



## THE EFFECT ON MYCOTOXIN DEVELOPMENT OF COMBINED ORGANIC ACID ADDITIVE AT DIFFERENT LEVELS IN DAIRY FEED

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**Abstract:** Organic acid treatment to prevent deterioration of feeds by exposure to mycotoxins and to extend their storage life is the most important requisite of natural, safe and wholesome feed production. (1) Background: The aim of this study was to determine the effects of an organic acid (OA) combination added in incremental levels to commercial dairy feed, on mycotoxin development in feeds stored for varying lengths of time; (2) Methods: For the trial, a total of 5 treatment groups were formed as control, 10 lt water only (without OA), and 10 lt water with respectively 0.2, 0.3 and 0.4 kg/ton OA addition. The trial conducted in a commercial feed mill for a span of 3 months from March through May, was set-up in a 3x5 factorial plan, taking into account the effect of groups and storage duration in months. Deoxynivalenol, zearalenone, aflatoxin, fumonisin B1, T-2 toxin, ochratoxin, fumonisin B2 and HT-2 levels were measured in the feed samples taken at certain control points of the feed manufacturing process, that is to say after the mixer, conditioner and cooler, and stored for three months; (3) Results: It was determined on the basis of the results that, deoxynivalenol and aflatoxin levels significantly decreased ( $P<0.05$ ) with the addition of both 0.3 and 0.4 kg/ton OA+water into the compound feed, whereas fumonisin B1 levels decreased ( $P<0.001$ ) with the addition of 0.3 kg/ton OA+water. Mycotoxin content was further affected by the length of storage, with the highest contamination detected in May ( $P<0.001$ ). Ochratoxin, fumonisin B2, T-2 and HT-2 remained below thresholds of detection throughout the trial; (4) Conclusions: Adding 0.3 or 0.4 kg/ton OA+water may be beneficial in reducing the development of mycotoxins in feed, however, more research is needed on the subject.

**Keywords:** Mycotoxin, Organic acid, Feed toxicity, Feed storage, Dairy

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### 1. Introduction

Efficient and sustainable animal husbandry has become an area of increasing scientific interest. In addition, there is an increasing demand for safe, healthy and residue-free animal production for human and animal consumption. Besides efficient and sustainable livestock production, the growing demand for wholesome and residue-free animal production safe for human and animal consumption has become an area of intensifying scientific focus. Among feed additives used in recent years as alternatives to antibiotics for preventing bacterial infections, especially contagious epidemics, and for growth promoting purposes, organic acids, their salts and/or blends have gained popularity. Organic acids are organic carboxylic compounds containing amino acids and fatty acids with the general structural formula R-COOH and their acidity is connected to the carboxyl group (-COOH). They are weak acids which dissolve in water to release a hydrogen ion

(H<sup>+</sup>) and a carboxylate ion (-COO<sup>-</sup>) (Lim et al., 2015). The most widely used organic acids are short-chain fatty acids such as formic, lactic, malic, citric, acetic, butyric and propionic acids. As they are substances synthesized by the animal itself, occurring as a result of natural biochemical metabolism, they are recognized as environmentally friendly, residue-free, safe and natural feed additives which do not present any risks when used in feeds (Humer et al., 2016; Gül and Tekce, 2017; Dijksterhuis et al., 2019). Improvements in terms of performance and feed efficiency have been reported in studies on organic acid supplementation in ruminants (Wang et al., 2020; Gallo et al., 2021), swine (Lei et al., 2017; Hossain et al., 2018; Nguyen et al., 2020) and poultry (Adil et al., 2010; Khan and Iqbal, 2016) diets. Furthermore, it has also been suggested that organic acids may regulate intestinal bacterial populations, reduce gas emission, improve nutrient digestibility and gut health in animals. However,



