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FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

**EFFECT OF T-2 TOXIN ON LIPID PEROXIDATION,
GLUTATHION REDOX SYSTEM AND THE
IMMUNOLOGICAL FUNCTION IN BROILER
CHICKEN**

Thesis of Ph.D. dissertation

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1. INTRODUCTION

Mycotoxins are potentially toxic substances in nature, produced as secondary metabolites of moulds. Due to their prevalence and diversity, they potentially endanger all monogastric animals and humans as well.

Among the so-called trichothecene mycotoxins of *Fusarium* moulds T-2 is the most common toxin of feed base grains in the temperate zone and in our country too, that depending on its quantity can cause massive acute or chronic toxic reactions.

Nervous system degeneration and dermatotoxicity are to be underlined from its toxic effects, as well as protein synthesis inhibition which decreases the detoxifying, xenobiotic transforming ability of the liver via reduced microsomal monooxygenase activity in hepatocytes. Additionally it causes immune function decrease. The effects on the nervous system also manifest in lymphatic organ failure, according to literal data.

In the European Union there is not even a suggested value for T-2 toxin in feeds or feed stocks, only a few countries (Hungary too) determined a value for the given country, that - considering its gravity - is a great control deficiency.

Biological / pathophysiological effects of a given toxin quantity detected in a given forage lot are affected positively or negatively by several other factors, some of these are the subject of my dissertation.

Therefore, the prevention of toxin-load and the development of methods reducing detrimental effects of toxins on productive parameters and product quality, in severe case the toxic symptoms are highly important scientific missions, and the determination of non harmful/health safe toxin concentrations for animals and consumers (on the bases of scientific results) is also essential.

2. OBJECTIVES

1. Estimation of sublethal T-2 toxin dose effect – close to practical levels – on the forage uptake of broiler chicken and the change of some parameters of lipid peroxidation and the biological antioxidant system.
2. According to the results examination of high-dose antioxidant (vitamin E and selenium) supplementation effects – used for antioxidant status improvement – on the lipidperoxid and antioxidant state of poultry in case of sublethal T-2 toxin load.
3. Determination of T-2 toxin impact on the systemic distribution of vitamin E, the most widely used lipid dissolved antioxidant in feed.
4. Analysis of the decrease in T-2 toxin induced lipid peroxidation and biological antioxidant protection system damage caused by a proposed trichotecene mycotoxin-adsorbent (yeast cell wall extract) during feed.
5. Investigation of the effect of sublethal dose T-2 toxin load immune response ability in broiler chicken during repeated vaccination against Newcastle disease by measuring antibody formation.
6. Additionally I studied the effect of vitamin E simultaneously applied with sublethal dose T-2 toxin on the immune response ability of broiler chicken during repeated vaccination against Newcastle disease.

3. MATERIALS AND METHODS

Sample collection

Blood samples were taken from the jugular veins (*aa. carotis ext. et int., v. jugularis*) during debleeding and collected in anticoagulant-containing tubes (0,05 ml 0,2 mol/L EDTA-Na₂ / 1 ml blood) and native blood collection tubes to gain blood serum.

Post mortem tissue samples (liver, kidney, spleen, bursa Fabricii) were collected in the course of dissection. Liver, kidney and spleen samples were stored at -18°C until biochemical analysis, bursa Fabricii was kept in 8 % (v/v) formaldehyde until histological section.

Tissue samples were homogenized in phosphate buffer saline (1:9). Native homogenizates were used for malondialdehyde concentration determination, while further biochemical analysis was performed from the 10.000g supernatant of the homogenizates (Mézès, 1999).

T-2 toxin – purified and partially purified – was dissolved in acetone or acetonitrile, then pulverized – evenly dispersed – on granulated forage dissolved in a small amount of solvent (5 ml/10 kg forage) in experiment 1-3. Further on the toxin (T-2 toxin and its natural metabolites) containing substrate was mixed directly to the granulated forage. Toxin dose applied in the experiment was determined on the basis of T-2 toxin levels measured in mould-contaminated inland corn samples (0,16-0,48 mg/kg) (Kovács, 2001).

