Hungarian University of Agriculture and Life Sciences

Investigation of the effects of important mycotoxins on DNA and the expression of certain elements of damage repair enzyme systems in chicken and common carp

Rubina Tünde Szabó

Gödöllő

2022

Name of the doctoral school:	Doctoral School of Animal Biotechnology and Sciences
Branch of science:	Animal sciences
Head:	Dr. Miklós Mézes full professor, member of the Hungarian Academy of Sciences Hungarian University of Agriculture and Life Sciences, Szent István Campus, Institute of Biology and Feed Science, Department of Feed Safety
Supervisors:	Dr. Mária Kovács-Weber associate professor, Ph.D. Hungarian University of Agriculture and Life Sciences, Szent István Campus, Institute of Animal Husbandry, Department of Animal Production Technology and Animal Welfare Dr. Balázs Kovács senior research fellow, Ph.D. Hungarian University of Agriculture and Life Sciences, Szent István Campus, Institute of Aquaculture and Environmental Safety, Department of Molecular Ecology

.....

approval of the head of school

.....

approval of the supervisor

.....

approval of the supervisor

1 Introduction

Mycotoxins, the secondary metabolites of microscopic fungi, can be found in the area of agricultural fields, nutrition and feeding. The literature about their effects is widespread like degradation of performance and other non-specific symptoms in farm animals. The well-known effect of mycotoxins (T-2, DON, aflatoxin and sterigmatocystin) is oxidative stress but little is known about its connection with DNA damage. The DNA repair mechanisms are responsible for the specific repair of DNA damages with several pathways. Little information is available about the relationship between DNA damage and DNA repair mechanisms; therefore, these processes should be investigated together to get accurate information on the molecular level. DNA is the building block of life, the basis for maintaining life, and it is essential for the development of biological functions. Any modification or alteration of the DNA can lead to an interruption of the continuity of life, a change in the genetic information. Damage to DNA can be caused by internal changes such as ageing, or by external influences such as xenobiotics, chemotherapeutic agents or mycotoxins ingested from feed and food (Dizdaroglu 2012). A commonly used method for testing genotoxic substances is the comet assay, which can be applied to almost all species and tissue types. In addition to its advantages, it has a number of disadvantages and limiting factors, which I have encountered in my examinations (Klaude *et al*. 1996, Olive & Banáth 2006). LORD-Q PCR was originally developed for the analysis of human cell lines. My aim was to adapt and use this procedure to study mycotoxin-induced DNA damage in chicken and carp species.

The body struggles to repair DNA every day, as it is estimated that 10.000 DNA damages occur in a cell every day even under natural conditions (Carusillo & Mussolino 2020). The DNA repair system is responsible for repairing and eliminating this damage, through a number of pathways that carry out damage-specific repair.

Our understanding of the relationship between DNA damage and the mechanisms that repair it is rather incomplete, although a combined study of these processes can provide an accurate picture of the changes that occur at the molecular level. Thus, I have made it a priority to explore these relationships through the objectives set out in my investigations.

1.1 Objectives

The objective of my doctoral research was to estimate the DNA damaging effect of DON, T-2, aflatoxin B1 and sterigmatocystin mycotoxins in common carp and of T-2, aflatoxin B1 and sterigmatocystin mycotoxins in chicken.

My further aim was to adapt and apply an objective, rapid PCR based method for both species and compare the application of it with comet assay in chicken species after the adaptation.

My purpose was to evaluate the change of the DNA repair mechanisms in the case of mycotoxin contamination. For this reason, I selected DNA repair genes for common carp and chicken species; the real-time PCR reactions were optimized, and the most perfect housekeeping gene was identified.

In chicken species, my further goal was to detect the liver pathology changes by aflatoxin B1 and sterigmatocystin.

2 Material and methods

2.1 Experimental design in chicken – comet assay

T-2 toxin was produced under controlled laboratory conditions on corn grits by F. sporotrichoides NRRL 3229 strain by the method of Fodor *et al*. (2006). The animals were fed with commercial broiler starter feed. T-2 toxin-contaminated corn grit was mixed with the feed before the trial. Measured mycotoxin content of the experimental diets were control <0.02 mg/kg, THT1 group 0.127 mg T-2 + 0.088 mg HT-2/kg, THT2 group 0.235 mg T-2 + 0.149 mg HT-2/kg, THT3 group 0.989 mg T-2 + 0.57 mg HT-2/kg. A total of 20 Cobb 540 male broiler chickens at 14 days of age were investigated in four groups.

2.1.2 Comet assay

Liver samples were diluted with phosphate buffer solution. Hydrogen peroxide (10 µl, 3% v/v) was used to create the positive control samples. First, the slides were covered with 0.8% (w/v) normal melting point agarose. When the first layer had solidified 1% (w/v), low melting point agarose was spread on the slides, diluted with the same volume of cell suspension. This layer was allowed to solidify onto the slides by placing them on melting ice for 5 min. Finally, the third layer of 0.5% (w/v) low melting point agarose gel was added to cover the second layer and again, allowed to solidify for 5 min. Slides were immersed to a freshly prepared lysis solution at 4 °C for at least 1 hr. The slides were transferred to an electrophoresis tank filled with electrophoresis buffer After the electrophoresis (20 V, 300 mA, and 24 min), the slides were rinsed with a neutralizing solution. Each slide was stained with 50 µl of ethidium bromide (0.05 mM) and was covered with a coverslip. Digital images of the slides were made by a QCapture MicroPublisher camera attached to a fluorescent microscope equipped with a 20× magnification objective. At least 150 cells were analysed in each control and treated group. DNA damage was evaluated by CometScore software (TriTek, Tritek Corporation, VA, USA), and percent DNA in the tail was used as the principal parameter. Comets were evaluated individually on a 0–4 scale during the visual analysis, based on the quantity of DNA fragments in the tail of the comet. At least 100 cells in each group were evaluated as was proposed by Horvatovich *et al*. (2013).

2.2 Experimental design in common carp – LORD-Q

T-2 toxin was produced by Fusarium sporotrichioides (NRRL 3299) and DON by Fusarium graminearum (NRRL 5883) strains on corn substrate by the methods of Fodor *et al.* 2006. The animals were fed with extruded, slowly sinking growth feed for carp (Aqua *Garant* Classic™). The mycotoxin concentrations of the feed were: control <0.02 mg/kg, T-2 mycotoxin group 4.11 mg T-2 + 0.49 mg HT-2/kg, DON group5.96 mg DON + 0.33 mg 15-acetil DON/kg. One-year-old

common carps (*Cyprinus carpio* L.) (n = 108) were obtained. The sampling was from *hepatopancreas* weekly (week 1,2 and 3).

2.3 Experimental design in common carp – gene expression

Aflatoxin was produced in ground corn, which was artificially infected with an aflatoxin producing Aspergillus flavus strain (ZT80), isolated by Dobolyi *et al*., 2013. Sterigmatocystin was purchased from Romer Labs Diagnostic GmbH with high purity (99.0±1.0%). aflatoxin-containing fungal culture was mixed with ground and extruded growth feed for carp (GARANT Aqua Classic™). The applied doses were A group 0.4 mg aflatoxin B1/kg, Ster1 group 1 mg sterigmatocystin/kg, Ster2 group 2 mg sterigmatocystin/kg, Ster4 group 4 mg sterigmatocystin/kg. One-year-old common carps (*Cyprinus carpio* L.) (n = 96) were obtained. *Hepatopancreas* were collected after 8, 16 and 24 hours of dosing.

2.4 Experimental design in chicken– gene expression

The aflatoxin B1 was produced with *Astergillus flavus* strain (ZT41), the sterigmatocystin was produced with *Aspergillus creber* 2663 train (Dobolyi *et al.* 2013). Sterigmatocystin was purchased from Romer Labs Diagnostic GmbH with high purity (99.0±1.0%). ™). The applied doses were AFLATOXIN group 149.1 µg aflatoxin B1/kg, STER group 1590 µg sterigmatocystin / kg, USTER group 1570.5 µg sterigmatocystin /kg. Sampling was on Day 1, 2, 3, 7. and 14.

