium* and *Aspergillus* are two specific genera of mold that commonly colonize cereal crops such as rice, corn, wheat, barley, and oats (Haschek et al., 2002). These crops are fundamental ingredients in many animal foods. Thus, if they become contaminated by *Penicillium* or *Aspergillus*, they can cause an array of negative health effects on large populations, and have even induced mold poisoning epidemics in the past (Meggs, 2015). Mold contamination is generally dangerous due to mycotoxins: toxins that certain molds produce which cause a variety of adverse health effects to both humans and animals (World Health Organization, 2018). Even at extremely low dosages, mycotoxins have the ability to cause severe health problems. Recently, there has been increasing concern regarding the health risks that mycotoxin contaminated pet foods pose on dogs. These concerns are justified in toxicological data, which shows that even naturally occurring levels of mycotoxins have harmful effects on farm and laboratory animals (Böhm et al., 2010). Because companion animals, like dogs, live longer when compared to laboratory animals, they consume more food throughout their lifetime, thus, making them more vulnerable to chronic mycotoxin exposure. While killing mold in animal food doesn't necessarily eliminate mycotoxins that may already be present, it does stop the creation of new mycotoxins that might proliferate during the transportation and storage of the food (Boermans & Leung, 2007). Thus, it is important to explore new methods of limiting mold growth in dog food, and in turn mycotoxin production, to ensure dogs safety.

Literature Review

As mentioned above, mold contamination in dog food is primarily harmful due to mycotoxins. Mycotoxins are defined as “secondary fungal metabolites (i.e., metabolites¹ not essential to the normal growth and reproduction of the fungus) that cause biochemical, physiologic, and/or pathologic changes in other species, including animals, plants, and other microbes” (Haschek et al., 2002). Dogs have a high risk of experiencing the adverse effects of mycotoxins because so much of their diet consists of cereal foods, like corn and wheat, that are susceptible to mycotoxin contamination.

The most common mycotoxins found in dog food are Fumonisin, Aflatoxin, and Ochratoxin. Depression is the most noticeable symptom of aflatoxicosis² in dogs; Although in some cases sudden deaths have also been reported. Aflatoxin is also a known liver carcinogen³ that causes cancer by inducing DNA adducts that lead to genetic changes in liver cells (Hamid, 2013). Typically, aflatoxins infiltrate dog food through contaminated corn in the manufacturing process (Bastianello, 1987). However, in some cases contamination occurs due to improper preparation of cereal based dog foods. In these cases aflatoxin outbreaks could last for months before being diagnosed meaning many dogs come in contact with these dangerous toxins (de Koe, 2002). This leads to large scale developments of mycotoxicosis. For example, in 2005, over 100 dogs died in the eastern United States after consuming aflatoxin contaminated corn (Stenske, 2006). Although many years have passed since this incident, little innovation has taken place within the dog food industry to prevent events like this in the future. As a result, a similar incident occurred in 2020 when a Sportmix pet food manufactured by Midwestern Pet Foods was found to have deadly amounts of Aflatoxins. Before it was recalled, the food caused the death of at least 28 dogs and made 8 others severely sick (FDA, 2022). This clearly demonstrates the apparent need for better mold inhibitors and or processing methods for dog food to prevent tragedies like this from occurring in the future.

Similarly to Aflatoxins, Fumonisin and Ochratoxins are also dangerous mycotoxins that commonly proliferate in dog food. Fumonisin is reported to have toxic effects on the liver and kidneys. Additionally, FB1, a specific type of fumonisin, is correlated with hepatocarcinoma, suppression of the immune system, defects in the neural-tube, and nephrotoxicity (Kamle et al., 2019). Similarly, when ingested by dogs, Ochratoxins inhibit protein synthesis, damage DNA, and most notably, cause kidney damage (Battacone et al., 2010). Outbreaks of both Fumonisin and Ochratoxins have been reported across the world including the United States, Germany, and Britain (Little et al., 1991). Compared to aflatoxin outbreaks, Fumonisin and Ochratoxin outbreaks are less harmful because these mycotoxins are less toxic in low dosages. However, this doesn't mean the toxic effects of these mycotoxins should be overlooked. In fact Dr. Leung states that “Ochratoxin A and Fusarium mycotoxins including trichothecenes, zearalenone, and Fumonisin may have chronic effects on the health of companion animals.” (Leung et al., 2006).

