THESES OF DOCTORAL (Ph.D.) DISSERTATION

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES
KAPOSVÁR CAMPUS

INSTITUTE OF PHYSIOLOGY AND ANIMAL NUTRITION

Head of Doctoral School

PROF. DR. SZABÓ ANDRÁS, DSc

Supervisor

DR. TÓTH TAMÁS, Ph.D.

Co-Supervisor

PROF. DR. FÉBEL HEDVIG, CSc

STUDY OF THE USE OF COMPOUND FEEDS WITH DIFFERENT CELLULOSE-, HEMICELLULOSE- AND LIGNIN CONTENT WITH GROWING- AND FINISHING PIGS THROUGH DIGESTIVE-PHYSIOLOGICAL AND MYOGRAPHIC MODEL INVESTIGATIONS AND PERFORMANCE EXPERIMENTS

DOI: 10.54598/001060

Written by

JÁRÓ-NAGY KATALIN

Kaposvár

2021

1. THE BACKGROUND AND THE OBJECTIVES OF THE RESEARCH

There is an increased competition for the cereal- and protein sources used in the course of feeding our farming livestock, in which not only the animal breeding- and keeping industry, but the consumerism of the world's growing population also participates. In the area of pig feeding, a possible alternative could be the increased addition of by-products to the formulas, as well as the more conscious investigation of the fibre content of feed compounds and its sophisticated use.

As a result of the breeding attempts of recent decades, high performance hybrid lines dominate the pig-breeding industry branch. The feeding of these intensively growing hybrid genotypes is characterised by the use of by-products. Due to the breeding particularities, selection subserved those animals, whose digestive system has adapted to compound feeds with high fibre content. On the increased intestine surface, a larger amount of microbe population is able to perform more effective microbial fermentation, thus more intensive short chain fatty acid (SCFA) synthesis. The above mentioned indirect selective pressure greatly contributes to the fact that compound feeds, whose crude fibre content is reasonably higher than the common values used in domestic practice, can be effectively used in intensive pig production systems.

The allocation of the physiological effects and digestibility of diverse fibre sources purely on the basis of monomer composition is quite hard. For precise classification, the investigation of digestible and net energy content, solubility, viscosity, physical structure and water holding capacity (*Asp* et al., 1996), the capability to fill the gastrointestinal tract, as well as the acid detergent fibre (ADF) and neutral detergent fibre (NDF) content is inevitable. These characteristics fundamentally determine the biological development, namely the body weight gain (BWG) of the animal, just as the frequency and measure of feed intake.

Nowadays, several analytical methods are available in order to receive more information concerning the nutritional values of the selected raw materials. The crude fibre content of the feed ingredients used for pig feeding is routinely checked, and in domestic practice these data are still used in the course of the calculation of feed and complete feed compounds too. At the same time, the allocation of the quantity and quality (solubility) of fibre fractions and certain carbohydrates becomes more and more necessary. Beyond the crude fibre content of the used ingredients, the awareness about the quantity of certain fibre fractions and the allocation of their digestibility are also determinative factors in the course of the formulation of compound feeds. Domestically, there are not any recommendations concerning fibre fractions for different pig age groups. The presently available official

reference *(Hungarian Feed Codex*, 2004*)* includes recommendations concerning the nutrition content of compound feeds, thus about the crude fibre content according to pig genotypes. The genotypic classification of the reference does not mention any differentiations concerning recommended crude fibre content (g/kg as in feed), (standardized in 35g/kg as in feed), and also does not define minimum or maximum values neither in case of metabolic (ME_s) nor digestive (DE_s) energy evaluation systems.

However, we know that in case of modern pigs genotype, the energy system has a strong determinative influence on how the formula changes affect the natural production indexes of the animals. The decreasing fattening results in case of the use of compound feeds containing fibre-rich ingredients probably stem from the higher crude fibre-, NDF- and ADF content, as well as from the use of certain metabolic or digestive energy values. Because of this, in case of the use of fibre-rich ingredients, the suggested formulation of compound feeds is based on net energy (NE) values. Net energy can be considered as the most precise factor concerning the energy values of feed, since it also takes the heat production caused by bacterial fermentation into account (*Noblet* et al., 1994). We know that DE_s or ME_s energy values cannot be used safely when calculating the energy values of diverse raw materials with high fibre content (*Shi* and *Noblet*, 1993), since their composition can be different due to their fermentable fibre and protein content. These energy evaluation systems overestimate the energy content of ingredients containing high fibre- and protein ratios (*Noblet* and *Milgen*, 2004).

The presence and the fibre content of the diverse components of feed compounds – among others, the by-products inserted into the formula – have various different effects on the physical changes of consumed feed in the gastrointestinal tract. The digestibility of feeding material, especially the fermentation of fibres is fundamentally determined by the exact amount of time, which the consumed feed spends in certain organs of the gastrointestinal tract. Therefore, the amount of time that the bacteria of the large intestine have for the fermentation of various fibre fractions, depends on the transit time. According to the diverse available sources, the effects of fibre sources on the operation of the digestive system and on the degradation of crude fibre is not completely clear, and rather contradictory. Various research endeavours suggest that crude fibre sources (both soluble and insoluble fibre) either slow down (*Miquel* et al., 2001; *Van Leeuwen* et al., 2006); does not affect (*Rainbird* and *Low*, 1986), or speed up (*Potkins* and *Lawrence*, 1984; *Guerin* et al*.,* 2001) the passage of the contents through the digestive system of porcine species.

Electromyography measures the electric activity of the smooth muscle tissue of the organs by being capable of recording electric impulses that participate in the creation of muscle movement which presents us an image about the characteristics of the work occurring in muscles. A special kind of electromyography (SMEMG - gastrointestinal smooth muscle electromyography), that investigates the smooth muscle tissues of the organs composing the gastrointestinal system (stomach, small- and large intestine) provides us previously unknown data about the physical changes occurring in the gastrointestinal tract. These results can greatly contribute to realistic motility investigation, and also could provide further opportunities for the real-time (non-estimated) measurement of intestinal transit time in the future.

The most important objectives in the course of research were as follows:

(1) Analytical investigation of typical fibre ingredients and fibre content of domestic feed compounds for growing- and finishing pigs.

