Thesis of the Ph.D. dissertation

POSSIBILITIES TO IMPROVE SHEEP INDUSTRY: ADAPTATION OF A HUMAN DIAGNOSTIC TOOL AND IMPROVING THE QUALITY OF LAMB MEAT WITH FEED SUPPLEMENTATION

Author:
Anikó Nagy
Ph.D. candidate

Supervisors:
Dr. András Jávor C.Sc.
Dr. Csiki Zoltán M.D., Ph.D.

UNIVERSITY OF DEBRECEN
Doctoral School of Animal Husbandry

Debrecen, 2015

TABLE OF CONTENS

I.BACKGROUND AND AIMS OF THE PH.D. STUDY 2
II.MATERIALS AND METHODS 5
2.1.Breath hydrogen measurement 5
2.2.The selenium and magnesium supplementation for lambs on quality of meat and consumers' health 5
III.RESULTS 10
3.1.Breath hydrogen measurement 10
3.2.Effects of supplementations on lamb health and the quality of carcass 11
3.3.Effects of supplementations on meat quality 12
3.4.Clinical and laboratory measurements 14
3.5.Results of questionnaires 14
IV.NEW STATEMENTS OF THE THESIS 16
V.PRACTICAL APPLICABILITY OF THE RESULTS 17
VI. REFERENCES 18
VII.LIST OF PUBLICATIONS RELATED TO THE DISSERTATION 20

I.BACKGROUND AND AIMS OF THE PH.D. STUDY

Diet-related disorders are one of the disease groups that have the highest morbidity and mortality worldwide (SATIA, 2009), still consumers do not exactly know the recommendations of healthy diet. It is difficult to interpret all of the food claims, although, more and more people think that it is important and they try to understand this claims. They became more concerned about their health and pay more attention to the healthiness of their diet. These trends set up new challenges for the food industry: food companies need to develop new products that have beneficial effects on human organism.

Meat has an important role in balanced diet; it contains essential amino acids in right quantity and ratio. Furthermore, it contains a lot of micronutrients as well, for example zinc and iron (SANTAELLA et al., 1997). One of the most valuable types of meat is lamb meat, which can support the prevention of colorectal polyps and carcinoma (KOTZEV et al., 2008).

The situation of sheep industry in Hungary is unfortunately not reflecting the excellent geographical facility of the country. Sheep meat is the least consumed meat in Hungary as well in the European Union. In order to develop this sector, we have to improve the efficiency of production by monitoring and improving animal health.

The hydrogen breath test is a simple, non-invasive and inexpensive method for estimating small bowel transit time, detecting the existence of excess bacteria in the small intestine and demonstrating carbohydrate maldigestion or malabsorption (WASHABAU et al., 1986). The rationale of hydrogen breath tests is based on the concept that parts of the gas produced by colonic bacteria fermentation diffuse into the blood and is rapidly excreted by breath (D'ANGELO et al., 2013). One of the exhaled substances is hydrogen, which can be measured relatively easily with the help of handy breath test devices. Hydrogen in the exhaled air only generated during anaerobic metabolism, consequently, the hydrogen measured in the exhaled breath sheds light on the quantity and the metabolic activity of anaerobic bacteria in the gut (MASTROPAOLO and REES, 1987). Unabsorbed dietary carbohydrates which reach the colon are metabolized by bacteria to hydrogen, methane and short chain fatty acids (GASBARRINI et al., 2009). Hydrogen measurement is routinely used in human medicine to investigate gastrointestinal function. Until now, little is known about the patterns of breath hydrogen excretion in suckling lambs. Importantly, postnatal growth in ruminant animals is divided into two physiologically distinct stages: the preruminant phase (milk-fed) and the postweaning ruminant phase. In the first phase, the digestive system is comparable to the ones

in monogastric animals or humans, and later, weaning stimulates rumen development and microbial fermentation (BELLVER et al. 1995). As the hydrogen concentration measured in the exhaled air is always a reflection of the mass of bacteria and of the bacterial metabolic activity in the intestines, hydrogen breath test can be a useful method for screening and investigating the suckling lambs' intestinal health and fermentation. Intestinal health of the lambs is a very serious point and a „weak link" during nursing the animals.

Raisers have to focus not just on weight control and animal welfare, but developing healthy and popular food as well, to support the boost of sheep industry. Lamb meat has special contain, so it is a good material to increase a bioactive components via feeding.

Human organism needs a lot of minerals and trace elements to normal functions. From the important minerals magnesium (Mg) is an essential one for the human body; it plays an important role in the regulation of cellular processes and functions as a cofactor of enzymes involved in energy metabolism. Many enzymes catalyzing phosphorylation and dephosphorylation reactions are activated by the formation of $MgATP^{+2}$ complexes. Magnesium deficiency often correlates with alterations in carbohydrate homeostasis, or with cardiovascular diseases. In addition, hypomagnesaemia has been reported to induce anaemia, reduce osmotic fragility, lead to echinocytosis, increase susceptibility of red blood cells (RBCs) to free radical injury, and enhance oxidative stress. Magnesium supplementation is used to decrease the symptoms of stress and prevent the diseases caused by stress, such as depression, sleeping disorders, headache and anxiety. Mg has effect on the central nervous system, so it also improves learning skills and memory (VIRÁG et al., 2011). Some epidemiological studies have suggested that adequate magnesium intake also reduces risk of development of type 2 diabetes (SALES et al., 2011).

One of the essential trace elements is selenium (Se). Selenium is an essential trace element, which is necessary for the synthesis of several proteins, such as glutation peroxidase, deiodase and thioredoxin reductase as well. Glutation perixidases play a role in reducing hydrogen peroxide and lipid peroxides to harmless products, thereby dampening the propagation of damaging reactive oxygen species. Se deficiency is characterized by several pathological conditions, such as growth retardation, skin lesions, hair loss, visual defects, reproductive disorders, pancreas atrophy and dystrophy of heart muscle as well as an increase of immature erythroid cellular elements. Plant foods are the major dietary sources of Se, consequently, the Se content of meat depends on the Se content of the soil in which plants are grown on animals are raised.

