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**EVALUATION THE EFFICIENCY OF
NUTRITIONAL METHODS TO DECREASE THE
AMMONIA EMISSION OF PIG FATTENING**

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1. Background and objectives

Ammonia emission is a major air quality concern at regional, national, and global levels and animal production is the main source of ammonia emission. The emissions from farm animals are a critical factor. It is estimated that 80-90% of ammonia (and nitrogen) emissions in the European Union are related to animal husbandry. In the case of Hungary, this value is around 70%, and the pig sector is responsible for 21% (LOVAS 2015). To improve air quality in the EU, the Directive 2016/2284 has been developed to reduce the national emissions of certain atmospheric pollutants. According to this, the member states must reach certain reduction. In Hungary after 2030, 32 percent reduction in ammonia emissions is expected compared to that of 2005 (NEC 2016).

There are a number of feeding options available to reduce N-excretion in animals. This can be achieved by multi-phase feeding (BOISEN et al. 1991, KOCH 1990, VAN DER PEET-SCHWERING and VOERMANS 1996) or by feeding low protein (LP) diets with crystalline amino acid supplementation (BAT 2015, BITTMAN et al. 2014, CHAN et al. 1998a, CARTER and KIM 2013, DUBLECZ 2011, GATEL and GROSJEAN 1992, KERR 2003, NIYAZOV and OSTRENKO 2020, POWERS et al. 2007, SCHUTTE et al. 1993, LI et al. 2015, WANG et al. 2018). Various studies have shown that if crystalline amino acid supplementation takes place, the crude protein content of feed can be reduced by 30-40 g/kg without affecting the performance of the animals (NIYAZOV and OSTRENKO 2020, CHAN et al. 1998b, DOURMAD et al. 1993, LENIS and SCHUTTE 1990, HAN and LEE 2000, KERR et al. 2003, CARPENTER et al 2004, ZHAO et al. 2019). According to the literature data, 1% protein reduction can decrease ammonia emissions by about 10%

(DUBLECZ 2011, WANG et al. 2018, FVM 2008, GAY 2008, FIGUEROA et al. 2000), but there is limited information on the impacts of the genotype and the age of fattening pigs when LP diets are fed. Presumably the relationship between the protein reduction and the excretion of the different N forms are not linear. There are also limited information available on that. Nitrogen balance studies have also shown that crude protein reduction has greater effect on urinary than faecal N excretion (CHAN et al. 1998a, PORTEJOIE 2004, O'CONNELL et al. 2006, O'SHEA et al. 2009). The main source of the so called total ammoniacal nitrogen (TAN) is the urinary N, mainly urea (HUTCHINGS 2015).

Additional feeding options that affect ammonia emissions are to reduce the proportion of urinary N excretion or decreasing the pH of the urine (BITTMAN et al. 2014, DUBLECZ 2011, GAY 2008, AARNINK and VERSTEGEN 2007). Reducing the amount of N excreted in the urine can be achieved by increasing the amount of fermentable non-starch polysaccharides (NSP) in the feed. Such carbohydrates promote the bacterial fermentation in the colon and this way more ammonia is converted into faecal bacterial protein, which is more slowly converted to ammonia in the manure (O'SHEA et al. 2009, AARNINK and VERSTEGEN 2007, LOW 1985, PHILIPPE et al. 2011, PHILIPPE et al. 2015). However, higher NSP levels above a certain level can also have negative effects by reducing the digestibility of nutrients (BITTMAN et al. 2014, SZABÓ and HALAS 2011). So, there are no such fermentable NSP levels and NSP sources defined, that are effective in ammonia emission without affecting the production traits.

The pH of urine depends on the ratio of acidic and basic substances excreted from the body (BITTMAN et al. 2014, DUBLECZ 2011, PHILIPPE et al. 2011, SZABÓ and HALAS 2011). The acidity of the urine can be acidified, for example, by adding acidifying Ca salts (CaSO_4 , CaCl_2 , Ca-benzoate) to the feed instead of the basic calcium carbonate (CaCO_3) (BITTMAN et al. 2014, DUBLECZ 2011, PHILIPPE et al. 2011, SZABÓ and HALAS 2011, NØRGAARD et al. 2010). In this case, the pH of the urine decreases by 1.6–1.8%, resulting in a 26–53% reduction in NH_3 emissions from urine and slurry (DUBLECZ 2011). The above-mentioned alternatives of CaCO_3 however, can also cause electrolyte imbalances and this way reduced feed intake and impaired growth.

In the course of this research our goal was to gain more accurate data on the N metabolism of the most common pig genotypes of the country and to use these results to improve the accuracy of the recent ammonia inventory calculations. Our further objective was to find out differences between the N-retention and excretion of fattening pigs of different live weight categories. Among the nutritional strategies the effects of feeding low protein diets, using 6 fattening phases instead of the most common 4 phases, feeding sugar beet pulp as a fermentable fibre source and Ca-benzoate as a potential acidifier of the urine was examined. Beside the individual treatments their combination was also investigated. In our animal trials it was an important condition, that the treatments should have been fit into the practical range, without affecting the growth of the animals.

2. Materials and methods

2.1. First trial

In the first experiment, we examined the impacts of age, genotype and feeding low-protein diets on the N-balance parameters of fattening pigs. We carried out our experiments according to the methodology of Nitrogen balance studies formulated by Czakó J. (REGIUSNÉ MŐCSÉNYI, 1982).

2.1.1. Animals, housing and treatments

Altogether 60 weaned male piglets, 30–30 of two genotypes were selected to have similar live weight and placed into 6-floor pens of 10 pigs per pen. The size of pens was 3.5 x 3.4 m. Wheat straw was used as bedding material, and the pens were equipped with self-feeders and drinkers. The manure was removed daily. Half of the piglets were Hungarian Large White (HLW), belonging to the late-maturing types of meat-type pigs, with high growth potential until a high body weight. The remaining 30 animals were crossbred pigs (Topigs 20 x Danbred Duroc) (DB) and represented the early maturing types of meat pigs, with more intensive growth potential and higher protein requirements in the early phases of fattening. Four phases fattening was carried out in the live weight categories of 20–30 kg, 30–40 kg, 40–80 kg and 80–110 kg. Starter, grower I, grower II and finisher diets were fed between days 53–71 and 72–80 and; 81–127 and 128–162, respectively. The diets of both groups were composed according to the requirements (Magyar Takarmánykódex 2004) of the intensive crossbred (DB) and semi-intensive Hungarian Large White (HLW) genotypes. Diets were formulated on a standardized ileal digestible (SID) amino acid basis. The SID amino acid contents of feedstuffs were evaluated by NIR. Besides a normal crude protein-containing control diet (C), two low-protein (LP)

diets were fed. The protein reductions in each phase were 1.5 (T1.5) and 3% (T3). The diets contained maize, wheat and extracted soybean meal as main ingredients. All feeds contained crystalline lysine, methionine and threonine. Tryptophan supplementation was needed only in the low-protein diets. As the protein content of the diets reduced, the proportion of maize, sunflower oil and crystalline amino acids increased, while the amount of extracted soybean meal decreased. The proportion of soybean meal in T3 diets was on average 10% lower, compared with the control diets. The diets were formulated to be isocaloric, and the main difference in their nutrient content was only in crude protein.

