FINAL REPORT

In vivo examination of the correlations between hen's egg composition, hatchability and hatched chick's development and production by means of computer tomography in dual-purpose genotypes

OTKA NNF 78840

As first step of this study, the methodology of computer tomograph (CT) examination of the hen's eggs was developed. During this procedure the optimal technical parameters and the number of simultaneously scanable eggs were determined in order to obtain CT images with the highest quality for the evaluation.

For this purpose 120 eggs per genotype were scanned by CT. Eggs were scanned first in egg holders (*Figure 1*) and then on egg trays (*Figure 2*), so 2 or 5 eggs were scanned simultaneously (*Figure 3 and 4*).

Figure 1.
Eggs in egg holder

Figure 2.
Eggs on egg tray

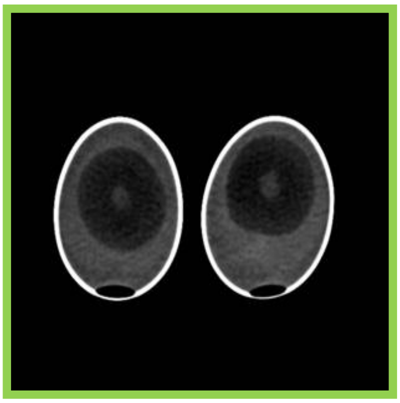

Figure 3.
Cross-sectional image of 2 simultaneously scanned eggs

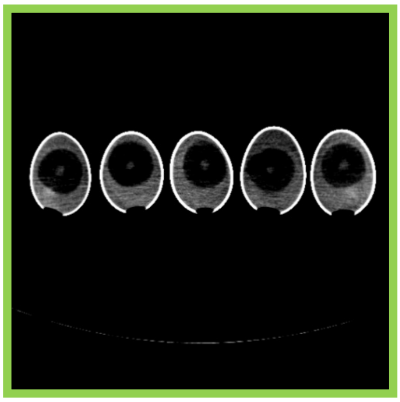

Figure 4.
Cross-sectional image of 5 simultaneously scanned eggs

In both arrangement the following 9 technical parameters were tested: 80 kV – 40 mAs, 80 kV – 80 mAs, 80 kV – 120 mAs, 110 kV – 40 mAs, 110 kV – 80 mAs, 110 kV – 120 mAs, 130 kV – 40 mAs, 130 kV – 80 mAs, 130 kV – 120 mAs.

After the CT examinations all of the eggs were broken and their yolk and albumen were separated. After weighing the yolk, its ratio to the whole egg was calculated. For predicting the egg yolk ratio *in vivo*, prediction equations were created by means of the linear regression method using the CT data as independent variable in the model.

As first step of the evaluation, the X-ray density values of the pixels (elements of CT images) were used to determine the yolk ratio in the hen's eggs *in vivo*. Using this evaluation method it was established that the X-ray density values of the egg yolk clearly differ from that of the animal's fatty tissues. While X-ray density values of the animal's fatty tissues ranges from -200 to -20 the so-called Hounsfield-scale, the values of the egg yolk varies between -10 to +30. The reason of this could be the higher water and protein content of the yolk, which results in an increase of the X-ray density values in the egg yolk. Because of the overlapping X-ray density values of yolk and albumen, this evaluation method was not useful for the determination of egg yolk ratio in the hen's eggs *in vivo* (*Figure 5*).

