THESIS OF DOCTORAL (PhD) DISSERTATION

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MOSONMAGYARÓVÁR
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INCREASING OF CONJUGATED LINOLEIC ACID CONTENT OF BROILER MEAT AND EGG BY FEEDING

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1. INTRODUCTION

As far as human feeding is concerned, the fat content and fatty acid structure of the animal based food products bear particular importance. A considerable number of studies proved the different fatty acids to have a number of effects on our health, based on their different biological roles (Manilla and Husvéth, 1999). That is why manipulation of the fatty acid content of the fatty tissue of animal origin through feeding has long been a matter of researches. Nowadays some of the researches which aim to alter the fatty acid content, are primarily investigating the opportunities of increasing the conjugated linoleic acid (CLA) content in fat.

This tendency largely depends of the fact that linoleic acid plays a wide parallels range of different roles in the organism. If it comes to these roles, the c9,t11 and t10,c12 CLA isomers are the most important ones. In considerable amount, the human organism initiates its CLA intake by consuming food products, made of ruminants (milk, meat), or by the consumption of different food supplements. In Hungary, beef and lamb consumption per head is insignificant, the yearly milk intake per head also fails to exceed 200 liters, which is, compared to the EU average, is rather low. That is why it would be advisable to increase the CLA content of such food materials - originally low at CLA - which are widely consumed in large amount. Broiler meat and egg could be such materials; of which CLA increase via feeding manipulation got to become the aim of our study.

2. THE INVESTIGATIONS OF THE AUTHOR

2.1. Stating an aim for the research

In the recent years, the University of West Hungary, Faculty of Agriculture and Food Sciences, Department of Animal Feeding and Nutrition Sciences housed extensive researches which aimed to alter the fatty acid consistency of animal based food products by feeding, in order to synchronize it with the human needs.

The choice of my topic was partly influenced by the fact that the department has extensive knowledge and experience of the subject, furthermore satisfactory facilities for laboratorial work and animal tests for the researches are given. On the other hand, however, increasing CLA content in broiler meat and egg, how and what extent could it be altered by feeding manipulation is not widely reported in the international reports and journals, and almost never mentioned in Hungarian works. This tendency also urged me to direct my focus on the topic.

Apart from the investigation of increasing CLA content, we also dealt with the question whether the blissful effect of CLA on the oxidation stability of the meat samples could be boosted by the vitamin E supplementation added to the feed.

Throughout the researches on broiler chickens we aimed to investigate the following:

- What effect the supplementation - containing 53.5% conjugated linoleic acid – of the feed has on daily weight gain, feed energy and protein utilization of broiler?
- Whether the linoleic acid supplementation affects the digestibility of nutrition, and the N-retention of broilers.

- What effects the supplementation, containing conjugated linoleic acid has on the fatty acid consistency of broiler fat, and most importantly on the conjugated linoleic acid content?
- What relationship is to be found between the amount of supplementation and CLA content of the broiler fat?
- What is the difference – if any – between the fatty acid consistency of the fat of the meat from different parts of the animal (breast, leg etc.).
- Apart from the c9,t11-C18:2 variant what other isomers appear in broiler fat.
- What the fat content of the broiler is going to be, could it be decreased by the supplementation containing CLA of various isomers?
- What effects CLA containing supplementation has on the oxidation stability of broiler fat?
- Does CLA supplementation affect the organoleptic features of broiler meat products?
- What effects the different preparing methods (avoiding adding fat, or baking with sunflower oil or lard) have on the fatty acid consistency of the food?

During the experiments, made on egg laying hens, answers for the following questions were sought:

- What effect CLA supplementation has on egg production?
- Does CLA containing supplementation to the feed have any effect on the weight of the egg, or the color of egg yolk?

- What effect CLA supplementation to the feed has on the fatty acid consistency of eggs, most importantly on the conjugated linoleic acid content?
- What other isomers appear in eggs apart from the c9,t11-C18:2 variant?
    - What effect CLA supplementation has on the organoleptic features of egg based food products?

2.2. MATERIALS AND METHODS

2.2.1. Experiments on broiler chicken

During my PhD work three fattening, one digesting, and one N-retention experiments were carried out on the animal test site of the Department of animal studies of the Agricultural and Food Sciences, Faculty of University of West Hungary. All of the experiments were carried out with replications.

The broiler fattening experiments were carried out on fifty Ross 308 genotype broiler roosters in all cases. Until their 21st day the cockerels were fed starting feed; between 22-35 days of age they were fed raising feed; until the end of the research – that is to say till the 42nd day – they were fed finishing feed. The feeds were of the same energy and protein content, therefore the feed of the treatments only differed in the fed oil sources and the added vitamin E supplementation.

