SZENT ISTVÁN UNIVERSITY

THESES OF PHD DISSERTATION

STRESS SENSITIVITY AROUND GATHERING FEATHERS AND ADAPTIVE VARIATION IN EGG PRODUCTION BY YEAR AND AGE IN GEESE

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1. Premises and objectives

The goose in Hungary has been kept for its meat and valuable feathers and down mainly under extensive conditions. Feathers used to gather two to three times from young and breeder geese after the growing and egg laying period till the slaughter, respectively, utilising the periodical natural moulting of the domestic goose. Feather gathering is a method to stops egg laying that synchronises the hormonal status (PÁLFFY 1980).

Gathering feathers from live geese is allowed in our country [MARD Decree 178/2009. (XII. 29)]. Yet, some animal rights groups campaigned lately vigorously against this practice in the media saying that it can cause to geese pain, skin injuries, bleeding, joint dislocation, bone fractures and even death. All these are true only if immature feathers are plucked (KOZÁK et al. 2010, KOZÁK et al. 2011). In consequence of these attacks, gathering feathers discontinued in 80% of the domestic goose stock that reduced the buying up of meat goose (KOZÁK 2012).

The future trend may be the formation of rearing systems based on intensive keeping and feeding technologies without gathering feathers or change the breed.

The goose unlike other poultry species has retained largely its ancient habits, however. It tolerates badly the enclosed, intensive or large-flock keeping and more sensitive to the environmental changes. Egg production of the breeder stocks is more uncertain and very low already under semi-intensive keeping as the goose under natural photoperiod is a seasonally breeding species.

During research of the two (actual to now) themes indicated by the title of my dissertation I focused on the following objectives and questions to be responded:

1.To test stress-sensitivity to gathering feathers in growing and/or adult breeder geese during their natural moulting period by detecting the changes in five stress indicator blood parameters namely, plasma corticosterone, two thyroid hormones, white blood cell count and the heterophil granulocita/lymphocyte (H/L) ratio.

1.1. To examine whether expert manual feather gathering causes distress more than catch, take in hand the geese or blood withdrawal do.

1.2. To assess whether an antistress mixture containing essential amino acids and vitamins given in drinking water prior to gathering feathers can affect the stress reactions of geese?

2. To analyse the adaptive changes in egg production parameters (onset and length of laying period; yield, quality and intensity of egg production; layer mortality) based on the two year data set of commercial breeder goose flocks kept semi-extensively (without gathering feathers), by year.

2.1. To assess the correlations of laying intensity with the climatic variables (day length, air temperature) recorded, by year.

2.2. To analyse the adaptive changes in the egg production parameters by flock age.

2.Materials and method

2.1. Testing stress sensitivity of geese around gathering feathers

The experiments were conducted at the goose farm of the Babatpuszta Goose Breeding Research Station in the period between 2004 and 2008.

2.1.1. Animals, keeping and feeding

Growing and/or adult breeder geese of Babat White Hungarian Upgraded breed were examined during their first true and post-breeding moulting period, respectively. Geese were housed in pens on deep litter system with yard access and bathing facility and fed ad libitum a growing and maintenance ration, respectively. Drinking water was always available.

2.1.2. Test protocol

Geese were assigned to five treatment groups 6 days before the first blood sampling: 1 control (naturally moulting); 2 gathered; 3 given antistress mixture 5 days before gathered; 4 sham gathered, 5 given antistress mixture 6 days before sham gathered. The antistress mixture containing essential animo acids and vitamins was given in the drinking water at a dose of 1 ml/goose/day.

2.1.3. Procedure of gathering and sham gathering feathers

During feather gathering the goose was positioned dorsally and upside down, meanwhile feathers and down were removed from the lower belly, the flanks and the area not covered by the wings. Subsequently, the goose was turned on its ventral side and feathers were removed from the back (SZENTIRMAY 1968). This procedure (inclusive catching) lasted for about 10 minutes per goose. Sham feather gathering was done similarly without removing any feather and it lasted for 4-5 minutes.

2.1.4. Examination of stress indicator blood parameters

2.1.4.1. Taking and handling blood samples

Blood (3 ml/goose) was taken from the wing vein by puncture. For measuring plasma hormones the blood was collected in heparin tubes. Plasma samples separated by centrifugation were stored at -20°C until analysed. For counting total white blood cells the blood was collected in tubes added an avian blood diluent (HORVÁTH 1979) and two slide blood smears were prepared per goose to appraise the differential count.

2.1.4.2. Test methods

<u>Plasma corticosterone</u>

Changes in its levels I examined in the five treatment groups in 25, 9 week old growing geese (n=5). Blood samples were taken between 13:00–17:00 p.m. (before maximum in plasma corticosterone level at the beginning of dark; BEUVING and VONDER 1977): right before, during and 5 min, 1hr and 3 hrs after gathering and sham gathering feathers. Geese were held in hand till the third blood sampling then they were replaced in the sheds and caught again before the blood samplings come due 1hr and 3 hrs later. Plasma hormone level was determined by radioimmunoassay using corticosterone double antibody RIA kit according to PEDERSEN et al. (2000).

<u>Plasma thyroid hormones</u>

Changes in their levels I examined in the five treatment groups in 50 adult breeder geese (n=10; 5♂, 5♀). Blood samples were taken between 9:00–12:00 a.m. (to eliminate any diurnal variation in thyroid hormones; NEWCOMER 1974): 1 hr before gathering/sham gathering feathers and 1 hr post-procedures. Plasma thyroxin (T_4) and triiodothyronin (T_3) levels were determined by radioimmunoanalysis according to PETHES et al. (1978).

White blood cell count, heterophil/lymphocyte (H/L) ratio

Their changes I examined in the five treatment groups in 50, mixed sex 8-9 week old growing geese (n=10) and 50 adult breeder geese (n=10; 5♂, 5♀). Blood samples were taken 24 hrs and 7 days after the procedures of gathering and sham gathering feathers (as in chickens exposed to a short-term physical stressor H/L ratios peaked in 20 hrs and returned to pre-stress values after 30 hrs; GROSS 1990).

Total white blood cell count was determined in a Bürker chamber and differential count was appraised from 100 cells of May-Grünwald-Giemsa stained slide blood smears prefixed in methanol (using a Zeiss type microscope). From this I calculated the H/L ratios = total percentage of heterophil granulocyte/total percentage of lymphocyte.

Statistical analysis

During statistical evaluation of the blood parameters' data (Microsoft Office Excel 2007) I used analysis of variance (ANOVA) and the paired Student's t-test to reveal the significance of the between or within-group differences in the means (SVÁB 1981).

2.2. Analysis of egg production data of breeder goose flocks by year and age

I have analysed daily egg production and mortality data for four breeder flocks of Hortobágy White goose breed recorded in 2012 and 2013 by year and flock age.

2.2.1. Flock management

The geese hatched in June–July 2007, 2008, 2010, 2011 and 2012 were in their first to fifth production year. The flock size differed between and within years, too.

The flocks were formed in November by age (with a sex ratio of 3♀:1♂, and 4♀:1♂ in one flock) and housed separately in sheds with deep litter system and yard access at the Tiszabábolna stock goose farm of the Hortobágy Goose Breeding Zrt., located outside the Tisza river floodplain (**Fig. 1**).

Fig. 1. Geographical location of Tiszabábolna

Geese were given a granulated laying feed ad libitum throughout the laying season. Drinking water was always available. They were observed daily and the number of eggs produced and that of dead geese were recorded in the flock diary. After the elapse of the laying period, the flocks were kept on pasture on the Hortobágy until the next November.