Experiment	Control		T-2 toxin contaminated forage	
	estimated	determined	estimated	determined
Experiment 1	0	0,23	2	2,12
Experiment 2	0	<0,01	1	0,877 + 0,015 HT-2
Experiment 3	0	<0,01	2	2,05 + 0,045 HT-2
Experiment 4	0	<0,01	1	1,17 + 0,02 HT-2
Experiment 5	0	<0,01	4	4,481 + 0,035 HT-2
Experiment 6 (initial)	0	<0,01	1	1,04 + 0,489 HT-2
6. experiment (final/terminator)	0	<0,01	1	0,12 + 0,016 HT-2

Table 1. Estimated and HPLC-determined T-2 toxin contamination (mg/kg) of control and T-2 toxin contaminated forages

Concentration of T-2 toxin and its natural metabolites was determined by HPLC in the Department of Chemistry, Central Veterinary Institute according to Trichothecene-HPLC-1:2000 in every experiment concerning artificially contaminated and control forages. Tissue sections were prepared from bursa Fabricii samples and stained with hematoxilin eosin for further histopathological analysis.

Haemagglutination inhibition tests to detect antibody production levels were carried out in the Department of Serology, Central Veterinary Institute according to the 92/66/EGK principle of the European Committee.

Biochemistry

Thiobarbituric acid reactive agents (malondialdehyde) were measured in the plasma, red blood cell (RBC) haemolysate and tissue homogenizates with colorimetry according to the protocol of Placer et al. (1966), modified by Matkovics et al. (1988).

Reduced glutathione concentrations of the plasma, RBC haemolysate and tissue homogenizates were determined on the basis of complex formation of free non protein SH-groups with 5,5'dithiobis-2-nitrobenzoic acid (Sedlak and Lindsay, 1968).

Glutathione peroxidase activity was measured in the plasma, RBC haemolysate and tissue homogenizates using the endpoint direct assay of Matkovics et al. (1988).

Enzyme activity data were correlated to protein concentration determined by Biuret reaction for the plasma and RBC haemolysate and Folin phenol reagent for tissue homogenizates (Lowry et al., 1951).

Reagent kits of Diagnosticum Ltd. Budapest were used to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactic acid dehydrogenase (LDH) enzyme activities, glucose and triglycerid concentration.

Vitamin E concentration of plasma and liver samples were determined on the basis of HPLC (McMurray et al., 1980) by the Department of Animal Physiology and Animal Nutrition, Faculty of Georgikon Faculty of Agriculture, University of Pannonia.

Ascorbic acid concentrations in the plasma and liver samples were measured according to the colorimetric method described by Omaye et al., (1979).

Mathematics and statistics

Arithmetic mean and standard deviation (S.D.) was calculated for all parameters based on the results.

Antibody response was correlated to the mean titer (geometrical average of individual HAG titers) for each group to evaluate the haemagglutination inhibition tests. The latter was determined as log₂ for the sake of statistical analysis.

Significance levels were calculated on the basis of Duncan's multiple range test.

Experimental arrangement

Animals were given a commonly traded complete forage mix – to model common technologies.

Water was provided ad libitum.

Esvex Oral Solution® (Dunavet-B Ltd., Dunaföldvár) was added to the water as antioxidant (vitamin E and selenium) supplementation, in the recommended 1 ml/L concentration. Considering the average water consumption, animals were given an average 10,5 mg/individual/day extra vitamin E and 0,045 mg/individual/day extra selenium.

For T-2 toxin adsorption, I used a glucomannane based adsorbent (Mycosorb®, Alltech) in the recommended dose (2g/kg).

For vaccination, attenuated Newcastle disease vaccine (Nobilis ND Clone 30®, Intervet, Boxmeer) was dropped to the eye according to technical recommendations.

Cage (experiment 1-5) and deep litter (experiment 6) housing systems were used concerning the housing densities recommended by animal welfare regulations.

Experiment 1. Analysing the effect of experimental purified T-2 toxin load and antioxidant supplementation on the lipid peroxidation and antioxidant state of TETRA H egg layer hybrids

65 TETRA H hybrid roosters were examined from 18 days of age. Feed was contaminated with 2 mg/kg purified T-2 toxin (Sigma, St. Louis).

Individuals were classified randomly into 4 groups. The control group (K) (n=20) was given control forage and drinking water. Group 2 (T) (n=15) was provided toxin contaminated (2 mg/kg) forage and water, Group 3 (EK) (n=15) was given control forage and vitamin E+selenium supplementation (1 ml/l) provided through drinking water. Group 4 (ET) (n=15) was given toxin contaminated (2 mg/kg) forage and Vitamin E+selenium supplementation (1 ml/l) provided through drinking water.

