

THESES OF DOCTORAL (PhD) DISSERTATION

UNIVERSITY OF WEST HUNGARY
FACULTY OF AGRICULTURAL AND FOOD SCIENCES
MOSONMAGYARÓVÁR
DEPARTMENT OF FOOD TECHNOLOGY AND MICROBIOLOGY

Leader of the Doctoral School:

Prof. Dr. Dr. h.c. János Schmidt

DSc, Member of the HAS

Program leader and supervisor:

Prof. Dr. habil. Jenő Szigeti

CSc

**EXAMINATION OF THE EFFECT OF SOME TRACABLE
CHEMICAL RESIDUES ON PRODUCT QUALITY OF
POULTRY (SHAVER 579 LAYING CHIKEN HYBREDS,
JAPANESE QUAIL)**

Written by

KATALIN REISINGER

Mosonmagyaróvár

2007

1. RESEARCH AIMS

Inside the nowadays frequently examined food safety topics, this dissertation demonstrates the possibilities of some pesticide residues to onset into animal organisms. During our research the toxic effects of seeds and baits containing carbendazim and cholrophacinone were examined on Japanese quails and Shaver 579 laying chicken hybrids. The examination includes clinical symptoms, body weights, food consumptions, umber of eggs, their weight and deformations, follicles and the weight of liver, pectoral muscle, and ovary. Liquid chromatographic method was aimed to detect the incidental active ingredients in liver, pectoral muscles, ovaries and eggs. Based on the examinations the connecting quality control systems was to be complemented.

2. MATERIALS AND METHODS

2.1. Experimental materials

“Kolfugo Szuper” (Chinoïn Inc. and Agro-Chemie Ltd.) liquid fungicide and “Redentin 75 RB” (Reanal Inc.) rodenticides were used as baits. The “Kolfugo Szuper” contained $20 \pm 1.0\%$ carbendazim and the “Redentin 75 RB” contained $0,0075 \pm 1.0\%$ cholrophacinone.

2.2. Test animals and their food

During the experiments Japanese quails (*Coturnix coturnix japonica*) and Shaver 579 laying chicken hybrids were used for the experiments. Protein enhanced poultry premix was used for feeding livestock, supplied by the “Szekszárd” Agricultural Inc. Its ingredients (dry matter, crude protein, crude fats, crude fiber, crude ash, calcium, phosphorus, sodium, NaCl) were measured by the “Labocontrol” laboratory of “Minerag” Ltd.

2.3. Experimental circumstances

The examinations were carried out under GLP circumstances (“Ökotoxikológiai Laboratórium” - Eco-toxicological Laboratory, Fácánkert). In our research besides the not-treated control group an additional treated group was formed. The number animals in each group were 24. The feeding period took four weeks followed by an additional four-week observation period. At the beginning of the experiments the number of animals in the coops and the deep littered stables were four.

2.4. Determination of concentration, feed analysis

The quantity of the agents that has to be mixed in the feed was determined so that avoiding its repellent effect, The animals consume it until the end of the examination period. After preparing the animal feed analytical samples were taken from the homogenized mixture (containing the chemical agents) and also from the control feed (not containing any agent) for determination of contents and liquid chromatographic examinations of homogeneity. The stability of mixed agents was checked also at the last day of exposition by measuring the agent content. The experimental animals could consume food and water ad libitum. The food – after preparation – contained 0.5 mL/kg carbendazim and 3.75 mg/kg cholrophacinone.

2.5. Examination Methods

2.5.1. Examinations of the effects on test animals and their bodies

Continuous clinical examinations were carried out during the measurements (Weeks 1-8), while the weights, the pathological deformations of the weekly three sacrificed birds and the reproduction data (number of eggs, the weights of intact, ruptured and broken eggs and the thickness of eggshells) were registered weekly. The weight of liver, ovary and pectoral muscle were measured of the CO₂

exterminated animals. The classifications of ovary follicles were carried out by measuring its diameters by a caliper. The eggs were collected, measured, classified and candled daily. The results were recorded continuously in the egg-production register. The thickness of the air-dried eggshells was measured weakly with micrometer.

2.5.2. Analytical Examinations

The carbendazim was extracted from the samples by using ethyl acetate and after cleaning it was determined by HPLC on 278 nm. The cholrophacinone was extracted with acetonitrile and after Florisil cleanup, the extract is injected into a high pressure liquid chromatograph for reverse phase chromatography on a polar NH₂ column and measured at 288 nm.

