

**THESES OF DOCTORAL (PhD)
DISSERTATION**

**UNIVERSITY OF KAPOSVÁR
FACULTY OF ANIMAL SCIENCE**
Department of Poultry and Companion Breeding

Head of the Doctoral School:
DR. PÉTER HORN
Ordinary Member of the Hungarian Academy of Sciences

Supervisor:
DR. FERENC BOGENFÜRST PhD
University Associate Professor

**THE FACTORS INFLUENCING THE LIVER
PRODUCTION ABILITY AND QUALITY IN GEESE**

Written by:
SZILVIA ÁPRILY

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1. INTRODUCTION AND OBJECTIVES OF THE RESEARCH

More criticism and attack is addressed to fattening by force-feeding despite of it that many research groups have verified by ethological and physiological examinations that force-feeding does not cause bigger stress and more pain in the waterfowls than any other technological procedure. Due to the strengthening animal protection movements cramming has been prohibited in many member states of the European Union as well as Israel. Therefore force-feeding is applied mainly in Hungary. In accordance with the Animal Protection Act effective in Hungary now, force-feeding carried out considerably is not prohibited. According to the predictions, the goose liver production by force-feeding shall be stopped after 2011 (TÓÁSÓ ET AL, 2006). Despite of it, that this is the product that represents the highest value in the poultry and waterfowl sector and provides the first position in the world to Hungary with the yearly 1800 tons production.

The actuality of this topic comes from the animal protection rules become stricter and stricter. Therefore the traditional goose fatty liver production shall be based on uniform and reproducible technology being applicable under closed and intensive keeping conditions. At the same time the physiological changes that the technology induces in the animal body shall be known.

In the research aims are as follows:

- How does the body weight, feed consumption, body weight gain, liver weight and mortality of geese in various genotypes and sexes having different appetite and weight gain, change in the various keeping periods and at the end of the force-feeding period?
- Is there any difference in the liver production of the geese, having to various genotypes and sexes and having different appetite?

- What is the effect of the preparation before the force-feeding period on the liver production of the geese (on the average liver weight)?
- Is any genetic difference detected in the geese of various sexes and of different appetites depending on the genotypes?
- What effects do the body weight of the lean goose (body weight before force-feeding) and fattening during the force-feeding period (body weight gain) have on the weight of fatty liver?
- How do the lipid content in the blood plasma as well as the level and composition of the fatty acids change in the raising and force-feeding period?
- What morphologic and physiologic changes occur in oesophagus and liver in the preparation and in the force-feeding period.

Four trials were set to answer the previous questions. The following aims were drawn in the experiments.

In the **pre-trial** the appetite relation to the feed consumption and important production parameters were set in liver and meat type goose hybrids.

In the **Experiment 1** the aim was to define the effects of the genotype, the sex and the different preparation methods on the liver weight.

In the **Experiment 2** the DNA originated from geese with different sexes and appetite was examined to searching for genetic markers relation to appetite. Further aims were the correlation between the body weight before the force-feeding and the weight of fatty liver, furthermore the weight gain during the force-feeding and the weight of fatty liver.

In the **Experiment 3** the main aim was to study the effects of the preparation and force-feeding on the fattening of the liver as well as to observe the changes in the lipid fraction of the blood plasma. Furthermore I wondered what morphological and structural changes occurred in the oesophagus and liver.

My finally objective was to develop a new growing and preparing technology which reproduces in close, intensive keeping.

2. MATERIAL AND METHODS

1. Study of the production parameters in goose with different genotypes and sexes (preliminary-trial)

The pre-trial there was in the close goose-house of the Training and Research Plant at Faculty of Animal Sciences of Kaposvár University. Based on the feed consumption of two different utilization type goose hybrid there were examined the appetite, the body weight, the body weight gain and mortality, furthermore the average weight of fatty liver after force-feeding.

Animal and experimental design

In this trial there were 504 geese of liver-type and 504 geese of meat-type hybrid tested. The goslings were separated by sex and utilization and kept in closed, intensive system in goose-house. The geese of both genotypes were kept in the same conditions up to age of eight weeks. During this time the feeding was completed as follows: in the first three weeks ad libitum starting feed; from 3 to 8 weeks age ad libitum growing feed. The body weight and weight gain was checked by weighing once a week. Between 4th and 5th two groups were created based on the individual feed uptake: group of the animals having good appetite and another group with geese having poor appetite. The individual feed consumption was calculated on the basis of the differences in the live weight gain weighed before providing the morning feed and after two-hours feeding. The weekly means of the values obtained were calculated for each test geese and the means were arranged in increasing order. The animals showing higher value than the mean were grouped in the group having good appetite while those showing values below the mean were put in the group of poor appetite. Two weeks were enough for adapting the new conditions. After that – at the age between 8 and 11 weeks – the geese were fed in accordance with restriction (with

periodical feeding program (fed with time limitation). Between 10th and 11th week the individual feed uptakes were weighed again and the groups were divided into sub-groups on the basis of the mean body weight gain. As a result there were four groups by genotypes and sexes: good appetite with large body weight gain (NÉNR); good appetite with small body weight gain (NÉKR); poor appetite with large weight gain (KÉNR) and poor appetite with small weight gain (KÉKR). The heads of the animals in the different group, were marked with different colours making their identification easier.

The force-feeding of the geese was begun at their age of 11 weeks; it was carried out for 18 days, twice a day using hydraulic force-feeder machine with mixture of corn, cramming feed and water in ration 27.5, 36.5 and 36.0 %, respectively suggested by DUBOIS AT AL (1994).

The following parameters were measured in the pre-trial: daily feed consumption, weekly the body weight. The body weight gain was calculated from the body weight data, furthermore there was recorded the mortality during the whole experimental period. The geese were killed and the liver was obtained and weighed one day later to the last force-feeding.

Statistical analysis

Experimental data were evaluated t-test and ANOVA (Tukey-test) was used ($P < 0.05$) with SPSS 10.0 for Windows software.

2. Effects of the genotypes, sex and the periodical feeding (pregavage) method on the weight gain and liver production in geese (Trial 1)

This trial was set in the Training and Research Plant at Faculty of Animal Sciences of Kaposvár University. The aim was to observe the effect of the

genotype, sex and different preparation methods before force-feeding on the liver weight in closed, intensive keeping in different goose hybrid.