During the experimental period (12 days) T-2 toxin content of control and contaminated forages was measured, and resulted the required 0,23 and 2,12 mg/kg concentrations.

number	label	T-2 toxin	Vitamin E+selenium
1.	K	-	-
2.	T	+	-
3.	EK	-	+
4.	ET	+	+

Table 2 Arrangement in Experiment 1.

Samples were collected on day 0, 3, 7 and 12. 5-5 individuals were exterminated each time, blood and tissue – liver and bursa Fabricii – samples were collected. Body weight, forage uptake, liver weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity were measured in the plasma, RBC haemolysate and liver homogenizate.

Experiment 2. Analysing the effect of experimental T-2 toxin and natural metabolite load, antioxidant and glucomannane supplementation on the lipid peroxidation and antioxidant state of broiler chicken

Ross 308 hybrid roosters were taken into the experiment at 21 days of age. Individuals (n=75) were classified into 5 groups.

Forage was contaminated with a 1 mg/kg dose of T-2 toxin and its natural metabolite produced by *Fusarium trincintum* on corn. In the original extract, HT-2, T-2 triol and T-2 tetraol were present.

There was no detectable T-2 toxin or metabolite in the control, while the toxin contaminated forage contained 0,877 mg/kg T-2 toxin. HT-2 toxin in 0,015 mg/kg concentration was also detected, while T-2 triol and T-2 tetraol were under the detectable level.

Vitamin E+selenium supplementation (1 ml/liter water) and glucomannane adsorbent (2 g/kg forage) were applied in the amount recommended by the manufacturer.

Individuals were classified into 5 groups, consisting of 15 roosters each. The control group (K) (n=20) was given control forage and water. The toxin loaded group (T) was given toxin contaminated forage and water, while the vitamin E+selenium supplemented and toxin loaded group (ET) was given toxin contaminated forage and vitamin E+selenium supplementation (1 ml/l) provided through drinking water. The toxin loaded and adsorbent treated group (KT) was given contaminated forage with glucomannane based toxin adsorbent and drinking water. The vitamin E+selenium supplemented and toxin and adsorbent treated group (EKT) was given toxin contaminated forage with toxin adsorbent and vitamin E+selenium supplemented water.

Samples were collected on day 3 and 7 following extermination.

number	label	T-2 toxin	Vitamin E+selenium	Toxin adsorbent
1.	K	-	-	-
2.	T	+	-	-
3.	ET	+	+	-
4.	KT	+	-	+
5.	EKT	+	+	+

Table 3. Arrangement in Experiment 2.

On day 3 5-5 individuals, whereas on day 7 10-10 individuals were exterminated, blood and tissue – liver, spleen and bursa Fabricii – samples were collected

Body weight, forage uptake, liver and spleen weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity was measured in the plasma, RBC haemolysate and liver and spleen homogenizate. Ascorbic acid content in the plasma was also measured.

Experiment 3. Analysing the effect of experimental T-2 toxin and natural metabolite load on lipid peroxidation antioxidant state and antibody production of broiler chicken following vaccination against Newcastle disease

Ross 308 hybrid roosters were taken into the experiment at 21 days of age. Individuals (n=65) were classified into 4 groups.

Forage was contaminated with a 2 mg/kg dose of T-2 toxin and its natural metabolite produced by *Fusarium trincintum*. In the extract, HT-2, T-2 triol and T-2 tetraol were also present.

There was no detectable T-2 toxin or metabolite in the control, while the toxin contaminated forage contained 2,05 mg/kg T-2 toxin and 0,045 mg/kg HT-2 toxin.

The control group consisted of 20, the experimental groups of 15-15 individuals. The control group (K) was the control, the toxin loaded group (T) was given toxin contaminated forage and drinking water. The vaccinated group (V) was given control forage and water, but was submitted to repeated attenuated Newcastle disease virus vaccination, while the toxin loaded and vaccinated group (VT) was given toxin contaminated forage and was subjected to repeated vaccination.

number	label	T-2 toxin	vaccination
1.	K	-	-
2.	T	+	-
3.	V	-	+
4.	VT	+	+

Table 4. Arrangement in Experiment 3.

