

# Zootechnical, bacteriological, and histometrical effects of a combination mycotoxin binder-acidifier in broiler chickens

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## Abstract

Mycotoxins are present in food and cannot be eliminated even by using modern technological processes in food manufacture; they can cause pathologies and economic losses in the poultry industry. To combat their effects, this study aimed to test the incorporation of a mycotoxin binder (MICOTEC 0.1%) associated with an acidifier (TECAVIAR 0.1%), both marketed in Algeria, in the feed distributed to broilers. During this study, a beneficial effect was found in subjects consuming the supplemented food compared to the control subjects. In addition to the significant decrease in mortality (51.47%), test subjects showed an average weight of 3308.1 g, which was significantly higher than control subjects (2876.25 g). The data revealed

a significant reduction in the number of total and faecal coliforms, with prevalence ranging from 3.1 to 36.5% vs 63.2 to 96.9% for the control group. An increase in the length of the small intestine (24.13 cm), increase in the weight of the Fabricius bursa (FB; 1.25 g), weight of the gizzard (10.25 g) and finally an increase in the weight of the wishbone (153.87 g) were recorded in the test subjects. The results indicate that the association of mycotoxin binder-acidifier in broiler chickens can improve growth performance. Further experiments are required to confirm more effects (with different doses, different strains, and other organs).

**Key words:** *mycotoxin; binder; acidifier; coliforms; histometry*

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## Introduction

Mycotoxins are fungal metabolites commonly found in foods that pose a health risk to the consumer (Liu et al., 2020; Székács, 2021). The most belong to the genera *Aspergillus*, *Penicillium*, and *Fusarium* (Petruzzi et al., 2014; Guerre, 2020). They can lead to carcinogenicity, immune toxicity, teratogenicity, neurotoxicity, nephrotoxicity, and hepatotoxicity (Jard et al., 2011).

Maximum permitted levels for major mycotoxins in food have been established worldwide. Good agricultural practices, plant disease management and proper storage conditions limit the levels of mycotoxins in the food chain, but do not completely eliminate them because in practice, all the ideal conditions are not always met (climatic hazards, choice of raw materials (Manafi and Khosravinia, 2012; Balqees et al., 2020). Besides, even at lower doses, they can lead to cumulative impact resulting in chronic health effects (Tola et al., 2016).

Studies have shown that food quality and labelling influence the occurrence of certain diseases such as coccidiosis, *E. coli* infections, enteritis, and mycotoxicosis (Yunus et al., 2009).

Poultry farming is the fastest growing agricultural sub-sector, especially in developing countries. The global poultry sector is expected to continue to grow as demand for meat and eggs is driven by growing populations, rising incomes and urbanisation (Mottet and Tempio, 2017). In Algeria, the sector is expanding rapidly, particularly following the national economic policies aiming at developing this sector. As a result, alternative avenues have been explored, in this case improving the quality of feed to allow a "natural" improvement in the health and welfare of the chickens without the use of antibiotics (Heidari et al., 2018).

The inclusion of binding agents in food has received considerable attention

as a strategy to reduce dietary exposure to mycotoxins. These adsorbents have high binding capacities in vitro, have been further tested in animals, and some adsorbents have been shown to be suitable for mitigating the toxic effects of specific mycotoxins (Pappas et al., 2014; Agboola et al., 2015)

The aim of this study was to improve the health status of broilers by improving the quality of feed by adding a mycotoxin binder and an acidifier, and to determine the effects of these products on zootechnical parameters. A bacteriological study and the weighing of certain organs were also performed.

## Materials and methods

### Animals and livestock

The study was conducted on a private poultry farm, in Boutlilis, in the ORAN region in the coastal zone, 415 km west of the capital Algiers. It took place from 30 December 2019 to 12 February 2020.

Chicks were reared in a poultry greenhouse (48 x 10 m), with a surface area of 480 m<sup>2</sup> containing a 32 m<sup>2</sup> SAS also used for food storage, the building is of the dark type which is cleaned and disinfected followed by a sanitary vacuum every 45 days. Ventilation is dynamic and ensured with pad cooling (4 x 1.5 m), with three extractors.

In total, 1280 day-old chicks of the Cobb500 strain (male and female) from the same hatchery were weighed and divided into two groups ( $n=640$ ) of equal weight, and then further into four subgroups to comprise eight replicates of 80 birds each (density of 6.4 birds/square meter).

