THESIS OF DOCTORAL (PhD) DISSERTATION

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INCREASING OF N-3 FATTY ACID CONTENT AND IMPROVEMENT OF OXIDATIVE STABILITY IN ANIMAL PRODUCTION BY FEEDING

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1. INTRODUCTION

Feeding comprehensively influences animal originated food composition (e.g. fat content, protein content, fatty acid profile, vitamin content) and so nutrition value. Having known the role of different substances in each metabolism process there is a possibility to produce so-called functional food products, and due to their special nourishing substances some illnesses can be delayed or its development can be avoided by eating them.

Among nutrients lipoids have come into the limelight for a decade, which is in connection with the extensive role of the fatty acid in the organism. It is particularly true for polyunsaturated fatty acid (PUFA), mostly for n-6 and -3 fatty acids.

Hungarian people's fatty acid supply using sunflower seed oil and pork-fat based on the Hungarian traditional cooking does not meet the food requirements. First of all, n-3 fatty acid supply falls behind the accepted level, and as a result of it the proportion of n-6/n-3 is significantly wider than the optimum and the ratio is usually over 28-30:1 (*Rodler, 2005*).

One possibility to improve Hungary's fatty acid supply is to add n-3 fatty acid to the diet of the farming animals and this way n-3 content in animal originated food can be increased. It can be solved in a biological way. To prove the above mentioned theory experiments were carried out with broiler, geese and rabbits.

2. OWN EXPERIMENTS

2.1. The aim of the experiments

Numerous experiments were carried out how to increase n-3 fatty acid content in some animal originated food at University of West-Hungary, Faculty of Agricultural and Food Sciences, Department of Animal Nutrition. So there are lot experiences in this subject and there are appropriate conditions for experiment and laboratory analysis. Considering the fact that there are few data about other animal species (e.g. goose, rabbit) in Hungarian as well as in the international scientific literature the research was expanded on two other animal species like goose and rabbit.

The oxidative stability is also an important point of view, when increasing the n-3 fatty acid content of meat. During the experiments the oxidative stability was examined in animal originated food while n-3 fatty acid was increased and the animals were fed from different originated vitamin E in different doses, or on the other hand whether their degradation significantly could be slowed down.

Consumers' important questions were taken into consideration as well, namely cooking methods (frying, boiling) could not damaged by the increased n-3 fatty acid, and whether changing fatty acid content influenced adversely the organoleptic properties of the prepared food.

According to the above mentioned elements the following questions were answered:

- Is it possible to increase n-3 fatty acid content in goose products (goose-meat, goose-liver, goose fat, goose crackling) by feeding them with forage?
  - Is it possible to increase n-3 fatty acid content in goose products by feeding them with green forage?
  - What are the results (e.g. daily weight gain, FCR, energy- and protein utilization) of feeding meat-type hybrid geese with green forage mixed with other substances?
  - How can n-3 fatty acid content be increased in geese products if they are fed linseed oil supplemented diet and green forage?
  - Are there any differences in fatty acid composition considering different parts of the body (breast, thigh), or liver, abdominal or subcutaneous fat?
  - How can n-3 fatty acid content be increased by feeding the goose with linseed oil supplemented diet in the last period of their fattening?
- Is it possible to increase n-3 fatty acid content in rabbit products by feeding?
  - What is the effect of increasingly linseed oil supplementation on the average daily weight gain FCR, energy- and protein utilization of rabbits?
  - How can n-3 fatty acid content be increased by feeding rabbits with linseed oil supplemented diet?

  - What is the effect of increased n-3 fatty acid content on organoleptic properties of the food made from them (taste, smell, colour, state, etc.)?
- How does oxidative stability of the fat with increased n-3 fatty acid content change?
- Effect of added vitamin E on the oxidative stability of different meat samples:
  - Stating the amount of vitamin E supplementation in order to decrease oxidation stability.
  - Measuring the effects of the natural originated d-α-tocopherol and dl-α-tocopherol-acetate produced by industrial way.
- Results of cooking methods (frying and fat sources) on n-3 fatty acid content in food.

2.2. Material and methods

2.2.1.Experimements with geese

Four experiments were conducted at University of West-Hungary, Faculty of Agricultural and Food Sciences, Department of Animal Nutrition Research Farm. The first experiment was carried out with 100 Lipsitsch-iXL meat-type hybrid geese, while the second one with 132 Gourmaud Si-14 liver-type hybrid geese. Another experiment was carried out in 2006 with the same hybrids of which number was 154 in each experiment.