3. RESULTS

3.1. Results of carbendazim examinations

3.1.1. Clinical symptoms

During the research behavior and the external deformations of the animals were being watched. We determined that carbendazim did not have any negative effect on hen hybrids. On the 17th day melaena of a quails among the control group was detected, which was vanished on the following day. The other quails did not show any symptoms.

3.1.2. Tendency of feed consumption

There was no significant difference in the weakly measured feed consumption between treated and control groups of quails statistically calculated by two-samples paired t Test. However, the feed consumption of hen hybrids decreased

statistically ($P < 0.05$) between the control and treated groups in the four week period of degradation.

3.1.3. Tendency of body weight

There was no significant difference in the weekly measured body weight changes between the treated and control groups of quails. The measured data clearly showed significant ($P < 0.05$) differences in the body weight increase of treated hens compared to the control group.

3.1.4. Tendency of quality and quantity parameters

Considering that the number and weight of eggs are decreasing drawing to the end of experiment, these values were weakly analyzed by weighted two-samples t Test. In case of quails there was significant ($P < 0.001$) decrease in the number of eggs comparing to the control group. There was also significant ($P < 0.001$) decrease in the weight of eggs comparing to the control group.

In case of hen hybrids there was significant ($P < 0.001$) increase in the number of eggs in the first four-week period, while in the second four-week period there was significant ($P = 0.01$) decrease comparing to the control group.

There was no significant difference in deformation data of quails calculated by Chi^2 probe. In the control group the occurrence of damaged eggs was 0 – 17.4%, while it was 0 – 6.2% in the group treated with carbendazim. During sorting of the hen eggs the deformity date showed significant increase calculated by Chi^2 probe. During sorting of eggs the examinations extended to the formation of limy eggshells and pigment deformation, egg without hard shell, shape and size defects and ruptured eggs. In the control group the deformed eggs occurrence was 0 – 13.8%, while in the treated group it was 0-52%.

Two-samples without repetition t statistical test was carried out of the averaged measured data of eggshell thickness. We did not find significant differences throughout the eight-week examination period between control and treated groups.

As a result of the treatment there was concrete correlation ($P < 0.05$) in changes of eggshell thickness of hen hybrids. As a result of variance analysis the treated animals' eggshell significantly increased compared to the control groups.

3.1.5. Tendency of weight changes of liver, pectoral muscle and ovary

There were not significant differences in weight changes of liver, pectoral muscle and ovary between the control and the treated quails groups neither in the feeding nor in the degradation period.

There was a significant difference ($P < 0.01$) in the weekly measured weight changes of liver of hen hybrids between the control and treated groups in both the feeding and degradation period evaluated by Student t probe. Concerning pectoral muscles there was not significant differences between treated and control groups. There was significant decrease in $P < 0.01$ level in average ovary weight between the treated and control groups in the feeding period.

3.1.6. Tendency of follicles numbers

There was not observable chemical agent effect on the numbers of follicles of quails. The accomplished Chi^2 probe did not prove any significant differences in the number changes of follicles between control and treated groups.

3.1.7. Pathological results

The completed autopsy did not showed any deformation on liver, pectoral muscle and the other giblets. On the other hand such F1 egg fundaments were found in both the control and also the treated groups that did not developed limy eggshells. In the control group among F2 follicles there were two dark grayed, 15 and 17 mm diameter, deceased egg fundaments and one 37 mm diameter deceased follicle-containing grayish colored liquid. After autopsy a dried F1 egg fundament was found.

3.1.8. Comparison the effect of carbendazim between Japanese quails and Shaver 579 hen hybrids

Table 1 summarizes the effects of 0.5 mL/kg carbendazim feeding on Japanese quails and Shaver 579 hen hybrids.

Table 1. Comparison the effect of carbendazim between Japanese quails and Shaver 579 laying chicken hybrids

	Japanese quail	Shaver 579 laying chicken hybrids
Average food consumption	=	-
Average body weight	=	+
Number of eggs	-	at the 1 st period + at the 2 nd period: -
Weight of eggs	-	-
Deformation	=	+
Thickness of eggshell	=	+
Average liver weight	=	-
Average weight of pectoral muscles	=	=
Average weight of ovary	=	-
Number of follicles	=	=

+: increased due to the agent
-: decreased due to the agent
=: no change

In the case of egg numbers and egg weight only the examined tendency of quails can be a notification that is on the occasion of carbendazim treatment the number and weight of quails' egg decreased in small scale. Although significant differences were proved differences in chicken hybrids but this cannot be considered as biological effect. The measured treated values did not reach 10% of the compared control values that is the threshold of biological values.

