

## **Assessment of certain nitrogen metabolism indicators, enteric CH<sub>4</sub> and CO<sub>2</sub> emitted through manure related to different diets in barrow**

**Hăbeanu Mihaela <sup>\*1</sup>, Gheorghe Anca <sup>1</sup>, Lefter Nicoleta Aurelia <sup>1</sup>,  
 Untea Arabela <sup>1</sup>, Idriceanu Lavinia <sup>1</sup>, Ranta Mirela Felicia <sup>2</sup>**

\*Corresponding author: mihaela.habeanu@ibna.ro

<sup>1</sup>National Research Development Institute for Animal Biology and Nutrition (IBNA), Calea Bucuresti No.1, Balotesti, 077015, Ilfov, Romania;

<sup>2</sup>University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Agriculture, Department of Plant Culture, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania.

### ABSTRACT

The present work was planned to test the effects of 2 protein-oil rich ingredients which replaced an important part of classical soybean meal, on nitrogen (N) metabolism indicators. Simultaneous we aimed to assess performances, enteric methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) emitted through manure. A balance test was conducted with 15 barrows, 36 ± 0.24 Kg initial body weight, assigned in three groups in metabolism cages: 1. SC diet based on a classical diet (soybean meal, 13%); 2. SP diet based on peas *Tudor* variety 16% that replace 49% of SM; 3. SPF diet containing 8% soybean meal, 12% peas and 4% linseed *Lirina* variety. Faecal and urine were quantitatively collected in two balance periods. A semiautomatic Kjeldahl method was used for assessing the N. The Spotchem EZ SP-4430 was used for determining plasma protein and urea nitrogen (BUN). The regression equations were used to determine N metabolism indicators, CO<sub>2</sub> emitted and enteric CH<sub>4</sub>. An increase of 1.16 times of feed intake was noticed in the SP group compared to SC. The lipid intake was slightly higher in the SP fed group which contributed to an increase of energy intake compared to SC group (>1.17 times) and vs SPL fed group (>1.2 times). The average daily gain (ADG) decreased highly significant in the SP diet compared to the SC diet. Linseed adds in SPL diet contributed to obtaining performances closed to SC group. SP diets increased the N level slightly in faeces and urine. BUN concentration has a linear relation with urinary N. CO<sub>2</sub> emitted represent 28-32% of CO<sub>2</sub> exhalation. CO<sub>2</sub>

increased in the SP diet ( $P < 0.10$ ) due to the fact that HP value increased as well. No significant influence was noticed for  $\text{CH}_4$  estimated value, although a slight increase was observed in the SP diet. In conclusion, although certain N metabolism indicator was affected by peas diet, only a little part was significantly modified. An oil-rich ingredient such as linseed could successfully complement peas by diminishing its adverse effects.

**Keywords:** nitrogen, barrows, metabolism, excreta,  $\text{CO}_2$ ,  $\text{CH}_4$

## INTRODUCTION

For many years, due to the environmental concern, the pigs farm management targets to cut down pollutant level through nutritional tools. Nitrogen is one of the most important nutrients for piglets that is necessary for a large quantity throughout their life, mainly for building protein and the synthesis of amino acids and nucleotides. The nitrogen, as part of dietary protein, participates in various metabolic processes but is one of the most expensive nutrients in the feeding of the pigs and a critical contributor to the negative impact of the environment. An essential part of nitrogen input is lost by excreta in organic and inorganic form, and along with nitrogen the other undigested and indigestible dietary components are eliminated through the excretory system. Two-third from nutrients excreted are represented by nitrogen (Millet et al., 2018).

During the transport of the manure to the storage platform in contact with the air form ammonia ( $\text{NH}_3$ ) which volatilises in an amount depending on inorganic compounds. A large part of nitrogen from manure is stored in the soil and groundwater. The surplus of nitrogen which is not used by the plant can be subjected to processes of leaching and denitrification. Through denitrification, the nitrates are transformed by a microorganism into  $\text{N}_2$  gas and nitrous oxide ( $\text{N}_2\text{O}$ ). From a practical point of view, this process closed the nitrogen cycle (Wang et al., 2018).

One of the ways to minimize the nitrogen level in excreta consists of nutritional approach (Monteiro et al., 2010; Dourmad et al., 2017; Wang and al., 2018). In a previous study (Dourmad et al., 2017; Millet et al., 2018), it was shown that only about 32% up to 46% of nitrogen intake is retained by pigs. A common practice used was to adapt the diet to the pig's requirements (Millet et al., 2018) or reduce the dietary protein while the essential amino acids covered the nutritional requirements (Hăbeanu et al., 2015).