2.5 Gene expression experiments

2.5.1 RNS isolation, cDNA synthesis

Hepatopancreas samples were extracted using Trizol reagent. The integrity and quality of the RNA samples were verified by agarose gel electrophoresis. The complementary DNA (cDNA) was performed with RevertAID reverse transcriptase and random hexamer.

2.5.2 qRT-PCR experiment

In common carp experiment, EF1α, β-actin, 40S (Hermesz & Ferencz 2009, Mráz 2012) control genes and four target genes were selected: OGG1 (Mustafa *et al*. 2015), HSP70 (Stolte *et al*. 2009), GADD45AA and genes (Primer3Plus). The initial incubation temperature was 95^{o}C for 12 min. This was followed by 40 cycles at 94^{o}C 10s, 55^{o}C 20s, 72^{o}C 30s for HSP70, at 94^{o}C 10s, 57^{o}C 20s, 72^{o}C 30s for GADD45 and p53, at 94^{o}C 10s, 60^{o}C 20s, 72^{o}C 30s for OGG1, EF1α, β-actin and 40S. In chicken experiment, there were 6 target genes (RAD51, REV1, BRCA2, XPA, GADD45, MSH6) and three control genes (GADPH, β-actin, UB) were involved. The initial incubation temperature was 95^{o}C for 12 min. This was followed by 40 cycles at 94^{o}C 10s20mp 59^{o}C, 30mp 72^{o}C for RAD51, REV1, BRCA2, XPA, GADD45, MSH6, GADPH and β-actin.

After the 95ºC for 12 min incubation, it was followed by 40 cycles denaturisation, then throughout 40 cycles10s at 94ºC, 20a at 61ºC and 30s at 72ºC for UB.

PCR reactions were carried with StepOnePlus™ Real Time PCR System. The PCR reaction mix was conducted using 40 ng of cDNA as a template, 5x Hot FirePol® EvaGreen® qPCR Supermix reaction mixture, 6.6 pM/µl primers in 10 µl final volume per reaction. The threshold cycle (Ct) of genes were determined. The relative quantification was analysed by the Pfaffl method (Pfaffl, 2001).

2.6 LORD-Q PCR

DNA was purified using MagAttract® HMW DNA Kit. The isolated DNA showed high purity (A260/A280 > 1.8). The LORD-Q PCR was carried out in a StepOnePlus™ Real Time PCR System. The PCR reaction mix consisted of 5× Hot FirePol® EvaGreen® qPCR Supermix reaction mixture, 3.3 pM/µL primers and 10 ng of template DNA in a total volume of 10 µL per well. The cycling conditions were as follows in the common carp experiment: a pre-incubation phase of 95 °C for 12 min was followed by 40 cycles of 10 s at 94 °C, 20 s at 59 °C, and 30 s at 72 °C (small fragment) or 4 min at 72 °C (large fragment). The cycling conditions were as follows in chicken experiment: a pre-incubation phase of 95 °C for 12 min was followed by 40 cycles of 10 s at 94 °C, 20 s at 59 °C, and 30 s at 72 °C (small fragment), and a pre-incubation phase of 95 °C for 12 min was followed by 40 cycles of 10 s at 94 °C, 20 s at 60ºC, 3min 40s at 72ºC (large fragment). The DNA damage was calculated for lesion per 10 kb DNA by including the size of the long fragment as described by Rothfuss *et al*. 2010.

2.7 Experimental design in chicken - preparation and evaluation of histological sections

Three liver samples per group from the same anatomical site were subjected to histological examination, and the sections were prepared at the Department of Pathology of the University of Veterinary Medicine. Sections of 3-4 µm thickness were made from the paraffin blocks. Histological analysis was performed by light microscopy after hematoxylin-eosin staining for primary evaluation of the sections. Sections were scanned using a Panoramic MIDI II (3DHistech) scanner with CaseViewer software (3DHistech), which allows for further magnification and image capturing.

2.8 Statistical analyses

The statistical analysis was carried out using the R 3.6.2. software package (R Core Team, 2013). In the LORD-Q and gene expression experiments, the different groups were compared with one-way ANOVA tests followed by Tukey post hoc. The DNA% in tail values of groups after comet assay were compared with non-parametric Kruskal–Wallis test with Tukey and Kramer (Nemény) test. A level of significance was $p \leq 0{,}05$.

3 Results and discussions

3.1 Comet assay results – chicken

When evaluated with CometScore® programme, a significant difference was found between the control and positive control groups, and between the control (K) and THT1 and THT2 treated groups. The THT1 group had the highest DNA % and was significantly different from the two controls and the other two treated groups. In visual evaluation, the control group was statistically different from the positive control and all three treated groups. The THT2 group had the lowest score of the treated groups, which was significantly different from the THT1 and THT3 groups. Rezar *et al*. (2007) did not detect significant DNA fragmentation in chicken spleen leukocytes at a dose similar to that of the THT3 group in my experiment (1.5 mg T-2/feed kg). However, a higher DNA % value was measured in the tail (16.75) than the THT3 group using CometScore evaluation. In contrast, significantly different DNA fragmentation was observed between the THT1 (0.215 mg T2+HT-2/feed kg) and THT2 (0.384 mg T-2+HT-2 toxin/feed) groups in my experiments.

3.2 LORD-Q PCR results - carp

At the first sampling, the number of lesions was significantly increased in both treated groups compared to the control ($p<0.001$). There was a significant difference between the DON and T-2 mycotoxin treated groups, with the same trend between the treated groups and the control at week 2. At week 3, there was no significant difference in the amount of lesions between treated and untreated groups ($p=0.164$). When changes over time were monitored, the lesion volume measured at week 1 significantly decreased by week 2, which was no longer statistically different from the lesion volume at week 3 for the T-2 mycotoxin-contaminated group. For the DON mycotoxin group, the lesion values for week 1 and week 3 were statistically different, with a stepwise decrease in lesion values over time.

3.3 Gene expression results - carp

In the case of the **OGG1 gene**, the gene expression values of none of the groups tested were significantly different at 8 h compared to the control group. The Afla group had the highest gene expression value at hour 16. At hour 24, the gene expression values of all four experimental groups were significantly different from those of the control and from each other. In my study, no significant OGG1 expression change was observed at either hour 8 or 16 in response to aflatoxin B1 or sterigmatocystin treatment. Liu *et al*. (2018) also showed this result at 0.01 μM AFB1 concentration in human liver cell lines. Then at 24 h, our results showed a significant increase in expression in the Afla and Ster4 groups.

Guindon-Kezis *et al*. (2014) also showed a significant increase in relative OGG1 protein levels following 50 mg/kg AFB1 administration in both mouse lung and liver tissue.

An increase in **HSP70 gene** expression was detected at 8 h in Ster1 and Ster2 groups, and at 16 h in Afla and Ster4 groups, respectively. Also, an increase in the expression of this gene was found in *Trachyderma hispida* specimens from textile area by El Gendy *et al*. (2020). My gene expression results measured at 24 h showed a significant increase in all groups. Kócsó *et al*. (2021) showed a significant increase in HSP70 protein in liver tissue at 24 h after treatment with 150 µg/rat/day zearalenone, 150 µg/rat/day fumonisin B1 and 30 µg/rat/day DON alone and in combination.

When **p53 gene** expression was examined at 8 h, only the Ster1 group showed a significant increase in expression compared to the control group. No significant difference was detected at hour 16. Then, at hour 24, an increase in gene expression was detected in the Afla and Ster4 groups, respectively, and a significant decrease was detected in the Ster1 and Ster2 groups compared to the control. The gene expression of HSP70 and p53 were similar in our results at 8 and 24 h, respectively, confirming the finding of Yang *et al*. (2009) that HSP70 is part of the p53 DNA repair pathway.

A significant decrease in **GADD45AA gene** expression was observed in the Afla group at hour 8. At hour 16, there was no significant change, while at hour 24, all treated groups were significantly different from the control. For carp, an increase in expression was observed in the Ster4 group at 8 h, but this did not prove to be statistically significant. An increase in gene expression was measured at hour 24 except for the STER1 group. Li *et al*. (2013) also showed an increase in GADD45α expression at 1000 ng/ml DON concentration in mouse thyroid cells.