The first group (control group) was fed with the standard basic feed adapted for each rearing phase: starter feed distributed from day 1 (d1) to d20

**Table 1.** Prophylaxis Plan

| Age (days) | Vaccination and treatment   |
|------------|---|
| 1          | Anti-stress for 5 days  |
| 3          | Vaccination against Newcastle disease and infectious bronchitis (B1-H120)             |
| 7          | AD <sub>3</sub> E Vitamins  |
| 9          | Vaccination against infectious bronchitis (IB mass)                                   |
| 14         | Vaccination against Gumboro IBD+vit E for 4 days                                      |
| 18         | Anticoccidial treatment for 48 hours  |
| 19         | Newcastle booster vaccination   |
| 23         | Gumboro disease booster vaccination IBD   |
| 30         | Booster vaccination against Newcastle disease and infectious bronchitis (HB1)         |
| 32         | Anticoccidial treatment for 48 hours  |
| 35         | Antibiotic treatment (doxycycline 300 g/1000 L) + (colistin 250 mL/1000 L) for 4 days |

**Table 2.** Mycotoxin binder composition (Micotec, 0.1%)

| COMPOSITION  |                           |
|--|---------------------------|
| E-282  | Calcium propionate        |
| E-281  | Sodium propionate         |
| E-238  | Calcium formate           |
| E-237  | Sodium formate            |
| E-202  | Potassium sorbate         |
| E-562  | Sepiolite                 |
| 1M558i   | Bentonite montmorillonite |
| E551c  | Kieselgur                 |
| Yeast cell wall extract MOS and [1.3] [1.6] Beta glucans |                           |
| E-321  | BHT                       |
| E-320  | BHA                       |
| E-310  | Propyl gallate            |
| E-333  | Calcium citrate           |

(metabolizable energy: 2930 kcal/kg; crude protein: 21.63%); growth feed from d21 to d39 (metabolizable energy: 2941 kcal/kg; crude protein: 20.83%), and finishing feed from d40 to d44 (metabolizable energy: 2958 kcal/kg; crude protein: 9.01%).

The second group received the same formula of feed for each phase with the same ingredients, with the addition

of a mycotoxin binder (MICOTEC, 0.1%; SARL Adicales, Oran, Algeria) and an organic acidifier (TECAVIAR PROMOTER, 0.1%; SARL Adicales, Oran, Algeria) added to the feed.

Throughout the trial, chickens were fed and watered ad libitum and were kept in the same building to ensure uniform rearing conditions.

## Breeding health programme

The sanitary protocol followed in our farm is presented in Table 1. It should be noted that all vaccinations are administered per os in the drinking water.

## Treatments administrated

### Mycotoxin binder: MICOTEC (0.1%)

MICOTEC is a mycotoxin sequestrant designed for all species that combines two actions, sequestering the toxins in the animal's body and as a powerful antifungal in the raw material (Table 2).

*Description:* Mycotoxin sequestrant, antifungal, and antibacterial

At the food level: A combination of propionic acid salts effectively combat aspergillus fungus which causes the formation of aflatoxins

In the body: The combination of propionic acid salts + sepiolite + bentonite + yeast cell walls allow a decrease in the rate of mycotoxins in food. MICOTEC's adsorption technique consists of trapping mycotoxins by steric and polar attraction to reduce their bioavailability during digestion in the intestinal tract and before passing into the bloodstream.

Organic acidifier: TECAVIAR PROMOTER (0.1%) in the feed.

*Description and composition:* Natural growth promoter that combines butyric, organic and inorganic acids in salt and free acid form.

## Mechanism of action

It works by acidifying the digestive tract, preventing the proliferation of salmonella, causing a sanitary effect in the animal, also keeps the pH low in the pro ventricle in birds, promoting efficient protein digestion; it stimulates the secretion of pancreatic enzymes, amylase, lipase and protease, as well as hormones that improve digestion; the formula contains sodium butyrate salt that acts as a powerful inhibitor of pathogenic microorganisms, decreasing

their intracellular pH, causing a decrease in the loss of energy to recover the osmotic balance.

## Effects

High productivity in zootechnical indexes; sodium butyrate gives the animal extra energy; better absorption of nutrients by the intestinal mucosa; better intestinal digestibility; bactericidal action in the animal body.

## Parameters measured

In addition to mortality and weight monitoring, two major parameters were studied during this trial: bacteriological and histometric study.

## Mortality and weight

Weighing was done for each rearing phase and all mortalities were also noted.

## Bacteriological study

A total of 16 subjects were randomly recovered from each group (experimental and control) on d20, d39 and d44. Autopsy was conducted at the laboratory of avian pathology, National High School of Veterinary Medicine (NHSVM, Algiers, Algeria) and samples of matrix (liver and intestine) were taken and sent to the bacteriology laboratory of the NHSVM for bacteriological examinations relating to the research and the isolation of *E. coli* and *Salmonella*. Samples were taken from 16 livers of chickens (8+8) sacrificed by bleeding and performed by swabbing these organs (Brugere-Picoux, 1992; Leconet, 1992).