The nitrogen balance trials represent a way to obtain information about net using of protein, the relation between protein intake and losses and about the link between protein and energy balance.

Usually, a classical diet for pigs consists of corn and soybean meal. The latter is a by-product rich in protein, lysine and have an energy level close to corn, but is expensive and in Romania, the most important part comes from import. On the other hand, the fodder peas (*Pisum arvense*) usually had a superior crop in the same area, reaching in the world in 2018 years up to 21.22 Mtonnes/ha (FAOSTAT, 2018) with a quite good capitalization price; however, the area cultivated with fodder peas is fluctuating, depending on demand. The fodder peas are grown mainly for animal feed and can be a profitable plant if certain conditions are met. The peas provide the vast majority of the nitrogen requirement of the soil in symbiosis with the fixing bacteria. From a health perspective, linseed (*Linum usitatissimum*) may be an appropriate option, due to their unsaturated fatty acids (FA) composition (>50% are n-3 FA, Hăbeanu et al., 2017), crude protein and fibre (lignin); however, linseed is not a traditional ingredient for pigs. But recently, there has been a growing interest from researchers in the use of linseed, mainly due to its functional property (Hăbeanu et al., 2019). It was also hypothesized that an oil-rich ingredient could have the effect of reducing greenhouse gas (GHG) level (Doreau et al., 2013).

Carbon dioxide (CO<sub>2</sub>) is a waste product that also resulted from the oxidation of fat and proteins. In pigs farm, the emissions of CO<sub>2</sub> come from exhalation by pigs and release from manure (Philippe & Nicks, 2014). If we take into consideration CO<sub>2</sub> from manure it comes from urea which hydrolyzed into NH<sub>3</sub> and CO<sub>2</sub>; also it comes from anaerobic fermentative processes of OM into intermediate volatile FA, methane (CH<sub>4</sub>) and CO<sub>2</sub>; the third source is the aerobic degradation of OM (Philippe & Nicks, 2014). European Parliament resolution of 14 March 2019 on climate change following the Paris Agreement (2019/2582(RSP)) mention that the average annual concentration of CO<sub>2</sub> increased from 400 ppm in 2015 to 403 ppm in 2016.

Methane is a volatile organic compound, resulted through feed digestion process throughout the digestive tract of the animal as well as from manure (Liu et al., 2014).

This study was carried out in male piglets first to assess the effects of three different diets on N excretion in relation with other indicators of nitrogen metabolism such as N retained (NR), N digested (ND), N digestibility, total N output (TNO), the biological value of feed protein (BVFP), net protein utilization (NPU), coefficient of metabolizability (CAM), N clearance rate (CR), blood urea nitrogen (BUN) and their correlation. Secondly, we aimed to examine the simultaneous effects of diets on performances and feeding efficiency, and enteric CH<sub>4</sub> and CO<sub>2</sub> emission through manure as well.

## MATERIALS AND METHODS

*Ethical procedure.*

The experimental protocol for balance test was approved by INCDBNA Balotesti Ethical Committee being in according to European Legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU). The biological test was running on to IBNA Balotesti Biobase.

*Experimental design and feeding*

During three weeks, a biological test was carried on with 15 crossbred barrows Topigs [(female Large White × Hybrid (Large White × Pietrain) × male Talent, mainly Duroc)],  $36 \pm 0.24$  Kg initial body weight (BW), assigned randomly in three groups using steel metabolism cages.

Three different oleo-proteinous ingredients were used in this study: 1. soybean meal is known as a classical by-product use in pigs feeding regime; 2) peas *Tudor* variety that replaces 53%, respectively 66% of soybean meal; 3. linseed *Lirina* variety known as an unsaturated oil-rich ingredient. In Table 1 is presented the comparative chemical composition of these ingredients.

**Table 1.** Chemical composition of soybean meal, peas and flaxseed

Item* %	Soybean meal	Peas <i>Tudor</i>	Flaxseed <i>Lirina</i>
DM	89.0	89.53	94.10
EM (MJ /kg )	13.38	13.77	16.98
CP	44.0	20.68	20.52
CPD	36.96	18.41	17.03
Fat	1.33	0.52	29.3
Cellulose	7.5	22.0	22.95
Ca	0.2	0.03	0.04
P	0.60	0.52	0.63
Lys.	2.77	1.80	0.93
Met. + Cys.	1.23	0.98	1.32

\*DM – dry matter; EM– metabolizable energy; PB = crude protein; D – digestible; Lys – lysine; Met+Cys – methionine + cystine.