(2) Evaluation – in the course of experiment feed – of how various fibre sources affect the performance parameters of growing- and finishing pigs, and what is the suggested rate of diverse fibre-rich by-products (maize-DDGS, soybean hull, wheat bran, sugar beet pulp) in the compound feed; and in what is the suggested ratio of supplementing extracted soybean meal with these by-products.

(3) Demonstrating the effects of fibre source on the gastrointestinal system by the use and adaptation of a new research method (smooth muscle electromyographic - SMEMG), which previously was never used on pigs based on our knowledge.

2. MATERIALS AND METHODS

In the course of my doctoral studies, experimental activities were divided into two thematic subjects. These were as follows: the effects of feeding industrial by-products based compound feeds containing high fibre-, NDF- and ADF ratios on the performance parameters of growing- and finishing pigs; within this we estimated the typical crude fibre content and fibre fractions of domestic compound feeds for growing pigs, and we investigated what kind of effects does the feeding of various by-products (maize-DDGS, soybean hull, wheat bran, sugar beet pulp) have on the performance parameters of growing- and finishing pigs under high NDF content feeding. Furthermore, I dealt with the electromyographic investigation of smooth muscle tissue of the gastrointestinal system (Smooth muscle electromyography - SMEMG) with growing pigs, under fibre-rich feeding. The animals investigated in the myographic experiment were treated according to the European Communities Council Directives (2010/63/EU) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). The myographic experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: VIII-I-001/01854-0005/2014).

2.1. ANALYTICAL INVESTIGATIONS CONCERNING THE CRUDE FIBRE CONTENT AND THE QUANTITY OF SPECIFIC FIBRE FRACTIONS IN THE HUNGARIAN GROWING AND FINISHING PIG COMPOUND FEEDS

Feed compounds samples were collected (between October 2016 and March 2017) in cooperation with the leading Hungarian feed producing and distribution companies. The feed samples came from large-scale (minimum 250 sows and their litters) breeding- and fattening farms, which used their respectively produced compounds, and also from the dominant Hungarian feed producers, as typical compounds (n=22). The genotypes of the farms that provided feed samples were classified into categories defined by the *Hungarian Feed Codex* (2004): there were genotype “A” (n=15, hybrids and other high performance crossover lines) and genotype “B” (n=7, pure bred and their crossover lines).

The allocation of the fibre content of the collected samples was implemented by chemical and NIRS (near infrared spectroscopy) methods. The crude fibre content of the samples was measured according to the XII. Method (MSZ 6830-7) included in Appendix 10 of the FVM regulation Nr. 44/2003 (IV.26.). For the evaluation NDF-, ADF- and acid

detergent lignin (ADL) content we used the methods described in the 8.2. chapter of *Hungarian Feed Codex* 1990. II. The crude fibre content of each sample was also determined by the use of FOSS NIRS ^{TM} DA 1650 (distributor: Servitec Kft., Hungary) feed analyser, in a 1100-1650 nm spectral range.

The one-way analysis of variance (Kolmogorov-Smirnov test, Levene-test, one-way ANOVA) and the correlation investigation (Pearson's correlation coefficient) between the calculated, chemical and NIRS generated crude fibre data was implemented by the use of SPSS 21.0 for Windows software (IBM Corp., ARMONK, NY, USA). The chosen significance level in case of each statistical analysis was $p \leq 0.05$. We determined the quality of the relation between chemical and calculated crude fibre content by the method described by *Bibby* and *Toutenburg* (1977). By the allocation of mean square prediction error (MSPE) value, we determined the error ratio of the model, namely the difference between chemical and calculated crude fibre content, and by the use of relative MSPE (relMSPE) value, we allocated the rate (%) of the error.

2.2. THE EFFECTS OF FEEDING VARIOUS BY-PRODUCTS (MAIZE-DDGS, SOYBEAN HULL, WHEAT BRAN, SUGAR BEET PULP) ON THE PERFORMANCE PARAMETERS OF GROWING- AND FINISHING PIGS WITH HIGH NDF CONTENT EXPERIMENTAL FEED

In the course of our experiment, we examined the effects of feeding various by-products among high-performance growing- and finishing pigs. In wheat- and barley-based feed we supplemented the extracted soybean meal and wheat grains (wheat, barley, corn) with different ratios of by-products, thus increasing the NDF content of the used feed compounds. Upon the formulation of the compounds, the net energy values of specific ingredients were taken into account.

The experiment was carried out in accordance with the European Commission Council Directives (86/609/EEC) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). The performance experiment was carried out by the use of growing pigs kept in individual pens [(Danish Landrace × Danish Large White) × Danish Duroc] (n=60, 30 gilts, 30 barrows, age: 86 days). Each of the open roof individual pens had $1m^2$ area with slatted concrete flooring (Schauer Agrotronic GmbH, Prambachkirchen, Austria), and each of them were equipped with individual feeder and drinker, for whose use

the pigs were granted free access. The pens had automatic ventilation (Microfan Bravo-E, Schauer Slc, BM2 Arcotherm GA / N 45 C, Lubing Top, Schauer Agrotronic GmbH, Prambachkirchen, Austria), and an artificial lighting program was applied with equal 12-hour long light and dark periods (natural and artificial light sources).

The starting body weight of the growing pigs was 40.9±2.2 kg. According to the consumed experimental diets, the animals were divided into three groups:

1. control, no by-products supplementation (CTR);
2. experimental feed compounds with a moderate level of by-products (MLB);
3. experimental feed compounds with high level of by-products (HLB).

The control feed was a compound feed based on wheat, barley and extracted soybean meal, and did not contain any by-products, while in the groups with moderate and high level of by-product the rate of contained by-products was 14.8% and 26.8% during the growing phase, and 19.7% and 32.8% in the finishing phase. The content ratio of extracted soybean meal was reduced from 13.4% (CTR) to 6.1% (MLB) and 3.5% (HLB) in the growing phase, while in the finishing phase it was reduced from 10.5% (CTR) to 1.8% (MLB) and 1.7% (HLB).

Both the control and experimental feeds were formulated according to the net energy value (NE_s) of the components, while taking the recommendation of SEGES (2016, 2020) into account. The standardized, ileal digestible (SID) amino acid content (lysine, methionine+cysteine, threonine, tryptophan) was adjusted to conform *NRC* (2012) recommendations, according to ideal protein concept. Due to the applied fat- and synthetic amino acid supplementations (L-lysine-HCl, L-threonine, L-tryptophan, DL-methionine) the net energy value (NE_s) and crude protein content of the fed compounds was almost identical.

