THESIS OF THE DOCTORAL (PhD) DISSERTATION

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DIETARY INFLUENCE OF FIBER ON THE ENERGY AND AMINO ACID DIGESTIBILITY AND ITS CONSEQUENCES FOR DIET FORMULATION IN GROWING PIG

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1.Introduction, aims of the study

The use of by-products in swine nutrition will always remain important to reduce feed costs in swine production. Increasing demand for cereals in human consumption forces the animal nutritionist to use more by-product in diets of monogastric animals. The limited availability and increasing cost of energy has created a competition between food and feed for ingredients. In the future the feed industry will have to compete not only for grain but for the by-products as well.

The process by which by-products are produced concentrates the fiber fraction. To use these ingredients the feed industry has to evaluate them precisely, considering the nutritive value of the by-products and the influence of interaction between nutrients in order to supply the nutrient requirement of livestock adequately. A better understanding of these factors will mean the use of by-product in feed formulation will not impair the animal performance, moreover reduced feed cost can be expected.

Fiber is extremely heterogonous and the behavior of different types of dietary fiber in the digestive processes is particularly dependant on their physico-chemical properties such as solubility and viscosity. Literary data show that soluble fiber reduces the apparent and true ileal digestibility of protein and amino acids more than insoluble fibers; however, fecal digestibility of energy is compromised by insoluble rather than soluble fiber content of the diet. It is necessary to develop regression equations for practical use that compute the reduction in ileal digestibility of nutrients as a function of different types of fiber, when fiber rich ingredients are used in diet formulation. Due to fiber-fat interactions, there may be significant differences between calculated and measured energy content of pig diets in the case of high fiber and high fat content. In order to do a precise and accurate diet formulation of a pig diet, further studies are required for the quantification of the effect of fiber-fat interaction on the digestible nutrient supply.

Therefore, the following objectives were determined:

1) to evaluate and quantify the effect of different dietary fiber and fat level on the total tract digestibility of nutrients and gross energy.

2) to determine the effect of increasing fiber content from different sources (from wheat bran and soy hulls) on the SID (standardized ileal digestibility) of selected amino acids (lysine, methionine, cystine threonine, and tryptophan).

3) to develop regression equations that can be used to predict the SID of selected amino acids applicable for different fiber sources commonly used in hog.

4) to obtain essential data for application in practical diet formulation when fiber rich components are used.

2. Materials and methods

In order to achieve these aims we conducted 2 trials:.

Diets, feeding and experimental procedure of the Trial 1

A total of 125 castrated hybrid (Large White x Landrace) growing pigs, 5 animals/treatment with no replicates with an initial body weight of 39 kg were used in the serial trial. The experiment was carried out with 25 dietary treatments. Pigs were fed with diets based on five different wheat bran levels (0, 150, 300, 450 and 600 g/kg) and five added fat levels (0, 25, 50, 75 and 100 g/kg) in a 5 x 5 factorial arrangement. Feed was supplied at the level of 2.6 times maintenance requirement of energy. Due to the different energy content of the feeds, the daily feed intake was different, but the energy intake and the ileal digestible protein, lysine, methionine, cystine and threonine intake were the same in each treatment. A 9-day adaptation period was followed by a 5-day collection period, during which faeces were collected quantitatively. The animals were housed in metabolic cages during both the adaptation and collection periods. Feed intake was recorded daily and occasional feed refusal was collected and measured. Fresh faeces production homogenized and stored below -18˚C until analysis.

Diets, feeding and experimental procedure of the Trial 2

The trials were conducted with total of 40 castrated hybrid (Large White x Landrace) growing pigs, 4 hybrid barrows per treatment, in 2 replicates (8 animals/treatment), with an initial mean live weight of 30 kg. Before starting the trial the animals were fitted with PVTC-cannula. The surgical operations were performed in accordance with van Leeuwen et al. (1991). With this surgery operation a specially shaped T-cannula is sucked into the cannula by vacuum generated and with the control of ileo-caecal valve the entire digesta is voided outside. In the digestibility study 10 dietary treatments were used with increasing wheat bran (WB) or soyhulls (SH) inclusion rates. The basal diet was a corn-soybean meal diet that was supplemented with 0, 25, 50, 75 and 100 g/kg wheat bran (WB-0, WB-25, WB-50, WB-75, WB-100, respectively) or soyhulls (SH-0, SH-25, SH-50, SH-75, SH-100, respectively). The analyzed NDF content of the feeds ranged from 135 to 167 g/kg in the wheat bran diets and from 135 to 179 g/kg in the soyhulls diets. The daily feed allowance covered 2.6 times of the pigs' maintenance energy requirement. The individual daily feed intake was recorded by gram precision. The trial consisted of a 5 days adaptation and a 3 x 12 hours collection period. In the collection phases the total amount of the digesta was collected and thereafter 30 % of the total collected volume was freeze-dried after homogenization.