Animals and experimental design

In the trial there were 250 geese of liver-type hybrid (a₁: ANABEST G) and 250 geese of meat-type (a₂: ANABEST W) hybrid, determined their sex (b₁: ganders; b₂: females) at age of one day and marked individually, housed separately by sex and genotype. Body weight was weighed once a week in the whole rearing period. The geese were kept in the same conditions up to age of six weeks (from 0 to the 3th starting period, feeding: starting feed and 3 weeks growing period; feeding: growing feed).

Following the growing period the geese were located in 16 boxes each having 15 geese (2.2 geese/m² density). They were separated by genotypes and sex prepared with four different methods (c₁-c₄) for force-feeding. (Method c₁: restriction in the quantity: for 21 days; method c₂ and c₃: time limitation for 21 days; feeding twice a day and continuously decreasing up-taking time – the difference between the methods is the days when the restriction is stopped; method c₄: time restriction for 35 days, giving feed once and twice a day, alternately).

During the preparation period geese were fed with mixture of raising and cramming feed in ratio 50:50, the feed consumption and body weight of the geese was recorded.

For force-feeding the geese were placed into group force-feeding cages (four geese/cage). At the age of 9th and 11th forty females and forty males with body weight of at least 4.0 and 4.5 kg. The force-feeding was carried out for 18 days, twice a day using a hydraulic force-feeder machine with mixture of corn, cramming feed and water in ratio 27.5, 36.5, 36.0 %, respectively (DUBOIS ET AL, 1994). On the 19th day all geese were killed, the liver was removed from

their bodies and qualified one day later in accordance with the Codex Alimentarius Hungaricus.

The following parameters were measured in the trial: body weight and body weight gain during raising period; the average daily feed consumption in the preparation period, body weight at the beginning and at the end of force-feeding period; body weight at the end of force-feeding and weight gain during the force-feeding period and the liver weight after killing.

Statistical analysis

Data were processed with SPSS 10.0 for Windows statistical software. To compare the means of the examined parameters within the block ANOVA (Tukey-test) was used ($P < 0.05$). To evaluate the effects of the treatments multivariate analysis (GLM) was applied ($P < 0.05$). In the statistical model the preparation method was fix factor, while genotype and sex were random factor.

3. Examination on DNA of the geese of different sex and appetite (searching for genetic markers related to the appetite); examination on relationship between the body weight gain in the force-feeding period and weight of fatty liver (Trial 2)

Selecting and breeding geese with good appetite is suggested the one of the alternative method of the goose fattening by MARCILLOUX AND AUFRAY (1982).

Since it has been observed in the preliminary trial that appetite strongly influences the liver weight I wonder if any genetic differences in the genotypes having various appetites can be detected. In addition, the effects of the live weight before force-feeding as well as of the body weight gain on the liver weight were examined.

3. 1. Examination of DNA of various genotypes and sexes goose (searching gene markers relation to appetite)

Animals and experimental design

For molecular biological examinations 400 liver-type hybrids (n= 200 ganders, n= 200 females) were kept from one-day age separated by sex, under intensive keeping conditions in the Training and Research Plant at Faculty of Animal Sciences of Kaposvár University. From 0 to the 3th lasts the starting period, from 3 to 6 weeks of age lasts the growing period.

To determine the appetite of the geese following the growing period the feed uptaking time was limited to two hours twice a day for a week (adapting period). Individual feed uptake was measured in one week difference between the sexes (starting with the ganders followed by the females one week later). In the mornings (after 12 hours starving) before giving the feed as well as after the feed was taken up all geese were weighed. During the feeding period the geese were not allowed to access to water. In the evenings only the average feed consumption was calculated for each sex. The morning values obtained in the week were averaged for each geese and the means were arranged in increasing order. 15 % of the animals showing the highest and another 15 % the lowest value than the average were selected (28:28 ganders and 27:27 females). Without any re-grouping they were located in another box to determine the daily average feed consumption. After that periodical feeding program was applied for three weeks providing feed twice a day, with continuously decreasing feed consumption time (it was decreased from 2×2 hours to 2×0.5 hours in three weeks). In this period the respective daily average feed consumption of the sexes was weighed.

Sampling

Blood sample

At the end of the periodical feeding time 25 ganders having good appetite and 25 ones with poor appetite were selected randomly. Blood samples were taken 5-6 hours after feeding from the wing vein into a 2 ml Eppendorf vial containing heparin. The blood samples from the females were taken one week later than from ganders. The samples were stored at -20 °C temperature before the analysis. DNA was extracted from the blood samples according to the GENTRA SYSTEMS INC. (2003) protocol.

The eucariotic genome is big and complex enough and therefore there must be some loci randomly, which are located in proper distance and direction in relation to one another and are complementary of each other, which make the amplification with PCR. There were used 10-12 bp long primers. The polymorph products resulted from the amplification is random amplified polymorphic DNA (RAPD) markers. In this study there were 20 primers of various basics, applied according the PCR protocol described by manufacturer. DNA fragments were visualised on gel with dye fluorescing in UV light. Amplification of the DNA with PCR was completed at the Molecular Laboratory of the University of Kaposvár.

3. 2. Examination of relationship between the weight gain during force-feeding period and weight of fatty liver

Animals and experimental design

The effects of the live weight before force-feeding and weight gain during the force-feeding period on the liver weight were studied on 50 liver-type ganders. The rearing period consists of 3 weeks starting period, growing period from 3 to 6 weeks age and preparation period before the force-feeding from 6 to 9 weeks age- The preparation was completed with a 21-day long

time restriction period when the time for feed consumption was decreased from 2×2 hours to 2×0.5 hours. The restriction was solved on 4th, 11th and 19th day. The average daily feed consumption and body weight gain of 50 ganders were examined.

At the age of 9th weeks the geese were weighed and 48 of them were selected randomly and placed into a group force-feeding cages (4 geese/cage). The force-feeding was carried out for 16 days, twice a day using hydraulic force-feeder machine with mixture of corn, cramming feed and water in ratio as described by DUBOIS ET AL (1994).

Statistical analysis

Relationship between weight gain during force-feeding and weight of fatty liver was carried out by correlation calculation ($P < 0.05$). The relationship of the two variables showing the best fitting was determined by regression analysis.