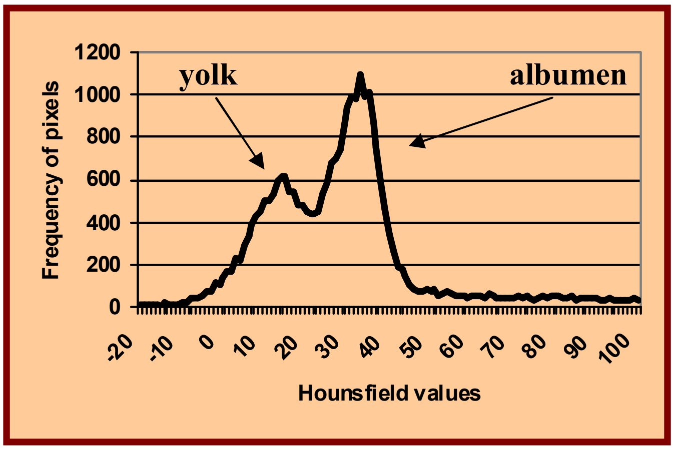

Figure 5.
X-ray density values of egg yolk and albumen on the Hounsfield-scale

As another method of the evaluation, the surface of the egg yolk was determined on the cross-sectional images for predicting the egg yolk content *in vivo* (*Figure 6*).

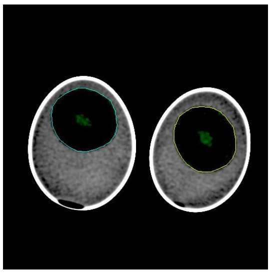

Figure 6.
Determination of the surface of egg yolk on a cross-sectional CT image of a hen's egg

As first step of this evaluation only one scan per egg was used for testing the accuracy of prediction. Using the scan taken at the germinal disc resulted in a 69.3% accuracy of prediction (*Eq. 1*).

***Eq. 1*: Egg yolk ratio in the hen's egg (%) = 6.490 + 0.499 x egg yolk ratio on the CT scan**
(R^2=0.693, P<0.001)

Only a slightly better accuracy was obtained, when the two (*Eq. 2*) or four (*Eq. 3*) neighbouring (±1 or ±2) scans were also involved into the evaluation:

***Eq. 2*: Egg yolk ratio in the hen's egg (%) = 3.911 + 0.593 x egg yolk ratio on the CT scans**
(R^2=0.729, P<0.001)

***Eq. 3*: Egg yolk ratio in the hen's egg (%) = 4.068 + 0.659 x egg yolk ratio on the CT scans**
(R^2=0.741, P<0.001)

This accuracy of prediction is better, than it was formerly by means of the TOBEC method, but the manual determination of the yolk's surface is very time consuming. Therefore, as third step of the evaluation, a new egg-separation and segmentation software was developed, which can be used to determine the whole volume of the yolk, albumen and shell automatically by separating them with the determination of their borders using all of the scans from the eggs.

Based on the results of using this software in the evaluation it was established that the correlation between predicted and measured yolk content is better, when only two eggs are scanning simultaneously. In this case the correlation coefficient is about 0.79, depending on the technical parameters of the scanning, while it is about 0.65, when five eggs are scanning simultaneously. The difference between the results can be explained by the better resolution of the scans, when eggs are examined in egg holders instead of egg trays. In this case an egg is represented by more pixels and therefore the separation of the anatomical structures is more exact by the edge detecting algorithm.

From the nine tested version of the technical parameters higher correlations were found, when 110 or 130kV was used (r=0.78-0.79) instead of 80kV (r=0.75-0.76). The change in the values of mAs had no effect on the correlations.

After testing the technical parameters of the examinations 3.500 eggs per genotype – 7.000 in total – were scanned by CT in order to determine their yolk content *in vivo*. Based on the yolk ratio determined from the cross-sectional images, eggs with the highest (10%), lowest (10%) and average (10%) yolk ratios were separated (n=350 per group per genotype). These eggs were incubated thereafter. After placing eggs from the incubator into the hatching machine, pedigree-hatching was used, which allowed the exact identification of which chick hatched from which egg.

After hatching, the weight of the chicks was recorded and all of the animals were assigned with wing tags individually. Twenty chicks per group and sex were randomly selected for chemical analysis of their body composition.

Based on the measured values it was established that the hatching weight of the chicks – except the cocks in Genotype 2 – decreased with increasing the yolk ratio in the eggs (*Figure 7*).

