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Influence of the combined application of probiotic, hypochlorite and the lignin-coal sorbent on alive mass of chickens

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The purpose. To study influence of the combined application of probiotic, hypochlorite and the lignin-coal sorbent on alive mass of chickens of breed rod-island at chronic fusarial toxicosis. **Methods**. Influence of application of probiotic Bacillus subtilis 44-P, sodium hipochlorite, lignin-carbonic sorbent and low densities fusariotoxines (T-2 toxine and zearalenon, 2 mg/kg of feedstuff) on alive mass of chickens is studied. **Results.** Injection of mycotoxines did not call statistically significant lowering of alive mass. Application of probiotic and sodium hipochlorite increased alive mass irrespective of injection of mycotoxines in feedstuffs. Simultaneous application of lignin-coal sorbent together with probiotic and hypochlorite reduced efficiency of the last at injection of mycotoxines. **Conclusions.** Sodium hipochlorite and probiotic Bacillus subtilis 44-P stimulate increase of alive mass of chickens, especially in the age of 4 – 6 weeks, however, their simultaneous application together with lignin-coal sorbent on the background of mycotoxicosis can be inefficient.

Key words: *probiotics, sorbents of mycotoxins, T-2 toxine, zearalenon, Bacillus subtilis 44-P, sodium hipochlorite, lignin, activated carbon.*

The problem of feed contamination with mycotoxins (toxic secondary metabolites of micromycetes) remains relevant due to the wide dissemination of mycotoxins, their role in the etiology of various health disorders in farm animals and poultry. Hundreds of mycotoxins are known, but only a few, e.g. aflatoxins, ochratoxins, trichothecenes, fumonisins, and zearalenone are considered the most dangerous. Ever increasing global demand for feeds, difficulties associated with the large-scale implementation of decontamination procedures, create a need for a simple method of decreasing animal sensitivity to the consumption of mycotoxin-contaminated feeds, such as the use of “anti mycotoxin feed additives” (AMA), which gained considerable popularity.

The direct inactivation of mycotoxins in the gastrointestinal tract (GIT) is widely believed to be the main principle of action of these additives. However, some works suggest that “unspecific” (not involving direct inactivation of mycotoxins by the additive) principles of action might underlie the ability of AMA to enhance the productivity of animals fed fusariotoxin-contaminated feeds [1-3]. Among possible mechanisms of action of AMA, the physical binding of mycotoxins within the GIT (hence the name “mycotoxin binders”), their biological inactivation (by microorganisms or enzymes), chemical inactivation (e.g. by oxidizing agents like hypochlorite), as well as the modulation of immunological reactions, intestinal microbiome, digestive functions, chyme viscosity, parameters of the intestinal unstirred layer are frequently mentioned.

Such diversity of possible mechanisms of action, along with contradictions, seen in the literature, makes it difficult to determine the exact mode of action of many AMA, as well as to predict their efficacy. For example, the supplementation of yeast glucomannans prevented the symptoms of chronic afla- and T-2 toxicoses [4], carryover of aflatoxins to milk [5], and decreased T-2 toxin concentration in the intestinal chyme [6]. But, in other works, yeast glucomannans appeared to be less effective [7], prevented neither carryover of aflatoxins to milk [8], nor the decrease of cytochrome P450 activity caused by T-2 toxin [9], and even increased the bioavailability of vomitoxin and doxycycline [10, 11]. Similar contradictions are also known for the clay-based AMA – namely bad correlation between their *in vitro* and *in vivo* efficacy, the possibility of potentiation of mycotoxins detrimental effects caused by the addition of these AMA [12, 13, 14] etc.

Such uncertainty creates both theoretical (classification of AMA, understanding their interactions) and practical (development, safety and efficacy assessment of AMA) difficulties, particularly pronounced in the case of multicomponent AMA. Investigation of the interactions between different AMA might also contribute to the search of their optimal combinations.

Objective. To study the influence of BPS-44 probiotic, sodium hypochlorite, a lignin-charcoal based mycotoxin binder, and moderate levels of fusariotoxins on the live weight of male Rhode-island chickens.

