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Article

The *in vivo* crude protein digestibility of soybean species cultivated in Hungary

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ABSTRACT - The purpose of the trial was to use the mobile nylon bag technique to determine the crude protein digestibility and calculate the digestible protein yield of soybean varieties cultivated in Hungary. The trial was carried out with 10, double cannulated (duodenal- and PVTC-cannula) hybrid barrows with initial live weights of 40±3.5 kg. The experimental basal diets were formulated on a corn-wheat-barley-soybean basis according to the requierement of growing pigs (Tybirk, 2015). A total of 20 soybean varieties were tested in this experiment. After simulating gastric digestion the nylon bags were inserted into the duodenum of ten barrows through simple duodenal T-cannulae. Ten bags were administered to each pig daily. A total of 200 (10 samples/soybean variety) bags were inserted over a 4-day period. The mean, standard deviation, minimum and maximum values of the crude protein digestibility of soybean samples were calculated. The protein yields per hectare according to the crop yield and the protein content values and also the digestible protein yield values were calculated. To examine the relationship between crop yield and crude protein yield and also between crop yield and digestible crude protein yield regression analysis were used. The overall results of this experiment indicate that the average crude protein digestibility of the tested soybean varieties was 76.0%, with an absolute difference of 17.3% between the best and the least digestible varieties. ES Mentor variety reached the best digestible crude protein yield, with 1305.4 kg/ha. The variety with the lowest digestible crude protein yield was Boglár, with 752.3 kg/ha. The difference in digestible crude protein yield between these two varieties was 173.5%. In the correlation analysis between digestible crude protein content and yield for soybean varieties our results show that there is no correlation between the two factors. However, Aires, Prestopro, and ES Mentor should be highlighted among the varieties with above trend line results, as they have the best yield (4020 kg/ha; 4100 kg/ha, 4510 kg/ha) and digestible crude protein content (31%, 30.3%, and 28.9%). ES Mentor produced the fourth best digestible crude protein content (28.9%) with the best yield (4510 kg/ha).

***Keywords*:** soybean, digestibility, nylon bag, yield

INTRODUCTION

In Hungary, soybean are the most important protein source for the production of feed mixes. In the EU, the nutritional importance of soybean meal is shown by a calculation of protein use: livestock production uses 43 million tones of protein per year (*Popp et al.,* 2015). One of the most important feed ingredients are cereals, which, although not considered as a source of protein, account for 40% of the protein content of compound feed. Soybean meal accounts for 34%

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of the protein supply, rapeseed meal 11%, sunflower meal 7% and other oilseeds 2%. Its extracted meal plays an important role in the mix of all intensive livestock species and is essential for the amino acid supply of animals, especially in poultry and pig meat production.

Economical, environmentally friendly and sustainable pork production is unthinkable without a balanced animal feed. It has long been known that feed costs account for around 70-75% of the cost of pig meat production. Therefore, to maintain economical production, it is necessary to develop and apply feeding technologies based on the digestible nutrient content of feed components. In this way, they are able to provide appropriate nutrients to the needs of the animals in order to enable them to reach their genetic potential.

The years 2020 and 2021 brought unaccustomed challenges for both the livestock sectors and the feed industry. For some farms, survival has already been a major challenge, due to the already present market and climate change related difficulties, enhanced or coupled with the african swine fever (ASF) and the emerging pandemic situation. Despite these setbacks, the yields of corn and sunflower exceeded those of recent years. However, soybean yields were decreased compared to the data monitored during 2019. The evolving situation has caused a steady increase in the price of autumn-sown crops, resulting a sharp rise in the purchase price of feed materials for pork production, with doubled sunflower prices compared to the previous year (*Gregosits*, 2021). In Hungary there are about 60 cultivated soybean varieties with difference crude protein content and yield values. But there is no information about the digestible crude protein digestible of these varieties. Due to the drastic increase in feed prices, the use of soybean varieties with the highest digestible crude protein content as feedstocks is inevitable in the feed industry, to improve the efficiency of economic production.