The tested diets were administered to three groups of barrows: 1. Diet SC contained soybean meal (13%); 2. Diet SP had soybean meal < 47% than SC diet and peas *Tudor* variety 16%; 3. Diet SPF contained 8% soybean meal, 12% peas and 4% linseed *Lirina* variety (Table 2).

In our study, 4.5 Kg of soybean meal and 11.4 Kg of corn were replaced with 15.9 Kg peas *Tudor* in the SP diet. Linseed *Lirina* was included in a proportion of 4% in SPL diet with 12% peas *Tudor* that together replaced 41% of soybean meal and 9.91 of corn quantity.

**Table 2.** Ingredients and chemical composition of the diets

Item*	Diets***		
	SC	SP	SPF
<b>g/kg (as-fed basis)</b>			
Corn	292.3	178.3	193.2
Wheat	320	320	320
Rice flower	150	150	150
Soybean meal	130	85	80
Peas <i>Tudor</i>	0	160	120
Linseed	0	0	40
Sunflower meal	50	50	50
Soybean oil <i>Onix</i>	19	20	10
DL -methionine	0.7	0.1	0
L-Lysine HCl	3.1	1.5	2
Carbonate calcium	18.4	18.6	18.5
Monocalcium phosphate	1.5	1.5	1.3
Salt	4	4	4
Premix choline	1	1	1
Vitamin/mineral premix**	10	10	10
<b>Calculated chemical composition g/kg (as-fed basis)</b>			
DM	886.2	885.5	885.7
Metabolizable energy (EM, Mj/kg)	12.96	12.95	12.88
Net energy (NE, Mj/kg)	10.82	10.82	10.74
CP	155.3	155.4	155.2
Lys.	9.7	9.7	9.7
Lys D.	8.1	8.2	8.2
Met + Cys.	6.1	6.1	6.1
Met + Cys. D	5.1	4.9	4.9
Ether extract	54.2	52.2	54
NDF	200.9	202.7	200.9
ADF	75.9	79.1	76.2
ADL (lignin)	20.6	20.2	20.3
Starch	321.2	319	307
Sugar	41.9	42.40	42.60
Cellulose	61.3	64.4	62.6
Hemicellulose	125	123.6	124.7
Calcium	8	8	8
Phosphorus total	7.8	7.9	7.7
Phosphorus D.	2.4	2.6	2.5

\*CP – Crude protein; D – digestible; NDF – neutral detergent fibre; ADF – acid detergent fibre.

\*\*Vitamin/mineral premix provided per kg diet: 6000 IU vitamin A; 800 IU vitamin D3; 20 IU vitamin E; 1 mg vitamin K3; 1 mg vitamin B1; 3.04 mg vitamin B2; 10 mg vitamin B3; 6.3 mg vitamin B5; 1.5 mg vitamin B6; 0.03 mg vitamin B7; 0.3 mg vitamin B9; 0.02 mg vitamin B12; 30 mg Mn; 80 mg Fe; 25 mg Cu; 100 mg Zn; 0.22 mg I; 0.22 mg Se; 0.3 mg Co; 60 mg antioxidant.

\*\*\*SC – control diet; SP – soybean meal: peas diet; SPF – soybean meal: peas: linseed diet.

The linseed was characterised by a higher content in methionine + cystine such as it was not necessary to add DL-methionine in SPF diet to adjust the level of this limiting amino acid. Peas *Tudor* was provided by SCDA *Turda* and linseed *Lirina* of INCDA Fundulea.

Diets were balanced for nutritional requirements of pigs and adjusted for amino acids, Ca and P needs by adding L-Lysine and DL-methionine, carbonate calcium, and monocalcium phosphate.

#### *Balance trial*

The three groups of pigs subjected to testing were kept in an atmosphere-controlled room, in individual metabolism cages. The first week was an adaptation period followed by two balance period. In balance period, faecal and urine were quantitatively collected, at 08.00–08.30h, weighed and processed as described in our previous work (Hăbeanu et al. 2019). A quantity of 10% from faecal samples pooled over successive days, weight, and mixed previously were stored at 5°C up to processing and analyses. Daily urine samples were taken, recorded daily, and 10% were preserved at -18°C up to analyses. It was used H<sub>2</sub>SO<sub>4</sub> with 25% concentration in each urine container for preservation.