Materials and methods. Newborn male chicks were selected by vent sexing at a local hatchery. The chicks were wing-banded and allotted to four treatments, 25 birds each. The chicks were fed once a day with a compound feed, prepared in accordance with [15], water was available *ad libitum*.

Mycotoxins were obtained according to Dr. Kotyk [16, 17], purified, using the column chromatography, and crystallized from diethyl ether. In order to obtain the desired concentration of mycotoxins in the feed, a fraction of it was sprinkled with mycotoxins solution in isopropanol, dried for a couple of days, and mixed with the feed. The concentrations of T-2 toxin and zearalenone were 2 milligrams per kilogram of feed each.

The lignin-charcoal based binder was obtained by mixing activated charcoal with purified lignin in ratio 1:2. Sodium hypochlorite was obtained by electrolysis of sodium chloride water solution on carbon electrodes. The concentration of sodium hypochlorite was determined by titration. BPS 44 probiotic was purchased through the distribution network. The binder was mixed into feeds (15 g/kg). Sodium hypochlorite (30 mg/l) and BPS 44 (8 mg/chick/day) were alternatively added to drinking water, in 7-day cycles, since the 1st until the 8th week of the experiment.

Statistical analysis was performed using the Scheffe's method [18].

Two experiments were conducted sequentially, using the same battery cage.

Experiment 1. The influence of moderate levels of fusariotoxins, sodium hypochlorite and BPS 44 on the live weight of chickens. The mycotoxin-contaminated feeds were fed according to the table 1, since the 3rd until the 56th day of the experiment.

1. The study of the influence of fusariotoxins, sodium hypochlorite and BPS 44 on the live weight of chickens (*The first experiment*)

Group	Mycotoxins	Sodium hypochlorite	BPS 44
1-Control	-	-	-
2-Mycotoxins	+	-	-
3-Additives	-	+	+
4-Mycotoxins & additives	+	+	+

Experiment 2. The influence of the combined addition of the lignin-charcoal binder, sodium hypochlorite, BPS 44 and fusariotoxins on the live weight of chickens. The binder and mycotoxins were added according to the table 2, since the 4th until the 45th day of the experiment, according to the table 2.

2. The study of the influence of the combined addition of sodium hypochlorite, BPS 44, the lignin-charcoal binder, and fusariotoxins on the live weight of chickens (*The second experiment*)

Group	Mycotoxins	Sodium hypochlorite	BPS 44	Binder
1-Control	-	-	-	-
2-Mycotoxins	+	-	-	-
3-Additives	-	+	+	+
4-Mycotoxins & additives	+	+	+	+

Results and discussion. During the first experiment, 3 chickens died: 1 in the second and 2 in the fourth group. Also, 11 female chickens were removed – 3 in the first, 4 in the second, 2 in the third, and 2 in the fourth group. During the second experiment, 6 chickens died: 1 in the first, 1 in the second, 2 in the third and 2 in the fourth group. Also, 21 female chickens were removed – 3 in the first, 5 in the second, 7 in the third, and 6 in the fourth group.

In both experiments, oral lesions were noted among birds fed mycotoxin-contaminated feeds (with or without additives).

The concentration of T-2 toxin, used in the present work, when fed in the form of "field contaminated" feeds, often causes severe impairment of productivity [19]. Nevertheless, the first experiment failed to demonstrate any decrease in the chicken live weight, caused by the addition of mycotoxins (Table 3). In the second experiment (Table 4), the live weight of chickens, fed mycotoxin-contaminated feeds, since the 4th until the 9th week of the experiment, was only 6-7% lower, than in the control group. The reason for this could be the use of highly purified mycotoxins, which are known to exhibit lesser toxicity than their equivalent amounts, fed in the form of mold-infested grain [20].