4. The effect of preparation and the force-feeding on fattening of the liver, on fatty acid content and composition in the liver and on the changes of lipid fractions of blood plasma; Macroscopic and microscopic examination of the oesophagus and liver (Trial 3)

The purpose of the trial 3 was to study the effects of preparation and force-feeding on the oesophagus and liver as well as to observe the changes in the lipid fraction on the blood plasma. The daily feed consumption during the preparation period and the weight gain before and during the force-feeding period was examined. Changes in size of the oesophagus (length and lumen) and weight of the fatty liver were measured. In this trial the force-feeding preparation (*pregavage*) and its physiological effects on the goose body were studied. I studied the morphological and histological changes in the oesophagus

and liver, changes in weight and quality of the liver as well as changes of lipid fraction in the blood plasma and fatty acid content in the liver.

Animals and experimental design

150 ANABEST G ganders – liver type hybrid – marked individually were located in goose-house under closed keeping conditions. The growing period was as same as specified before. After raising period geese were divided into two groups. For group 1 preparation method provided the best results for liver in trial 1 was selected and used to prepare the animals for cramming. The other group was control, for which no the feed was limited. Both groups were fed with mixture of raising and cramming feed in ratio of 50:50 %. After preparation period all geese were weighed then forty geese were selected randomly for each group and placed into cramming cages (4 geese/cage). The force-feeding was carried out for 14 days, twice a day using a hydraulic force-feeding machine with mixture of corn, cramming feed and water in ratio 27.5, 36.5 and 36.0 %, respectively (DUBOIS ET AL, 1994).

Sampling

Blood samples were taken in the 6th, 9th and 11th week from 15 geese of each group. The same 15 geese were sampled at each sampling time. The blood samples were taken 5-6 hours after the feeding to the animals from the wing vein into a blood tube containing heparin. At sampling times 5 geese were killed for test. Before killing the animals were weighed and oesophagus was prepared. The length and the diameter of the proximal and distal section of the oesophagus were measured by slide-gauge. Ratio of proximal to distal section was calculated; macroscopic examinations were carried out and the changes were recorded. Liver was removed from the body; it was weighed, qualified as well as sampled to determine its fatty acid composition and content and to have microscopic tissue sections. The fatty liver was qualified

by Codex Alimentarius Hungaricus. Liver and oesophagus samples were fixed in 8 % neutral formalin and stored until preparing tissue sections. Feeds and nutrients applied in the preparation and force-feeding periods also were sampled to determine their fatty acid composition and content. Lipid profiles of the blood samples and fatty acid composition and content in liver and feed samples were determined at Animal Breeding and Feeding Research Institute in Herceghalom. The sections were prepared and coloured with hematoxylin and eosin at Department of Pathology at Kaposi Mór Training Hospital. The sections were examined with Axioskope microscope.

Statistical analysis

Experimental data were evaluated t-test and ANOVA (Tukey-test) was used ($P < 0.05$) with SPSS 10.0 for Windows software.

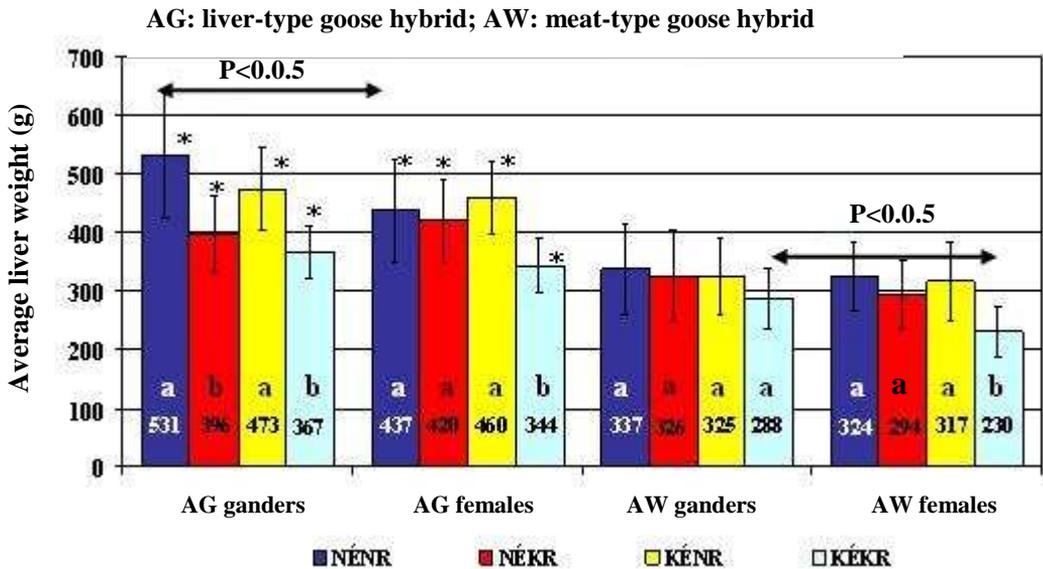
3. RESULTS

3.1. Production parameters of different genotypes and sexes goose (Pre-trial)

In the pre-trial – according to the aims – the main production parameters (average body weight gain in the different raising periods, feed consumption and – on the basis – the appetite) of the liver-type and meat-type goose hybrids were studied. The average body weight of the liver-type geese is lower than one of the meat-type geese. The average of the females is less than one of the ganders in every raising period and in both genotypes. The females – despite of their smaller body weight than one of the ganders – achieved more weight gain in the 18-days force-feeding period than the ganders (the body weight gain of liver-type geese: for females 1991 ± 293.5 g, for ganders 1888 ± 243.0 g, while in meat-type: for females 2322 ± 209.7 g and for ganders 2228 ± 200.3 g).

In the different genotypes the average daily consumption of the groups with various appetites was significantly different. In the groups with the same appetite the feed consumption of the two sexes can be considered the same.

The rate of the death was very small in all growing periods and even in the cramming period it was not more than 5 % although in fattening of the geese of meat-type by force-feeding higher rate of mortality was expected (*Figure 1*).



NÉNR: group with good appetite and great weight gain; **NÉKR:** group with good appetite and small weight gain; **KÉNR:** group with poor appetite and great weight gain; **KÉKR:** group with poor appetite and small weight gain;

*Significant difference between the same sex liver- and meat type geese having same appetite and weight gain ($P < 0.05$); $n=10/\text{group}$ (total 160 geese)

Figure 1. The average liver weight in the liver- and meat-type geese with difference appetite and weight gain

Liver production of the geese with different appetite was different. As expected, the liver production of the liver-type geese was much higher than one of the meat-type hybrids: the average liver weight of the liver-type group, having the smallest liver weight (KÉKR) was higher than in case of the meat-type group having the best liver productin (group NÉNR) in both sexes.

