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ESTIMATION OF FEED PROTEIN QUALITY BY NITROGEN BALANCE EXPERIMENTS

PROCJENA KAKVOĆE BJELANČEVINA POKUSIMA RAVNOTEŽE DUŠIKA

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SUMMARY

Feed, proteins vary in the amino acid content and this one is never ideal or sufficient for the animal supply. The quality of proteins could be determined by some chemical methods, but to answer the question about the availability of the proteins to animals it is necessary to perform some animal experiments. In the studies of true digestibility, biological value and net protein utilisation two defined varieties of Slovenian wheat (Ana and Rezka), six defined varieties of barley (Rabur, Union, Barley 22, Barley 26, Križanec and Berta), two samples of barley of undefined origin, three defined hybrids of maize (Lj-275/6, E-Lj-15/76 and E-Lj-277/25), one sample of maize of undefined origin and one sample of oats and buckwheat were included. The results of true digestibility and biological value of those cereals are in many cases inferior to cited in the literatur. In barley samples big variability was found, especially in true digestibility (66.61%-93.2%) and net protein utilisation (54.32%-76.9%) and some smaller variability in biological value (79.89%-90.6%). Wheat proteins are on average more digestible than barley proteins, but biological value of wheat proteins was lower than of barley proteins. The variability in true digestibility and biological value of different maize samples is very big. The influence of supplementation one (lysine) or two (lysine and methionine) amino acids in barley Gotic and wheat Rezka, both of Slovenian origin, on true digestibility, biological value, net protein utilisation and nitrogen excretion was determined. Supplementation with only two essential amino acids gives a better biological value and protein utilisation, which is in connection with improved nitrogen utilisation and the reduction of nitrogen excretion.

Key words: wheat, barley, oats, buckwheat, maize, amino acids, protein, true digestibility, biological value, net protein utilisation

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INTRODUCTION

The primary function of dietary protein is to furnish a mixture of amino acids of the proper pattern for the synthesis of tissue proteins and for body maintenance. Any method for measuring the value or quality of food proteins must, directly or indirectly, evaluate these functions. Nonessential amino acids can be synthesised in the animal organism in excess of the requirements. Essential amino acids cannot be synthesised, or cannot be synthesised at a sufficient rate to permit optimal growth, reproduction or health of animal. Allthough amino acids in both categories are needed at the physiological or metabolic level, normal simple - stomached animals and human diets contain adequate amounts of nonessential amino acids or amino groups for their synthesis. This was confirmed even for low-protein diets that were supplemented with crystalline amino acids (Brudevoid and Southern, 1994). Therefore the dietary provision of essential amino acids in correct amounts and proportions determines the adequacy of a dietary protein concentrate.

Only chemical methods, and even amino acid analyses, are not suitable to estimate the protein usefulness to the animal. Before the protein becomes available to the animal it must undergo digestion. The digestible protein in a feedstuff may be determined by in vivo digestibility trials. Such trials give figures for "apparent" and "true" protein digestibility. Up to the present amounts of digestible protein were used in diet calculations, supported by estimated biological value of proteins. The concept of biological value of protein was based on the nitrogen gained or lost by an animal determined by an analysis of food, faeces and urine in nitrogen balance experiments (Mitchell, 1923-1924; Allison, 1955, cit. by McLaughlan and Campbell, 1969).

The fraction of resorbed nitrogen retained in the body has been defined as the "biological value" of the protein. The biological value of protein depends upon the number and kind of digestible amino acids present in the molecule. The nearer the food protein approaches the body proteins in amino acid make-up, the higher will be its blological value. The biological value reflects the content of the limiting amino acid in the protein. Biological value is a suitable method for the evaluation of protein quality, but it is obvious that no single figure for it will suffice as a measure of the nutritional value of a feed protein for different animals and different functions. The consequent need for multiple figures limits the use of the biological value concept in practice.

Besides biological value many other methods for the evaluation of protein quality have been developed: Protein efficiency ratio, Net protein ratio, Net protein utilisation, Net protein value, Chemical scoring methods, Liver protein and enzymes, Rat repletion methods, Microbiological methods, Blood amino acid methods and different methods to determine availability of amino acids. These methods have been reviewed frequently (McLaughlan and Campbell, 1969, McDonald et al. 1995).

Today a fundamental concept in determining amino acid requirements is that it is possible to obtain an optimal dietary pattern among essential amino acids that corresponds to the needs of the animal. This optimal dietary pattern is often called "ideal protein" and is based on methods and results of measuring feedstuf amino acid ileal digestibility (NRC, 1998; Babinszky et al., 2001). After the "ideal protein" concept the use of cristallinic essential amino acids is getting more and more efficient.