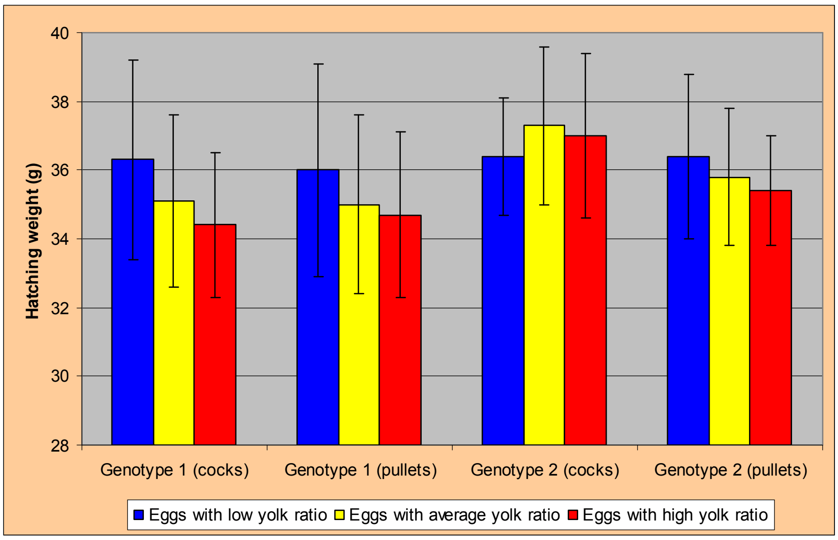

Figure 7.
Hatching weight of the chicks hatched from eggs with low, average and high yolk ratio

In spite of this the ratio of the heart and liver to the liveweight in the chicks of Genotype 1 was slightly increasing with increasing the yolk ratio in the eggs (*Figure 8 and 9*).

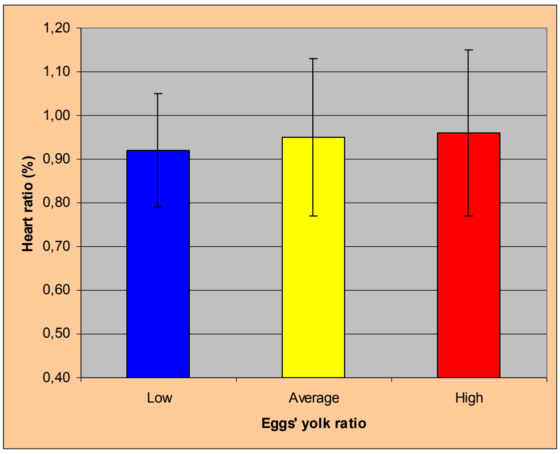

Figure 8.
Ratio of the heart to the hatching weight in chicks hatched from eggs with low, average and high yolk ratio

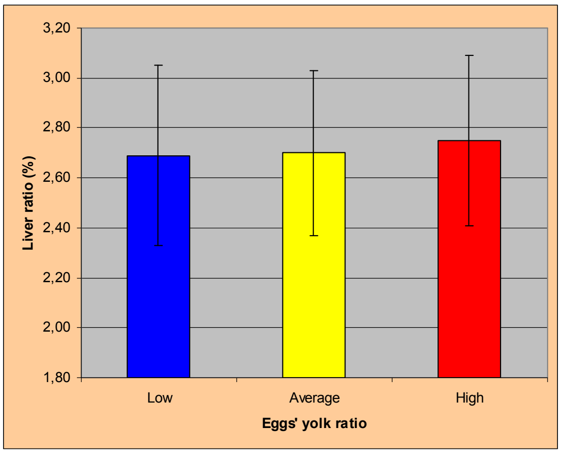

Figure 9.
Ratio of the liver to the hatching weight in chicks hatched from eggs with low, average and high yolk ratio

In Genotype 2 the heart and liver ratio to the hatching weight was not calculated. In this genotype the composition of the whole body was chemically analyzed immediately after hatching. Based on the results of this analysis it was pointed out that the protein content of the body decreased, while its fat content increased with increasing the yolk ratio in the eggs (*Figure 10 and 11*).