The geese showing good appetite and fattening ability (group NÉNR) having liver weight in both sexes than animals of the other three groups except in the liver-type females. It is remarkable that the average liver weight of the group having poor appetite but good fattening ability (group KÉNR) was similar to – and in liver-type females sometimes higher than – the liver

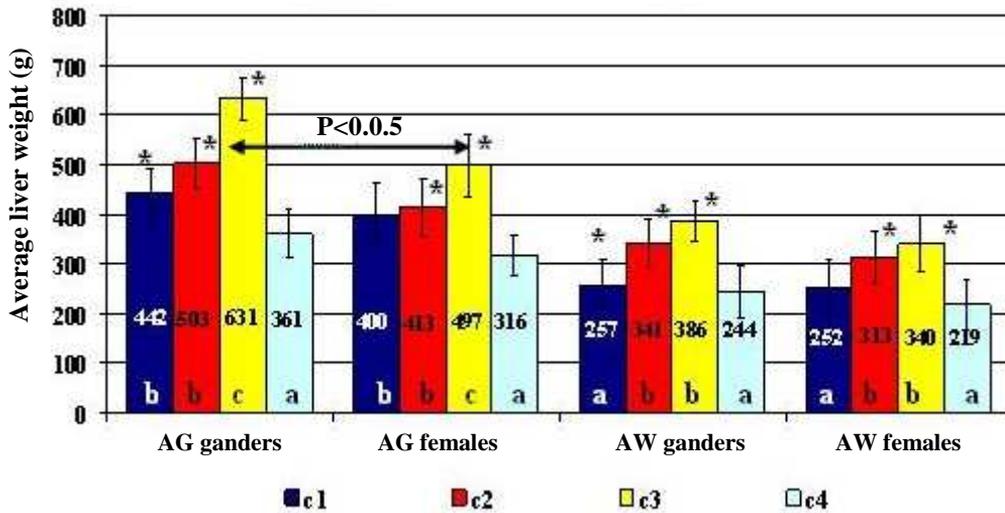
weight of the group with good appetite and fattening ability. This relationship is important from economic aspect because it indicates that the liver-type hybrid with poor appetite but well-fattening shows higher weight gain from less feed and higher average liver weight is produced.

3.2. Effect of the genotype, sex and periodic feeding (during the preparation period) on the weight gain and liver production in geese (Trial 1)

Body weight of ganders was more than one of the females both at the beginning and end of the preparation period and at the end of force-feeding period. As the liver weight changes as function of the body weight, it can reach 10 % of the body weight and the difference of the body weight between males and females is 8-10 % up to the ganders (PINGEL ÉS SCHNEIDER, 1981; BÖGRE, 1991), therefore bigger liver weight was expected the ganders than from the females. It corresponded with my expectations and with data of the literature; the average liver weight of ganders and females was 484.0 ± 113.0 g and 406.0 ± 83.0 g.

LARZUL ET AL (2000) observed similar difference in the average liver weight between the sexes. It was associated with difference in the body weight by the data from the literature the adult female geese are more inclined to put on weight and have better ability to produce liver than the adult ganders. In case of young animals the situation is reversed (PÁLFFY, 1980; BOGENFÜRST, 1992). Results of the liver production in the various preparation methods are shown in *Figure 2*.

AG: liver-type goose hybrid; AW: meat-type goose hybrid



a, b, c: significant difference in the same genotype and in the same sex; * significant difference between the various genotypes in the same sex ($P < 0,05$ szinten)

Figure 2 The average liver weight related to the genotypes, sexes and the different preparation methods

The biggest liver weight was resulted by the 21-day long time restricted feeding method (c₃) in both genotypes while the smallest liver weight was achieved by c₄ method applying 35-day long time restricted feeding method.

When quantitative restriction was applied, the average liver weight was lower in both sexes than in case of any methods in which hourly restriction was applied, in accordance with ROBIN AND CASTAING (2002) LEPRETTRE ET AL (1997) results.

The treatments and the interactions between the treatments were studied by multivariate analysis (GLM). The effect of the sex was significant on all studied parameters except the body weight gain in the force-feeding period. In this trial the preparation method showed significant effect on all parameters examined except the live weight measured at the end of the preparation period. Preparation and sex showed joint effects on the weight

gain and liver weight in the preparation period only. It is interesting that the liver weight was significantly ($P < 0.05$) influenced by the treatments both separately and double interaction but no the statistical test confirmed the significant effect in case of genotype \times sex \times preparation method interaction.

3. 3. Examination on DNA of the geese of different sex and appetite (searching for genetic markers relation to the appetite); examination on relationship between weight gain in the force-feeding period and the weight of fatty liver (Trial 2)

3. 3. 1. Examination on DNA of the geese of different sexes and appetite (searching for genetic markers relation to the appetite)

To determine the appetite measurements were completed for a week. The average feed consumption weighed in the mornings was 140 g and 80 g for gluttonous females and females having poor appetite, respectively. Regarding the ganders, the gluttonous geese consumed 108 g feed as an average in 2 hours. The feed consumption of the ganders with poor appetite was less than 100 g (98 g). The feed consumed in the evenings was always above the feed consumed and measured in the morning. The evening feed consumed by the geese was not weighed one by one but average feed uptake was calculated. The average values were 200 g and 160 g for ganders and females, respectively. The daily feed consumption of the gluttonous ganders was 380 g; in the geese having poor appetite it was less than 300 g. The feed consumption of females was 300 g and 240 g for gluttonous birds and ones having poor appetite, respectively.

Studying the daily average feed consumption of the total stock in preparation period in 21 days, the average was 300 g and 267 g for ganders and females, respectively. The feed consumption of ganders was a little higher while one of the geese was a little lower than the values obtained in the Trial 1.

In this trial the progeny of parents not being selected by their appetite, hence (as it could be expected) there was no genetic difference between the progenies having different appetite.

At the beginning all geese were examined in separately reactions. Each samples of geese showed the same RAPD profile, no differences could be observed (*Figure 3*).