Aims

The purpose of the trial was to use the mobile nylon bag technique to determine the crude protein digestibility and calculate the digestible protein yield of soybean varieties cultivated in Hungary. Using the protein digestibility and the available yield results our further objective was to rank the tested species according to the digestible protein yield.

MATERIAL AND METHOD

The trial was carried out with 10, double cannulated (duodenal- and PVTC cannula) hybrid (DanBred) barrows with initial live weights of 40±3.5 kg. During the entire experimental period the animals were kept in special individual

pens (2 m^2/animal). The room temperature and relative humidity were regulated in accordance with the requirements of growing pigs (*Tybirk,* 2015). During the trial animals received a coarse meal diet ad libitum. Water was offered *ad libitum* via automatic drinkers.

Before the start of the trial (ethitcal permission number: SOI/31/00659-14/2018) we implanted a duodenal T-cannula to the proximal part of duodenum and a PVTC-cannula onto the ileocecal valve (*Sauer et al.,* 1982; *Steiner et al.,* 2011, *Van Leeuwen et al.,* 1991). After the surgery the animals had a 14 day long regeneration period before the trial.

The basal diets were formulated on a corn-wheat-barley-soybean basis according to the requierement of growing pigs (*Tybirk,* 2015). The composition and nutrient content of the basal diet are summarized in *Table 1*.

Table 1
The composition and calculated nutrient content of the basal diet

Ingredients	g/kg	Nutrients	g/kg
Corn	302.95	Dry matter	885.6
Soybean meal	160.0	DE (MJ/kg)**	13.8
Wheat	250.0	Crude protein	150.3
Barley	250.0	Crude fat	26.4
Vegetable oil*	4.0	Crude fiber	32.5
MCP	9.2	Crude ash	25.0
Limestone	10.3	Lysine	9.2
NaCl	3.6	Methionine+Cystine	5.2
Lysine-HCL	3.6	Threonine	6.0
DL-Methionine	0.4	Thryptophan	1.9
L-Threonine	0.8	Calcium	6.4
L-Tryptophan	0.2	Phosphorus	5.3
Premix 0,5%***	5.0		
Total	**1000.0**		

* Sunflower oil; ** Calculated value; *** 1 kg premix contain: Vit. A: 1,750,000 IU, Vit. D3: 350,000 IU, Vit. E: 8,750 mg, Vit. K3: 350 mg, Vit. B1: 262.5 mg, Vit. B2: 875 mg, Vit. B3: 2,100 mg, Vit. B6: 700 mg, Vit. B12: 4,375 mg, Biotin: 21 mg, Folic acid: 105.07 mg, Cholin: 24,000 mg, Fe: 19,175 mg, Zn: 20,001 mg, Mn: 6,488.3 mg, Cu: 2,225 mg, Co: 6.5 mg, I: 65 mg, Se: 67.75 mg.

The crude protein digestibility of the relevant soybean varieties cultivated in Hungary (n=39) were determined by an *in vitro* method in a pre-experiment. According to these results 20 varieties with the best crude protein digestibilty

value were chosen to test by *in vivo* method. During the trial the *in vivo* crude protein digestibility of the samples were determined by mobile nylon bag technique (*Sauer et al.,* 1982, *Quiao et al.,* 2004, *Steiner et al.,* 2011). Feed was ground through a 1 mm screen and 1 g samples (10 samples/soybean species) were enclosed in 25 × 40-mm monofilament nylon bags (50-μm mesh). At first step the samples were pre-digested by an *in vitro* gastric-digestion method (*Babinszky et al.,* 1990, *Cone,* 1993, *Boisen et al.,* 1995). The bags were grouped in blocks of 10 and placed in a 1000 ml beaker containing 500 ml of a solution made up of deionized water, 0.01 N HCl and one g of purified activated pepsin powder. The beaker was then placed into a shaking water bath (65 oscillations/min) and incubated for 4 h at 40 °C to simulate gastric digestion. After incubation, the bags were removed from the beaker, washed with deionized water and frozen (-20 °C) in small plastic bags until required. In the second step prior to insertion, nylon bags were removed from the freezer and thawed for 5 min in a 37.8 °C water bath. The nylon bags were inserted through the duodenum of ten barrows through simple duodenal T-cannulae 30 minutes after the morning feeding. Two bags were introduced 30 minutes apart (i.e. two bags 30 minutes after the morning feeding and further pair of bags in every 30 minutes). Ten bags were administered to 5 pig daily. A total of 200 (10 samples/soybean variety) bags were inserted over a 4-day period.