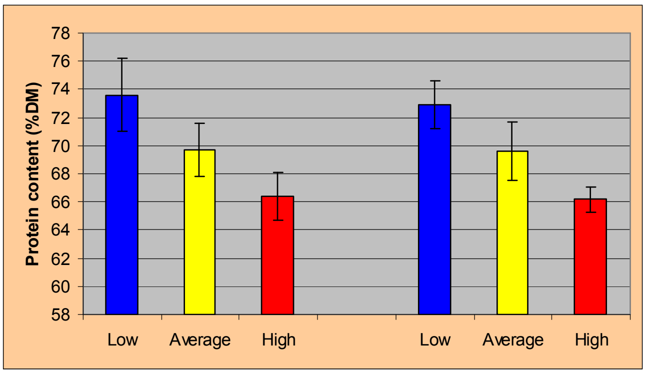

Figure 10.
Protein content of the whole body of hatched chicks hatched from eggs with low, average and high yolk content

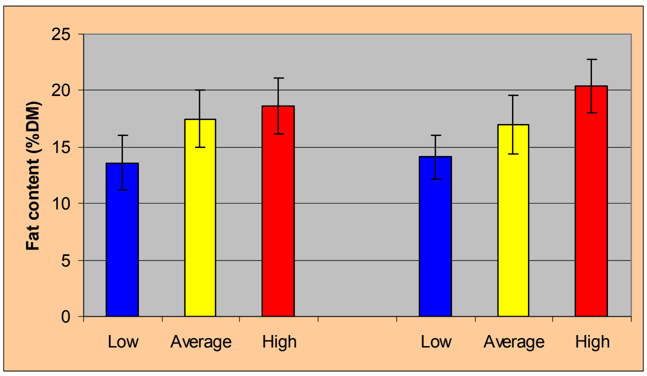

Figure 11.
Fat content of the whole body of hatched chicks hatched from eggs with low, average and high yolk content

Chicks, which were not killed after hatching, were placed into semi-intensive conditions and were fed *ad libitum* with a commercial pelleted diet according to their requirements. Drinking water was also continuously available from self-drinkers.

By measuring the liveweight of the animals it was established that – except the pullets in Genotype 1 – chicks hatched from eggs with low yolk content reached the highest liveweight at the end of the rearing period (*Figure 12*).

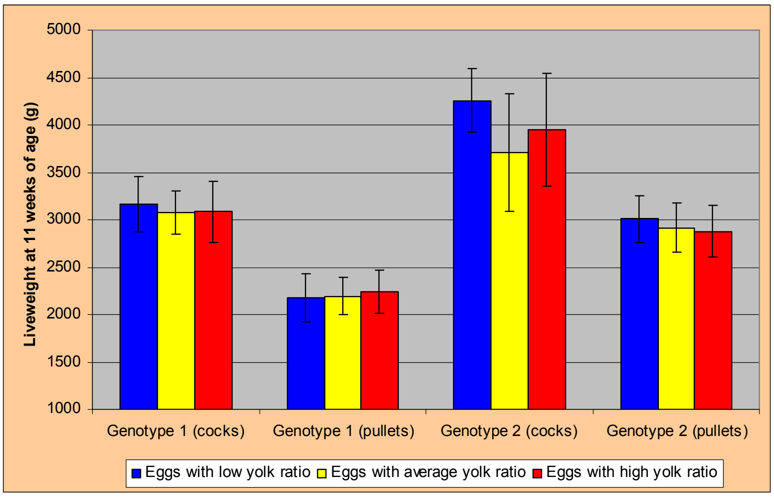

Figure 12.
Liveweight at 11 weeks of age in chicks hatched from eggs with low, average and high yolk ratio

For measuring the changes in the body composition of the chicks during the rearing period CT examinations were done on 20 randomly selected birds per group and sex in both genotype between 5 and 11 weeks of age. For fixing these animals during the measurement procedure a special plexi container – developed earlier – was used, which allowed the parallel examination of three animals at the same time (*Figure 13*).