Feeding of the animals was the same in all 4 experiments during the first 5 weeks.

In 2005 the first experiment was conducted with meat-type hybrid geese. At 6 weeks of age, geese were moved to a finisher building and randomly assigned to one of four groups:

Dietary treatments between 6-11 weeks of age	
1. group	finisher diet
2. group	70 % of finisher diet plus ad libitum forage
3. group	finisher diet supplemented with 4 % linseed oil
4. group	70 % of finisher diet supplemented with 4 % linseed oil plus ad libitum forage

Four geese from each group were slaughtered at the age of 11 weeks, and fatty acid composition of breast, thigh, liver, abdominal and subcutaneous fat was measured.

Feeding of the rest of geese was standardized, so all of them fed diet supplemented with 4% linseed oil in the amount of 250g/day plus ad libitum green forage. At the end of the experiment, on the 17th week, four geese from each treatment were randomly selected and samples collected to determine the chemical composition and fatty acid profile. The experiment results were stated with the same method used during the 11th week.

The geese were fed with linseed oil and green forage (wheat-vetch, green alfalfa and grass) to increase n-3 fatty acid in geese products.

The 2nd experiment in this year was carried out with liver-type hybrid with the same aims as of the meat-type hybrid geese. At 6 weeks of age, geese were moved to a finisher building and randomly assigned to one of six dietary treatment groups:

1. group	finisher diet
2. group	70 % of finisher diet plus ad libitum forage
3. group	50 % of finisher diet plus ad libitum forage
4. group	70 % of finisher diet supplemented with 4 % linseed oil plus ad libitum forage
5. group	50 % of finisher diet supplemented with 4 % linseed oil plus ad libitum forage
6. group	finisher diet supplemented with 4 % linseed oil

Feeding of the geese was changed between the 12th and 15th week so they were fed with goose growing's compound feed or goose growing's compound feed supplemented with 4% linseed oil, but dietary treatment groups were the same. As green forage animals were fed with chopped corn mixture ad libitum during the whole period of the experiment.

Most of the animals were slaughtered after the 15th week and samples of 3 animals from each group were analyzed in the same way as it happened in the first experiment. The rest of the geese (30 altogether) were fed forcibly. The animals were assigned into two groups: control geese were selected from 1-3 dietary treatment groups, the animals of other group were selected from 4-6 dietary treatment group and got 4 % linseed oil supplementation during force-feeding.

A fattening machine was applied for force-feeding. The force-feeding was performed with 4 feeding events a day (at 7 and 9 o'clock in the morning, at 3 and 5 o'clock in the afternoon) during 17 days. The portion of each feeding events was 20:30:20:30 %. The daily portion was 600g at the beginning of the force-feeding period and by the end it was raised to 1960g.

Other experiments were carried out with the same hybrid geese (Lipitsch-iXL, Gourmaud Si-14) in 2006. The main aim of these

experiments was to analyze the oxidative stability in geese products and to improve it by feeding. According to the aim of the trials geese diet were supplemented with linseed oil and vitamin E. The source of synthetic vitamin E supplementation was the dl-α-tocopherol-acetate, and the natural vitamin E source was fatty acid distillate, a by-product of the oil industry which contains 16-17000 mg d-α-tocopherol/kg.

As the experiments were conducted simultaneously in both hybrid, so the same dietary treatments were applied. At the age of 5 weeks geese were moved into a finisher building where they fed the following 7 experimental diets from the 6th week:

Dietary treatments between 6-11 weeks of age	
1. group	finisher diet
2. group	80 % of finisher diet plus ad libitum forage
3. group	80 % of finisher diet supplemented with 2 % linseed oil plus ad libitum forage
4. group	80 % of finisher diet supplemented with 2 % linseed oil plus ad libitum forage + 150 mg dl-α-tocopherol-acetate / kg diet
5. group	80 % of finisher diet supplemented with 2 % linseed oil plus ad libitum forage + 250 mg dl-α-tocopherol-acetate/kg diet
6. group	80 % of finisher diet supplemented with 2 % linseed oil plus ad libitum forage + 150 mg d-α-tocopherol from fatty acid distillate/kg diet
7. group	80 % of finisher diet supplemented with 2 % linseed oil plus ad libitum forage + 250 mg d-α-tocopherol from fatty acid distillate/kg diet

Feeding was changed into goose growing's compound feed during the 11-14th week, but linseed oil and vitamin E supplementation and green forage remained the same proportion in each groups.