#### *Chemical analyses*

A quantity of 0.4 g was weighed from each sample (accuracy was of  $\pm 2 \times 10^{-4}$  g) and was processed for assessing the N content using a semiautomatic Kjeldahl method (Kjeltec Auto 1030 Analyzer, Hillerod, Denmark). A blank digest was carried out in the same way. The samples were digested using H<sub>2</sub>SO<sub>4</sub> in the presence of catalysers, followed by distillation and titration. Class A glassware was used for transvasation, dilution and storage. All reagents used were supplied by Merck (Darmstadt, Germany).

At the end of the experiment, blood samples were collected by jugular venipuncture in heparin tubes, centrifuged (3000 rpm for 15 min) for plasma separation (Hăbeanu et al., 2019). A chemistry analyser (Spotchem EZ SP-4430) was used to assess protein and urea nitrogen.

#### *Statistical Analysis and Calculation*

To determine the animal response to the treatment applied a multivariate general linear model through SPSS software, (2011) was used in this study. The data were presented as the mean and standard error of the mean (SEM). The diets were considered main, fixed factors while the responses to the treatment in term of performance, nitrogen metabolism data, and CO<sub>2</sub> emitted by manure and enteric CH<sub>4</sub> were considered dependent variables. For determining the relationship between indicators, Pearson's correlation, Software Statistical Package SPSS, was used. The differences between

treatment applied were considered significant if  $P < 0.05$ , highly significant when  $P < 0.001$  or  $P < 0.0001$  and the treatment was considered to have a tendency to influence the results if  $0.05 < P < 0.10$ .

The equations of Moreira et al., (2004), Kohn et al., (2005), White et al., (2015), were used to determine NR, ND, N digestibility, TNO, BVFP, NPU, CAM, CTTAD, CR as follow:

$$\text{NR} = \text{NI} - \text{TNO}$$

$$\text{ND} = \text{NI} - \text{N faecal}$$

$$\text{N digestibility} = 100 \times (\text{N intake} - \text{N faecal}) / \text{N intake}$$

$$\text{TNO} = \text{N faecal} + \text{N urine}$$

$$\text{BVFP} = \text{NR} / \text{ND}$$

$$\text{CAM} = ((\text{N intake} - \text{N faecal output} - \text{N urinary output}) / \text{N intake})$$

$$\text{CTTAD} = ((\text{N intake} - \text{faecal N output}) / \text{N intake})$$

$$\text{CR} = \text{urinary N} / \text{BUN}$$

were:

CTTAD = coefficient of total tract apparent digestibility

The quantity of lipids in faeces and lipid digested equation was calculated by the relationship proposed by Le Goff and Noblet (2001).

CO<sub>2</sub> emitted was determined starting with heat production (HP, KJ/day) based on 0,163 l/h CO<sub>2</sub> per watt of heat (International Commission of Agricultural and Biosystems Engineering (CIGR), 1984), corrected for density (22.4 l / mol) and molecular weight 44 g / mol).

$$\text{HP} = 360 \times \text{BW}^{(42/100)} + 0,25 \times \text{EM intake (Noblet, 1994)}$$

Enteric CH<sub>4</sub> was calculated starting from feed intake multiply with 18.6 (MJ GE/kg DM intake) and 0.007 (methane conversion factor of gross energy) all divided to 55.22 (PigGas: Pork Industry Greenhouse Gas Calculator User Guide).

## RESULTS AND DISCUSSION

The reduce of nitrogen excretion mean a better cope with the problem of the negative contribution of pigs to the “nitrogen cycle” on a farm. When we reviewed the main factors of variation of nitrogen flow, it has been observed that the feeding regime can have a significant influence related to the excessive amount of N excreted. The N in faeces and urine (g/d) varying with the structure of the diets used, directly depending on DM intake. The dietary protein level, undigested N originated from a microorganism, and endogenous N is the source of excreta N. Although the small intestine has microbial flora, it has a limited role in nutrient degradation.

Due to their specificity of the digestion, pigs are adapted to a type of feed with a lower level of cellulose. The diets consist mainly on concentrated fodder, corn being the component with the highest share in the ration.

Complementary, the protein and oil-rich ingredients are added for balanced diets adjusted for met nutritional requirements.

*Nutrients and energy intake per head and per day*

In our study, the nutrients and energy intake are shown in Table 3.

