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Research Article

Piceid as a Preservative; Efficacy against Mold and Yeast in Poultry Feed

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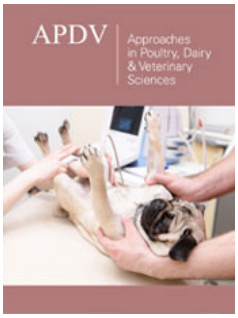

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Abstract

There has been an increased interest in investigating safer alternatives for chemical preservatives. It has been known that chemical preservatives have negative effects on the health of the consumer. The objective of this study was to investigate the efficacy of piceid, either alone or in combination with berberine, against mold and yeast in poultry feed. Piceid was mixed into the feed at a concentration of 0.5g/kg or in combination with berberine at a concentration of 0.5g/kg picied and 0.03g/kg berberine. A cocktail of yeast and mold was added to each bag. The concentration of mold and yeast was determined at different time points. In addition to that, the stability of the concentration of piceid was determined using HPLC. The 0.5g/kg concentration and the combination group with berberine showed significant preservative properties against mold and yeast. Furthermore, the concentration of piceid was stable in the feed for tested period.

Keywords: Piceid; Natural preservative; Antimicrobial; Stability; Efficacy

Introduction

The preservation of food is defined as applying different techniques to prevent spoilage and deterioration of food to extend the shelf life. In addition to that, is must be free of pathogenic micro-organisms Sancho [1]. Natural ways of preserving food without any additives can be achieved by boiling, dehydrating, smoking, pasteurizing and freezing food Sharma [2]. However, using preservatives as additives to extend the shelf life of a product is much more common. Chemical preservatives are most often used but there are some known harmful effects that are caused by certain kinds of preservatives. Nitrites and nitrate are being linked to stomach cancer (but with conflicting reports), benzoates may cause allergies and sorbates/sorbic acids have been associated with contact dermatitis [3-7]. Therefore, a natural preservative without harmful effects is preferable.

Piceid, or polydatin, was originally found and isolated from the root and rhizome of Polygonum cuspidatum and is found in peanuts, grapes and chocolate products [8]. There are several derivates of piceid; trans-polydatin, trans-reservatrol, cis-polydatin and cis-reservatol. A study performed by Mikulski & Molski [9] showed that the trans-isomers have an increased bioactivity in comparison with the cis isomers Mikulski & Molski [9]. Berberine has been described previously Geerlofs et al. [10]. The aim of this study was to test the stability of piceid in poultry feed for 14 days followed by a challenge study of picied either alone or in combination with berberine against mold and yeast strains to investigate the efficacy of piceid as a preservative in poultry feed.

Material and Methods

Feed

The feed was manufactured at SPR feed mill and the feed samples were provided by Southern Poultry Research, Inc. Each batch of feed for this study was mixed and bagged separately. Pelleted feed was produced from mash via steam conditioning (5" diameter x 36" length) and subsequently use of a pellet mill (Model CL5 California Pellet Mill Co., Crawfordsville, IN) equipped with a 5/32"x 7/8" die for broiler diets. Mash feed was placed in the hopper of the pellet mill and the feeder was set at a constant rate to achieve approximately 2lbs. per min-

ute. The target conditioning temperature of a 185F was achieved by adjusting (increasing) steam addition and conditioning time was approximately 30 seconds. Pellets were collected in cooling trays as they exited the pellet die. Pellets were cooled with ambient air for approximately 10 minutes in counter-flow cooler. A 20lb flush was run between each of the treatments.

Stability

The samples with the certificates of analysis were provided by Aspen and the samples are listed in Table 1. Each sample of piceid was dissolved in 80:20 methanol: water solution. Three samples of the individual stock standards were prepared and combined to create a combined standard. That standard was used to prepare the dilutions in methanol for the experiment. The samples used were originally in pellet form but were crushed with a mortar and pestle. To extract 1g of sample, 10mL of methanol was used. The samples were shaken for at least half an hour and allowed to settle for 10 minutes. Afterwards, the methanol was removed and transferred into a separate vial. Piceid (0.5g/kg) and the combination group of berberine (0.03g/kg) and piceid (0.5g/kg) were diluted in 10:1 in methanol. All samples were filtered through a 0.2mm PTFE syringe prior to analysis. All standards and samples were injected to a LCMS using conditions in Table 2. Samples from each concentration were taken on day 0,7 and 14.