Laboratory analysis in the trials

The nutrient (dry matter, crude protein, crude fat, fatty acids, crude fiber, N-free extract, crude ash and NDF) and amino acid content of the feeds and the amino acid content of digesta samples were determined according to AOAC (2000). In the trial 1 the GE content of the diet and faeces was determined with adiabatic bomb calorimeter (IKA-C-4000).

Calculations and statistical analysis of trial 1

The digestible energy content (DE) of the feeds was calculated according to Schiemann et al. (1972) as follows:

$$DE\ (MJ/kg) = (24.2 * dP) + (39.4 * dEE) + (18.4 * dF) + (17.0 * dNfe)$$

where:

dP	:	digestible crude protein (g/kg)
dEE	:	digestible crude fat (g/kg)
dF	:	digestible crude fiber (g/kg)
dNfe	:	digestible N-free extract (g/kg)

The experimental data were evaluated by means of ANOVA (SAS, 2004). For determination of the mean effect on digestibility coefficients two-ways ANOVA was used with the following general model:

$$Y_{ijk} = \mu + A_i + B_j + (AxB)_{ij} + e_{ijk}$$

where:

Y_{ijk}	:	dependent variables
μ	:	overall mean
A_i	:	effect of fiber level, i=5 (0, 150, 300, 450 and 600 g/kg wheat bran)
B_j	:	effect of fat level, i=5 (0, 25, 50, 75 and 100 g/kg of added fat)
$(A \times B)_{ij}$	:	interaction of fiber and fat level
e_{ijk}	:	residual error

The contribution of the main effects (fat, fiber, fat x fiber) to the variance of the dependent variables was calculated by the VARCOMP procedure of SAS (2004). The goal of VARCOMP procedure is to estimate the contribution of each of the random effects to the variance of the dependent variable. Multiple linear regressions were used to predict the digestibility of nutrients and energy as a function of crude protein, crude fat, crude fiber and N-free extract (SAS, 2004).

Calculations and statistical analysis of trial 2

Standardized ileal digestibility (SID) of each amino acid was computed by the following equation:

$$\text{SID \%} = \left[\frac{\text{AA intake [g]} - (\text{ileal AA outflow [g]} - \text{endogenous AA [g]})}{\text{AA intake [g]}} \right] * 100$$

AA loss was taken from a pilot study being 406, 52, 103, 592 and 204 mg/kg dry matter intake for lysine, methionine, cystine, threonine and tryptophan, respectively.

The experimental data was analyzed separately for treatments containing wheat bran and soyhulls by using a one-way ANOVA (SAS,2004). The relationship between wheat bran and soyhulls level of the diet and standardized ileal digestibility of amino acids was examined with regression analysis separately in each fiber source (SAS, 2004). The following regression analyses were carried out:

linear $(Y = a_0 + a_1X)$,

quadratic $(Y = a_0 + a_1X + a_2 X^2)$ and

linear-plateau with sharp transition $(Y = a_0 - a_1 * \ln[1 + \exp(a_2 - X)$;

where

X is level of wheat bran or soyhulls in the diet [g/kg] and

Y is SID of the amino acid [%]).

3. Results

3.1 The impact of dietary fiber and fat levels on total tract digestibility of energy and nutrients in growing pigs and its consequence for diet formulation

Many of the diets in the 25 treatment study (dietary wheat bran increased from 0 up to 600 g/kg combined with supplemented dietary fat from 0 to 100 g/kg) are far from the normal feeding practice; however, extreme combinations are also required to quantify the magnitude of fiber x fat interaction.

Digestibility of crude protein

Variance component analysis of digestibility of crude protein showed that the fat content of diets contributed a mere 0.9 %, and the impact of fiber was 53.3 % Table 1.. The impact of interaction between fat and fiber was limited (6.7 %) that refers to relative consistent changes in protein digestibility to increased wheat bran and fat inclusion. Up to about 39.1 %, however, digestibility was influenced by other factors, which cannot be explained with the treatment effects. It can be concluded that increasing fiber content clearly decreased the protein digestibility; however, it was also influenced by other factors independent from dietary treatments.