The results of previous trials were considered to determine the dose of linseed oil supplementation and diet restrictions. Liver- and meat-type hybrid geese were fed chopped grass mixture for green forage during the whole period of the trial.

At the end of the experiment, on the 14th week, the meat- and liver-type hybrid geese were slaughtered and samples were collected from 3 animals in each group for the chemical analysis.

The rest of the liver-type hybrid geese (40 altogether) were force feeding similarly to the previous year's method. Animals were chosen from the 1st, 3rd, 5th and 7th dietary treatment groups. The fattening forage equals to the forage used in the first experiment. The diet contained 2% linseed oil and the above detailed tocopherol supplementation in the 2nd, 3rd and 4th fattening groups. The force feeding methods (mechanical fattening, feeding events time and proportion) were performed with the same procedures as it was during the 1st experiment technologies.

In the case of liver hybrid geese, sample killing was fulfilled with 3 animals in each group after fattening them.

As the aims of the experiments suggested the oxidative stability of meat samples was measured as well. TBARS value was determined by taking breast and thigh meat samples from 3 animals of each group at the end of the experiment on the 14th week. The samples were stored frozen on -16 ^{0}C for 1 and 2 months long. TBARS value was determined similarly to the method described and used with meat-type hybrid geese.

2.2.2. Experiments with broiler

Earlier results of the Department's researches with boiler chicken were taken into consideration therefore diet of the experimental groups was supplemented with 2% linseed oil and synthetic or natural vitamin E (250mg/kg feed).

During 42 days feeding 200 Ross meat hybrid chicken were divided into 1 control and 3 experimental groups. The applied treatments are shown in the following table:

	Starter diet	**Growing and finisher diet**
1. group	2% sunflower oil (NFO)	4% NFO
2. group	2% linseed oil (LO)	2% NFO + 2% LO
3. group	2% LO+250 mg SE/kg diet.	2%NFO+2% LO+250 mg SE/kg diet
4. group	2% LO+250 mg TE/kg diet	2%NFO+2% LO+250 mg TE/kg diet

SE=dl-α-tocopherol-acetate TE= d-α-tocopherol from fatty acid distillate

At the end of the experiments the animals were slaughtered and the samples of 3 geese from each group were collected for chemical analyses. The drawn body was boned, thigh and breast meat without skin were minced and homogenised, and after it samples were collected to determine fatty acid and vitamin E content as well as TBARS value. The oxidative stability was measured by TBARS value form fresh meat and after 1 and 2 months of storage on-16 ^{0}C.

2.2.3. Experiments with rabbits

The experiment with rabbit was performed together with Research Institute for Animal Breeding and Nutrition in Gödöllő. The 1. experiment was conducted using 250 Pannon White rabbits (mixed sex). Animals were

randomly assigned to one of the five treatment groups (50 rabbits/treatment) and fed the experimental diets between 35 and 80 days of age.

1. csoport	negative control (no added oil)
2. csoport	positive control (4 % sunflower oil)
3. csoport	1 % linseed oil + 3 % sunflower oil
4. csoport	2 % linseed oil + 2 % sunflower oil
5. csoport	4 % linseed oil

Composition of diets was determined according to Lebas' recommendation (2004).

At 84 days of age all the rabbits were slaughtered and 10 rabbits from each treatment were randomly selected and samples were collected to determine fatty acid profile of meat and liver.

In the 2nd experiment (conditions were the same like in the previous experiment) 300 New Zealand White rabbits (mixed sex) were randomly assigned to one of the six treatment groups: a negative control diet with no added oil and 5 diets supplemented with 2% sunflower and 2% linseed oil were used. In addition to the 60 mg/kg dl-α-tocopherol-acetate content, no more vitamin E (2.group), 90 or 150 mg/kg synthetic vitamin E (dl- α-tocopherol-acetate) (3. and 4. group), 90 or 150 mg/kg natural vitamin E (NE) were added to the oil supplemented diets. Therefore, the diets supplemented with vitamin E contained a total of 150 or 300 mg/kg vitamin E. Our trial was to evaluate the effect of oil and different vitamin E supplementation on the oxidative stability and α-tocopherol content of rabbit meat.