In the present study, we measured the breath hydrogen excretion in dorper lambs, furthermore, we supplemented the drinking water of merino lambs with Mg and Se in order to develop a healthy food. We observed the health of lambs during the study period, investigated the quality of meat, assessed the beneficial effects of its consumption for human health, and evaluated the changes in consumers' attitude.

AIMS

The aims of this study were:

- to assess the patterns of breath hydrogen excretion in lambs before and after feeding ewe's milk, and to evaluate pathological and/or physiological alterations in lambs' gastrointestinal function.

- to investigate the effects of magnesium and selenium supplementation on animal health and the quality of carcass.

- to measure the magnesium and selenium level of the meat from supplemented lamb groups.

- to assess, whether magnesium or selenium supplementation to lambs can change the fatty acid ratio of meat.

- to investigate, whether magnesium or selenium supplementation to lambs can influence the quality of meat (color, taste, popularity, toughness) or the health status of consumers.

- to evaluate the consumers' attitude to lamb meat and assess the changes during the study period.

II.MATERIALS AND METHODS

2.1.Breath hydrogen measurement

Animals

A total of 52 black head dorper breed lambs were included in the study. The age of study group was 15.4 ± 1.6 days, and their weight was 6.59 ± 1.74 kg. All animals were considered healthy according to veterinary clinical examination. No animal had evidence of systemic disease or had received antibiotics in two weeks prior to the study. The animals and their dams were kept on an experiment farm. The lambs were fed ewe's milk only. The mother animals were fed separately from their lambs thus lambs had no access to solid feed.

Breath collection and sampling

Breath samples were collected by using portable breath hydrogen monitor device (Gastro^{+} Gastrolyser, Bedfont Scientific Ltd., Rochester, Kent, ME1 3QX, England). The collection system composed of flatpak mouthpiece, D-piece, facemask and Y-piece. According to the lambs face and mouth size, appropriate facemask was used. The facemask was fitted towards the lambs' mouth, and they were allowed to breathe normally through the mask for 30 seconds. The instrument measures hydrogen concentration in parts per million (ppm) in concentration range 0-500 ppm. Before use, the hydrogen monitor was calibrated with Bedfont 100 ppm hydrogen in air gas.

Before the day of measurements, the lambs were weaned from their dams for 12-14 hours overnight fasting. The first measurement was performed before the dams were allowed to breast-feed their lambs. The feeding took 30 minutes, thereafter the lambs were separated again from their dams, and we carried out the second measurement, which was followed by two further measurements with 30 minutes interval.

There was a two-week follow-up after the measurements, in order to assess the gastrointestinal health of the lambs. The vet done general clinical examinations and investigated the clinical sings of diarrhea in all lambs daily.

2.2.The selenium and magnesium supplementation for lambs on quality of meat and consumers' health

In the study, we enrolled 51 lambs from the Research Institute of Karcag of the University of Debrecen. The lambs were divided into three study groups: Group 1 received no supplementation, Group 2 got magnesium (in a form of MgO), and Group 3 got selenium supplementation. We supplemented lambs with nano-selenium form (ESZENYI et al., 2011), because of its low toxicity (BENKO et al., 2012). The health status of the lambs was

followed-up regularly by a vet. After slaughtering the animals, we collected meat (sirloin) samples of ten animals from each group and analysed fatty acid ratios. We also measured the selenium and magnesium content of the ready-to-eat meat.

Thirty-nine healthy volunteers consumed the study food from lambs prepared at the university restaurant managed by Bükkvidéki Vendéglátó Ltd. Food dishes were made of a variety of cooking process according to a healthy nutrition. The individuals ate study food (150 g meat + garnish) for lunch three times a week (Monday, Wednesday, Friday) for 6 weeks.

The subjects were randomly divided into three corresponding groups and they were blind to study food type. We analysed the changes in general physical state, blood parameters and gastrointestinal health, as well as the attitude to study food of the healthy participants.

Animals and supplementation

Fifty-one newborn Hungarian Merino lambs were randomly allocated into 3 groups. Lambs in Group 1 (n=16; 9 males, 7 females) drank non-manipulated drinking water. Lambs in Group 2 (n=18; 9 males, 9 females) was watered with MgO enriched water only at a concentration of 0.6 g/L MgO. Lambs of Group 3 (n=17; 9 males, 8 females) got selenium nanoparticles, which were mixed into drinking water at a concentration of 20 µg/L. Importantly, a mono-diet system was applied. The experimental diets were formulated to meet the nutritional requirements for lambs. The lambs drank the study drink only ad libitum until they were slaughtered with an average live animal weight of 28.141 ± 2.983 kg (86 ± 3 days of age, mean ± SD). A lamb drinks approximately 2.2 L water daily; consequently, the study animals were supplemented with circa 1.32 g Mg in Group 2 and 44 µg Se in Group 3 daily.

All animals were considered healthy according to veterinary clinical examination. None of the animals had evidence of systemic disease or received antibiotics during the study.

Table 1: The lamb groups and the quantity of supplementations

Groups	**n = 51** **F / M**	**Supplementation in 2.2 L water** **(animal weight 30 kg)**
Group 1.	7/9	No supplementation
Group 2.	9/9	1.32g Mg
Group 3.	8/9	44 µg Se

Evaluation of carcass

The qualification of carcass was made on the basis of the statute „vidékfejlesztési miniszter 139/2011. (XII. 22.) VM rendelete vágójuhok vágás utáni minősítéséről és kereskedelmi osztályba sorolásáról szóló 16/1998. (IV.3.) FM rendelet módosításáról és a 16/1998. (IV.3.) FM rendelet".