## Histometric study of the intestine and organs development

Histometric studies of the intestine were carried out on d20 (end of start-up), d39 (end of growth) and d44 (finishing) on eight control and eight experimental chickens (1 subject per subgroup having a weight representative of its batch).

**Table 3.** Weight evolution (in grams) by group and by phase

| Control | start-up | growth  | finishing | Experimental | start-up | growth  | finishing |
|---------|----------|---------|-----------|--------------|----------|---------|-----------|
| c 1     | 946.50   | 2670    | 2935      | exp 1        | 1042.10  | 2765    | 3257.50   |
| c 2     | 933.85   | 2592.50 | 2827.50   | exp 2        | 1023.25  | 2735    | 3210      |
| c 3     | 947.05   | 2667.50 | 2780      | exp 3        | 1021.20  | 2767.50 | 3330      |
| c 4     | 939.90   | 2510    | 2805      | exp 4        | 1051.15  | 2865    | 3302.50   |
| c 5     | 935.10   | 2600    | 2800      | exp 5        | 1068.40  | 2882.50 | 3460      |
| c 6     | 959.15   | 2720    | 2902.50   | exp 6        | 1075.95  | 2860    | 3490      |
| c 7     | 953.75   | 2665    | 2872.50   | exp 7        | 1068.90  | 2830    | 3325      |
| c 8     | 972.65   | 2672.50 | 3087.50   | exp 8        | 1042.05  | 2635    | 3090      |

These chickens were weighed and then sacrificed by bleeding. The entire intestine (from the junction with the gizzard to the colon) and the two caeca (detached at the ileo-caecal junction) were juxtaposed and the total length measured.

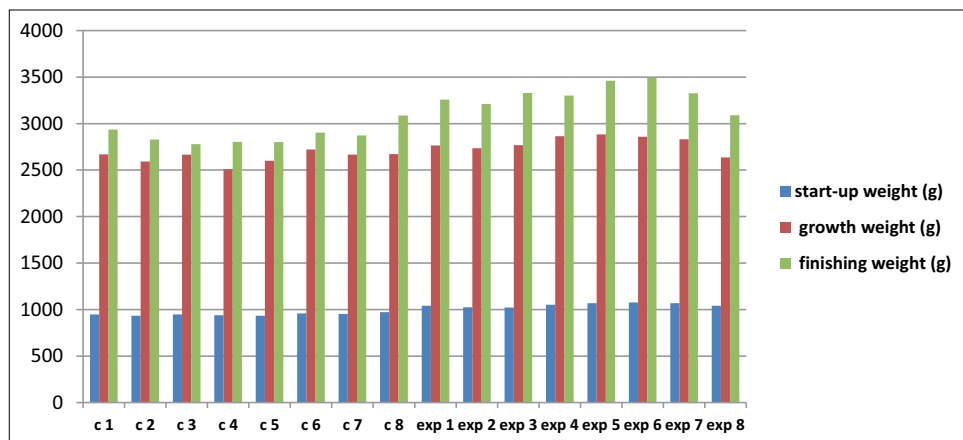
The weights of the carcass, wishbone and Fabricius bursa (FB) of sacrificed animals were also weighed at the end of each rearing period; the weight of the empty gizzard and the weight of the proventricle were also recorded.

From the beginning of the trial, at the end of the start-up period, at the end of the growth period and at the end of

the finishing period, a representative sample of live chickens (25%) from each group was weighed, and the statistical data was analysed. All experiments were performed in compliance with the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

### Statistical study

For the statistical analysis of results, the Chi-square test of independence was performed using SPSP statistics software, with a significance level *P* set at 5%.



**Figure 1.** Weight evolution by phase

## Results

### Weight evolution

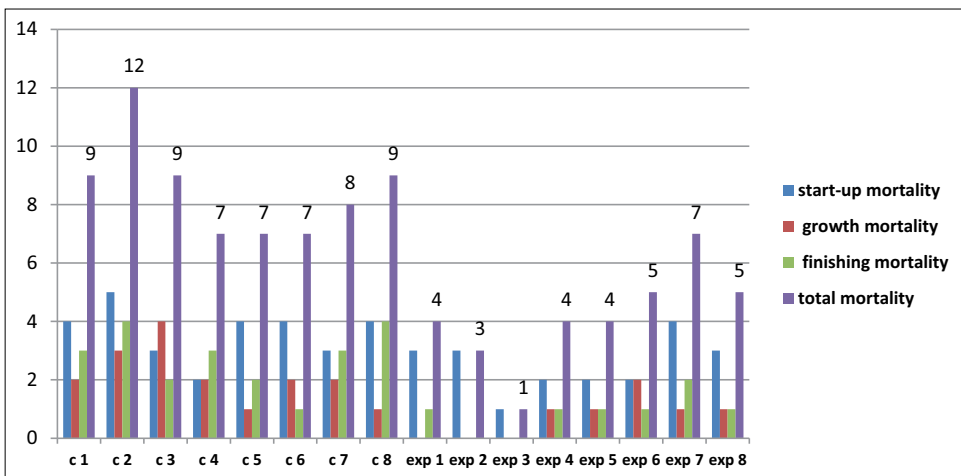
At the end of each phase, we weighed 20 subjects in each group (control and experimental) at random, and we obtained the recorded averages (table 3). It was observed that the weight averages for all subjects in the control group ranged from 933.85 to 972.65 g in starting,