The major nutrition content of the compounds applied in the experiment was verified with a proximate analysis, whose recorded values were synchronous with the calculated values. The dry matter-, crude protein and crude fibre content of the compound feed was measured according to Hungarian standards; for the evaluation of dry matter content MSZ ISO 6496:2001 was used, and for crude protein and crude fibre content the 152/2009/EK disclosure. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) content analysis was carried out by the use of Van Soest method (1979), according to the methods described in 8.2. chapter of *Hungarian Feed Codex* 1990. II.

In the course of the 67 day long experiment the growing- and finishing pigs were individually weighed in two week intervals. The amount of consumed feed was continuously evaluated by each pen, which facilitated the allocation of daily feed consumption. According to the measured data average daily feed intake (FI, kg/day/pig), and average daily gain

(ADWG, kg/day/pig) of the animals, as well as the weight gain/feed intake ratio (G:F) of the pigs (kg/kg) were calculated.

The following statistical methods for the evaluation of the performance data were used: Kolmogorov–Smirnov test, Levene-test and GLM-General Linear Model (SPSS 26.0, IBM, Armonk, NY, USA). In the course of GLM (Univariate) test we evaluated the effects of feed, gender, and gender × feed interactions upon the investigated performance parameters. The multiple comparison of the observed averages was carried out by the use of Bonferroni post hoc test. The values included in the table are presented as mean value ± SEM (standard error of the means). The chosen significance level was $p \leq 0.05$.

2.3. INVESTIGATION OF THE APPLICABILITY OF MYOGRAPHIC MEASURING TOOL FOR GROWING PIGS

We conducted our pilot study with the use of one animal (n=1, Hungarian Landrace × Hungarian Large White barrow, (age: 48 days, weight: 25 kg). Before and after the measurement, the experimental animal was placed into an individual pen ($2m^2$/animal). Myographic measurements were conducted under anaesthesia. Before and after the measurement, the subject received its daily feed portion in two parts (7.00 am and 2 pm), which consisted of traditional extracted soybean and corn based growing pig feed. Drinking water was provided *ad libitum*.

The myographic measurement were carried out under general anaesthesia (Stresnil injection, A.U.V., producer: Janssen Pharmaceutica Ltd., distributor: Lifescan Janssen-Cilag, Pharmaceutical Trade Ltd.). Under anaesthesia, both outer sides of the abdominal wall as well as the area of left thigh were shaved and disinfected. The electrical waves were detected by the use of disposable Ag/AgCl electrodes (Electrode PE Foam Solidgel, Bio Lead-Lok B Sp. Zo.o, Józefów, Poland). At first, the measuring electrodes were fixed on the left side of the abdominal wall, while the neutral electrode was attached to the left thigh. The data from the different located electrodes - fixed on the right and left sides, were recorded and evaluated separately (1^{st} measurement: left side, 2^{nd} measurement: right side). The electrode pairs (2 electrodes) were fixed to the right and left sides of the abdominal wall, 10 cm lateral from the spine, while the neutral electrode was placed on the right thigh at a distance of 5 cm from the tail. The myographic measurement was carried out by the use of EGIG holter device (MSB-MET Kft., Balatonfüred, Hungary). The holter device and the electrodes on the animal's skin surface were connected by wires. The holter collected and stored the recorded electric signals;

during the course of the examination the data were on display by a computer screen, and the curve of the collected electric waves was constantly monitored.

The first measurement (1st measurement) was carried out on the left side of the experimental animal. Following the fixing of electrodes we recorded a state of dormancy, which is typical to the gastrointestinal tract. It was followed by the intramuscular application of 5 ml Buscopan compositum A.U.V. injection (producer: Boehringer Ingelheim Vetmedica Gmbh, Germany; distributor: Boehringer Ingelheim RCV GmbH & Co. KG Hungary Ltd., Hungary). The applied product reduces the activity of the smooth muscle tissue in the gastrointestinal tract as well as intestine motility due to its hioscine butyl bromide + dypirone agents, thus we were able to record a "slowing" phase. The duration of the measurement was 10 minutes.

After the recording of the "slowing" phase, the effects of 25 mg/kg metoclopramide (Szelenyi et al., 1994) was tested agent by the use of the myographic measurement tool, during a "speeding" phase. The selected substance increased motility and the activity of the smooth muscle tissue in the gastrointestinal tract, thus "speeding" phase could be detected. The duration of the measurement was also 10 minutes.

The second trial measurement (2nd measurement) was carried out according to the methodology of the 1st measurement. The medical substances and the duration of the measurement were identical to the 1st measurement. The green and blue electrodes were placed on the right side of the animal, the yellow and red electrodes remained on the left side in accordance with the measuring outlines of the 1st measurement; the neutral electrode also remained on the left thigh.

Following Fast Fourier Transformation (FFT analysis), the measured and recorded myoelectric primary waves were evaluated by ISO/Myo software (MDE GmbH, Walldorf, Germany). In the course of the measurement, the recorded waves were visualised in both 2D and 3D, and were evaluated in ISO/Myo software.

2.4. SMOOTH MUSCLE ELECTROMYOGRAPHY (SMEMG) MEASURMENT OF THE SMOOTH MUSCLE TISSUE OF THE GASTROINTESTINAL SYSTEM AMONG AWAKE PIGS UNDER HIGH FIBRE CONTENT FEEDING IN METABOLIC CAGES

The experiment was conducted with three animals (n=3, Hungarian Landrace × Hungarian Large White barrows, age: 40±2 days, weight: 27±1 kg) while feeding two

compounds with different fibre content. 4% Opticell C5 (Agromed Austria GmbH, Kremsmünster, Austria) was used in order to obtain higher fibre content in the experiment feed. The adaptation period was 5 days both in the control and the experiment dietary treatments. The compound feed was fed to the animals in two portions (7.00 am, 12.00 am); in both phases, drinking water was provided *ad libitum*.

During the adaptation period, the animals were placed into individual pens ($2m^2$/animal). During the myographic measurement the animals participating in the measurement were in metabolic cages.

In the course of the myographic measurement, for the sake of the precise recording of feed intake times, the test animal was fed in two different times (7.00 am, 12.00 am). The myographic measurement was carried out individually, with one animal per day, between 7.00 am and 1.00 pm; the duration of the measurement was 6 hours uniformly, in case of each pigs. In both dietary treatments (control and experiment), the myographic measurement was repeated three times at each animal.