2.1.2. Sampling

In each age category, 6 pigs per pen with similar live weight were selected and transferred to a different room containing specific balance cages. The cages were equipped to collect separately the total amounts of urine and faeces. The amount of daily feed was calculated as 95% of the ad libitum feed intake. Daily rations were distributed in two equal portions and given to the animals at 7.00 a.m. and 3.00 p.m. Water was provided ad libitum. In the balance cage room, heat blowers were used for heating and an exhaust air chimney for ventilation. The room temperature was 18 ± 2 °C. Nine hours of the daily light period was applied with 80 lux. The NH₃ and CO₂ air concentrations were measured daily with a Draeger equipment (Draeger x-am 5600). The ranges of CO₂ and NH₃ air concentrations were 400–1100 and 0–2.6 ppm, respectively. The balance experiment took 7 days. After 2 days adaptation in the subsequent 5 days, the total amounts of faeces and 100 mL of urine were collected daily and stored at - 20 °C. To reduce the nitrogen loss from the urine, 20 mL of 5% sulfuric acid was

poured into the urine containers. Before the analytical procedure, daily faeces and urine samples from every pig were mixed, homogenized, and a representative sample of about 500 mL urine and 500 g faeces was used for nitrogen analysis.

2.1.3. Chemical analyses

From the faeces and urine samples, their N contents were determined according to the Kjeldahl method with a Foss–Kjeltec 8400 Analyzer (Nils Foss Allé 1, DK-3400 Hilleroed, Denmark), and the most important N-balance parameters were calculated. TAN percentage was calculated as the ratio of urinary N in the total N-excretion.

2.1.4. Feed Analyses

The experimental diets were analysed for dry matter (152/2009/EK III. appendix A), crude protein (152/2009/EK III. appendix C), crude fat (MSZ 6830-19:1979), crude fibre (152/2009/EK III. appendix I), raw ash (152/2009/EK III. appendix M), amino acid (159/2009/EK III. appendix F), N (MSZ EN ISO 5983-2), total P (ISO 6491:2001) and Ca (ISO 6869:2001) content.

2.1.5. Statistical Analyses

All data were analysed using the SPSS 22.0 software. The general linear model and univariate ANOVA was used with genotype, live weight and dietary protein content as main factors. Levene's test was used to test the equality of error variances. If the homogeneity of variance was adequate, Tukey's tests were used for runs with the same number of elements, while Hochberg's and Gabrielle's tests were used for different numbers of elements. If the Levene's test was significant Dunnett T3 post hoc test was

used. Linear regression was used to investigate the effect of N-intake on N-excretion within the genotypes and diet groups. Besides the significant effect of the main factors, their determination on the investigated parameters was also investigated using the partial Eta squared values of the univariate ANOVA. Our TAN results were also compared with the 70% default value, currently used in the Hungarian ammonia emission inventory calculation. The significance of these differences was investigated with one-sample T-test.

2.2. Second trial

In the second experiment the effect of different feeding factors and their combinations on the N-balance parameters of fattening pigs was examined. This experiment was also carried out according to the methodology of nitrogen balance studies developed by Czakó J. (REGIUSNÉ MŐCSÉNYI, 1982).

2.2.1. Animals, housing and treatments

During the experiment, the control groups contained twenty, while the different treatment groups 10-10 crossbred (Topigs 20 x Danbred Duroc) (DB) weaned male piglets. The animals were selected to have similar live weight and placed into 8 floor pens of 10 pigs per pen. The conditions of the keeping were completely identical to those described in Chapter 2.1.1. The experimental diets were fed in 3 phases (30-40 kg; 40-80 kg; 80-110 kg). The different treatment groups were as follows:

- control (average protein, maize, soybean meal based diet) (C)
- 2% reduced crude protein diets with crystalline amino acid supplementation (P)
- feeds containing sugar beet pulp (10%) (S)

- mixing of Ca-benzoate to partially replace CaCO₃ (0,5%) (B)
- reduced crude protein content + sugar beet slices (PS)
- reduced crude protein content + Ca-benzoate (PB)
- reduced crude protein content + Ca-benzoate + sugar beet slices (PBS)

2.2.2. Sampling

In the second feeding phase, 12 animals from the control group and 6 animals from the other treatment groups were transferred to specific balance cages. The methodology of the nitrogen balance tests was identical to that described in Chapter 2.1.2. An additional test was performed in this trial, which was the measurement the pH of the fresh urine. The fresh urine required for this was collected during the morning feeding. The pH measurements were performed with an Adwa Waterproof AD12 type instrument.

2.2.3. Chemical and feed analysis

The analysis during the second trial was the same as described in the 2.1.3. and 2.1.4. chapters.

2.2.4. Statistical analysis

The statistical evaluation was performed using the SPSS 22 program. One-way analysis of variance was used to test whether there was a significant difference between the treatments for each parameter ($p < 0.05$). If the homogeneity of variance was adequate the Tukey, if the Levene's test was significant, the Dunnett T3 post hoc test was used.

2.3. Third trial

In the third experiment, the effect of the number of fattening phases on the production traits and N-balance parameters of fattening pigs was examined.

2.3.1. Animals, housing and treatments

During the experiment, to the different feeding groups 35-35 crossbred (Topigs 20 x Danbred Duroc) (DB) weaned male piglets were selected to have similar live weight and placed into 5-5 floor pens of 7 pigs per pen. The housing and care of the animals were identical to those described in the case of group housing described in Chapter 2.1.1. During their feeding, in the case of the control groups four phases (20-30 kg; 30-40 kg; 40-80 kg; 80-110 kg), while in the case of the experimental groups, 6-phase feeding (20-30 kg; 30-40 kg; 40-60 kg; 60-80 kg; 80-100 kg; 100 kg-) were used.