**Table 3.** Nutrients and energy intake calculated /head /day

<b>Item**</b> g/head/day	<b>SC</b>	<b>SP</b>	<b>SPF</b>	<b>SEM</b>	<b>P-value</b>
Feed intake	2460	2860	2410	90.6	0.083 <sup>T</sup>
DM	2170	2540	2140	80.3	0.084 <sup>T</sup>
OM	1980	2310	1960	73.1	0.098 <sup>T</sup>
<b>Protein</b>					
Protein intake	420	499	415	18.36	0.061 <sup>T</sup>
N	67.21	79.85	66.48	2,51	0.061 <sup>T</sup>
<b>Carbohydrate</b>					
Hemicellulose	310	250	230	11.2	0.107
Lignin	50	40	40	1.84	0.108
Polysaccharide	460	420	370	17.0	0.068 <sup>T</sup>
NDF	490	580	480	18.36	0.064 <sup>T</sup>
Cellulose	150	170	140	5.8	0.027*
Starch	787	916	740	29.0	0.037*
Sugar	100	120	100	3.8	0.083 <sup>T</sup>
<b>Lipids and energy (Mj/head/day)</b>					
Lipid	132	140	130	4.76	0.206
EM intake	31.77	37.2	31.08	1.17	0.075 <sup>T</sup>
EMm, %	29.67	25.28	30.37	1.28	0.238*
EN intake	23.51	27.53	22.95	0.87	0.072 <sup>T</sup>
EN/EM	2.17	2.54	2.14	0.08	0.084 <sup>T</sup>

\*\*OM, organic matter; DM, dry matter; EM, metabolizable energy; EN, Net energy; EMm energy metabolizable for maintenance calculated as % from intake EM; NDF, neutral detergent fibre.

\*P < 0.05 significant difference; T, tendency of influence at 0.05 < P < 0.10.

Although the diets tested were similar concerning protein, energy, amino acids and cellulose, the intake of nutrients registered (expressed as g head<sup>-1</sup> day<sup>-1</sup>) tended to be influenced by the diets. In SP diets, a higher level of DM and OM intake compared to SC and SPL diets were noticed. While hemicellulose and lignin were closed in both SP and SPL fed group vs SC group, the cellulose and starch intake were significantly higher in the SP fed group than the SPL group. Unexpected, the lipid intake was slightly higher in the SP fed group which contributed probably to an increase by 1.17 times of EM and EN intake



compared to SC group and by 1.2 times than SPL fed group. A possible explanation could also be given a higher consumption of total carbohydrates and protein. Such as in diets used in this study, the composition of modern diets had been affected by replacing the classical ingredients with mostly fibrous or oil-rich ingredients. The feed has become more fibrous while was containing less starch. Pigs compensate for the lower energy density by consuming more feed. Thus, an increase of 1.16 times of feed intake was noticed in the SP group compared to SC and by 1.18 times than the SPL group. Cellulose and most of the hemicellulose are a soluble fraction of non-starch polysaccharides (NSP). It is known that the monogastric do not have enzymes that degrade NSP, which can impact performances.

Maintenance energy, as basal energy necessary for supporting body function, temperature and activity of animals (Liu et al., 2019), as expected, was not affected significantly by the diets. In metabolism cage used in this test, the pig's activity was closed to 0. EMm is not depended on the dietary level of energy. Metabolizable energy intake tended to be higher in the SP group compared to SC and SPL groups. An explanation consists in the fact that the EM intake is high significantly, positively correlated ( $r = 0.99$ ) with cellulose, starch, sugar, NDF, lignin, polysaccharides and lipids intake. Implicit, the EN was strongly positively correlated with carbohydrates and lipids intake.

#### *Growth performances*

The animals were individual weight at the beginning and the end of biological testing. The growth performance data are presented in Table 4.

**Table 4.** Feed intake and growth performance

Item*	SC	SP	SPF	P-value*
Feed intake (Kg)	2.56	2.88	2.46	0.08 <sup>T</sup>
Gain: feed (g:g)	0.40	0.32	0.39	0.06 <sup>T</sup>
Initial BW (Kg)	37.0	37.0	36.0	0.29
Final BW (Kg)	53.0	50.0	51.4	0.07 <sup>T</sup>
ADG (kg)	1.03	0.92	0.97	0.004*

\* P < 0.05 significant difference; T, tendency of influence at 0.05 < P < 0.10

The growth performance registered a decline in SP fed group due to an increase of fibrous component intake despite the higher level of lipids intake and metabolizable energy. The explanation consists of strongly negative correlation between feed efficiency and carbohydrates and energy, lipids consumed ( $r > 0.9$ ) although this category of pigs has the ability to digest a high content of fibre in the diet. The peas added in the SP diet increase the

feed intake by 1.17 times while the feed efficiency decrease 1.31 times compared to SC diet. The lower feed intake was registered in SPF fed group (<1.04 times than SC group). The ADG decreased highly significant in the SP diet compared to the SC diet. Although in a lower quantity, linseed adds in SPL diet contributed to obtaining growth performances values closed to SC group.