With these relevant health concerns surrounding mycotoxins, it's clear a solution is needed. The main mycotoxin prevention strategy currently being explored is nutrient supplementation. Nutrient supplementation is when a compound is added to the diet of an animal with the hopes of counteracting the harmful effects of ingested mycotoxins. The main compounds currently being explored for nutrient supplementation are a type of protein called selenium and vitamins A, C, and E (Galvano et al., 2001). These compounds are being explored due to their high antioxidant properties which act as superoxide anion scavengers and protect cell membranes from mycotoxin-induced damage (Atroschi et al., 2002). However, despite the protective effects of nutrient supplementation, there is very limited clinical evidence to prove its effectiveness in limiting mycotoxicosis in animals (Leung et al., 2006).

¹ “A substance made or used when the body breaks down food, drugs or chemicals, or its own tissue” (NCI Dictionary of Cancer Terms, 2023).

² “Aflatoxicosis is a fungal toxicosis that may affect all species of animals” (Aflatoxicosis | Business Queensland, 2016).

³ “A carcinogen is a substance, organism or agent capable of causing cancer” (Carcinogen, 2023).

While killing mold in dog food doesn't necessarily eliminate mycotoxins that may already be present, it does stop the creation of new mycotoxins that might proliferate during the transportation and storage of the food. As stated by Dr. Leung “preventing fungal growth in pet food can certainly minimize the risk of mycotoxicoses.” Because of this, it's important to explore strategies of limiting mold growth both during and after the processing of the food. Current strategies used to prevent mold in dog food include washing, pearling, ozonation, and acid-based mold inhibition (Boermans et al., 2007). Despite these current methods of reducing mold growth, dog food that reaches consumers still frequently contains mold. For instance, one study found, through mycological determination techniques, that out of 20 cereal dog foods tested, 12 (60%) had mold contamination (Martins, 2003). Another study found that out of the 18 cereal based dog foods tested, 6 (33%) had mold contamination (Kazimierska et al., 2021). This is likely because the small amounts of mold left over after utilizing these mold prevention strategies have the ability to rapidly multiply. Thus, new strategies to prevent mold growth throughout both transportation and storage of dog food are needed.

The most common mold inhibitors used on dog food are acid based preservatives like Propionic acid. Acid based preservatives work by reducing the pH of food, which slows mold and bacterial growth. A study conducted by Dr. Acott tested acid based preservatives effectiveness at limiting the growth of two common molds: *Aspergillus glaucus* and *Aspergillus niger*. The study found that few of the compounds alone or in combination could limit the growth of both molds (Acott et al., 2006). Additionally, low concentrations of acid based mold inhibitors have previously been metabolized by mold which, in some cases, stimulates mold growth and mycotoxin production (Al-Hilli, 2017). This is a significant issue as adding mold inhibitors in too little concentrations or unevenly could make dog food more dangerous if ingested. Another problem with these compounds is that they require significant coverage of the food to be successful. Inadequate coverage can easily lead to contamination and mold growth (Marin, 1999). In an analysis of post-harvest contamination control strategies, Dr. Magan states “There is thus interest in finding alternative compounds to either enhance or to replace such [existing] compounds” (Magan, 2007). Additionally, Dr. Moon asserts that “there is growing interest to develop alternatives to propionic acid [a commonly used mold inhibitor],” due to its “high concentration of addition and foul smell,” (Moon et al., 2018).

With this evidence it's clear that there's a need for new mold inhibitors in dog food. Natural compounds like herbs and vinegar could be a great option to fill this role. While herbs like cumin, astragalus, ginger, and rosemary, along with other natural compounds like apple cider vinegar show high antimicrobial properties, it's not clear to what extent these compounds can effectively limit mold growth in dog food, especially when given in safe amounts. Because of this, it's important to explore these natural compounds' efficacy as mold inhibitors. With this in mind, this research questions: *Can natural compounds, added in safe amounts, effectively act as mold inhibitors and limit mold growth in dog food?*

Materials and Method

Overview

Through direct experimentation, this research aims to measure and compare mold growth between groups of dog food treated with no additional compounds (control) and experimental groups of dog food treated with additive natural compounds. For this experiment, I first grew mold that was used as the model organism. After growing the mold, I divided it equally into multiple sections on a petri dish. These sections were then placed into containers with dog food. Each container of dog food was treated with a different compound like ginger or turmeric. There was also a control batch with no added compounds. After a set period of time, I removed the mold and analyzed how much it grew in each sample of dog food. If a sample of dog food grew less than the control sample, then it can be concluded that the compound acted as an effective antifungal. This information will deepen the understanding of these compounds' antifungal properties and explore their efficacy as mold inhibitors in dog food.