The body weight of the broilers were measured individually at the 21st and 42nd days, furthermore we determined the feed intake as well. For the laboratorial experiments 8 animals of each treatment were slaughtered at the end of the research. The leg and breast meat of the broilers were ground

with skin on it. The ground meat was homogenized carefully, after that samples were taken for investigating the fatty acid consistency, and the oxidation stability (TBARS measure). The oxidation stability of the meat was measured on the day of slaughter from fresh sample; and from 1 and 2 months old samples stored in -16^{o}C.

2.2.1.1. First broiler trial

During the first fattening experiment one control and three experimental groups were formed. The feed of the groups contained the following oil supplementation:

- control group: 4% sunflower seed oil
- 1st experimental group: 1% CLA product (0.535%CLA)+3% sunflower oil
- 2nd experimental group: 2% CLA product (1.07% CLA)+2% sunflower oil
- 3rd experimental group: 4% CLA product (2.14% CLA)

The primary aim of the research was to determine the effect of different doses of CLA products on the most important production measures of broilers and on the oxidation stability of the fatty acid content of broiler meat.

2.2.1.1.1. Digesting and N-retention experiments on broilers

In the 4th week of the 1st fatting experiments 8 broilers from every group were taken in pairs into metabolism cages in order to investigate the effect of CLA products on the digestibility of nutrition and on the N-

retention of broilers. The metabolism-cage enabled to determine the feed consumption and the amount of faeces. At the end of the experimental period sample was taken from the faeces in order to determine the digestibility of the nutrition. The cockerels in the metabolism-cages were consuming the same feed (raising feed) as the individuals in the booths. In 5 days the individuals were got accustomed to the metabolism-cages, and then a 5 days long period came during which the feed consumption and faeces amount was measured.

2.2.1.2. 2nd experiment on broilers

During the 2nd experiment – relying on reports, journals and the results of our previous researches – we intended to investigate whether the positive effect of CLA on meat oxidation stability could be improved further by adding vitamin E (in our case it was DL-alpha-tocopheryl-acetate) to the feed. During the fattening experiment we used the following treatments:

- control group: 4% sunflower oil
- 1st experimental group: 1% CLA product (0.535% CLA) + 3% sunflower oil
- 2nd experimental group: 1% CLA product (0.535% CLA) + 3% sunflower oil + 100 mg DL-alpha-tocopheryl-acetate / kilogram of feed.
- 3rd experimental group: 1% CLA product (0.535% CLA) + 3% sunflower oil + 200 mg DL-alpha-tocopheryl-acetate/kilogram of feed.

2.2.1.3. 3rd experiment on broilers

According to several sources and to the outcomes of our researches, carried out as the 1st and 2nd experiments, besides its positive effect on the CLA content of fat, CLA supplementation to the feed increases the amount of saturated fatty acid (SFA) in the fatty tissue of the broilers, meanwhile decreases the total amount of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). That is why during the 3rd experiment on broilers we were investigating whether this negative effect of CLA could be at least partially compensated by combining CLA supplementation with linoleic oil supplementation. We chose linoleic oil for the purpose because our sources reported it to aid the fatty acid consistency of broilers, that is to say it increases the proportion of unsaturated fatty acid (primarily the proportion of n-3 fatty acid) in the product (Schmidt et al., 2008). Apart from that, from the oils available for feeding purposes, linoleic oil possesses the highest level (55-57%) of alpha linoleic acid (C18:3). That is why it is the most sufficient oil for the purpose of considerably decreasing the proportion of n-6/n-3 fatty acids in broiler fat (Chanmugam et al., 1992; López-Ferrer et al., 2001; Haemmal et al., 2001; Pálfy et al., 2007)

During the experiment the following four treatments were used:

- control group: 2% sunflower oil +2% linoleic oil
- 1st experimental group: 2% sunflower oil +2% linoleic oil +200 DL-alpha-tocopheryl-acetate/feed kilogram
- 2nd experimental group: 1% CLA product + 1% sunflower oil + 2% linoleic oil
- 3rd experimental group: 1% CLA product + 1% sunflower oil + 2% linoleic oil +200 mg DL-alpha-tocopheryl-acetate/feed kilogram

2.2.2. Experiments on laying hen

The feeding experiments on laying hens were carried out on the plant of a raiser (Halászi). 40 caged Shaver-576 laying hybrids were involved into every treatments of the experiment. The oil supplementation of the feed was the following:

- control group: 3% sunflower oil
- 1st experimental group: 1% CLA product +2% sunflower oil
- 2nd experimental group: 1% CLA product +1% sunflower oil + 1% linoleic oil.