Geese were kept under natural climatic conditions. Daily air temperature and relative air humidity values were recorded at the automated weather station of Hungary Meteorological Service (HMS) set up at Poroszló 12 km apart from Tiszabábolna. Day length data I collected from the calendar (*http://calendar.zoznam.sk/sunset-hu.php*). The daily data I averaged for the weeks of the laying period per year and per flock (**Fig. 2a, b**).

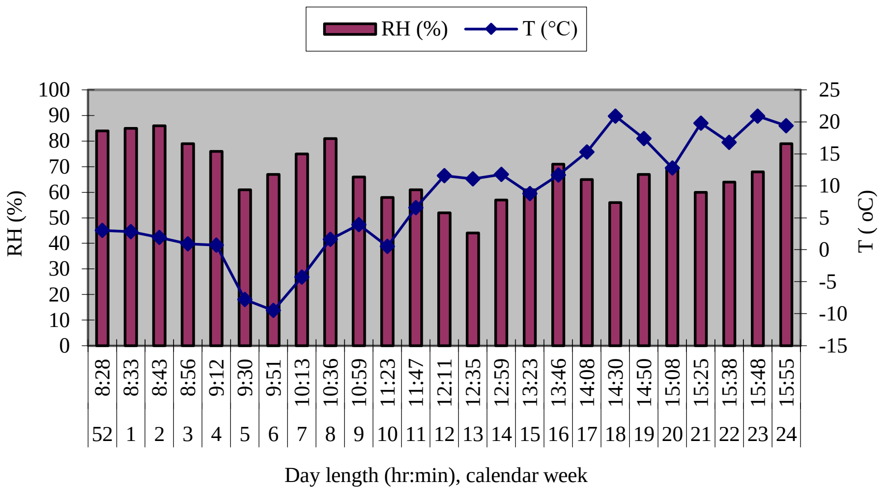

Fig. 2a. Variation in climatic factors in 2012
(Source: Own calculations from HMS and calendar data.)

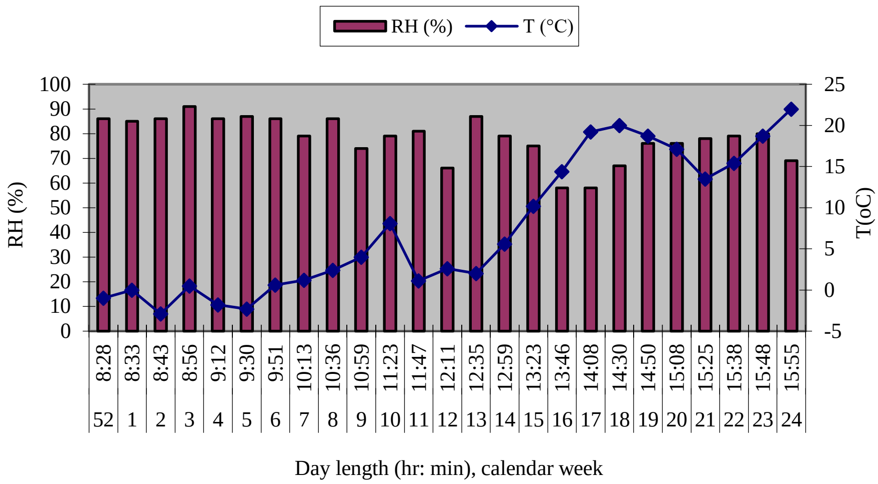

Fig. 2b. Variation in climatic factors in 2013
(Source: Own calculations from HMS and calendar data.)

Comparison of the two figures show that day lengths agreed throughout the calendar weeks, but air temperature was higher and relative humidity was lower in 2012 than in 2013, except for week 5-9, 15-20 and 24.

2.2.2. Production parameters

From the flock diary data I determined values for the following parameters:

- onset and duration of egg laying period;
- average weekly egg yields/surviving layers;
- egg quality (percentage of defected eggs);
- laying Intensity (average weekly egg yields/7 x 100);
- mortality rate of layers.

Performances of one year old and two to five year old flocks I compared by the weekly cumulative egg production, laying intensity and layer mortality graphs.

Statistical analysis

During statistical evaluation of the production and climatic variables (Microsoft Office Excel 2003, SP3) I used analysis of variance (ANOVA) and the paired Student's t-test to reveal the significance of the between and within-year differences in the means. The pairwise correlation coefficients for the selected variables I determined by simple regression analysis (SVÁB 1981).

3. Results

3.1. Testing stress sensitivity in geese around gathering feathers

Plasma corticosterone

In 9 week old growing geese corticosterone level was high in all five groups at the first blood sampling, prior to gathering and sham gathering feathers (for control 164±33, for treatments 212±48mmol/l).

At the second sampling (during procedures) the hormone level dropped significantly ($P < 0.01$) in all group and it decreased slightly ($P > 0.10$) at the third sampling (5 min post-procedures).

At the fourth and fifth sampling (1hr and 3 hrs post-procedures when geese were caught again) the corticosterone level went up in all five groups. It was significant after 1 hr (in control and group 3 at $P < 0.001$, 2.-4. at $P < 0.05$ and in group 5. at $P < 0.01$, respectively), but non-significant ($P > 0.10$) 3 hrs later (**Fig. 3**).

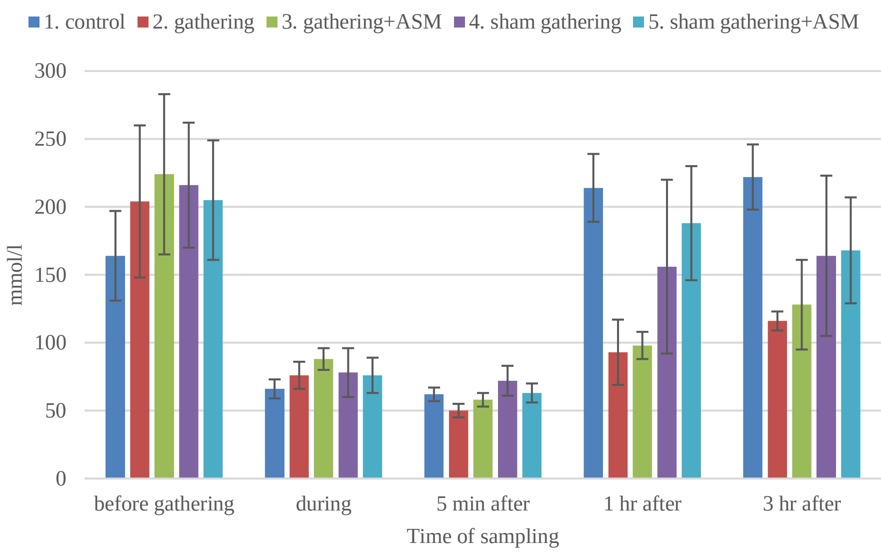

Fig. 3. Plasma corticosterone levels by group and sampling time in growing geese

ASM= antistress mixture containing essential amino acids and vitamins

Plasma thyroid hormones

Thyroxine (T_4) and triiodothyronine (T_3) levels varied similarly in breeder geese (**Fig. 4a, b**). Plasma T_4 and T_3 levels showed no significant between-group difference 1 hour before gathering/sham gathering feathers. The values decreased in all five groups 1hr post-procedures significantly in T_4 in group 3, 5 ($P < 0.10$, $P < 0.05$) and in T_3 in group 2, 4 and 5 ($P < 0.05$).