Selection of Tested Compounds

The compounds tested in this study were chosen due to their ability to safely be ingested by dogs and high antifungal properties. Based on this criteria, 5 compounds were chosen: turmeric powder, astragalus root powder, ginger powder, cumin powder, and apple cider vinegar (ACV). All compounds were purchased from Walmart shopping center. The ginger, turmeric, cumin, and apple cider vinegar were produced by the Great Value brand and the astragalus root powder was produced by BareOrganics. Each of these compounds have high antifungal properties that have been extensively tested in various studies. Each compound also has a different recommended amount that can be safely ingested by dogs per day as shown in table 1. The amount of each compound given depends on the dog's weight. For example, a 10-pound dog wouldn't be able to consume as much ginger as a 100-pound dog. For the purposes of this study, the veterinarian recommended amounts were calculated based on the weight of a 60-pound dog. 60 pounds was chosen as the model weight because it reflects the weight of the most popular dog breeds including the Labrador Retriever and Golden Retriever.

Table 1. Recommended Daily Amounts of each Compound Based on the Weight of a 60 Pound Dog.

	ACV	Ginger	Turmeric	Cumin	Astragalus
Recommended amounts	10 milliliters	1.4 grams	1.2 grams	4 grams	6 grams

Mold Growth

The first step in this experiment was to cultivate mold. When determining the type of mold to use, it was important to consider what mold commonly contaminates dog food. *Aspergillus niger* (*A. niger*) most commonly contaminates dog food and produces multiple harmful mycotoxins including Fumonisin and Ochratoxin (Martins, 2003). For these reasons, it was chosen as the model organism for this experiment. I purchased a quick start mold culture of *Aspergillus niger* from Carolina Biological. Quick start cultures are freeze dried cultures of mold or bacteria that must be rehydrated before use. To rehydrate the culture of *Aspergillus niger*, I followed the manufacturer's recommendation detailed in appendix 1. After rehydration, the *Aspergillus niger* was ready to be inoculated on petri dishes. Using a 10 μ L inoculation loop, I gathered a film of rehydrated *Aspergillus niger* pores on the loop by dipping the loop in the test tube containing the mold. During this process I went down to the bottom of the tube to ensure that any spores at the bottom would be caught on the loop. Once I had a layer of rehydrated *Aspergillus niger* on the loop, I applied it to 5 potato dextrose agar petri dishes (PDA). Each petri dish was inoculated using the inoculation pattern shown in fig 1. This inoculation pattern allows for even coverage of the whole petri dish. PDA was chosen as the growth medium for this experiment because *A. niger* has demonstrated the ability to prosper on PDA (Dynowska et al., 2011). After each petri dish was inoculated, a new film was gathered on the inoculation loop by redipping it in the test tube of rehydrated *A. niger*. This was done to ensure that each petri dish had even and consistent coverage. Once each petri dish was inoculated, they were left to incubate in the dark for 4 weeks at 23 °C.

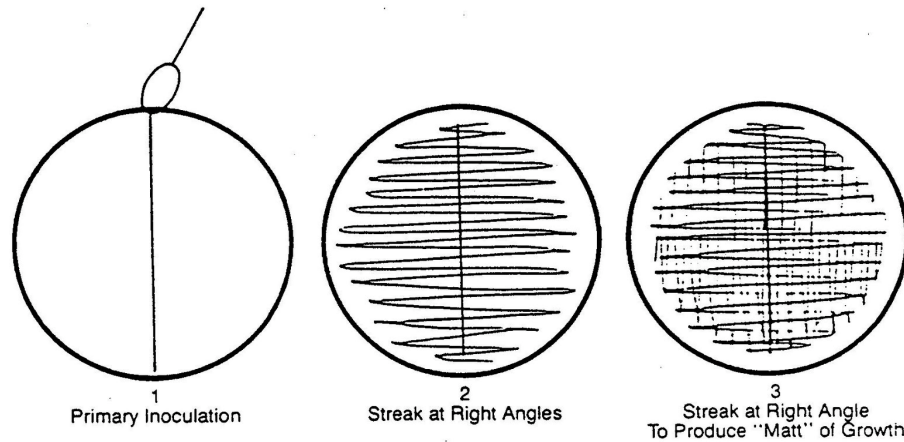


Figure 1. Source: Streaking Agar Plates: 4 Quadrant Streak Method, 2023

After 4 weeks of incubation, the petri dish that had the most even growth of *A. niger* was chosen. To collect the *A. niger* samples that would be used in the experiment, the "Hole Punched Plate" method was used. This method involves using a cork borer to "punch a hole" in the PDA, allowing multiple uniform samples of *A. niger* to be collected from one petri dish (Siede, 2018). I used a 25 mm cork borer to collect 6 mold samples from one petri dish (figure 2).