Laboratory analyses of the meat

Meat samples were collected after the slaughtering from ten randomly selected lambs of each group. We investigated the fatty acid content of lamb meat (sirloin) with gas chromatography (5890 Series II GC, Hewlett-Packard Company, Palo Alto, CA, USA) according to ISO standard 5508 (1995).

For the measurement of Se and Mg, we used 23 samples of ready-to-eat meat from each food groups. Se levels were determined with Millenium Merlin Atomic Fluorescence Spectometer (P S Analytical, Kent, UK). Mg contents were measured with Thermo Scientific ICE 3000 Atomic Absorption Spectometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Healthy consumers

Thirty-nine healthy individuals (24 females and 15 males, mean age: 37.79 ± 8.96 years, range: 19-55) were enrolled in the study. They were selected on a basis of an interview carried out by a dietician in order to evaluate their dietary habits and ensure appropriate homogeneity of consumers groups. Exclusion criteria were the follows: a) any chronic diseases b) pregnancy or lactating c) recent history of alcohol abuse d) use of any dietary supplements e) aversion to lamb meat or their products f) followed any specific diet, such us vegetarian, vegan, macrobiotic. Subjects were instructed to maintain their normal dietary and lifestyle habits during the six-week-long study period.

Participants were randomly divided (by using a computer random number generator) into three groups according to the study diet. The subjects were blind to the type of study food.

The individuals in lamb meat group (Group L; n=13) received meat of untreated lambs (Group 1), the magnesium group (Group Mg; n=13) consumed the meat of lambs with magnesium supplementation (Group 2), while selenium group (Group Se; n=13) received meat of lambs treated with selenium (Group 3).

Informed written consent was obtained from the healthy volunteers enrolled in the investigation, and the study has been approved by the Ethics Committee of the University of Debrecen and the Policy Administration Services of Public Health of the Government Office

of Hajdú-Bihar County (protocol number: FL01_2010). All experiments carried out were in compliance with the Declaration of Helsinki.

Clinical and laboratory investigations

We evaluated the height (mean: 171.77 ± 9.09 cm) and body weight (mean: 73.77 ± 17.9 kg) of the patients to calculate body mass index (BMI, mean: 24.55 ± 3.58 kg/m^2). Fasting peripheral blood samples were collected after an overnight fasting at the beginning and the end of the study period. Peripheral blood white blood cell count, red blood cell count, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet cell count, mean platelet volume, platelet distribution width, mean peroxidase index, and ratios of lymphocytes, monocytes, neutrophils, eosinophils and basophils were evaluated using an Advia 2120 hematology analyzer (Siemens Medical Solutions Diagnostics Europe Limited, Dublin, Ireland). The Cobas 8000 modular analyzer series (Roche Diagnostics GmbH, Mannheim, Germany) was used to evaluate blood of participants for cholesterol, high- and low density lipoprotein (HDL & LDL), triglycerides, c-reactive protein, iron, glucose, glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase, and lactate dehydrogenase. The aforementioned analyses were conducted at the Department of Laboratory Medicine, University of Debrecen.

Questionnaires

Participants filled out questionnaires after every single study lunch. Questionnaires were made by a responsible researcher; for the quality of food 1-3 point scale was used, where 1 represented the worst and 3 the best value. We asked about toughness, taste, smell and appreciation of study food.

Gastrointestinal health questionnaires were answered before the beginning and after the end of study. We investigated the changes in constipation, abdominal pain/discomfort, bloating and diarrhea with a 1-3 point scale.

Finally, by analyzing the questionnaire on consumer habits, we evaluated the changes in the attitude of subjects toward lamb meat consumption.

Statistical analyses

Statistical analyses were performed using SPSS software (Chicago, IL, USA) version 20.0. To assess the distribution of the data Kolmogorov-Smirnov test was used. In cases of normal distribution, we determined mean ± standard deviation (SD) values and used two-sample t-test for statistical comparison of the experimental data. In cases of distributions different from that

of normal, median, minimum and maximum values were calculated, and Mann-Whitney test were used. According to the distribution, paired samples t-test or Wilcoxon test was used to evaluate the changes in distinctive human parameters. Differences were considered statistically significant at $p < 0.05$.

III.RESULTS

3.1.Breath hydrogen measurement

During the follow-up period, clinical signs of diarrhea developed in six lambs. Therefore we divided lambs into two groups before the statistical evaluation. Group A consisted of 46 lambs without any signs of diarrhea. The median level of baseline breath hydrogen of healthy lambs (Group A) was 1.00 ppm (minimum: 0.00 ppm, maximum: 2.00 ppm). We compared baseline values with the results measured 30 minutes [median: 1 (0.00-6.00) ppm], 60 minutes [median: 1(0.00-7.00) ppm] and 90 minutes after the start of feeding [median: 4 (0.00-7.00) ppm]. Based on our observations, the elevation in breath hydrogen levels became significant at 60 minutes after feeding ($p = 0.004$) (Figure 1).

Six lambs showing clinical signs of diarrhea formed Group B. In this group we compared baseline values [median: 7.5 (7.00-8.00) ppm] with the results measured 30 minutes [median: 7.5 (7.00-8.00) ppm], 60 minutes [median: 8 (7.00-9.00) ppm] and 90 minutes after the start of feeding [median: 9 (7.00-10.00) ppm]. Based on our observations, the elevation in breath hydrogen levels became significant at 90 minutes after feeding in Group B ($p = 0.046$) (Figure 1).