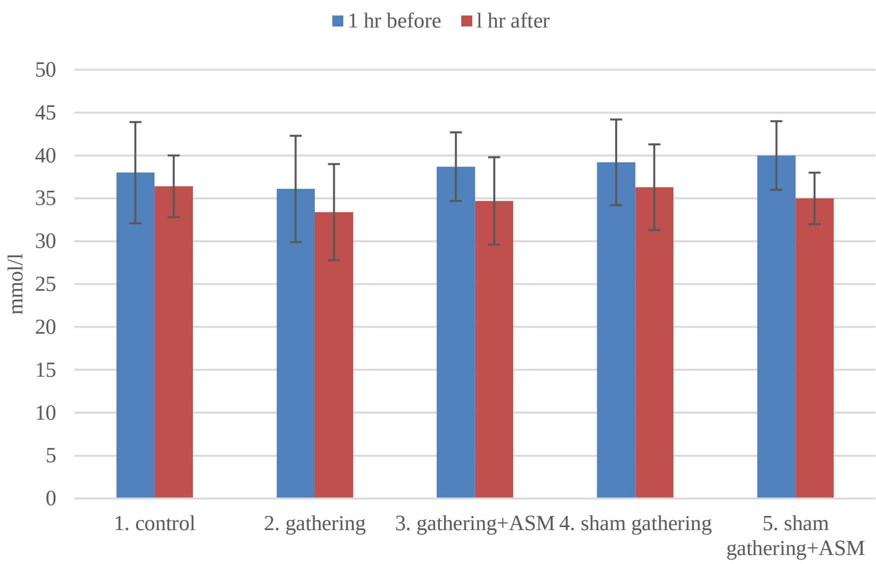

Fig. 4a. Plasma thyroxin levels by group and sampling time in breeder geese

ASM= antistress mixture containing essential amino acids and vitamins

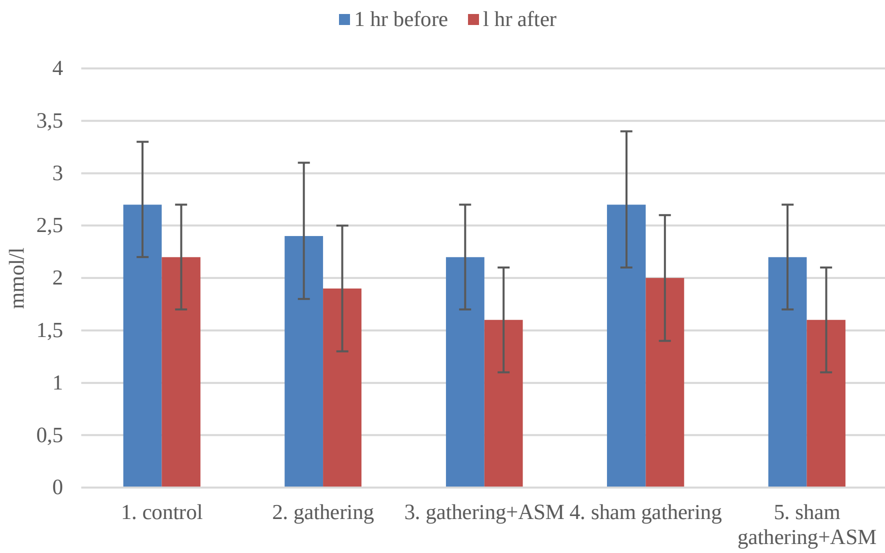

Fig. 4b. Plasma triiodothyronine levels by group and sampling time in breeder geese

ASM= antistress mixture containing essential amino acids and vitamins

White blood cell count, heterophil/lymphocyte (H/L) ratios

In growing geese total white blood cell count was similar in all five groups 24 hrs after gathering and sham gathering feathers (**Fig. 5a**). The values decreased 7 days later in all five, that was significant in group 2, 3 (P <0.01, P <0.05) and group 5 (P <0.10).

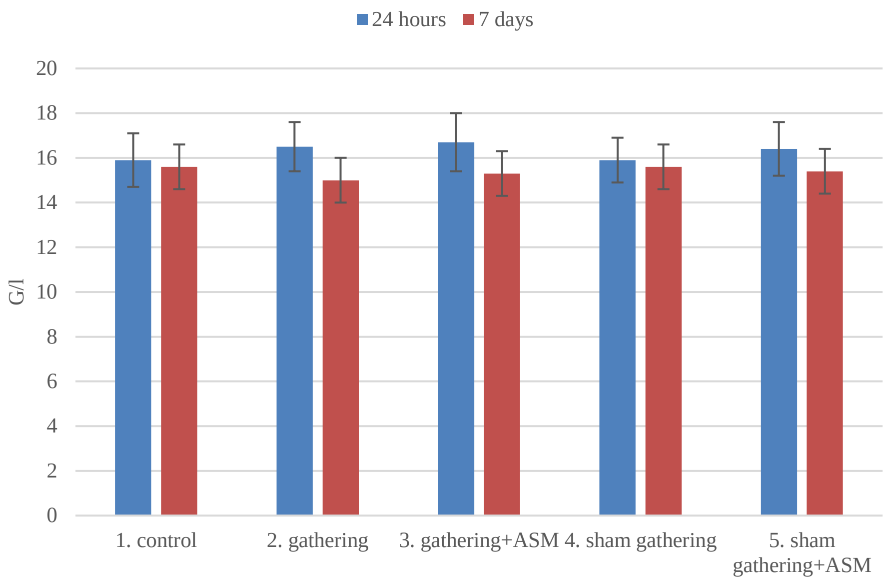

Fig. 5a. Total white blood cell count by group and sampling time in growing geese

ASM= antistress mixture containing essential amino acids and vitamins

Percentage of heterophil granulocytes was higher than in the control 24 hrs post-procedures (**Fig. 5b**). The values decreased in all groups significantly 7 days later at level of $P < 0.05$ (control), $P < 0.001$ (group 2, 3) and $P < 0.01$ (group 4, 5).

Percentage of lymphocytes was lower 24 hrs post-procedures than in the control (**Fig. 5c**). The values went up 7 days later significantly in group 2, 3 ($P < 0.001$) and 4, 5 ($P < 0.05$, $P < 0.01$).