Figure 2.

Dog Food Preparation

"Purina Dog Chow Real Chicken Dry Dog Food" was used as the model dog food in this experiment due to its consumer popularity and high contents of corn, which is extremely susceptible to *A. niger* contamination (Soares et al., 2013). Before starting the experiment, the amount of dog food per batch and the amount of its respective additive compound had to be determined for each trial. For the purpose of this experiment, three cups of food were used for each trial. This amount was decided upon because it's the recommended daily amount of food for a 60-pound dog.

The amount of each compound added to a batch of dog food was individually determined for every compound. This is because the compounds have different recommended amounts that can be safely ingested by dogs per day.

Once the amount of each compound was determined per batch, the additives had to be mixed to each 3-cup sample of dog food. In total the experiment used 6 batches of dog food, 5 of which contained the additive compounds (turmeric powder, astragalus root powder, ginger powder, cumin powder, and apple cider vinegar) and one that contained no additive compounds, which acted as the control. All powdered compounds (turmeric, astragalus, ginger powder, and cumin) were added to the dog food and then mixed in by hand in uniform motions until homogeneous. The apple cider vinegar was added to the dog food using a spray bottle and then was mixed in by hand until homogeneous. Each batch of dog food was stored in a lidded 5.2 cup tupperware container with the dimensions 6.37 x 6.98 x 5.07 Inches. A tupperware container was used for storage to simulate the conditions that dog food is transported in and the conditions that dog owners typically store food.

Transferring *A. niger* to Dog Food Samples and Measuring Growth

Once the six 25 mm diameter *A. niger* samples were collected and each batch of dog food had its respective compound, *A. niger* samples were ready to be placed in each batch. Samples were buried 2 inches under the dog food and placed in the center of each tupperware container. Once each batch had an *A. niger* sample, the container was closed and left to grow in the dark for 4 weeks at 23 °C to give the mold enough time to evenly grow. After 4 weeks, each *A. niger* sample was removed from every batch and was measured and weighed. Any *A. niger* that had grown onto surrounding pieces of dog food was first measured in mm using a ruler and then cut off using a scalpel to be weighed in mg on a digital scale.

For the purposes of this study, a percent growth difference greater than 30% between the control batch and the experimental batches will affirm that the tested compounds effectively acted as mold inhibitors in the dog food.

$P > 30\%$

P = percent difference between control and experimental batches

Percent difference was calculated using the equation below:

$$p = |a - b| \div ((a + b) \div 2) \times 100$$

a = *A. niger* growth in experimental batch

b = *A. niger* growth in control batch

Results

Data on the mold growth of each sample can be found in Table 2. Final weight including the *A. niger* PDA disks represents the weight of the mold growth onto surrounding pieces of dog food in addition to the final weight of the *A. niger* PDA disk initially placed in each dog food batch. Similarly, final length including *A. niger* PDA disks represents the length of the mold growth onto surrounding pieces of dog food in addition to the length of the initial *A. niger* on the PDA disks. As shown by the table, each *A. niger* PDA disk initially weighed .07 g and was 25 mm in length.

Table 2.

	Initial weight (g)	Initial length (mm)	Final weight including PDA (g)	Final weight excluding PDA (g)	Final PDA weight (g)	Final length including PDA (mm)	Final length excluding PDA (mm)
Control	.07	25	.25	.21	.04	40	15
ACV	.07	25	.11	.06	.05	32	7
Ginger	.07	25	.04	0	.04	25	0
Turmeric	.07	25	.14	.09	.05	34	9
Cumin	.07	25	.23	.17	.06	40	15
Astragalus	.07	25	.21	.16	.05	39	14