2510 and 2720 g in growth, and 2780 and 3087.5 g in finishing. These values are largely inferior to those recorded for the experimental group; with average weights recorded ranging from 1021.20 and 1075.95 g in starting, 2635 and 2882.5 g in growth, and 3090 g and 3460 g in finishing (Figure 1).

This difference in favour of the experimental group was confirmed by the

**Table 4.** Comparison of average weights between control and experimental groups

| Control          |        |          |         | Experimental |        |         |         |
|------------------|--------|----------|---------|--------------|--------|---------|---------|
| <b>Start up</b>  |        |          |         |              |        |         |         |
| Max              | 972.65 | 948.49   | average | Max          | 1076   | 1049.10 | average |
| Min              | 933.85 | 13.08    | gap     | Min          | 1021.2 | 20.82   | gap     |
| Mod              | 38.8   | 171.21   | var     | Mod          | 54.75  | 433.57  | var     |
| <b>Growth</b>    |        |          |         |              |        |         |         |
| Max              | 2800   | 2518.75  | average | Max          | 2900   | 2631.30 | average |
| Min              | 2250   | 198.09   | gap     | Min          | 2250   | 217.02  | gap     |
| Mod              | 550    | 39241.10 | var     | Mod          | 650    | 47098   | var     |
| <b>Finishing</b> |        |          |         |              |        |         |         |
| Max              | 3087.5 | 2876.25  | average | Max          | 3490   | 3308.10 | average |
| Min              | 2780   | 100.93   | gap     | Min          | 3210   | 129.12  | gap     |
| Mod              | 307.5  | 10187.50 | var     | Mod          | 280    | 16673   | var     |



**Figure 2.** Mortality evolution by phase

variance and deviation test (Table 4); this group obtained better weight averages during the three phases of the rearing cycle than in the control group (1049.1 g, 2631.3 g, and 3308.1 g vs 948.494 g, 2518.75 g, and 2876.25 g).

### Mortality evolution

At the end of each phase, we counted the number of dead subjects. A difference in the totals was observed, with the control group recording more deaths during all phases (Figure 2).

At the end of the rearing period, the number of deaths was 68 subjects in the control group and 33 in the experimental group, for a difference of 35 subjects, thus giving an improvement of 50.47%.

## Bacteriological results

### Intestinal counts

For each phase of the breeding cycle, we prepared a separate summary table. Significantly higher total and faecal intestinal coliform counts were found in the control group, with significant differences (Tables 6 and 7) during the growing and finishing phases and a highly significant difference in the starter phase (Table 5).

These values show the beneficial effect of the mycotoxin binder and acidifier on the level of intestinal coliforms in broilers; they reduce the harmful bacteria to lower values than those found in the control group.

**Table 5.** Intestinal counts in the start-up phase

| Organ     | Bacteria         | Group   | Number    | Prevalence (%) | P     |
|-----------|------------------|---------|-----------|----------------|-------|
| Intestine | Total coliforms  | EXP     | 1090909.1 | 10.08          | 0.001 |
|           |                  | Control | 9727272.7 | 89.91          |       |
|           | Faecal Coliforms | EXP     | 927272.73 | 4.99           |       |
|           |                  | Control | 50909091  | 95.01          |       |

**Table 6.** Intestinal counts in the growth phase

| Organ     | Bacteria         | Group   | Number     | Prevalence (%) | P      |
|-----------|------------------|---------|------------|----------------|--------|
| Intestine | Total coliforms  | EXP     | 5590006.09 | 36.5           | 0.0027 |
|           |                  | Control | 9723572.73 | 63.49          |        |
|           | Faecal Coliforms | EXP     | 1927272.73 | 3.07           |        |
|           |                  | Control | 60907090.9 | 96.93          |        |

**Table 7.** Intestinal counts in the finishing phase

| Organ     | Bacteria         | Group   | Number     | Prevalence (%) | P       |
|-----------|------------------|---------|------------|----------------|---------|
| Intestine | Total coliforms  | EXP     | 4573477.02 | 34.78          | 0.00097 |
|           |                  | Control | 8577337.03 | 65.22          |         |
|           | Faecal Coliforms | EXP     | 4007272.73 | 33.63          |         |
|           |                  | Control | 7909090.97 | 66.37          |         |

**Bacteriological analysis of the liver**

Testing for bacteria in the liver gave the following results (Tables 8, 9, 10).

