THESIS OF DOCTORAL (PhD) DISSERTATION

KOVÁCS PÉTER

MOSONMAGYARÓVÁR

2011

THESIS OF DOCTORAL (PhD) DISSERTATION

UNIVERSITY OF WEST-HUNGARY
FACULTY OF AGRICULTURAL AND FOOD SCIENCES
MOSONMAGYARÓVÁR
INSTITUTE OF ANIMAL SCIENCES

UJHELYI IMRE ANIMAL SCIENCES SCHOOL OF DOCTOR'S

Chairman of the School of Doctor's:
DR. BENEDEK PÁL
professor

Supervisor:
DR. SCHMIDT JÁNOS
professor emeritus, academician

THE USE OF GLYCEROL GENERATED DURING THE BIODIESEL PRODUCTION FOR FEEDING PIG

PÉTER KOVÁCS

MOSONMAGYARÓVÁR

2011

1. INTRODUCTION

The available supply of non renewable (fossil) energy is finite on our Earth, while the demand for energy continues to grow as a result of the world's economic development (Ekéné, 2004; Kállai, 2007)

This has two important trains that are inevitable:

- the price will be growing continuously
- solution shall be find urgently to the exploitation of alternative renewable energy sources (Vajda, 2001)

Meanwhile the usage of fossil energies burdens our environment in a great extent (air pollution → greenhouse effect → global warming), on the course of the utilisation of renewable energy sources, which can be found in the nature (sun, water, wind, bioenergy), do not arise or only in a moderate quantity metabolism products increasing the proportion of one of the constant components in the circulation of the nature, burdening the environment. (Alföldi, 2008; Németh and Sevella, 2007; Szulmanné, 2007).

The biodiesel, which it is possible to manufacture from different materials, belongs to the renewable energy sources. In Hungary, the rapeseed (Brassica napus var. arvensis) and the sunflower (Helianthus annuus) are those two oil plants from which biodiesel can be manufactured in the largest quantity, economically (Sinóros at al., 2007), but it can be manufactured from Soya bean (Glycine soya), flax seed (Linum usitatissimum), coconut oil, palm fat and animal fat, too (Fledderus, 2000).

In chemical reactions taking place in the course of the production of the biodiesel 100 litres of biodizel and 10 litres of glycerine are formed from 100 litres of oil and 10 litres of methanol. (Friedrich, 2004; Barcsik, 2008). According to other authors 7.9 litres of crude glycerol generated for every 100 litres of biodiesel produced (Thompson and He, 2006).

An exceptionally huge amount of glycerine, which its use causes a problem worldwide, is produced in his totality in USA, in Europe and on the world (NBB Statistics, 2010; EBB Statistics, 2010). According to some estimates onto 2010 the production of 1 million tons of glycerine expected (Rick, 2006). Therefore, in recent years an increasing number of experiments were carried out to determine how to integrate the glycerine in monogastric animals (pigs, poultry) feeding. Namely, one of the possibilities for the utilisation of the glycerine being produced in a growing quantity is its utilisitation as animal feedstuff. (Bartelt, 2002; Südekum, 2002; Józsa, 2006; Barta, 2009).

The continuously growing supplies of glycerines will reduce, presumably, of its price, what adds an opportunity to the economical use of the glycerine as animal feed.

2. OWN EXPERIMENTS

2.1. The aim of the experiments

The use of the huge amount of glycerine arising as the by-product of biodiesel production turned into an important task worldwide. Lately, researches have been focused on experiments, in which ones examine the glycerine as a feed component in the livestock feeding. The glycerol has been used for decades with good results for dairy cows to improve the energy supply of the animals. However, it is still not widespread in practice in the case of monogastric animals (broiler chicken, laying hen, pig).

Several studies were carried out both in the USA and Europe to investigate how the glycerine could be inserted as an energy source into the feeding system of monogastric animals. Related studies have not yet been reported in Hungary. Therefore author examined under domestic relations that glycerol under what conditions can be used in the feed ration of pigs. In the interest of consumers in mind, it was also considered that how influences the pigs' feed supplementation with glycerol the chemical composition of pork, the sensory properties and the cooking characteristics.

The experiments were carried out to answer the following questions:

- What is the chemical composition of the feed grade glycerol, produced from crude glycerol, arising during biodiesel production?
- What quantities of glycerol can be fed to pigs without any negative impact?