2.3.2. Sampling

The feed consumption was measured on pen basis every day, while the live weight of the animals was measured every week on Monday. Live weight was measured using a digital animal scale with an accuracy of 0.1 kg. From the data the weight gain and the feed conversion ratio were calculated. The N-retention of the animals was calculated from the values of N-intake and weight gain for the given phase. The average N content of the pig carcass - in accordance with the international literature - was taken into account as 2.56% (DÄMMGEN et al. 2013).

2.3.3. Chemical and feed analysis

The analysis during the third trial was also the same as described in the previous 2.1.3. and 2.1.4. chapters.

2.3.4. Statistical analysis

The obtained data were evaluated using the SPSS 22 program using a two-sample T-test (Independent Samples T Test) ($p < 0.05$).

3. Results and discussions

The results of the most important N-balance parameters received during the first experiment are shown in Table 1.

Table 1. N-flow parameters of fattening pigs as influenced by live weight, genotype and dietary protein content.

		N intake (g/day)	Faecal N (g/day)	N digestibility (%)	Urinary N (g/day)	TAN (%)	Total N excretion (g/day)	N retention (%)
Live	20-30	38.22 ^d	5.53 ^d ±0.24	85.47 ^a ±0.4	10.38 ^c ±0.65	61.50 ^{ab} ±1.15	15.91 ^c ±0.62	58.67 ^a ±1.07
	30-40	43.98 ^c	7.25 ^c ±0.24	83.51 ^b ±0.4	10.32 ^c ±0.64	57.56 ^b ±1.13	17.56 ^c ±0.61	60.33 ^a ±1.05
	40-80	63.00 ^b	9.86 ^b ±0.25	84.56 ^{ab} ±0.4	16.81 ^b ±0.69	61.31 ^{ab} ±1.21	26.67 ^b ±0.65	58.96 ^a ±1.13
	80-	73.28 ^a	12.61 ^a ±0.25	82.92 ^b ±0.4	24.49 ^a ±0.67	65.04 ^a ±1.81	37.10 ^a ±0.64	50.22 ^b ±1.10
Diet	C	62.92 ^a	10.64 ^a ±0.21	83.31 ^b ±0.4	19.62 ^a ±0.57	63.34 ^a ±1.01	30.27 ^a ±0.54	53.24 ^b ±0.94
	T 1.5	52.80 ^b	7.98 ^b ±0.21	85.26 ^a ±0.4	14.45 ^b ±0.58	62.53 ^a ±1.02	22.43 ^b ±0.55	59.10 ^a ±0.95
	T 3	48.14 ^c	7.81 ^b ±0.21	83.77 ^b ±0.4	12.42 ^c ±0.57	58.19 ^b ±1.00	20.23 ^c ±0.54	58.80 ^a ±0.93
Genotype	DB	60.87 ^a	9.67 ^a ±0.17	84.16 ^a ±0.3	20.09 ^a ±0.46	66.27 ^a ±0.80	29.75 ^a ±0.43	51.63 ^b ±0.75
	HLW	48.37 ^b	7.95 ^b ±0.18	84.07 ^a ±0.3	10.91 ^b ±0.48	56.44 ^b ±0.85	18.87 ^b ±0.46	62.47 ^a ±0.79
	Poole	1.564	0.327	0.266	0.803	0.850	1.049	0.890
p-values								
Live weight		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Genotype (G)		0.0001	0.0001	0.824	0.0001	0.0001	0.0001	0.0001
Diet (D)		0.0001	0.0001	0.001	0.0001	0.001	0.0001	0.0001
GxLW		0.0001	0.0001	0.001	0.0001	0.001	0.0001	0.0001
GxD		0.0001	0.0001	0.0001	0.276	0.042	0.016	0.479
LWxD		0.0001	0.0001	0.061	0.009	0.037	0.0001	0.413
GxLWxD		0.0001	0.0001	0.0001	0.013	0.001	0.001	0.05

a–d: Mean values in one column within a main effect not sharing a common letter differ significantly ($p < 0.05$)

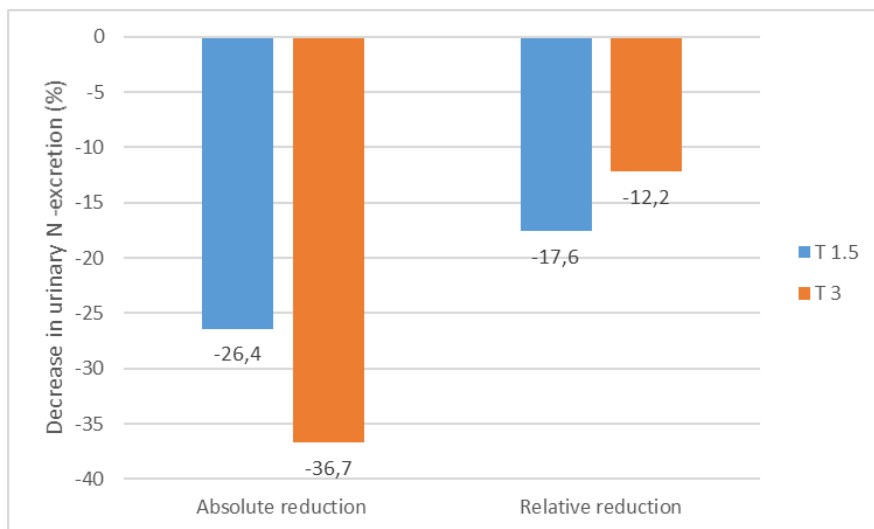
DB: Topigs 20 x DanBred Duroc; HLW: Hungarian Large White; C: control diets

T1.5: Low protein diet with 1.5% protein reduction; T3: Low protein diet with 3% protein reduction.

Based on our results, it can be stated that all the investigated main factors, the genotype, the age of pigs and the protein content of the diets have significant effects on the N-balance of fattening pigs. Among the N-balance traits, N-digestibility was hardly influenced. The urinary N ratio of the total excreted N, which is responsible mostly for the ammonia emission from

the manure, showed the highest variance in response to the treatments. The TAN content of excreted N until 80 kg live weight is about 60% but increases afterward to 65%. In our balance trial, HLW pigs of the semi-intensive genotype were more favourable from the N-and TAN excretion point of view, which means less ammonia emission. Regarding the dietary treatments, compared with the control the TAN, was decreased only at a 3% protein reduction from 63.3% to 58.2%. Remarkably, a 10% difference was found in this trial between the TAN of the two different genotypes. The reason for this was mostly the significantly higher urinary N excretion of the DB pigs. Feeding pigs an LP diet is one of the most efficient ways to mitigate ammonia emission. From our results, however, it can be concluded that the effect of dietary protein reduction is not linear (Figure 1.).