**Table 8.** Results of *E. coli* and *salmonella* analysis in the liver (start-up phase).

| <i>E. coli</i> |     | <i>Salmonella</i> |     |
|----------------|-----|-------------------|-----|
| control        | Exp | control           | Exp |
| +              | -   | +                 | -   |
| +              | -   | +                 | -   |
| +              | +   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | +   | -                 | -   |
| +              | -   | -                 | -   |
| 100%           | 25% | 25%               | 0%  |

The chi-square test showed a statistically significant difference ( $P=0.0019$ ) between the liver positivity for *E. coli* between the experimental and control groups. The  $P$ -value of Chi-square independence ( $P=0.1306$ ) showed that there was not a significant difference between the positivity of the control and experimental groups concerning the presence of *Salmonella* in the liver.

**Table 9.** Results of *E. coli* and *Salmonella* in the liver (growth phase).

| <i>E. coli</i> |      | <i>Salmonella</i> |     |
|----------------|------|-------------------|-----|
| control        | Exp  | control           | Exp |
| +              | +    | -                 | -   |
| +              | +    | -                 | +   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| +              | +    | -                 | -   |
| 100%           | 100% | 0%                | 0%  |

No difference between the two groups.

**Table 10.** Results of *E. coli* and *Salmonella* in the liver (finishing phase).

| <i>E. coli</i> |     | <i>Salmonella</i> |     |
|----------------|-----|-------------------|-----|
| control        | Exp | control           | Exp |
| -              | +   | -                 | -   |
| +              | +   | -                 | -   |
| +              | +   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | -   | -                 | -   |
| +              | +   | -                 | -   |
| -              | +   | -                 | -   |
| 75%            | 62% | 0%                | 0%  |

The  $P$ -value of Chi-square of independence ( $P=0.1604$ ) indicates that there is no significant difference between the positivity of the control and the experimental group concerning *E. coli* carriage in the liver. Data revealed the absence of *Salmonella* in both groups.

**Antibiogram**

We carried out an antibiogram for the 16 (as mentioned in the bacteriological study) samples of the two groups (Table 11). It was noted that the bacteria found in the control group are more resistant to all antibiotics (ATB) than those found in the experimental group (figure 3).

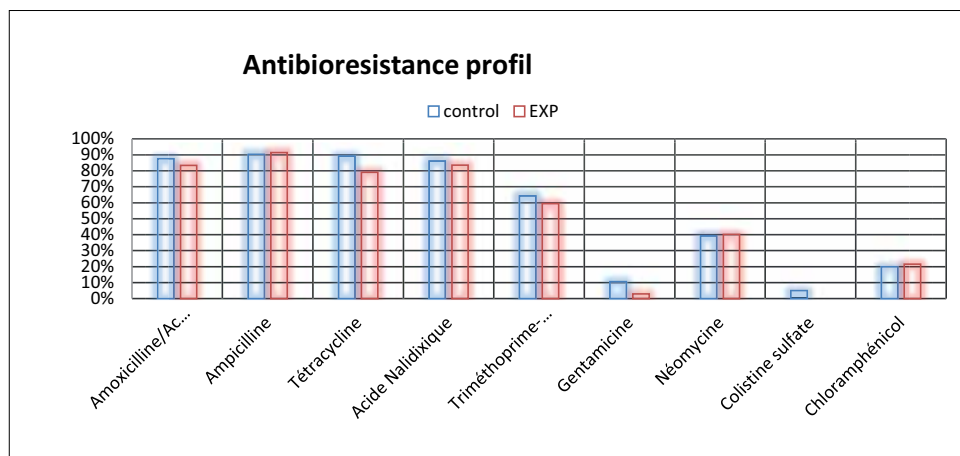
**Study of the measurements of the intestine and the development of certain organs**

The results relating to the evolution of weight of different parts of the carcasses per group are reported in Tables 12, 14 and 16. These results were averaged by group and rearing phase and reported in Tables 13, 15 and 17 to facilitate comparison.



