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INVESTIGATION OF THE USE OF GLYCEROL SUPPLEMENTATION IN THE NUTRITION OF LACTATING SOWS

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1. THE BACKGROUND AND THE OBJECTIVES OF THE RESEARCH

In terms of animal product manufacturing it is an important objective to meet the energy- and nutrient requirements of the swine hybrids with high genetic potential optimally, in addition, the high level and safe use of industrial by-products has become of great importance nowadays.

The nutrition of lactating sows is affected from both aspect, because moderating the negative energy balance during lactation has an important role considering the reproduction and lifetime performance of the breeding animals. Because of its role in the intermediary metabolism, glycerol (by-product from the biodiesel production, min. 85% glycerol content) could be an alternative for ensuring the appropriate energy supply.

Concerning the use of glycerol for animal nutrition, it is important to note that its quality may vary by production batch, and also largely depends on production technology. Methanol and mineral salt (NaCl, KCl) residues from the technological processes could be a potential source of risk to the health of the herd. All of these, as well as other potential risk sources concerning the use of by-products providing valuable feeding solutions, always have to be estimated and avoided, especially in case of breeding animals with high genetical value.

Based on the toxicity of methanol, monitoring the methanol levels of glycerol products is necessary, and it has to be implemented by the use of such analytical methods that provide fast and precise results. For this, the application of the electronic nose, which is used for the detection of volatile materials, could be a possible solution.

The objectives of my research were to gain information concerning the following aspects:

(1) What is the concentration of hazardous contaminants (especially methanol residues) in the glycerol sources that are used in Hungary?
(2) Could electronic nose (e-nose), as a rapid analytic method, provide a useful method for the determination of methanol contamination in glycerol?
(3) How does affect *powdered glycerol on solid carrier applied at 1%* in the feed of lactating sows:
    - the performance of the sows
    - the value of some blood parameters (glucose, cholesterol, triglycerides)?
(4) How does affect *liquid glycerol applied at 5%* in the feed of lactating sows:
    - the performance of sows and piglets
    - the value of some blood parameters (total protein, albumin, glucose, cholesterol, triglycerides, ALT, AST, GGT)
    - the amount, nutrient content and fatty acid profile of sow milk
    - the apparent total tract digestibility of nutrients?

2. MATERIALS AND METHODS

During my doctoral studies, the investigations were directed towards the safely use of glycerol in the nutrition of lactating sows. According to this, the research was divided into two main parts, which included the chemical analysis of glycerol samples of different quality, consistency and origin, in connection with this a method development conducted with electronic nose (e-nose) and feeding trials with lactating sows.

2.1. CHEMICAL ANALYSIS OF THE GLYCEROL AND METHANOL CONTENT OF THE GLYCEROL PRODUCTS AVAILABLE IN THE HUNGARIAN MARKET

During preliminary chemical analysis it was evaluated how the potential source of risk associated with feeding glycerol (especially methanol content) is present in the Hungarian practice. During the chemical analysis the glycerol and methanol content of 9 glycerol samples of different origin (2 from farms and 7 from feed producers), quality (2 food grade and 7 feed grade) and consistency (1 solid and 8 liquid) was evaluated by using Biotronik 2000 HPLC instrument (Biotronik Wissenschaftliche Geräte GmbH, Germany) with the fallowing settings: Bio-Rad Aminex®HPX-874, size 300 mm × 7,8 mm; split temperature: 45 °C, eluent: 0,005 M H_2SO_4; pump: flow: 0,85 ml/min., pressure 77 kg/cm^2.