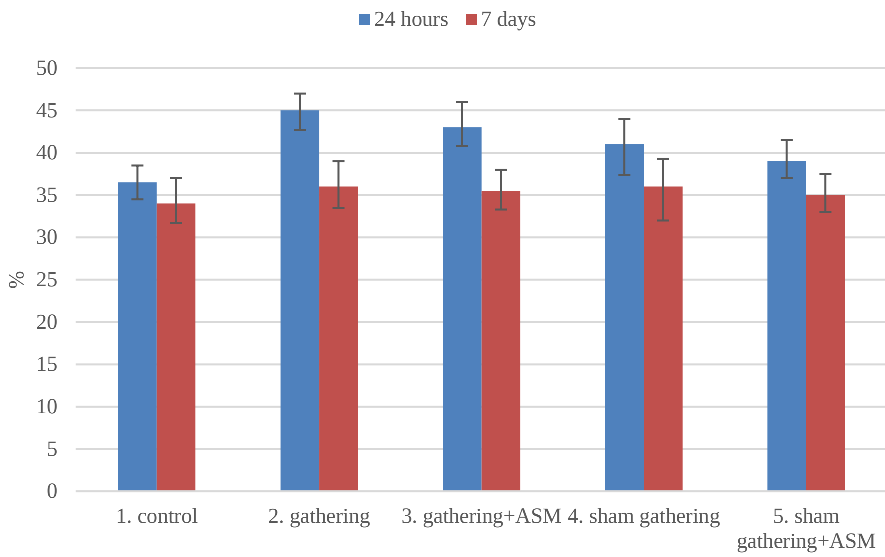

Fig. 5b. Percentage of heterophils by group and sampling time in growing geese

ASM= antistress mixture containing essential amino acids and vitamins

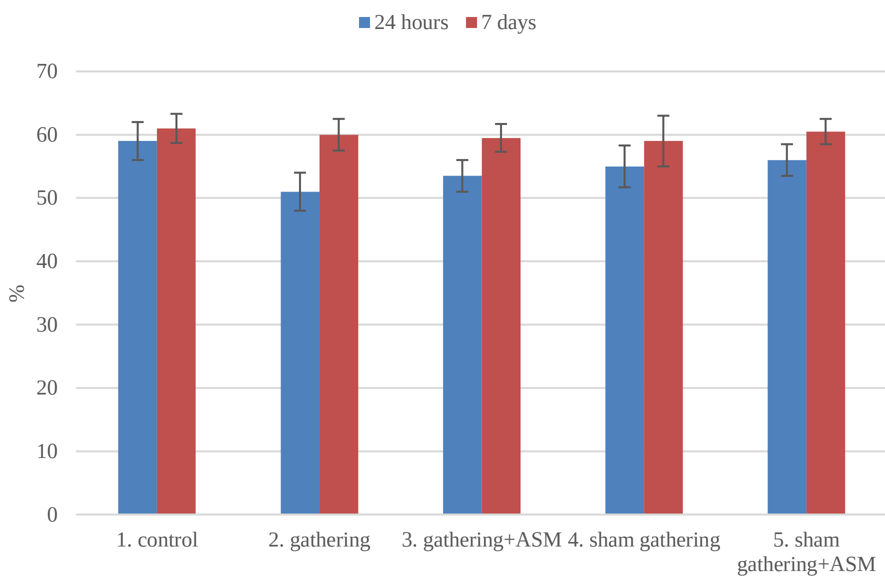

Fig. 5c. Percentage of lymphocytes by group and sampling time in growing geese

ASM= antistress mixture containing essential amino acids and vitamins

The H/L ratios ranged between 0.62–0.88 and 0.56–0.61 at the first and second sampling, respectively (**Fig. 5d**).

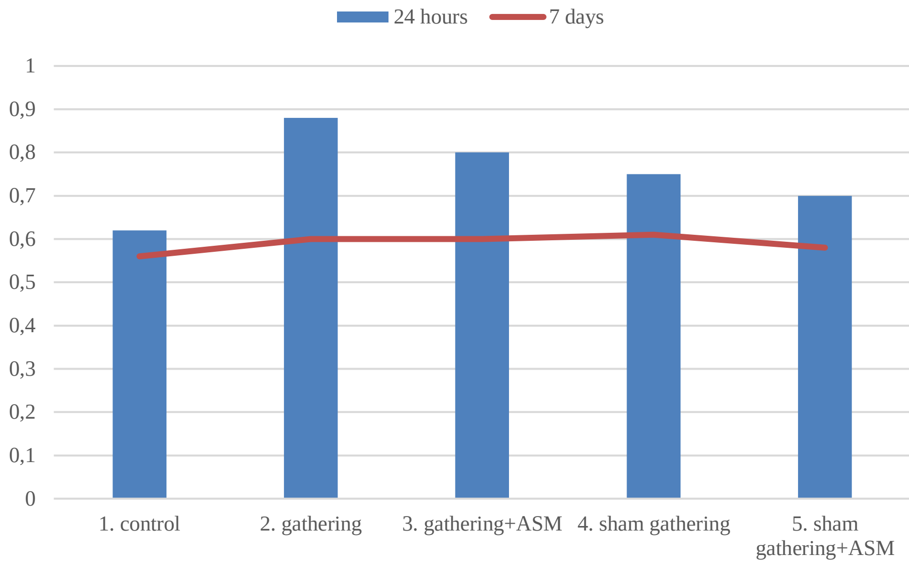

Fig. 5d. H/L ratios by group and sampling time in growing geese
ASM= antistress mixture containing essential amino acids and vitamins

In breeder goose groups, total white blood cell count was comparable 24 hrs as well 7 days after gathering and sham gathering feathers (**Fig. 6a**).

Percentage of heterophils and lymphocytes little differed among the five groups' 24 hr post-procedures. Seven days later percentage of heterophils increased in the control but decreased in the rest and that of lymphocytes changed inversely or not changed (**Fig. 6b, c**).

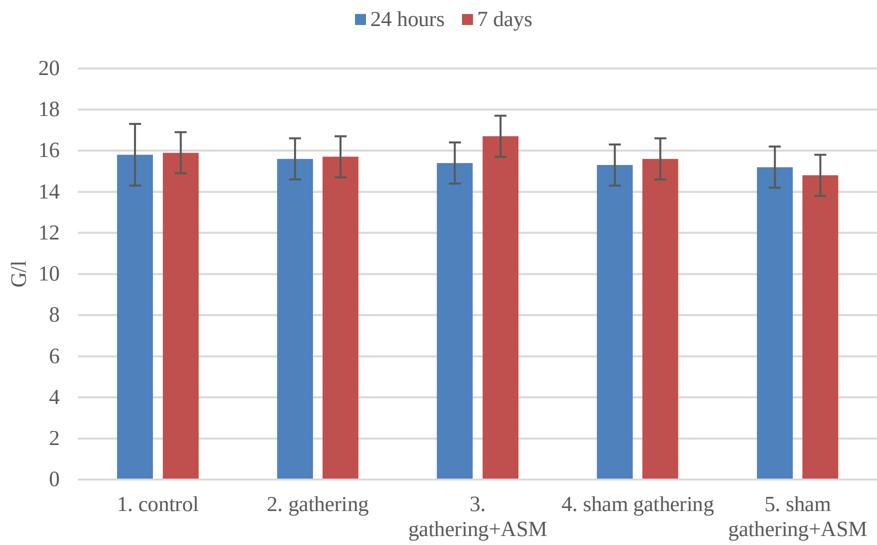

Fig. 6a. Total white blood cell count by group and sampling time in breeder geese