3.4 LORD-Q PCR results- chicken

On Day 1, Day 2 and Day 7, all three treatment groups were significantly different from the control ($p<0.0001$) and from each other. The aflatoxin B1 treated group had the most lesions on Day 1, while the sterigmatocystin treated group had the most lesions on Days 2 and 7. On Day 3, the USTER group was significantly different from the control and the STER and AFLATOXIN groups. The control was statistically different from the STER and AFLATOXIN groups, which were not significantly different from each other. On Day 14 sampling, the control group was not significantly different from the STER group ($p<0.166$) but was statistically different from the USTER and AFLATOXIN groups ($p<0.001$).

When comparing the toxicity of aflatoxin B1 and sterigmatocystin, the highest lesion number was measured on Day 1 in the AFLATOXIN group. A similar result was obtained by Abd-Allah *et al*. (1999) who found significant DNA damage by comet assay in response to aflatoxin B1 (0.5 mg/kg body weight) at 24 h in rainbow trout liver.

.

3.5 Gene expression results - chicken

Regarding the **RAD51 gene**, the STER and USTER groups were significantly different compared to the control group, and the STER group had significantly higher gene expression levels compared to the aflatoxin B1 contaminated group on Day 1. No statistical difference was detected between the groups on Day 2. On Day 3, compared to the control group, the STER group had a significantly lower value, while the USTER and AFLATOXIN groups had a significantly higher value, with statistically demonstrable differences between the treated groups. By Day 14, all treated groups showed a decrease in gene expression, which was significant for the STER and USTER groups. The results of Huang *et al*. (2020) suggest inhibition of transcription of the DNA repair genes tested, whereas in my study, the doses used likely enabled DNA repair by increasing repair gene expression.

For the **REV1 gene**, the AFLATOXIN group was significantly different from the other two treated groups and from the control group on Day 1. On Day 2, a decrease in expression was detectable in the treated groups, which was significant for the USTER and AFLATOXIN groups. In contrast, on Day 3, there was a statistically demonstrable increase in expression in the USTER and AFLATOXIN groups compared to the control and STER groups, respectively. The STER group was significantly decreased compared to the control and the other two treated groups. On Day 7, the gene expression values in the USTER group showed a similar trend to the previous day, but a significant increase in the STER value and a significant decrease in the AFLATOXIN group gene expression compared to the control. All treated groups showed a statistically proven decrease in gene expression compared to the control group. Dumstorf *et al*. (2009) also detected a decrease in REV1 gene expression in mouse fibroblast cells after 48 hours of treatment with benz(a)pyridine (0.150 μmol/L).

BRCA gene expression was significantly increased in the USTER and AFLATOXIN groups compared to the control and STER groups on Day 1, respectively. There was no statistically detectable difference between groups on Day 2. The gene expression values of the treated groups showed a significant decrease compared to the control, with the USTER group showing a significant difference in BRCA gene expression compared to the other two treated groups. On Day 7, the expression values of the STER and USTER groups showed a significant increase, while the AFLATOXIN group showed a significant decrease, and the treated groups were statistically different from each other. On Day 14, a significant decrease in gene expression was seen in all treated groups compared to the control. Moradi *et al*. (2015) presented data showing a decrease in expression in human cell line (HMEC) after 24 h AFB1 (15, 25, 35 μl) treatment.

XPA gene expression was significantly decreased in USTER and AFLATOXIN groups compared to control and STER groups, respectively. On Day 2, no differences were found between groups similar to the results for RAD51

and BRCA genes. On Day 3, all treated groups were significantly different from each other, but only the USTER and AFLATOXIN groups were significantly different from each other compared to the control. On Day 7, the AFLATOXIN group showed a statistically proven decrease in gene expression compared to the other two treated groups and the control groups. Similar to the gene expression shown so far, XPA expression was also reduced in all treated groups compared to the control on Day 14. The expression of the XPA gene was very similar to that of the GADD45α and MSH6 genes. The large decrease in gene expression after Day 3 may be due to a reduction in DNA damage, but in the case of aflatoxin B1, it is possible that DNA repair mechanisms are inhibited (Shen & Ong 1996).

The expression of the **GADD45 gene** was significantly increased only in the AFLATOXIN group compared to the other two treated and control groups. On Day 2, only the AFLATOXIN group differed significantly compared to the control, with the STER group having the highest expression compared to the other two treated groups. There was a significant increase in the expression of the GADD45 gene in the treated groups compared to the control, with all three treated groups differing. By Day 7, gene expression levels had decreased, with the USTER and AFLATOXIN groups statistically different from the control. Unlike the other genes tested, only the STER group showed a significant decrease in expression on Day 14. On Day 1, an increase in GADD45α expression was only measured in response to applied aflatoxin B1 contamination, similar to the results of Ayed-Boussema *et al.* (2008), where an increase in expression of the gene was observed in human hepatocyte cell lines after 24 h in response to zearalenone (80 μM).

No detectable difference in the expression of the **MSH gene** compared to the other genes tested was observed compared to the control group on Day 1. On Day 2, only the AFLATOXIN group was significantly different from the control. On Day 3, the USTER and AFLATOXIN groups had significantly increased MSH gene expression compared to the control. The three treated groups were also statistically different from each other. A decrease in the AFLATOXIN group and an increase in the STER and USTER groups were statistically proven. With the exception of the GADD45 gene, the expression of all three treated groups was significantly lower than the control, similar to the other genes tested.

3.6 Histopathological results of chicken species

In the liver samples of the control group taken on Day 1 the hepatocytes with intact parenchyma were isolated and the cellular compartments were intact. Exceptionally, lymphocytic-plasmacytic inflammatory foci were visible. The STER group showed severe regressive liver degeneration with the presence of lymphocytic-plasmacytic inflammatory foci. The USTER group had histological findings similar to the STER group, but also showed signs of dilatation (dilated blood vessels). The AFLATOXIN group also showed severe diffuse hepatocellular damage and enlargement. Liver samples on Day 2 showed normal liver cell lines and intact cells in the control group. Samples from the STER group

were characterized by moderate fatty infiltration, mild to moderate regressive liver degeneration. The histological results of the USTER group were similar to those of the STER group. Moderate degrees of fatty infiltration, severe regressive hepatic cell change and enlargement were found in liver samples from the AFLATOXIN group. On Day 3, liver samples from the control group showed no lesions. The STER group also showed mild fatty and vacuolar degeneration. In the USTER group, moderate diffuse hepatocellular damage was observed. Moderate to severe diffuse hepatocellular injury was present in liver samples from the AFLATOXIN group. On Day 7, mild to moderate regressive liver degeneration and lymphocytic-plasmacytic inflammatory infiltration characterized liver samples in the A STER group. In the USTER group, all liver samples showed a moderate degree of regressive liver degeneration. The histological results of the AFLATOXIN group were in agreement with those of the STER group. On Day 14, the STER and USTER groups showed the same degree of lesions on liver cells with extensive lymphocytic infiltration. Liver samples from the AFLATOXIN group showed moderate to severe regressive liver degeneration.

4 Conclusions and recommendations

4.1 Comet assay examination – chicken species

The standard protocol for the comet assay recommends a dilution of the cells to be tested of 1:1 or 1:10 (Devaux *et al*. 1997). Sokolovic *et al*. (2007) have established a dilution of 1:200 for chicken blood samples to achieve the most optimal cell density. Nevertheless, in my preliminary experiments I considered a dilution of 1:100 to be appropriate for chicken liver samples.

The lowest dose used in the experiment (0.215 mg T2+HT-2/feed kg) was close to the maximum recommended dose in the European Union (2013/165/EU) and half of the tolerable limit for poultry (hen species) as recommended by Eriksen & Pettersson (2004). At this dose, more than 4% fragmented DNA was found in the caudal section of the liver of chickens with high variance. In the THT2 group (0.384 mg T-2+HT-2 toxin/kg feed), the adverse effect in terms of DNA fragmentation was significant. However, the highest dose THT3 group (1.559 mg T-2+HT-2 toxin/feed kg) did not differ significantly from the control group in terms of DNA fragmentation, as expected, when evaluated by CometScore software. The visual evaluation provided more consistent results with lower standard deviation values compared to the selected software evaluation, with all three treated groups scoring significantly different from the control group.