The myographic measurement was conducted by the use of EGIG holter device (MSB-MET Kft., Balatonfüred, Hungary), in an awaken state. The detection of the waves was implemented by disposable Ag/AgCl electrodes (Electrode PE Foam Solidgel, Bio Lead-Lok B Sp. Zo.o, Józefów, Poland).

The electrodes were fixed to the shaved and disinfected abdominal wall, 10 cm lateral from the spine, and the neutral electrode was placed on the right thigh at a distance of 5 cm from the tail. The holter device was connected to the electrodes attached to the skin of the pig by wires; the device was placed onto the top of the metabolic cage, above the animal.

Following FFT analysis, the measured and recorded myoelectric primary waves were evaluated by ISO/Myo software (MDE GmbH, Walldorf, Germany).

Statistic evaluation was conducted by the use of SPSS 19.0. software package (IBM, Armonk, NY, USA). In the course of the analysis of the results, descriptive methods, graphs and test statistics were used. In case of constant variables, the descriptive statistics included case number, average, standard deviation, minimum, median and maximum. In case of categorical variables, case number and occurrence frequency were included. During the measuring sequence of each animal, the experiment and control data sequences were compared by the use of Wilcoxon-test. In order to compare control and experimental feed, repeated variance analysis was applied; the animal and the measuring sequence were included in the model as variables.

2.5. EFFECT OF GASTROINTESTINAL SMOOTH MUSCLE ELECTROMYOGRAPHY (SMEMG) MEASUREMENT FOR GROWING AWAKE, FREE-MOVING PIGS FED WITH HIGH FIBER DIET

The myographic measurements were conducted with 9 (n=9, [(Danish Landrace × Danish Large White) × Danish Duroc], age: 72±3 days, average body weight: 30±3 kg) barrows. The animals were placed into individual pens (1 animal/pen, 1m^2/pen), without litter; drinking water was provided *ad libitum*.

The control feed was a traditional feed compound with extracted soy and corn basis. The experimental feed contained 4% Opticell fibre supplementation. The animals received the daily feed amounts in two equal portions, the amount of feed was 2,8 times higher than the standard maintenance energy requirement among pigs (450 kJ ME_s /$kg^{0.75}$/ day). The amount of daily feed was calculated on the basis of the body weight of the animals (the animals in the experiment were weighed individually each week, their individual feed amount was adjusted to their body weight). The pigs were fed at 7.00 and 12.00 am.

The myographic measurements were conducted with two devices in parallel; 4 animals participated in measuring process each day (at 7.00 and 12.00 am), 2 animals/treatment/day in case of each dietary treatment. The duration of the measuring process was 4 hours. In a completely randomized design (CRD), the animals were alternately fed control and experimental feed, in two repeats per treatment. In case of both dietary treatments, the adaptation period was 5 days. The miographic measurement started at the time of individual feed portioning (7.00 or 12.00. am). Before the measurements, the epigastric area of the abdominal wall and the thigh were gently cleaned and shaved. The Ag/AgCl electrodes (Electrode PE Foam Solidgel, Bio Lead-Lok B Sp. Zo.o, Józefów, Poland) were fixed on the surface of the skin with adhesive plaster (Leukoplast 5 cm, BSN medical GmbH, Hamburg, Germany),without surgery. Ten20 EEG conductive gel (Bio-Medical Instruments, USA) was used on the skin surface to provide the proper conductancy for the electrodes. The standard electrode pairs (2 electrodes) were fixed to the right and left sides of the abdominal wall, 10 cm lateral from the spine, while the neutral electrode was placed on the right thigh at a distance of 5 cm from the tail. A special belt was designed to fix and protect the electrodes, and hold the holter device which recorded and stored the myoelectric signals.

Following the FFT analysis, the measured and recorded myoelectric primary waves were evaluated by ISO/Myo (MDE GmbH, Walldorf, Germany) software.

The electric signals were recorded and analysed with an on-line computer, and were amplifier of the S.P.E.L. Advanced ISOSYS Data Acquisition System software (MDE GmbH, Walldorf, Germany). SMEMG signals were amplified by using a custom-made amplifier designed by MDE Ltd. (Budapest, Hungary). In order to reduce electric noise ("artefact") double filtering system was used. All analogue signals were pre-filtered with a first-order Bessel-type lowpass filter and were converted into digital signals at a sample rate of 2 Hz with a slop of 80dB/decade. Then, the pre-filtered myoelectric signals were further filtered by Bessel-type bandpass filters with a frequency of 0-30cycles per minute with a slope of 140dB/decade. The recorded signals were analysed by Fast Fourier transformation (FFT). Electric activity was described by cpm (circle per minute) values, and the magnitude of the electric activity was described as maximum of power spectrum density (PsD_{max}). The allocated cpm and PsD_{max} values were compared by t-test, by the help of Prism 5.0 computer software package (GraphPad Software, USA). Each statistical investigation was conducted with $p \leq 0.05$ significance level.

3. RESULTS

3.1. ANALYTICAL INVESTIGATIONS CONCERNING THE CRUDE FIBRE CONTENT AND THE QUANTITY OF SPECIFIC FIBRE FRACTIONS IN THE HUNGARIAN GROWING AND FINISHING PIG COMPOUND FEEDS

In case of 19 compound feeds out of the investigation 22, there were measurable efforts to provide at least 35 g/kg (as in feed) crude fibre content (*Hungarian Feed Codex,* 2004). In the majority of the samples, the calculated crude fibre content was above the recommended level.

According to the data of chemical analyses, the crude fibre content of the samples varied between 22.2 and 73.9 g/kg (as in feed), with an average of 36.1±10.4 g/kg (as in feed), which corresponds with the recommendations of *Hungarian Feed Codex,* (2004). Upon measurement with NIRS, crude fibre content was between 24.0 and 76.7 g/kg (as in feed), with an average of 39.5±12.3 g/kg (as in feed). Between the calculated crude fibre content values and the results of chemical analysing, there was -6.2g/kg difference in absolute value, while between the calculated crude fibre content and NIRS values there was 7.3 g/kg (n=22) difference.