ASM= antistress mixture containing essential amino acids and vitamins

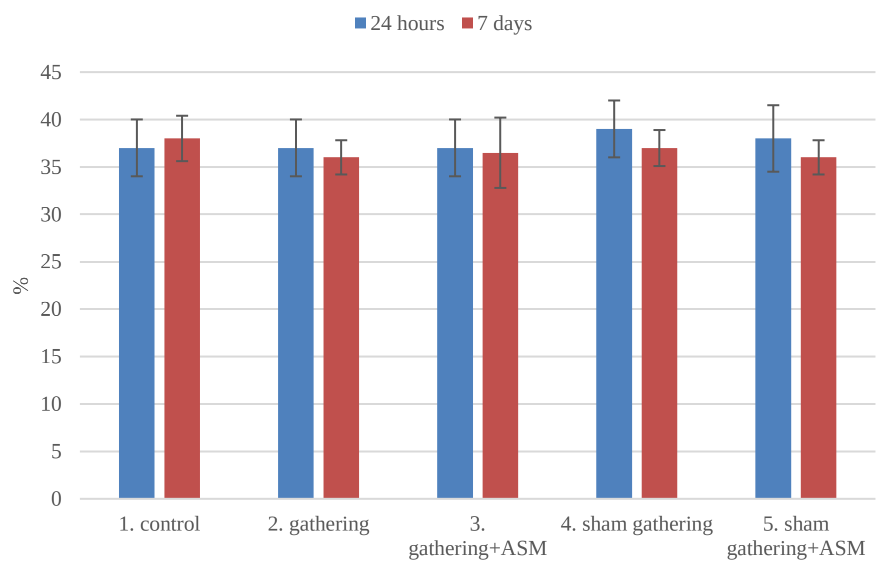

Fig. 6b. Percentage of heterophils by group and sampling time in breeder geese

ASM= antistress mixture containing essential amino acids and vitamins

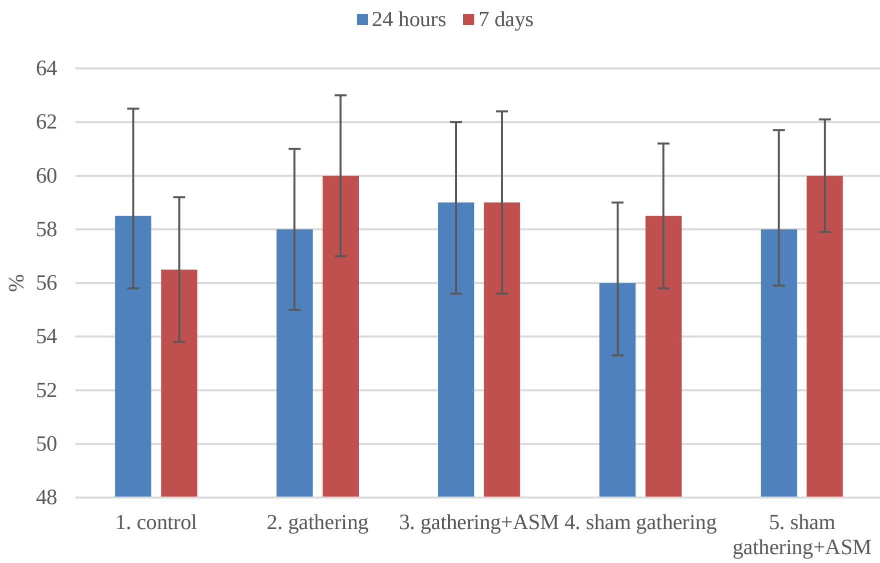

Fig. 6c. Percentage of lymphocytes by group and sampling time in breeder geese

The H/L ratios ranged between 0.63–0.70 and 0.60–0.67 at the first and second sampling, respectively (**Fig. 6d**).

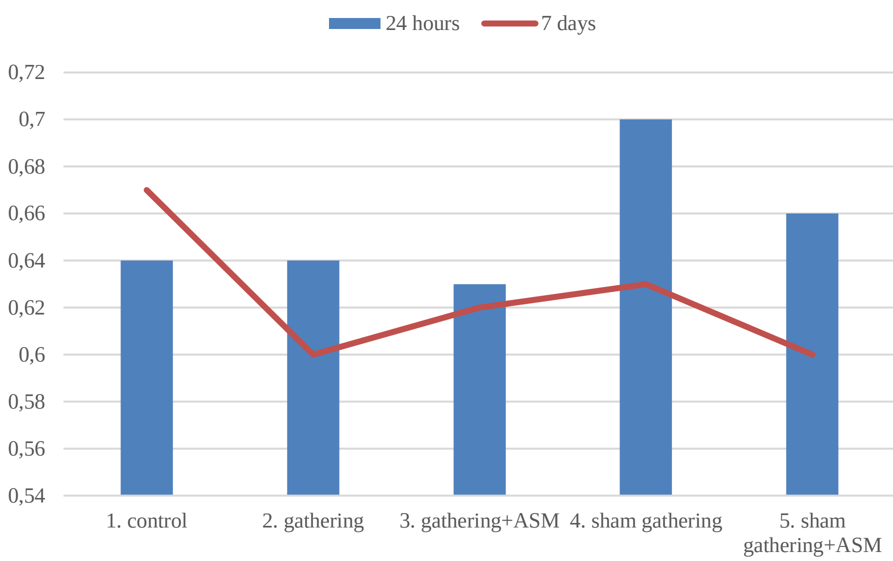

Fig. 6d. H/L ratios by group and sampling time in breeder geese
ASM= antistress mixture containing essential amino acids and vitamins

3.2. Variation in the production parameters of geese by year and flock age

3.2.1. Variation by year

Onset and duration of laying period. In one year old flocks the laying period lasted in 2012 for 18 weeks (11.II.–16.VI.) and for 15 weeks in 2013 (28.I.–12.V.). In elder flocks it lasted for 21 weeks in 2012 (21.I.–17.VI.) and for 20 weeks in 2013 (25.XII.–12.V.).

Egg production and quality. In 2012, the one year old flock laid 7 eggs more and 6% less defective eggs per goose than in 2013. Two to five year old flocks laid 8 eggs more and 2% less defective eggs per goose in 2013 than in 2012 (**Fig. 7a, b**).

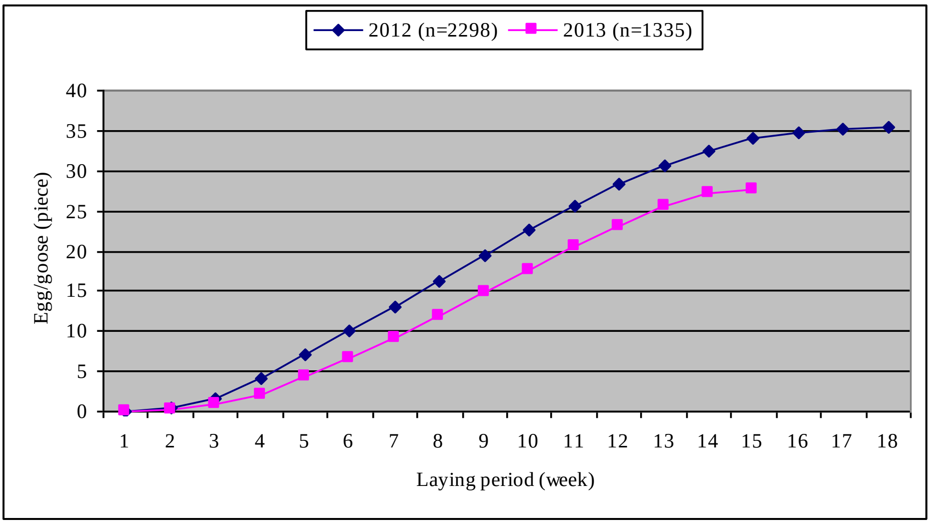

Fig. 7a. Cumulative egg production in one year old goose flocks

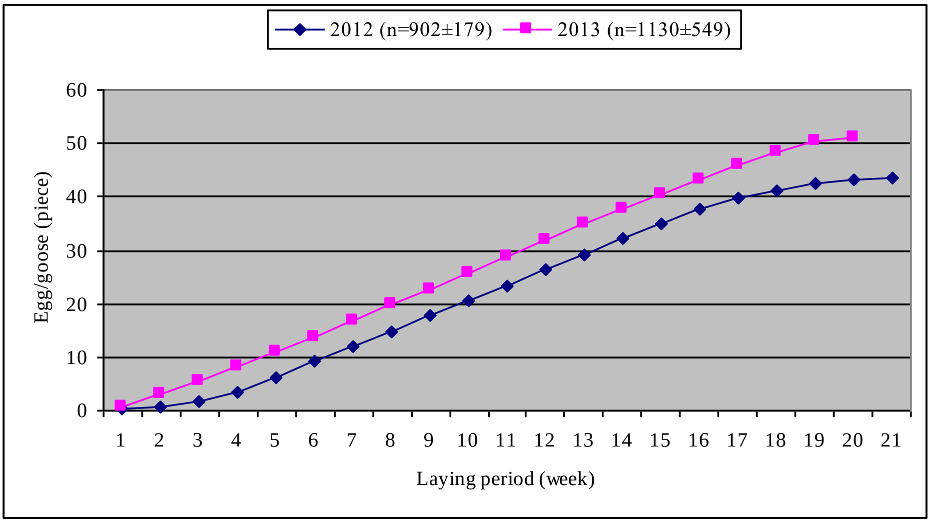

Fig. 7b. Cumulative egg production in two to five year old goose flocks

<u>Laying intensity</u>. In 2012, the one year old flock had 1% higher laying intensity than that in 2013. In one year old flocks, laying intensity in 2012 and 2013, respectively, increased until week 4 and 7, reaching a plateau (of 44 vs. 40%) across weeks 5-11 and 8-12, respectively, and then decreased (**Fig. 8a**). In two to five year old flocks laying intensity in 2012 and 2013 increased until week 5, maintaining a plateau (of 41 vs. 42%) across weeks 6-15 and 6-13, respectively, and then decreased (**Fig. 8b**).