Figure 1. Effects of feeding LP diets on the reduction in urinary N excretion.



T1.5: Low-protein diet with 1.5% protein reduction; T3: Low-protein diet with a 3% protein reduction.

In the case of 1.5% dietary protein, a decrease of 17.6% TAN and ammonia emission reduction can be calculated for each percentage protein decrease. This decrease is lower (12.2%) at a 3% dietary protein reduction. It should be considered if the effects of LP diets on ammonia emissions are calculated. The measured N-balance parameters - N-excretion, TAN-content of the excreted N and N-retention of pigs - are more favourable than the default values which can be found in the official recommendations (Figure 2-3.). Using the more detailed, national, measured parameters can help to reach the ammonia mitigation goals of the EU member states.

Figure 2. Total N-excretion (kg/animal/year) as influenced by genotype, age, and dietary crude protein content.



The currently used default values are visible above the red lines.

a-c: The different letters indicate a significant difference within the given factors ($p < 0.05$)

A" genotype: Topigs 20 x Danbred Duroc; „B" genotype: Hungarian Large White; Treatment 1: - 1.5% crude protein; Treatment 2: 3% crude protein

Figure 3. Total ammoniacal nitrogen (TAN) content of the total excreted nitrogen (%) as influenced by genotype, age, and dietary crude protein content.



The currently used default value is visible above the red line.

a, b: The different letters indicate a significant difference within the given factors ($p < 0.05$)

"A" genotype: Topigs 20 x Danbred Duroc; „B" genotype: Hungarian Large White; Treatment 1: - 1,5% crude protein; Treatment 2: 3% crude protein

In the second experiment, beside feeding LP diets (2% reduction in protein), the effects of using benzoic acid, sugar beet pulp and their combinations were studied. The results of this trial can be found in Table 2. In this trial the balance experiment was carried out in the 55-66 kg live weight category. Feeding sugar beet pulp at 10% inclusion rate decreased N-digestibility, but resulted the lowest urinary N-excretion and TAN %. It means, sugar beet pulp, and its fermentable fibre content can push the N-excretion of animals toward the faecal excretion. Feeding the LP diet in this trial decreased the TAN excretion by about 10%. Benzoic acid at 0.5% inclusion rate failed to modify the N-balance parameters and the pH of the urine. The modulatory effects of the treatments were not additive.

Table 2. N-flow parameters of fattening pigs as influenced by different feeding factors and their combinations.

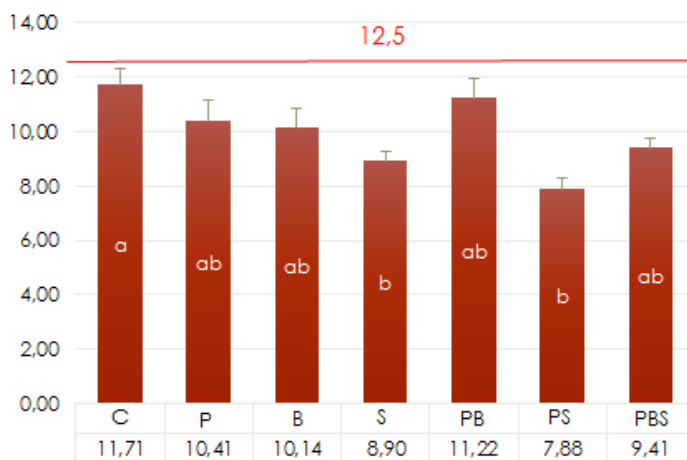
Treatments	C	P	B	S	PB	PS	PBS
Faecal N	7.57	9.13	7.27	9,56	9.00	7.29	8.76
(g/day)	±0.43	±0.43	±0.23	±1,08	±0.53	±0.39	±0.51
N Digestibility	86.58 ^a	83.41 ^{ab}	85.72 ^a	81,1 ^b	83.43 ^{ab}	83.31 ^{ab}	84.15 ^{ab}
(%)	±0.73	±0.78	±0.44	±2,13	±0.74	±0.90	±0.93
Urinary N	24.5 ^a	19.38 ^{ab}	20.5 ^{ab}	14,83 ^b	21.73 ^{ab}	14.3 ^b	17.01 ^b
(g/day)	±1.52	±2.15	±1.77	±1,00	±1.87	±1.03	±0.53
Total N	32.07 ^a	28.51 ^{ab}	27.77 ^{ab}	24,39 ^b	30.74 ^{ab}	21.60 ^b	25.77 ^{ab}
Excretion	±1.72	±2.09	±1.87	±1,02	±1.99	±1.13	±0.92
(g/day)							
TAN (%)	76.14 ^a	67.04 ^{abc}	73.40 ^{ab}	60,98 ^c	70.29 ^{abc}	66.00 ^{bc}	66.10 ^{bc}
	±1.18	±2.95	±1.54	±3,94	±2.03	±1.85	±1.18
N Retention	43.50	48.19	45.45	51,80 [±]	43.62	50.56	53.37
(%)	±2.11	±3.79	±3.67	2,01	±2.36	±2.58	±1.66
Urine pH	8.66 ^{a±}	7.71 ^b	8.49 ^a	8,87 ^a	7.68 ^b	8.65 ^a	8.63 ^a
	0.09	±0.16	±0.07	±0,11	±0.09	±0.06	±0.07

a–c: The different letters indicate a significant difference within the given parameter ($p < 0.05$)

C: control; P: -2% crude protein; B: +0,5% benzoic acid; S: + 10 g/kg fermentable NSP; PB: reduced crude protein content + benzoic acid; PS: reduced crude protein content + fermentable NSP; PBS: reduced crude protein content + benzoic acid + fermentable NSP

In Hungary, N-excretion is currently calculated as 12.5 kg/animal/year for pigs over 50 kg in the national ammonia inventory. The N-excretion of the animals in the control group was close to this value, but it can be seen that by changing the composition of the feeds or using feed supplements, these N-excretion data become significantly more favorable (except for treatment PB) (Figure 4.). During the inventory calculation, we currently calculate with 70% TAN for all age groups and utilization types for pigs. Except for the PBS treatment our results hardly differ from this value(Figure 5.).