**Table 11.** Expression of ATB resistance

|                               | control | EXP    |
|-------------------------------|---------|--------|
| Amoxicillin/Ac clavulanic     | 87.50%  | 83.36% |
| Ampicillin                    | 90.25%  | 91.45% |
| Tetracycline                  | 89.15%  | 79%    |
| Nalidixic acid                | 86.12%  | 83.47% |
| Trimethoprim-sulfamethoxazole | 64.23%  | 59.45% |
| Gentamicin                    | 10.56%  | 3%     |
| Neomycin                      | 39.24%  | 40.12% |
| Colistin sulfate              | 5%      | 0%     |
| Chloramphenicol               | 19.99%  | 21.50% |



**Figure 3.** Antibiotic resistance profile

### Start-up phase

Anatomically, the remarkable effect of the binder and the acidifier was observed on the weight of the FB, the gizzard and the brevis; in fact, there was an increase in the treated subjects of these weights (0.73, 26.69, and 208 g, respective in the control group, vs 1.36, 38, 230.86 respectively in the experimental group). Similarly, we noticed an increase in the length of the small intestine with 160.50 cm for the control group and 174.38 cm for the experimental one.

### Growth phase

An increase was also observed during the growth phase, where we note in the control group average weights of 2.75 g of the FB, 44.5 g of the gizzard, and 618.75 g of the wishbone, as compared to 3 g, 60.38 g and 782.875 g respectively for the experimental group. An increase was noted in the length of the small intestine with 193.63 cm for the control group and 212 cm for the experimental group.

**Table 12.** Results on the evolution of carcass, intestine, Fabricius bursa (FB), gizzard and knuckle weights.

| Group     | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|-----------|-----------------|---------------|--------------------|---------------------|
| control 1 | 126             | 0.5           | 11.5               | 189                 |
| control 2 | 158             | 0.8           | 18                 | 233                 |
| control 3 | 177             | 0.7           | 17                 | 224                 |
| control 4 | 157             | 0.6           | 38                 | 206                 |
| control 5 | 159             | 1             | 31                 | 188                 |
| control 6 | 176             | 0.8           | 33                 | 221                 |
| control 7 | 154             | 0.4           | 31                 | 205                 |
| control 8 | 177             | 1             | 34                 | 198                 |
| exp 1     | 180             | 1             | 31                 | 205                 |
| exp 2     | 156             | 1             | 32                 | 215                 |
| exp 3     | 155             | 0.9           | 35                 | 216                 |
| exp 4     | 158             | 1             | 34                 | 214                 |
| exp 5     | 165             | 1             | 47                 | 246                 |
| exp 6     | 198             | 2             | 52                 | 251                 |
| exp 7     | 185             | 2             | 40                 | 269                 |
| exp 8     | 198             | 2             | 33                 | ND                  |

**Table 13.** Average intestine length and weight of FB, gizzard and wishbone per group

| Group   | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|---------|-----------------|---------------|--------------------|---------------------|
| control | 160.50          | 0.74          | 26.69              | 208                 |
| Exp     | 174.38          | 1.363         | 38                 | 230.86              |
| Gain    | 13.88           | 0.638         | 11.31              | 22.86               |

**Table 14.** Results on the evolution of carcass, intestine, bursa, gizzard and Wishbone weights

| Group     | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|-----------|-----------------|---------------|--------------------|---------------------|
| control 1 | 208             | 4             | 47                 | 700                 |
| control 2 | 210             | 3             | 44                 | 665                 |
| control 3 | 204             | 5             | 48                 | 586                 |
| control 4 | 170             | 2             | 41                 | 521                 |
| control 5 | 174             | 1             | 43                 | 554                 |
| control 6 | 182             | 4             | 40                 | 603                 |
| control 7 | 211             | 2             | 53                 | 672                 |
| control 8 | 190             | 1             | 40                 | 649                 |
| exp 1     | 196             | 1             | 50                 | 737                 |
| exp 2     | 220             | 4             | 54                 | 817                 |
| exp 3     | 223             | 4             | 75                 | 889                 |
| exp 4     | 213             | 2             | 55                 | 704                 |
| exp 5     | 214             | 4             | 65                 | 919                 |
| exp 6     | 200             | 3             | 63                 | 737                 |
| exp 7     | 210             | 4             | 62                 | 760                 |
| exp 8     | 220             | 2             | 59                 | 700                 |

**Table 15.** Average intestine length and weight of FB, gizzard and wishbone per group and per phase

| Group   | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|---------|-----------------|---------------|--------------------|---------------------|
| control | 193.63          | 2.75          | 44.5               | 618.75              |
| exp     | 212             | 3             | 60.38              | 782.88              |
| Gain    | 18.37           | 0.25          | 15.87              | 164.13              |

### Finishing phase

At the end of the finishing period, we noted an increase of the same parameters, Fabricius bursa 2.875 g, gizzard 60.38 g, wishbone 788.25 g for the control group,

as compared to Fabricius bursa 4.125 g, gizzard 70.63 g, wishbone 942.13 g for the experimental group. Small difference in the intestine length (206.5 cm control vs 230.63 cm experimental).