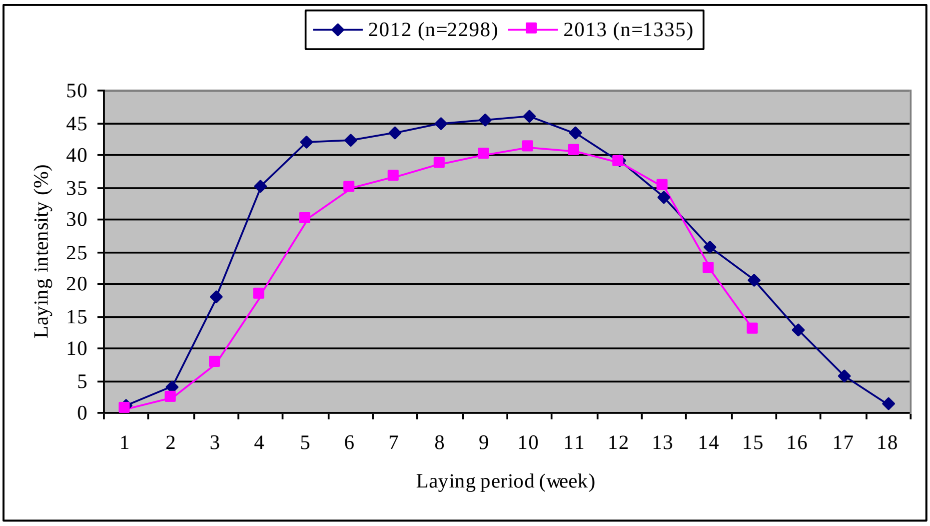

Fig. 8a. Laying intensity in one year old goose flocks

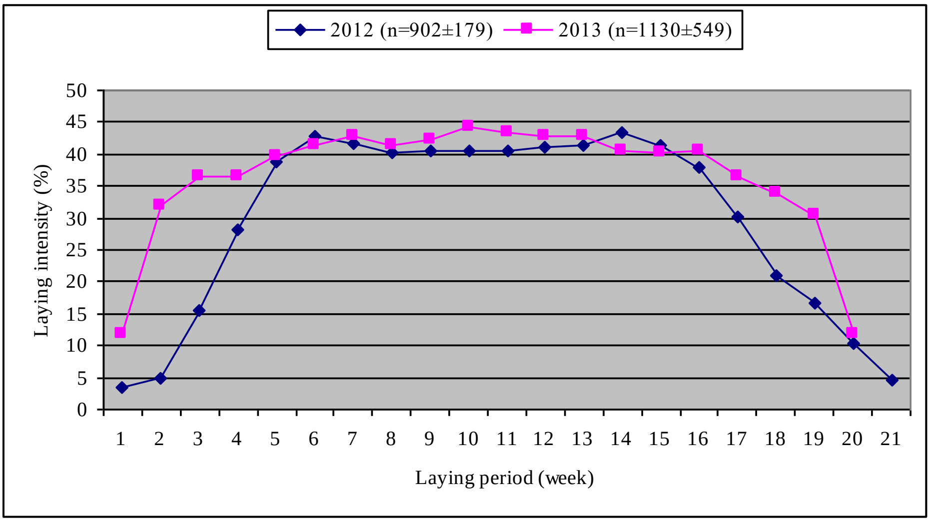

Fig. 8b. Laying intensity in two to five year old goose flocks

Based on the degree of laying intensity the laying period could be divided into a rapid increase, a plateau and a rapid decrease phase, still during the increasing period of day length.

The correlation of laying intensity with day length was thus positive until the decrease phase and then negative. Its value for one year old flocks was r_{2012}=0.85 vs. -0.99, P <0.001; r_{2013}= 0.90 vs. -0.998, P <0.001 and for two to five year flocks was r_{2012}=0.76 vs. -0.995, P <0.001; r_{2013}=0.63, P <0.05 vs. -0.85, P <0.001. The correlation with air temperature was of similar sign, except the decrease phase of the one year old flock in 2013 being positive.

Mortality rate of layers. Mortality of one year old layers was 13% less in 2012 than in 2013 with 48% vs. 64% share in total mortality between week 4-8 and 4-7, respectively (**Fig. 9a**). In two to five year old layers it was 7% less in 2013 than in 2012 with 27% vs. 22% share in total mortality between week 4-7 and 4-6, respectively (**Fig. 9b**).

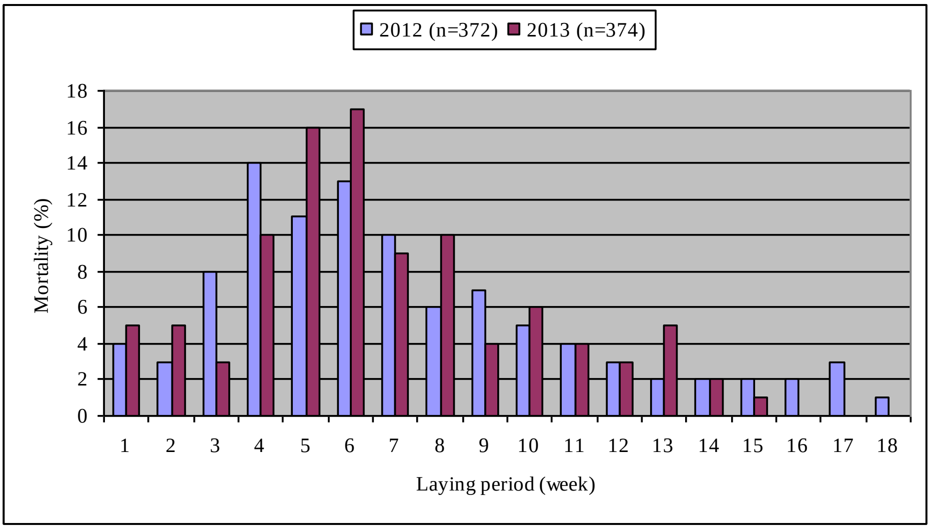

Fig. 9a. Distribution of layer mortality in one year old goose flocks

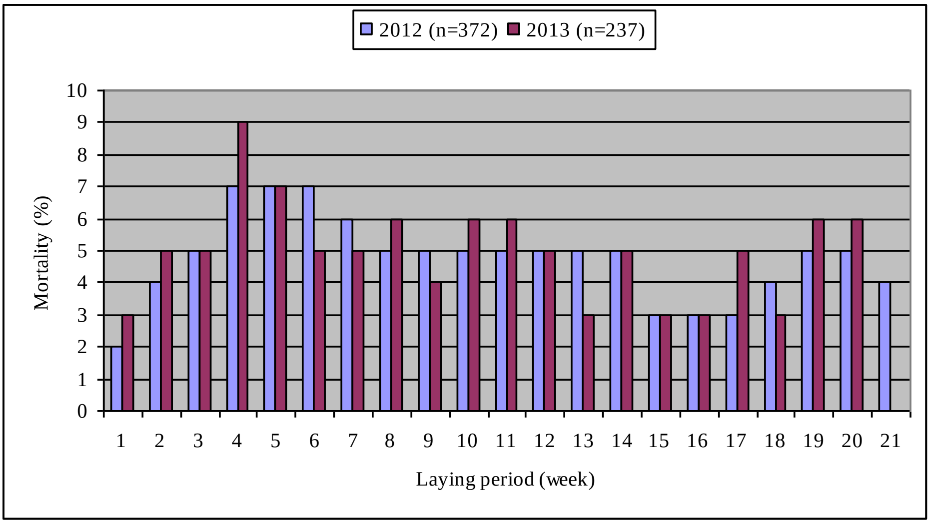

Fig. 9b. Distribution of layer mortality in two to five year old goose flocks

3.2.2. Variation by flock age

Onset and duration of laying period. In two to five year old flocks egg laying began 3-5 weeks earlier and lasted 3-5 weeks longer, respectively, than in one year old flocks.