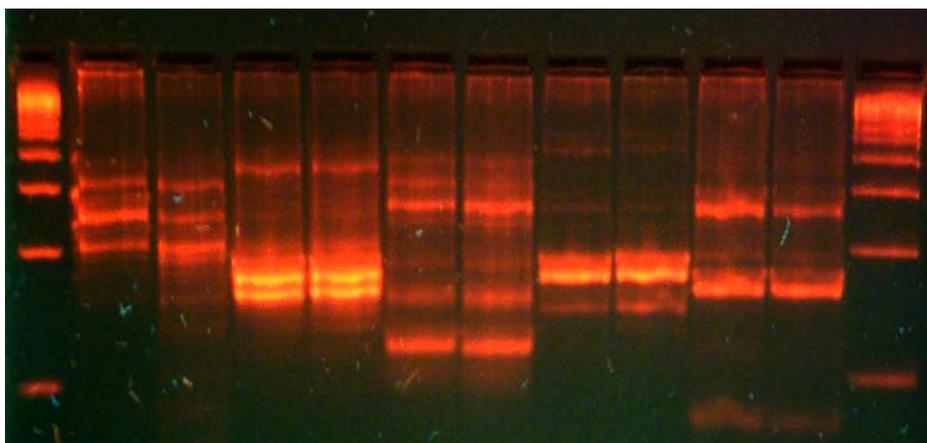


Figure 3 The homogenous RAPD profile of the X-1, X-2, X-3, X-4 and X-5 primers in the pooled samples

Later – due to the facts mentioned above – pooled samples were applied where the samples were separated by sex but within the samples of the geese having both good and poor appetite were mixed. RAPD profile for each marker was the same in both sexes which means no genetic differences could be detected between the groups of good and poor appetite.

3. 3. 2. Examination onrelationship between weight gain during force-feeding and weight of fatty liver

Average body weight of the birds selected for force-feeding was 5323 ± 510 g and 7351 ± 456 g at the beginning and end of the force-feeding period, respectively. The average weight gain and average weight of fatty liver were

2031 ± 317 g and 603.3 ± 130 g, respectively. It is remarkable that this liver weight was achieved when the geese were fattened by force-feeding in approximately 40 %.

The correlation between the variables is very close: $r=0.794$. SZIGETI ET AL (1999) also found close correlation between these parameters in case of another three liver-geese genotype but the correlation coefficient was higher ($r=0.98$) The relationship between the parameters was described with linear curve.

If there is a significant relationship between two parameters, the one variable is known the other one can be determined. The liver weight and the body weight is increasing up to a certain degree. The connection is analogue with the growing therefore it can be defined by a logistic function rather than a linear one. Fattening of the geese was carried out at young age (before they reached the body weight typical for the full-grown birds) therefore an exponential function can be fitted best to the relationship between the variables. The function is in the lower section of the logistic curve.

In case of the 45 geese studied the liver weight can be estimated with 68,1 % accuracy on the basis of body weight gain occurred during the force-feeding period. The estimation equation can be described with the following formula:

$$\text{Liver weight (g)} = 202.155 * e^{(0.000535 * \text{weight gain during force-feeding period})}$$

In case of the geese which were fattened by force-feeding at late age relation between weight gain and liver weight changes and can be described by a saturation function which is in the section above section changing point of the logistic curve.

In this trial there was no relationship between the body weight weighed before force-feeding and liver production (liver weight) in contradiction to observations of KATZ ET AL (1997), PENKOVA AND BÓDI (1995) and SHALEV ET AL (1986).

The results are in contradiction SHALEV ET AL (1986) namely when suggest for the liver weight is made the body weight measured before force-feeding shall be focused on rather than the body weight at the end of the fattening (force-feeding) period.

It is remarkable that average weight of fatty liver was achieved when the geese were fattened with weight gain 40 % according to the body weight before force-feeding. The positive changes can attribute to the genetic advance, preparation method and technology of force-feeding.

3. 4. Examination of the effect of the preparation and force-feeding period on the fattening of liver; examination of the macroscopically and microscopically of the oesophagus and liver (Trial 3)

The average body weight of the geese at the age of 6 weeks was 3600 ± 329 g. The average body weight at the age of 9 weeks and feed intake of the two groups were significantly different: weight of the geese being prepared for force-feeding and of the control group was 4567 ± 436.4 g and 4984 ± 444.5 g, respectively. The average of weight gain of the group with restricted feed intake was 1 kg while the other group showed 1.2 kg weight gain in the same period. Feed intake of the geese prepared for force-feeding showed increasing tendency in the preparation period; 151 g daily feed consumption at the beginning increased to 310 g in 3 weeks (the mean was 243 g in the studied period). The daily mean feed intake of the control group was 320-330 g in the three weeks. At the same time the hourly feed consumption of the geese having been prepared for force-feeding was ten times higher than one of the control geese. The body weight at the end of force-feeding, the

body weight increase during the force-feeding, the liver weight and its ratio to the body weight at the end of force-feeding of both experimental groups was studied and significant difference was observed between the groups and confirmed by statistical tests, except the body weight gain. The control group had higher body weight during the whole force-feeding period.

Results of the blood plasma examination

Changes of lipid fractions in the blood plasma are represented in *Table 1*.

Table 1 Changes of the lipid fraction in blood plasma of geese prepared and have not been prepared for force-feeding

Group	Sampling time (week)	Triglyceride (mmol/l)	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Total lipid (g/l)
Geese had not been prepared	6.	3.22 ± 0.43 b	3.43 ± 0.39 a	1.99 ± 0.36 a	5.84 ± 0.91 a
	9.	2.30 ± 0.52 a	3.65 ± 0.27 a	2.17 ± 0.18 b	5.29 ± 0.90 a
	11.	4.38 ± 0.61 c	4.34 ± 0.66 b	3.00 ± 0.52 b	8.08 ± 1.47 b
Geese prepared for force-feeding	6.	2.71 ± 0.49 b	3.46 ± 0.45 a	2.10 ± 0.34 a	5.30 ± 0.73 b
	9.	0.81 ± 0.12 a*	3.87 ± 0.31 b	2.56 ± 0.19 b	3.74 ± 0.55 a*
	11.	4.15 ± 0.62 c	4.65 ± 0.51 c	3.40 ± 0.34 c	8.27 ± 1.15 c

n=15/sampling time; a, b, c: significant difference in consecutive sampling time; *significant difference between the two groups in same lipid-fraction and sampling time

The dominant lipid fraction was the total cholesterol in blood plasma of both groups and showed continuously increasing tendency; in parallel the HDL cholesterol level also changed. The amount of the triglyceride decreased at the second sampling time in both groups; more decrease was observed in groups having been prepared for force-feeding. Triglyceride amounts followed the changes of the total lipid content in both groups and changed in

parallel with it. In the samples taken at the 6 weeks of age the parameters examined were not different in the two groups. Differently from my expectations, triglyceride content in the blood, taken at second sampling time from the animals having been prepared for force-feeding, decreased significantly in comparison to both the value measured at 6 weeks of age and the value measured in the control group. Significant difference ($P < 0.05$) between triglyceride contents in blood plasma of the two groups was verified by a statistical test. In the samples taken after force-feeding period the amounts of all lipid metabolites increased and no statistically verified difference was observed between parameters of the groups. The total lipid content decreased by the 9th week in both groups, however at the end of force-feeding it increased to one and half times of the value having been measured in the basic status.