There are more significant postulations, which support the implementation of ideal protein concept especially in swine nutrition. A closer balance between digestible amino acids supplies in feed and animal response potential will lead to optimal animal growth and, at the same time, reduce the quantity of nitrogen excreted into the environment. Not negligible is the fact that after BSE problems the use of meat and bone meal in animal nutrition is prohibited. We specially support low plant protein feedstuffs production in Slovenia. But also produced corn, barley and wheat contain proteins. Those raw materials are the primary ingredients of most swine diets and usually provide 30 to 60 percent of the total amino acid requirements. Justified use of crystalline amino acids can improve the utilisation of home produced cereal grains and reduce the import of other sources of protein, such as soybean meal.

Tables of amino acid ileal digestibility are presented (NRC, 1998; Jonderville et al., 1995).

There is opinion that the amino acid composition of a given feed protein will be relatively constant (McDonald et al., 1995). The use of tabular presentation of results of ileal aniino acid digestibility also presumes the same values for the cereal grains produced under different conditions. That cannot be true. Not only in the protein content but also in the protein quality and ileal amino acid digestibility of the same kind of cereal grains significant variability can be expected.

After several trials performed in Slovenia in the last 20 years and in comparison to results cited in the literature we can demonstrate the differences in digestibility and biological value of grain proteins.

MATERIAL AND METHODS

True digestibility (TD), biological value (BV) and net protein utilisation (NPU) were investigated in young male laboratory rats of the Wistar strain in the body mass between 90 and 120 g. The experiments were done by the Thomas-Mitchell balance method in the metabolic cages of our own construction. Each sample of the diets was tested on four animals. The pre-experimental period lasted five days, also the experimental period in which the feed intake and the amount of faeces and urine were measured. During the experiments the room temperature was practically the same (about 21°C) with special moisture conditions (about 60%). The light was regulated automatically during the experiment to obtain the same conditions every day (12 hours light/dark regime).

Diets for animals were prepared from milled cereals or maize with the addition of minerals and vitamins in order to meet rat requirements. Nitrogen in samples of cereals or maize, tested diets, faeces and urine were determined by the Kjeldahl method in the chemical laboratory of the Institute of Nutrition in Rodica.

From the data obtained was calculated:

True digestibility (TD, %) = (N intake - (N faeces - N endogenous))/ N intake

Endogenous N faeces (ENF, mg) = 15.2 x (average body mass/ 0.075)

Endogenous N urine (ENU, mg) = 0.081 x (average body mass) + 3. 01

$$\text{Biological value (BV, \%)} = \frac{N_{intake} - (N_{faeces} - MNF) - (N_{urine} - ENU)}{N_{intake} - (N_{faeces} - MNF)}$$

Net protein utilisation (NPU, %) = BV x TD

RESULTS AND DISCUSSION

In Table 1 the results of true digestibility, biological value and net protein utilisation of two varieties of Slovenian wheat, Ana and Rezka are presented. In literature could be higher values for true digestibility of wheat, between 88.8% to 93.4% found (Nerhing and Bock, 1961), in comparison to our results. Lang and Schoen (1952) give a biological value for wheat protein at 67% and Nerhing and Bock (1961) found 69%. These values are similar to those obtained in the experiment with Slovenian wheat variety Ana and Rezka; on the other hand these values are superior to the value obtained by Nerhing and Bock (1961) -60%.

Table 1. True digestibility (TD), biological value (BV) and net protein utilisation (NPU) of two defined varieties of wheat

Tablica 1. Stvarna probavljivost, biološka vrijednost, neto iskoristivost bjelančevina dviju određenih kultivara pšenice

	True digestibility Stvarna probavljivost	Biological value Biološka vrijednost	Net protein utilisation Neto iskoristivost bjelančevina
Ana*	79.12±3.22	68.21±6.04	53.49±5.07
Rezka*	84.06± 3.22	67.85± 6.04	56.51±5.07

* Tomšič, 1997

Wunsche and Bock (1965) found true digestibility of barley 89.3% and biological value 75.7%. In different samples of barley we found some smaller values for true digestibility with big variability among the samples. Values vary between 66.61% and 93.2%. From Table 2 it can be seen that two samples of barley of undefined origin have very high values for true digestibility. Barley Križanec and Berta have the smallest values for true digestibility, but biological value is not much different from that in other samples of barley, which is between 79.89% and 90.6%. These values are higher than the values obtained by Wunsche and Bock (1965) -75.7%. In the values of net protein

utilisafion of different varieties of barley again variability increased between 54.32% and 76.9% can be seen, which is the result of variability in true digestibility.