Bags were collected via PVTC-cannulas wich were opened 2.5 hours later than the first inserting. The collected samples were stored at −80°C until analysis.

The crude protein content of the original and the collected soybean samples were determined in accordance with the *AOAC* (1989) recommendations.

The apparent crude protein digestibility of soybean samples was calculated according to the protein content of the original samples and the protein contect of the collected samples as the following:

$$\text{CP digestibility (\%)} = \frac{CPin - CPout}{CPin} x100$$

CPin = CP in original sample (g)

CPout = CP in collected sample (g)

Means and standard deviations for digestible crude protein were calculated using Microsoft Excel (*Microsoft,* 2016). The protein yields per hectar according to the crop yield and the proteint content values and also the digestible protein yield values were determined.

Regression analysis was carried out to examine the relationship between crop yield and crude protein yield and crop yield and digestible crude protein yield. The univariate linear regression modell was performed as the follows: Y = aX + b.

Y = CP conetent (g/kg) or digestible CP content (g/kg)

X = crop yield (t/ha)

RESULTS AND DISCUSSION

The crude ptotein content, crude protein digestibility and the digestible crude protein content data of the 20 soybean varieties are shown in *Table 2*. The results show that the Bólyi 1117 soybean variety had the highest apparent crude protein digestibility (84.3%), while the lowest value was observed in Bólyi 612 (67.0%), 20.5% less than the best performing variety Bólyi 1117. The average in vivo crude protein digestibility of the tested soybean varieties is 76.0%, with an absolute difference of 17.3% between the best and the least digestible varieties. In our study 60% of the soybean varieties had the apparent crude protein digestibility between 74.0% and 82.0%. *Boisen et al.* (1995) and *Cone et al.* (1993) determined higher apparent crude protein diegestibility (78.1% and 82.1 to 83.8%) than our results, but it is not relevant to compare these results because they studied different varieties from different cultivation area.

The distribution of in vivo protein digestibility data for the studied soybean varieties is shown in Figure 1. 15-15% of the samples were rated in the in vivo crude protein digestibility interval of 66-74%. 60% of the tested samples fell within the range from 74% to 82%. 10% of the samples can be taken in the in vivo crude protein digestibility interval of 84 ± 2,0%.

Table 2
The crude protein content, the crude protein digestibility and the digestible crude protein content of different soybean varieties (%)

Variety	Crude protein content	Crude protein digestibility	Digestible crude protein content
Bólyi 1117	35.1	84.3	29.6
Suedina	38.2	82.4	31.5
Aires	37.9	81.8	31.0
Bólyi 27	38.3	79.7	30.5
ES Gladiator	36.5	78.8	28.8
Pannónia Kincse	35.0	78.8	27.6
Seka	35.8	78.6	28.1
ES Advisor	37.0	78.0	28.9
Boglár	34.6	77.7	26.9
Prestopro	39.6	76.5	30.3
Bahia	34.0	76.4	26.0
Speda	40.5	76.4	30.9
ES Mentor	38.2	75.8	28.9
Sponzor	35.1	74.6	26.2
ES Mediator	37.7	73.8	27.8
ES Comandor	38.1	72.2	27.5
Stumpa	34.4	71.9	24.7
Borbála	38.9	68.2	26.5
Navigator	37.1	67.4	25.0
Bólyi 612	35.2	67.0	23.6
mean	**36.9**	**76.0**	**28.0**
St. deviation	*1.9*	*4.8*	*2.3*
difference	*6.5*	*17.3*	*7.9*
minimum	*34.0*	*67.0*	*23.6*
maximum	*40.5*	*84.3*	*31.5*

In *Table 3* the crop yields and the digestible crude protein yields of soybean varieties per hectare are presented. Based on our results, the ES Mentor variety reached the best digestible crude protein yield, with 1305 kg/ha. The variety with the lowest digestible crude protein yield was Boglár, with 752 kg/ha. The difference in digestible crude protein yield between these two varieties was 173.5%.