it was emphasized that the type of organic acid, its dosage and overall feed formulation may affect the response to the use of dietary organic acids (Nguyen et al., 2020). On the other hand, feed ingredients such as cereals, by-products thereof and oilseeds carry a potential contamination risk due to the emergence of various mycotoxins and toxic metabolites, usually produced by a range of fungi or mold species (Pearlin et al., 2020). The use of acids is among several tools employed in good management practices to reduce the microbial contamination risk during storage of the feeds. It has been reported that the effect of salts or combinations of organic acids in feeds is mainly related to their protective action, especially against mycotoxin development (Kaya et al., 2014; Humer et al., 2016; Moon et al., 2018). The synergistic effects of pKa values, which is an indication of their undissociated forms, and their strong antimicrobial properties depending on the cell membrane structure and pH range are particularly important for protein-rich feed ingredients such as soybeans (Khan and Iqbal, 2016; Lei et al., 2017). Organic acids, of which antimicrobial effects increase in parallel with their pKa values, suppress the proliferation of pathogens by increasing the acidity of cellular pH through the released hydrogen ions and by disrupting bacterial cellular metabolism (Nguyen et al., 2020). On the other hand, Humer et al. (2016) reported that organic acids, which are frequently used in feed preservation and processing, improve the nutritional properties of feeds by promoting the breakdown of antinutritional factors, enriching the feed with healthier ingredients, thus increasing their health benefits in animal and human nutrition. It has been emphasized that commonly used mold inhibitors contain organic acids in various levels and combinations (Eissen et al., 2010). Surfactants can be added to organic acid blends in liquid form in order to increase their effectiveness, by ensuring that mold inhibitors and water are evenly distributed among the feed particles. Surfactants, which basically consist of mixtures of wetting agents, are compounds that reduce the surface tension at the interfaces between phases insoluble in one another (oil-water, solid-water) by virtue of their hydrophilic and hydrophobic groups (Rosen and Kunjappu, 2012). Surfactants also improve the water binding capacity of feed ingredients and reduce water activity in feeds (Eissen et al., 2010). Mycotoxins that cause various clinical infections in animals, depending on the storage conditions and duration of feeds, are toxic substances produced by certain fungal species such as *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* (Gül and Tekce, 2017). The most common mycotoxins with mutagenic, teratogenic and carcinogenic effects are aflatoxin, zearalenone, deoxynivalenol, T2, HT-2 and fumonisins (Yang, 2019; Jubeen et al., 2020; Yang et al., 2020). Although there are various physical, chemical and biological methods to reduce mycotoxin contamination, not all approaches are suitable for feed manufacturers (Čolović et al., 2019). In the feed milling industry, mycotoxins need to be eliminated or neutralized

by easy to store, practical to apply and economical solutions without compromising the technological properties and nutritional value of the products (Zhu et al., 2017; Huss et al., 2018). The aim of this study was to determine the effects of an organic acid (OA) blend on mycotoxin development in stored feeds for dairy cattle.

## 2. Materials and Methods

This study was conducted at an independent and privately owned commercial feed mill over a period of three months (March, April and May). The material used in the trial was commercial compound feed for dairy cattle in mash form, produced in the same factory with 2790 kcal/kg metabolizable energy (ME) and 21% crude protein content (Table 1).

**Table 1.** Nutrient composition of the commercial compound feed\*

Nutritional composition	%
Crude ash	7.75
Starch	25.5
Crude fiber	Max 9
ADF	11
NDF	22
Lignin	2
Crude protein	21
ME (kcal/kg)	2790
NE <sub>L</sub> (kcal/kg)	1813.5

\*Ingredients= Wheat bran, corn, corn DDGS, sunflower seed meal, full fat soybean, rapeseed meal, molasses, limestone, salt, vitamin & mineral premixes, zeolite

With a view to examining the effects of incremental doses of organic acid blend (OA) on the mycotoxin toxicity of the compound feed, a total of 5 treatment groups were formed: A control group with no addition whatsoever, another group to account for the effect of water addition (10 lt without OA), and three treatment groups with 10 lt water plus 0.2, 0.3 and 0.4 kg/ton OA addition respectively. The OA doses used were determined by taking into account the company's recommendation. The trial was planned in a 3x5 factorial model considering the effect of groups and storage duration in months. OA (Fylax Forte-HC liquid) used in the trial was sourced from Trouw Nutrition TR Gıda Tarım Hayvancılık San. ve Tic. A.Ş., Türkiye. The said OA product consisted of a mixture of 80% organic acids (sorbic, formic, acetic, lactic, propionic acids and ammonium propionate) and 20% 1,2-Propanediol as surfactant. Throughout the trial, 10 liters of water was added to all groups, except for the control, in order to ensure moisture optimization of the compound feed and to improve production performance. The specified OA doses were added to that 10 liters of water and the solution thus obtained was added to the compound feed at the mixer