2.2. RAPID ANALYSIS METHOD DEVELOPMENT BY THE USE OF ELECTRONIC NOSE

As the first step of our rapid analysis method development dilution was made from high purity materials (glycerol, ≥99.5 %; methanol, ≥99.9 %, GC Ultra Grade, Carl Roth GmbH, Karlsruhe, Germany) according to different methanol concentrations (0%, 0.05%, 0.10%, 0.20%, 0.30%, 0.40%, 0.50%). The aroma profile of the different mixtures was determined in 3 replicates by Alpha MOS Heracles NEO electronic nose (Alpha MOS, Toulouse, France), which is an ultra-fast dual-column gas chromatograph, equipped with automatic sample handling unit, two flame ionisation detector (FID-1, FID-2) and trap. During the model study, the 1 ml samples were pretreated at 50 °C with incubation time of 5 minutes, thereafter 1 ml of gas sample was injected into the analyser from the steam by Hamilton syringe, which was preheated to 60 °C.

2.3. GLYCEROL FEEDING TRIALS WITH LACTATING SOWS

2.3.1. FEEDING TRIAL WITH LACTATING SOWS BY THE USE OF GLYCEROL ON SOLID CARRIER

The experiment was conducted at a Hungarian Large White×Hungarian Landrace breeder farm in Rábacsécsény. The test was approved by the Committee on Test Animal Welfare (MÁB). The trial was conducted with multiparous sows (313±24.9 kg; n=2×5) of almost identical parity (control: 2.8; experimental: 3.4) according to randomized block design. The trial was started on the 106th day of gestation, when the sows were moved into the farrowing room, and was finished when the piglets were weaned at the age of 21 days. After farrowing the sows had

ad libitum access to feed and water. Within the first 24 hours after farrowing, litters were cross fostered within treatments, the litter size was adjusted to 12 piglets/sow.

Control and experimental diets were formulated on wheat-barley-corn-soybean meal based according to the recommendation of the NATIONAL RESEARCH COUNCIL (2012) on standardised ileal digestible (SID) amino acid basis. In the experimental diet 1% of glycerol on solid carrier was used. The glycerol and methanol content of the glycerol source was evaluated before the formulation according to the methods described in the analytical analysis of the glycerol products (glycerol: 71.92%, methanol: 0%). The nutrient content of the diets fed in the experiment was checked by proximate chemical analysis, which confirmed that the nutrient content of the control and experimental diets was almost the same. Dry matter, crude protein, ether extract, crude fiber and crude ash content were determined according to the methods described in the Hungarian Feed Codex (2004) (MSZ ISO 6496: 1993, MSZ 6830-4: 1981, MSZ 6830-6: 1984, MSZ 6830-7, MSZ ISO 5984).

On the 106th day of gestation and the 21st day of lactation, sows body weight, backfat thickness were recorded. The back fat thicknesses were determined behind the last rib arch, 10-10 cm to the right and left of the spine line, at the so called P2 point (Lean-Meater, Renco Corp.). The feed intake of the sows was registered individually and continuously during the trial with 0.1 kg accuracy. Sows were monitored for estrous after weaning daily.

Litter weights were measured at birth and the 21st day of lactation. Blood samples were collected from the sows from the superior vena cava on the first, second and third week after farrowing in 3 hours after feeding.

Glucose, cholesterol, triglyceride content of the blood samples was measured by Beckman Coulter AU480 analyser with Beckman Coulter (Brea, California, USA) and Diagnosticum (Diagnosticum Zrt. Hungary) reagent kits.

2.3.2. FEEDING TRIAL WITH LACTATING SOWS BY THE USE OF LIQUID GLYCEROL

The experiment was conducted at the Product Development and Monitoring Research Centre of Kaposvár University (at present: Hungarian University of Agriculture and Life Sciences, Kaposvár Campus). The ethics approval for this study was issued by the National Scientific Ethical Committee on Animal Experimentation, Hungary, prior to the initiation of the experiment (approval number: SOI/31/446-6/2014). The trial was conducted with multiparous Danish Large White×Danish Landrace (DanBred Genetics, Denmark, Copenhagen) sows (323±17.0 kg; n=2×12) of almost identical parity (control: 2.7; experimental: 2.6) according to randomized block design. The trial was started on the 106th day of gestation, when the sows were moved into the farrowing room, and was finished when the piglets were weaned at the age of 28 days. During lactation, sows were kept in individual farrowing crates (2.4 m long ×1.8 m wide×0.7 m high) equipped with individual boxes (1.0 m long×1.2 m wide×0.7 m high) for the piglets.