As far as I know, the effect of mycotoxins on DNA damage in broiler chicken liver has not been investigated by comet assay. The 1:100 cell dilution I determined proved to be appropriate, as it allowed me to avoid overlapping of comets due to the high density, which would also hinder visual evaluation. By adjusting the optimal cell density, the method could also be applied to chicken liver. The choice of the method of evaluation is essential to draw the right conclusions. In the case of software-based manual evaluation, the evaluator needs to mark and separate the parts of the comet (head and tail). In the case of the highest dose group (THT3), it was not feasible to separate and distinguish the comet parts, as only the tail part of the comet was visible in most cells. As a consequence, the evaluation by CometScore software could not be fully adequate in this group. Knowing this, I had to choose another evaluation method to be able to judge the results correctly. I have chosen the visual evaluation because it does not have the limiting factors mentioned above (Horvatovich *et al*. 2013). Both methods demonstrated that T-2/HT-2 mycotoxin has DNA damaging effects, which can be detected at a dose as low as 0.215 mg T2+HT-2/feed kg.

4.2 LORD-Q PCR examination- carp

I have successfully adapted the LORD-Q PCR technique to carp species to objectively monitor the DNA damaging effects of individual xenobiotics.

Several parameters such as GST, GPx, GR, catalase or TBARS levels are indicators of oxidative stress, and their elevated levels indicate a state of oxidative stress, one of the consequences of which may be DNA damage. Deng *et al*. (2019) showed statistically proven increased levels of GST in tilapia on Days 16 and 20 when fed T-2 mycotoxin (10.8 mg/kg). Matejova *et al*. (2017) obtained significantly elevated TBARS, catalase and GST levels in both carp liver and kidney on Day 28. Compared to these results, in my own experiments in carp, the lesion values of the T-2 treated group were significantly elevated on Days 7 and 14, followed by a smaller non-significant difference from the control group on Day 21.

My experiment with carp showed that both trichothecene mycotoxins tested form DNA lesions during prolonged exposure (1-2 weeks), but that this exposure is no longer able to significantly affect DNA by Week 3. This may be due to DNA repair mechanisms that are activated by DNA damage, which may be inhibited during the first 2 weeks or unable to keep up with the level of damage, but then become more effective by Week 3. Further DNA repair genes or proteins would need to be tested to prove this.

4.3 Gene expression examination- carp

In the case of the **OGG1 gene**, AFB1 is able to significantly increase 8-OH-Gua and 8-hydroxycytosine (Guidon-Kezis *et al.,* 2014), which triggers BER repair processes, of which OGG1 is a part. This correlation may also be behind my results, which will require future measurements of 8-OH-Gua and 8-oxoG from the tissue samples tested to prove this. Significant expression increase was seen at hour 24, thus the formation of these lesions in carp species reaches a critical level only after one day, at which the expression of OGG1 gene is increased to repair the DNA damage that is formed. The difference in the effect of AFB1 was shown, significant increase was observed in Afla and Ster4 groups compared to control and STER1 and STER2 groups. The two additional doses of sterigmatocystin resulted in a significant decrease in OGG1 expression, thus presumably failing to produce substantial amounts of lesions, and hence DNA damage.

An increase in **HSP70** gene expression was measured at all time points in all treated groups, which was significant with a few exceptions. There was a tendency for the expression of Afla and Ster4 groups to be identical, so that the tenfold difference in effect of AFB1 versus sterigmatocystin was not clearly demonstrated for this gene. AFB1 or sterigmatocystin exerted their DNA damaging effects after 8 hours, as suggested by the increase in expression of the HSP70 gene. To clarify the extent of this relationship, it will be necessary in the future to study both the amount of lesions and the expression of repair genes. In my studies, over time, the Afla group showed a significant increase in HSP70 gene expression by hour 24, whereas in the Ster4 group gene activation occurred only at hour 16. During this time course, AFB1 can develop a more prolonged

damage that persists over time, in response to which gene expression shows a prolonged increase over time.

P53 plays a critical role in the development of the cellular stress response and is thus associated with several genes. These include OGG1, which regulates its transcription levels. This interaction is supported by my results. HSP70 is also involved in the repair of p53 DNA, which is supported by my results, and in the future, it will be necessary to study these genes together. One of the regulators of p53 is the GADD45α gene, which is supported by my results and by data from Ayed-Boussema *et al*. (2008), so this needs to be investigated further. AFB1-epoxide can create mutations in the p53 gene sequence, which has been demonstrated in zebrafish (Santacroce *et al*. 2008), and it may be worthwhile to follow up on this by investigating the negative regulator of p53, the mdm2 gene. The interaction of p53 with MSH2 in mismatch repair has been demonstrated (Williams & Schumacher 2006). Accordingly, it would be important to extend my studies to the MSH2 gene in the future to infer the DNA damaging effects of individual xenobiotics more accurately. In my experiments, the difference in effect of aflatoxin B1 was shown to be greater than tenfold at hour 24, but a tenfold difference in effect of aflatoxin B1 and sterigmatocystin was shown at hours 8 and 16.

GADD45α is regulated by p53, a correlation supported by my results. Another regulator is BRCA1, so it would be important in the future to study this gene, which can both activate and inhibit GADD45α. Significant expression changes at hour 8 occurred only in the Afla group in the negative direction, but at hour 24 in the Ster2 group, an increase in expression was detected. Presumably, the increased DNA damage in these groups had already reached a level at which activation of GADD45α was required. Li *et al.* (2013) found a link between GADD45α and mdm2, so it would be beneficial to include the mdm2 gene in my future studies not only on the p53 side but also through GADD45α.

4.4 LORD-Q PCR examination- chicken

I have successfully adapted the LORD-Q PCR technique to chicken species to objectively monitor the DNA damaging effects of individual xenobiotics.

Balogh *et al.* (2019) found an activation of the glutathione redox system in broilers under the same mycotoxins and doses, mainly due to the effect of AFB1. For the genes tested (GSS, GPx4, GSR), AFB1 was under-expressed on Day 1, followed by an increase in expression over time and a decrease again by Day 14. In contrast, the amount of lesions I measured was highest on Day 1, indicating a state of oxidative stress, and then a decrease on Day 2 could be due to the efficiency of the antioxidant defence system. On Day 14, the AFLATOXIN group showed a significant increase in lesions in addition to the USTER group. Longer-term (12 weeks) AFB1 (100 μg/kg) feeding resulted in increased expression of GST and GPx genes in the treatment group, indicating a state of

oxidative stress (Bacou *et al*. 2021). Results obtained on Day 14 also suggest the development of oxidative DNA damage in response to both USTER and AFLATOXIN treatment. Future investigation will be needed to examine the individual members of the antioxidant defence system together to confirm this. Based on my results, a tenfold difference in the effect of aflatoxin B1 against sterigmatocystin in chicken is likely. In contrast, Schroeder and Kelton (1975) found a sixteenfold difference in toxicity in favour of aflatoxin B1 in chicken embryos.

4.5 Gene expression examination- chicken

RAD51 results show that gene expression follows the rate of DNA damage. The exceptions to this are Days 1-2 in the STER group, when gene expression decreased while lesion levels increased, and Days 7-14 in the USTER group, when I also found a decrease in expression with an increase in lesions. In this context, the RAD51 gene could effectively participate in the elimination of lesions. To confirm that two-stranded DNA repair by RAD51 also occurs in chicken species, future studies should be complemented with two-stranded DNA fragmentation markers such as the γ-H2AX protein.

In the case of **REV1**, gene expression and lesion abundance changes in the STER group moved predominantly together, and then on Day 14 there was a significant decrease in both gene expression and lesion abundance, suggesting that the gene had completed its task. In contrast, in the USTER group, these two parameters also moved together, but on Day 14, gene expression showed an underactivation and the number of lesions a significant increase, which could be due to gene depletion. In the AFLATOXIN group, the number of lesions decreased significantly by Day 2, and its level did not differ significantly over time in the following days but was always significantly higher compared to the control. In contrast, the REV1 gene was overexpressed on Days 3 and 7, with a significant decrease in gene expression on Day 14, suggesting gene depletion.