- How much is the apparent digestible (DE) and metabolizable energy (ME) content of the glycerol when fed to pigs?
- How the increasing doses of glycerine in the diet of fattening pigs effect the digestibility of the nutrients and the N-utilisation of the animals?
- How the glycerine influences the fattening performance of the animals?
- What is the impact on the slaughter properties of glycerol (e.g. percentage of lean meat, lean meat: fat ratio)?
- What is the effect of dietary glycerol
  - on the crude protein, crude fat, crude ash content and the fatty acid composition of the meat?
  - on the rate of dripping, melting and cooking losses?
  - on the sensory properties of the meat?

2.2. Material and methods

2.2.1. Animal trials

Digestion and N-metabolism experiments

Digestibility and N-metabolism experiments were used for the determination of the apparent digestible (DEs) and metabolizable energy (MEs) content of feed grade glycerol deriving from biodiesel production as well as its effect on the digestibility of nutrients and on the N-utilization in pigs.

The studies were conducted with two groups of pigs of different weight (25-47 kg and 57-85 kg). Two experiments were carried out with the smaller and one with the bigger weight category. In each experiment 12 barrows, altogether 36 animals were included into the experiments. Feed rations were supplemented with glycerol.

The glycerol used in experiments contained 86.76 % glycerol, 0.05% crude fat and 5.4 % crude ash, the majority of the latter was NaCl (5.2 %).

Animals were placed into individual metabolism cages, thereby allowing the individual measurement of the feed intake as well as the faeces and urine excretion. Each experiment consisted of 3 periods. In each period the following treatments were fed: 0, 5 or 10 % glycerol supplementation with basal diet. In order to avoid the effect of weight gain of pigs on the results, the three diets were fed according to the rules of the Greco-Latin square arrangement.

The pigs were adapted to the metabolism cages in the first 10 days period. The experimental period was 5 days long, during which the excreted urine and faeces were collected and measured individually. Before that was a pre-feeding period, which also was 5-days long.

The collected faecal samples were dried and the chemical composition and energy content of each sample was determined. In the case of the urine samples we defined beside the energy content the N content as well.

Farm experiments

The pig fattening experiment on farm scale served more purposes. Primarily, the results of the farm experiment (weight gain, feed conversion ratio) may confirm, whether the digestible and metabolizable energy values determined for glycerol in the digestibility and N-utilization experiments, are realistic. Secondly in the framework of the farm experiment it was also assessed what kind of effect the glycerol feeding has on the quality of the meat samples and some important cooking characteristics of the meat.

The farm experiments were conducted with 100 pigs (Norwegian landras x Duroc) at the pig farm of the Bezenyei Sertés Kft. Pigs were divided into two groups (control and experimental), each group consisting of 50-50 animals. The gilt:barrow proportion was identical inside the groups and the two groups was nearly the same initial average body weight (30,0 ± 3,8 and 30,2 ± 2,5 kg).

The 2 groups were placed in 4-4 boxes in a concrete grid floor stable. For feeding dry-food self feeders, for watering tipped (Nipple) self watering devices were used.

Animals received until 70-75 kg of live weight a growing diet, and then till to the end of the experiment a finishing diet. The experimental group's diets contained 5 % glycerine, with which a part of the corn proportion was replaced. We decided for the replacement of the corn because this crop is available in the largest quantity in our homeland, and its digestible energy content (14.18 MJ/kg; Sauvant et al., 2002) falls very near to the DE value reported in this study of the glycerine used in the experiments. The small decrease in crude protein deriving from the smaller proportion of corn in the diet was compensated by a corn gluten supplement. The animals were individually weighed at the beginning of the experiment, then when changing the feed mixture (on the 57th day of the experiment, 70-75 kg live weight) and at the end of the trial (105-110 kg live weight). After the experiment all animals were slaughtered in Kapuvár Meat Co. slaughterhouse and rated according to SEUROP rules.

2.2.2. Methods of the chemical analysis

Analysis of the chemical composition

The dry matter, raw protein, raw fat, raw fibre and raw ash content of the feeds, the faeces samples and the pork, further the N-content of urine samples were determined with the methods suggested in the Hungarian Feed Codex (2004). The raw fat content of the glycerine was determined, after the dissolution of the glycerine with water, according to the Soxlet procedure (with Soxtec equipment). The energy content of the diets, the glycerine, the excreted faeces and the urine was measured with a bomb calorimeter (type IKA C2000).

The glycerol content of the glycerols fed in the experiments was determined according to the ISO EN 14106 (2003) standard. The methanol content of the glycerol, as a by product of methyl ester production from rapeseed oil, was analysed as described in the ISO EN 14110 standard.