In order to determine at which production and storage stage the moisture loss and toxin formation occurs, three sets of 2x500 g samples were taken at the beginning of February to represent each trial month from the exits of the mixer, conditioner and cooler. The samples were placed in storage in plastic zipper bags under room conditions and sent for analysis at the beginning of March, April and May to monitor the change as a function of storage duration. Deoxynivalenol, zearalenone, aflatoxin, fumonisin B1, T-2 toxin, ochratoxin, fumonisin B2 and HT-2 analyzes were performed on those feed samples stored for one, two or three months, preparing composite laboratory samples from four different points of each monthly sample. Mycotoxin analyzes were performed following the procedures described in ISO 17125 Dutch Accreditation Council NEN-EN 17194 (2017). According to the procedure; the samples are desiccated for 4 hours at 103°C. The loss in mass is determined by weighing. Measurement is based on gravimetric method by LC-MS/MS. The mycotoxins are extracted using 79% acetonitrile with 1% formic acid. Following the addition of the internal standard, evaporation and reconstitution in mobile phase, the extract is analyzed by ultra-high performance liquid chromatography on a reversed phase column with a triple quadrupole mass spectrometer. For each mycotoxin a precursor is selected and fragmented to two daughter ions, after which the ratio is used as confirmation.

### 2.1. Statistical analysis

Variance analysis is conducted to establish whether there was a difference in terms of toxicity between three dosages of OA, untreated water addition and control groups after three different periods of storage (analyzed in March, April, May). In addition, Duncan's multiple comparison test was used to determine the differences between doses and months for the characteristics deemed to be significant. The following mathematical model (equation 1) was used for the variance analysis.

$$y_{ijk} = \mu + a_i + b_j + e_{ijk} \quad (1)$$

$y_{ijk}$  : Measured value for any characteristic

$\mu$ : Expected average for the analyzed characteristic

$a_i$  : i. Dosage effect (i = Group I, Group II, Group III, Group IV and Group V)

$b_j$  : j. Storage duration effect (March, April, May)

$e_{ijk}$  : Normally distributed error effect with a mean of zero and variance of  $\sigma_e^2$

Moreover, HT 2 levels are counted as either below 10  $\mu$ /kg, or above 10  $\mu$ /kg (11 and 12  $\mu$ /kg) and Chi-squared test was performed. Since the expected values for this test were too small, the OA+Water groups were integrated to perform a 3x2 Chi-squared test with OA+Water, Control and Untreated Water rows, and below and above 10  $\mu$ /kg columns (SPSS, 2008).

### 3. Results

The average mycotoxin contents obtained from compound feed samples collected after mixer, conditioner and cooler are given in Table 2. The effect of treatment and storage duration was found to be significant between the groups in terms of post-mixer mycotoxin contents ( $P < 0.01$ ). The lowest post-mixing mycotoxin content of 11.7  $\mu$ g/kg was observed in Group III with 0.4 kg/ton OA+water addition and was significantly to all other treatments (Table 2). The differences between Group I, untreated water and control groups in terms of post-mixing mycotoxin content were not significant ( $P > 0.05$ ). As to group II, it was found to be higher than the group III but lower than the other groups with statistical significance. In terms of post-mixing mycotoxin content, the lowest average mycotoxin content was 12.1  $\mu$ g/kg in April and the highest was 12.5  $\mu$ g/kg in May ( $P < 0.01$ ).

The effect of group and month on the mean mycotoxin content in compound feed at the exit of the conditioner was statistically significant ( $P < 0.01$ ). The lowest mean value was 14.5  $\mu$ g/kg in Group III, which was the one with 0.4 kg/ton OA+water addition, and this value was significantly lower than the other groups ( $P < 0.01$ ). On the other hand, the differences between the other four groups were not significant ( $P > 0.05$ ). The lowest mean mycotoxin content after conditioner was observed in March and the highest in May and the differences between the three months were significant ( $P < 0.01$ ).