Figure 13.
CT examination of three chickens at the same time

By the evaluation of the obtained cross-sectional images it was observed that the body fat content of the pullets hatched from eggs with high yolk ratio was higher during the whole rearing period than that of the pullets hatched from eggs with average or low ratio in both examined genotype (*Figure 14 and 15*).

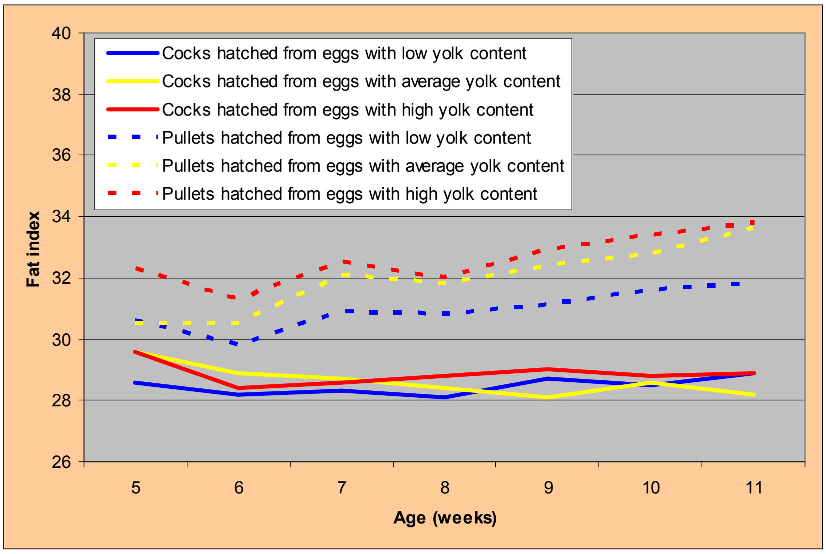

Figure 14.

Changes in the fat index of the chicks hatched from eggs with low, average and high yolk content (Genotype 1)

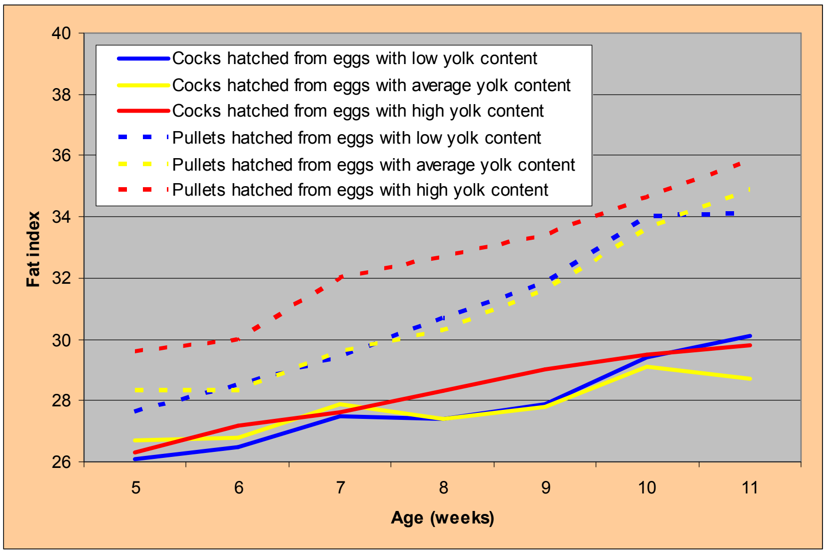

Figure 15.