### *Nitrogen balance*

The N balance data is shown in Table 5.

**Table 5.** Effects of diets on N balance

Item	SC	SP	SPL	SEM	P-value
g/head /day					
N intake	67.20	79.85	66.48	2.51	0.061 <sup>T</sup>
Urine + faeces	1895	2011	1811	55.6	0.33
N in faeces	8.84	10.08	9.03	0.52	0.63
Urinary N	13.64	14.36	12.44	0.42	0.36
TNO	22.48	24.43	21.47	0.74	0.26
NR	44.72	55.42	45.0	2.431	0.15
ND	58.36	69.68	57.45	2.46	0.08 <sup>T</sup>
N digestibility %	84.52	83.64	82.82	0.72	0.67
N excreted as % of intake	35.35	33.04	34.47	1.57	0.85
NPU, %	64.64	66.96	65.52	1.57	0.85
BVFP, %	74.49	77.07	76.08	1.33	0.76
CR, %	0.76	0.55	0.66	0.03	0.04*
Lipid digested	39.72	38.05	39.56	0.09	
Total serum protein g/dL	4.6	5.8	5.9	0.09	<0.0001**
BUN mg /dL	18.44	24.25	19.33	0.9	<0.0001**

\*P < 0.05 significant difference < T= p < 0.10 tendency of influence; \*\* P<0.0001 highly significant differences

Total nitrogen output (TNO); retained nitrogen (NR); biological value of feed protein (BVFP); net protein utilization (NPU); coefficient of total tract apparent digestibility (CTTAD); coefficient of metabolizability (CAM); clearance rate of waste N (CR), blood urea nitrogen (BUN).

During the experiment, no gastrointestinal disorders were noticed. Although the SP diets increased the nitrogen level slightly in faeces (>1.08 times compared to SC diet) and urine (>1.05 times in urine, respectively), which led to an increase of TNO with 8% than SC group and NR level also with 19%, no significant differences were observed. Except was noticed for ND, which tended to be influenced by peas added in the diet. We found a highly significant positive correlation between carbohydrates fraction and NR ( $r >$

0.96), % of N digestibility ( $r > 0.4$ ), NPU ( $r > 0.74$ ) and BVFP ( $r > 0.78$ ). A positive high significant correlation was noticed between EM, EN and nitrogen balance indicators ( $r > 0.8$ ). The data obtained in our study are comparable with that presented in one of our previous work (Hăbeanu et al., 2019) related to N in faeces and urine, TNO; however, a higher value we noticed for ND, % of N digestibility, NPU and BVFP in the present paper. The N intake was lower except for the SP diet. Unexpected, NR value was higher in SP diet while % of N excreted decreased in SP diet with 6% compared to SC and with a lower extend decreased in SPL diet (3%) compared to SC diet. Usually, the level of dietary protein is elevated in traditional diets for meeting the needs of lysine as first amino acids limiting in pigs (Wood et al., 2018). In our study, the protein level was 15% that represent the minimum necessary for the Topigs hybrids from the weight category studied. Instead, the level of lysine, methionine and cystine was ensured at the level of the specific requirements of this category.

Plasma protein and BUN concentration were significantly higher in the SP diet compared to the SC diet. BUN level ranged within the physiological interval (8.2–25 mg/dL; Hăbeanu et al., 2019). BUN concentration has a linear relation with urinary N.

CR, defined by Kohn et al., (2005) as liters of blood cleared entirely of urea per day, decreased in SP diets ( $P = 0.04$ ). Irrespective the diets, CR in our diets was lower than that estimated by Kohn et al., (2005). No other results we found in the literature on CR in pigs.

#### *CO<sub>2</sub> emitted through manure and enteric CH<sub>4</sub>*

It is known that the endogenous enzymes degrade an important part of carbohydrates at small intestine level; the dietary fibre fraction undergoes a fermentative process in the gut. From these processes result in short-chain fatty acids such as acetic, propionic and butyric acid, but also gases like CO<sub>2</sub>, H<sub>2</sub> and CH<sub>4</sub>, urea and heat. In the stomach and small intestine, there is also microorganism, responsible for the production of H<sub>2</sub> (Jørgensen et al., 2011). We calculated emission of CO<sub>2</sub> and enteric CH<sub>4</sub> expressed as well as equivalent CO<sub>2</sub> associated with the feed.

