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Article

Dietary Effects of Mycotoxins Adsorbents Mycostop Premium® and Mycostop Duplo® on Piglets Productive Performance and Blood Serum Enzyme Activities

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Abstract: This study aimed to investigate the dietary effects of mycotoxin adsorbents Mycostop Premium® and Mycostop Duplo® on piglets' productive performance and serum enzyme activities. A total of 100 weaned piglets (28 days old) were randomly assigned to five dietary treatment groups (20 piglets in each group) for a duration of 42 days. The dietary treatments included: control group C with no mycotoxin adsorbent; group E1 supplemented with Mycostop Premium® adsorbent in amount of 1kg/t of feed; group E2 supplemented with Mycostop Premium® adsorbent in amount of 2.5kg/t of feed; group E3 supplemented with Mycostop Duplo® adsorbent in amount of 1kg/t of feed; and group E4 supplemented with Mycostop Duplo® adsorbent in amount of 2.5kg/t of feed. All diets were formulated to be isocaloric and isonitrogenous. The results revealed significant improvements in productive performance parameters, including average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR), in piglets fed diets containing mycotoxin adsorbents. Serum enzyme activities were also assessed to gauge the impact of mycotoxin adsorbents on piglets' health. The activities of liver enzymes aspartate transaminase (AST), gamma-glutamyltransferase (GGT), and gut health-related enzymes (amylase and lipase) were evaluated. Piglets consuming diets supplemented with mycotoxin adsorbents exhibited a reduction in the activities of liver enzymes, indicating lower hepatic stress, and an enhancement in the activity of gut health-related enzymes, reflecting improved nutrient digestion and absorption. In conclusion, the inclusion of mycotoxin adsorbents in piglet diets resulted in significant improvements in productive performance and serum enzyme activities.

Keywords: Mycotoxins; piglets; mycotoxin adsorbents; productive performance; serum enzyme activities; swine nutrition; mycotoxin mitigation.

1. Introduction

Mycotoxins in pig production are a significant concern as they can have adverse effects on pig health, performance, and overall economic viability [1,2]. Mycotoxins are toxic secondary metabolites produced by molds (fungi), and they can contaminate various components of pig diets, including grains, forages, and feed ingredients [3]. Mycotoxin contamination can occur in the field during crop growth, during storage, or in the feed manufacturing process. Common mycotoxins found in pig feed include aflatoxins, deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A, fumonisins, and others. These mycotoxins are produced by molds such as *Aspergillus*, *Fusarium*, and *Penicillium* [4].

Mycotoxins can have a range of negative health effects on pigs, including gastrointestinal disorders, immunosuppression, and organ damage [5]. Some mycotoxins, like aflatoxins, are carcinogenic and can lead to long-term health issues. Mycotoxin-contaminated feed can lead to reduced growth rates, decreased feed intake, and poor feed conversion efficiency in pigs. Pigs exposed to mycotoxins may exhibit reduced weight gain and increased susceptibility to diseases [6]. Some mycotoxins, such as ZEN, can lead to reproductive problems in sows. This may include infertility, embryonic death, and the development of abnormal or stillborn piglets [7].

Mycotoxins can suppress the pig's immune system, making them more susceptible to infections and diseases. This can result in increased veterinary and medication costs. Mycotoxin contamination can result in significant economic losses for pig producers due to reduced productivity, increased veterinary costs, and increased mortality rates [1].

To manage mycotoxin risk in pig production, several strategies can be employed such as frequent testing of feed ingredients and finished feed for mycotoxin contamination to identify and manage contaminated batches, as well as usage of mycotoxin binders and adsorbents designed to bind mycotoxins in the gastrointestinal tract, preventing their absorption and reducing their harmful effects [4]. Various countries have established regulations and guidelines regarding acceptable levels of mycotoxins in animal feed [8]. Pig producers need to comply with these regulations to ensure animal and food safety [9].