Changes in the fat index of the chicks hatched from eggs with low, average and high yolk content (Genotype 2)

In the case of the cocks no differences were found in the body fat content of the birds hatched from eggs with different egg yolk ratio.

Parallel with the CT examinations blood samples were taken from 10 of the examined birds in each group in order to determine the blood's total lipid and total protein content. The analysis of the blood samples is already done, data are waiting for the evaluation.

At 11 weeks of age 20 birds per group and sex were slaughtered and dissected according to the practice in slaughterhouses. Based on the results it was established that the weight of the different body parts (grill-ready weight, thigh with skin and bones, breast with skin and bones and breast fillet) was higher in chicks hatched from eggs with low yolk ratio. The reason of it was the higher slaughter weight of these animals. When the ratio of the different body parts to the slaughter weight was calculated no differences were found between the chicks hatched from eggs with different yolk ratio. However, the ratio of abdominal fat to the slaughter weight was highest in those chicks, which were hatched from eggs with high yolk ratio (*Figure 16*).

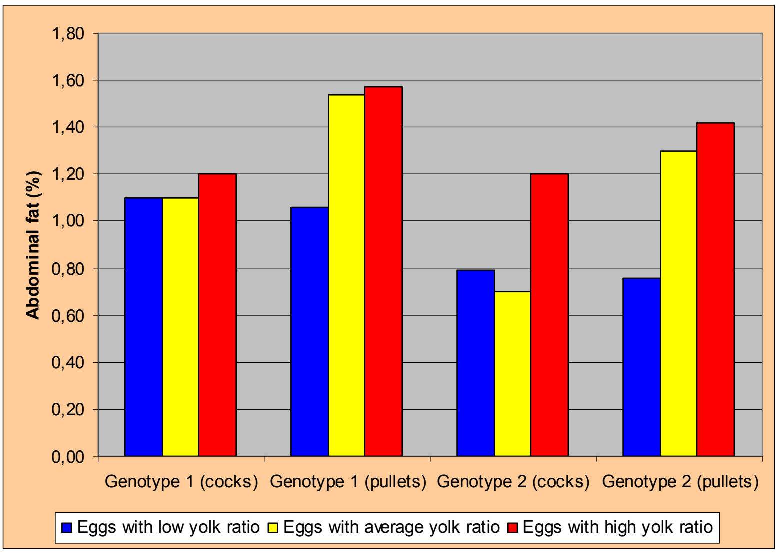

Figure 16.
Ratio of abdominal fat to the slaughter weight in chicks hatched from eggs with low, average and high yolk ratio

After weighing the valuable meat parts and the abdominal fat, the breast and the legs were stored at -18°C till the transportation to the Italian partner. The chemical composition of the breast and thigh muscles was analyzed in the laboratory of the University of Padova. These analyses were already done, data are waiting for the evaluation.

Pullets not slaughtered at 11 weeks of age were reared further. Altogether 120 of them (20 per group in both genotype) were examined by CT first at 20 weeks of age and monthly thereafter for the *in vivo* determination of changes in their body composition. Based on the results it was established that pullets hatched from eggs with high yolk ratio had higher body fat content also at starting the laying period (*Figure 17*).

