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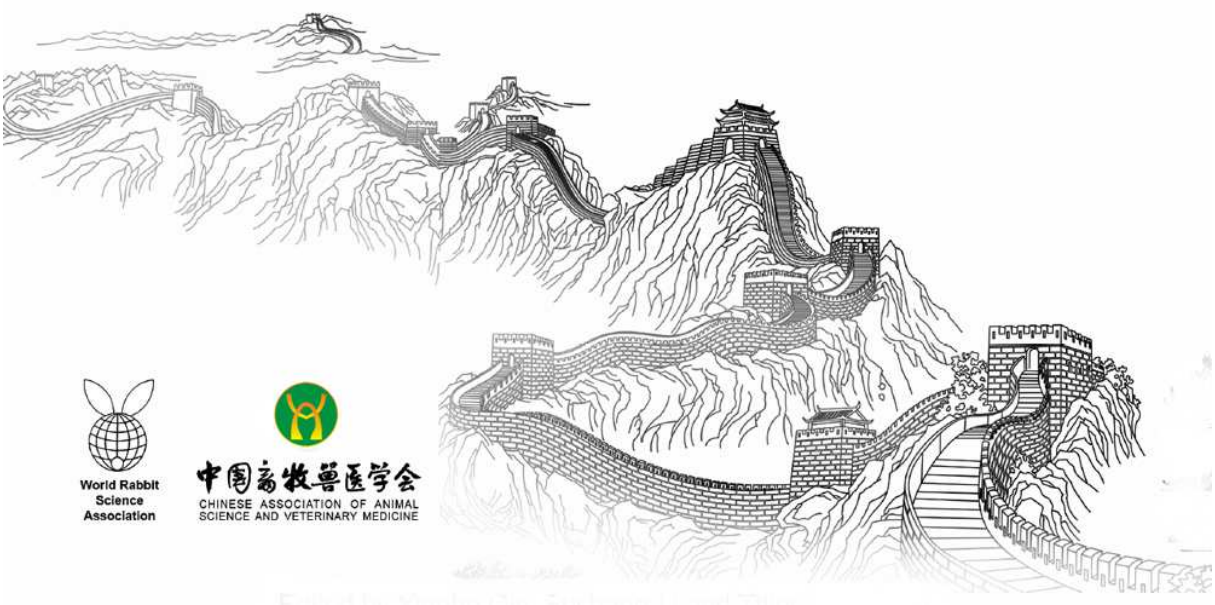

INFLUENCE OF A MYCOTOXIN BINDER COMPOUND, DEFITOX®, ON THE PERFORMANCES AND SOME BIOCHEMICAL AND HISTOLOGICAL CHARACTERISTICS OF GROWING RABBITS FED BY MYCOTOXINS CONTAMINATED FEED

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ABSTRACT

516 weaned 36d old rabbits (Hyplus) received feeds contaminated with mycotoxins (DON 0.70 ppm + ZEA 0.32 ppm + tenuazonic acid 0.90 ppm). They were split into two treatments, one control, the other one supplemented with Defitox®, a mycotoxin binder associating bentonite, bacterial walls, active charcoal and an extract of plant with liver protective functions. Mortality was lower in Defitox group (13.9 *vs* 18.4% for 36-74d old period, P<0.01) with a lower incidence of digestive pathologies. Weight gain was increased mostly during the early growing period (40.7 *vs* 36.8 g/d, P<0.001). Some biometric and biologic parameters were lower for Defitox group: relative weight of the vermiform appendix (0.28 *vs* 0.37 %, P= 0.03); leucocytes number (5.9 *vs* 7.7 .10^3 /µl, P= 0.01) – monocytes (1.6% *vs* 1.9 %, P= 0.01); serum glucose concentration (61 *vs* 84 mg/dl, P=0.01); alkaline phosphatase (189 *vs* 286 UI/l, P= 0.02). The incorporation of a complex mycotoxin binder as Defitox® in a mycotoxin contaminated rabbit feed would alleviate the effects of mycotoxins and improve the viability and rabbit performances. These first results must be confirmed using a design with a negative control group without mycotoxin contaminated feed.

Key words: Rabbits, Defitox®, Mycotoxins, Toxin-binder, Bentonite, Charcoal, MOS, Tenuazoic acid.