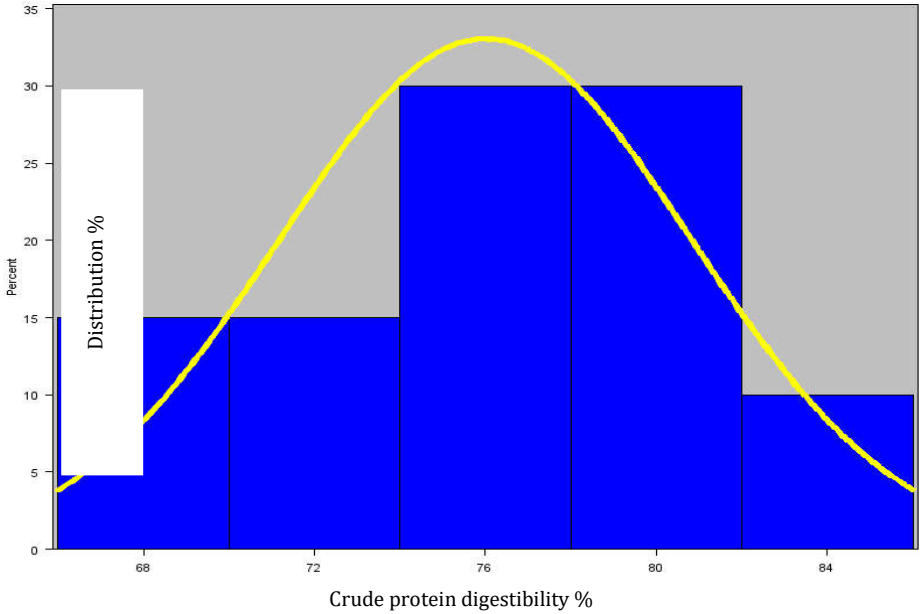

Figure 1: Distribution of apparent protein digestibility in soybean varieties

Table 3
Digestible crude protein yield of different soybean varieties (kg/ha)

Variety	Crop yield	Digestible crude protein yield
ES Mentor	4510	1305
Aires	4020	1247
Prestopro	4100	1242
Seka	3990	1122
Bahia	4260	1107
Pannónia Kincse	4000	1104
ES Gladiator	3780	1088
Sponzor	3940	1032
Borbála	3810	1011
Bólyi 612	3900	920
Bólyi 27	2670	815
Boglár	2800	752

Figure 2 shows the correlation between crude protein content and yield for soybean varieties. Our results indicates, that there is no relationship between the two factors (R^2=0.0013). *Malik et al.* (2006, 2007) and *Jagtap et al.* (1993) also found no correlation between crude protein content and yield. The Prestopro variety had the highest crude protein content of 39.6% and this excellent crude protein content was associated with the second best yield with 4100 kg/ha. The Borbála variety also had a remarkable crude protein content (38.9%) and a yield with 3810 kg/ha but on the other hand the digestible crude protein yield value is quite low (1011 kg/ha). A favourable combination of crude protein content and yield was also observed for the variety ES Mentor, with a yield of 4510 kg/ha and a crude protein content of 38.2%.