The expression of **BRCA2** and RAD51 were not correlated in my studies for Days 1-3, but their expression showed complete concordance on Days 7 and 14. On Day 7, this confirms the likely repair of double-stranded DNA breaks in the STER and USTER groups, homologous recombination. The AFLATOXIN group showed a decrease in expression and this trend was observed for all three groups on Day 14, suggesting gene depletion or inhibition. When comparing the amount of lesions, it can be seen that the expression of the BRCA2 gene in the AFLATOXIN group follows the change in the amount of lesions at all time points. In the STER group, this was realized only between Days 3-7 and 7-14, while in the USTER group, these two parameters described opposite trends.

In the STER group, the activation of the **MSH6** gene occurred on Day 7 and by Day 14 it significantly reduced the number of lesions formed, thus significantly decreasing expression. In the USTER group, the activation of the gene occurred as early as Day 3 and effectively reduced the number of lesions by

Day 7, but by Day 14, the gene was depleted due to an increase in the number of lesions due to the underexpressed state. In the AFLATOXIN group, this depletion occurred much more rapidly, with a large increase in expression on Day 3, but this had no effect on the number of lesions.

On Day 1, there was a significant increase in **GADD45α** expression in the AFLATOXIN group, which can be attributed to a significant increase in lesions, followed by a significant decrease in expression levels and number of lesions on Day 2. On Day 3, the gene expression increased significantly, but no significant increase in the number of lesions was detected. On Days 7 and 14, there was no change in the number of lesions, while the expression dropped below the control level on Day 7, indicating the exhaustion of the process, and reached the control level on Day 14. In the USTER group, changes in gene expression over time were consistent with changes in the number of lesions formed, whereas the same could not be said for the trends in the STER group. The results of Ayed-Boussema and colleagues (2008) also point to the linkage between p53 and the GADD45 gene, and future studies of the p53 gene in chicken species are needed.

In the STER group, **XPA** expression and lesion number followed each other only between Days 7-14. In the USTER group, the effective reduction in lesion number caused by XPA was achieved by Day 7, but gene depletion may have occurred on Day 14, as there was a significant increase in lesion number by Day 14. The AFLATOXIN group showed a decrease in lesion number by Day 2 due to a non-significant increase in expression, but the overexpressed state on Day 3 did not result in a further decrease in lesion number. On Days 7 and 14, depletion or possible inhibition of the XPA gene was evident in my study.

4.6 Histopathological results- chicken

On Day 14, both forms of sterigmatocystin showed mild lesions on liver cells with extensive lymphocytic infiltration. Solcan *et al*. (2013) in a pathological study of chicken liver fed 54 μg aflatoxin B1/kg body weight on Day 14 observed diffuse hydrocortical degenerative lesions, vascular sections with haematomas and the initial formation of new bile duct sections. Yang *et al*. (2012) found mild lesions in 83.3% of the livers examined and more severe lesions in 16.6% of the livers after 21 days of feeding 75% of the diet contaminated with aflatoxin. Similarly to these results, in my study I observed mild to moderate regressive liver degeneration in the AFLATOXIN group on Day 7 liver samples. On Day 14, the livers I examined showed moderate to severe regressive liver degeneration. My histopathological results and the data in the literature confirm that both sterigmatocystin and aflatoxin B1 predominantly induce lesion formation after several weeks of mycotoxin exposure, which may be associated with other lesions (fatty degeneration, inflammatory infiltration). The amount of lesions I examined was also significantly higher on Day 14 in the USTER and AFLATOXIN groups, so the cellular damage was not abrogated, as shown by the histological results.

5 NEW SCIENTIFIC ACHIEVEMENTS

1. I have successfully adapted the comet assay to the liver cells of chicken species and determined the optimal 1:100 cell density. I demonstrated the DNA damaging effect of mycotoxin T-2 by comet assay.
2. I successfully adapted and applied the LORD-Q PCR technique for the first time to carp and chicken species. I optimized PCR reactions for both the long and short DNA stretches.
3. I demonstrated that lesions were formed in the carp genome by DON and T-2 mycotoxins, but a decreasing trend in the number of lesions was observed over time for both mycotoxins.
4. I successfully designed and optimized detection procedures for OGG1, HSP70, p53, GADD45α genes in carp species, which were successfully applied to the impact of aflatoxin B1 and sterigmatocystin.
5. I successfully designed and optimized a detection procedure for RAD51, GADD45α, REV1, BRCA2, MSH6, XPA genes in chicken species, which were successfully applied in the aflatoxin B1, sterigmatocystin and purified sterigmatocystin impact assessment.
6. With few exceptions, aflatoxin B1 resulted in a predominant decrease in expression of the genes tested in both species and a significant increase in lesion number in the chicken species.
7. I observed that in carp species, sterigmatocystin had a variable effect on the expression of the genes tested, but no dose-dependent correlation was observed. In chickens, both naturally produced and purified sterigmatocystin were able to form lesions and affected the expression of the genes tested.
8. Histopathological studies showed that aflatoxin B1 caused greater lesions compared to sterigmatocystin. Natural and artificial sterigmatocystin caused similar levels of lesions.

9 PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

Scientific publications in journals:

1. Szabó, R. T., Kovács-Weber, M., Balogh, K. M., Mézes, M., & Kovács, B. (2021). Changes of DNA Damage Effect of T-2 or Deoxynivalenol Toxins during Three Weeks Exposure in Common Carp (Cyprinus carpio L.) Revealed by LORD-Q PCR. *TOXINS*, *13*(8). http://doi.org/10.3390/toxins13080576
2. Szabó, R. T., Kovács-Weber, M., Erdélyi, M., Balogh, K., Fazekas, N., Horváth, Á., … Kovács, B. (2019). Comet Assay Study of the Genotoxic Effect of T-2 and HT-2 Toxins in Chicken Hepatocytes. *BIOLOGIA FUTURA*, *70*(4), 330–335.

Publications in conference volumes:

1. Szabó, R. T., Kovács-Weber, M., Vlaskality, S. D., Balogh, K., Erdélyi, M., Mézes, Mi., & Kovács, B. (2019). DNS repair gének expressziójának vizsgálata aflatoxin, és szterigmatocisztin etetés hatására csirkében (Gallus gallus). In *VII. Gödöllői Állattenyésztési Tudományos Nap* (p. 43).
2. Szabó, R. T., Kovács-Weber, M., Kövesi, B., Balogh, K., Mézes, M., & Kovács, B. (2019). Mycotoxins induced DNA damage in common carp (Cyprinus carpio): investigations using LORDQ-PCR and DNA repair gene expression analyses. In *54th Croatian &14th International Symposium on Agriculture: Book of Abstracts* (p. 175).
3. R T, S., M, K.-W., Á, H., M, M., & B, K. (2018). Investigations on the effect of T-2 toxin on chicken liver cells with comet assay. In *17th Alps-Adria Scientific Workshop* (pp. 32–33).
4. Szabó, R. T., Kovács-Weber, M., Kövesi, B., Balogh, K., Mézes, M., & Kovács, B. (2018). DNS javító gének expressziójának változása aflatoxin, illetve szterigmatocisztin hatására pontyban (Cyprinus carpio L.) = Effect of aflatoxin and sterigmatocystin on DNA repair gene expression changes in common carp (Cyprinus carpio L.). In *XXXVII. ÓVÁRI TUDOMÁNYOS NAPOK "FENNTARTHATÓ AGRÁRIUM ÉS KÖRNYEZET AZ ÓVÁRI AKADÉMIA 200 ÉVE – MÚLT, JELEN, JÖVŐ" Összefoglalói* (pp. 94–94).
5. Szabó, R. T., Kovács-Weber, M., Mézes, M., & Kovács, B. (2017). DNS károsodás vizsgálata ponty (Cyprinus carpio) fajban LORDQ-PCR technikával = Evaluation of DNA damage in common carp (Cyprinus carpio) by LORDQ-PCR technique. In *6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia* (pp. 39–39).
6. Szabó, R. T., Kovács, B., Bencsik, D., Kovács, R., Horváth, Á., Mézes, M., … Weber, M. (2016). A CometScore programmal, illetve vizuálisan

történő kiértékelés összehasonlítása comet-assay esetében. In *XXXVI. Óvári Tudományos Nap - Hagyomány és innováció az agrár- és élelmiszergazdaságban* (p. 61).