At 84 days of age all the rabbits were slaughtered and 10 rabbits from each treatment were randomly selected and meat samples were

collected to determine fatty acid profile, vitamin E content and oxidative stability of thigh and loin.

2.2.4. Organoleptic examinations

Organoleptic properties were investigated during the trials with geese and rabbits. Tasting board with 20 members checked 5 features (state, smell, colour, taste and general impression) without knowing the origin of the samples and they evaluated the outcome with grades from 1-5. The given grades for each property was averaged and the effect of the different dietary treatments was investigated by reason of these results.

In the case of meat- and liver-type hybrid geese fried geese meat without adding fat were examined by taste.

Two ready-to-eat foods, namely scrambled chicken (fried in pork fat or sunflower oil) and fried chicken without adding fat was prepared for organoleptic examination in trial with broiler chicken.

Rabbit meat from different oil supplemented groups (1st experiment) were used to cook stew and fried meat with bacon, onion and paprika.

Taking care of applying the same preparation method and treatment was a question.

2.2.5. Experiments on impact of cooking methods

During the experiment other factors were paid attention as well heating affect of boiling (in a cooking pot or pressure-cooker) and fat source (sunflower oil, pork fat) of frying on original the fatty acid composition of meat.

To determine the fatty acid profile the same methods were used as the raw meat.

2.3. Chemical analysis

Chemical composition of diets was measured by Hungarian Feed Codex (1990) Volume 2. The fatty acids from diets and meat were extracted and measured as methyl esters with a gas chromatograph (HP Agilent Technologies 6890N). The α-tocopherol concentration in meat was determined using high performance liquid chromatography (HPLC) according to 44/2003 (IV.26.) MARD order 10. appendix. To evaluate the oxidative stability of meat samples were kept refrigerated -16 ^{0}C and oxidation (TBARS) was measured (Ramanathan *and Das*, 1992) after one and two months of storage.

2.4. Statistical Analysis

Data were analyzed by analysis of variance by using the one-way ANOVA in the SPSS 12.0 for Windows (SPSS Inc.). Homogeneity of variances between groups was previously verified by the Levene's test. The Bonferroni post hoc test was used when variances were equal between groups and/or Dunnett's T3 was used if variances were not equal. Statistical significance was considered at $P<0.05$.

3. NEW SCIENTIFIC RESULTS

According to the carried out trials the following new scientific result can be stated:

1. Feeding animals with green forage or diet supplemented with 4% linseed oil significantly decreased MUFA and increased PUFA content in tissues of geese and rabbits. The amount of α-linolenic acid and arachidonic acid furthermore EPA and DHA in the liver increased in the greatest degree. Effect of linseed oil supplementation and green forage on the MUFA and PUFA content cumulated. Both treatments decreased n-6/n-3 fatty acid ratio for the experimented species. The effect of linseed oil is considerably greater than that of the green forage.
2. Geese force-feeding increases oleic acid, and significantly descrease the proportion of PUFA in the liver. Changes in the fatty acid content during force-feeding were influenced by the diet fed the geese before force-feeding.
3. The fatty acid composition of the diet has different affect on the fatty acid profile of fat from different parts of the body in geese.
4. D-α-tocopherol of fatty acid distillate as source of natural form of vitamin E was a more effective antioxidant in the case of meat-type hybrid geese, broiler chicken and rabbits than the dl-α-tocopherol-acetate produced by industrial way.
5. Organoleptic properties of the food prepared from the meat of rabbits and geese fed diet supplemented with 2% linseed oil were not influenced by dietary treatment.

6. The fatty acid composition of meat with high n-3 fatty acid content is not changed by cooking the products for 2 hours in a traditional way or boiling it in a pressure-cooker for 20 minutes, and frying it without adding fat. Frying the meat in porkfat or sunflower oil for 15-20 minutes long means some change in fatty acid content but it does not result absolute n-3 fatty acid content descreases.

4. LIST OF PUBLICATIONS IN THE THEME OF THE DISSERTATION

Book chapters:

Schmidt J., Perédi J., Tóth T., ***Zsédely E***. (2008): A takarmányozás hatása az állati eredetű élelmiszerek összetételére c. fejezet A jövő élelmiszerei és az egészség c. könyvben (Szerk.:Nagy J., Schmidt J., Jávor A.). Debreceni Egyetem Agrár- és MűszakiTudományok Centruma, Debrecen, 11-48.p.