Table 6 shows the mean values of CO<sub>2</sub> and enteric CH<sub>4</sub> calculated according to our data. CO<sub>2</sub> released from manure tended to be 9% higher in the SP diet compared to SC diet while in SPL diet was 2% lower than SC group. Our data related to the proportion of CO<sub>2</sub> emitted from CO exhalation ranged between 28–32%, which are closed of that mentioned by Philippe & Nick, (2014). However, as he noted, there are the data much lower in literature. In our study, the SP diet tended to increase the CO<sub>2</sub> due to the fact that HP value increased as well; these indicators being strongly correlated. CH<sub>4</sub> was strong

positive significantly correlated with feed intake, DM intake, carbohydrates and CO<sub>2</sub> ( $r > 0.89$ ).

**Table 6.** Main values of CO<sub>2</sub> emitted and enteric CH<sub>4</sub>

Item*	SC	SP	SPL	SEM	P-value
HP (Mcal/head/day)	3.80	4.10	3.74	0.07	0.09 <sup>T</sup>
CO <sub>2</sub> emitted (g/head/day)	0.33	0.36	0.33	0.006	0.09 <sup>T</sup>
E-CO <sub>2</sub> (Kg/head/day)	1.17	1.15	1.16	0.005	0.31
CO <sub>2</sub> emitted from CO <sub>2</sub> exhalation %	28.76	31.57	28.69	0.53	0.054 <sup>T</sup>
Enteric CH <sub>4</sub> g/head/day)	5.06	5.81	5.25	0.18	0.14
CH <sub>4</sub> eq CO <sub>2</sub>	0.12	0.14	0.12	0.004	0.08 <sup>T</sup>

HP, heat production; E-CO<sub>2</sub> exhalation (E-CO<sub>2</sub>, pig, in kg CO<sub>2</sub> day<sup>-1</sup>, calculated according Philippe & Nick, 2014).

No significant influence was noticed for CH<sub>4</sub> estimated value, although a slight increase was observed in the SP diet. According to the Intergovernmental Panel on Climate Change (IPCC, 2007) established as a conversion factor in equivalent CO<sub>2</sub> for CH<sub>4</sub> to be 25, which means the global warming potential of 25 for CH<sub>4</sub>. Expressed in the term of equivalent CO<sub>2</sub>, we noticed a tendency to increase CH<sub>4</sub> level in the SP diet compared to SC and SPL diets.

## CONCLUSION

Taking into account the results obtained in this study, we can conclude that both peas and linseed had the valuable potential for replacing a part of the soybean meal. Due to a higher ingest of some carbohydrates fraction, peas associated with soybean meal led to a decline in growth performance. Still, a mix between peas, soybean meal and linseed contributed to the cancellation of these differences. It is important to mention that the dietary protein level was insured at the minimum threshold corresponding to the nutritional requirements irrespective the diet. Despite this, in pigs group fed peas diet, DM and OM intake were higher. Although peas incorporated in the diet SP increased significantly the total concentration of BUN, the N metabolism indicators were not significantly affected. CO<sub>2</sub> and CH<sub>4</sub> value registered a slightly increased by dietary addition of peas.

## ACKNOWLEDGEMENTS

*This study was supported by the Ministry of Agriculture and Rural Development of Romania through Sectorial project ADER 9.1.4.*

## REFERENCES

Directive 2010/63/EU

Dourmad, J.Y, Garcia-Launay F., Narcy A. 2017. Pig nutrition: impact on nitrogen, phosphorus, Cu and Zn in pig manure and on emissions of ammonia, greenhouse gas and odours. Batfarm European Workshop Reconciling Livestock Management to the Environment. Mar 2013. Rennes. France. fhal-01594359f. HAL Id: hal-01594359 <https://hal.archives-ouvertes.fr/hal-01594359>.

European Parliament resolution of 14 March 2019 on climate change – a European strategic long-term vision for a prosperous, modern, competitive and climate neutral economy in accordance with the Paris Agreement (2019/2582(RSP)).

FAOSTAT.ORG. <http://www.fao.org/faostat/en/#data/QC/visualize>.

Hăbeanu, M., Lefter, N.A., Gheorghe, A., Tabuc, C., Untea, A., Surdu, I., Ciurescu, G., Balan, C.G., Dragomir, C. 2015. Changes in certain serum and faeces parameters in weaned piglets as a response to nutritional stress. *S Afr J Anim Sci.* 45 (2), 164-172.