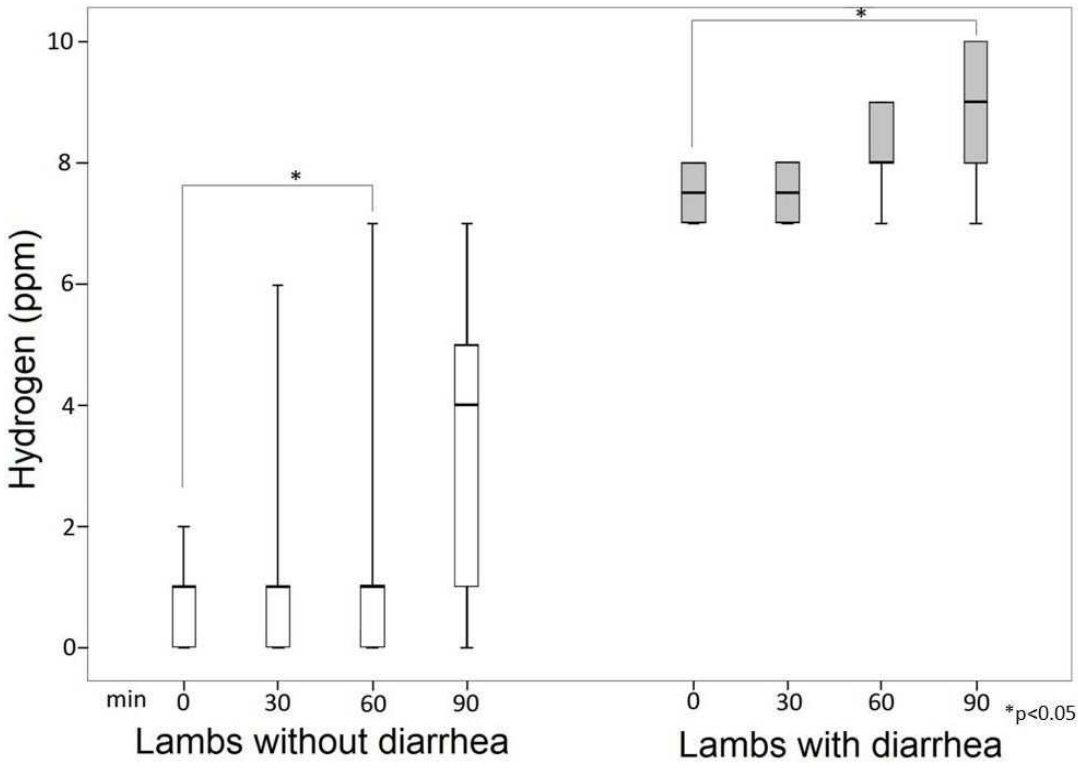

Figure 1: Effects of feeding on breath hydrogen levels in healthy lambs

Interestingly, when we compared the results measured in the two animal groups, we found that lambs in Group B had significantly higher baseline levels of breath hydrogen compared to the lambs without any signs of diarrhea ($p < 0.001$). That significant difference between Group A and B remained stable at each time point after feeding, as well ($p < 0.001$) (Figure 1).

3.2.Effects of supplementations on lamb health and the quality of carcass

The lambs were regularly followed-up by a vet in order to ensure that all of the animals were healthy during the study period.

Table 2 shows the muscle mass evaluation and Table 3 shows the tallow mass evaluation on the basis of S/EUROP system.

Table 2: Muscle mass evaluation on the basis of S/EUROP system

Groups	**Muscle mass evaluation on the basis of S/EUROP system**	
	U	**R**
Group 1 (Control)	31.25 %	68.75 %
Group 2 (Mg)	22.22 % [a]	77.77 % [b]
Group 3 (Se)	58.82 % [a]	41.17 % [b]

[a,b] $p<0.05$

According to statistical evaluation, there is a significant difference between group 2. and group 3 ($p=0.029$). There were no significant differences between group 1 and group 2 or between group 1 and group 3 ($p>0.05$).

Table 3: Tallow mass evaluation on the basis of S/EUROP system

Groups	**Tallow mass evaluation on the basis of S/EUROP system**		
	1	**2**	**3**
Group 1 (Control)	12.50 %	75.00 %	12.50 %
Group 2 (Mg)	0.00 % *	55.55 % *	44.44 % *
Group 3 (Se)	17.64 %	76.47 %	5.88 %

*$p<0.05$

There is a significant difference between group 2. and group 3. ($p=0.003$). In group 2, there is higher level of value 3, contrarily in group 3, there the highest level of evaluation is value 2.

Furthermore, the evaluation of tallow mass in group 2 is significantly higher than in group 1 ($p = 0.021$). There is no significant difference between the evaluation of tallow mass in group 1 and group 3 ($p > 0.05$).

3.3.Effects of supplementations on meat quality

After slaughtering lambs, we measured the fat content in the raw meat (sirloin) in each group. We found significantly higher fat content in the meat of Group 2, compared to the values measured in Group 1 (14.472 ± 3.428 g/100 g vs. 7.004 ± 1.973 g/100 g, respectively, $p < 0.001$). Similarly, in Group 3, we found significantly higher fat content than in Group 1 (10.625 ± 4.003 g/100 g vs. 7.004 ± 1.973 g/100 g, $p = 0.019$).

The level of saturated fatty acids (SFA) was significantly higher in the meat from Group 2 compared to the meat from Group 1 (6.696 ± 2.072 g/100 g vs. 3.371 ± 1.076 g/100 g, respectively, $p < 0.001$), but between the level of Group 1 and Group 3, there was only a non-significant difference (3.371 ± 1.076 g/100 g vs. 4.785 ± 1.902 g/100 g, respectively, $p = 0.056$).

Monounsaturated fatty acids (MUFA) were increased in both supplemented group compared to control group (Group 2: 5.764 ± 1.947 g/100 g vs. Group 1: 2.572 ± 0.814 g/100 g, $p < 0.001$; and Group 3: 4.457 ± 1.233 g/100 g vs. Group 1: 2.572 ± 0.817 g/100 g, $p = 0.001$).