In case of crude fibre content allocated and calculated by chemical methods, MSPE value was 67.06; relative error (relMSPE) was 0.227. If MPSE value is small, the model estimates the investigated index with high accuracy. RelMSPE value reflects the difference between the indexes, thus, the calculated and measured data altogether included 22.7% error. The average of measured and calculated data only deviates from each other in a small extent (A=13.0%) and the regression component of errors was also relatively small (R=14.5%), thus the relative error was primarily caused by the non-comparative nature of the calculated and measured values.

Chemical analysing implied that in 9 samples, and the use of NIRS device showed that in 7 samples crude fibre content was below the 35 g/kg (as in feed) value recommended by *Hungarian Feed Codex* (2004) for growing and finishing pigs.

Upon evaluating the outcomes of chemical and NIRS analysis by genotypes, was found that according to both measuring methods, crude fibre content in the feeds of genotype "B" was below the recommended 35 g/kg (as in feed) value. In the feed samples of genotype "A", the calculated value was also a bit higher (39.8 *vs.* 37.4 g/kg as in feed), however, upon measurement by the use of both analytical methods, their crude fibre content was massively higher. We found especially large deviations between the values received by NIRS analysis (42.5 and 33.1 g/kg as in feed). It is important to highlight that due to the large standard deviations, there was no significant difference ($p=0.44$) between calculated, chemical and NIRS average values.

The correlation coefficient between the calculated crude fibre values and the outcomes of chemical examination was $R=0.737$ ($p<0.01$). Upon comparison of calculated and NIRS values the allocated correlational coefficient was $R=0.719$ ($p<0.01$), while between chemical and NIRS results it was $R=0.690$ ($p<0.01$).

In case of genotype "A", 9 out of the 15 complete feed compounds contained at least one by-product. Out of the 7 compound feeds of genotype „B", there were 4 feed formulas with at least one by-product. In the formulas of the 22 feed samples, the rate of by-products deviated between 3.8% and 14.45%. In the compound feeds that contained by-products, the typical number of these was 1 (10 feed samples) or 2 (5 feed samples).

3.2. THE EFFECTS OF FEEDING VARIOUS BY-PRODUCTS (MAIZE-DDGS, SOYBEAN HULL, WHEAT BRAN, SUGAR BEET PULP) ON THE PERFORMANCE PARAMETERS OF GROWING- AND FINISHING PIGS WITH HIGH NDF CONTENT EXPERIMENTAL FEED

In the growing phase (0-42 days) average daily weight gain was 0.85 kg in the control group, 0.84 kg in the group with medium by-products level, and 0.83 kg by the animals in the group with high by-product level. In the finishing phase (42-67. days), these parameters were changed as follows: 1.48 kg (CTR), 1.45 kg (MLB) and 1.53 kg (HLB). Average daily feed intake in the growing phase at the animals of the control and moderate level by-products dietary treatment was 2.00 kg, in case of high level by products was 2.1 kg. In the finishing phase, the uniform average daily feed intake was 3.40 kg in all three treatments. There was no statistically differences between the results at any of the investigated parameters ($p>0.05$). In the growing phase, weight gain/feed intake ratio (kg/kg) was similar in each of the control and experiment dietary treatments (MLB, HLB): 0.43 kg/kg, 0.42 kg/kg, and 0.40 kg/kg. In the finishing phase, this parameter slightly changed, but any significant differences were not found between the dietary treatments: 0.44 kg/kg (CTR), 0.43 kg/kg (MLB) and 0.45 kg/kg (HLB). There was no significant differences were found between the investigated performance parameters or sex.

3.3. INVESTIGATION OF THE APPLICABILITY OF MYOGRAPHIC MEASURING TOOL FOR GROWING PIGS

During the pilot study, the placement of measuring electrodes on the left and right sides of the abdominal wall as well the attachment of the neutral electrode on the left thigh proved to be successful. All of the signals recorded by the electrodes were constantly measurable and visible through 2D and 3D computer visualisation. The organs of the gastrointestinal tract were clearly recognizable by frequency range-based filtering, which could also be displayed in computer –generated 3D graphs.

3.4. SMOOTH MUSCLE ELECTROMYOGRAPHY (SMEMG) MEASURMENT OF THE SMOOTH MUSCLE TISSUE OF THE GASTROINTESTINAL SYSTEM AMONG AWAKE PIGS UNDER HIGH FIBRE CONTENT FEEDING IN METABOLIC CAGES

In the experiment which was conducted with three animals (n=3), the measurement time and the dietary treatment also affected the number of contraction of the smooth muscle, measured in cycles per minute (cpm). In two cases out of the three repeat of the measuring occasions, this number changed significantly in case of all animals (M1, M2, M3). The changes in the animals' cpm values were not uniform neither in the control, nor in the experiment treatment.

Feeding high fibre content experimental feed caused changes in the maximum frequency value of the smooth muscle contractions (Y max) in case of each animal. However, the direction of the changes did not point out a uniform tendency – it decreased in one animal while increasing in another. Concerning the frequency values, significant changes at each measurement only at one animal (M3) was observed upon examining the effects of two different dietary treatments.

In case of M3 pig, significant changes was observed in case of each measurement (measurement 1st, 2nd and 3rd) between the treatments (M3, 1st. measurement: 85.79 *vs*. 31.95; M3, 2nd. measurement: 122.78 *vs*. 20.64; M3, 3rd. measurement: 87.34 *vs*. 14.98; respectively, control, test treatment, $p<0{,}001$).

Based on the *multivariate analysis* of the effects of the dietary treatment (control, experiment) was observed that neither the treatment, nor the animal had significant effects ($p>0.05$) on cpm values, however, in contrast with that, significant measurement sequence effects was observed. The treatment×animal ($p<0.001$); treatment×animal×measuring sequence ($p<0.001$) and the animal×measuring sequence ($p=0.002$) had significant difference.

The treatment, the tested animal, and the measuring sequence all had significant effects on Y max values. The treatment×animal ($p<0.001$); treatment×measuring sequence ($p=0.044$) sequence; and the treatment×animal×measuring sequence ($p<0.001$) and the animal×measuring sequence ($p<0.001$) interactions also had significant difference, which was implied that the effects of certain variables were not independent of each other.