Mycotoxin adsorbents are added to piglet diets to bind mycotoxins and prevent their absorption in the gastrointestinal tract [10]. By reducing mycotoxin exposure, these adsorbents can lead to improved growth performance in piglets [11]. This improvement is manifested through increased average daily gain (ADG) and better feed conversion ratios (FCR). Piglets on mycotoxin adsorbent-supplemented diets tend to grow faster and more efficiently, resulting in higher body weights and overall production performance [12]. Having in mind that mycotoxins can cause feed refusal or reduced feed intake in piglets due to their unpalatable and toxic nature, mycotoxin adsorbents help make the feed more palatable and safer, which can encourage piglets to consume their full feed allowance [13]. This increased feed intake is essential for optimal growth and development. Mycotoxin adsorbents can alleviate this immunosuppressive effect by reducing mycotoxin-related stress on the immune system. This helps piglets maintain a healthier immune response, reducing the risk of illness and the need for antibiotic treatments. By binding mycotoxins and preventing their harmful effects on the intestinal lining, these adsorbents contribute to a healthier and more functional digestive system [14]. This, in turn, improves nutrient absorption, leading to better growth and development. In addition to improving piglet growth, mycotoxin adsorbents can also play a role in reducing reproductive issues in sows [15]. By preventing mycotoxin-related reproductive problems, such as infertility and abnormal piglet development, these adsorbents contribute to the long-term sustainability of the pig production system.

Besides mycotoxin binders, antibiotics, on the other hand, are substances used to treat bacterial infections [16], and they have no direct effect on mycotoxins. However, there are some indirect ways in which antibiotics can indirectly impact mycotoxin contamination and exposure, because they are known to alter the composition and balance of the gut microbiota [17]. This can have downstream effects on the host's immune system and metabolism, potentially affecting how the body processes mycotoxins. Some research suggests that a healthy gut microbiome may help detoxify mycotoxins and reduce their harmful effects [18], so changes in the microbiota due to antibiotic use could theoretically impact this detoxification process [19].

Mycotoxin adsorbents are valuable tools in piglet production, as they effectively reduce the negative impact of mycotoxin contamination on growth, health, and overall performance. By improving growth, immune system function, gut health, and reducing health costs, mycotoxin adsorbents contribute to the well-being of piglets and the profitability of pig production operations [20].

Mycotoxin contamination in pig production is a significant challenge that can have adverse effects on pig health and productivity. Proactive management and prevention strategies, including proper testing, mycotoxin binders, and good agricultural and storage practices, are crucial for mitigating the risks associated with mycotoxins in pig production.

2. Materials and Methods

Pigs, feeding, and experimental design

Animal welfare and animal protection principles were followed throughout the experimental protocol, which was approved by the University Ethics Committee (EC22/08-123). A total of 100 weaned piglets (28 days old) were randomly assigned to five dietary treatment groups (20 piglets in each group) for a duration of 42 days.

The experimental design is presented in Table 1.

Table 1. Experimental design with dietary mycotoxin adsorbents supplemented to piglets.

Mycotoxin adsorbent (kg/t of feed)	**Control (C)**	**Experimental group 1 (E1)**	**Experimental group 2 (E2)**	**Experimental group 3 (E3)**	**Experimental group 4 (E4)**
Mycostop Premium®	0	1.0	2.5	0	0
Mycostop Duplo®	0	0	0	1.0	2.5

Mycotoxin adsorbents are added on top of a basic diet in all experimental treatments.

All piglets in the experiment were provided with *ad libitum* access to feed and drinking water. Also, the unconsumed feed mixture was monitored. All diets were formulated to be isocaloric and isonitrogenous (Table 2). Concentrations of mycotoxins in the complete feed samples used in piglets' diet (μg/kg of feed), in the control and experimental groups are presented in Table 3.

Table 2. Analyzed nutrient composition of experimental diets (as-fed basis).

Nutrient levels	%
Crude protein	20.0
Crude fat	6.50
Crude fibre	3.0
Lysine	1.52
Methionine	0.47
Threonine	0.91
Tryptophan	0.23
Calcium	0.86
Total phosphorus	0.63
Sodium chloride	0.30
Digestible energy, MJ/kg	15.0

Digestible energy is calculated value. Other nutrient levels in the table are analyzed values.

Table 3. Concentrations of mycotoxins in the complete feed samples used in piglets' diet (μg/kg).