PUFA were also significantly higher in Group 2, compared to the levels measured in Group 1 (Group 2: 2.011 ± 1.180 g/100 g vs. Group 1: 0.988 ± 0.522 g/100 g, respectively, $p = 0.022$); while Group 3 and Group 1 had similar values (1.468 ± 1.478 g/100 g vs. 0.988 ± 0.522 g/100 g, $p = 0.346$). Figure 2 shows the fat and fatty acids content in raw meat of each group.

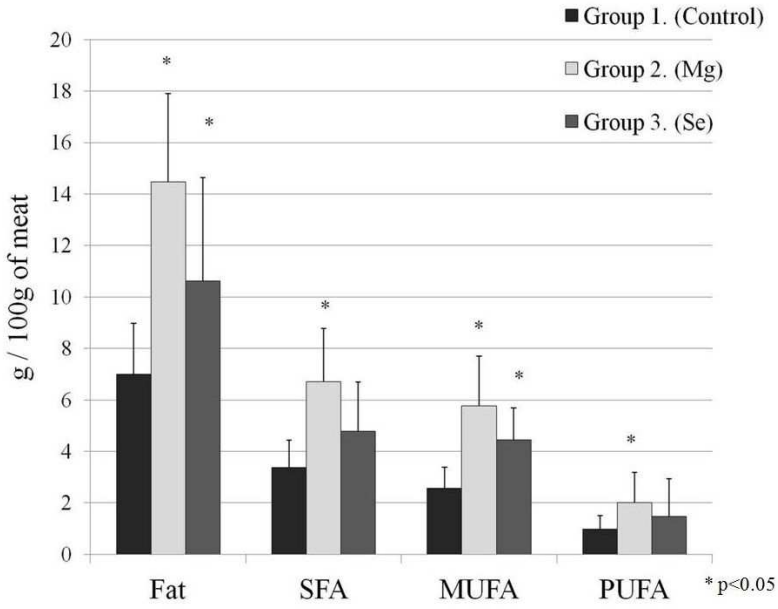

Figure 2: The total fat, SFA, MUFA and PUFA content of raw meat in each group

We also investigated the ratio between the level of total fat and saturated fatty acids, total fat and monounsaturated fatty acids, and total fat and polyunsaturated fatty acids in each group. The ratio of MUFA/total fat was significantly higher in Group 3, compared to Group 1 (43.27 ± 5.8 % vs. 36.5 ± 5.04 %, p = 0.014), on the contrary, Group 2 did not differ from Group 1 (39.6 ± 6.9 % vs. 36.5 ± 5.0 %, p = 0.269). The ratios of SFA/total fat and PUFA/total fat were similar in each group (SFA/total fat: Group 1: 48.6 ± 7.9 %, Group 2: 45.6 ± 6.1 %, Group 3: 45.00 ± 5.9 %; PUFA/total fat: Group 1: 14.8 ± 4.6 %, Group 2: 14.6 ± 7.9 %, Group 3: 12.1 ± 8.46 %)(Figure 3).

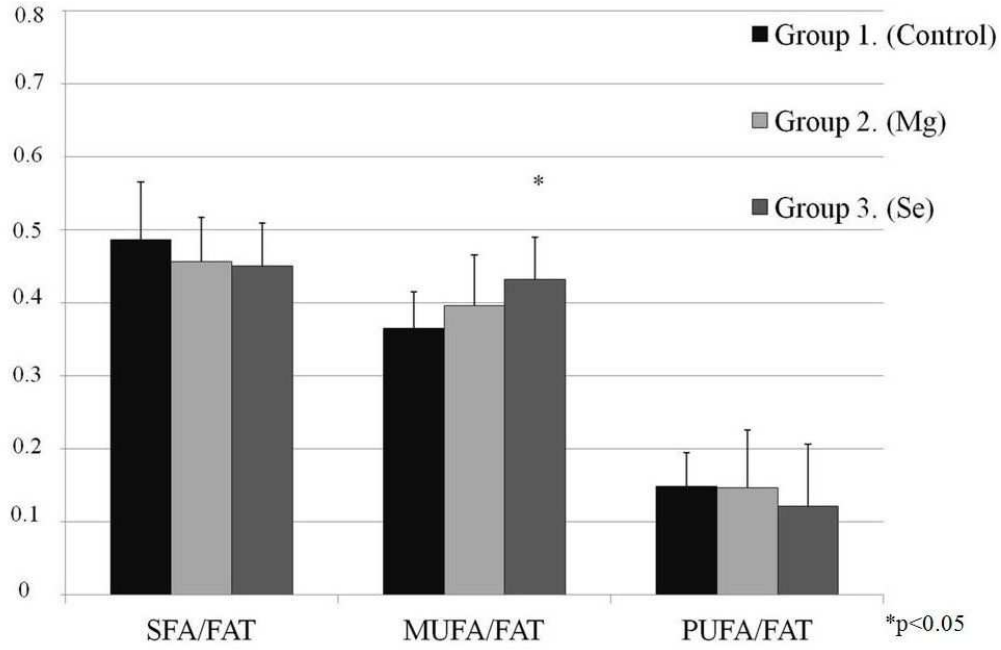

Figure 3: Ratio of SFA and total fat, MUFA and total fat and PUFA and total fat in each group

As the next step, we measured the levels of selenium, magnesium in the ready-to-eat meat of each animal group. The meat from control group's dishes (150g) contained 35.71 ± 13.18 mg Mg and 12.44 ± 5.95 µg Se. This Mg value is 9.52 % of the recommended daily intake in the EU [9] and 22.6 % of Se RDI according to the National Research Council [16]. In Mg supplemented group there was 37.74 ± 9.85 mg Mg in 150g meat (10.06 % RDI), in addition in Se supplemented group there was 19.96 ± 13.22 µg Se (36.3% RDI) in the meat.