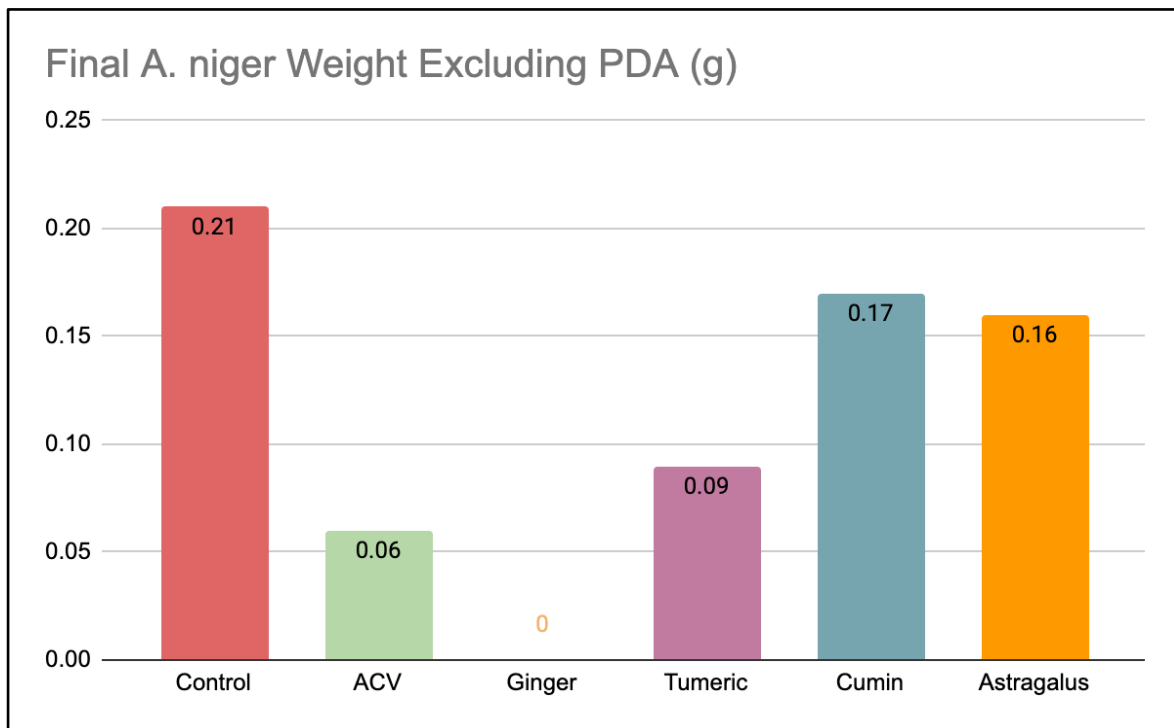


Figure 3.