3.1.9. The measured carbendazim agent residues in Japanese quails and eggs at the fourth and fifth weeks

At the fourth week of measurements the carbendazim residue in the different organs of both the treated quails and chickens were above the detectable level but under the allowed contamination level (0.02 mg/kg). Individual egg collection cannot be carried out due to the group keeping of the animals, therefore the concentration of carbendazim was detected from the sum of eggs collected on the fourth week (quails: 0.0338 mg/kg, laying chickens: <0.02 mg/kg, Tables 2-3).

Table 2. The measured carbendazim residues at the forth week in quails and eggs

Sample type	Control (mg/kg)	Measured agent content (mg/kg)	The allowed maximal contamination (MRL: mg/kg)
Liver	<0.020	0.0224	0.1
Liver	<0.020	0.0308	0.1
Liver	<0.020	0.0253	0.1
Pectoral muscle	<0.020	<0.020	0.1
Pectoral muscle	<0.020	0.0229	0.1
Pectoral muscle	<0.020	0.0279	0.1
Eggs	<0.020	0.0338	0.1

Table 3. The measured carbendazim residues at the forth week in hens and eggs

Sample type	Control (mg/kg)	Measured agent content (mg/kg)	The allowed maximal contamination (MRL: mg/kg)
Liver	<0.020	0.0971	0.1
Liver	<0.020	0.0427	0.1
Liver	<0.020	0.0570	0.1
Pectoral muscle	<0.020	0.0655	0.1
Pectoral muscle	<0.020	0.0310	0.1
Pectoral muscle	<0.020	0.0485	0.1
Eggs	<0.020	<0.020	0.1

3.2. Results of cholrophacinone examinations

3.2.1. Clinical symptoms

During the research behavior and the external deformations of the animals we determined that cholrophacinone in our dose did not have any negative effect on chicken hybrids. At the 18th day one of the cholrophacinone treated quails (# 341) died off, while the other animals did not show any symptoms.

3.2.2. Tendency of feed consumption

The cholrophacinone contained feed consumption did not changed in the group of quails according to the statistical analysis (two sampled paired t probe) compared to the control group. The average feed consumption did not change between the treated and control chicken groups in the four-week period of degradation that could have been statistically verified.

3.2.3. Tendency of body weight

The weekly measured weight changes of treated quails were not significantly different from the control groups of quails. Significant in $P < 0.05$ level differences in the body weight increase of treated chickens compared to the control group.

3.2.4. Tendency of quality and quantity parameters

During the examination of egg number changes in the four-week period of feeding a significant ($P < 0.001$) decrease was experienced compared to the control group. By analyzing the weight of eggs there were significant decrease due to the cholrophacinone treatment both in the feeding and also in the degradation period in significance levels of $P < 0.001$ and 0.05 , respectively. During the ranking of eggs there was not found any significant differences between the treated and the control

groups. In the control group the deformed eggs occurrence was 0 – 13.8%, while in the treated group it was 3.6 – 20.87%.

In case of laying chickens the number of eggs significantly ($P < 0.001$) increased throughout the eight weeks. In contrast to this the weight of egg at the first four weeks significantly increased ($P < 0.001$) while in the degradation period it shows significant decrease. In the feeding period the deformed eggs number significantly ($P < 0.05$) increased. There was not found significant differences between the treated and the control group in the degradation period.

As a result of variation analysis the eight weeks examination period there was significant decrease in level of $P < 0.05$ of the thickness of egg shells compared to the untreated control animals. According the first four-week examination period concrete increase was detected in laying chicken eggs thickness due to the treatment ($P < 0.01$). In the data of degradation period there was no significant differences between the treated and the control groups.

3.2.5. Tendency of weight changes of liver, pectoral muscle and ovary

Statistically evaluating the average weight of liver during the degradation period there were significant ($P < 0.05$) decrease compared to the control group. There were not significant differences in the average weight changes of pectoral muscles and ovaries between the treated and control group of quails neither in the feeding period nor the degradation period. There was not found any statistical differences in different organs of chickens.

3.2.6. Tendency of follicles numbers

There were not significant differences in ovary and follicles data neither in quails nor in chickens between the treated and control group.

3.2.7. Autopsy results

During the autopsy we determined that the cholrophacinone treated quails showed hemophiliac symptoms in several times. At the end of the first period we have found an animal (#312) the liver was extremely enlarged and fatty (11.3 g).