Hăbeanu M., Lefter N.A, Gheorghe A, Tabuc C., Dumitru M, Ciurescu G., Palade M.. 2017. Effects of dietary peas mixed with linseed (3:1) on the growth performance, enteritis and certain serum parameter in weaned piglets. *Food and Feed Research.* 44 (2), 173-180. DOI: 10.5937/FFR1702173H

Hăbeanu, M., Lefter, N.A., Gheorghe, A., Untea, A.E., Ropotă, M., Grigore, D.M., Varzaru, I., Toma, S.M. 2019. Evaluation of performance, nitrogen metabolism and tissue composition in barrows fed an n-3 PUFA-rich diet. *Animals.* 9, 234.

IPCC, 2006. Guidelines for national greenhouse gas inventories.

IPCC, 2007. Climate change 2007: The physical science basis. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel.

International Commission of Agricultural and Biosystems Engineering (CIGR), 1984.

Jørgensen, H., Theil, P.K., Knudsen, K.E.B. 2011. Enteric Methane Emission from Pigs. *Planet Earth 2011–Global Warming Challenges and Opportunities for Policy and Practice.* 605-622.

Kohn, R.A., Dinneen, M.M., Russek-Cohen, E. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *J. Anim. Sci.* 83, 879–889.

Le Goff and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *J. Anim. Sci.* 79, 2418–2427.

- Liu, Z., Powers, W., Liu, H. 2014. Greenhouse gas emissions from swine operations: Evaluation of the Intergovernmental Panel on Climate Change approaches through meta-analysis. *J. Anim. Sci.* 91, 4017–4032. doi:10.2527/jas2012-6147.
- Liu, H., Chen, Y., Li, Z., Li, Y., Lai, C., Piao, X., van Milgen, J., Wang, F. 2019. Metabolizable energy requirement for maintenance estimated by regression analysis of body weight gain or metabolizable energy intake in growing pigs. *Asian-Australas J Anim Sci.* 32, (9), 1397–1406. <https://doi.org/10.5713/ajas.17.0898>.
- Millet, S., Aluwé, M.A., Van den Broeke, Leen, F., De Boever, J., De Campeneere, S. 2018. Review: Pork production with maximal nitrogen efficiency. *Animal.* 12(5), 1060–1067. doi:10.1017/S1751731117002610.
- Monteiro, D.O., Pinheiro, V.M.C., Mourão, J.L.M., Rodriguez, M.A.M. 2010. Strategies for mitigation of nitrogen environmental impact from swine production. *R. Bras. Zootec.* 39, 317–325.
- Moreira, I., Fraga, A.L., Paiano, D., Oliveira, G.C., Scapinello, C., Martins, E.N. 2004. Nitrogen balance of starting barrow pigs fed on increasing lysine levels. *Braz. Arch. Biol. Techn.* 47, 85–91.
- Noblet, J., Fortune, H., Shi, X.S., Dubois, S. 1994. Prediction of net energy value of feeds for growing pigs. *J. Anim. Sci.* 72, 344–354.
- Noblet, J., Bellego, L.L., van Milgen, J., Dubois, S. 2001. Effects of reduced dietary protein level and fat addition on heat production and nitrogen and energy balance in growing pigs. *Anim. Res.* 50, 227–238.
- PigGas: Pork Industry Greenhouse Gas Calculator User Guide.
- Philippe, F.X., Nicks, B. 2014. Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. *Agriculture, Ecosystems and Environment* 199, 10–25. <https://doi.org/10.1016/j.agee.2014.08.015>.
- Wang, J., Chadwick, D., Cheng, Y., Yan, X. 2018. Global analysis of agricultural soil denitrification in response to fertilizer nitrogen. *Sci Total Environ.* 616–617, 908–917. <https://doi.org/10.1016/j.scitotenv.2017.10.229>.
- Wang, Y., Junyan, Z., Wang, G., Cai, S., Zeng, X. Qiao, S. 2018. Advances in low-protein diets for swine. *J Anim Sci Biotechnol.* 9:60. <https://doi.org/10.1186/s40104-018-0276-7>.
- White, G.A., Smith, L.A., Haudijk, J.G.M., Homer, D., Kyriazakis, I., Wiseman, J. 2015. Replacement of soya bean meal with peas and faba beans in growing/finishing pig diets: Effect on performance, carcass composition and nutrient excretion. *Anim. Feed Sci. Tech.* 209, 202–210.