PUBLICATIONS ON A TOPIC OTHER THAN THAT OF THE DISSERTATION

Scientific publications in journals:

7. Abayné, H. E., Bokor, B., Szabó, R. T., Kovács-Weber, M., Pajor, F., & Póti, P. (2021). Evaluation of selected parameters of carcass quality of intensively fattened Hungarian merino and German mutton merino lambs. *ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS*, *70*(2), 147–155.
8. Pap, T. I., Szabó, R. T., Drobnyák, Á., & Kovács-Weber, M. (2021). A baromfitartásban alkalmazott LED megvilágítás hatásának összefoglaló elemzése (Irodalmi áttekintés). *ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS*, *70*(2), 123–132.
9. Zimborán, Á., Kovács-Weber, M., Szabó, S., Szabó, R. T., Drobnyák, Á., & Erdélyi, M. (2021). Effect of different oils supplementation on broiler chicken performance. *ANIMAL NUTRITION AND FEED TECHNOLOGY*, *21*, 205–211.
10. Akos, B., Jozsef, S., Rubina, T. S., Peter, P., Istvan, E., & Ferenc, P. (2020). Feeding Experiences of Paulownia Spp. Leaves: Potential Forage Source for Domestic Animals. *INTERNATIONAL JOURNAL OF ZOOLOGY AND ANIMAL BIOLOGY*, *3*(1), 1–4. http://doi.org/10.23880/izab-16000212
11. Drobnyak, A., Phuong, T. N. L., Heincinger, M., Kustos, K., Almasi, A., Szalay, I. T., … Weber, M. (2019). The Positive Effect of Crossing Speckled Hungarian Breed with Commercial Lines in Term of Meat Production and Meat Quality. *INTERNATIONAL JOURNAL OF POULTRY SCIENCE*, *18*(6), 249–254.
12. Drobnyák, Á., Bódi, L., Heincinger, M., Kustos, K., Szalay, I. T., Almási, A., … Weber, M. (2019). The effect of the crossing of Yellow Hungarian chicken breed with different commercial lines on meat production and meat quality. *BULGARIAN JOURNAL OF AGRICULTURAL SCIENCE*, *25*(6), 1281–1286.
13. Gerencsér, Z., Matics, Z., Szabó Rubina, T., Kustos, K., Mikó, A., Nagy, I., … Szendrő, Z. (2019). Aggressiveness, Mating Behaviour and Lifespan of Group Housed Rabbit Does. *ANIMALS*, *9*(10). http://doi.org/10.3390/ani9100708
14. Drobnyák, Á., Szabó, R. T., Bódi, L., Kustos, K., Almási, A., Liptói, K., & Weber, M. (2018). Egg parameters of two Hungarian indigenous chicken breeds. In *WORLD'S POULTRY SCIENCE JOURNAL* (p. 466).

15. Németh, T., Podmaniczky, B., Szabó, R. T., Bodnár, Á., Póti, P., Kenéz, C., & Kovács-Weber, M. (2017). Mikroalga takarmánykiegészítés hatása brojlercsirkéknél. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *13*(2), 107–115.
16. Bognár, B., Mézes, M., Balogh, K., Kovács-Weber, M., Szabó, R. T., Könyves, L., & Jurkovich, V. (2016). Szubklinikai paratuberkulózis fertőzöttség hatása tejelő tehenek egészségére és teljesítményére. *HOLSTEIN MAGAZIN*, *24*(1), 38–40.
17. Jurkovich, V., Bognár, B., Balogh, K., Kovács-Weber, M., Fornyos, K., Szabó, R. T., … Mézes, M. (2016). Effects of subclinical Mycobacterium avium ssp. paratuberculosis infection on some physiological parameters, health status and production in dairy cows. *ACTA VETERINARIA HUNGARICA*, *64*(3), 301–312.
18. Kustos, K., Abayné, H. E., Balláné, E. M., Fazekas, N., Heincinger, M., Kovács-Weber, M., … Somodi, B. (2016). 4% és 8% kakukkfű kiegészítés vizsgálata a növendék és a hízónyulak termelésére és egészségi állapotára. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *12*(2), 80–90.
19. Szabó, R. T., Bódi, L., & Weber, M. (2016). A baromfitakarmányozás és a húsminőség egyes összefüggései. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *12*(1), 43–50.
20. Szabó, R. T., Mézes, M., Szalai, T., Zajácz, E., & Kovács-Weber, M. (2016). Colour identification of honey and methodical development of its instrumental measuring. *COLUMELLA: JOURNAL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES*, *3*(1), 29–36. http://doi.org/10.18380/SZIE.COLUM.2016.3.1.29
21. Drobnyák, Á., Erdélyi, M., Mézes, M., Szabó, R. T., Szöllősiné, B. R., Horvainé, S. M., & Weber, M. (2015). The effect of oregano supplement on turkey meat quality, lipid peroxidation and antioxidant status. *WORLDS POULTRY SCIENCE JOURNAL*, *71*(Suppl 1), 171.
22. Drobnyák, Á., Kovács, A., Szabó, R. T., & Weber, M. (2014). Bivaly és marhahús fogyasztói szemmel, objektíven. *AGRÁRÁGAZAT*, *5*, 138–141.
23. Szabó, R. T., Bodnár, Á., Pajor, F., Póti, P., & Weber, M. (2014). Mikroalga alapú takarmánykiegészítők alkalmazási lehetőségeinek feltérképezése. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *10*(2), 157–169.
24. Weber, M., Jukovich, V., Hadfi, Z., Szabó, R. T., & Végh, Á. (2014). Állatjólléti igények a tojótyúk és brojler tartásban: termelők kontra fogyasztók az állatokról. *AGRÁRÁGAZAT*, *2*, 142–144.

25. Bálint, C., Kende, Z., Tóth, E., Szabó, V., Szabó, A. K., Tóth, T., … Bálint, C. (2013). *Agrár- és vidékfejlesztési tanulmányok*. Gödöllő: SZIE Vidékfejlesztési Szakkollégium.
26. Drobnyák, Á., Szabó, R. T., Kustos, K., & Weber, M. (2013). A csincsilla tenyésztés Magyarországon. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 117–121.
27. Kerényi, V., Weber, M., Erdélyi, M., Apáti, N. G., Ábrahám, C., Szabó, R. T., & Mézes, M. (2013). Hízósertések termelésének és húsminőségének vizsgálata gyógynövénnyel dúsított takarmányok etetésének hatására. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 183–188.
28. Körözsi, V., Weber, M., Erdélyi, M., Apáti, N. G., Ábrahám, C., Szabó, R. T., & Mézes, M. (2013). Pecsenyeludak termelésének vizsgálata gyógynövénnyel dúsított takarmányok etetésének hatására. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 214–218.
29. Szabó, R. T., Kovács, A., Kokavecz, G., Heincinger, M., Horvainé, S. M., Ábrahám, C., … Weber, M. (2013). Vörösáru /párizsi/ objektív és szubjektív húsminőség vizsgálata. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 337–343.
30. Szabó, R. T., Weber, M., Weidel, W., & Szalai, T. (2013). A méz minősége és színe. In *Agrár- és vidékfejlesztési tanulmányok* (pp. 66–66).
31. Szöllősiné, B. R., Erdélyi, M., Mézes, M., Szabó, R. T., Koczka, N., Horvainé, S. M., … Weber, M. (2013). Illóolaj tartalmú készítmény alkalmazása növendék pulykák termelési paramétereinek fokozására. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 355–361.
32. Takács, A., Weber, M., Szabó, R. T., Kustos, K., Fazekas, N., & Liptói, K. (2013). Korai embrióelhalás összehasonlítása SPF (speficied pathogen free) és normál tyúktojásnál. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 363–369.
33. Weber, M., Sidó, I., Apáti, N. G., Ábrahám, C., Szabó, R. T., Mézes, M., & Erdélyi, M. (2013). Tenyészludak termelési paramétereinek javítása gyógynövény-kiegészítésekkel. *ANIMAL WELFARE ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA / ANIMAL WELFARE ETHOLOGY AND HOUSING SYSTEMS*, *9*(3), 393–400.