The effect of group on post-cooler mycotoxin content in compound feed was found to be significant ( $P < 0.01$ ), while the effect of month (storage duration) was not significant ( $P > 0.05$ ). Similar to the results of post-conditioner samples, the lowest mycotoxin content was found to be 11.7  $\mu$ g/kg in the 0.4 kg/ton OA+water supplemented feed group. However, the differences between the other four groups were not significant ( $P > 0.05$ ).

The mean values observed for the analysed mycotoxin types (Deoxynivalenol, zearalenone, fumonisin B1 and aflatoxin) in compound feed samples are presented in Table 3. In terms of deoxynivalenol, group effect was found to be significant ( $P < 0.01$ ), whereas the month effect was not significant statistically ( $P > 0.05$ ). Although the highest mean deoxynivalenol content was found in the Control group, the differences between Control and Group I, were not significant, and the deoxynivalenol content was not affected by the addition of OA ( $P > 0.05$ ). In contrast, deoxynivalenol findings were lower in Group II than in the Control, and lower in Group III than both Water Group and Control ( $P < 0.01$ ). The differences observed between the trial groups in terms of zearalenone level were not found to be statistically significant ( $P > 0.05$ ). However, significant differences were found between the trial months. While there was no statistically significant difference between March and May, the mean of April was found to be 41.9  $\mu$ g/kg, nearly 30% higher than the other two months ( $P < 0.01$ ).

The effects of group and month were found to be

significant for Fumonisin B1 ( $P<0.01$ ). The highest mean Fumonisin B1 level was observed in the Water group and the Control group, while the lowest was in Group III and the differences between these groups were statistically significant ( $P<0.01$ ). On the other hand, there was no difference between Group I and Group II, while these two groups were among the high and low groups and were found to be similar to them. The lowest mean fumonisin B1 value was found in May. While the differences between March and April were not significant ( $P>0.05$ ), the differences between these two months and May were statistically significant ( $P<0.01$ ). The highest aflatoxin level was found in the Control Group at 1.45  $\mu\text{g/kg}$ , with the lowest ones were found at 1.04 and 0.98  $\mu\text{g/kg}$  in Groups II and III respectively. The difference between Group II and Group III was not statistically significant, but the difference between these groups and

the Control group was significant with lower values ( $P<0.01$ ). However, the levels of aflatoxin observed in the Water Group group and OA added groups were similar ( $P>0.05$ ). The aflatoxin content of all 10 samples analyzed in March could not be determined as it was lower than 5  $\mu\text{g/kg}$ . The aflatoxin content determined in May was 0.99  $\mu\text{g/kg}$ , which was approximately 50% lower than in April ( $P<0.01$ ).

The amounts of T-2, ochratoxin and fumonisin B2 could not be detected in all groups for the three months of trial as they were below 10  $\mu\text{g/kg}$ . However, the results of the Chi-squared test for HT-2 amounts are given in Table 4. The analysis results indicated no difference between the groups in terms of HT-2 levels ( $P>0.05$ ). In another way of saying, there was no difference between OA+Water, Control and water only groups in terms of HT-2 levels being below 10  $\mu\text{g/kg}$  or above 10  $\mu\text{g/kg}$ .

**Table 2.** Average mycotoxin values found in compound feed samples taken after the mixer, conditioner and cooler ( $\mu\text{g}$ )