The level of Se was significantly higher in the meat given to Group Se, 19.96 ± 13.22 µg/150 g compared with the one given to Group L, 12.44 ± 5.95 µg/150 g (p = 0.011). There were no significant differences between the groups in level of Mg (Table 4).

Table 4: Magnesium and selenium content of the study meat (150g) given to different groups of consumers

	Magnesium (mg, mean ± SD)	Selenium (µg, mean ± SD)
Group L	35.71 ± 13.18	12.44 ± 5.95
Group Mg	37.74 ± 9.85	13.32 ± 7.93
Group Se	35.24 ± 8.62	19.96 ± 13.21*

3.4.Clinical and laboratory measurements

At the end of the study, we assessed the body mass index of the individuals, and found no significant difference from the values determined at the beginning of our investigation (Group L: 22.38 ± 2.96 kg/m^2; Group Mg: 25.52 ± 3.28 kg/m^2, Group Se: 25.92 ± 3.48 kg/m^2). Additionally, there was no significant difference between the blood parameters measured before the beginning and after the end of the study.

3.5.Results of questionnaires

The subjective quality of study food

Based on the result of the food quality questionnaire, there was no significant difference between Group Mg and Group Se, regarding the smell, toughness, taste and overall impression of study food. However, consumers eating non-supplemented meat (Group L) gave significantly higher rates to all of the investigated parameter, compared to the consumers of Group Mg and Group Se.

Gastrointestinal symptoms

We found significantly lower incidence of constipation after the consumption period compared to the beginning values in each group (Group L before: 1.538 ± 0.776 points, after: 1.076 ± 0.640 points, respectively, p = 0.034; Group Mg before: 1.769 ± 0.599 point, after: 1.000 ± 0.816 point, respectively, p = 0.020; Group Se before: after: 1.538 ± 0.660 points, after: 1.000 ± 0.577 points, respectively, p = 0.020). The consumption of study food did not affect the other investigated parameters (abdominal pain/discomfort, bloating and diarrhoea).

Consumer attitudes toward study food

We also evaluated the questionnaire on consumer attitudes. Interestingly, 72.22 % of the volunteers did not eat lamb meat regularly before the beginning of the project. The assessment shed light on the reasons: 64.28 % of these subjects could not buy lamb meat in their environment, 28.57 % found this type of meat too expensive, and 7.14 % did not like the taste of lamb meat. Surprisingly, most of the individuals answering that they cannot buy lamb meat

live in a village (55.55 %), 27.77 % of them in a city and 16.66 % in a small town. We also construed the data according to the education of volunteers. 53.84 % of the participants who never ate lamb meat were skilled workers and 46.15 % of them were graduated. In contrast to consumers who had already eaten lamb meat regularly before our study, 60 % of this subject had a university degree and 40 % were skilled workers.

We assessed the changes of attitude to lamb meat in each group. In Group Se, 71.43 % of the consumers declared an improvement in their attitude to lamb during the study period, and 28.57 % of them reported no change. In Group Mg, 42.86 % of the consumers found a positive change in their attitude, while 57.14 % of them felt no change. In the control Group L, 57.14 % of the individuals indicated a positive change, and 42.86 % of them indicated no change. None of the enrolled individuals reported worsening attitude.

Regarding the long-term plans of consumers, 42.85 % of individuals belonging to Group Mg plan to eat this food weekly, and 57.14 % of them plan this monthly. In Group Se, 28.57 % of the volunteers plan to consume lamb meat weekly and 71.48 % monthly. In Group L, we observed ratios similar to the ones in Group Se. Importantly, there was nobody who wanted to eat lamb meat more rarely than monthly.

Finally, we evaluated the percentages of individuals, who would like to prepare a meal from lamb meat at home. 95.23 % of participants want to make the study dishes at home, and only 4.76 % had a negative answer.

IV.NEW STATEMENTS OF THE THESIS

1) Based on our observations, the median level of baseline breath hydrogen of healthy lambs was 1.00 ppm (minimum: 0.00 ppm, maximum: 2.00 ppm). The elevation in breath hydrogen levels became significant at 60 minutes after feeding maternal milk [median: 1(0.00-7.00) ppm, $p = 0.004$)].

2) During the two weeks long follow-up six lambs showing clinical signs of diarrhea. In this group we established the median level of baseline breath hydrogen, 7.50 ppm (minimum: 7.00 ppm, maximum: 8.00 ppm). The elevation in breath hydrogen levels became significant at 90 minutes after feeding maternal milk in this group [median: 9 (7.00-10.00) ppm, $p = 0.046$)]. That significant difference between the two groups remained stable at each time point (0’ 30’ 60’ 90’) after feeding, as well ($P < 0.001$).

3) Selenium supplementation in water at a concentration of 20 µg/L Se, can lead to increased Se concentration in lamb meat with 60.45 % during 86 ± 3 days.

4) Selenium supplementation in water at a concentration of 20 µg/L Se, can lead to increased MUFA ratios in lamb meat during 86 ± 3 days.

5) Based on our observations, lamb meat containing 19.96 ± 13.21 µg Se did not lead to any adverse reaction in the consumers, and all of the investigated laboratory parameters remained in normal range during the six week long study period.

V.PRACTICAL APPLICABILITY OF THE RESULTS

1) We measured the level of breath hydrogen of lambs before and after feeding maternal milk. According to our results, the high breath hydrogen level may indicate an intestinal bacterial overgrowth, so the breath hydrogen monitor could be a useful method in veterinary medicine as well. Furthermore our results are useful as a reference value to other research.

2) In my thesis I draw attention to importance of selenium intake. In Hungary, we do not have a database about the selenium content of different food. In this study we measured the selenium content of lamb meat (8.30 ± 3.97 µg/100g), which data could be a start of a new investigation.