Determination of the fatty acid composition

The examinations covered also the question, whether the feed supplemented with glycerol alters the fatty acid composition of pork. For this purpose tissue samples were taken from 10-10 animals by groups in Darnóhús meat processing plant, following the slaughtering, from the following parts: pork chop, thigh, back fat, lard. The fatty acid composition of fat in the samples was analysed by gas chromatography (chromatograph: Agilent Technologies 6890N Network).

<u>2.2.3. Measurement of the parameters of meat quality</u>

For the consumers, such as restaurants, canteens and households, it is an important question, what the cooking and sensory properties of pork derived from animals fed glycerol. The parameters listed, were determined in part (dripping loss, baking loss, shearing strength, colour and sensory properties) by the experts of the National Meat Industry Research Institute, using by groups 5-5 pork chop samples, which we cut from the 11. rib section. Another part of the laboratory tests (freezing, defrosting and cooking loss) was performed by the laboratory of Department of Animal Nutrition.

Dripping loss

The accurately weighed pork chop slices were stored in polyethylene bags at 4 ^{0}C, the same way as in standard packaging with protective gas, so that the meat does not come into contact with the entire surface of the foil. After 6 days the meat slices were taken out from the bags and having them soaked up, they were weighed. Dripping loss is the difference between the initial and final weight of the samples, which is expressed as a percentage of raw weight. The lower this value the better the water-holding capacity of the meat.

Freezing or melting loss

Experiments were performed with meats stored at two different temperatures: at temperatures between -12 and -20 ^{0}C degrees and at -40 ^{0}C degrees. The two freezing methods were tested separately because of the freezing process takes place otherwise at the different temperatures. The freezing between -12 and -20 ^{0}C degrees modelled the freezing environment in the deep-freezers of households and catering establishments, which makes up a large volume of frozen meat. At this freezing method the meat reaches just slowly the desired core temperature and the long process of freezing results in the intra and extracellular liquid (water) the formation of large ice crystals, which damage the cell membrane and consequently the melting loss will be greater when thawing.

In the case of industrial "shocking" freezing (- 40 C), during which the product will rapidly rich the desired core temperature (later on it can be stored at higher temperature of -20 C with less energy cost) small ice crystals are formed which are less damaging to the cell membrane and therefore resulting at the time of regular defrosting less freezing loss and economic damage. Both method of freezing was tested by 5-5 pork chop samples.

Both freezing method was studied in terms of melting losses of frozen meat. All frozen samples were put into a refrigerator of 4 ^{0}C degrees for 24 hours. The weight of the frozen and thawed samples was measured. The percentage difference between the two values indicates the loss of defrosting (melting).

Cooking loss

The thawed samples were used for measuring the cooking loss. 100 g meat slices in sealed plastic bags were cooked in water bath a further 2 hours having reached the 75 ^{0}C degrees core temperature. Weight of samples was measured before and after cooking. The percentage difference between the two values gives the cooking loss.

Baking loss

Pork chop samples were cut into slices, their mass was measured. Then the slices were baked in a contact grill oven one by one until the reaching of the core temperature of 72 ^{0}C degrees. The temperature was controlled by a core thermometer. We left the fried samples cool down to room temperature (20 ^{0}C degrees), then soaked them up with filter paper and weighed again. The difference between the initial and the post frying weight is the baking loss, which is expressed as a percentage of the initial weight. The greater baking losses indicate increased intramuscular fat content.

Colour

The colour measurements were performed with portable chromameter (Typ: Minolta Chromameter CR-300) on the fresh cutting surface of the raw pork chops in six parallel repetitions. The colour measurement happened in a CieLab system, with the application of diffuse illumination (D65 light-source, pulsing xenon arclamp), 0 degrees viewing angle and a gauge head with an 8 mm diameter measuring hole. (The device measures both the incident and the reflected light.)

The instrument measures on a three-dimensional scale the chromatic properties of the reflected surface. The x-axis measures the red (+a*) and green (-a*) colours, the y-axis the yellow (+b*) and green (-b*) ones and the z axis measures the degree of the lightness. Measurements were performed in daylight. By the help of the measured chromatic pattern the hue (*a*/b**) and intensity (colour strength and saturation) can be characterized. Chroma = $\sqrt{a*^2 + b*^2}$.

Shearing strength

The tenderness of meat, i.e. the shearing strength value, was measured with TA-XT2i strength metering device (Stable Micro

Systems) and Warner-Bratzler blade. The 72 ^{0}C degrees core temperature baked slices were soaked up with filter paper and cooled to room temperature. Then we cut cylinders of 11 mm diameter, perpendicular to the fibres of the meat. From all meat parts 5 parallel measurements were performed. The higher values are measured the harder, the smaller values are measured the softer, tenderer is the meat. The instrumental tenderness measurement gives numerical data on the substance of the meat. The results are always evaluated in comparison with the results of organoleptic assessment.