Egg yield and quality. Over the two years egg yield per goose averaged 27.5 in the one-year old, 50 in the two years old, 46.5 in the three to four years old and 44 in the five year old flocks. Percentage of defected eggs was 9%, 6%, 4% and 5%, respectively (**Fig. 10a, b**).

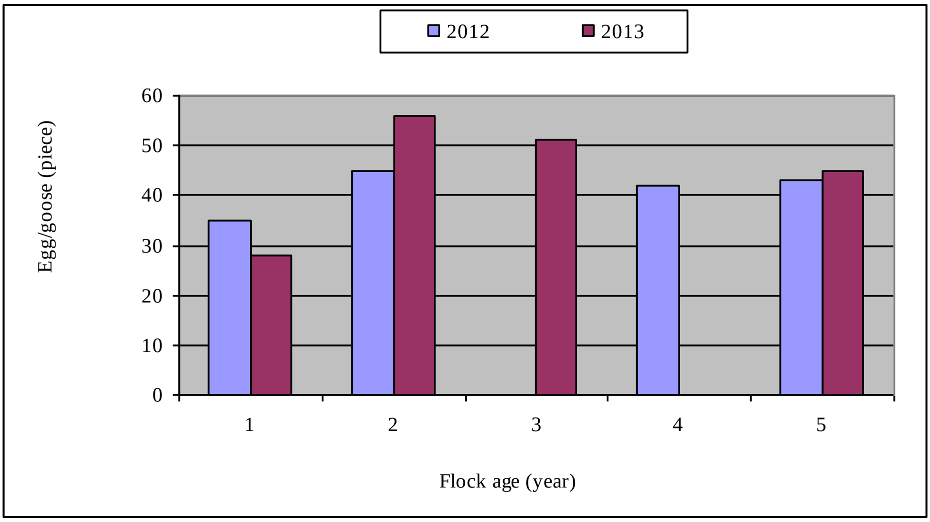

Fig. 10a. Variation in egg yield by flock age

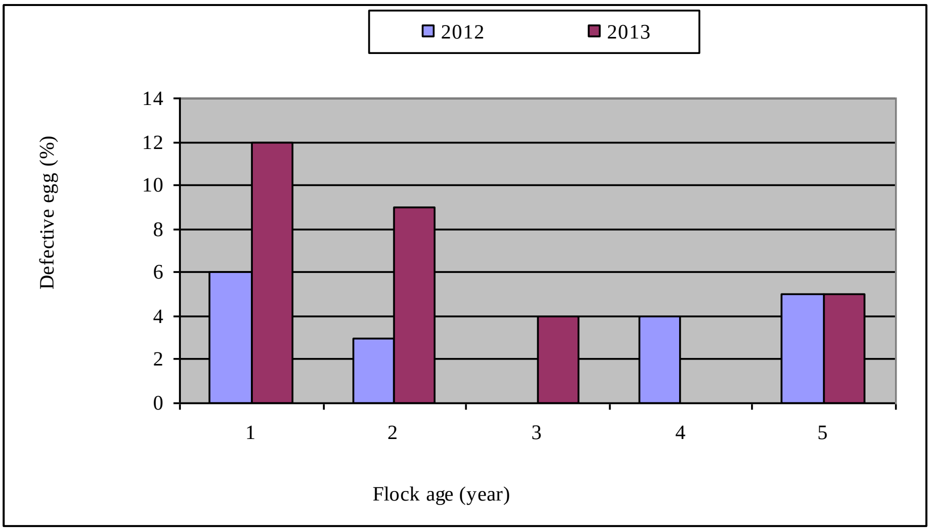

Fig. 10b. Variation in percentage of defective eggs by flock age

<u>Laying intensity</u>. Over the two years laying intensity averaged 27.5% in the one year old, 35.5% in the two years old, 32.5% in the three to four year old and 30.5% in the five year old flocks (**Fig. 11**).

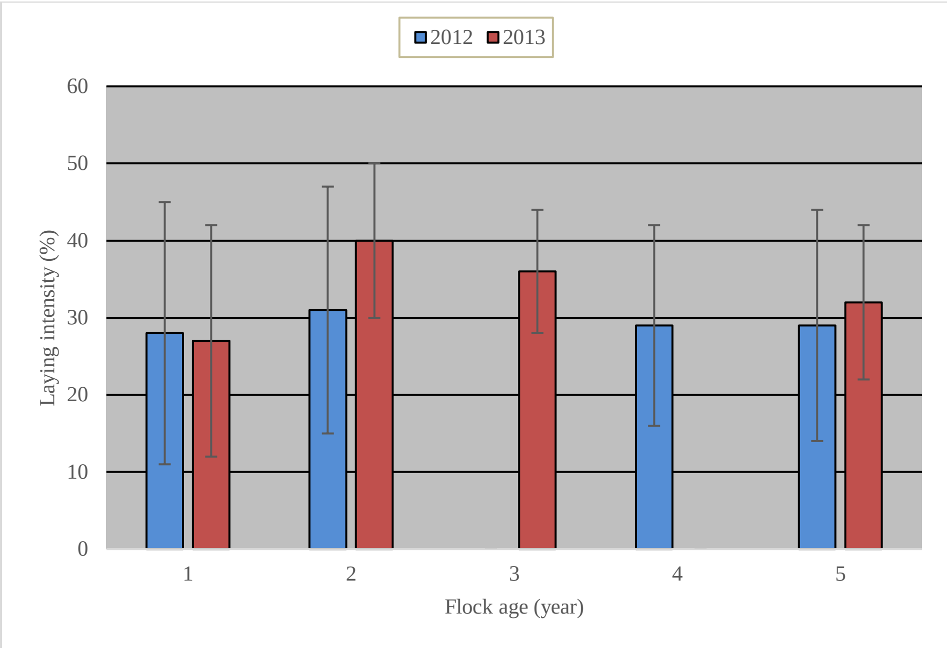

Fig. 11. Variation in laying intensity by flock age

Layer mortality rate. Over the two years mortality averaged 21.5% in the one year old, 8% in the two years old, 12.5% in the three to four years old and 11% in the five years old flocks (**Fig. 12**).

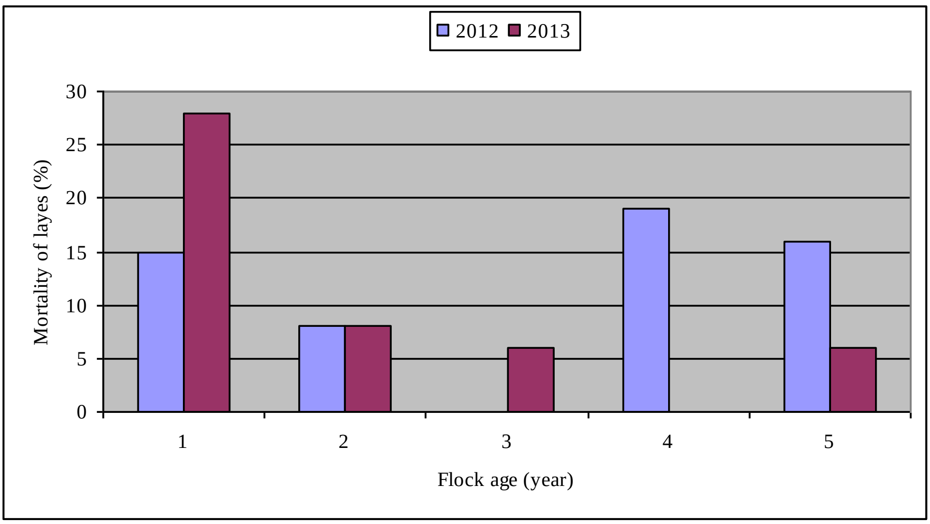

Fig. 12. Variation in layer mortality rate by flock age

3.3. New scientific results

1. I have shown the changes in five stress indicator blood parameters in growing and/or adult breeder geese of Babat Hungarian Upgraded breed subjected to feather gathering and sham gathering compared to controls.