Samples were collected on day 0, 3, 7 and 14. 5-5 individuals were exterminated each time, blood and tissue – liver, spleen, kidney and bursa Fabricii – samples were collected.

Body weight, forage uptake, liver and spleen weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity was measured in the plasma, RBC haemolysate and liver, spleen and kidney homogenizate. Ascorbic acid content of the plasma and liver samples was also measured. Haemagglutination inhibition tests to detect antibody production levels indicating the effectiveness of the vaccination was also carried out.

Experiment 4. Analysing the effect of experimental T-2 toxin and natural metabolite load on lipid peroxidation, antioxidant state and antibody production of broiler chicken following repeated vaccination against Newcastle disease

Ross 308 hybrid roosters were taken into the experiment at 22 days of age. Animals (n=125) were classified into 8 groups.

Forage was contaminated with T-2 toxin and its natural metabolites produced by *Fusarium trincintum*. HT-2 was observed in the original extract as a natural metabolite.

There was no detectable T-2 toxin or metabolite in the control, while in the toxin contaminated forage 1,17 mg/kg T-2 toxin and 0,020 mg/kg HT-2 toxin was detected. T-2 triol and T-2 tetraol were under the detectable level.

Individuals were classified into 8 groups. The control group consisted of 20, while the experimental groups consisted of 15 individuals each, arranged as follows (Table 5.):

Number	Label	Forage	Vaccination	Vitamin E+selenium
		control: - toxin loaded: +	vaccinated: + not vaccinated:-	treated: + untreated:-
1.	K	-	-	-
2.	T	+	-	-
3.	E	-	-	+
4.	V	-	+	-
5.	ET	+	-	+
6.	EV	-	+	+
7.	VT	+	+	-
8.	ETV	+	+	+

Table 5. Arrangement in Experiment 4

Samples were collected on day 0, 3, 7 and 14. 5-5 individuals were exterminated each time, blood and tissue – liver, spleen, kidney and bursa Fabricii – samples were collected.

Body weight, forage uptake, liver and spleen weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity was measured in the plasma, RBC haemolysate and liver, spleen and kidney homogenizate. Ascorbic acid content of the plasma and liver samples was also measured.

Haemagglutination inhibition test of the plasma indicating the effectiveness of vaccination was carried out.

Histological sections were cut from bursa Fabricii samples.

Experiment 5. Analysing the effect of high-dose experimental T-2 toxin and natural metabolite load and antioxidant supplementation on lipid peroxidation, antioxidant state and antibody production of broiler chicken following repeated vaccination against Newcastle disease

26 days old Ross 308 hybrid roosters were taken into the experiment. Individuals (n=40) were classified randomly into 8 groups.

Forage was contaminated with T-2 toxin produced by *Fusarium trincintum*.

There was no detectable T-2 toxin or metabolite in the control, while in the toxin contaminated forage 4,481 mg/kg T-2 toxin was detected. T-2 triol and T-2 tetraol were under the detectable level.

Experimental groups were submitted to antioxidant treatment, vaccination and toxin load. Individuals were treated as described previously, treatment combinations are described in Table 5.

Samples were collected on day 0, 3, 7 and 14. On day 0, 3 and 7 blood samples were collected from the wing vein (v. cubitalis) from all individuals for haemagglutination inhibition tests. On day 14 all individuals were exterminated, blood and tissue – liver, spleen, kidney and bursa Fabricii – samples were collected.

Body weight, forage uptake, liver and spleen weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity was measured in the plasma, RBC haemolysate and liver, spleen and kidney homogenizate. Ascorbic acid content of the plasma and liver samples was also measured.

Haemagglutination inhibition test of the plasma indicating the effectiveness of vaccination was carried out.

Histological examination of bursa Fabricii samples was done, sections were cut and submitted to histopathological analysis.

Experiment 6. Analysing the effect of long-term, low-dose experimental T-2 toxin and natural metabolite load and antioxidant supplementation on productive parameters, lipid peroxidation and antioxidant state of broiler chicken

Day-old Ross 308 hybrid roosters were taken into the experiment. Individuals (n=20) were classified into 2 groups. Control (K) group was given commonly traded forage according to breeding state. The toxin-loaded group was given the same forage contaminated with T-2 toxin and its natural metabolites produced by *Fusarium trincintum*

There was no detectable T-2 toxin or metabolite in the control (initial and final) forages, while the toxin contaminated forage contained T-2 toxin in 1,04 mg/kg (initial) and 0,012 mg/kg (final) concentrations. HT-2 toxin was also detected in 0,489 mg/kg concentration in the initial and in 0,016 mg/kg concentration in the final forage.