Figure 4. Total N-excretion (kg/animal/year) as influenced by different feeding factors and their combinations.

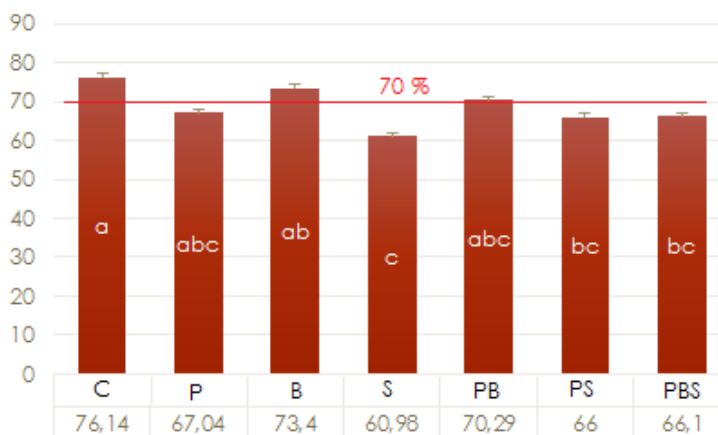


The currently used default value is visible above the red line.

a, b: The different letters indicate a significant difference ($p < 0.05$)

C: control; P: -2% crude protein; B: +0,5% benzoic acid; S: + 10 g/kg fermentable NSP; PB: reduced crude protein content + benzoic acid; PS: reduced crude protein content + fermentable NSP; PBS: reduced crude protein content + benzoic acid + fermentable NSP

Figure 5. Total ammoniacal nitrogen (TAN) content of the total excreted nitrogen (%) as influenced by different feeding factors and their combinations.



The currently used default value is visible above the red line.

a–c: The different letters indicate a significant difference ($p < 0.05$)

C: control; P: -2% crude protein; B: +0,5% benzoic acid; S: + 10 g/kg fermentable NSP; PB: reduced crude protein content + benzoic acid; PS: reduced crude protein content + fermentable NSP; PBS: reduced crude protein content + benzoic acid + fermentable NSP

The third trial was focusing on the effects of using different number of fattening phases (Table 3). The common 4 phases feeding was compared with a 6-phase fattening. The more diversified nutrient supply resulted less N-intake and more favourable, about 3.8% higher N retention in the 6-phase group. No significant differences in the production traits (feed intake, growth rate and feed conversion) were found. Multi-phase feeding can also decrease the feed prices. Using more diets can generate additional logistical costs, however, it can be compensated by the lower feed prices.

Table 3. Effect of multiphase feeding on the N-flow parameters

		1. phase	2. phase	3. phase	4. phase	whole fattening
N intake (kg)	4-phase treatment	0.61±0.02	0.27±0.00	2.53 ^a ±0.01	3.52 ^a ±0.03	6.93 ^a ±0.04
	6-phase treatment	0.60±0.02	0.27±0.00	2.48 ^b ±0.01	3.31 ^b ±0.03	6.66 ^b ±0.02
N retention (kg)	4-phase treatment	0.28±0.01	0.12±0.01	1.01±0.01	1.18±0.01	2.59±0.03
	6-phase treatment	0.27±0.01	0.13±0.01	1.00±0.01	1.19±0.03	2.59±0.02
N retention (%)	4-phase treatment	46.36±2.18	45.09±3.21	39.84±0.52	33.64 ^b ±0.34	37.46 ^b ±0.46
	6-phase treatment	44.59±1.65	48.01±3.45	40.55±0.49	35.84±0.68 ^a	38.88 ^a ±0.35

a, b: The different letters indicate a significant difference ($p < 0.05$)

4. Conclusion

From our results, it can be concluded that there are many feeding options available to reduce N and TAN excretion in fattening pigs. Based on our tests, among these options, feeding diets with reduced crude protein supplemented with crystalline amino acids is the most effective. Our results make it possible to clarify the effect of feeding protein-reduced feeds on the excretion of N and TAN in fattening pigs. The generally applied and accepted 10% N- and TAN excretion reduction per 1% dietary protein reduction is acceptable for Danbred fattening pigs, although there are significant differences between the excretion values of each weight category. However, in the Large White animals, belonging to the "B" genotype, a 1% reduction in dietary protein results in a 20% reduction in excretion. For this genotype, a reduction exceeding 1.5 already causes a smaller, 10% reduction in excretion.

Increasing the fermentable fibre content of the diet is an effective method to decrease the urinary N excretion., but in this case the digestibility of the nutrients can also decrease. It would therefore be worthwhile to test the effect of sugar beet pulp and other feeds rich in fermentable fibre under farm conditions, during fattening experiments, in order to see how it affects production parameters. Currently, there is not enough information available to consider the effect of the fermentable fibre content of feeds in reducing ammonia emissions. The effect of benzoic acid at a mixing ratio of 0.5% is not sufficient to change the pH of urine. It can be considered an important result that there is no meaningful interaction between the feeding of protein-reduced diets, the addition of benzoic acid and the feeding of sugar beet pulp when the treatments are used together.

In the case of using more fattening phases compared with the number of phases most often used in our country, the N intake of animals can be reduced without affecting the production parameters. Although this may generate additional logistics costs, it may even pay off economically due to the reduction of feed costs. However, it would be worth to repeat the tests with larger numbers of animals, also under farm conditions, because in this way it would be possible to find out whether the use of the method is plays off.

In relation to the efficiency test of the mentioned methods, it is important to note that repeating the experiments, performing them with more animals, adapting them to practical conditions and performing farm tests are extremely important. Nevertheless, I believe that our results provide a great help in clarifying the Hungarian ammonia emission situation and in order to improve the accuracy of the Hungarian ammonia emission inventory. During our experiments, we determined the N-balance parameters of the most common breeds, generally used in Hungary in the different live weight categories. The data obtained during our tests show that it would be worth to take the different genotypes into account when calculating the inventory, and to use more weight categories when calculating the N- and TAN excretion of fattening pigs.

It would also be necessary to review the recommendations of the Hungarian Feed Code, because the actual version was published in 2004, i.e. an 18-year-old work. During this time, there has been so much progress both the science and practice of pig fattening that innovation is inevitable. It is important for farmers not only to get recommendations from the feed

manufacture and breeding companies, but also independent from recommendations/guidelines.