3.5. EFFECT OF GASTROINTESTINAL SMOOTH MUSCLE ELECTROMYOGRAPHY (SMEMG) MEASUREMENT FOR GROWING AWAKE, FREE-MOVING PIGS FED WITH HIGH FIBER DIET

In the miographic measurements 1-1 electrodes were placed on the abdominal wall of the pigs and the neutral electrode was attached to the thigh. Due to this, we were able to efficiently reduce the number of electrodes. It made the technical execution of the measurement with awake, free-moving animals much easier. During the measurements, we were able to avoid the use of a metabolic cage; the animals were able to move free in the pen, thus the attachment and wearing of the measuring tool did not hinder their movement, feed- and water intake. The duration of the measurement was reduced to 4 hours.

The absolute values for PsD_{max} (mV^2) varied in the different segments of the gastrointestinal tract (stomach, small intestine, large intestine) and in the different treatments (control and experiment) indicating changes in the intensity of the gastrointestinal tract smooth muscle contractions. The PsD_{max} values measured during the feeding experiment diet different from the outcomes of the animals fed by control feed. Significant changes were found in the small intestine, higher PsD_{max} values ($p \leq 0.001$) were measured at every animal in case of feeding higher fibre content diets. There were no significant differences between the PsD_{max} values in the stomach and the large intestine.

The PsD_{max} values measured during the feeding experiment diet different from the feed of control dietary treatment. Significant increase in the PsD_{max} values (mV^2) were found only for the small intestine ($p \leq 0.001$).

Feeding control feed PsD_{max} values (mV^2) varied between 101.3 and 350.3 for stomach; between 20.4 and 67.2 for small intestine, and between 44.0 and 362.9 for large intestine. As an effect of experiment feed, PsD_{max} (mV^2) deviated between 102.8 and 355.6 for stomach; between 82.9 and 264.0 for small intestine; and between 64.8 and 385.8 for large intestine.

Significant differences were not found during the investigation of the measured cpm values in the different segments of the gastrointestinal tract when using feeds with different fibre content. Identical values were measured for the stomach (control: 4.06 *vs*. experiment: 4.06). Very similar values were detected for the small intestine (control: 22.06 *vs*. experiment: 22.10) and in the large intestine (control: 2.11 *vs*. experiment: 2.13).

4. CONCLUSIONS

In 19 compound feeds out of the collected and analysed by chemical methods 22, there were measurable efforts to provide at least 35 g/kg (as in feed) crude fibre content (*Hungarian Feed Codex,* 2004). In the majority of the samples, the calculated crude fibre content was above the *Hungarian Feed Codex* (2004) recommended level. It implies that domestic practice considers the Hungarian recommendation as a minimum value, and calculates in the formulation and feeding production with a bit higher crude fibre content for pigs in the growing phase in case of both, but especially for genotype "A" appears to be a general preference. This could suggest the reassessment of the fibre concentration recommended by the *Hungarian Feed Codex* (2004) in the future.

Upon further investigation of the feed compound samples, it was found that in case of crude fibre content allocated and calculated by chemical methods, the MSPE value was 67.06; relative error (relMSPE) was 0.227, thus, the calculated and measured data altogether included 22.7% error. The average of measured and calculated data only deviate from each other in a small extent (A=13.0%) and the regression component of errors was also relatively small (R = 14.5%). The relative error was primarily caused by the non-comparative nature of the calculated and measured values. According to this, we can confirm that the size of the error concerning the crude fibre content calculated in the course of formulation is independent of the crude fibre content allocated by chemical measurement.

There could be several reasons for the difference between the results of available measurement methods (chemical, NIRS) and the crude fibre content calculated during the formulation of the compound feed. We can assume that during formulation, neither the feed producer- and distributor companies, nor the pig farms with respective mixing facilities do necessarily reconsider the formula for each new ingredient, even in spite of the available results of different measurements (chemical, NIRS). Due to this, the nutrition value of the ingredients – including the by-products – can deviate by each item and also could be affected by the production technology. The hemicellulose- and soluble fibre content affects the outcomes and the precision of the measurement, thus another reason for the deviation of calculated and measured values could stem from the inconsistency of analytics, and especially susceptible to the hemicellulose content of the given by-product and to the content ratio of the given by-product in the formula. Due to this, the investigation and correction of the crude fibre-, NDF- ADF- and ADL content of ingredients according to available research outcomes is highly recommended in the course of feed formulation.

According to the correlation investigation of the diverse testing methods (allocation of fibre content by calculation and by chemical measurement; calculated, chemical and NIRS nutrition value investigation) we can declare that there is a moderate-strength correlation between the investigated parameters. According to this, the NIRS method could be an alternative measurement tool for the local verification and rapid measurement of the real nutrition contents of the formulas and feed mixtures in case of swine feeding.

According to the examination of feed samples and the comparison of evaluation methods we can declare that upon formulation, the Hungarian nutrition experts dealt with the crude fibre content of compound feeds cautiously. Thus, the compound feeds based on formulas with relatively smaller fibre content that only marginally meets the recommended standard (at least 35 g/kg crude fibre content) will contain only a small amount of crude fibre content in production. Upon looking at the outcomes we can see that the calculated crude fibre content did not always meet the real crude fibre content outcomes collected by chemical analysis. In most of the cases, the real crude fibre content was lower than the calculated value. Due to this, we recommend the adjustment of higher crude fibre values in case of feed compounds for growing and finishing pigs as well as the continuous monitoring of nutrition content values of feed mixtures either by chemical or by NIRS method.

The NDF-, ADF- and ADL-content of the analysed feed compounds as well as their calculated hemicellulose- and cellulose content presented significant differences. This could be explained by the use of diverse basic ingredients and by the different ratio of the included by-products. The content ratio of by-products in the formulas deviated between 3.8% and 14.45%. We can declare that domestic practice still deals with the subject of by-products and their applied quantity cautiously in the course of feed production.

In our performance experiment with [(Danish Landrace × Danish Large White) × Danish Duroc] hybrid genotypes, the content ratio of extracted soybean meal in the compound feeds produced according to net energy value (NE_s) or standardized ileal digestible (SID) amino acids, could be significantly reduced from 13.4% to 3.5% in the growing phase (0-42 days) and from 10.5% to 1.7% in the finishing phase (42-67 days). The reduction of soybean meal ratio could be implemented by the increase of the by-product ratio, in the growing phase from 0% to 26.8%, and in the finishing phase from 0% to 32.8%. Our outcomes confirm the possibility that the ratio of “traditional protein sources” just as extracted soybean meal could be radically reduced in the formulas, upon their supplementation with various by-products. The fact, that our formula contained 4% and 2% extracted soybean meal in the growing and the finishing phase (respectively), confirmed that the composition of our compound feed

provided appropriate nutrition support for the high-performance hybrid genotypes (average daily weight gain 840 g in the growing, and 1480 g in the finishing phase). It projects the supposition that extracted soybean meal is not a complimentary component of the feed of pigs with high-performance genotype. These outcomes prove that the diverse by-products (even several by-products simultaneously) could be successfully included in the feed formulas of growing- and finishing pigs upon the use of feed rations formulated by the calculation of net energy value (NE_s) and standardized ileal digestible amino acids (SID).