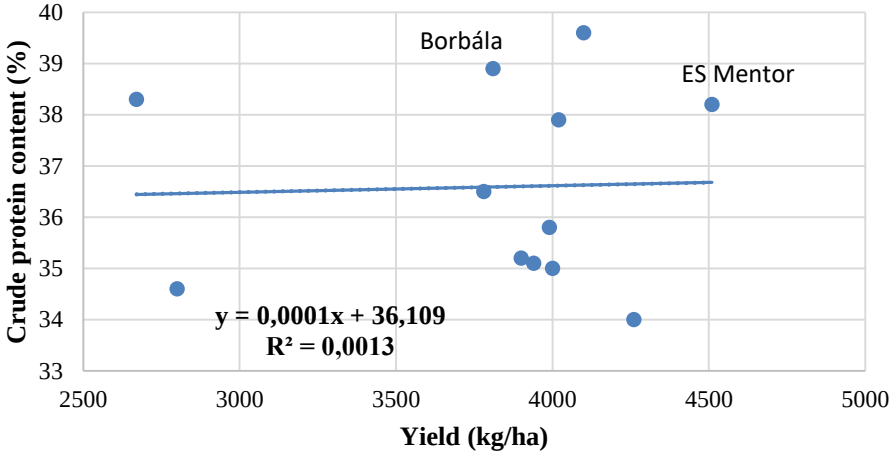

Figure 2: Correlation between the crude protein content and yield for different soybean varieties

Figure 3 shows the test results of the correlation between digestible crude protein content and yield for soybean varieties (R^2=0.0114). No correlation was found. However Aires, Prestopro, and ES Mentor should be highlighted among the varieties with above trend line results, as they have the best yield (4020 kg/ha; 4100 kg/ha, 4510 kg/ha) and digestible crude protein content (31%, 30.3%, and 28.9%). ES Mentor produced the fourth best digestible crude protein content (28.9%) with the best yield (4510 kg/ha).

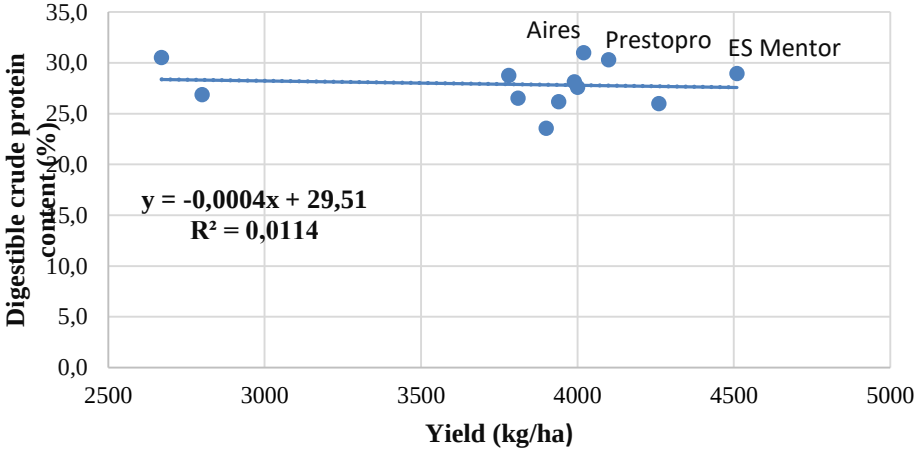

Figure 3: Correlation between the digestible crude protein content and yield for soybean varieties

CONCLUSIONS AND RECOMMENDATIONS

The following main conclusions can be drawn from the results of our study. It would be useful to include the digestible crude protein yield per hectare among the plant breeding judging aspects, which could be used as a quality indicator for the use of the crop for feed. In addition, variety selection by the grower should be based on the digestible crude protein yield values if the crop will be used or sold for feed. Our results provide a good basis for selecting varieties that are able to produce the expected yields and have an acceptable digestible crude protein content. It would be an incentive effect for plant growers, if the digestible crude protein content will be among the aspects of the forming of the purchase price so that they would have a greater interest to choose varieties that can produce higher digestible crude protein yields. It would be justifiable to perform this experiment with extracted soybean meal, to determine the extent of difference in digestibility of untreated soybean varies with heat treatment and steeping, and to determine the difference between extracted meals of varieties with different protein digestibility and the extent of the difference.

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