Control and experimental diets were formulated on wheat-barley-corn-soybean meal based according to the recommendation of the NATIONAL RESEARCH COUNCIL (2012) and the breeding company (DANISH PIG RESEARCH CENTRE, 2017) on standardised ileal digestible (SID) amino

acid basis. 5% corn was replaced by liquid glycerol in the experimental diet.

The glycerol and methanol content of the glycerol source was evaluated before the formulation according to the methods described in the analytical analysis of the glycerol products (glycerol: 86.95%, methanol: 0.02%). The nutrient content of the diets fed in the experiment was checked by proximate chemical analysis, which confirmed that the nutrient content of the control and experimental diets was almost the same. By the determination of the dry matter, crude protein, ether extract, crude fiber, crude ash, calcium, phosphorus and sodium content of the feed samples the fallowing methods were used: MSZ ISO 6496:2001, MSZ EN ISO 5983-2:2009, MSZ EN ISO 11085:2015 method "A", MSZ EN ISO 6865:2001, 152/2009/EK III. M appendix, MSZ EN15510:2008.

The amount of feed per sow was 3.5 kg/day from the 106th day of gestation to the farrowing. After farrowing the amount was increased until the 5th day of lactation gradually, between the 5th and 28th lactation days restricted feeding was replaced by *ad libitum* feeding.

On the 106th day of gestation and the 28th day of lactation, sows body weight, backfat thickness were recorded. The back fat thicknesses were determined behind the last rib arch, 10-10 cm to the right and left of the spine line, at the so-called P2 point (Lean-Meater, Renco Corp.). The feed intake of the sows was registered individually and continuously during the trial with 0.1 kg accuracy. Sows were monitored for estrous after weaning daily.

Within the first 48 hours after farrowing, litters were cross fostered, the litter size was adjusted to 12 piglets/sow. Piglet weights were measured at birth, after cross-fostering and at the 28th day of lactation individually.

Milk production was estimated with weigh suckling weigh (WSW) method on the 14th, 21st and 28th days of lactation (RENAUDEU AND NOBLET, 2001).

After milk production estimation, 10 IU oxytocin (Oxytocine NCP, Kela) was injected intramuscularly 65 minutes after the last suckling to induce milk let down and samples were collected by hand (n=2×36). The dry matter, protein, fat, lactose content and fatty acid profile of the milk samples were determined according to the methods described by the relevant standards (MSZ ISO 6496:2001; MSZ EN ISO 5983-2:2009; MSZ 6830-19:1979; MSZ 6830-26:1987; MSZ EN ISO 12966-2:2011).

Blood samples were collected from the sows from the superior vena cava on the 28th day of lactation. Plasma glucose, cholesterol, triglyceride, total protein, albumin content, and activity of different liver enzymes (ALT, AST, GGT) were measured by Beckman Coulter AU480 analyser with Beckman Coulter (Brea, California, USA) and Diagnosticum (Diagnosticum Zrt. Hungary) reagent kits.

To determine the apparent total-tract digestibility (ATTD) of nutrients indicator method was used. The ATTD of nutrients were calculated using titanium-dioxide (TiO_2) as an indigestible marker at 0.5% (as-fed basis) in both diets. Faecal samples were collected twice daily between the 18th and 23rd days of lactation. The dry matter, crude protein, ether extract, crude fiber content of the feed and faecal samples were analysed according to the methods described by the Hungarian Standard (MSZ EN ISO 5983-2:2009, MSZ EN ISO 11085:2015 A method, MSZ EN ISO 6865:2001). The TiO_2 content of the samples was determined spectrophotometrically (UV-160, Shimadzu Co., Japan) at 410 nm after addition concentrated sulfuric acid and a specific color reagent (35 cm^3 of

concentrated sulfuric acid, 15 cm^3 of 85% phosphoric acid, 13.2 cm^3 of 30% hydrogen peroxide).