5. New scientific results

1. The genotype of the fattening pigs, irrespectively form the age categories and protein content of the diets, has significant effect on the N-excretion of the animals. The Danbred pigs with higher growth potential excrete more urinary N, compared with the Hungarian Large White pigs.
2. Feeding low protein diets decrease the both the faecal and urinary N excretion. However, the decrease in N-excretion is not linear. When higher protein reduction is used, the decrease in N-excretion is relatively lower.
3. The measured N-excretion values below 80kg live weight and the total ammoniacal nitrogen (TAN) percentage of all live weight categories are lower, than the values used in the calculation of the Hungarian ammonia emission inventory.
4. Feeding 10% sugar beet pulp, as fermentable fibre source, decrease significantly the urinary N excretion of the fattening pigs in the 40-80 live weight category.
5. Using Ca-benzoate, as a feed additive at 0.5% in the diets of fattening pigs in the 40-80 kg live weight category, does not decrease the pH of the urine.
6. Using 6-phase fattening instead of 4 phases, the protein intake of pigs can be reduced without affecting the production traits. It results increased N-retention mostly in the live weight categories of 80-100 kg and above 100 kg.

Bibliography

1. AARNINK, A.J.A., M.W.A., VERSTEGEN (2007): Nutrition, key factor to reduce environmental load from pig production. *Livestock Science*, 109 194–203
2. BAT (2015): Best Available Techniques (BAT) Reference Document for the Intensive Rearing of Poultry or Pigs; European Commission, GG_KG/EIPPCB/IRPP_Final_Draft August 2015
3. BITTMAN, S. M., DEDINA C. M., HOWARD O., OENEMA M. A., SUTTON (2014): Options for Ammonia Mitigation Guidance from the UNECE Task Force on Reactive Nitrogen. CEH
4. BOISEN, S., J. A. FERNANDEZ, A. MADSEN (1991): Studies on ideal protein requirement of pigs from 20 to 95 kg live weight; P. 299 in Proc. 6th Int. Symp. Protein Metab. Nutr., Herning, Denmark
5. CANH, T.T., A.J.A. AARNINK,, Z. MROZ, W. JONGBLOED, J.W. SCHRAMA, M.W.A.VERSTEGEN (1998a): Influence of electrolyte balance and acidifying calcium salts in the diet of growing finishing pigs on urinary pH, slurry pH, and ammonia volatilisation from slurry. *Livestock Production Science*. 56. 1-13.
6. CANH, T.T., AARNINK, A.J.A., SCHUTTE, J.B., SUTTON, A.L., LANGHOUT, D.J., VERSTEGEN, M.W.A., SCHRAMA, J.W. (1998b): Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing, finishing pigs; *Livestock Production Science* 56, 181–191
7. CARPENTER, D. A.; O’MARA, F. P. AND O’DOHERTY, J. V. (2004): The effect of dietary crude protein concentration on growth performance, carcass composition and nitrogen excretion in entire grower-finisher pigs; *Irish Journal of Agricultural and Food Research* 43:227-236
8. CARTER S.D., HJ. KIM (2013): Technologies to reduce environmental impact of animal wastes associated with feeding for maximum productivity; *Animal Frontiers* Vol. 3, No. 3, 42-47
9. DÄMMGEN U., A. BERK, C. OTTEN, W. BRADE, N. J. HUTCHINGS, H-D. HAENEL, C. RÖSEMANN, S. DÄNICKE, M. SCHWERIN (2013): Anticipated changes in the emissions of greenhouse gases and ammonia from pork production due to shifts from fattening of barrows towards fattening of boars; *Landbauforschung Volkenrode* 63(1):47-60

10. DOURMAD, J.Y., HENRY, Y., BOURDON, D., QUINIOU, N., GUILLOU, D. (1993): Effect of growth potential and dietary protein input on growth performance, carcass characteristics and nitrogen output in growing-finishing pigs; Proceedings Congress on Nitrogen Flow in Pig Production and Environmental Consequences, Wageningen 8–11 June, pp. 206–211
11. DUBLECZ K. (szerk.) (2011): Takarmányozástan; Az Agrármérnöki MSc szak tananyagfejlesztése TÁMOP-4.1.2-08/1/A-2009-0010 projekt;
https://regi.tankonyvtar.hu/hu/tartalom/tamop425/0010_1A_Book_13_Takarmanyozastan/adatok.html; Keresőprogram: Google, kulcsszavak: sertéstakarmányozás lekérdezés időpontja: 2021.02.18.
12. FIGUEROA J. L., A. J. LEWIS, P. S. MILLER (2000): Nitrogen balance and growth trials with pigs fed low-crude protein, amino acid-supplemented diets; Nebraska Swine Report
13. FVM Vidékfejlesztési, Képzési és Szaktanácsadási Intézet megbízásából (2008): Az állattartás környezeti hatásai, helyzete és viszonya a hazai és EU szabályozáshoz. Szerződésszám: VKSZI/188/2008, GreenCapital-4/2008.
http://www.greenflow.hu/wp-content/uploads/2012/03/FVM_GREEN_%C3%A1llattart%C3%A1s-kv-i-hat%C3%A1sai.pdf; Keresőprogram: Google, kulcsszavak: állattenyésztés környezeti hatása, lekérdezés időpontja: 2016.09.05.
14. GATEL F. AND F. GROSJEAN (1992): Effect of protein content of the diet on nitrogen excretion by pigs; *Livestock Production Science*, 31 (1992) 109-120 Elsevier Science Publishers, B.V., Amsterdam
15. GAY, S. W. (2008): Ammonia Emissions and Animal Agriculture; Virginia Cooperative Extension
16. HAN, I. K., J. H. LEE (2000): The role of synthetic amino acids in monogastric animal production; *Asian Australasian Journal of Animal Sciences* 13 (4):543-560.
17. HUTCHINGS N., B. AMON, U. DÄMMGEN, J. WEBB (2015): EMEP/EEA emission inventory guidebook 2013 update July 2015 – Manure Management
18. KERR, B. J. (2003): Dietary manipulation to reduce environmental impact. In: 9th International Symposium on Digestive Physiology in Pigs, May 14–17, 2003, Banff, Alberta, Canada. p. 139–158