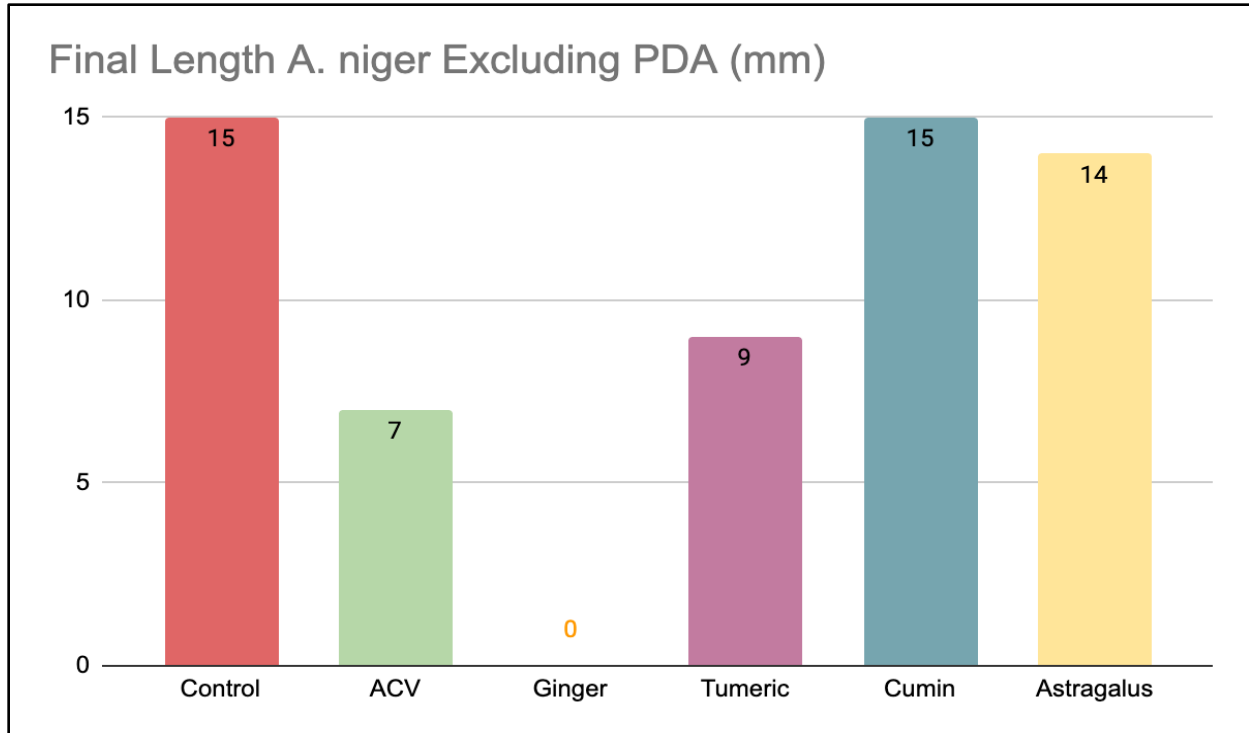


Figure 4.

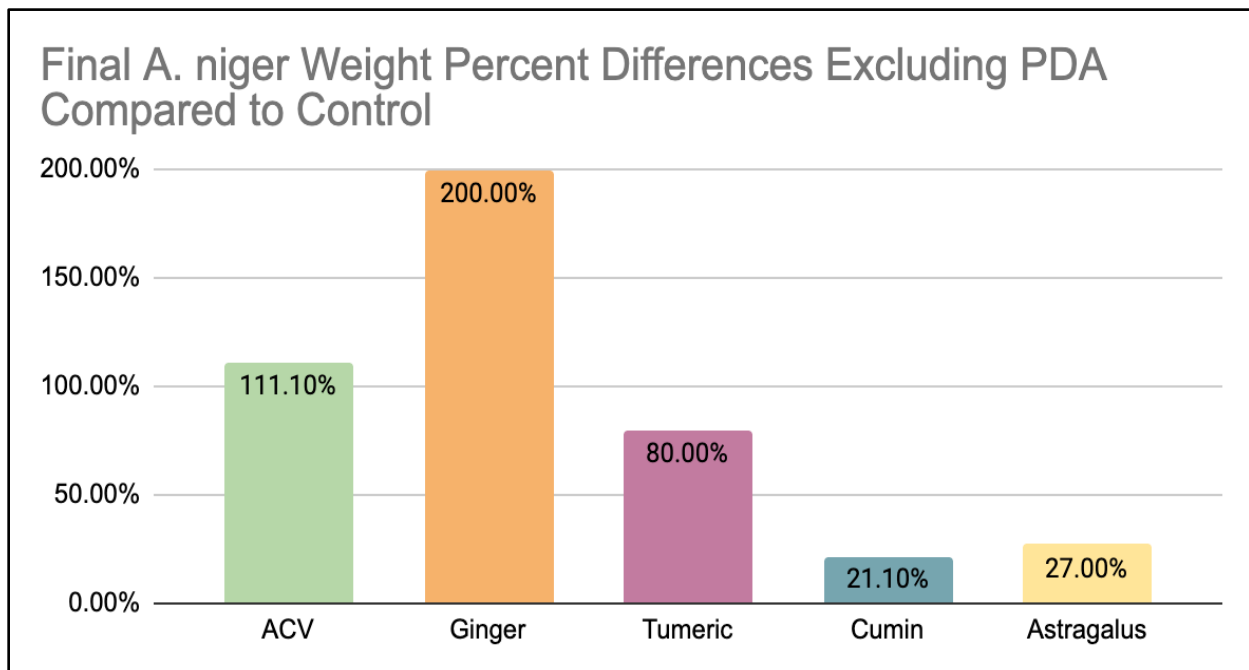


Figure 5.