3) Selenium supplementation in water at a concentration of 20 µg/L Se, can lead to increased Se concentration and changed fatty acid ratio in lamb meat during 86 ± 3 days.

4) Supplementation with magnesium in water at a concentration of 0.6 g/L MgO, cannot lead to increased Mg concentration in lamb meat during 86 ± 3 days, but can increase the tallow ratio of carcass.

5) In general, Hungarian people eat a very low quantity from lamb or sheep meat. Our results supported, that Hungarian people do not know lamb meat, and they could have a neutral or even a negative feeling toward this type of meat; but upon tasting it, the positive experience may have a deep impact on the former attitude. The attitude of consumers toward lamb meat improved significantly during the study period, which highlights that it will be important to disseminate more information on healthy foods in the future.

6) The assessment shed light on the reasons of low lamb meat consumption: 64.28 % of the subjects who do not eat lamb meat could not buy lamb meat in their environment, 28.57 % found this type of meat too expensive, and 7.14 % did not like the taste of lamb meat.

VI. REFERENCES

1) BELLVER, S.P., BEERMANN, D.H., BELL, A.W., VAN TASSELL, C.P., HOGUE, D.E., MCLAUGHLIN, C.L. (1995): Effects of exogenous somatotropin on whole-body glycemic response to insulin in young preruminant and ruminant lambs. Domest Anim Endocrinol. 12, 143-156.
2) BENKO I., NAGY G., TANCZOS B., UNGVARI E., SZTRIK A., ESZENYI P., PROKISCH J. BANFALVI G. (2012): Subacute toxicity of nano-selenium compared to other selenium species in mice. Environ Toxicol Chem. 31, 2812-2820.
3) D'ANGELO, G., DI RIENZO, T.A., SCALDAFERRI, F., DEL ZOMPO, F., PIZZOFERRATO, M., LOPETUSO, L.R., LATERZA, L., BRUNO, G., PETITO, V., CAMPANALE, M.C., CESARIO, V., FRANCESCHI, F., CAMMAROTA, G., GAETANI, E., GASBARRINI, A., OJETTI, V. (2013): Tricks for interpreting and making a good report on hydrogen and 13C breath tests. Eur Rev Med Pharmacol Sci. 17, Suppl 2, 90-8.
4) ESZENYI, P., SZTRIK, A., BABKA, B., PROKISCH, J. (2011): Elemental, nano-Sized (100-500nm) selenium production by probiotic lactic acid bacteria. Int J Biosci Biochem Bioinforma. 2, 148-152.
5) GASBARRINI, A., CORAZZA, G.R., GASBARRINI, G., MONTALTO, M., DI STEFANO, M., BASILISCO, G., PARODIM, A., USAI-SATTA, P., VERNIA, P., ANANIA, C., ASTEGIANO, M., BARBARA, G., BENINI, L., BONAZZI, P., CAPURSO, G., CERTO, M., COLECCHIA, A., CUOCO, L., DI SARIO, A., FESTI, D., LAURITANO, C., MICELI, E., NARDONE, G., PERRI, F., PORTINCASA, P., RISICATO, R., SORGE, M., TURSI, A. (2009): Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. Aliment Pharmacol Ther. 29, S1:1-49.
6) HANSEN, B.C. (1999): The metabolic syndrome X. Ann N Y Acad Sci. 892, 1-24.
7) KOTZEV, I., MIRCHEV, M., MANEVSKA, B., IVANOVA, I., KANEVA, M. (2008): Risk and protective factors for development of colorectal polyps and cancer (Bulgarian experience). Hepatogastroenterology. 55, 381-387.
8) MASTROPAOLO, G., REES, W.D. (1987): Evaluation of the hydrogen breath test in man: definition and elimination of the early hydrogen peak. Gut. 28, 721-725.

9) SALES, C.H., PEDROSA, L.F., LIMA, J.G., LEMOS, T.M., COLLI, C. (2011): Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. Clin Nutr. 30, 359-364.
10) SANTAELLA, M., MARTÍNEZ, I., ROS, G., PERIAGO, M. J. (1997): Assessment of the role of meat cut on the Fe, Zn, Cu, Ca andMg content and their in vitro availability in homogenised weaning foods. Meat Sci. 45, 473–483.
11) SATIA, J.A. (2009): Diet-related disparities: understanding the problem and accelerating solutions. J Am Diet Assoc. 109, 610–615.
12) VIRÁG V., MAY Z., KOCSIS I., BLAZOVICS A., SZENTMIHALYI K. (2011): Magnéziumpótlás hatása a kalcium- es magnéziumszintekre, valamint a redox-homeosztázisra normolipidaemiás és alimentárisan előidezett hyperlipidaemiás patkányokban. Orv. Hetil. 152, 1075–1081.
13) WASHABAU, R.J., STROMBECK, D.R., BUFFINGTON, C.A., HARROLD, D. (1986): Use of pulmonary hydrogen gas excretion to detect carbohydrate malabsorption in dogs. J Am Vet Med Assoc. 189, 674-679.

VII.LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY

Registry number: DEENK/210/2015.PL
Subject: Ph.D. List of Publications

Candidate: Anikó Nagy
Neptun ID: YQSE5S
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10040537

List of publications related to the dissertation

Hungarian book chapter(s) (1)

1. Veressné Mile M., **Nagy A.**, Csiki Z.: Szelénnel dúsított joghurt egészségre, életminőségre kifejtett hatásai- fogyasztói pilot vizsgálat.
   In: Fiatal kutatók az egészséges élelmiszerért : tudományos ülés. Szerk.: Bódi Éva, Fekete István, Kovács Béla, Debreceni Egyetem Hankóczy Jenő Növénytermesztési, Kertészeti és Élelmiszertudományok Doktori Iskola, Debrecen, 281-286, 2013. ISBN: 9789634736011

Hungarian scientific article(s) in Hungarian journal(s) (3)

2. **Nagy A.**, Jávor A., Takácsné Hájos M., Borbélyné Varga M., Soltész P., Csiki Z.: Céklalé készítése során fellépő beltartalmi változások, alkalmazhatósága állati eredetű funkcionális élelmiszer fejlesztésére.
   Agrártud. Közl. 65, 53-57, 2015. ISSN: 1587-1282.