3. The influence of mycotoxins, sodium hypochlorite and BPS 44 on the live weight of chickens (*The first experiment*) (M±s)

Age, weeks	1-Control	2-Mycotoxins	3-Additives	4-Mycotoxins & additives
	Live weight, g			
2	90 ± 12^{a}	98 ± 10^{a}	96 ± 12^{a}	95 ± 7^{a}
4	252 ± 32^{a}	$270 \pm 20^{a,b}$	282 ± 35^{b}	$275 \pm 28^{a,b}$
6	492 ± 60^{a}	497 ± 49^{a}	551 ± 54^{b}	546 ± 44^{b}
8	804 ± 69^{a}	844 ± 56^{a}	852 ± 88^{a}	836 ± 64^{a}
10	1139 ± 79^{a}	1207 ± 80^{a}	1185 ± 114^{a}	1185 ± 91^{a}
12	1405 ± 91^{a}	1447 ± 140^{a}	1447 ± 154^{a}	1363 ± 126^{a}

$^{a\text{-}b}$ Here, and in the following table, means in a row with different superscripts differ significantly at $P \geq 0,05$.

4. The influence of the combined addition of the lignin-charcoal binder, sodium hypochlorite, BPS 44 and mycotoxins on the live weight of chickens (*The second experiment*) (M±s)

Age, weeks	1-Control	2-Mycotoxins	3-Additives	4-Mycotoxins & additives
	Live weight, g			
2	$79 \pm 9^{a,b}$	$84 \pm 10^{a,b}$	87 ± 12^{a}	77 ± 9^{b}
4	$210 \pm 34^{a,b}$	197 ± 27^{b}	229 ± 31^{a}	194 ± 31^{b}
6	463 ± 67^{a}	436 ± 60^{a}	473 ± 57^{a}	418 ± 66^{a}
9	943 ± 92^{a}	873 ± 128^{a}	890 ± 111^{a}	880 ± 95^{a}
12	1317 ± 111^{a}	$1363 \pm 118^{a,b}$	1427 ± 119^{b}	$1340 \pm 119^{a,b}$
13	$1399 \pm 97^{a,b}$	$1422 \pm 93^{a,b}$	1476 ± 128^{a}	1350 ± 121^{b}

The use of BPS 44 and sodium hypochlorite increased the live weight of chickens in the presence of mycotoxins as well as in their absence: since the 2nd till the 6th week of the first experiment live weigh of these chickens was 9-12% higher, than in the control group. This effect was most pronounced at the end of the 6th week of the experiment, which could be related to the higher growth intensity of chickens during the first 6 weeks of growth.

The simultaneous use of the lignin-charcoal binder with BPS 44 and sodium hypochlorite in the second experiment did not cause any statistically significant changes in the live weight, however, initially, during the 2nd and the 4th weeks, live weight of the chickens, which received this treatment was 9-10% higher, than in the control group.

The simultaneous addition of fusariotoxins, the lignin-charcoal binder, BPS 44 and sodium hypochlorite degraded the growth stimulating properties of the latter two, seen in the first experiment: since the 2nd until the 6th week of experiment live weight of these chickens was 8-17%, and final live weight 9% lower than in the third group, which received this additives without fusariotoxins. Since this effect was most pronounced in presence of fusariotoxins, it might denote that considerable amount of the added fusariotoxins was not inactivated, despite the addition of AMA. This is somewhat paradoxical because hypochlorite and BPS-44 were rather effective in experiment 1, and the lignin-charcoal binder had previously shown comparatively high *in vitro* activity [21]. This might be explained by the possibility of synergism between the side effects of the used mycotoxin binder and the effects of the addition of fusariotoxins (effects of T-2 toxin addition involve alteration of the intestinal epithelium, thus creating the basis for synergistic interaction with antinutritional factors, like the addition of unspecific binder), as well as by the ability of factors, present in the GIT to impair the direct antitoxic activity of the additives used (comparatively to *in vitro* conditions).

Conclusions

The combined use of sodium hypochlorite and BPS 44 has shown growth-stimulating properties, which were most pronounced at the age of 4-6 weeks, regardless of the presence of fusariotoxins in feeds. The use of the lignin-charcoal binder degraded growth-stimulating properties of sodium hypochlorite and BPS 44 in the presence of fusariotoxins. The reasons for such effect might require additional investigation.

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