INTRODUCTION

Mycotoxins are secondary metabolites of moulds which grow on plant (Ténier and Colin, 2010). They impact negatively the performance of animals such as rabbits (Mézes, 2008). Even though only few researches have been done about the effect of mycotoxins on rabbits, they all showed very negative impact of mycotoxins (Farahat et *al.*, 2009; Ténier and Colin, 2010). Three concepts can be used to reduce mycotoxin effects (Ténier and Colin, 2010): biotransformation of mycotoxins into a non toxic form, binding of mycotoxins, liver protection and immunity preservation. Defitox®, the mycotoxin binder used in this trial is designed to respond to the latter two concepts previously mentioned.

MATERIALS AND METHODS

Animal and experimental design

The trial was carried out from 30th June 2015 to 6th August 2015, in an experimental farm (EARL 3L) that has not been using any form of antibiotics since 2003. 516 Hyplus PS19 x PS40 hybrid rabbits of both sexes were pre-weaned one week before the weaning (29d old): does and their litter were moved all together from the maternity to the fattening building. At weaning (36d old) rabbits were allotted in two groups according to litter origin and parity number of the doe: control or Defitox (Table 1) with 54 and 50 cages respectively. Rabbits were housed in collective cages (5-6 animals as described by Teillet et *al.*, 2012). The slaughter age was 74d old.

Table 1 Experimental diets

Diets	Defitox®	Number of cages	Number of rabbits
Control	0 kg/t	54	272
Defitox	1 kg/t	50	244
TOTAL			**516**

Table 2 Mycotoxins in raw materials

Mycotoxins in ppm	Alfalfa	Corn
Deoxynivalenol (DON)	0.120	6.795
Zearalenone (ZEA)	0.030	1.370
Fumonisin	0.020	0.050
Tenuazonic Acid	0.360	<0.050

Experimental diets

The feeding plan was done according to Teillet et *al.* (2012). To reach a high dietary mycotoxin content, alfalfa and corn were ship from regions usually having high contamination levels (Table 2). The two experimental feeds were manufactured at the farm and contained respectively 15 and 30% of the contaminated corn and alfalfa, to reach a level of mycotoxin higher than those found in the standard feed used in the farm (Table 3). Defitox feed contained 1 kg per ton of Defitox®, a mycotoxin binder elaborated by MG2Mix, a French premixer, gathering three components to bind mycotoxins: Bentonite (Magnoli et *al.*, 2008), Mannanes oligo-Saccharides from bacteria walls (Zaghini et *al.*, 2005), and activated charcoal (Abeer et *al.*, 2014). The product also contains plant extracts to protect the liver (Shaker et *al.*, 2009).

Measured criteria

The performances indexes, the causes of mortality and Eimeria counting and identification were assessed according to Teillet et *al.* (2012) and Colin et *al* (2013). The biological impact of the mycotoxin binder was estimated on eight animals from each group, by biometrics (relative weight) and histological parameters of some organs (liver, kidneys and vermiform appendix), white blood cells number and characteristics (SKIL analyzer) and blood biochemistry (Alkaline phosphatase (ALP) and serum glucose) (SPOTCHEM analyzer). Blood samples were taken on living animals in a laboratory. Organs were collected after the euthanasia by the veterinary.

Statistical Analysis

All the parameters were treated with an Analysis of Variance (ANOVA) except mortality which was treated according to the method described by Savietto et *al.* (2015).

RESULTS AND DISCUSSION

Experimental diets and feed intake

Both feeds contained mycotoxins in higher proportion than the standard (Table 3). One relevant point is the high level of Tenuazonic acid: this mycotoxin has never been studied in the rabbit. Feed and water intakes were similar among the two groups (Table 4).

Table 3 Mycotoxins in the feeds

Mycotoxins (ppm)	Control	Defitox®	Standard feed
Deoxynivalenol	0.660	0.720	0.095
Zearalenone	0.215	0.225	0.015
Fumonisin	0.025	0.025	<0.020
Tenuazonic Acid	0.945	0.870	0.410

Table 4 Feed intake

Treatments	Control	Defitox®
Feed intake (kg/rabbit)	4.19	4.48
Water/feed ratio	2.41	2.47
Economical FCR	3.80	3.67
Technical FCR	2.93	2.89

Mortality

The mortality (36-74d old period) was 4.5 units lower in Defitox compared to the control ($P<0.01$, table 5), with enterocolitis (REE) as a main cause. The incidence of digestive pathologies seems lower in

Defitox than in control group (Figure 1). Negative impact of mycotoxins on digestive diseases has been demonstrated in human (Bhat *et al*., 1997) and in domestic animals (Antonissen *et al*., 2014). The positive action of Defitox® on mortality can be explained by the reduction of mycotoxins' effects due to their binding with the components of the product.