Throughout the experiment the following issues were examined

- Daily egg yield
- 10 eggs were randomly picked from every treatment every day to measure them in order to calculate the average weight of eggs (g/egg)
- Once a week (always on the same day) 10 eggs from each treatments were broken and their yolk examined on the basis of a DSM color scale (from 1 to 15)
- Simultaneously, the weight of the egg yolk and egg white, then the fatty acid content of the yolk was measured.

2.2.3. Organoleptic examinations

During the experiments on broilers and laying hens we also carried out tasting in order to clarify how the organoleptic features of animal based foods of modified fatty tissue consistency (leg, eggs) are altered by different treatments. The ten members of the tasting committee were investigating 5

features in case of meat: consistency, smell, color, taste and overall impression. The committee graded the features in a 1 to 5 scale. The points, given to the features were averaged, that is how we determined the reputation of the food.

In case of broilers nature meat (prepared without added fat) and chicken tights (baked with added sunflower oil or swine fat) were provided for tasting. The compared meats of the investigation came from the following treatments of the previously initiated fattening experiments:

- control group: 4% sunflower oil
- 1st experimental group: 3% sunflower oil +1% CLA product
- 2nd experimental group: 1% sunflower oil +1% CLA product + 2%linoleic oil.

The organoleptic features of scrambled and boiled eggs were examined in the case of the treatments mentioned in chapter 2.2.2. The tasting committee evaluated four features of the egg based meals: smell, color, taste and overall impression. Tasting and grading happened in the same manner as reported in the case of the organoleptic examination of meat.

2.2.4. The effect of preparing methods on the fatty acid consistency

The samples, undergoing the organoleptic examination, were also examined on the basis of how the fatty acid consistency of broiler meat (leg) is changed by such preparing methods like frying with or without different kind of fats or oils (such as sunflower oil and lard).

During the preparing test 3-3 legs were fried from each treatment, without any added fat, or with added sunflower oil or lard. The meat with

skin on were placed one by one into aluminum trays, then with or without 50 g of fat were fried for 90 minutes in 180°C oven. The fat content and fatty acid consistency of the meats prepared as such were determined in a same manner.

2.2.5. The method of producing CLA products used for the experiments

For the purpose of isomerization, the linoleic acid or the fat containing linoleic acid has to be heated with dissolvent and alkali in presence. During our experiment 5.0 kilogram sunflower oil (~60% linoleic acid containing, feed grade) was isomerized by being stirred for 3,5 hours in 150-160C with KOH containing propylene glycol tincture in a sealed caldron, equipped with stirring and heating devices. During the process we drove N2 as a protecting gas over the substance in the caldron.

After being kept in temperature, the material was cooled to room temperature then 15% HCl substance was added to neutralize it. The fatty acid, exuded from the neutralized material was isolated than it was washed from hydrochloric acid by distilled water. In order to get rid of water the fatty acid was stirred with dry Na2SO4 then it was filtered by a filter paper.

2.2.6. Chemical methods

The chemical content of the feed (dry matter, crude protein, crude fat, crude fiber, crude ash, Ca and P content), and the dry matter, crude protein, crude fat and crude ash content of feces samples and raw or prepared meat was investigated with the following methods:

dry matter: MSZ ISO 6496:2001

crude protein: MSZ 6830 - 4:1981

crude fat: 44/2003. (IV. 26.) FVM rendelet

crude fiber: MSZ EN ISO 6865:2001

crude ash: MSZ ISO 5984:1992

Ca: MSZ ISO 6490-2:1992

P: Magyar Takarmánykódex (1990)

The fatty acid consistency of the fed oil supplementations, the fats used for frying, the slaughtered product and of the fried meat was determined with an HP Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Inc. Headquarters Santa Clara, US). The type of the colonna was Supelco SPTM 2560 (100m × 0,25mm × 0,2μm). H2 was used as protecting gas. Pressure: 176,8 kPa. Detector: FID. Speed of stream: 35ml/min nitrogen, 300 ml/min air. Split proportion: 10:1. Temperature: injector: 240C, thermostat: 170-215C (170C for two minutes then raised to 200C with 1C/min tendency, then with the tendency of 5C/min it was raised further to 215C which it held for 20 days.) Detector: 205C. Amount of sample: 1ul. The saponification of the fat happened with the aid of methanol solved in 1NaOH. Esterification was made with the use of 10% boron trifluoride solved in methanol, picking the sample was made with hexane.

The oxidation stability (TBARS number) was investigated with the Ramanathan and Das method (1992). The principle of determination is the following: the malondialdehyde in the trichlor acedic acid extract of the meat gives a red color reaction with thiobarbituric acid on 95-97C; which could be measured with spectrum-photometer on 532nm. As a standard, malondialdehyde was used, produced from the acidic hidrolisys from 1,1,3,3 tetraetoxi propane.