Treatment	Mean Mycotoxin Values ( $\mu\text{g/kg}$ )		
	Post-Mixer	Post-Conditioner	Post-Cooler
Group	*	*	*
Group I (0.2 kg/t OA+Water)	12.4 $\pm$ 0.04 <sup>a</sup>	15.3 $\pm$ 0.09 <sup>a</sup>	12.9 $\pm$ 0.08 <sup>a</sup>
Group II (0.3 kg/t OA+Water)	12.3 $\pm$ 0.04 <sup>b</sup>	15.1 $\pm$ 0.09 <sup>a</sup>	12.8 $\pm$ 0.08 <sup>a</sup>
Group III (0.4 kg/t OA+Water)	11.7 $\pm$ 0.04 <sup>c</sup>	14.5 $\pm$ 0.09 <sup>b</sup>	11.7 $\pm$ 0.08 <sup>b</sup>
Water	12.4 $\pm$ 0.04 <sup>a</sup>	15.2 $\pm$ 0.09 <sup>a</sup>	12.7 $\pm$ 0.08 <sup>a</sup>
Control	12.4 $\pm$ 0.04 <sup>a</sup>	15.2 $\pm$ 0.09 <sup>a</sup>	12.9 $\pm$ 0.08 <sup>a</sup>
Months (Storage Duration)	*	*	ns
March (one month)	12.2 $\pm$ 0.03 <sup>b</sup>	14.5 $\pm$ 0.07 <sup>c</sup>	12.6 $\pm$ 0.07
April (two months)	12.1 $\pm$ 0.03 <sup>c</sup>	14.9 $\pm$ 0.07 <sup>b</sup>	12.6 $\pm$ 0.07
May (three months)	12.5 $\pm$ 0.03 <sup>a</sup>	15.8 $\pm$ 0.07 <sup>a</sup>	12.6 $\pm$ 0.07

\* $P<0.01$  Means with different superscript within the same column differ significantly; ns= non-significant.

**Table 3.** Mean values of Deoxynivalenol, Zearalenone, Fumonisin B1 and Aflatoxin observed in compound feed samples ( $\mu\text{g/kg}$ )

Treatment	Mycotoxin Types ( $\mu\text{g/kg}$ )			
	Deoxynivalenol	Zearalenone	Fumonisin B1	Aflatoxin
Group	*	ns	**	*
Group I (0.2 kg/t OA+Water)	644.8 $\pm$ 13.8 <sup>abc</sup>	35.5 $\pm$ 2.3	265.0 $\pm$ 8.7 <sup>ab</sup>	1.27 $\pm$ 0.12 <sup>ab</sup>
Group II (0.3 kg/t OA+Water)	643.0 $\pm$ 13.8 <sup>bc</sup>	33.0 $\pm$ 2.3	262.0 $\pm$ 8.7 <sup>ab</sup>	1.04 $\pm$ 0.12 <sup>b</sup>
Group III (0.4 kg/t OA+Water)	622.0 $\pm$ 13.8 <sup>c</sup>	32.3 $\pm$ 2.3	238.7 $\pm$ 8.7 <sup>b</sup>	0.98 $\pm$ 0.10 <sup>b</sup>
Water	667.8 $\pm$ 13.8 <sup>ab</sup>	37.3 $\pm$ 2.3	281.0 $\pm$ 8.7 <sup>a</sup>	1.32 $\pm$ 0.16 <sup>ab</sup>
Control	686.7 $\pm$ 13.8 <sup>a</sup>	39.5 $\pm$ 2.3	283.7 $\pm$ 8.7 <sup>a</sup>	1.45 $\pm$ 0.10 <sup>a</sup>
Months (Storage Duration)	ns	**	**	**
March (one month)	645.0 $\pm$ 10.7	34.4 $\pm$ 1.8 <sup>b</sup>	268.9 $\pm$ 6.8 <sup>a</sup>	-
April (two months)	654.7 $\pm$ 10.7	41.9 $\pm$ 1.8 <sup>a</sup>	284.4 $\pm$ 6.8 <sup>a</sup>	1.44 $\pm$ 0.09 <sup>a</sup>
May (three months)	658.9 $\pm$ 10.7	30.3 $\pm$ 1.8 <sup>b</sup>	244.9 $\pm$ 6.8 <sup>b</sup>	0.99 $\pm$ 0.07 <sup>b</sup>

\* $P<0.05$ , \*\* $P<0.01$  Means with different superscript within the same column differ significantly; ns= non-significant.