In the second period the liver of the 314th animal was pale and 2-3 times bigger (18.4 g) than the normal. On its surface there was a lentil-sized bloody infiltration. There were bleedings of the 321st bird at the wing root, beck, oral cavity, pharynx, gullet, crop, small and large intestines in the 14th day of the second experimental period. At the same day the peritoneum, liver, kidney and abdominal cavity of the 322nd numbered animal was filled with blood or covered with blood.

The 341st animal deceased on the 18th day of the third period. The reason of death was bleeding extended to the peritoneum, ovary, oviduct, kidney, liver and abdominal cavity. In the 21st day of the third period there was an extended haematoma in the muscle of left leg of the 324th animal. Meanwhile on the left upper side of the pectoral muscle of the 353rd bird there was a removable, olive-drab, solid synopsis.

During the autopsy of quails in the third period the 341 numbered one had one with soft shelled, black colored F1 egg and three black and bloody F3 eggs were found. In the fifth period the lime shell did not formed of the F1 egg fundament of the 341st quail. In the fifth period the lime shell of the F1 egg fundament of the 342nd quail did not formed. Besides of mature follicles in the ovary of the 351st bird in the sixth period there was a 16 mm long and 5-6 mm wide deceased egg remains. There was a deceased egg fundament in the 321st laying hybrid in the second period.

3.2.8. Comparison the effect of cholrophacinone between Japanese quails and Shaver 579 hen hybrids

Table 4 shows the effects of 3.75 mg/kg cholrophacinone feeding of the examined Japanese quails and Shaver 579 laying chickens. The cholrophacinone experiments had negative indicator results for the quality and quantity of eggs and

liver weight only of quails but not of laying chickens. The results of chicken experiments are questionable. In case of liver examinations we found several times deformation that together with the liver weight statistical analysis could indicate hepatotoxic effect.

During the experiments from the measurements toxic effect could not be determined in case of laying chickens.

Table 4. Comparison the effect of cholrophacinone between Japanese quails and Shaver 579 hen hybrids

	Japanese quail	Shaver 579 laying chicken hybrids
Average food consumption	=	=
Average body weight	=	+
Number of eggs	-	-
Weight of eggs	-	at the 1 st period + at the 2 nd period: -
Deformation	=	+
Thickness of eggshell	-	+
Average liver weight	-	=
Average weight of pectoral muscles	=	=
Average weight of ovary	=	=
Number of follicles	=	=

+: increased due to the agent
 -: decreased due to the agent
 =: no change

3.2.9. The measured cholrophacinone agent residues in Japanese quails and eggs at the fourth and fifth weeks

HPLC examinations were carried out of quails eggs above the detection limits, meanwhile at the fifth week below the limit (Table 3).

The cholrophacinone residue was above the detection level (0.01 mg/kg) of eggs and bodies of the treated laying chickens at the fourth week. At the fifth week only the eggs were examined and resulted the lower values than the threshold (Table

4). We could detect cholrophacinone only from egg samples due to methodological errors.

The MRL level of cholrophacinone was not measured because of its high toxicity.

Table 5. The measured cholrophacinone agent residues in quails and eggs at the fourth and fifth weeks.

Sample type	Control (mg/kg)	Measured agent content (mg/kg)	The allowed maximal contamination (MRL: mg/kg)
Sample type	<0.010	Not measured	–
Liver	<0.010	Not measured	–
Liver	<0.010	Not measured	–
Liver	<0.010	Not measured	–
Pectoral muscle	<0.010	Not measured	–
Pectoral muscle	<0.010	Not measured	–
Pectoral muscle	<0.010	0.073 (4 th week) <0.010 (5 th week)	–

Table 6. The measured chlorphacinone agent residues in hen hybrids and eggs at the fourth and fifth weeks

Sample type	Control (mg/kg)	Measured agent content (mg/kg)	The allowed maximal contamination (MRL: mg/kg)
Liver	<0.010	0.218* (4 th week) Not measured (5 th week)	–
Liver	<0.010	0.219* (4 th week) Not measured (5 th week)	–
Liver	<0.010	0.148* (4 th week) Not measured (5 th week)	–
Pectoral muscle	<0.010	0.015* (4 th week) Not measured (5 th week)	–
Pectoral muscle	<0.010	0.008* (4 th week) Not measured (5 th week)	–
Pectoral muscle	<0.010	0.006* (4 th week) Not measured (5 th week)	–
Eggs	<0.010	0.107 (4 th week) <0.010 (5 th week)	–

*: The measured agent in liver and muscles is only informative!