Table 2. True digestibility (TD), biological value (BV) and net protein utilisation (NPU) of defined varieties of barley and two samples of barley of undefined origin.

Tablica 2. Stvarna probavljivost, biološka vrijednost i neto iskoristivost bjelančevina dvaju određenih kultivara ječma i dva uzorka ječma neodređenog podrijetla

	True digestibility Stvarna probavljivost	Biological value Biološka vrijednost	Net protein utilisation Neto iskoristivost bjelančevina
Stock from Homec*	85.3	85.1	72.3
Stock from Emona*	84.6	81.4	68.8
Rabur*	93.2	82.5	76.9
Union*	73.6	82.7	60.9
Barley 22*	73.8	85.5	63.2
Barley 26*	77.5	90.6	70.2
Križanec**	68.89±4.00	83.81±7.51	58.78±6.30
Berta**	66.61±4.00	79.89±7.51	54.32± 6.30

* Orešnik and Cvirn, 1984a

** Tomšič, 1997

In Table 3 the results of one sample of oats and buckwheat and four samples of maize can bee seen. The result of true digestibility for oats is a little smaller than literature data, which are between 78 and 83% (Novus, 1992), but biological value is in agreement with the literature data (McDonald et al., 1995). Buckwheat proteins are well known for the good biological value. Eggum (1980) found in the same variety of buckwheat for true digestibility, biological value and net protein utilisation were 79.9%, 93.1% and 74.3%, respectively, which is very similar to our results.

True digestibility of different maize was between 86.5% and 91.1%, which is connected with different crude protein content of samples (Orešnik and Cvirn, 1984b). The variability in biological value of those maize samples is even higher, between 42.9% and 84.5%. According to Kellner and Becker (1976) and Kirchgessner (1980) the biological value of different maize samples varies between 54 and 60%.

Table 3. True digestibility (TD), biological value (BV) and net protein utilisation (NPU) of defined variety of oats, buckwheat and three defined varieties of maize and one sample of maize of unidentified origin.

Tablica 3. Stvarna probavljivost, biološka vrijednost i neto iskoristivost određenog kultivara zobi, hmelja i tri određena kultivara kukuruza i jednog uzorka neodređenog podrijetla

	True digestibility Stvarna probavljivost	Biological value Biološka vrijednost	Net protein utilisation Neto iskoristivost bjelančevina
Oats 96*	71.23±3.22	63.20±6.04	46.00±5.07
Buckwheat **	80.8	91.2	73.3
Maize			
Stock from Homec***	86.5	84.5	73.2
Lj - 275/6***	90.6	63.8	57.8
E - Lj - 15/76***	88.2	42.9	37.8
E - Lj - 277/25***	91.1	71.1	64.8

*Tomšič, 1997

** Orešnik and Cvirn, 1984a

*** Orešnik and Cvirn, 1984b

Amino acid composition of different cereals is well known and presented in many nutrition tables (Souci et al, 1994; NRC, 1998; Jonderville et al, 1995; Kunachowicz et al, 1998). The utilisation of cereal proteins as well as maize protein is limited by low lysine content. The total amount of amino acids in some defined samples of Slovenian cereals was determined not long ago (Pirman and Orešnik, 1999). The concentrations of essential amino acids in wheat, barley, oats or maize are inferior to the results of Souci et al., (1994), but in some cases superior (NRC, 1998). Supplementation of limiting amino acids in the diets based on cereals could improve the biological value and net protein utilisation of proteins.

In Table 4 the influence of supplementation of one or two limiting amino acids in wheat or barley proteins is presented. According to those results, wheat proteins were much better digestible than barley proteins. Contrary to that the biological value of wheat protein was lower than that of barley protein. The calculation of average essential amino acid index shows higher values for barley as compared to wheat, 0.64 and 0.58, respectively (Pirman and Orešnik, 1999), which is the confirmation of better biological value of barley proteins, as can be seen in Tables 1 and 2. The net protein utilisation obtained in the control group and in the group with one amino acid supplemented was similar.

Supplementation of diets with lysines lightly improved the true digestibility of wheat and barley proteins and did not have an influence on the biological values and net protein utilisation. Supplementation of two limiting amino acids improved the net protein utilisation, more in wheat than in barley proteins. It is well known that the biological value is not a very good method for getting the best answer to the positive effect of essential amino acids added. In Table 4 it could be seen that only the third group of wheat (supplemented with lysine and methionine) gives a real better biological value. Glem-Hansen and Eggum (1972) show that of non-essential amino acids might effect the biological value. This is the reason why biological value is not dependent only on the first limiting amino acids, but on the whole aminogram. This could be a reasons, why the supplementation of only lysine in wheat protein has no effect on the improvement of protein utilisation, two amino acids are much more effective.