3. **Nagy A.**, Csiki Z., Horváth Z., Monori I., Jávor A.: Minőségi bárányhús a humán táplálkozásban, termékjellemzők, elfogadottság.
   M. Gasztroent. 2014 (2 különszám), 14-19, 2014. ISSN: 1788-1145.

4. **Nagy A.**, Csiki Z.: Élelmiszer eredetű polifenolok kardiovaszkuláris hatásai.
   M. Gasztroent. 2014 (2 különszám), 40-44, 2014. ISSN: 1788-1145.

Foreign language scientific article(s) in international journal(s) (1)

5. Jávor, A., **Nagy, A.**, Papp-Bata, Á., Vass, N., Oláh, J., Csiki, Z.: Bacterial overgrowth can be detected by breath hydrogen measurement before clinical manifestations in suckling lambs. *Acta Vet. Brno. 84* (3), 243-247, 2015. ISSN: 0001-7213.
DOI: http://dx.doi.org/10.2754/avb201584030243
IF:0.469 (2014)

Hungarian abstract(s) (1)

6. **Nagy A.**, Jávor A., Csiki Z.: Minőségi bárányhús mint funkcionális élelmiszer: Fejlesztési lehetőségek.
Orv. Hetil. 155 (45), 1808, 2014. ISSN: 0030-6002.

List of other publications

Hungarian scientific article(s) in Hungarian journal(s) (7)

7. Bézi I., **Nagy A.**, Csiki Z., Jávor A., Soltész P.: A polyphenolok szív- és érrendszerre kifejtett hatásai.
Érbetegségek. 21 (4), 137-143, 2014. ISSN: 1218-3636.

8. **Nagy A.**, Lugasi A., Novák J., András C., Csiki Z.: Gyulladásos ízületi megbetegedések és a táplálkozás.
Immunol. Szle. 5 (1), 13-18, 2013. ISSN: 2061-0203.

9. **Nagy A.**, Takáts A., Papp G., Csiki E., Novák J., Kraszits I., Csiki Z.: Probiotikumok alkalmazása a háziorvosi gyakorlatban.
Gyermekorv. továbbk. 12 (5), 228-231, 2013. ISSN: 1589-0309.

10. **Nagy A.**, Takáts A., Papp G., Csiki E., Novák J., Kraszits I., Csiki Z.: Probiotikumok alkalmazása a háziorvosi gyakorlatban.
Háziorv. Továbbk. Szle. 18, 180-183, 2013. ISSN: 1219-8641.

11. Csiki Z., Husi K., **Nagy A.**, Altorjay I.: A hasnyálmirigy akut gyulladása.
Metabolizmus. 11 (1), 55-60, 2013. ISSN: 1589-7311.

12. Szendi S., Körmendi B., **Nagy A.**, Csiki Z., Tömösközi S.: Gabonafélék komponenseivel végzett vizsgálatok és kísérlet.
M. Gasztroent. 2012 (1 különszám), 3-7, 2012. ISSN: 1788-1145.

13. **Nagy A.**, Csiki Z.: Arabinoxylánban gazdag lisztkeverékből készült száraztészta vizsgálata.
M. Gasztroent. 2012 (1 különszám), 17-21, 2012. ISSN: 1788-1145.

Foreign language scientific article(s) in international journal(s) (1)

14. Csiki, Z., Papp-Bata, Á., Czompa, A., **Nagy, A.**, Bak, I., Lekli, I., Jávor, A., Haines, D.D., Balla, G., Tósaki, Á.: Orally delivered sour cherry seed extract (SCSE) affects cardiovascular and hematological parameters in humans.
Phytother. Res. 29 (3), 444-449, 2015. ISSN: 0951-418X.
DOI: http://dx.doi.org/10.1002/ptr.5273
IF:2.66 (2014)

Hungarian conference proceeding(s) (2)

15. **Nagy A.**, Fekete I., Csiki Z.: A céklalé rendszeres fogyasztásának terápiás jelentősége vazospasztikus megbetegedésekben:Fogyasztói pilot vizsgálat.
In: Fiatal kutatók az egészséges élelmiszerért : tudományos ülés. Szerk.: Bódi Éva, Fekete István, Kovács Béla, Debreceni Egyetem Hankóczy Jenő Növénytermesztési, Kertészeti és Élelmiszertudományok Doktori Iskola, Debrecen, 259-263, 2013.

16. Halasi S., **Nagy A.**, Csiki Z.: Porított meggymagbél-tartalmú minőségi élelmiszer értékelése - fogyasztói pilot vizsgálat.
In: Fiatal kutatók az egészséges élelmiszerért : tudományos ülés. Szerk.: Bódi Éva, Fekete István, Kovács Béla, Debreceni Egyetem Hankóczy Jenő Növénytermesztési, Kertészeti és Élelmiszertudományok Doktori Iskola, Debrecen, 183-189, 2013. ISBN: 9789634736011

Foreign language abstract(s) (1)

17. Czompa, A., **Nagy, A.**, Bak, I., Hendrik, Z., Lekli, I., Csiki, Z., Tósaki, Á.: Consumer investigation and toxicological analysis of sour cherry seed kernel extract.
Acta Physiol. 211 (Suppl 697), 84, 2014. ISSN: 1748-1708.
DOI: http://dx.doi.org/10.1111/apha.12362

Total IF of journals (all publications): 3,129
Total IF of journals (publications related to the dissertation): 0,469

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

01 October, 2015