**Table 4.** Chi-squared test results for HT-2

Group		Below 10 µ/kg	Above 10 µ/kg	Total
OA+Water	n	16	2	18
	Row	88.9%	11.1%	100.0%
	Column	66.7%	33.3%	60.0%
Control	n	4	2	6
	Row	66.7%	33.3%	100.0%
	Column	16.7%	33.3%	20.0%
Water only	n	4	2	6
	Row	66.7%	33.3%	100.0%
	Column	16.7%	33.3%	20.0%
Total	n	24	6	30
	Row	80.0%	20.0%	100.0%
	Column	100.0%	100.0%	100.0%

Pearson Chi-Square = 2.222. P=0.329

#### 4. Discussion

Beneficial bacteria survive in a lower pH environment than pathogenic bacteria. The antimicrobial effect of OAs can be attributed to the decrease in pH they cause by virtue of their penetration through the bacterial cell membrane into the cytoplasm, forming protons and anions that are toxic to cells, and the subsequent death of permeabilized cell due to the energy loss (Youssef et al., 2017; Markazi et al., 2019). Therefore, the addition of OAs to feeds to lower pH inhibits, reduces or prevents the colonization of pathogenic bacteria and creates favorable conditions for the proliferation of beneficial bacteria (Qui, 2023). In the present study, it was observed that 0.4 kg/ton OA+water addition to the compound feed during the production process significantly reduced feed toxicity in terms of both total mycotoxin content and individual mycotoxins (deoxynivalenol, zearalenone, fumonisin b1, aflatoxin), while mycotoxin content did not change with respect to the Control Group with the addition of water only. The average mycotoxin levels observed after the mixer, conditioner and cooler decreased significantly with the addition of 0.4 kg/ton OA + water to the compound feed, and at the same time, the addition of 0.3 kg/ton OA + water also ensured a drop in mycotoxin levels in the post-mixer samples compared to the other trial groups. It can be stated that this decrease was in line with longer storage durations. It has been suggested that organic acid blends have positive effects on the storage life of feeds, as one of the most effective feed additives in mycotoxin prevention (Jouany, 2007; Samli et al., 2008). In contrast to the present study, it was reported that the protective effect of 0.2% propionic acid supplementation to layer breeder and broiler compound feeds stored for 15, 30, 45 and 60 days under normal room conditions decreases as the storage period lengthens, leading to a consequent increase in aflatoxin and fumonisin values (Erdoğan and Kaya, 2022). Similarly, Samli et al. (2008) reported that mycotoxin formation was significantly reduced in protein-rich feed ingredients treated with 3 g/kg organic acid blend for 30

days of storage, and that this effect may vary depending on the nature of the feed ingredients, initial microorganism populations and components of the organic acid mixture. Since lactic and acetic acids are among organic acids having very strong antibacterial activities, it is emphasized that the use of bio-preservatives containing a blend of these organic acids as antimicrobial agents could be a potential solution approach (Hu et al., 2019; Rad et al., 2021). In a study on the antifungal properties of eight organic acids (propionic, acetic, formic, lactic, tartaric, citric, oxalic and malic acids) against the growth of four fungi species (*Aspergillus flavus*, *Penicillium purpurogenum*, *Rhizopus nigricans* and *Fusarium oxysporum*), acetic acid (10%) and lactic acid (10%) were reported to have the highest inhibitory effect on *A. flavus* and *F. oxysporum* toxins (Hassan et al., 2015). The presence of acetic acid and lactic acid in the organic acid blend used in this study may have played an active role in the reduction of toxins throughout the 3-month storage period. On the other hand, although the average mycotoxin levels decreased to a certain extent in April, they have shown an increase in May compared to March and April. This increase can be attributed to the rising temperatures of the feeds stored at room temperature during the trial which continued into warmer months. It can also be argued that the storage temperature should not exceed the maximum room temperature on average. In general, it is known that molds remain inactive if grains are stored below 20°C (Mousa et al., 2013). Seasonal weather conditions are reported to be the main variable in determining mycotoxin concentration, with a significant correlation observed between them (Qu et al., 2024). Moreover, it has been suggested that excessive moisture and high water activity in feeds are the foremost causes of mold growth as far as the feed industry is concerned, and that the moisture content of grains should not exceed 13% during storage, and the water activity of raw materials, feed mixtures and finished feeds should be kept below 0.8% (Mohapatra et al., 2017; Dijksterhuis et al., 2019). In