2.4. STATISTICAL ANALYSIS

During the analysis conducted by the use of electronic nose, Kováts' retention indexes were assigned to the chromatograms created from the samples, according to the specifications of AlphaSoft controlling and analyser software (Alpha MOS, Toulouse, France). The aroma profile describing multivariable data was analysed with principal component analysis (PCA) and guided classification methods (discriminant function analysis, DFA; soft independent modelling by class analogy; SIMCA). A quantitative calibration model, based on the partial least squares regression (PLSR), was assigned to the known methanol content and on the signals of the sensors which are describing the aromatic profiles.

Data of the feeding trial conducted with 1% glycerol on solid carrier were analysed using independent-samples t-test and non-parametric tests (Kolmogorov-Smirnov test, Mann-Whitney test) (SPSS, IBM, Armonk, NY). In case of each statistical analysis, the selected significance level was $p \leq 0.05$.

Data of the feeding trial conducted with 5% liquid glycerol were analysed using the Kolmogorov-Smirnov test, Levene-test and two sample t-test for sow performance and the fatty acid profile of milk samples. The data of milk yield, milk composition and the amount of nutrients were analysed using GLM procedure (SPSS, IBM, Armonk, NY). The statistical model included effects of diet, week and week×diet. Multiple comparisons of the observed means were based on the Bonferroni Post Hoc test. In case of each statistical analysis, the selected significance level was $p \leq 0.05$.

3. RESULTS

3.1. CHEMICAL ANALYSIS OF THE GLYCEROL AND METHANOL CONTENT OF THE GLYCEROL PRODUCTS AVAILABLE IN THE HUNGARIAN MARKET

During the chemical analysis it was found that the glycerol content of the glycerol samples (n=9) from different origin varied between 64% and 100%. 44% of the glycerol samples had higher methanol content compared to the declared value on the product specification and by 33% of the samples the methanol content also exceeded the limit established by the current European Union regulation (<0.5%). The highest methanol content was 2.68%. All of the samples of farm origin had higher methanol content compared to the declared value on the product specification.

3.2. RAPID ANALYSIS METHOD DEVELOPMENT BY THE USE OF ELECTRONIC NOSE

During the method development using the electronic nose well identifiable and high intensity peaks appeared at the two column of the e-nose at the retention indexes of 436 and 502 even by the chromatogram of glycerol sample with 0.05% methanol content.

According to the result of principal component analysis (PCA) conducted with multivariate data measured by e-nose, the samples were well separated along the principal component fallowing the methanol concentration. The result of the discriminant function analysis (DFA) of the aroma profile, similar to the PCA result, showed the separation of the sample groups according to the methanol content *(Figure 1)*.