Mycotoxin	**Mean value**	**EC permitted/guidance level** [2]
Aflatoxin	19.8	20.0
Zearalenone	2892.3	2000.0
Ochratoxin	244.6	250.0
Fumonisin	72748.1	60000

Piglets were weighed on the 1st, 21st, and 42nd day of the experiment. The average daily feed intake and feed conversion were monitored for the respective experimental periods of piglet's weight control. The mortality of piglets was recorded daily.

Blood samples from five animals from each group were collected during the last day of an experiment via the jugular vein into EDTA tubes and 10 mL heparinized vacuum tubes, then aliquots were centrifuged at 3000 × g for 10 min to collect plasma, which was then frozen at -20°C until analysis.

Blood samples analysis

Blood samples were collected to determine aspartate transaminase (AST), and gamma-glutamyltransferase (GGT). Spectrometric analyses were performed by a biochemical analyzer (Thermo Fisher Scientific) and commercially available reagents.

After blood samples preparation, assay reagents, standard curve, sample assay, enzymatic reaction, and termination reactions, a spectrophotometer was used to determine amylase and lipase activity, measuring the absorbance of each sample and standard at 540 nm (amylase) and 405 nm (lipase).

Statistical analysis

The one-way ANOVA analysis was performed to assess data differences between various groups using Statistica software version 13 (StatSoft Inc. 2013; USA). The data means were considered different at $p<0.05$.

3. Results and Discussion

Feed efficiency during the experiment

The average scores of feed intake, conversion, daily weight gain, and mortality were compared to evaluate feed efficiency. During all monitored periods (1st day, 21st day, 42nd day), statistically significant differences ($p<0.05$) in efficiency between the control and experimental groups were observed. All results of piglets' efficiency traits are presented in Table 4.

From the results shown in Table 4, it can be seen that the piglets entered in experiment with uniform body weights between 7.26 and 7.42 kg, without statistically significant differences ($p>0.05$). Further, at the end of the first period of the experiment dietary addition of both mycotoxin adsorbents in both concentrations showed significant differences ($p<0.05$) in piglets' body weight when compared with the control group, as well as between experimental groups. Piglets in group E4 achieved a statistically significant ($p<0.05$) higher body weight of 16.60 kg in comparison to other groups. The same tendency of mycotoxin adsorbents has been recorded at the end of the experiment. The highest body weight of piglets was recorded in group E4 (27.38 kg) supplemented with Mycostop Duplo® in the concentration of 2.5 kg/t, following the groups E3 (26.08 kg), E2 (25.83 kg), E1 (25.03 kg), with statistically significant differences ($p<0.05$). Between groups E3 and E2 supplemented with Mycostop Duplo® and Mycostop Premium® in the concentration of 1.0 and 2.5 kg/t, significant numerical or statistical differences were not recorded ($p>0.05$). The lowest effects were recorded in treatment E1 with the supplementation of the adsorbent Mycostop Premium® in the concentration of 1.0 kg/t, but with statistically significant differences ($p<0.05$) in comparison to a control group without any addition of mycotoxin adsorbents (Table 4).

The results obtained in our study confirm and support previous studies that feed contaminated with mycotoxins is toxic to weaning pigs and poor growth is the first and most consistent sign of mycotoxin intoxication [21,22]. The addition of adsorbents to mycotoxin-contaminated diets restores the productive performance to that of mycotoxins non-contaminated diets [23].

The addition of both mycotoxin adsorbents recorded statistically significant differences ($p<0.05$) in both experimental periods, and overall results regarding average daily gain and average daily feed intake, compared between the experimental groups and with control group, respectively. The highest overall ADG was recorded in group E4 (0.48 kg) following E3 (0.45 kg), E2 (0.44 kg), and E1 (0.42 kg), while significantly lowest ADG for the overall experimental period was recorded in the control group C (0.39 kg) of piglets. Groups E1, E2, and E3 didn't show any statistically significant differences between themselves ($p>0.05$) when it comes to ADFI. The highest ADFI was recorded in group E4, and the lowest in group C, with statistically significant differences ($p<0.05$). The present results are also consistent with the results of other researchers [24–26], who found that ADG was lower when piglets were fed a diet contaminated with mycotoxins, and the addition of adsorbents resulted in the recovery of the growth loss for more than 60% [27]. Decreased feed consumption is often observed in piglets fed mycotoxins contaminated diets, and supplementation of adsorbents to the diet has been shown to improve feed intake [4].