Results of the oesophagus examination

In the oesophagus samples of geese at the age of six and nine weeks no difference – either in the total length or in diameter of proximal and upper sections or their ratio – between the groups was observed. At the age of 9 weeks, more remarkable changes in the oesophagus distal section were expected at the end of preparation period but there was no significant difference between two groups. Diameter of the distal section of the oesophagus was bigger in case of the group having been prepared for force-feeding. At the end of the force-feeding period there was significant difference in the diameter of the distal section of the oesophagus. The oesophagus parameters measured and calculated during the force-feeding was evaluated with ANOVA. The results summarised in *Table 2*.

Table 2 Some parameters of oesophagus and the liver weight of control geese and of geese prepared for force-feeding

Group prepared for (n=5/sampling time)	Sampling times		
	6 weeks of age	9 weeks of age	11 weeks of age
Total length of the oesophagus (mm)	388.0±13.5 a	401.6±9.9 a	442.4±11.2 b
Diameter of the prox.section of oesophagus (mm)	13.5±1.7 a	31.8±3.2 b	37.4±3.5 c
Diameter of the dist.section of oesophagus (mm)	19.2±0.85 a	64.0±2.2 b	75.6±3.5 c*
Ratio prox/dist section (%)	70.7±7.1 b	50.1±7.0 a	49.7±4.8 a
Average liver weight (g)	113.8±12.3 a	118.6±17.1 a	568.4±63.9 b
Group control (not prepared for force-feeding) (n=5/sampling time)	6 weeks of age	9 weeks of age	11 weeks of age
Total length of the oesophagus (mm)	366.4±17.3 a	372.8±18.5 a	412.6±16.9 b
Diameter of the prox.section of oesophagus (mm)	13.1±1.5 a	34.6±3.1 b	38.0±5.9 c
Diameter of the dist.section of oesophagus (mm)	18.6±2.4 a	61.2±2.6 b	62.2±6.5 b
Ratio prox/dist section (%)	56.6±6.6 b	62.3±4.7 ab	56.6±3.9 a
Average liver weight (g)	96.4±12.4 a	102.8±14.4 a	413.0±52.3 b

a, b, c: significant differences (P<0.05 level); ANOVA, Tukey-test; *: significant difference between two groups in the same time (P<0.05)

Based on the data of *Table 1* the proximal section of the oesophagus became triple of the basis status in case of both groups. Tendency of the diameter of the distal was similar for both groups; however this section was definitely wider in geese prepared for force-feeding than in the control geese. The difference was verified by a statistical test, too (P<0.05).

Results of the macroscopically and microscopically examination of the oesophagus

In oesophagus of the control geese wound and blood-stain, mainly in the distal section, occurred sometimes after force-feeding. In the other group neither the preparation nor the cramming caused any visible wounds. In samples taken at the end of cramming thickening of epidermis covering the mucosa was observed frequently in both groups, especially in the proximal section of the oesophagus. The intensive wrinkledness typical for the

oesophagus at the beginning became moderate especially in case of the geese prepared for force-feeding and sometimes it disappeared due to the widening of the oesophagus. Plethora and intensive salivation was observed in oesophagus of the force-fed geese.

The intensive wrinkledness typical for the oesophagus at the beginning became moderate especially in case of the geese prepared for force-feeding and sometimes it disappeared due to the widening of the oesophagus. Thickened epithel, paraceratosis and acanthosis was observed in oesophagus sampled at the end of force-feeding. In the oesophagus of the geese not being prepared for force-feeding occasional symptoms of inflammation in the adventitia could be observed (indicated by the great number of the eosinophyl cells in the tissues). In the proximal section of the oesophagus extended mucosal glands and lymphatic cells (typical for the waterfowls) could be observed. The number of the mucosal glands in the proximal section was significantly higher than in the distal section. In both groups much mucosa was observed in the epidermis of the oesophagus in samples taken at the end of force-feeding. Plethora and intensive salivation was observed in the oesophagus of the force-fed geese.

Evolution of the fatty acid content and composition of the liver

Fatty acid content and composition in the liver reflected the fatty acid profile of the feed.

In case of the liver samples taken at the first sampling time no differences in amounts of the most important fatty acids were observed between the groups. Amounts of certain fatty acids in samples taken at the second sampling time were significantly different. Palmic acid and oleic acid occurred in more ratios in liver of the control geese than in the other ones while stearic acid, linolic acid and arachidonic acid were found in liver of geese prepared for force-feeding. In samples taken at the end of force-

feeding the level of all fatty acids except linolic acid increased in livers of both groups but the difference was not significant. After force-feeding 5 geese of each group were killed for test. None of the control birds reached the level of prepared groups: either in the weight or in the quality of the liver. After killing the all geese the average weight of the geese liver prepared for force-feeding was 624.6 g, much higher than one of the control geese (518.7 g).

Results of macroscopically and microscopically examination of liver

In the structure of liver sampled after the growing and the preparation period no macroscopic changes were observed at any sampling times and in any experimental groups. At the age of 9 weeks the colour of the liver in group having been prepared for force-feeding was lighter, the difference became higher contrast at the end of the force-feeding period. Symptoms of neither fattening nor inflammation were observed in the samples taken at the end of the growing period; the liver tissue has entired and unchanged structure (*Figure 4*).

In the liver of geese prepared for force-feeding the fat occurred as microvesicular steatosis (*Figure 5*), while the liver samples taken from geese without preparing was the same as one of the control at age of 6 weeks (*Figure 6*). The sections showed the fat accumulation began in the liver of the geese having been prepared.

In the liver samples taken at the end of force-feeding period strong fattening can be observed. In the liver tissue of both groups fat drops could be seen but characteristic of the fattening was different. In the group prepared for force-feeding macrovesicular steatosis (*Figure 7*) could be seen while in the liver of the force-fed (without preparing) geese mixed type fattening occurred (*Figure 8*).