The liquid chromatographic examination of the liver and pectoral muscle of laying hybrids was not properly evaluated due to a methodological error: the recovery value was not adequate (10-30%), therefore they are only informative. A foreign expertise was asked for solving the methodological problems, in spite of this the examination could have been carried out because of the storage time of quail samples had expired.

4. SUGGESTIONS

We suggest taking into account by quality control systems of seeding technologies serious supervision when seeding treated seeds in nearby animal farms in order to avoid exposition of wild and domestic animals.

During every aspect of storage and feed mixing (upload, unloading, handling, etc.) it has to be considered the possibilities of contaminations with agents, especially the environmental contamination. We stress the importance that the treated seed and food should be all the time separated from each other, because in feed storage there is not allowed to use water and chemical cleaning hence the treated seed and treated materials can be easily mixed with the feed stuffs.

During the seeding technology more rigorous control is advised at the autumn and spring rodent exterminations, because the wild animals (pheasants, quails, fawns and deer) and domestic animals at the open farms close to the agricultural fields can intake the chemical agents and hence can be poisonous for humans, too.

We stressed out more during the storage and mixing of seeds the uploading and emptying operations (storage, handing over, etc.)

In the chicken keeping technologies we emphasize and control the importation of rodent control chemical agents by rodents.

5. NEW SCIENTIFIC RESULTS (THESIS)

1. It was determined that 0.5 mL/kg carbendazim feeding resulted decrease in numbers and weights of eggs by quails, but did not affected the behavior and average feed consumption, average body weight, deformation of eggs, thickness of eggshells, the average weights of liver, pectoral muscle, ovary and the number of follicles.

After the four-week period carbendazim traceable in liver, pectoral muscle and egg samples. Although the measured values were under the allowable limits, it is possible that the chemical agent could get into the food chain.

2. It was determined that after the four week feeding period the Shaver 579 laying hybrids fed with 0.5 mL/kg carbendazim there was detectable chemicals in liver and pectoral muscle but only under the allowed limits. In egg samples the chemical agent concentration was under the traceable level.

3. It was determined that 3.75 mg/kg cholrophaconone feeding resulted decrease of egg numbers, weights, and egg shell thickness and the average liver and pectoral muscle weights in Japanese quails. The chemical agent did not affected by the behaviors, the average feed consumption, average body weight, the deformation of eggs, the average ovary weights and the number of follicles. Cholrophaconone was detected in the egg samples at the end of the fourth period by HPLC analytical examinations. At the end of the fifth period we measured it under the detectable level in egg samples.

4. Analytical examinations was be used to detect in eggs of Shaver 579 hen hybrids feed with 3.75 mg/kg cholrophaconone at the fourth week and under the determination point level at the fifth week.

6. LIST OF PUBLICATIONS

1. **Reisinger, K., Szigeti, J. (2005):** A karbendazim hatása Shaver 579 tojóhibridre. Növényvédelem. 41(5). 215-221.
2. **Reisinger, K., Szigeti, J., Várnagy, L. (2006):** Determination of carbendazim residues in the eggs, liver and pectoral muscle of Japanese quail (*Coturnix coturnix japonica*). Acta Vet. Hungarica. Vol. 54.(1). 127-133.
3. **Reisinger, K., Szigeti, J. (accepted):** A karbendazim és a klórfacinon hatása japán fűrjre (*Coturnix coturnix japonica*). Magyar apróvad közlemények.

Proceedings:

1. **Reisinger, K. (2002):** Mikotoxinok, mint az élelmiszerek minőségének egyik káros tényezője. VIII. Ifjúsági Tudományos Fórum, Konferencia Kiadvány, Keszthely
2. **Reisinger, K. (2004):** A karbendazim hatása a japán fűrj (*Coturnix japonica*) testsúlyára, tojásának értékmérő tulajdonságaira, a petefészkek, a máj és a mellizom makroszkópikus tüneteire és súlyára, 50. Növényvédelmi Tudományos Napok, Konferencia Kiadvány, Budapest
3. **Reisinger, P., Reisinger, K. (2005):** Informatikai fejlesztések a növényvédelemben. XV. Keszthelyi Növényvédelmi Fórum, Konferencia Kiadvány, Keszthely
4. **Reisinger, K. (2005):** Karbendazim és klórfacinon hatóanyag-maradék vizsgálatok madarak tojásaiban, 51. Növényvédelmi Tudományos Napok, Konferencia Kiadvány, Budapest

Poster:

1. **Reisinger, K., Szigeti, J. (2005):** Effects of carbendasim on the parameters of organs and eggs of poultry, 56th International Symposium on Crop Protection, Gent, Belgium