Samples were collected before forage swap (21 days of age) and at the end of the fostering period (39 days of age). Blood samples were collected from 10-10 individuals during extermination, then tissue samples from the liver, spleen, kidney and bursa Fabricii were taken.

Body weight, forage uptake, liver and spleen weight and weight of the bursa Fabricii was measured.

Findings and observations during dissection were noted.

MDA, GSH and protein concentrations and GSHPx activity was measured in the plasma, RBC haemolysate and liver, spleen and kidney homogenizate. Glucose and triglyceride content, AST, ALT and LDH activity of the plasma was also determined.

4. DISCUSSION

One of the main objectives was to study the effects of T-2 toxin on the forage uptake of broiler chicken. In the course of two week exposure, no feed rejection was observed to the used doses, so presumably other factors may also play a role when feed rejection occurs. In the course of previous observations describing feed rejection toxin concentrations multiple to the natural were used.

However, when long-term toxin load with lower T-2 toxin dose was used, body weight was lower than the control (Experiment 6) so animals could not compensate for the toxin load during the whole fostering period and its resulting differences, consequently, longer but lower toxin exposure could evoke symptoms of a short term, high level load.

Other clinical symptoms were also observed as a response to the used doses – except for the lowest, 1mg/kg dose. Mucosal burn, enteritis, diarrhoea, clay-yellow liver were also observed, which abated following toxin adsorbent (yeast cell wall extract) or antioxidant (Vitamin E, selenium) treatment, their combination or vaccination under the given research conditions.

Atrophy of Bursa fabricii, as a general indicator of immune-system damage in accordance with literature could not be proven.

Occasionally, T-2 toxin had a significant effect on some lipid peroxidation and biological antioxidant system parameters. It was found, that if the examined parameters were not statistically verifiable, then T-2 toxin effect on the tendencious change of certain parameters would refer to the overload of the given process.

Change of biochemical parameters differed also in direction and degree in the examined tissues. Moderate lipid peroxidation inducing effect of T-2 toxin was detected in the liver at 2,05 mg/kg dose.

Trichotecene mycotoxins could induce free radical production burdening the biological antioxidant system. This kind of oxidative stress was observed along with the change of vitamin C and E levels, as T-2 toxin reduces the amount of these antioxidant vitamins.

Results also pointed out that high dose antioxidant (Vitamin E, selenium) supplementation to improve antioxidant status could favour to lipid peroxid and antioxidant status of poultry even if exposed to sublethal doses of T-2 toxin. Supplementation with a proposed toxin-binding substance (yeast cell wall extract) to bind trichotecene mycotoxins could moderately improve T-2 toxin induced lipid peroxidation and its effects on the investigated components of the antioxidant system.

It could also be concluded that the applied toxin adsorbent helps to reduce simultaneous effects of T-2 toxin and its natural metabolites, confirmed by histopathological observations. Significant changes were observed in the systemic metabolism and transport of vitamin E as a result of antioxidant (vitamin E and selenium) supplementation and T-2 toxin load. Vitamin E content of the liver was much lower in chicken subjected to vitamin E and selenium supplementation or T-2 toxin load along with vaccination, compared to those subjected exclusively to vitamin E and selenium supplementation, probably as the result of enhanced free radical production reducing the level of vitamin E, a small molecular weight antioxidant.

I also concluded that T-2 toxin induced remarkable stress reactions in birds, reducable under practical conditions by the toxin adsorbent or antioxidant supplementation or by the combination of these two, this latter one being the most effective.

As the result of low T-2 toxin supplementation during the whole fostering period (39 days), lipid peroxidation processes and the load of antioxidant protecting system increased after 21 days. Significant, non-eliminable effects of T-2 toxin manifested in young birds may account for the continuous effect of the toxin, applied in different doses.

Along with the change of lipid peroxidation and antioxidant parameters moderate change in the carbohydrate and lipid metabolism, probably controlled by other protection systems.