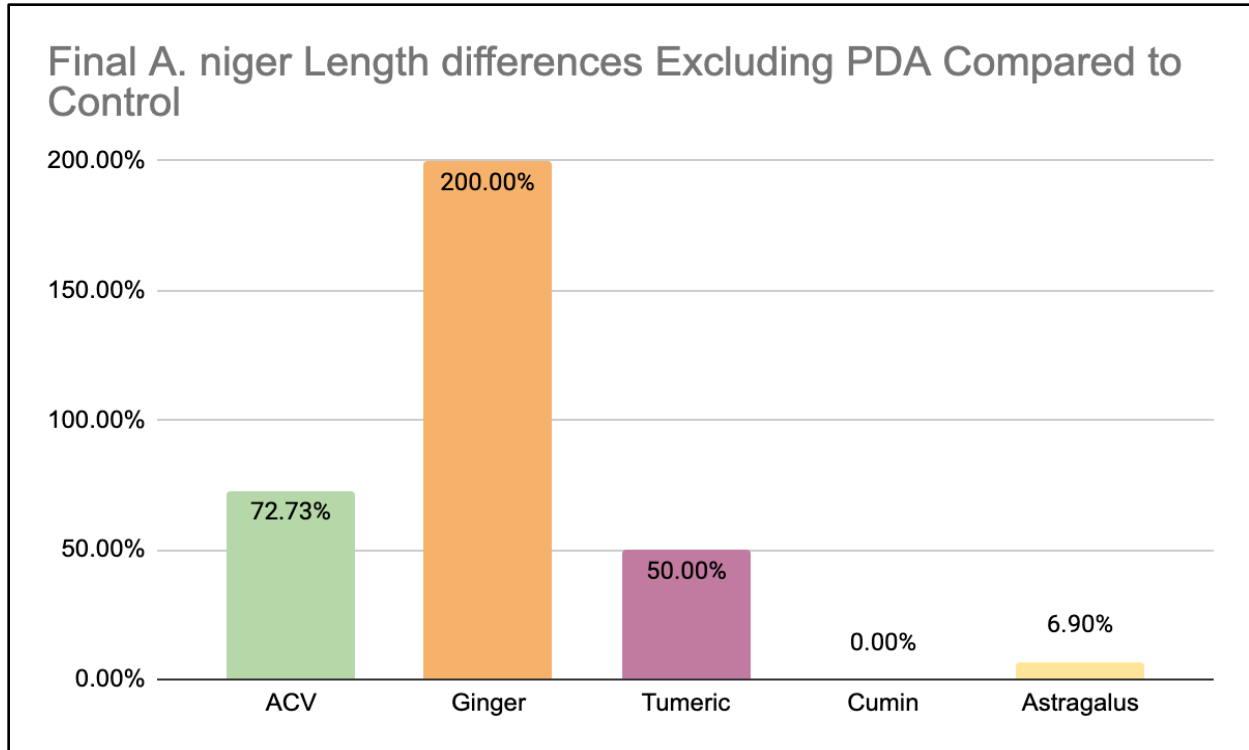


Figure 6.

$P > 30\%$

P = percent difference between control and experimental batches

Discussion

As shown in Table 1, Figure 3, and Figure 4, almost every experimental group had less mold growth, by both weight and length when compared to the control group. The exception to this was cumin which grew the same length of the control group. Despite the cumin *A. niger* growing the same length as the control group, its final weight excluding PDA still weighed .04 g less than the control group meaning, cumin, may to some extent, limit mold growth. While cumin slightly outperformed the control group, other compounds, most notably ginger, had significantly lower *A. niger* growth both in length and weight compared to the control group. What's most impressive about ginger is that it completely limited the spread of any mold growth from the *A. niger* disk to the surrounding pieces of dog food. This is why ginger has zero growth for both weight excluding PDA and length excluding PDA (Figure 3 and 4).

When analyzing the percent growth differences of the compounds, it's important to note that a larger percent difference indicates that a compound acted more effectively as a mold inhibitor. While all compounds limited mold growth when compared to the control batch, not all compounds had a percent growth difference greater than 30% ($P > 30\%$) when compared to the control. Once again, for the purposes of this experiment, a compound with a percent growth difference less than 30% ($P < 30\%$) when compared to the control is considered an ineffective mold inhibitor. Based on this qualification, Cumin and Astragalus were ineffective mold inhibitors as their percent differences were lower than 30% for both weight and length (fig 5 and fig 6). Although Cumin and Astragalus had a growth percent difference less than 30%, Ginger, Apple Cider Vinegar (ACV), and Turmeric all had percent growth differences greater than 30% ($P > 30\%$) when compared to the control. It's most notable that ginger had a 200% growth difference when compared to the control, which is the highest possible percent difference that can be yielded from the equation used. This difference was so high because ginger had zero growth off of the PDA disk. While apple cider vinegar

(ACV) and turmeric didn't have percent differences as large as ginger, they all had percent differences greater than 30% when compared to the control for both weight and length, meaning they all effectively acted as mold inhibitors in the dog food.