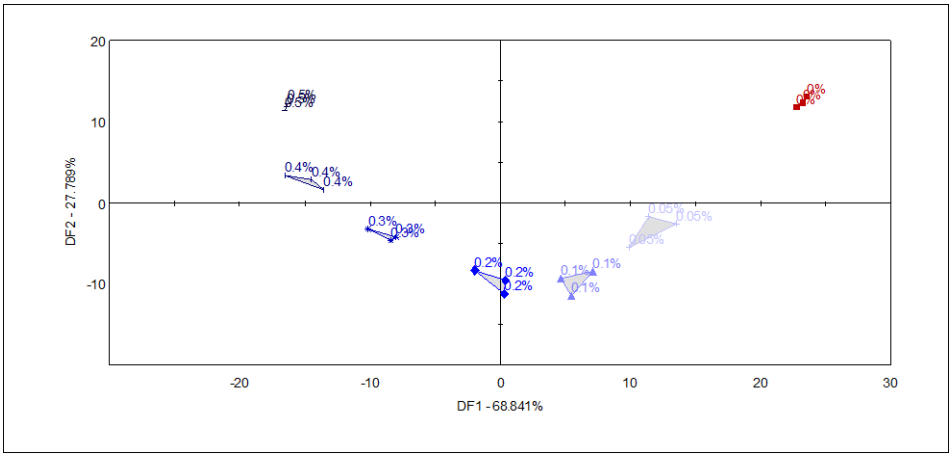

Figure 1: The values of the discriminant function analysis (DFA) determined according to the aroma profiles with the results of the samples of different methanol concentrations (0%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%) in 3 replicates

During the soft independent modelling by class analogy (SIMCA) samples with 0.05% methanol content fell outside the range of acceptability, which was determined based on the variance of the aroma profile of the samples of high purity. This method may be able for the development of a rapid quality control protocol in the future.

The coefficient of determination of the calibration based on partial least squares regression (PLSR) showed high reliability (R^2=0.91), the method proved to be suitable for the rapid and accurate detection of methanol contamination of glycerol in samples of high purity *(Figure 2).*

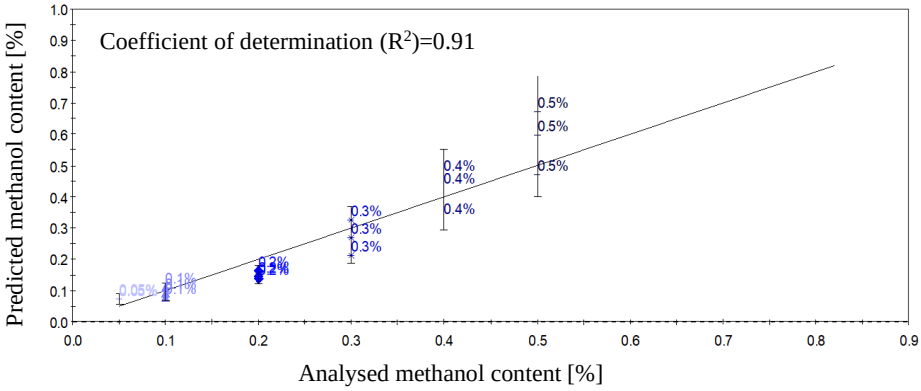

Figure 2: The result of the calibration fitted to the methanol content according to the aroma profile

3.3. GLYCEROL FEEDING TRIALS WITH LACTATING SOWS

3.3.1. FEEDING TRIAL WITH LACTATING SOWS BY THE USE OF GLYCEROL ON SOLID CARRIER

In the trial conducted with Hungarian Large White×Hungarian Landrace (313±24.9 kg; n=2×5) sows 1% glycerol on solid carrier (71.92% glycerol, 0% methanol) had no effect on the lactating sow's performance (live weight loss, feed intake, backfat thickness, return to estrous interval) and the glucose, cholesterol and triglyceride concentration of the blood ($p>0.05$).

3.3.2. FEEDING TRIAL WITH LACTATING SOWS BY THE USE OF LIQUID GLYCEROL

In the trial conducted with Danish Large White×Danish Landrace sows (323±17 kg; n=2×12) 5% liquid glycerol supplementation (87% glycerol, 0.02% methanol) had no effect on the lactating sow's

performance (live weight loss, feed intake, backfat thickness, return to estrous interval) and the weaning weight of the piglets ($p>0.05$). Glycerol addition increased the amount of sow's milk on the 21st day of lactation (control: 8.94±2.27 kg/day *vs*. 5% glycerol: 10.39±1.56 kg/day; $p<0.05$), but did not affect ($p>0.05$) the total milk production of the sows during the trial period (from 14th to 28th days of lactation). Glycerol supplementation reduced the protein content (%, m/m) in the milk on the 14th and 21st day of lactation and during the trial period ($p<0.05$). The ash content of the sow's milk was also decreased by adding 5% liquid glycerol (from 14th to 28th days of lactation, $p<0.05$). The feeding of glycerol did not affect the dry matter, fat and lactose content of sow's milk ($p>0.05$).