The lowest overall achieved feed conversion ratio was recorded in group E4 (1.45 kg/kg), following the rest experimental groups, with the highest FCR in the control group (1.50 kg/kg). Differences in FCR for both experimental periods, as well as the overall experiment, were statistically significant ($p<0.05$). Mycotoxin contamination of piglet feed often results in poor feed conversion efficiency [28]. Following other research, our study also shows a poor feed efficiency for the mycotoxins-contaminated diet, and the inclusions of 1.0 kg/t and 2.5 kg/t of Mycostop Duplo® and Mycostop Premium® in the mycotoxins-contaminated diet increased the feed conversion efficiency to that of the control group. The improvement of feed conversion efficiency through the inclusion of Mycostop Duplo® and Mycostop Premium® in the contaminated diet with mycotoxins in our study indicates that these adsorbents have an impact on the adverse effects of mycotoxins.

Table 4. Performances of piglets fed contaminated feed with two levels of mycotoxin adsorbents.

Measures	Groups					p-value
	C	E1	E2	E3	E4	
Body weight (BW), kg (x±SD)						
1st day	7.36±0.32^{a}	7.42±0.31^{a}	7.38±0.32^{a}	7.26±0.31^{a}	7.27±0.27^{a}	0.993
21st day	13.78±0.69^{d}	14.49±0.59^{c}	15.27±0.51^{b}	15.25±0.46^{b}	16.60±0.78^{a}	0.021
42nd day	23.59±0.63^{d}	25.03±0.69^{c}	25.83±0.69^{b}	26.08±0.74^{b}	27.38±0.48^{a}	0.034
Average daily gain (ADG), kg (x±SD)						
21st day	0.31±0.04^{d}	0.34±0.03^{c}	0.38±0.02^{b}	0.38±0.03^{b}	0.44±0.04^{a}	0.032
42nd day	0.47±0.04^{d}	0.50±0.05^{c}	0.51±0.05^{b}	0.51±0.04^{b}	0.52±0.05^{a}	0.046
Overall	0.39±0.02^{d}	0.42±0.02^{c}	0.44±0.02^{b}	0.45±0.02^{b}	0.48±0.01^{a}	0.041
Average daily feed intake (ADFI), kg (x±SD)						
21st day	0.54±0.04^{a}	0.52±0.05^{a}	0.51±0.04^{b}	0.52±0.05^{a}	0.54±0.05^{a}	0.010
42nd day	0.60±0.06^{c}	0.72±0.04^{b}	0.79±0.04^{b}	0.79±0.04^{b}	0.86±0.06^{a}	0.038
Overall	0.57±0.04^{c}	0.62±0.03^{b}	0.65±0.03^{b}	0.65±0.03^{b}	0.70±0.04^{a}	0.022
Feed conversion ratio (FCR), kg of feed/kg of gain (x±SD)						
21st day	1.82±0.31^{a}	1.55±0.19^{b}	1.37±0.12^{c}	1.37±0.17^{c}	1.22±0.17^{d}	0.047
42nd day	1.30±0.21^{a}	1.44±0.16^{b}	1.59±0.18^{b}	1.54±0.15^{c}	1.70±0.18^{d}	0.011
Overall	1.50±0.13^{a}	1.49±0.10^{b}	1.48±0.09^{b}	1.47±0.08^{c}	1.45±0.07^{d}	0.029

x-mean value; SD-standard deviation; The different lowercase letters indicate a statistically significant difference ($p<0.05$).

Biochemical parameters

The activities of liver enzymes aspartate transaminase and gamma-glutamyltransferase in piglets' blood at the end of the experiment are shown in Figure 1.