Based on the outcomes of a miographic pilot study, which aimed for the examination of the smooth muscle tissue of the gastrointestinal system, and was conducted with a growing pig in anaesthesia, we found the measuring method and the myographic measuring tool suitable for the detection of the myographic changes occurring in the smooth muscle tissue that composes the gastrointestinal tract of the pig. The hardware parts (holter device and wires), as well as the used ISO/Myo software was suitable for the evaluation and the visual display of the myoelectric signals. According to this we can declare that from the cluster of electrogastrographic methods (EGG), smooth muscle electromyography (SMEMG) – that deals with the examination of the myographic signals detected from the smooth muscle tissue of the different organs of the gastrointestinal tract – can be applied as a diagnostic method in case of pigs.

In case of the use of a feed compound containing 4% Opticell fibre supplementation, the maximum frequency value of the smooth muscle contraction (Y max), as well as the number of contraction of the smooth muscle, measured in cpm (cycles per minute) significantly deviated ($p<0.001$) from the control dietary treatment group (2.80% crude fibre content). Thus, the investigation of the maximum frequency value (Y max) of the action potentials generated by the smooth muscle tissue of the gastrointestinal tract, and the number of contraction cycles per minute (cpm: cycles per minute) proved to be effective for the myographic investigation describing the use of feed compound with 4% Opticell fibre supplement.

As a result of our feeding experiment with high fibre content feed adapted to growing pigs (*Szűcs* et al., 2016), conducted with the use of SMEMG testing method on free moving [(Danish Landrace × Danish Large White) × Danish Duroc] hybrid pigs, we determined that the effects of the use of a compound feed with increased (4.9%) crude fibre content could be confirmed upon the use of organ-specific filters in the course of the analysis of myographic results. In case of different organs of the gastrointestinal tract – stomach (3-5 cpm), small

intestine (20-25 cpm), and large intestine (1-3 cpm), the frequency ranges determined by *Szűcs* et al. (2016) – and in the course of human experiments – could also be effectively used with regards to pigs.

In comparison with the control, low fibre content (2.9%) diet, the absolute value of maximum power spectrum density (PsD_{max}, mV^2) significantly increased ($p<0.001$) by the use of experiment feed compounds with higher fibre content. Thus, according to the received PsD_{max} values, we can determine that the contractions of the smooth muscle tissue of the small intestine were more intensive in case of the use of experiment compound feed with increased fibre content. Both the presence of fibres in the experiment feed and the significant deviation between PsD_{max} values due to the effects of consuming experiment feed became detectable by SMEMG measuring method. Thus, we were able to detect real, measurable differences by the use of this diagnostic method. These outcomes could greatly contribute to the investigation of real motility, thus – in later research endeavours – to the precise measurement of transit times.

5. NEW SCIENTIFIC RESULTS

1. In the performance experiment, conducted with growing and finishing pigs [(Danish Landrace × Danish Large White) × Danish Duroc] between 40-110 kg body weight, up to 30% by-products (maize DDGS, soybean hull, wheat bran, sugar beet pulp), under 18% NDF content in growing- and 22% NDF content in finishing phase, the amount of extracted soybean meal could be reduced from13.4% to 3.5% in growing phase, and from 10.50% to 1.70% in finishing phase, without any statistically detectable deteriorations ($p>0,05$) in the important performance parameters (average body weight, daily feed intake, and weight gain/feed intake ratio). In case of higher ratio of by-products, we recommend the formulation of the compound feed according to net energy value (NE_s) and standardized ileal digestible (SID) amino acid content.

2. I confirmed that the electromyographic investigation of the smooth muscle tissue of the gastrointestinal system Smooth muscle electromyography (SMEMG), as a new non-invasive test method of the smooth muscle tissue of the gastrointestinal system is useful for the measurement of the activity of the different segment of the gastrointestinal tract (stomach, small- and large intestine) in case of free moving awake growing pigs *in vivo*.

3. By the use of SMEMG investigation method adapted to growing pigs, in a feeding experiment conducted with [(Danish Landrace × Danish Large White) × Danish Duroc] genotype hybrids (age: 72±3 days, average body weight: 30±3 kg, in individual pens, without moving limitations, it was determined that the use of a compound feed with increased crude fibre content (4.9%) significantly ($p<0.001$) increased the motility of the smooth muscle tissue in the small intestine. In comparison with the control, low fibre content (2.9%) diet, the absolute value of maximum power spectrum density (PsD_{max}, mV^2) significantly increased ($p<0.001$) by the use of experiment feed compounds with higher fibre content. Thereby, the contractions of the smooth muscle

tissue of the small intestine were more intensive in case of the use of experiment compound feed with increased fibre content.

4. By the use of the SMEMG investigation method adapted to growing pigs, among [(Danish Landrace × Danish Large White) × Danish Duroc] genotype hybrids, in individual pens, without moving limitations, it was determined that the effects of the use of a feed compound with increased level of crude fibre (4.9%) could be confirmed by the use of organ specific filters in the course of the analysis of myographic results. In case of certain organs – stomach (3-5 cpm), small intestine (20-25 cpm), and large intestine (1-3 cpm) – the frequency ranges determined by *Szűcs* et al. (2016) and in the course of human experiments, could also be effectively used with regards to pigs.

6. SCIENTIFIC PAPERS ON THE SUBJECT OF THE DISSERTATION

6.1. PEER-REVIEWED PAPERS PUBLISHED IN FOREIGN SCIENTIFIC JOURNALS

NAGY, K. – FÉBEL, H. – HALAS, V. – TÓTH, T.: The effect of inclusion of fibre-rich by-products on the performance of growing and finishing pigs (pilot study). *Acta Agriculturae Scandinavica, Section A - Animal science*, 2021. Volume 70, Issue 1. 23-30.
https://doi.org/10.1080/09064702.2020.1829697.