Publications in a conference volume:

1. Pap, T. I., Szabó, R. T., Varga, B., Podmaniczky, B., Pacz, M., & Kovács-Weber, M. (2021). LED és hagyományos (Wolfram szálas izzó) megvilágítás hatásai pecsenyecsirkék viselkedésére és termelési paramétereire (Előzetes etológiai vizsgálati eredményekkel). In *XXVII. Ifjúsági Tudományos Fórum* (pp. 36–41).
2. Pap, T. I., Varga, B., Szabó, R. T., Pacz, M., Podmaniczky, B., & Kovács-Weber, M. (2020). A megvilágítás hatása pecsenyecsirkék viselkedésére és az ezzel összefüggésbe hozható termelési paraméterekre. In *A Magyar Etológiai Társaság XXII. (online) konferenciája* (pp. 44–45).
3. Pap, T. I., Szabó, R. T., Pacz, M., Podmaniczky, B., & Kovács-Weber, M. (2020). Examination of the effect of light sources on meat quality parameters for broilers. In *55th Croatian & 15th International Symposium on Agriculture* (p. 233).
4. Drobnyák, Á., Heincinger, M., Kustos, K., Bódi, L., Szabó, R. T., Skrobár, S. C., … Kovács-Weber, M. (2019). The meat production of Yellow Hungarian Chicken breed in different keeping systems. In *XVII European Symposium on the quality of eggs and egg products and XXIV European symposium on the quality of poultry meat, Book of abstracts* (pp. 171–172).
5. Kovács-Weber, M., Kovács, D., & Szabó, R. T. (2019). Examining the objective quality parameters of pigeon meat. In *XVII European Symposium on the quality of eggs and egg products and XXIV European symposium on the quality of poultry meat, Book of abstracts* (p. 176).
6. Pap, T. I., Szabó, R. T., Pacz, M., Podmaniczky, B., Drobnyák, Á., Zimborán, Á., & Kovács-Weber, M. (2019). Eltérő fényforrások hatása pecsenyecsirkék egyes termelési paramétereire. In *VII. Gödöllői Állattenyésztési Tudományos Nap* (p. 40).
7. Szabó, R. T., Drobnyák, Á., Heincinger, M., Zimborán, Á., Bódi, L., Kustos, K., … Kovács-Weber, M. (2019). The comparison of production and meat quality of turkey crossbreds from Old Hungarian and commercial breeds. In *XVII European Symposium on the quality of eggs and egg products and XXIV European symposium on the quality of poultry meat, Book of abstracts* (p. 170).
8. Szabó, R. T., Lengyel, Á., & Kovács-Weber, M. (2019). Examination of the rearing ability of homing pigeon breeds in different housing technologies. In *18. BOKU-SYMPOSIUM TIERERNAHRUNG* (pp. 211–215).
9. Szabó, S., Szabó, R. T., Fehér, J., Podmaniczky, B., Zimborán, Á., & Kovács-Weber, M. (2019). Immunobiotikum hatásának vizsgálata brojlercsirke hústermelésére. In *VII. Gödöllői Állattenyésztési Tudományos Nap* (p. 44).
10. Szabó, S., Erdélyi, M., Kauser, J., Szabó, R. T., Mézes, M., Fehér, J., … Kovács-Weber, M. (2019). Haszonállatok termelési teljesítményét fokozó lehetőségek feltérképezése. In *XXV. Ifjúsági Tudományos Fórum*.

11. Szabó, S., Kauser, J., Szabó, R. T., Erdélyi, M., Mézes, M., Fehér, J., … Kovács-Weber, M. (2019). INVESTIGATION OF POSSIBILITIES OF ANTIBIOTIC REPLACEMENT IN BROILERS. In *Animal Breeding 2019* (p. 123).
12. SZABÓ, S., PÁLINKÁS, K., SZABÓ, R. T., ZIMBORÁN, Á., SZABÓ, B., & KOVÁCS-WEBER, M. (2019). Pecsenyecsirke szülőpárállományok teljesítményének vizsgálata üzemi körülmények között. In *Tavaszi Szél 2019 Konferencia. Nemzetközi Multidiszciplináris Konferencia* (pp. 84–84).
13. Zimborán, Á., Erdélyi, M., Szabó, R. T., & Kovács-Weber, M. (2019). A kémhatás és a fehérjék víztartó képességétől függő veszteségek összefüggéseinek vizsgálata kókusz-, pálma- és napraforgóolaj etetésének hatására. In *VII. Gödöllői Állattenyésztési Tudományos Nap* (p. 48).
14. Zimborán, Á., Erdélyi, M., Drobnyák, Á., Szabó, R. T., Szabó, S., Pétervári, S., … Kovács-Weber, M. (2019). Napraforgóolaj adagolás hatásának vizsgálata brojlerek termelésére és húsminőségére. In *XXV. Ifjúsági Tudományos Fórum*.
15. Drobnyák, Á., Skrobár, S. C., Szabó, R. T., Kustos, K., Heincinger, M., Almási, A., … Weber, M. (2018). Termelési és húsminőség vizsgálatok eredményei sárga magyar tyúkra alapozott keresztezésekben = Results of production and meat quality tests in crosses based on yellow Hungarian hens. In *Őshonos- és Tájfajták - Ökotermékek - Egészséges táplálkozás - Vidékfejlesztés - Minőségi élelmiszerek - Egészséges környezet: Az agrártudományok és a vidékfejlesztés kihívásai a XXI. században* (pp. 91–92).
16. Drobnyák, Á., Végi, B., Szabó, R. T., Bódi, L., Kustos, K., Kissné, V. É., … Liptói, K. (2018). Kendermagos magyar tyúkra alapozott keresztezések szaporodásbiológiai vizsgálata = Investigation of the reproduction parameters of speckled hungarian chicken crossbreeds. In *Őshonos- és Tájfajták - Ökotermékek - Egészséges táplálkozás - Vidékfejlesztés - Minőségi élelmiszerek - Egészséges környezet: Az agrártudományok és a vidékfejlesztés kihívásai a XXI. században* (pp. 47–48).
17. Szabó, S., Szabó, R. T., Fehér, J., Podmaniczky, B., & Kovács-Weber, M. (2018). Immunbiotikum hatásának vizsgálata brojlercsirke termelésére. In *XXXVII. ÓVÁRI TUDOMÁNYOS NAPOK "FENNTARTHATÓ AGRÁRIUM ÉS KÖRNYEZET AZ ÓVÁRI AKADÉMIA 200 ÉVE – MÚLT, JELEN, JÖVŐ" Összefoglalói* (p. 219).
18. Zimborán, Á., Kovács-Weber, M., Bócsai, A., Ancsin, Z., Szabó, R. T., Balogh, K., … Erdélyi, M. (2018). Kókusz- és pálmaolaj hatása a brojler csirke termelési paramétereire. In *XXXVII. ÓVÁRI TUDOMÁNYOS NAPOK "FENNTARTHATÓ AGRÁRIUM ÉS KÖRNYEZET AZ ÓVÁRI AKADÉMIA 200 ÉVE – MÚLT, JELEN, JÖVŐ" Összefoglalói* (p. 195).
19. Drobnyák, Á., Skrobár, S. C., Szabó, R. T., Kustos, K., Heincinger, M., Zimborán, Á., … Weber, M. (2017b). Vágási paraméterek és húsminőség