Scientific paper published in Hungarien language

1. *Schmidt J., Perédi J., Tóth T.,* ***Zsédely E***. (2006): Fontosabb állati eredetű élelmiszerek zsírsavösszetételének módosítása takarmányozással, I. Sertészsír és sertéshús. Élelmezési Ipar 2006 (LX. évf.), 10-11. 235-240
2. *Dublecz K., Pál L., Bartos Á.,* ***Zsédely E.****, Wágner L., Kovács G., Bányai A., Tóth Sz.*(2006): A takarmányozás hatása a baromfitermékek minőségére. Állattenyésztés és Takarmányozás. Vol 55. S71-S87
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4. *Schmidt J., Perédi J., Tóth T.,* ***Zsédely E***. (2007): Néhány állati eredetű élelmiszer zsírsavösszetételének javítása takarmányozás útján. Olaj Szappan Kozmetika. LVI. évf. 3. szám 89-95.
5. *Schmidt J., Tóth T.,* ***Zsédely E.*** (2008): A tojás n-3 zsírsav- és E-vitamin tartalmának növelése takarmányozás útján. Állattenyésztés és Takarmányozás 57. 4. 387-401.

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1. ***Zsédely Eszter***: A nyúlhúsω-3 zsírsavtartalmának növelése takarmányozás útján. XII: Ifjúsági Tudományos Fórum Keszthely, 2006.április 20.
2. ***Zsédely E.****, Tóth T., Eiben Cs., Gódor S-né, Végi B., Virág Gy.,Fébel H., Schmidt J.*:Napraforgó- és lenolaj és különböző E-vitamin-kiegészítések hatása a nyúlhús E-vitamin tartalmára és oxidatív stabilitására. 19. Nyúltenyésztési Tudományos Nap, Kaposvár 2007. május 23. 29-34. o.
3. *Eiben Cs., Gódor S-né, Végi B., Virág Gy., Fébel H.,* ***Zsédely E.****, Tóth T., Schmidt J.:* Napraforgó- és lenolaj és különböző E-vitamin-kiegészítések hatása a nyulak hízlalási és vágási mutatóira. 19. Nyúltenyésztési Tudományos Nap, Kaposvár 2007. május 23. 17-22.o.
4. *Virág Gy., Gódor S-né, Végi B., Eiben Cs., Fébel H.,* ***Zsédely E.****, Tóth T., Schmidt J.*: Napraforgó- és lenolaj és különböző E-vitamin-kiegészítések hatása a nyúl combhús pH-jára és színére. 19. Nyúltenyésztési Tudományos Nap, Kaposvár 2007. május 23. 23-27.
5. ***Zsédely E.,*** *Tóth T., Eiben Cs., Virág Gy., Fábián J., Schmidt J. (2008)*: Effect of dietary vegetable oil (sunflower, linseed) and vitamin E supplementation on the fatty acid composition, oxidative stability and quality of rabbit meat. Proceedings of 9th World Rabbit Congress, Olaszország, Verona, 2008. 06. 10-13. (CD kiadvány)

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2. *Eiben Cs., Tóbiás G., Gódor S-né, Végi B., Virág Gy.,* ***Zsédely E.****, Tóth T.*: Influence of sunflower and linseed oil addition in rabbit feed: 1. Growth and carcass traits. World Rabbit Science 2006, 14:272.
3. ***Zsédely E.****, Tóth T., Schmidt J.*: Zöldtakarmány-etetés és lenolajkiegészítés hatása a libahús kémiai összetételére. XXXI. Óvári Tudományos Nap, Mosonmagyaróvár 2006.október 5. (előadás) 87. o.

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5. *Eiben Cs., Gódor Surmann K., Végi B., Virág Gy., Fébel H., **Zsédely E.**, Tóth T., Schmidt J.* (2008): Effect of sunflower oil and linseed oil and vitamin E dietary supplementation on growing and slaughter performance of rabbits. World Rabbit Science, 16. 38-39. (abstract)

Poster peresentations in foreign language

*Fébel H., Czabai G., Balogh K., Szabó A., Eiben Cs., Tóth T., Mézes M., **Zsédely E.**, Schmidt J., Miklós Sz., Lugasi A., Blázovics A.* (2007): Dietary oils and added tocopherol: Effects on tissue fatty acid composition, tocopherol level and antioxidant defense system of rabbits. Proceedings of the 11th ESCVN Congress 2007 128.o.