2. I have shown that suitable indicator to detect stress sensitivity to feather gathering is plasma corticosterone level in growing geese, plasma levels of thyroxin (T_4) and triiodothyronine (T_3) in breeder geese and the H/L ratio in growing geese. The H/L ratio in breeder geese, used alone, is not a reliable stress indicator as they are already familiar with the procedure.

3. I have found that expert manual feather gathering does not cause more distress than catch and taking in hand the geese or the blood withdrawal do.

4. I have found that the antistress mixture given in the drinking water prior to gathering feathers has no significant effect on the blood parameters' values.

5. By analysing the egg production traits of Hortobágy White goose flocks I have shown that reproductive performance of two to five year flocks surpasses that of the one year old ones, irrespective of the year.

6. I have shown the correlations of laying intensity with day length and air temperature for the three phases (rapid increase, plateau and rapid decrease) of the laying period. Accordingly, the correlation of laying intensity with day length was positive until the decrease phase and thereafter negative in the one year old flocks r_{2012} =0.85 vs. -0.99, P <0.001; r_{2013}=0.90 vs. -0.998, P <0.001 as well in the two to five year old

flocks r_{2012}=0.76 vs. -0.995, $P < 0.001$; r_{2013}=0.63, $P < 0.05$ vs. -0.85, $P < 0.001$.

The correlation of laying intensity with air temperature was of similar sign, except for the decrease phase in the one year old flock in 2013 being weak positive.

4. Conclusions and suggestions

4.1. Conclusions deducible from stress indicator values during feather gathering

I examined stress sensitivity to gathering feathers by changes in five stress indicator blood parameters' values in growing and/or adult breeder geese of Babat Hungarian Upgraded goose breed according to a uniform test protocol, in five treatment groups.

Among blood parameters examined the elevated plasma corticosterone levels in 9 week old growing geese at their first and repeated catch, taking in hand and blood sampling indicated stress responses; the feather gathering procedure caused no higher increase in the hormone level, i.e., distress.

The parallel decreases in levels of plasma thyroid hormones namely thyroxine (T_4) and triiodothyronin (T_3) 1 hr after the feather gathering procedure indicated moderate distress in breeder geese.

In 8-9 week old growing geese total white blood cell count was significantly higher 24 hrs after the feather gathering procedure than 7 days later ($P < 0.01$, $P < 0.05$) and the heterophyl granulocyte to lymphocyte (H/L) ratio reached and even surpassed the value characteristic of strong stress (0.80). With them the H/L ratio can be used to indicate stress reactions to feather gathering as being a new stressor for them. In adult breeder geese total white blood cell count varied little and the H/L ratio was higher but lower the values characteristic of optimal (0.50) and strong stress, respectively, 24 hrs after feather gathering. Therefore the H/L ratio, used alone, is not a reliable indicator with them. The already known procedure elicited stress reactions weaker to affect the circulating white blood cells.

The antistress mixture given in the drinking water pre-procedures had no significant effect on the blood parameters' values of geese.

Basic values stated for the five blood parameters of Babat Hungarian Upgraded goose breed can be utilised to examine stress sensitivity in other goose breed.

4.2. Conclusions deducible from the analysis of the egg production of breeder geese

In the Hortobágy White breeder goose breed kept under natural climatic conditions, egg production is restricted only to one season coinciding with the increasing day length.

The egg production traits (onset and duration of egg laying, egg yields per goose, laying intensity and the mortality rate of layers) differed by year in one year old as well in two to five year old flocks.

Similar to the technical literary data, the production traits improved with the flock age. In two to five year flocks the laying period started earlier and lasted longer. During this time they produced more eggs with higher intensity, less defected eggs and layer mortality than the one year old flocks.

In agreement with earlier publications, it can be stated that based on the degree of laying intensity the laying period can be divided into a rapid increasing, a plateau, and a rapid decrease phase, irrespective of the year, even at the increasing time of day length.

The correlation between laying intensity and day length was positive until the decrease phase and thereafter negative. The correlation was of similar sign with air temperature except for the decrease phase in the one year old flock in 2013.

The method I used to evaluate the reproductive performance by the climatic factors of the year and by flock age can be adapted to analyse the production data of other semi-extensive goose breeds.

5. Publications related to the dissertation

1. Peer-reviewed full-text scientific articles

1.2. In English, published in international journals

P. TÓTH – J. JANAN (2016): Variation in some blood parameters of geese subjected to feather gathering. International Journal of Poultry Science. 15:(6) pp. 240-244.

J. JANAN – **P. TÓTH** – I. HUTÁS – Á. TREUER – J. PÁLI – B. CSÉPÁNYI (2015): Effects of dietary micronutrient supplementation on the reproductive traits of laying geese. Acta Fytotechnica et Zootechnica. 18: (1) pp. 6-9.

P. TOTH – J. JANAN – E. NIKODEMUSZ (2014): Variation in laying traits of Hortobagy white breeder geese by year and age. International Journal of Poultry Science. 13: (12) pp. 709-713

1.3. In English, published in Hungarian journal with impact factor

P. TÓTH – L. BÓDI – K. MAROS – E. SZŰCS – J. JANAN (2012): Blood corticosterone levels in growing geese around feather gathering. Acta Veterinaria Hungarica 60: (4) pp. 477-487 **[IF (2012): 1,173.**]

4. Conference proceedings

4.1. Full-text articles published in English

JANAN, J. – SZŰCS, E. – **TÓTH, P.** – BÉRES, A. – JÁRVÁS, K. (2010): Relationship between feather harvesting and some physiological parameters in geese. In E. Szűcs, Sz. Konrád, E. N. Sossidou (eds): Basics of animal welfare & product quality. Szent István University. Gödöllő. ISBN 978-963-269-143-5

JANAN, J. – BÓDI L. – KOZÁK J. – MÉZES M. – TÓTHNÉ MAROS K. – **TÓTH P.** (2002): A vér glükóz szint alakulása ludakban a termelés különböző szakaszaiban. XLIV Georgikon Napok Stabilitás és Intézményrendszer az Agrárgazdaságban 17. (Full text available on CD) [Blood glucose level of geese in different phases of production] ISBN 963 9096 78

4.2. Full-text articles published in Hungarian

JANAN, J. – **TÓTH P.** – SZŰCS E. (2010): A ludak plazma kortikoszteron szintjének változása a tollszedés hatására. XXXIII. Óvári Tudományos Napok, Mosonmagyaróvár, 2010. október 7. ISBN 978-963-9883-55-0

JANAN, J. – KOZÁK J. – RUDAS P. – TÓTHNÉ MAROS K. – **TÓTH P.** (2003): Állatvédelmi szempontok és a lúdtolltermelés. EU Konform Mezőgazdaság és Élelmiszerbiztonság Konferencia. Szent István Egyetem Mezőgazdaság- és Környezettudományi Kar – Debreceni Egyetem Agrártudományi Centrum, Mezőgazdaság-tudományi Kar, Gödöllő 2003. Június 5. II. kötet, 250-255 p. ISBN 963 9483 28 1, ISBN 963 9483 30 3

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5. Conference abstracts

5.3. One-page summary

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