T-2 toxin applied for a longer period of time damaged the kidney more than the liver according to the results (MDA concentration), probably due to the increased excretion of harmful metabolites through the kidney. Still, in case of longer T-2 load, liver remained the major indicator in studying the effects of toxin load, as it was seen in previous investigations of shorter, about two weeks time. These results show that 6-week, low-dose toxin load under certain conditions could even cause more serious damage than toxin load applied for two weeks in a higher dose.

It could also be appointed that certain tissues and organs react differentially to toxin load as well as to vaccination. In this respect, it was the liver that responded first and the most intensely, according to the results of all the examined parameters. On the basis of these, the examined organs could be ranked according to toxin sensitivity.

- In respect of GSHPx activity:
liver>rbc (red blood cell).>kidney>plasma>spleen,
- In respect of GSH:
liver>rbc (red blood cell).>plasma>kidney>spleen

The intensive GSHPx synthesis in the tubular system of the kidney may account for the different rank of the kidney and the plasma, as the GSHPx is transported to the plasma, while GSH production of the kidney is not considerable, but its metabolism is fast.

The results suggest that changes in the Vitamin C levels could be used as stress indicators since T-2 toxin load reduced vitamin C levels of the plasma.

One of the main objectives was to examine the effect of T-2 toxin load on broiler chicken immune response ability during repeated vaccination against Newcastle disease by measuring antibody formation. Furthermore the effect of vitamin E simultaneously loaded with sublethal T-2 toxin on the lipid peroxid and antioxidant state in the given tissues of broiler chicken during antioxidant supplementation was also examined.

I concluded that medium dose (2,05 mg/kg feed) T-2 load did not influence significantly the immune capacity during repeated vaccination against Newcastle disease

I also observed that the antioxidant status declined when T-2 toxin and vaccination were applied simultaneously. It could be first noticed in the kidney, while lipid peroxidation processes intensified in the kidney and in the spleen too.

Detected antibody levels obviously show that antioxidant supplementation stimulates, while T-2 toxin inhibits immune capacity.

Whereas, antibody levels (HAG titer) show that antioxidant supplementation has positive effects while high-dose T-2 toxin (4mg/kg forage) reduces antibody production. Therefore the effectiveness of repeated vaccination against Newcastle disease along with T-2 toxin load could be improved by high-dosage Vitamin E or selenium supplementation.

Whereas absolute/relative changes in the weight of bursa Fabricii and histopathological observations did not indicate immune system damage unambiguously.

5. NEW SCIENTIFIC RESULTS

1. I demonstrated that T-2 toxin does not influence the forage uptake of 2-week-old broiler chicken neither in purified form (2,12 mg/kg forage) nor with its metabolites (0,88-4,48 mg/kg T-2 toxin + 0,015-0,035 mg/kg HT-2 toxin). Toxin load applied in different doses (Day 1-21: 1,04 mg/kg T-2 + 0,48 mg/kg; Day 22-39: 0,012 mg/kg T-2 toxin + 0,016 mg/kg HT-2 toxin) during the whole fostering period reduced body weight gain despite forage uptake approximately equal to the control.
2. I concluded that antioxidant supplementation (8,75 mg vitamin E + 0,02 mg selenium / individual /day) provided through drinking water, yeast cell wall extract (2 g/kg forage) mixed to the forage or the combination of these two could reduce clinical symptoms (enteritis, diarrhoea, clay-yellow liver) induced by T-2 toxin + HT-2 toxin (1 mg/kg forage), the latter one being the most effective.
3. I found that T-2 + HT-2 toxin contaminated forage induced lipid peroxidation and loaded the glutathion system in the kidney and liver of broiler chicken.
In short-term (7-14 days) experiments toxins applied in 0,88-4,48 mg/kg dose induced tendencious change in 3-5-week-old chicken, whereas in case of long-term toxin load applied in 1 mg/kg dose from day-age, changes could be confirmed statistically. Short-term toxin load combined with antioxidant supplementation and/or yeast cell wall extract feeding, especially the combination of these two could reduce the glutathion redox system response significantly.
4. Studying the effects of antioxidant (vitamin E + selenium) supplementation I observed that T-2 + HT-2 toxin load affects the systemic transport of vitamin E.
5. I found that 2,05 mg/kg forage dose of T-2 toxin did not, while 4,48 mg/kg dose significantly decreased immune response ability during repeated vaccination against Newcastle disease, reducible by simoultaneously applied antioxidant (vitamin E + selenium) supplementation.

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