vizsgálata sárga magyar tyúkra alapozott keresztezésekben. In *6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia* (pp. 28–28).
20. Drobnyák, Á., Skrobár, S. C., Szabó, R. T., Kustos, K., Heincinger, M., Zimborán, Á., … Weber, M. (2017a). Hússzín- és porhanyósság-vizsgálatok kendermagos magyartyúkkal kialakított keresztezésekben. In *6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia* (pp. 27–27).
21. Szabó, R. T., Erdélyi, M., Drobnyák, Á., Szöllősiné, B. R., Balogh, K., Mézes, M., & Weber, M. (2017). Effect of different plant oils on some meat quality parameters of turkey. In *16. BOKU- Symposium Tierernahrung* (pp. 179–182).
22. Zimborán, Á., Meszlényi, Z., Bódi, L., Heincinger, M., Szabó, R. T., Drobnyák, Á., … Kovács-Weber, M. (2017). Őshonos magyar pulykafajtákra alapozott keresztezések vizsgálata. In *6th Scientific Day of Animal Breeding in Gödöllő - International Conference; VI. Gödöllői Állattenyésztési Tudományos Nap - Nemzetközi Konferencia* (pp. 33–33).
23. Zs, M., Zs, S., I, R., T P, F., R, K., L, K., … Zs, G. (2017). ANIHWA - Experimental results at Kaposvár University. In *20. Internationale Tagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere* (pp. 27–36).
24. Drobnyák, Á., Kustos, K., Szabó, R. T., Heincinger, M., Bódi, L., Petruska, E., & Weber, M. (2016). Tojáshéjminőség vizsgálata őshonos tyúkfajtákból létrehozott keresztezési konstrukcióknál. In *XXXVI. Óvári Tudományos Nap - Hagyomány és innováció az agrár- és élelmiszergazdaságban* (p. 62).
25. Drobnyák, Á., Szabó, R. T., Bódi, L., Pap, T., Zimborán, Á., Kustos, K., & Weber, M. (2016). Vedletett és nem vedletett őshonos magyar tyúkfajták tojáshéjminőségének összehasonlítása. In *Őshonos- és tájfajták - Ökotermékek - Egészséges Táplálkozás – Vidékfejlesztés* (pp. 45–45).
26. Gerencsér, Z., Kustos, K., Szabó, R. T., Mikó, A., Odermatt, M., Radnai, I., … Szendrő, Z. (2016a). MATING BEHAVIOUR OF RABBITDOES AND BUCKS INGROUPS (PRELIMINARY RESULTS). In *Proceedings of the 11th World Rabbit Congress* (pp. 675–678).
27. Gerencsér, Z., Kustos, K., Szabó, R. T., Mikó, A., Odermatt, M., Radnai, I., … Szendrő, Z. (2016b). Szexuális viselkedés vizsgálata csoportosan tartott anyanyulaknál. In *28. Nyúltenyésztési Tudományos Nap* (pp. 103–107).
28. Szendrő, Z., Matics, Z., Szabó, R. T., Kustos, K., Mikó, A., Odermatt, M., & Gerencsér, Z. (2016a). Agressivity and its effect on lifespan of group housed rabbit does. preliminary results. In *Proceedings of the 11th World Rabbit Congress* (pp. 719–722).

29. Szendrő, Z., Matics, Z., Szabó, R. T., Kustos, K., Mikó, A., Odermatt, M., & Gerencsér, Z. (2016b). Agresszív viselkedés vizsgálata csoportosan tartott anyanyulaknál. In *28. Nyúltenyésztési Tudományos Nap* (pp. 97–101).
30. Viktor, J., Barbara, B., Krisztian, B., Maria, K.-W., Rubina, T. S., Peter, K., … Miklos, M. (2016). The effects of subclinical M. avium ssp. paratuberculosis infection on the health and production parameters of dairy cows. In *The 29th World Buiatrics Congress, Dublin 2016 - Congress Proceedings* (pp. 529–529).
31. Balogh, S., Heincinger, M., Szabó, R. T., Bódi, L., Drobnyák, Á., Kustos, K., & Weber, M. (2015). Alternatív keresztezések tojásvizsgálata. In *V. Gödöllői Állattenyésztési Tudományos Napok Nemzetközi Konferencia* (p. 47).
32. Bognár, B., Balogh, K., Mézes, M., Weber, M., Szabó, R. T., Könyves, L., & Jurkovich, V. (2015). Szubklinikai Mycobacterium avium ssp. paratuberculosis fertőzöttség hatása tejelő tehenek egészségére és teljesítményére - előzetes eredmények. In *A Magyar Buiatrikus Társaság XXV. Nemzetközi Kongresszusa* (pp. 21–25).
33. Drobnyák, Á., Heincinger, M., Kustos, K., Szalay, I., Bódi, L., Szabó, R., … Weber, M. (2015). Comparing meat quality and production of F1 crossbreds between Speckled Hungarian chicken breed and two intensive chicken breeds. In *AGROBIODIVERSITY PROTECTION AND RESEARCH CONFERENCE* (p. 8).
34. Szabó, R. T., Drobnyák, Á., Héver, T., Farkas, G., & Weber, M. (2015). Különleges fogyasztói minőségű pecsenyecsirkék húsminőségének összehasonlítása. In *V. Gödöllői Állattenyésztési Tudományos Napok Nemzetközi Konferencia* (p. 54).
35. Szabó, R. T., Erdélyi, M., Drobnyák, Á., Körözsi, V., Balogh, K., Mézes, M., & Weber, M. (2015). The effect of thyme and rosemary supplement on roast goose meat quality. *WORLDS POULTRY SCIENCE JOURNAL*, *71*(1), 170–170.
36. Weber, M., Szabó, R. T., Drobnyák, Á., Bukovics, C., & Podmaniczky, B. (2015). A tojáshéjszilárdság tudományos és gyakorlati szemmel. In *V. Gödöllői Állattenyésztési Tudományos Napok Nemzetközi Konferencia* (p. 56).
37. Weber, M., Szabó, R. T., Ray, K., Drobnyák, Á., Bukovics, C., Podmaniczky, B., & Heincinger, M. (2015). Eggshell quality. *Worlds Poultry Science Journal*,*71*(1), 133.
38. Zimborán, Á., Heincinger, M., Szabó, R. T., Bódi, L., Drobnyák, Á., Kustos, K., & Weber, M. (2015). Őshonos magyar tyúkfajtákra alapozott nemesítés. In *V. Gödöllői Állattenyésztési Tudományos Napok Nemzetközi Konferencia* (p. 57).
39. Szabó, R. T., Weber, M., Erdélyi, M., Kerényi, V., Balogh, K., & Apáti, N. G. (2013). The effect of thyme and rosemary supplementation on the

lipid peroxidation in the liver of fattening pigs. (Kakkukfű és rozmaring adagolás hatásának vizsgálata a lipidperoxidációra hízósertések májában.). In *VIII. Kárpát-medencei Biológiai Szimpózium - I. Fenntartható fejlődés a Kárpát-medencében nemzetközi konferencia* (pp. 125–126).

40. Szöllősiné, B. R., Erdélyi, M., Mézes, M., Szabó, R. T., Balogh, K., & Weber, M. (2013). Analyzing the effect of plant extracts on lipid peroxide status of turkeys. (Növényi kivonatok hatásának vizsgálata pulykák lipidperoxid státuszára.). In *VIII. Kárpát-medencei Biológiai Szimpózium - I. Fenntartható fejlődés a Kárpát-medencében nemzetközi konferencia* (pp. 128–129).
41. Szőlősiné, B. R., Erdélyi, M., Mézes, M., Szabó, R. T., Koczka, N., Horvainé, S. M., … Weber, M. (2013a). Illóolaj tartalmú készítmény alkalmazása növendék pulykák termelési paramétereinek fokozására. In *Illóolaj tartalmú készítmény alkalmazása növendék pulykák termelési paramétereinek fokozására.* (p. CD-ROM).
42. Szőlősiné, B. R., Erdélyi, M., Mézes, M., Szabó, R. T., Koczka, N., Horvainé, S. M., … Weber, M. (2013b). *Illóolaj tartalmú készítmény alkalmazása növendék pulykák termelési paramétereinek fokozására.* Szent István Egyetem Mezőgazdaság- és Környezettudományi Kar.
43. Szabó, R. T., Weber, M., Weidel, W., & Szalai, T. (2012). Mézzel kapcsolatos fogyasztói ismeretek felmérése és érzékszervi minősítése. In *A magyar mezőgazdaság - lehetőségek, források, új gondolatok: XXXIV. Óvári Tudományos Nap* (pp. 291–297).