19. KERR, B. J.; YEN, J. T.; NIENABER, J. A. AND EASTER, R. A. (2003): Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs; *Journal of Animal Science* 81:1998-2007
20. KOCH F. (1990): Amino acid formulation to improve carcass quality and limit nitrogen load in waste; Proc. Carolina Swine Nutr. Conf., p 76-95., Carolina feed Ind. Assoc., Raleigh, NC
21. LENIS, N.P., SCHUTTE, J.B. (1990): Aminozuurvoorziening van biggen en vleesvarkens in relatie tot de stikstofuitscheiding; In: Jongbloed, A.W., Coppoolse, J. (Eds.), Mestproblematiek: aanpak via de voeding van varkens en pluimvee. Onderzoek inzake de mest en ammoniakproblematiek in de veehouderij, vol. 4. Dienst Landbouwkundig Onderzoek, Wageningen
22. LI Q.-F., N. TROTTIER, W. POWERS (2015): Feeding reduced crude protein diets with crystalline amino acids supplementation reduce air gas emissions from housing1; *J. Anim. Sci.* 2015.93:721–730, doi:10.2527/jas2014-7746
23. LOVAS, K. (2015): Mezőgazdasági kibocsátások a hivatalos leltárjelentésekben; Földművelésügyi Minisztérium, szakmai konzultáció, 2015. április 20.
24. LOW, A.G. (1985): Role of dietary fibre in pig diet; In: Haresign, W., Cole, D.J.A. (Eds.), Recent Advances in Animal Nutrition. Butterworths, London, UK, p. 87.
25. MAGYAR TAKARMÁNYKÓDEX, 2004
26. NATIONAL EMISSION CEILINGS (NEC) DIRECTIVE, (EU) 2016/2284 (2016)
27. NIYAZOV N.S.- AND A., K.S. OSTRENKO (2020): Effect of low-protein diets on the nitrogen balance and productivity of pigs; *Journal of Livestock Science* 11: 106-109
28. NØRGAARD J.V., J.A. FERNÁNDEZ, J. ERIKSEN, O.H. OLSEN, D. CARLSON, H.D. POULSEN (2010): Urine acidification and mineral metabolism in growing pigs fed diets supplemented with dietary methionine and benzoic acid; *Livestock Science* 134, 113–115

29. O'CONNELL, J.M., CALLAN, J.J., O'DOHERTY, J.V. (2006): The effect of dietary crude protein level, cereal type and exogenous enzyme supplementation on nutrient digestibility, nitrogen excretion, faecal volatile fatty acid concentration and ammonia emissions from pigs; *Animal Feed Science and Technology* 127, 73–88.
30. O'SHEA, C.J., LYNCH, B., LYNCH, M.B., CALLAN, J.J., O'DOHERTY, J.V. (2009): Ammonia emissions and dry matter of separated pig manure fractions as affected by crude protein concentration and sugar beet pulp inclusion of finishing pig diets; *Agriculture, Ecosystems & Environment* 131, 154–160.
31. PHILIPPE, F.X., CABARAUX, J.-F., NICKS, B. (2011): Ammonia emissions from pig houses: influencing factors and mitigation techniques. *Agric. Ecosyst. Environ.* 141, 245-260.
32. PHILIPPE F. X., M. LAITAT, J. WAVREILLE, B.AUDOUIN NICKS, J.-F. CABARAUX (2015): Effects of a high-fibre diet on ammonia and greenhouse gas emissions from gestating sows and fattening pigs; *Atmospheric Environment* 109, 197-204.
33. PORTEJOIE, S., DOURMAD, J.Y., MARTINEZ, J., LEBRETON, Y. (2004): Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs; *Livestock Production Science* 91, 45–55
34. POWERS W. J., S. B. ZAMZOW, B. J. KERR (2007): Reduced crude protein effects on aerial emissions from swine; *Engineering in Agriculture* Vol. 23(4): 539-546 _ 2007 American Society of Agricultural and Biological Engineers ISSN 0883–8542 539
35. REGIUSNÉ MŐCSÉNYI Á. (1982): Az anyagforgalmi kísérletek tervezése és lebonyolítása. p. 307-336. In: CZAKÓ J. (Szerk.): *Állattenyésztési kísérletek tervezése és értékelése*. Budapest: Akadémiai Kiadó, 545 p.
36. SCHUTTE, J. B., J. DE JONG, G. J. M. VAN KEMPEN (1993): Dietary protein is relation to requirement and pollution in pigs during the body weight range of 20-40kg; p: 259-263 in Nitrogen flow in pig production and environmental consequences – M. W. A Verstegen, L. A. den Hartog, G. J. M. van Kempen, J. H. M. Metz, ed. Pudoc Scienfitic Publishers, Wageningen, The Netherlands

37. SZABÓ CS., HALAS V. (2011): A takarmányértékelés alapjai, Oktatási segédlet az Állattenyésztő mérnöki, valamint a Takarmányozási és takarmánybiztonsági mérnöki (MSc) mesterszak hallgatói számára;
https://regi.tankonyvtar.hu/hu/tartalom/tamop425/0059_sertestakarmanyozas/adatok.html; Keresőprogram: Google, kulcsszavak: precíziós sertéstakarmányozás, lekérdezés időpontja: 2021.02.18.
38. VAN DER PEET-SCHWERING, C., M. VOERMANS (1996): Effects of feeding and housing on the ammonia emission of growing and finishing pig facilities; *Rep. Exp. Pig Stn., Rosmalen, The Netherlands* 10:17-19
39. WANG Y., J. ZHOU, G. WANG, S. CAI, X. ZENG, S. QIAO (2018): Advances in low-protein diets for swine; *Journal of Animal Science and Biotechnology* 9:60
40. ZHAO Y., G. TIAN, D. CHEN, P. ZHENG, J. YU, J. HE, X. MAO, B. YU (2019): Effects of varying levels of dietary protein and net energy on growth performance, nitrogen balance and faecal characteristics of growing, finishing pigs; *Brazilian Journal of Animal Science - R. Bras. Zootec.*, 48: e20180021

6. Publications related to the topic of the dissertation

Peer-reviewed technical article in foreign languages:

Geicsnek-Koltay I. A., Benedek Zs., Baranyai N. Hegedűsné, Such N., Pál L., Wágner L., Bartos Á., Kovács Á., Poór Judit, Dublec K. (2022): Impacts of Age, Genotype and Feeding Low-Protein Diets on the N-Balance Parameters of Fattening Pigs. Agriculture, 12: 1. 94. pp 15.

Peer-reviewed technical article in Hungarian:

Dublec K., Koltay I., Such N., Dublec F., Husvéth F., Wágner L., Péterné Farkas E., Márton A., Farkas V., Pál L. (2018): Lehetőségek a takarmányok nyersfehérje tartalmának csökkentésére monogasztrikus állatokban (Recent development on feeding low protein diets in monogastric animals), Állattenyésztés és Takarmányozás, 67.4 273-286.