Table 5 Mortality results

Treatments		Control	Defitox®	Stat. Sign.
Defitox® (kg/t)		0	1	
Number of rabbits		272	244	-
Mortality (%)	36-55 d	14.71	11.07	P<0.01
	55-74 d	4.04	2.87	P<0.01
	36-74 d	18.45	13.93	P<0.01

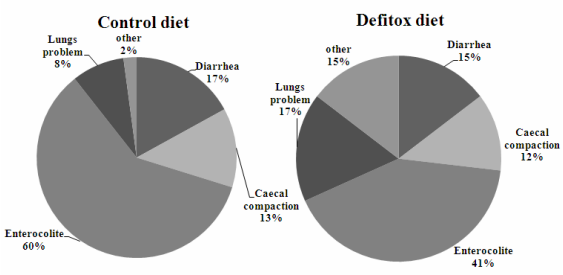

Figure 1 Causes of mortality

Weight and Growth

Weights of rabbits were 2% higher in Defitox group at 55 and 69d old, although their weaning weight was lighter (Table 6). Accordingly growth rate was 7% higher (36-55d old) in Defitox group. Results are in line with those of Khera et *al*. (1986) who showed that a high level of deoxynivalenol in the diet decreases the doe's weight.

Table 6 Weight and growth results

Treatments		Control	Defitox®	Stat. Sign.
Defitox® (kg/t)		0	1	
Weight (g)	36 d	876	833	P<0.01
	55 d	1575	1607	P<0.01
	69 d	2182	2226	P<0.01
Average Daily Gain (g/d)	36-55 d	36.8	40.7	P<0.05
	55-69 d	43.3	44.2	NS
	36-69 d	39.6	42.2	NS

Table 7 Biometric and biological analysis (parameters showing a difference between the treatments)

Treatments	Control	Defitox®	Stat. Sign.
Defitox® (kg/t)	0	1	
Vermiform appendix (% of Body Weight)	0.37	0.280	0.03
Leucocytes (10^3/µl)	7.7	5.9	0.01
Lymphocytes (%)	15.2	17.2	0.65
Monocytes (%)	1.9	1.6	>0.01
Glucose (mg/dl)	83.6	60.8	>0.01
ALP (UI/l)	285.8	189.3	0.02

Biological and Biochemical Analysis

The relative weight of the vermiform appendix, an important immune organ (Fortun Lamothe and Boullier, 2004) was 24% lower for Defitox (P=0.030, table 7). The total number of leucocytes was 23% lower in Defitox treatment (P=0.01), and the proportion of monocytes 16% lower (P<0.01). These results are in agreement with those of Farahat *et al*. (2009) who showed that the incorporation of a mycotoxins deactivating agent in the feed decreased the white cells number. However in our experiment the proportion of lymphocytes was not affected by treatments, which disagree with Farahat *et al*. (2009) who showed a decrease of lymphocytes with the addition of the mycotoxin deactivating agent in the feed.

Alkaline phosphatase (ALP) was 34% lower for Defitox treatment (P<0.01). Conkova *et al*. (2002) showed that zearalenone (ZEA) increase ALP, suggesting a preservation of the liver in Defitox group probably by alleviating the action of ZEA. Furthermore a 20% decrease of the glycemia was observed in Defitox group. Besides, eight rabbits were autopsied in each treatment. Three demonstrated nephritis in control diet and two in Defitox one.

Histological Analysis

Eight rabbits were autopsied in each treatment. Three demonstrated nephritis in control diet and two in Defitox® one. It would be interesting to repeat this measurement on a higher number of rabbits.

CONCLUSIONS

Our results demonstrate the interest to use a complex of mycotoxin-binders associated with a liver protector in rabbit feed contaminated by high levels of mycotoxins. In these contaminated conditions of feeding the high mortality rate was reduced by 20%, while post-weaning growth was improved. The weight of the vermiform appendix and the total number of lymphocytes was lower in Defitox group suggesting a lower stimulation of the immune system. These first results must be confirmed using a design with a negative control group without mycotoxin contaminated feed.

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