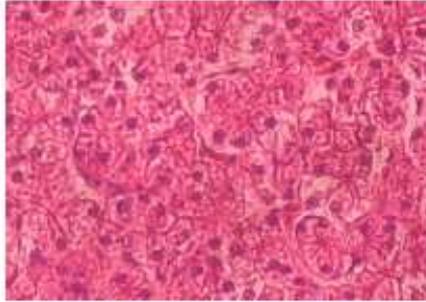


Figure 4. Normal liver tissue (control – 6 weeks of age)

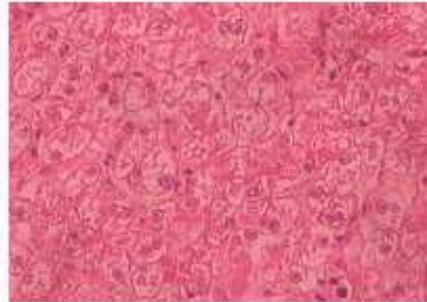
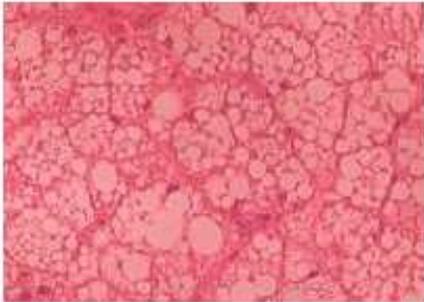


Figure 5-6. Microvesicular steatosis and normal liver tissue in 9-week-old geese prepared and had not been prepared for force-feeding, respectively

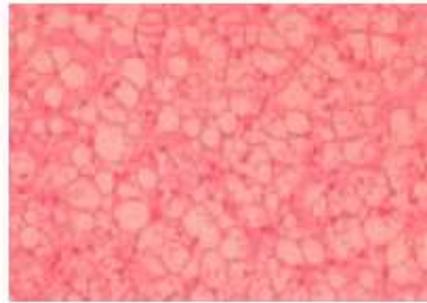
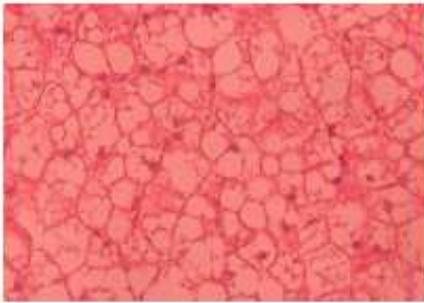


Figure 7-8. Macrovesicular steatosis and mixed type steatosis after force-feeding in geese prepared and had not been prepared, respectively

4. CONCLUSIONS AND RECOMMENDATIONS

4. 1. Production parameters

In the pre-trial for studying the main production parameters the obtained data show the body weight of the meat-type geese is higher than one of the liver-type hybrids and body weight of the females is less than one of the ganders, in both genotypes. Measuring the individual feed consumption makes possible to determine the appetite of geese. The liver weight is significantly influenced by genotype and the method of preparation for force-feeding – in addition to the sex. Significant difference in liver weight occurred between geese having different appetite.

Differences in the liver weight of the groups prepared different preparation methods may be resulted from the different feed conversion of the groups. It is characteristic especially for the females of meat-type prepared with 35-days restriction.

Despite of the relatively high weight gain of this group the 35 days restriction induced decreasing feed conversion. In comparison to the 21 days restriction, the limitation lasting two weeks longer does not give any reason of the luxury feed consumption of the females in meat-type, which does not result in significant difference either in the body weight at the end of force-feeding or in the liver weight. In the feed restriction (pregavage) period the feed conversion of the liver-type hybrids was better in both sexes than one of the meat-type geese. The liver weight was strongly influenced by genotype, sex and preparation method; however their common effect was not significant. It can be concluded that in both genotypes and sexes the 21-day time restriction preparation programme (c₃ method) resulted better average liver and body weight at the end of the force-feeding period; therefore – regarding the tested feed restriction methods – this method is recommended

to be applied in the practice. The preparation before force-feeding started fattening of the liver in the liver-type geese and fattening of the body in the meat-type hybrids. Fattening of the liver-type geese occurs mainly in the liver and less below the skin and in the abdominal cavity, while in the peripherals rather than in the liver in case of meat-type geese. The genetic background of this phenomenon seems reasonable for the further.

Results in Trial 2 justify that the weight of fatty liver is in closed relation to the weight gain during the force-feeding period. It means, that the weight of fatty liver could be predicted if weight gain during the force-feeding period is known. The geese produced 603 g average liver weight in the 16-day fattening period while rate of the weight gain was only 40 % in comparison to the weight at the beginning of the force-feeding.

The increase of the liver weight as the body weight gain is defined by a logistic function. In case fattening of young geese can be expected heavier liver weight relation to greater weight gain; the function is in the lower section of the logistis curve. An exponential function can be fitted best to the relationship between the examined variables.

In the traditional fattening technologies the geese are force-fed every 3-4 hours, which is straining for both the animals and the person carrying out the procedure. The results show as well that the force-feeding carried out twice a day can result suitable liver production with good quality. After 14-day long force-feeding period there was significant difference between the liver weights in geese prepared and had not been prepared for force-feeding. The average liver weight of the geese not prepared for force-feeding reached the first class quality as specified in the Food Alimentarius Hungaricus but due to its lower fat content and it had darker colour and therefore was qualified as third class, while 70 % of the livers of the prepared geese was qualified as first class.

4. 2. Conclusions about molecular biology examination

There was no genetic difference between the geese of various sexes and having different appetite was detected. Based on the results of the molecular biology examination, the difference between the geese having different appetite could be not originated from genetic reasons but any environmental factor or individual differences. The differences between the two sexes were not caused by the differences in the genom. Further examinations seem to be worth to select special parent lines on the basis of their appetite and to study their progenies with molecular biological investigations. In case of favourable results the gluttony could be used as selection parameters in the breeding of liver-type parent stock.

4. 3. Conclusions about laboratory examinations of blood and liver

The fatty acid composition of the liver is changed during the preparation period, the stearic-, linol- and arachidonic acid was increased correlated to the samples taken in 6 weeks of age. In the liver the dominating fatty acids were as same as those occurring mainly in the feed.

By the time of the preparation period the triglyceride concentration in the blood plasma drastically decreased in case of the group prepared for force-feeding. It indicates the triglycerides are accumulated in the liver and filling of the liver with fat started already in the preparation period (this is confirmed by the microscopically images of the liver sampled at the age of 9 weeks).