Limitations

The main limitation of this research is that the experiment consisted of only 6 trials. With such a limited number of trails, results can easily be skewed by a single outlier. To counteract this, future research could replicate the experimental process used in this study but include more trails for each compound tested. By increasing the number of trials tested for each compound, an average weight growth and length growth could be calculated, which decreases the ability of an outlier to significantly skew the data.

Another limitation of this research is that all the compounds were tested under the same ideal conditions with low humidity, low water activity, and a moderate temperature (23 °C). While having uniform conditions is vital to preventing confounding variables⁴ from effecting results, it limits our understanding of these compounds' effectiveness as mold inhibitors under different circumstances. For example, ginger was the most effective mold inhibitor in this experiment, but would that change if humidity was raised? Understanding how these mold inhibitors behave when other variables are manipulated is vital in growing our understanding of these compounds. Future research can address this by using the same experimental process but adding more trials that manipulate other variables such as humidity, water activity, and temperature.

Another limitation of this research is that only one species of mold, *A. niger*, was tested. While *A. niger* is the mold that most commonly proliferates in dog food, it's important to explore how other mold reacts to the addition of these compounds. This is important to explore because not all molds respond the same to every compound. This is clearly demonstrated by the current acid based preservatives used to limit mold growth in dog food. While these preservatives are beneficial in limiting growth of some species of mold, they can stimulate the growth of others (Al-Hilli, 2017). It's extremely important to explore if ginger, or any of the other compounds tested, has this same stimulating effect on other species of mold.

Future Research and Implications

These natural compounds, specifically ginger, apple cider vinegar, and turmeric, were able to effectively limit mold growth in dog food at a statistically significant level ($P > 30\%$). This can act as preliminary evidence that these compounds can be added into dog food to prevent mold growth. In this study, these compounds have proven to be more effective than the current acid based preservatives used to limit mold growth in dog food, which have little efficacy as mold inhibitors (Acott et al., 2006). This research can potentially be used as a starting point for other researchers to explore these compounds' efficacy more thoroughly as mold inhibitors. These results would hold a more robust level of significance if replicated in the future given the technical limitations of this project.

It is also crucial to explore if dog food with additive compounds would still be desirable for dogs to eat. While this research proves that natural compounds have efficacy as mold inhibitors in dog food, it doesn't test if dogs would still eat food with these compounds. This is extremely important to explore because if dogs won't eat food with these additive compounds, then they can't feasibly be added to dog food even if they do effectively limit mold growth. This is especially important to explore with compounds that have stronger scents such as apple cider vinegar and cumin which may be overwhelming to dogs' acute sense of smell.

⁴ A third variable that can affect the independent and dependent variables leading to distorted associations.

Lastly, it's important for future researchers to explore how these compounds, specifically ginger, could be implemented into the dog food manufacturing process on an industrial scale. Given that acid-based preservatives currently used in the manufacturing process are powder, similarly, ginger could, in theory, be implemented using existing infrastructure. If existing infrastructure could be used, adding ginger into the manufacturing process would be economically feasible and streamlined.

If dog food with these additive compounds is still desirable to dogs, these compounds (especially ginger) can feasibly act as mold inhibitors in dog food and in turn protect dogs from mycotoxin exposure. Considering how effective it was at limiting mold growth, ginger shows massive potential as an effective mold inhibitor in dog food. Taking into account that ginger also has health benefits for dogs like strengthening the immune system (Zhou et al., 2006), and it becomes clear that ginger has the power to significantly reduce the harm that mold poses to dogs. By adding ginger into dog food, dog owners will gain peace of mind that mold growth will be slowed, and dogs will be better protected from mycotoxins that proliferate due to harmful mold contaminated food. Additionally, adding ginger to dog food also has the potential to prevent future mycotoxin outbreaks in dog food by directly limiting mold growth. By preventing future mycotoxin outbreaks, ginger has the potential to save thousands of dogs' lives across the world. Thus, it's vital that further research is conducted to validate ginger's efficacy and feasibility as a mold inhibiting agent in dog food.

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