The investigation of the vitamin E content of the feeds was initiated in accordance with the MSZ EN ISO 6867 researching method.

3.2.7. Statistical evaluation

The statistical evaluation of the outcomes of the experiments made with the use of the SPSS 12.0 for Windows program (SPSS Inc. Chicago, US). After studying the data distribution (Kolmogorov-Smirnov test) in case of parameters showing normal distribution we initiated one factor analysis of variance (Levene's test, one-way ANOVA, Bonferoni test, Games-Howell test) while in the opposite cases we used non-parametric trials (Kruskal-Wallis test, Mann-Whitney test). The chosen significance level in all cases is $P \leq 0,05$.

The multi factor analysis of variance was made with the proc MIXED method of the SAS 9.2 program (SAS Institute Inc. Cary, NC. US).

3. NEW SCIENTIFIC ACHIEVEMENTS

On the basis of the outcomes of the experiments on broiler chicken and laying hens the following new scientific achievements could be stated:

1. 1 or 2% CLA product – containing 53.5%CLA, made by alcaline isomerisation of sunflower oil - of the feed significantly increases the daily weight gain of the broilers. 4% CLA product, however, hinders the weight gain.
2. The CLA supplementation of broiler feed does not affect considerably the digestibility of the nutrition, or N-retention. CLA supplementation furthermore does not change the crude protein or crude fat of breast and leg meat significantly.
3. The CLA supplementation of broiler and laying hen feed increases the CLA proportion of the broiler meats (leg, breast) and egg lipids. Regardless of the fact that c9,t11 and t10c12 isomers were present in the CLA product in an equal amount, the proportion of the c9,t11 variation in meats is 1.5; in eggs 4 times higher than of the t10,c12 isomer.
4. As a result of CLA feeding, the amount of saturated fatty acids in breast meat increases, the proportion of mono and poly unsaturated fatty acids decreases. In the lipids of egg yolk, besides the increase of the proportion of saturated fatty acids, the proportion of mono unsaturated fatty acids decreases. The direction of changes in the proportion of the main fatty acid groups, triggered by the linoleic oil, fed simultaneously with the CLA product, cannot be modified.

5. Our results proved that substituting the sunflower oil content of the feed with CLA product aids the oxidation stability of broiler meat. This favorable effect could be strengthened by the combination of CLA supplementation with vitamin E.

4. LIST OF PUBLICATION MADE IN THE THEME OF THE DISSERTATION

Studies to scientific journals under publication

1. **Tanai A.**, Perédi J., Zsédely E., Tóth T., Schmidt J. (2011): Erhöhung des Gehaltes an konjugierten Linolsäure im Broilerfleisch durch Fütterung. Archiv für Geflügelkunde, 75(2)
2. **Tanai A.**, Zsédely E., Perédi J., Tóth T., Schmidt J. (2010): Konjugált linolsav és lenolaj kiegészítés hatásai a brojlercsirkék zsírjának zsírsav-összetételére. A hús.

Studies, published in scientific journal

1. **Tanai A.**, Perédi J., Tóth T., Zsédely E., Schmidt J. (2010): A konjugált linolsav kiegészítés hatásai a brojlercsirke hízlalásban. 1. Konjugált linolsav és lenolaj együttes adagolásának hatása a brojler hús lipidjeinek zsírsav-összetételére. Állattenyésztés és Takarmányozás, 58. 2-3.
2. **Tanai A.**, Perédi J., Tóth T., Zsédely E., Schmidt J. (2010): A konjugált linolsav kiegészítés hatásai a brojlercsirke hízlalásban. 2. A konjugált linolsav hatása a brojlercsirkék termelésére, a táplálóanyagok emészthetőségére, valamint a hús kémiai összetételére. Állattenyésztés és Takarmányozás, 2010. 59. 2-3.

Presentation, delivered in scientific conferences, and published in full

1. **Tanai A.** (2009): A konjugált linolsav hatása a brojlerhús zsírsav-összetételére és a brojlercsirkék súlygyarapodására. XV. Ifjúsági Tudományos Fórum Keszthely, 2009. április 16. (CD kiadvány)

Presentation, delivered in scientific conferences, and published in abstract

1. **Tanai A.,** Tóth T., Schmidt J. (2009): A konjugált linolsav hatása a brojlerek termelési mutatóira és a brojlerhús zsírsav-összetételére. LI. Georgikon Napok Keszthely, 2009. október 1-2. (előadás) 142. o.
2. **Tanai A.,** Tóth T., Schmidt J. (2009): A konjugált linolsav hatása a brojler hús zsírsav-összetételére és oxidációs stabilitására. II. Gödöllői Állattenyésztési Tudományos Napok Gödöllő, 2009. október 16-17. (előadás) 86. o.