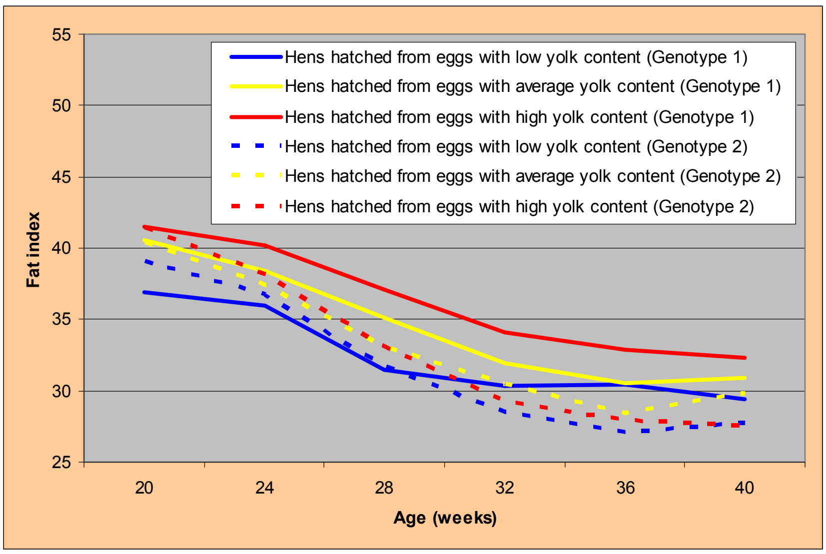

Figure 17.
Changes in the fat index of laying hens hatched from eggs with low, average and high yolk content

Because of the restricted feeding during the laying period the body fat content of the hens decreased till 32 weeks of age in all groups and genotypes.

Data of the laying hens are evaluated till 40 weeks age up to now. At this moment hens are 60 weeks of age old, but we try to keep them till 72 weeks of age in order to finish their first egg laying period. Till 60 weeks of age CT images were already taken, they are waiting for the evaluation.

Parallel with the CT examinations blood samples were taken from 10 of the examined hens in each group in order to determine the blood's total lipid and total protein content. These samples are collected and they will be analyzed together with the samples taken at 64, 68 and 72 weeks of age.

During the laying period also the eggs produced by the experimental hens were collected. Their yolk and albumen content was determined and also their chemical composition was analyzed. Data are waiting for the evaluation also in this case.

Summarizing the above mentioned results it can be established that predictability of the composition of hen's eggs is more accurate by means of the CT than it was formerly by means of the TOBEC method. The composition of the eggs has a significant effect on the liveweight and body composition of the chicks at hatching, which has an effect on their latter development and production. Therefore, based on these results it seems to be necessary to improve the accuracy of the *in vivo* prediction of egg's composition in order to clarify more precisely the correlations between egg composition, hatchability and hatched bird's development and production.

The results of this research project were published mainly in conference abstracts and proceedings up to now (see below). However, after finishing the evaluation of all of the data, publications in scientific journals are also planned.

PUBLICATIONS

1. **MILISITS, G. – DONKÓ, T. – SÜTŐ, Z. – BOGNER, P. – REPA, I. (2009):** In vivo predictability of egg yolk ratio in hen's eggs by means of computer tomography. *60th Annual Meeting of the European Association for Animal Production*, Barcelona (Spain), 24-27 August 2009., 166p.
2. **MILISITS, G. – DONKÓ, T. – SÜTŐ, Z. – ORBÁN, A. – PŐCZE, O. – UJVÁRI, J. – REPA, I. (2010):** Effect of computer tomography examinations on the hatchability of hen eggs. *45th Croatian and 5th International Symposium on Agriculture*, Opatija (Croatia), 15-19 February 2010., 226p.
3. **DONKÓ, T. – EMRI, M. – OPPOSITS, G. – MILISITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Development of new image evaluation software and its applicability in the in vivo prediction of egg yolk content in hen's eggs depending on some CT aquisition parameters. *Acta Agraria Kaposváriensis*, 14 (2), 289-293.
4. **DONKÓ, T. – EMRI, M. – OPPOSITS, G. – MILISITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Tyúktojások sárgája tartalmának becsülhetősége CT-vel a genotípus és az egyidejűleg vizsgált tojások számának függvényében. *IX. Wellmann Oszkár International Scientific Conference*, Hódmezővásárhely (Hungary), 22 April 2010., CD-ROM
5. **DONKÓ, T. – EMRI, M. – OPPOSITS, G. – MILISITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Effect of CT scanning method on the in vivo predictability of egg yolk content in hens' eggs. *XIIIth European Poultry Conference*, Tours (France), 23-27 August 2010., CD-ROM
6. **DONKÓ, T. – EMRI, M. – OPPOSITS, G. – MILISITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Predictability of egg content in hen's eggs by means of cross sectional image analysis. *2nd International Workshop on Computer Image Analysis in Agriculture*, Budapest (Hungary), 26-27 August 2010., 38-41.