By calculating with the results of the determination of the milk yield and the composition of the sow's milk the daily amount of nutrients secreted with milk was also established. The glycerol increased the daily amount of fat secreted with milk on the top of the lactation (21st day of lactation; $p<0.05$), the other parameters (dry matter, protein, lactose, gross energy) were not affected ($p>0.05$). The daily amount of dry matter, fat, protein and gross energy was significantly higher on the 21st day of lactation compared to the results of the 14th to 28th lactation days ($p<0.05$). The daily amount of lactose secreted with milk was not affected by the lactation days ($p>0.05$). During the trial there was no treatment×lactation days interaction found ($p>0.05$).

The effect of liquid glycerol supplementation on the milk yield, composition, and the daily amount of nutrients secreted with milk is summarized in *Table 1*.

Table 1: The effect of glycerol supplementation on the milk yield, composition of milk, and daily amount of the nutrients secreted with milk (5% liquid glycerol; n=2×36)

	Day 14			Day 21			Day 28			Day 14-28			p-value			
Glycerol (%)	**0%**	**5%**	**SEM**	**0%**	**5%**	**SEM**	**0%**	**5%**	**SEM**	**0%**	**5%**	**SEM**	**Day 14**	**Day 21**	**Day 28**	**Day 14-28**
Milk yield, kg/day	7.84	8.38^{AB}	0.44	8.94^{b}	10.39^{aA}	0.41	8.62	7.9^{B}	0.52	8.45	8.98	0.27	0.56	0.03	0.50	0.34
Dry matter, g/100 g	19.12	18.73	0.26	18.76	18.73	0.18	18.35	18.00	0.21	18.74	18.49	0.13	0.47	0.95	0.42	0.33
Fat g/100 g	7.17	7.09	0.26	7.25	7.60	0.19	6.73	6.62	0.18	7.05	7.10	0.04	0.40	0.38	0.75	0.85
Protein, g/100 g	5.12^{a}	5.02^{b}	0.05	5.45^{a}	5.18^{b}	0.06	5.42	5.24	0.10	5.33^{a}	5.15^{b}	0.13	0.87	0.03	0.38	0.04
Lactose, g/100 g	5.05	5.23	0.09	4.67	4.87	0.13	5.09	4.75	0.18	4.94	4.95	0.08	0.31	0.45	0.35	0.93
Ash, g/100 g	0.89	0.85^{A}	0.01	0.90	0.83^{A}	0.02	0.97	0.95^{B}	0.01	0.92^{a}	0.87^{b}	0.02	0.30	0.20	0.61	0.02
Gross energy (GE), MJ/kg	25.99	25.77	0.24	25.37	26.42	0.29	25.22	25.47	0.32	25.59	25.97	0.16	0.66	0.09	0.75	0.26
Dry matter[1], g/day	1499^{B}	1570^{B}	83.4	1677^{A}	1946^{A}	74.8	1582^{B}	1422^{B}	88.6	1584	1660	50.0	0.64	0.06	0.46	0.36
Fat[1], g/nap	562^{B}	594^{B}	42.4	648^{bA}	790^{aA}	34.9	580^{B}	523^{B}	31.9	596	638	23.5	0.71	0.03	0.43	0.29
Protein[1], g/nap	401^{B}	421^{B}	23.1	487^{A}	538^{A}	20.0	467^{B}	414^{B}	26.3	450	462	14.2	0.66	0.19	0.45	0.59
Lactose[1], g/nap	396	438	26.7	417	506	26.9	439	375	34.9	417	445	16.7	0.47	0.12	0.30	0.47
Gross energy[1], MJ/day	2038^{B}	2160^{B}	254.0	2268^{A}	2745^{A}	217.0	2174^{B}	2012^{B}	232.8	2162	2332	146.0	0.65	0.06	0.38	0.36