Table 1: Samples of berberine and piceid used to determine the stability of the concentration in the feed.

Sample Description	ARC ID	Date Received	Date(s) Extracted
Piceid (>98%)	52359-3	6/7/2018	Method dev and standard
Piceid; 0.5g/kg feed	52359-32	9/27/2018	9/28;10/5;10/12
Berberine 0.03g/kg + Piceid acid 0.5g/kg = 0.53g/kg	52359-34	9/27/2018	9/28;10/5;10/12

Table 2: The HPLC settings used to determine the stability of the concentration in the feed.

HPLC		
LC model	Agilent 1200 LC	
LC column	Luna 3u C18(2), 150mm x 2.0 mm	
LC flowrate	0.2ml/minute	
LC injection volume	5ml	
LC column temperature	40 °C	
LC eluent A	0.1% Formic Acid	
LC eluent B	0.1% Formic Acid in Acetonitrile	
LC Gradient		
Time minutes	**Eluant A%**	**Eluant B%**
0	90	10
5	90	10
20	5	95
35	5	95
37	90	10
Run time	37 minutes	
Post time	10 minutes	
Mass Spectrometer		
MS model	Agilent 6210 Time of Flight MS	
ESI (electrode ionization)	Positive mode	
ESI source	300 °C gas (N_2) at 8L/minute, 30psi nebulizer, 4000V Vcap, 110V fragmentor	
TOF (Time of Flight) range	50-1500amu (atomic mass unit)	

Efficacy

The mold and yeast organisms were cultured and accumulated according to the protocols provided by Deibel Labs and presented in Table 3. To form a spore suspension, the accumulated mold cultures were filtrated through a sterile cheese cloth. The separately harvested suspensions were combined to create an inoculation cocktail. 50µl of the inoculation cocktail was put onto a slide and lactophenol aniline was added to the slide. The combination of lactophenol aniline and the cocktail was then mixed, and the slide was sealed with a cover slip. The slide was analyzed under a microscope to determine the number of hyphae present. A significant number of hyphae could indicate that a spore culture wasn't achieved. The cocktail was filtrated for a second time if the amounts of hyphae was too high. After confirming the spore culture, the solution was diluted to attain the target concentration. Each sample consisted out of a sterile whirl-pak bag containing 50 grams of feed. The control group samples were bags with feed that did not contain piceid and the test samples were bags with feed that did contain piceid. The target concentration per bag was 102-103CFU/g. 0.25ml of the mold or yeast cocktail was added to each sample bag and was spread throughout the entire bag. The bags were massaged to ensure the cocktail covered all the product. The bags were closed by using the rolling technique. The bags were stored at room temperature in a closed chamber that had an open pan containing water to ensure high humidity.

Table 3: Official name and ATCC number of the used mold and yeast strains for the inoculation cocktail.

Mold	Organism ID/ATCC Number	Yeast	Organism ID/ATCC Number
Aspergilus flavus	USDA NRRL 453	*Candida albicans*	ATCC 869
Penicilium glabrum	USDA NRRL 766	*Zygosaccharomyces bailli*	ATCC 2227
Mucor plumbeus	ATCC 2630	*Zygosaccharomyces rouxii*	ATCC 12691
Rhizopus oryzae	ATCC 3652	*Saccharomyces cerevisiae*	ATCC 12632
Eurotium rubrum	USDA NRRL 5000	*Dekkera bruxellensis*	ATCC 1411
Aspergilus flavus	USDA NRRL# 5565 (Turkey feed mix)	Yeast spp.	DLI client isolate
Talaromyces sayulitensis	NRRL# 62280 (Chicken feed)		

Samples from the test bags were taken on day 0,7,14,21 and 28 and plated. Samples from the control bags were taken on day 0 and on day 28 to establish the presence of background flora. All samples were taken in triplicate. On day 7,14,21 and 28, 450mL of Butterfields Phosphate Buffered Diluent (BPB) was added to the sample. After 1-2 minutes the samples were plated. Those plates were then spread plated onto new plate. The samples that were inoculated with mold were plated onto a Potato Dextrose Agar plate with a chlortetracycline additive. The samples that were inoculated with yeast were plated onto a YM agar plate. The test and control plates were enumerated after incubation at 25 ºC for 5 days.

Statistics

To compare the test and control groups at every time point, an unpaired T-test was performed. The results were considered significant if $p < 0.05$. The graphs were generated in GraphPad Prism version 8 (GraphPad software Inc., USA).