Table 1. The contribution of fat, fiber and fat x fiber interaction in the total variance (%)

Component of variance	Crude protein	Crude fat	Crude fiber	Gross energy
Fat-effect	0.9	51.6	13.2	3.0
Fiber-effect	53.3	5.0	30.9	86.7
Fat * fiber interaction	6.7	36.2	35.0	5.2
Other effects	39.1	7.2	20.9	5.1
Total	100.0	100.0	100.0	100.0

Digestibility of crude fat

Analyzing the variance components of fat digestibility showed that the fat content of diets contributed to it by 51.6 %, and fiber content contributed by 5.0 %, the interaction between fat and fiber was 36.2 %. In conclusion, digestibility of crude fat increased consistently by fat supplementation and by inclusion of wheat bran in case of no added dietary fat. When diets were formulated with additional fat, the wheat bran inclusion did not change the digestibility of fat consistently. The effect of fiber is modified by fat content of the diet; therefore due to the fiber x fat interaction the digestible fat content is not accurately predictable by addition of digestible fat content of feed ingredients.

Digestibility of crude fiber

Approximately 13 % of the total variance of fiber digestibility is explained by fat supplementation. The fiber content of diets contributes to the total variance by 30.9 %. The result of this study suggests that the digestibility of fiber cannot be determined by any well defined factors, because the fat x fiber interaction distorts both fat or fiber effects.

Digestibility of energy

As it can be seen from the data of variance component analysis (Table 1), the changes in energy digestibility are primarily caused by the crude fiber content of the diets (86.7 %). Fat content of the diets contribute to the total variance by 3.0 % only, whereas the interaction between fat and fiber as well as other non defined factors contribute about 5 %.

Consequences of fiber x fat interaction on diet formulation

Determination of DE content of the feed is crucial in diet formulation. For that purpose Schieman’s equation for swine (Schieman et al. 1972) are widely used, however, the limitation of the formula’s application is that it implies digestible nutrient content. Moreover, DE content of the feed can also be calculated by multiplying the gross energy content of the feed by its digestibility coefficient.

The results of the multivariate linear regression presented in Table 2 show that the digestibility coefficients of crude protein, ether extract and crude fiber can be predicted with low accuracy ($R^2 < 0.50$).

Table 2. Relationships between crude fat (EE), crude fiber (CF) and N free extract (NFE) contents of the feed (g/kg) and nutrient and energy digestibility (%) using multiple linear regression

Crude protein digestibility	RMSE = 2.200	r^2 = 0.48	P<0.0001
	coefficient	se	P-value
intercept	90.296	0.698	<0.0001
EE	0.013	0.0062	0.0316
CF	-0.136	0.0128	<0.0001
Crude fat digestibility	RMSE = 10.256	r^2 = 0.50	P<0.0001
	coefficient	se	P-value
intercept	30.457	3.219	<0.0001
EE	0.284	0.0286	<0.0001
CF	0.186	0.0589	0.0019
Crude fiber digestibility	RMSE = 5.324	r^2 = 0.17	P<0.0001
	coefficient	se	P-value
intercept	1.417	16.549	0.93
EE	0.124	0.0340	0.0004
CF	-0.076	0.0308	0.0155
NFE	0.055	0.0256	0.0347
N-free extract digestibility	RMSE = 1.768	r^2 = 0.88	P<0.0001
	coefficient	se	P-value
intercept	90.995	02.192	<0.0001
CF	-0.288	0.0102	<0.0001
NFE	0.014	0.0037	0.0003
Energy digestibility	RMSE = 1.875	r^2 = 0.84	P<0.0001
	coefficient	se	P-value
intercept	92.208	0.589	<0.0001
EE	-0.021	0.0052	<0.0001
CF	-0.264	0.0108	<0.0001

This is a logical consequence of results of variance component analysis, considering that in case of protein digestibility the undefined effect or in cases of fat and fiber digestibility the effect of fiber x fat interaction was significant. Due to the fact that the impact of fat and fiber contributed approximately 90 % of the total variance of the data, the accuracy of the regression that computes the energy digestibility is quite high ($R^2 = 0.845$).

3.2 The effect of different levels of dietary wheat bran and soyhulls on the standardized ileal digestibility (SID) of amino acids in pigs and its consequence for diet formulation

According to data in this study the SID of lysine was not influenced by the wheat bran level ($P>0.05$). Significant differences ($P\leq0.05$) were obtained between diets wheat bran not included (WB-0) and wheat bran 75g/kg included (WB-75) in the case of methionine and between diets WB-0 and WB-50 in the case of cystine. Increasing the level of wheat bran decreased the digestibility of threonine and tryptophan, however, a significant difference was only obtained between treatments WB-0 and WB-100.