this study, although there was no change in the water group compared to the control, the improvement observed with organic acid supplementation can be considered as an indication that the optimum moisture balance was achieved with 10 liters of water addition. The results of the present study revealed that deoxynivalenol, fumonisin B1 and aflatoxin levels were significantly reduced by the addition of 0.4 kg/ton OA+water to the compound feed, and that the addition of 0.3 kg/ton OA+water led to lower levels of deoxynivalenol and aflatoxin compared to the control group. Humer et al. (2016) emphasized that the strongest mycotoxin reducing effect of organic acid treatment in soaked feed samples was on Deoxynivalenol and its derivatives compared to control feed samples, and that this effect was related to the duration of soaking and interaction with the organic acid. Besides, the claims of the researchers that the T2 level decreased below the detection limit at all soaking periods following organic acid treatment are congruent with the results of the present study. However, in contrast to the Zearalenone level, which was not affected by organic acid treatment in the present trial, they reported an increase in Zearalenone concentration in feed samples treated with acids for 24 hours, suggesting that this might be due to the release of masked zearalenone by the acid treatments. Nonetheless this suggestion needs to be clarified by further studies. In the present trial, aflatoxin levels in compound feeds supplemented with 0.3 or 0.4 kg/ton OA+water were found to be lower than the control group. Moon et al. (2018) reported that bacterial biocontrol agents might be suppressing aflatoxin production by means of inhibiting the expression of aflatoxin biosynthetic genes or due to increased antimicrobial activity of organic acids with longer aliphatic carbon chains such as butyric acid. The researchers also established that propionic acid completely inhibited the growth of *A. flavus* at a concentration of 0.5%, whereas sorbic acid, which was included in the organic acid mixture in that study, demonstrated two times stronger inhibitory activity compared to propionic acid. Hassan et al. (2012) found that organic acids can inhibit the growth of *A. flavus* strains, but only acetic, formic and lactic acids reduced growth and partially inhibit aflatoxin production. It has been reported that fumonisin and zearalenone, which are among the predominant toxins of fusarium species, usually infect raw materials before or immediately after harvest, while mycotoxin production of *Aspergillus* and *Penicillium* species are generally associated with foods during drying and storage (Qu et al., 2024). On the basis of these results, the positive effect of organic acids in reducing deoxynivalenol, fumonisin B1 and aflatoxin levels can be attributed to the effects of organic acids in suppressing pathogenic bacteria and enhancing the growth of beneficial ones (Pearlin et al., 2020). According to the findings of this study, the levels of Ochratoxin, Fumonisin B2, T-2 and HT-2 were relatively low in contaminated samples. Therefore, the decontamination potential of organic acid treatments on

the said toxins could perhaps be better judged in more heavily contaminated feed samples. Further research is required in this field.

## **5. Conclusion**

Organic acids are added to feeds to prevent mold or fungal growth during the feed production process, to evade decomposition of feeds by pathogenic microorganisms, to delay deterioration of feeds and to extend their storage life. Thus, by reducing bacterial toxins, the nutrient content of feed and animal health can be maintained and the quantity and quality of animal products can be improved. Safe feed raw materials for safe feed production are essential to ensure animal health/welfare and to supply safe animal products fit for human consumption. Mycotoxin formation is a major global problem that threatens animal and human health and causes substantial economic losses in the feed milling industry. Following a general evaluation of the results obtained from this study, it was concluded that 0.4 kg/ton OA+water addition to compound feed significantly reduced feed toxicity. More effective results can be achieved when multiple organic acids are used as blends. These findings suggest that OA blends can serve as an effective strategy for feed preservation and improved animal health. In addition, it was concluded that organic acids can also be effective even when compound feeds are stored for extended periods of time, but the storage ambient temperature should be kept at maximum room temperature on average. Addition of organic acid mixtures have positive effects on feed preservation in the feed storage process. However, the studies conducted on the subject to date are quite limited and new studies are needed. Currently, there is a clear need to deploy natural, environment friendly and safe solutions to reduce mycotoxin contaminations in feed. However, only a limited body of research is available on the efficacy and duration of the inhibitory power of antimicrobial organic acids, and further research is needed to evaluate their effects.

## Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	D.B.	K.B.
C	60	40
D	100	
S	100	
DCP		100
DAI		100
L	100	
W	100	
CR	50	50
SR	100	
PM	60	40
FA	100	

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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