Organoleptic evaluation

A trained committee of 7 members has completed organoleptic rating on the 72 ^{0}C degrees core temperature roasted pork slices. Each sample series were evaluated for flavour, taste, texture (hardness-softness) and overall impression (overall acceptability). Descriptive criticism and 0-10 point scale scoring was performed.

2.2.5. Statistical analysis

Statistical analysis was carried out by using GenStat.11R software (GenStat Procedure Library Release PL19.1, VSN International Ltd.)

- For the definition of the energy value of the glycerine we created a descriptive statistics. We defined the glycerine's DEs and

MEs content based it on the steepness of the regression relationship between the feed consumption and the digestible energy intake as well as the concerned feed consumption and the metabolizable energy (ME) intake.

- A single-factor variance-analysis was used to show the impact of the glycerine on the digestibility coefficients, where the feed treatment (control or experimental) was the basis of the examination.
- In farm scale trials single-factor variance-analysis was used concerning body weight and carcass traits, whereby the effect of treatments were analyzed.
- The chemical composition of different tissues, as well as the fatty acid composition was evaluated by using two-factor variance-analysis, in which the treatment, the location of sampling and the role of the interaction between the two was investigated.

When examining the meat quality parameters the following statistical tests were performed:

For the dripping, freezing, cooking and baking loss, colour, shearing strength, a single-factor, for the organoleptic test a two-factor variance-analysis was used. The Scheffe test was applied using the Statgraphics program (ANOVA) for pairwise comparisons. In the case of the two factor variance-analysis the treatment x reviewer interaction was considered as random factor, concerned for a comparison basis.

3. NEW SCIENTIFIC RESULTS

Based on the experimental data the following new scientific results can be formulated:

1. 14.01 MJ/kg was the apparent digestible energy content and 13.48 MJ/kg was the apparent metabolizable energy content of the 86.76 % feed grade glycerol tested, what amounts to 91.5 % and 88.0 %, respectively of the tested glycerine's gross energy content. This is counted to 100 % glycerine equal to a content of 16.43 MJ/kg apparent DE and 15.54 MJ/kg apparent ME, respectively.
2. The weight of the animals between 25-85 kg and the application rate of the glycerine between 5-10 % did not effect the utilization of glycerine.
3. Dose of 5 or 10% glycerol supplement in the diet does not affect the digestibility of feed nutrients either N-retention of pigs.
4. Feeding of 5% glycerine does not affect the results of fattening (weight gain, feed-, energy- and protein utilization), ie. glycerol can replace the corn in pigs' feed, based on its apparent digestible energy value.
5. 5% glycerol has only a small effect on the "de novo" fatty acid synthesis, resulting in a small change in the fatty acid composition of loins, thighs, back-fat and lard.
6. The glycerol feeding reduces the dripping loss of the meat, stored at a temperature between -12 and -20 ^{0}C degrees, after thawing.

4. LIST OF PUBLICATIONS IN THE THEME OF THE DISSERTATION

Scientific paper published in Hungarien language

1. **Kovács P.** *- Zsédely E. - Schmidt J. (2010):* Glicerin felhasználása a monogasztrikus állatok takarmányozásában. 1. Glicerin a hízósertések takarmányozásában. Állattenyésztés és Takarmányozás 59. 5-6. 441-455.

2. **Kovács P.** *- Zsédely E. - Kovács Á. - Tóth T. - Schmidt J. (2010):* A biodízel előállítás során keletkező glicerin etetésének hatása a sertéshús minőségére. A Hús, 1-2. 46-51.

Scientific paper published in foreign language

1. **Kovács P.** *- Zsédely E. - Kovács A. - Virág Gy. - Schmidt J. (2011):* Apparent digestible and metabolizable energy content of glycerol in feed of growing pigs. Livestock Science (javítás után benyújtva)

Oral peresentations in Hungarian

1. **Kovács P.** *- Zsédely E. - Schmidt J.* (2010): Glicerin felhasználása a monogasztrikus állatok takarmányozásában. LII. Georgikon Napok, Keszthely, 2010. szeptember 31-október 1.

2. **Kovács P.** *- Zsédely E. - Schmidt J.* (2010): Glicerin a hízósertések takarmányozásában. XXXIII. Óvári Tudományos Nap, Mosonmagyaróvár, 2010. október 7.