Results show significant ($p<0.05$) decreases in the mean serum activities of AST, and GGT enzymes in piglets fed the diet supplemented with Mycostop Duplo® and Mycostop Premium® compared to the control diet at the end of the experiment. The highest AST and GGT values were recorded in the control group (54.8, and 30.1 IU/L) with significant differences ($p<0.05$) in comparison to decreased values of all experimental groups. Generally, an increase in serum enzyme activities, such as AST, and GGT are indicators of inflammation or damage of the hepatic cells or biliary tract cells [29,30]. An increase in liver-specific serum enzyme activities may suggest that mycotoxins can cause morphologic changes in the liver of pigs that have ingested mycotoxins-contaminated feed [31,32].

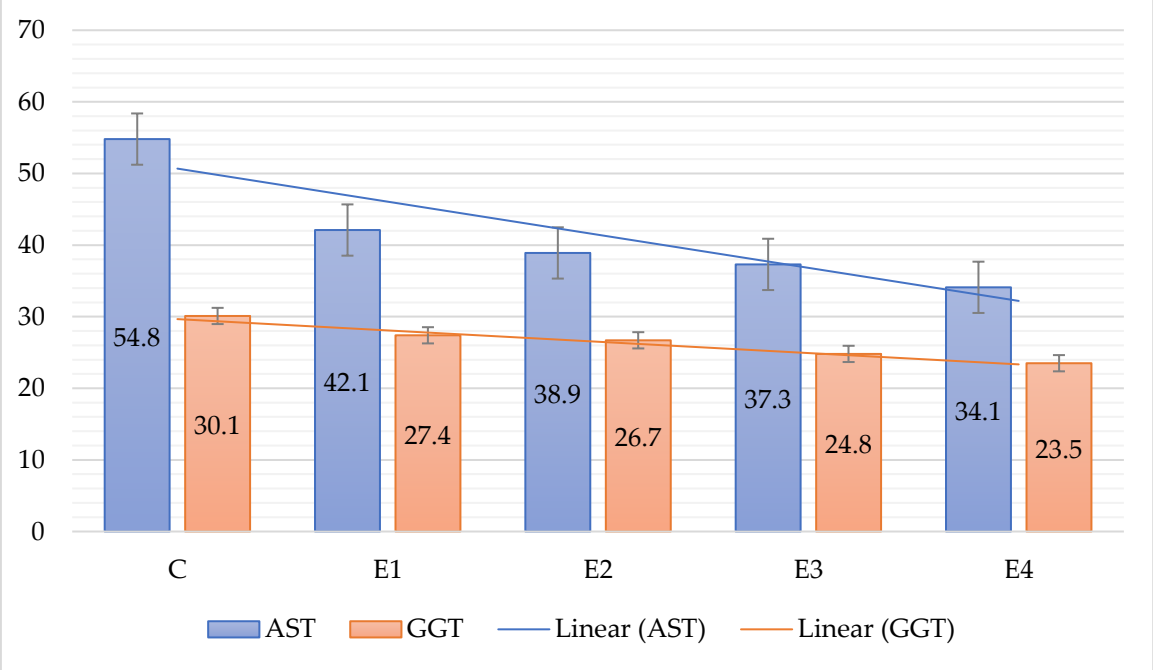

Figure 1. The activities of liver enzymes AST and GGT in piglets' blood on the 42nd day of the experiment (IU/L).

Results regarding the activities of amylase and lipase seen in Figure 2, show a similar tendency as AST, and GGT activity. Dietary addition of Mycostop Duplo® and Mycostop Premium® in our study in all experimental groups has significantly ($p<0.05$) decreased the activity of amylase and lipase. Synthesis of digestive enzymes in the pancreas is deficient during the beginning of the growing period of piglets and increases over time when the growing rate of the pancreas reaches its maximum [33,34]. However, as the piglet grows, its intestine and pancreas mature regarding the production of amylase and lipase, which are deficient at the beginning of the piglet's life. So, as the pigs become older, the digestibility of lipids and carbohydrates becomes better, consequently reducing the stimulus for pancreatic secretion of amylase and lipase. The highest activity of amylase and lipase in our experiment was recorded in control group C (5311, and 4998 UI/mg protein), with a linear decrease towards group E4 (2668, and 2150 UI/mg protein). The effects of mycotoxins on the activity of digestive enzymes are little studied and sometimes controversial [35].