4. 4. Conclusions about macroscopically and microscopically examinations

The preparation for force-feeding had an influence on the length and diameter of the force-feeding, either. The difference between two groups was significant only in the diameter of the distal section of the oesophagus. At the end of force-feeding the oesophagus was wider of geese having been prepared for force-feeding than geese having not been. Increase of the length and diameter of the oesophagus resulted from growing of the young birds mainly (active growth is typical between 6 and 11 weeks of age) and from the effects of the continuously increasing feed portion applied in the force-feeding.

The results show the preparation for force-feeding has effects on diameter of distal section and by this on the capacity (stronger effect on this than on the proximal section).

The preparation for force-feeding did not cause any wounds in the oesophagus. Oesophagus samples of the geese taken after the force-feeding, can be characterised by thickened epithel, significantly decreasing wrinkledness and strongly mucosa. The intensive mucosa in the oesophagus was resulted from the increased activity of the enlarged mucosa glands in both groups.

Results of the macroscopic and microscopic examinations of the liver show fattening of the liver starts already in the preparation period, the fat can be seen in the liver tissue in a small drop form (microvesicular steatosis). At the end of the fattening period, the character of the fattening is different in the two groups, the fattening is characterized with macrovesicular steatosis in geese having been prepared while in the liver of the geese not being prepared are typified with mixed-type fattening.

All experimental data and results have supported the necessity of the preparation before force-feeding. Its inclusion in the keeping technology is definitely favourable because the force-feeding period can significantly shortened to 14 days (in spite of 18-21 days) and the number of the daily force-feeding also can be decreased without significant decrease in liver weight and destruction of liver quality. The results of all trials have proved that the force-feeding can be started safely already at the age of 9 weeks. The preparation has very important economic advantages: the raising and force-feeding is less expensive and the feed utilisation of the younger animals is better than the elder ones.

The results prove that the preparation before force-feeding is definitely advantageous from point of view of both weight and quality of the liver. Applying it, the fattening period can significantly shortened, number of cramming can be decreased to two per day and the feed can be portioned simply by machines therefore is recommended for the practice.

1. Starting period:

- 0-3 weeks of age; density of livestock: 8-10/m² (in litter);
- feeding: ad libitum goose starter feed (granulated);
- heated goose house (temperature: 32 °C from the sited to 3rd day then it have to decrease with 2 °C every third day).

2. Growing period:

- 3-6 weeks of age; density of livestock: 5-6/m² (in litter);
- feeding: ad libitum goose growing feed (granulated) with vitamin and mineral elements supplement;
- heating does not necessary.

3. Preparation for force-feeding (pre force-feeding) period:

- 6-9 weeks of age; density of livestock: 4-5/m² (in litter);

- feeding: mixture of growing and cramming feed in ratio of 50 to 50 % with hourly restriction (recommend with method c₃)
- vitamin and mineral supplement is not recommended);
- advantageous is the cooled goose house.

4. Force-feeding period:

- 6-9 weeks of age (duration 14 or max. 16 days);
- group cages is force-feeding house;
- feed: mixture of corn, cramming feed and water;
- technology of force-feeding: once a day in the 1st day, twice a day from the 2nd day
- cooled force-feeding house.

In spite of the favourable results, since the capacity of cage in force-feeding house is not allowed to practice the technology in larger scale, testing of this technology is recommended.

5. NEW SCIENTIFIC RESULTS

1. In the examination of the goose hybrids various genotypes and sexes, the heaviest liver weight was resulted by the 21-day long time preparation method before force-feeding with timely-restriction.
2. The genotypes, sex and preparation method have significant effects as well as in double interactions on the liver weight. At the same time in the interaction of the genotype \times sex \times preparation method the statistical test did not show significant effect.
3. Close positive phenotype correlation ($r=0.794$) was founded between weight gain during force-feeding and weight of fatty liver in 9-week old geese force-fed 16 days. The relationship between two variables can be fitted the best an exponential function. The weight of fatty liver can be estimated with 70 % accuracy (in geese weighed between 1400 and 2650 g) if the weight gain during the force-feeding is known.
4. It has been proved that triglyceride concentration in blood plasma has drastically decreased as an effect of the preparation for force-feeding. It indicates that the tryglicerides are accumulated in the liver. The preparation changed the fatty acid composition in the liver; ratios of the stearic acid and arachidonic acid and oleic acid are dominant.
5. It was demonstrated by macroscopic and microscopic examinations that the preparation for force-feeding did not cause any injury in the oesophagus. The force-feeding caused expansion in the distal section of the oesophagus and paraceratosis and acantosis was observed in the samples. Fattening of the liver started already during the preparation period. After force-feeding period macrovesicular steatosis was observed in the group having been prepared for force-feeding while fattening of mixed-type occurred in the group not being prepared for cramming.

6. SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

Scientific papers

Scientific papers in Hungarian:

GYÖRFFY A. – RÓNAI ZS. – **ÁPRILY SZ.** – ZSARNOVSZKY A. – FRENYÓ V. L. – BOGENFÜRST F. – **RUDAS P.** – BARTHA T. (2008) A hízottmáj-termelés metabolikus és hormonális hátterének vizsgálata máj- és húshasznosítású lúdhibridekben; Magyar Állatorvosok Lapja; 2008/3; 156-164 o.

ÁPRILY SZ. (2008) A vér lipid-frakcióinak, valamint a máj zsírsavtartalmának és -összetételének vizsgálata májhasznú lúdhibridekben; Agrár és Vidékfejlesztési Szemle; Vol. 3. (közlésre elfogadva)

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ÁPRILY SZ. (2007) Animal welfare aspects of force-feeding and foie gras production in waterfowls (Review); Agrár- és Vidékfejlesztési Szemle; 2007/01. Vol. 2. 5-10. p.

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Full conference papers in proceedings:

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BOGENFÜRST F. – **ÁPRILY SZ.** (2005) A hízott lúdmáj-termelés és a töméses hizlalás állatvédelmi szempontjai; II. Állategészségtani Nap kiadványa; 28. o.

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Technical articles:

BOGENFÜRST F. – **ÁPRILY SZ.** (2004) A minőségi májtermelést és a töméses hizlalás jövőjét érintő kérdések a víziszárnyasoknál 1. rész; Baromfiágazat 2004/2; 32-39. o.

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ÁPRILY SZ. – BOGENFÜRST F. (2008) A tömés-előkészítés hatása a ludak májtermelő képességére és májminőségére; Agronapló, 2008/03.; 106-107. o.

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