Other peer-reviewed scientific publications:

Geicsnek-Koltay I. A., Zs. Benedek, N. Baranyai, L. Farkas, I. László, N. A. Such, L. Wágner, K. Dublec (2022): Effect of multiphase feeding on the production traits and nitrogen flow parameters of growing pigs. Ifjúság a tudományért Tanulmánykötet, Magyar Agrár- és Élettudományi Egyetem Georgikon Campus Keszthely, 143-148 ISBN: 978-615-6338-06-8

Geicsnek-Koltay I. A., Benedek Zs., Hegedűsné Baranyai N., Wágner L., Such N. A., Dublec K. (2022): A hazai sertésenyésztés takarmányozási aspektusai az ammónia emisszió tükrében. Ifjúság a tudományért Tanulmánykötet, Magyar Agrár- és Élettudományi Egyetem Georgikon Campus Keszthely, 154-158 ISBN: 978-615-6338-06-8

Geicsnek-Koltay I. A., Benedek Zs., Husvéth F., Farkas E., Such N. A., Dublec K. (2022): Az intenzív sertéstartás környezeti hatásai. Ifjúság a

tudományért Tanulmánykötet, Magyar Agrár- és Élettudományi Egyetem
Georgikon Campus Keszthely, 197-202 ISBN: 978-615-6338-06-8

Conference publication:

Koltay I. A., Benedek Zs., Hegedűsné Baranyai N., Such N. A., Farkas L., Nagy J., Szűcs K., Pál L., Wágner L., Dublec K. (2018): Csökkentett fehérjetartalmú tápok etetésének hatása sertések ammónia-emissziójára, Tavaszi Szél Konferencia, absztraktkötet 66.o. 2018. május 4-6. Győr ISBN:978-615-5586-26-2

Koltay I. A., Benedek Zs., Hegedűsné Baranyai N., Such N., Wágner L., Dublec K. (2018): A hazai sertésenyésztés az ammónia emisszió tükrében; Fókuszban a takarmányozás, XXIV. Ifjúsági Tudományos Fórum, 2018. május 24. Keszthely,

Koltay I. A.; Zs. Benedek; N. Hegedűsné Baranyai; Á. Kovács; S. Valásek; N. Such; V. Farkas; N. Török; L. Farkas; L. Wágner; K. Dublec (2019): The effects of genotype and age on the nitrogen excretion of young pigs. 18. BOKU-Symposium Animal Nutrition, Wien, 30. April, konferenciakötet 202-205

Koltay I. A., Benedek Zs., Husvéth F., Farkas E., Such N. A., Dublec K. (2019): Az intenzív sertésstartás környezeti hatásai. XXV. Ifjúsági Tudományos Fórum, május 23. Keszthely

Dublec K., Husvéth F., Márton A., Koltay I., Such N., M. A. Rawash, Mezőlaki Á., Pál L., Molnár A. (2019): Feeding low protein poultry and pigs diets- physiological, economic, and environmental aspects. International Symposium on Animal Science (ISAS). 20-29 ISBN: 978-86-7520-468-8

Koltay I. A. (2019): The effects of age and genotype on the nitrogen balance of fattening pigs; Proceedings conference of agronomy students with international participation, Čačak, Serbia; 14-16 august 2019.; ISSN 2334-9883; 373-380

Koltay, I. A.; Zs. Benedek; N. Hegedűsné Baranyai; L. Farkas; Ivett L.; N. Török; N. A. Such; L. Wágner; K. Dublec (2020): Effect of multiphase feeding on the production traits and nitrogen flow parameters of growing pigs. XXVI. Ifjúsági Tudományos Fórum, május 21. Keszthely. Konferencia iakiadvány 1-7. o. ISBN 978-963-396-143-8

Conference participation with poster:

I. A. Koltay; Zs. Benedek; N. Hegedűsné Baranyai; Á. Kovács; S. Valásek; N. Such; V. Farkas; N. Török; L. Farkas; L. Wágner; K. Dublec (2019): The effects of genotype and age on the nitrogen excretion of young pigs. 18. BOKU-Symposium Animal Nutrition, Wien, 30. April

Presentation in Hungarian:

Koltay I. A., Benedek Zs., Hegedűsné Baranyai N., Such N. A., Farkas L., Nagy J., Szűcs K., Pál L., Wágner L., Dublec K. (2018): Csökkentett fehérjetartalmú tápok etetésének hatása sertések ammónia-emissziójára, Tavaszi Szél Konferencia, 2018. május 4-6. Győr

Koltay I. A., Benedek Zs., Hegedűsné Baranyai N., Such N., Wágner L., Dublec K. (2018): A hazai sertésenyésztés az ammónia emisszió tükrében; Fókuszban a takarmányozás, XXIV. Ifjúsági Tudományos Fórum, 2018. május 24. Keszthely

Koltay I. A., Benedek Zs., Husvéth F., Farkas E., Such N. A., Dublec K. (2019): Az intenzív sertéstartás környezeti hatásai. XXV. Ifjúsági Tudományos Fórum, május 23. Keszthely

Koltay I. A. (2020): Ammónia emisszió a sertéstenyésztésben; SÜN (Sertés Ünnep) Konferencia 2020. február 19-21. Keszthely

Presentation in English:

Koltay I. A. (2019): The effects of age and genotype on the nitrogen balance of fattening pigs; Proceedings conference of agronomy students with international participation, 14-16. August, Cacak, Serbia.

Other:

Dublec K., Husvéth F., Wágner L., Dublec F., Hegyi O., Márton A., Bartos Á., Farkas V., Koltay I., Pál L. (2017): A fehérjetakarmányozás hatékonysága. Magyar Mezőgazdaság, 72. évfolyam, 2017. október 11., 28-31.

Research reports for the Ministry of Agriculture:

Authors: Hegedűsné Dr. Baranyai N., Dr. Dublec K., Benedek Zs., Koltay I. A.:

- „Sertéságazati kutatási feladatok elvégzése 2016-17.”
- „Sertéságazati kutatási feladatok 2017-18. A sertéságazatot érintő agrár-környezetvédelmi indikátorok meghatározása és tápanyag-hasznosítási kísérletek végzése 2017-2018”
- „A sertéságazatot érintő agrár-környezetvédelmi indikátorok meghatározása és tápanyag-hasznosítási kísérlet végzése 2018-2019”
- „Sertéságazati kutatási feladatok 2019 A sertéságazatot érintő agrár-környezetvédelmi indikátorok meghatározása.”