Results

Stability

Table 4: Concentration of piceid in feed and in the combination feed with berberine.

ARC ID	Sample ID	Sample Type	Piceid Conc (g/kg) T-0	Piceid Conc (g/kg) T-0 +7	Piceid Conc (g/kg) T-0 + 14
A52359 blank	Basal pellet	Pellet	N. D	N. D	N. D
52359-32A	Piceid 0.5g/kg feed	Pellet	0.29	0.33	0.37
52359-34A	Berberine 0.03g/kg + Piceid acid 0.5g/kg = 0.53g/kg	Pellet	0.28	0.28	0.31

The results of the testing are summarized in Table 4. The method used can detect 0.005g/kg of additive. If the response was below that, the compound was reported as not detected (N.D). The results showed that piceid is stable when stored at room temperature over the two-week storage time.

Efficacy

The pellet samples were tested, and the graphs are presented in Figure 1. The 0.5g/kg group (5A) showed that the test group was performing significantly better than control group in reducing the yeast concentration at day 14 ($p<0.001$) and day 28 ($p= 0.037$). Furthermore, the test group was performing better in reducing the mold concentration at day 14 ($p<0.001$) and 21($p<0.001$). The combination group of berberine and piceid (5B) showed that the control group was performing significantly better than the test group at day 7 ($p<0.001$) at reducing the yeast concentration. The test group performed better at reducing the yeast concentration at the end of the experiment. From day 14 until the end of the experiment, the test group had more effect against the mold than the control group.

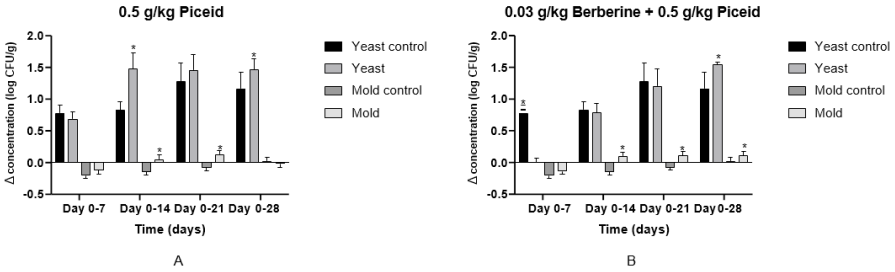

Figure 1: A- The effect of 0.5g/kg piceid on mold and yeast growth. The test group was performing better on inhibiting the mold and yeast growth at several time points.
B- The effect of berberine and piceid on the growth of mold and yeast. Over the full 28 days, the test group was significantly performing better than the control group at reducing the mold and yeast concentration.
All values are mean ±SD. *indicates a significant difference.

Discussion

There are two classes of preservatives: class I and class II. Class I preservatives are from a natural source and class II preservatives are chemical or synthetic preservatives. An item intended for consumption can have only one class II preservative otherwise there is a chance it might affect the overall health of the consumer Anand & Sati [11]. The harmful effects of chemical or synthetic preservatives in food and medicine have been well documented. Researchers have linked some preservatives to type 2 diabetes Tirosch et al. [12], prevalence of asthmatic attacks Nagel et al. [13], increased anxiety and motor impairment Noorafshan et al. [14]. All the industries that use preservatives in their products have been responding to the customers resistance and have been investigating the possibility replacing the harmful compounds with natural compounds [11]. Piceid could be one of the replacements compounds the results of this study showed that piceid does significantly reduces mold and yeas concentration at a concentration of 0.5g/kg incorporated into the feed. Furthermore, the combination group of berberine and piceid also decreased the concentrations significantly. In addition to that, the stability experiment showed that the concentration of piceid was stable for at least 14 days under the experimental conditions employed. This study has showed that piceid has a significant effect on the concentration of mold and yeast when incorporated in poultry feed and is stable. Good preservative effects were observed at a piceid concentration of around 0.5g/kg. Preservative effects were also observed with a berberine piceid combination, which was also found to be stable [15-19].

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Authors' Contributions

Zhiyong He designed the experiment. The study was conducted upon request from Sa Xiao and Zhi-Cheng Xiao. Lotte Geerlofs wrote the manuscript on consultation with Zhiyong He and made revisions when necessary. Lotte Geerlofs, Vivan Liu and Sa Xiao analyzed the data. All authors have read and approved the manuscript.

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