Different studies have reported an increase in the activity of pancreatic α-amylase, lipase, trypsin, and chymotrypsin across several poultry species such as broilers, breeder hens, and ducks after exposure to different mycotoxins levels [36]. Several researchers suggested that the increased activities of α -amylase and lipase were abnormal and pathologic, which may be ascribed to increased proenzyme released from the injured pancreas [37,38]. The results also suggested that the effects of mycotoxins on pancreatic enzymes could be concentration- and time-dependent [37].

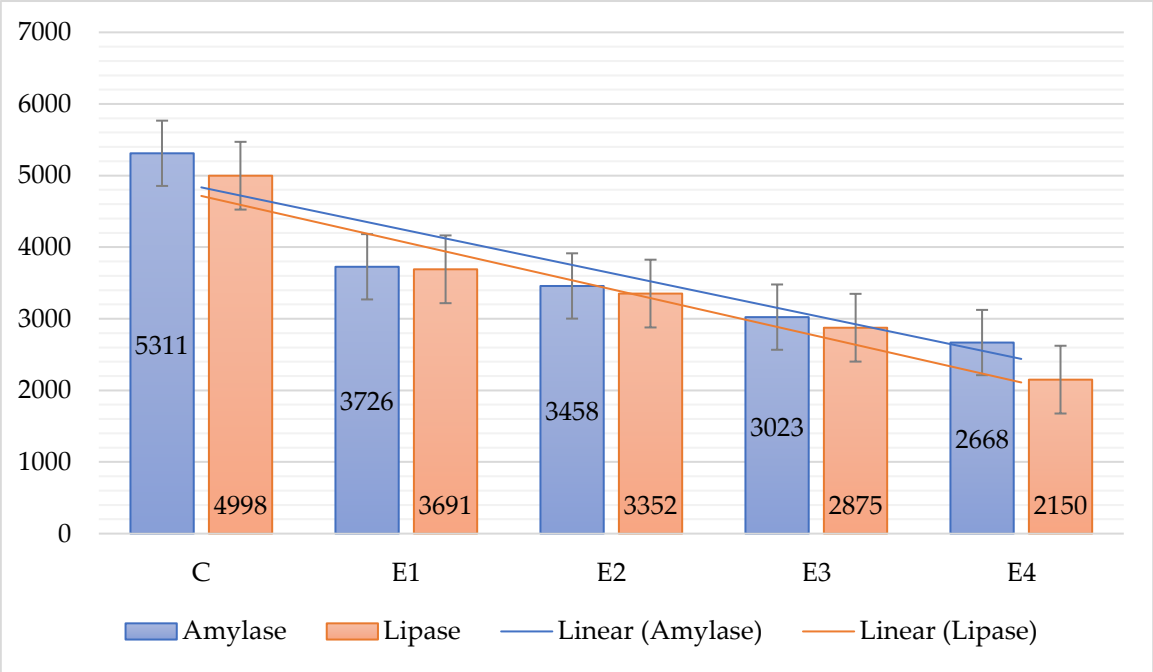

Figure 2. The activities of amylase and lipase on the 42nd day of the experiment (UI/mg protein).

4. Conclusions

In conclusion, the inclusion of mycotoxin adsorbents Mycostop Duplo® and Mycostop Premium® in piglet diets in concentrations of 1.0, and 2.5 kg/t resulted in significant improvements in productive performance and serum enzyme activities. The results of this study demonstrate that the presence of mycotoxins in the diet leads to impaired piglets' performance. In addition, feed contaminated with mycotoxins promotes an increase in the activity of endogenous enzymes in the pancreas, while pancreatic enzymes play a crucial role in the digestion and absorption of ingested macromolecules. Although mycotoxins increase digestive enzymes, it does not result in better performance, indicating that nutrient absorption, rather than digestion, is affected by its presence in the diet.

Our findings underscore the potential of mycotoxin adsorbents as effective tools in safeguarding piglet health and growth when faced with mycotoxin-contaminated feedstuffs.

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Conflicts of Interest: The authors declare no conflict of interest.

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