SEM= standard error of mean
a,b: indicates mean differences of treatments within lactation days at a $p<0.05$
A,B: indicates mean differences of lactation days within treatments at a $p<0.05$
[1]Calculated: (Milk yield[kg/day]×1000×nutrient [g/100g)])/100

Glycerol supplementation decreased the proportion of saturated fatty acids (SFAs) in sow's milk compared to the control group ($p < 0.05$). Within saturated fatty acids, the proportion of C14:0 (myristic acid) and C16:0 (palmitic acid) fatty acids decreased ($p<0.05$). Within monounsaturated fatty acids (MUFAs) glycerol supplementation increased the oleic (C18:1, n-9), vaccenic (C18:1, n-7) and eicosenoic acid (C20:1) proportion, however the ratio of MUFAs and polyunsaturated fatty acids (PUFAs) did not change ($p>0.05$).

Liquid glycerol supplementation did not affect the analysed blood parameters of lactating sows (ALT, AST, GGT, glucose, total protein, albumin, triglyceride, cholesterol, $p>0.05$).

5% liquid glycerol supplementation had no effect on the apparent total-tract digestibility of the dry matter, protein, fat, and fiber ($p>0.05$).

4. NEW SCIENTIFIC RESULTS

1. According to the results of the trial conducted by the use of electronic nose, in case of chemically pure samples, the PLSR (Partial Least Squares Regression) calibration applied on samples with 0.05-0.5% methanol content could be able for the detection of methanol contamination of glycerol. The determination coefficient of the calibration presents high reliability (R^2=0.91).
2. It was first confirmed that glycerol on solid carrier (72.92% glycerol content, methanol-free) could be safely used in lactating sow feeds, without deteriorating the performance (live weight loss, feed consumption, back fat thickness, length of the return to estrous interval) of lactating sows, and without altering certain blood parameters (glucose, cholesterol and triglyceride concentration) that indicate the energy status of sows.
3. 5% of liquid glycerol supplementation (87% glycerol and 0.02% methanol content) increased the amount of sow milk by the 21st day of lactation compared to the control group ($p<0.05$) however, it did not affect the milk production ($p>0.05$) concerning the investigated period of lactation (day 14 to 28).
4. Feeding 5% liquid glycerol (87% glycerol and 0.02% methanol content) reduced the partial ratio of saturated fatty acids (SFAs) in sow milk ($p<0.05$), while the ratio of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) did not change ($p>0.05$). By the use of compound feed with 5% liquid glycerol content it was measured significantly higher oleic acid, vaccenic acid and eicosapentaenoic acid ratio, along decreased myristic acid and palmitic acid rates.

5. SCIENTIFIC PAPERS ON THE SUBJECT OF THE DISSERTATION

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Vida, O., Fábián, J., Bazar, G., Egri, B., Tóth, T. (2019): The effect of dietary glycerol supplementation on milk production and composition, blood parameters and performance of lactating sows. *Livestock Science.* 230 Paper 103859.
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Vida, O., Egri, B., Tóth, T. (2018): The effect of feeding different glycerol sources on the performance of lactating sows. *Acta Agraria Debreceniensis*. 75:99-103.
DOI: https://doi.org/10.34101/actaagrar/75/1654

PEER-REVIEWED PAPERS PUBLISHED IN HUNGARIAN SCIENTIFIC JOURNALS

Vida, O., Egri, B., Tóth, T. (2020): A folyékony glicerinkiegészítés hatása a szoptató kocák teljesítményére, valamint a kocatej mennyiségi és minőségi paramétereire, *Állattenyésztés és Takarmányozás.* 69:375-386.

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Vida, O., Tóth, T., Egri, B. (2015): A glicerinetetés potenciális veszélyei. *Agro Napló.* 19:93-94.