**Table 16.** Results on the evolution of carcass, intestine, bursa, gizzard and Wishbone weights

| Group     | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|-----------|-----------------|---------------|--------------------|---------------------|
| control 1 | 210             | 4             | 67                 | 850                 |
| control 2 | 221             | 3             | 51                 | 828                 |
| control 3 | 206             | 3             | 52                 | 870                 |
| control 4 | 195             | 2             | 52                 | 784                 |
| control 5 | 200             | 3             | 56                 | 850                 |
| control 6 | 205             | 3             | 95                 | 718                 |
| control 7 | 220             | 3             | 61                 | 770                 |
| control 8 | 195             | 2             | 49                 | 636                 |
| exp 1     | 233             | 5             | 75                 | 810                 |
| exp 2     | 223             | 2             | 56                 | 1000                |
| exp 3     | 210             | 3             | 55                 | 879                 |
| exp 4     | 225             | 4             | 75                 | 900                 |
| exp 5     | 246             | 5             | 75                 | 1167                |
| exp 6     | 238             | 6             | 70                 | 850                 |
| exp 7     | 240             | 4             | 96                 | 1045                |
| exp 8     | 230             | 4             | 63                 | 886                 |

**Table 17.** Average intestine length and weight of FB, gizzard and wishbone per group

| Group   | Gut length (cm) | FB Weight (g) | Gizzard Weight (g) | Wishbone Weight (g) |
|---------|-----------------|---------------|--------------------|---------------------|
| control | 206.50          | 2.88          | 60.38              | 788.25              |
| exp     | 230.63          | 4.13          | 70.63              | 942.13              |
| Gain    | 24.13           | 1.25          | 10.25              | 153.88              |

## Discussion

In the current study, the addition of a mycotoxin binder with an acidifier in broiler feed modified the growth performance of chickens, which contribute to improving the feed quality and combatting the effects of mycotoxins. Its effects were observed on several parameters (intestinal and liver bacterial counts, intestinal measurement and weight of some organs).

These results indicate that the association binder-acidifier in broiler rearing induced a significant improvement in carcass weight, which agrees with the results reported by Aravind et al. (2003), where the addition of esterified dietary glucomannan was found to be effective in counteracting the toxic effects of naturally mycotoxin-contaminated feed, thus improving body weight, feed intake, and feed efficiency.

The same effect was observed by Hedayati et al. (2014), where it was noted on day 42 that the group receiving the binder alone recorded the best weight gain ( $2275.49 \pm 0.94$  g) compared to other groups, followed by the control group that received aflatoxin free feed and no binder ( $2207.93 \pm 0.53$  g); then the group that received the aflatoxin and the binder where the addition of the latter restored the weight gain at day 42 ( $2163.67 \pm 0.71$  g) that was far above the average of the group that received the aflatoxin alone ( $2052.60 \pm 0.18$  g).

In this study, the average weight was 3308.1 g on day 44, which is significantly higher than obtained in the above study. This may be due to the positive effect of the two products and could result in improved food processing efficiency by the additional effect of the acidifier. It is crucial that mycotoxins in contaminated feed have been found to retard growth, nutrient retention, and meat quality (Liu et al., 2011).

In this study, mortality in the subjects supplemented with mycotoxin binder and acidifier was reduced compared to the control group with an improvement rate of 50.47%, similar to the report by Le Bars (1992), where mortality due to mycotoxicosis is important and its incidence depends on the lethal dose specific to each toxin in chicken, e.g., 6.5 to 16.5 in the case of aflatoxin. This decrease in mortality may be due to a reduction in intestinal and hepatic bacteria (*E. coli* and *Salmonella*), which are factors in several infections such as colibacillosis that are most often subclinical and cause a reduction in food intake; its consequences are mainly economic (Lecoanet, 1992).

In birds, *E. coli* has "two faces"; it is found in the intestinal microbiota of healthy poultry, but also associated with extraintestinal diseases (Kuznetsova et al., 2020). *E. coli* in poultry is not highly involved in digestive pathologies but participates in various syndromes evolving in a septicemic or localised

form. Colibacillosis is sporadic and often caused by a superinfection. It can lead to fibrinous aerosacculitis, omphalitis, septicemia, salpingitis, and arthritis (Lecoanet, 1992).

The addition of the binder to feed significantly reduced the levels of *E. coli* in the intestine, indicating its value in combatting the risk of colibacillosis. Mycotoxins also seem to have an insidious effect on the immune response of chickens, since it was observed that a feed intake of subclinical doses of Aflatoxin significantly decreased the vaccine response to Newcastle disease and infectious bronchitis (Dahshan et al., 2012). This may explain our results concerning the reduction of intestinal and hepatic bacteria in subjects treated with both products. It is also important to know that aflatoxin increases the sensitivity of chickens to certain bacterial species (*Salmonella gallinarum*) and that an important biological effects is the reduction of resistance and immunity (Le Bars, 1992; Van Kol et al., 2011; Emami et al., 2013), which was confirmed by our results where we noted the absence of *Salmonella* in the experimental group.