Inclusion of SBH depressed the digestibility of all investigated amino acids. In the cases of lysine, methionine and cystine, the SID values were significantly lower (with 5.9, 4.3 and 5.8 % respectively) than in the control when soyhulls were not included in the diets. However, the level of soyhulls had no influence on digestibility of these amino acids. Increasing the level of soyhulls up to 50 g/kg (SH-50) reduced the digestibility of threonine and tryptophan from 77 to 69 % and from 87 to 82 %, respectively ($P\leq0.05$). Higher inclusion rates of fiber did not depress the digestibility of threonine and tryptophan.

The relationship between dietary wheat bran or soyhulls level and SID of amino acids was analyzed when a significant difference was obtained among dietary treatments (Table 3). In the case of methionine and cystine the best fit was found with linear-plateau curve.

To gain a better understanding the linear-plateau function with sharp transition was chosen:

$$(Y = a_0 – a_1 * \ln[1 + \exp(a_2 – X)])$$

in which a_0 is the value of the plateau on the Y axis, a_1 is the slope of the linear phase and a_2 is the transition point between the two phases on the X axis (Figure 1).

Table 3. Relationship between wheat bran or soyhulls level of the diet (X, g/kg) and the standardized ileal digestibility of amino acids (Y,%)

		$Y = a_0 + a_1X$			
X	Y	a_0	a_1		P-value
WB	Thr	77.3	-0.044		0.004
WB	Trp	86.8	-0.033		0.005
		$Y = a_0 + a_1X + a_2X^2$			
X	Y	a_0	a_1	a_2	P-value
SH	Thr	77.2	-0.221	0.0015	0.043
		$Y = a_0 – a_1 * \ln(1 + \exp(a_2 – X))$			
X	Y	a_0	a_1	a_2	P-value
WB	Met	84.7	-0.084	29.8	0.004
WB	Cys	77.2	-0.108	29.9	0.016
WB	Thr	73.5	-0.060	67.5	0.024
SH	Lys	77.8	-0.201	31.0	0.001
SH	Met	82.4	-0.154	29.0	0.013
SH	Cys	73.7	-0.171	37.2	0.001

WB: wheat bran, SH: soyhulls

$$Y = a_0 - a_1 * \ln (1 + \exp (a_2 - x))$$

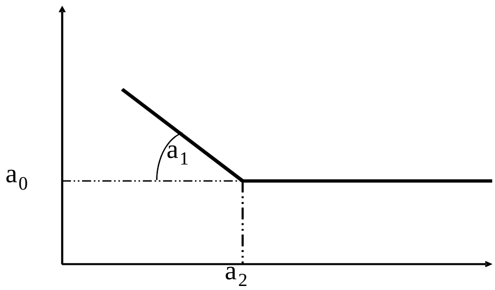

Figure 1. Linear-plateau function with sharp transition

The data from the experiment carried out shows that increasing the level of NDF from dietary wheat bran or soyhulls reduced the standardized ileal digestibility of amino acids. The reduction in digestibility coefficients was greater when soyhulls were included to the diet.

Quantification of the depression effect of fiber has significant importance in diet formulation. Based on the computed regressions the standardized ileal digestible amino acid content of the diets formulated with wheat bran and soyhulls inclusion can be calculated and from that data the required amino acid supplementation can be determined.

4. Conclusions and suggestions

The following conclusions can be drawn from the thesis:

1) Data from the experiment and the results of the variance component analysis show that among the factors of fiber, fat and fiber x fat interaction, the total tract digestibility of protein was mainly determined by the dietary fiber content (53.3 %).
2) Although fiber and fat digestibility were significantly ($P \leq 0.05$) influenced by fiber x fat interaction, the digestibility of energy was principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility was 87 % when wheat bran up to 60 % and supplemental fat up to 10 % were used in diet formulation.
3) Inclusion of wheat bran and soyhulls depress the standardized ileal digestibility of amino acids with different magnitude. Whilst 2.5 % soyhulls inclusion significantly decreases the digestibility coefficients in case of wheat bran, the statistically proven reduction in digestibility occurs at 5 % or higher level.
4) Among the selected amino acids the SID of threonine decreases mostly when fiber rich components are used like wheat bran and soyhulls. The reduction in digestibility coefficient of threonine was 4.3 % and 7.2 %, when 100 g/kg of wheat bran and soyhulls were included, respectively.
5) The relationships between wheat bran level and SID of methionine, cystine and threonine as well as between soyhulls level of the diets and SID of lysine, methionine and cystine could be described by the linear-plateau manner. In case of including wheat bran at 30 g/kg or above level the SID of methionine and cystine, and at 68 g/kg or above level

the digestibility of threonine do not decrease further. In case of soyhulls inclusion those thresholds occur at 30 g/kg for lysine and methionine, and at 37 g/kg for cystine.