6.2. PEER-REVIEWED PAPERS PUBLISHED IN HUNGARIAN SCIENTIFIC JOURNALS

NAGY, K. – FÉBEL, H. – TÓTH, T.: A rostellátás jelentősége a növendék sertések takarmányozásában: Irodalmi összefoglaló. Állattenyésztés és Takarmányozás, 2016. 65. 1-22.

NAGY, K. – FÉBEL, H. – HALAS, V. – TÓTH, T.: A hazai növendéksertés takarmánykeverékek jellemző nyersrosttartalma és rostösszetétele. Állattenyésztés és Takarmányozás, 2019. 68. 62-76.

6.3. PROCEEDINGS IN HUNGARIAN

NAGY, K.: A rostellátás korszerű megközelítése és új lehetőségei a sertés takarmányozásban. *Magyar Fajtatiszta Sertést Tenyésztők Egyesülete szakmai rendezvény*, Kaposvár - Gyomaendrőd, 2018.

NAGY, K. – FÉBEL, H. – SUDÁR, G. – TOSSENBERGER, J. – VIDA, O. – TÓTH, T.: A hazai növendéksertés-takarmánykeverékek nyersrosttartalmának és rostfrakcióinak vizsgálata. *XXIII. Ifjúsági Tudományos Fórum*, Pannon Egyetem Georgikon Kar, Keszthely. 2017. 1-6.

NAGY, K. – FÉBEL, H. – SUDÁR, G. – TOSSENBERGER, J. – GROSZ, GY. – TÓTH, T.: Miográfiás vizsgálatok alkalmazhatóságának lehetőségei növendék sertésekkel végzett kísérletben nagy rosttartalmú takarmány etetésekor (előzetes eredmények). *XXII. Ifjúsági Tudományos Fórum*, Pannon Egyetem Georgikon Kar, Keszthely. 2016. 1-7.

NAGY, K. – FÉBEL, H. – SUDÁR, G. – TOSSENBERGER, J. – GROSZ, GY. – TÓTH, T.: Emelt rosttartalmú takarmány etetésének hatása a gasztroenterális rendszer simaizom szövetében jelentkező akciós potenciálváltozásokra növendék sertésnél. *XXXVI. Óvári Tudományos Nap: Hagyomány és innováció az agrár- és élelmiszergazdaságban I-II.* Nyugat-magyarországi Egyetem Mezőgazdaság-és Élelmiszertudományi Kar, Mosonmagyaróvár. 2016. 419-425.

NAGY, K.: A rostellátás korszerű megközelítése és új lehetőségei a sertés takarmányozásban. *Agrifirm Magyarország Zrt. és Nuscience Hungary Kft. Sertéshús termelés gazdaságosan szakmai rendezvény*, Budaörs, 2015.

NAGY, K. – FÉBEL, H. – TÓTH, T.: A rost szerepe a növendék sertések takarmányozásában: Irodalmi összefoglaló. *XXI. Ifjúsági Tudományos Fórum*, Pannon Egyetem Georgikon Kar, Keszthely. 2015. 1-6.

6.4. INFORMATIVE PROCEEDINGS IN HUNGARIAN

NAGY, K. – HALAS, V. – FÉBEL, H. – TÓTH, T.: Melléktermékek alkalmazásának irányelvei a nagy teljesítményű növendék - és hízósertés takarmányozásában. *Agro Napló,* 2020. 26. 64-66.

NAGY, K. – FÉBEL, H. – TOSSENBERGER, J. – SUDÁR, G. – HALAS, V. - TÓTH, T.: A rostfrakcióra alapozott takarmányozás a növendék sertéseknél. *Agro Napló,* 2017. 21. 81-83.

NAGY, K. – SUDÁR, G. – FÉBEL, H. – TOSSENBERGER, J. – TÓTH, T.: A nyersrostellátás újszerű megközelítése a növendék-hízósertések takarmányozásában. *Agro Napló,* 2016. 20. 130-131.

SUDÁR, G. - NAGY, K. – FÉBEL, H. – TOSSENBERGER, J. – TÓTH, T.: A melléktermékek jelentősége a növendék-, hízósertések takarmányozásában. *Agro Napló,* 2016. 6. 110-111.

7. OTHER PUBLICATIONS

7.1. PROCEEDINGS IN HUNGARIAN

VIDA, O. – EGRI, B. – NAGY, K. –TÓTH, T.: Különböző glicerinforrások etetésének vizsgálata szoptató kocák takarmányozása során (Bevezető eredmények). *XXIII. Ifjúsági Tudományos Fórum*, Pannon Egyetem Georgikon Kar, Keszthely. 2017. 1-6.

NAGY, K.: Az antibiotikum felhasználás csökkentésének lehetséges alternatívái a sertés takarmányozásban. *Agrifirm Magyarország Zrt. és Nuscience Hungary Kft. Sertés Szakmai Nap*, Budaörs, 2016.

NAGY, K.: Egy vaskészítmény alkalmazásának hatása a malacok néhány termelési mutatójára és élettani paramétereire. XXXII. Országos Tudományos Diákköri Konferencia Agrártudományi Szekció. Szegedi Tudomány Egyetem, Hódmezővásárhely. 2015. 303.

NAGY, K. – ZSÉDELY, E. – RÓZSA, L. – FÉBEL, H. – TÓTH, T.: Egy vaskészítmény alkalmazásának hatása a malacok néhány termelési mutatójára és élettani paramétereire. *XXXV. Óvári Tudományos Nap: A magyar és nemzetközi agrár-és élelmiszer-gazdaság lehetőségei.* Nyugat-magyarországi Egyetem Mezőgazdaság-és Élelmiszertudományi Kar, Mosonmagyaróvár. 2014. 492-497.

7.2. PROCEEDINGS IN ENGLISH

NAGY, K. – ZSÉDELY, E. – CSAVAJDA, É. – RÓZSA, L. – FÉBEL, H. – TÓTH, T.: Effect of dietary iron supplementation on growth performance and haematological status in young pigs. *KRIMVA 2015: XXII Medunarodna Savjetovanje – 22nd International Conference*. Opatija, Croatia. 2015. Book of abstract, 45.

The doctoral dissertation was supported by the EFOP-3.6.3-VEKOP-16-2017-00005 project by the European Union and the European Social Fund.