Antibiotic susceptibility tests showed that the bacteria isolated from the control group were less sensitive to the antibiotics used, which may be due to the effect of the binder on the attenuation of the pathogenicity of the bacteria.

The gastrointestinal tract is the primary site where conversion and absorption of dietary components take place; it is the first organ to come into contact with food-borne mycotoxins and is expected to be affected with greater potency than other organs (Eftekhari et al., 2015; Guerre, 2020). It has been reported that the density of the whole intestine (weight/length) decreased after 3 weeks of dietary exposure to aflatoxin B1 at levels as low as 0.02 mg/kg and 0.7 mg/kg (Yunus et al., 2009). According to recent studies in broilers, it appears that

the unit absorptive surface area of the small intestine deteriorates upon chronic exposure to low levels of aflatoxin B1. However, it has been noted that broilers compensate for the reduced unit absorptive surface by increasing the length of the small intestine (Liu et al., 2011).

In this study, this phenomenon was observed and confirmed by the results. The length of the intestines of treated subjects was longer during all phases of rearing, and this difference was found to increase with time to reach a difference of 24.125 cm at the end of rearing.

It should be noted that dietary addition of a yeast cell wall adsorbent in non-transmissible diseases treatment showed a positive protective effect on liver and spleen relative weight at 21 days, and relative FB and thymus weight at 42 days. Chickens receiving contaminated feed without a yeast cell wall adsorbent had higher liver, FB and thymus relative weight (Liu et al., 2011). In this study also, an increase was obtained in the FB weight in the treated group of 1.25 g which is contrary to the results of the above study. The increase in chicken weight in the experimental group by reaching an increase in breast weight of 153.875 g can be related to the increase in body weight gain of 129.12 g at the end of the cycle.

## Conclusion

This study on the effects of the association of a mycotoxin binder (Micotec 0.1%) and an acidifier (Tecaviar 0.1%) showed positive effects on a broiler farm by improving zootechnical performances and reducing mortality. Data also revealed an increase in the average weight of breasts, an improvement in the development of the small intestine, an increase in the weight of the gizzard thus improving digestion; and an increase in the FB weight

that could imply an improvement in immunity. There was also a reduction in the number of coliform bacteria with an increase in non-pathogenic strains. New perspectives are opening up in order to adjust the concentrations of the molecules and to develop the bacteriological study by identifying the strains, and to deepen the histological study, especially of the digestive tract.

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## Zootehnički, bakteriološki i histometrijski učinci kombinacije veziva za mikotoksin-sredstva za zakiseljavanje u tovnih pilića

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Mikotoksini su prisutni u hrani i nije ih moguće eliminirati niti uporabom modernih tehnoloških procesa u prehrambenoj industriji; mogu prouzročiti patologije i ekonomske

gubitke u peradarskoj industriji. Za borbu protiv njihovih učinaka, odlučili smo ispitati alternativne načine inkorporiranjem veziva za mikotoksin (MICOTEC 0,1 %) u kombinaciji sa

sredstvom za zakiseljavanje (TECAVIAR 0,1 %), koji se prodaju na tržištu u Alžiru, u hranu koja se daje tovnm pilićima. Tijekom ove studije otkrili smo vrlo blagotvoran učinak na subjekte koji su konzumirali hranu s dodacima u usporedbi s kontrolnim subjektima. U stvari, zamijetili smo, uz značajno smanjenje mortaliteta (51,47 %), prosječnu masu od 3308,10 g, što je značajno više od one kontrolnih subjekata (2876,25 g). Uz to, podatci su otkrili značajno smanjenje broja ukupnih fekalnih koliforma, s prevalencijom u rasponu od 3,07 % do 36,50 % u usporedbi s 63,22 %

do 96,93 % za kontrolnu skupinu. Zamijetili smo i povećanje duljine tankog crijeva s 24,13 cm, povećanje mase Fabriciusove burze (FB) s vrijednošću od 1,25 g, mase želudca (10,25 g) te povećanje mase jada (153,87 g). Na temelju ovih podataka, moguće je rabiti kombinaciju veziva mikotoksina i sredstva za zakiseljavanje u tovnm pilića za poboljšanje rasta. Potrebni su dodatni eksperimenti za potvrđivanje više učinaka (s različitim dozama, različitim sojevima i drugim organima).

**Ključne riječi:** *mikotoksin, vezivo, sredstvo za zakiseljavanje, koliformi, histometrija*