7. **MILISITS, G. – DONKÓ, T. – EMRI, M. – OPPOSITS, G. – ORBÁN, A. – SÜTŐ, Z. – REPA, I. (2010):** Possibilities and deliminations in the in vivo determination of egg composition. *9th International Symposium "Prospects for the 3rd Millennium Agriculture*, Cluj-Napoca (Romania), 30 September – 2 October 2010., 271-277p.

8. **MILISITS, G. – DONKÓ, T. – EMRI, M. – OPPOSITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Evaluation of cross sectional CT images on hen's eggs. *Farm Animal Imaging Congress*, Rennes (France), 17 June 2010., 12p.

9. **DONKÓ, T. – EMRI, M. – OPPOSITS, G. – MILISITS, G. – SÜTŐ, Z. – ORBÁN, A. – REPA, I. (2010):** Effect of CT scanning method on the in vivo predictability of egg yolk content in hens' eggs. *World's Poultry Science Journal*, Volume 66, Supplement, 290p.

10. **MILISITS, G. – SZABÓ, A. – DONKÓ, T. – SÜTŐ, Z. – ORBÁN, A. – SZENTIRMAI, E. – PŐCZE, O. – UJVÁRI, J. – REPA, I. (2011):** Effect of yolk ratio in hen's eggs on the hatching weight and on the heart and liver ratio in chicks at hatching. *46th Croatian and 6th International Symposium on Agriculture*, Opatija (Croatia), 14-18 February 2011., 239p.

11. **MILISITS, G. – SÜTŐ, Z. – DONKÓ, T. – ORBÁN, A. – SZENTIRMAI, E. – PŐCZE, O. – UJVÁRI, J. – REPA, I. (2011):** Effect of yolk ratio in hen's eggs on the slaughter weight and slaughter characteristics of hatched chicks. *46th Croatian and 6th International Symposium on Agriculture*, Opatija (Croatia), 14-18 February 2011., 240p.

12. **MILISITS, G. – SZABÓ, A. – DONKÓ, T. – SÜTŐ, Z. – ORBÁN, A. – SZENTIRMAI, E. – PŐCZE, O. – UJVÁRI, J. – DALLE ZOTTE, A. – CULLERE, M. – REPA, I. (2011):** Effect of yolk ratio in hen's eggs on the hatching weight and on the heart and liver ratio in chicks at hatching. *X. Wellmann Oszkár International Scientific Conference*, Hódmezővásárhely (Hungary), 5 May 2011. (submitted)

13. **DALLE ZOTTE, A. – CULLERE, M. – SÜTŐ, Z. – DONKÓ, T. – MILISITS, G. (2011):** Effect of egg composition and genotype on the hatched broiler's meat traits and meat composition. *XIVth European Symposium on the Quality of Eggs and Egg Products and XXth European Symposium on the Quality of Poultry Meat*, Leipzig (Germany), 4-8 September 2011. (submitted)

14. **MILISITS, G. – DONKÓ, T. – SÜTŐ, Z. – ORBÁN, A. – PŐCZE, O. – UJVÁRI, J. – DALLE ZOTTE, A. – CULLERE, M. – REPA, I. (2011):** Changes in the liveweight and body composition of chicks hatched from eggs with different composition. *XIVth European Symposium on the Quality of Eggs and Egg Products and XXth European Symposium on the Quality of Poultry Meat*, Leipzig (Germany), 4-8 September 2011. (submitted)