6) There is a quadratic relationship between dietary soyhulls level and SID of threonine. The quadratic equation shows that SID of threonine has a minimum value at 74 g/kg of soyhulls inclusion.

7) SID of tryptophan decreased linearly with wheat bran inclusion ($P<0.05$), meanwhile in case of diets included soyhulls neither linear, quadratic nor linear-plateau curve could be fitted to the plots ($P>0.05$).

8) In diet formulation, to maintain the digestible amino acid content of the diets, the lysine, methionine, cystine and threonine supply should be increased up to the level to compensate for the depression of SID caused by wheat bran or soyhulls inclusion, but not thereafter. According to the data in the study the lysine and methionine supplementation should not be increased above 30 g/kg soyhulls inclusion. Using wheat bran, methionine and cystine supplementation should not be increased above 30 g/kg, and threonine supplementation above 68 g/kg wheat bran inclusion.

9) The results of the study suggest that the source of NDF has to be considered in the diet formulation, since the magnitudes of the reduction in SID of amino acids are different when wheat bran and soyhulls are used.

10) Based on the research carried out it can be concluded that further studies are required to quantify the effect of different fiber sources on the standardized ileal digestibility of amino acids in pigs.

5. New scientific achievements

1) Although fiber and fat digestibility are significantly ($P \leq 0.05$) influenced by fiber x fat interaction, the digestibility of energy is principally affected by dietary fiber content. The contribution of fiber effect within the total variance of energy digestibility is 87 % when wheat bran up to 60 % and supplemental fat up to 10 % are used in diet formulation.
2) Inclusion of wheat bran and soyhulls depress the standardized ileal digestibility of amino acids with different magnitude. Whilst 2.5 % soyhulls inclusion significantly decreases the digestibility coefficients, in case of wheat bran the statistically proven reduction in digestibility occurs at 5 % or higher level.
3) The relationships between wheat bran level and SID of methionine, cystine, and threonine, as well as between soyhulls level of the diets and SID of lysine, methionine and cystine can be described by linear-plateau manner. In case of including wheat bran at 30 g/kg or above level the SID of methionine and cystine, and at 68 g/kg or above level the digestibility of threonine do not decrease further. In case of soyhulls inclusion those thresholds occur at 30 g/kg for lysine and methionine, and at 37 g/kg for cystine.

6. Publications from the thesis studies

Papers related to the thesis:

1) Dégen, L., Halas, V., Babinszky, L., 2006. A nyersrost szerepe a hízósertések takarmányozásában: a klasszikus és az új rostelmélet (The role of the dietary fiber in hog grower and finisher diets, the classical and new fiber theory) (in Hungarian) TAKARMÁNYOZÁS 2, 9-13.

2) Dégen, L., Tossenberger, J., Halas, V., Babinszky, L., 2006. The effect of soybean hulls inclusion on the apparent and ileal digestibility of selected amino acids in growing pigs # 377 proceeding. J. Anim. Sci. Vol. 84 Suppl.

3) Dégen, L., Halas, V., and Babinszky, L., 2007. Effect of dietary fibre on protein and fat digestibility and its consequences on diet formulation for growing and fattening pigs: A review, Acta Agriculturae Scandinavica, Section A - Animal Sciences. 57:1, 1–9

4) Dégen, L., Halas, V. , Tossenberger J., Szabó, Cs., Babinszky, L., 2009. The impact of dietary fiber and fat levels on total tract digestibility of energy and nutrients in growing pigs and its consequence for diet formulation. Acta Agriculturae Scandinavica, Section A - Animal Sciences, 59:3, 150-160.

5) Dégen, L., Halas, V., Tossenberger J., Babinszky, L. The effect of different levels of dietary wheat bran and soyhulls on the standardized ileal digestibility of amino acids and their consequences for diet formulation. Act. Agraria Kaposvariensis (in press).

Oral presentations:

1) Dégen, L, Tossenberger, J., Halas, V., Babinszky, L., 2006. The effect of soybean hulls inclusion on the apparent and ileal digestibility of selected amino acids in growing pigs; ADSA ASAS Joint Annual Meeting Minneapolis, Minnesota 2006 July 11.