Table 4. Influence of supplementation of limiting amino acids on true digestibility, biological value and net protein utilisation of wheat and barley proteins (Orešnik and Blanchon, 1999)

Tablica 4. Utjecaj dodatka limitirajućih amino kiselina na stvarnu probavljivost, biološku vrijednost i neto iskoristivost bjelančevina pšenice i ječma

	True digestibility Stvarna probavljivost	Biological value Biološka vrijednost	Net protein utilisation Neto iskoristivost bjelančevina
Wheat Rezka			
Control	81.1	63.5	51.4
Control + Lys	82.9	62.7	51.9
Control + Lys + Met	84.6	67.8	57.4
Barley Gotic			
Control	66.1	75.9	50.2
Control + Lys	68.9	75.5	52.0
Control + Lys + Met	72.2	75.7	54.6

Table 5. Excretion of nitrogen per g of daily gain (Orešnik and Blanchon, 1999)

Tablica 5. Izlučivanje dušika na g dnevnog prirasta

	Excreted N in faeces and urine (mg) - Dušik izlučen fecesom i urinom (mg)	Daily gain - Dnevni prirast (g)	Excreted N per g of gain (mg) - Dušik izlučen po g prirasta (mg)	Difference Razlika (mg)	%
Wheat Rezka					
Control	118	2.45	48.2		
Control + Lys	139	3.00	46.8	-1.9	3.9
Control + Lys + Met	124	3.93	33.4	-16.6	34.4
Barley Gotic					
Control	131	2.66	49.2		
Control + Lys	117	2.78	42.1	-7.1	14.4
Control + Lys + Met	134	4.21	31.8	-17.4	35.4

Important item in the results of supplementation of the limiting amino acids in the diets based on wheat or barley proteins was the reduction of animal nitrogen excretion after diet amino acid supplementation (Table 5). Good results were obtained with both cereals. The two thirds of the groups of rats reduced their nitrogen excretion by 34.4% and 35.4% for wheat and barley, respectively. Both groups with supplementation of lysine only, reduced the nitrogen excretion in a smaller amount than the both groups with supplemented two limiting amino acids. Better protein utilisation is connected with the reduction of excreted nitrogen. This fact is important not only in animal nutrition, but also for prevention of environmental nitrogen load.

In all the trials performed a significant variability was demonstrated nitrogen balance studies obtained results. Also amino acid composition of cereal grain protein produced in Slovenian conditions differs from tabellary presented values. We can also expect such variability in ileal amino acid digestibility and for that reason tabellary presented results in the literature are certainly not the optimal solution for implementation of ideal protein concept in swine nutrition. Our statements support the demand for the determination of ileal amino acids digestibility of cereal grain proteins produced in our conditions.

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SAŽETAK

Bjelančevine u krmi variraju u sadržaju amino kiselina koji nije nikada idealan ili dovoljan za opskrbu životinja. Kakvoća bjelančevina može se odrediti nekim kemijskim metodama ali je potreban odgovor na pitanje o dostupnosti tih bjelančevina za provođenje pokusa na životinjama. U povećavanje stvarne dostupnosti tih bjelančevina, biološke vrijednosti i iskoristivosti neto bjelančevina- uključena su dva kultivara slovenske pšenice (Ana i Rezka), šest određenih kultivara ječma (Rabur, Union, Barley 22, Barley 26, Križanec i Berta), jedan uzorak kukuruza neodređenog podrijetla i jedan uzorak zobi i heljde. Rezultati stvarne probavljivosti i biološke vrijednosti tih žitarica u mnogo su slučajeva slabiji od navoda u literaturi. U uzorcima ječma nađena je velika raznolikost, osobito u stvarnoj probavljivosti (66.61% do 93.2%) i neto iskoristivosti bjelančevina (54.32% do 76.9%) te nešto manje raznolikosti u biološkoj vrijednosti (79.89% do 90.6%). Bjelančevine pšenice su prosječno nešto probavljivije od bjelančevina ječma ali biološka vrijednost bjelančevina pšenice niža je od bjelančevina ječma. Raznolikost stvarne probavljivosti i biološke vrijednosti različitih uzoraka kukuruza vrlo je velika. Određen je utjecaj dodavanja jedne (lizin) ili dvije (lizin i metionin) amino kiseline ječma Gotic i pšenice Rezka, obje slovenskog podrijetla na stvarnu probavljivost, biološku vrijednost, neto iskoristivost bjelančevina i izlučivanje dušika. Samo dodavanje dviju bitnih amino kiselina daje bolju biološku vrijednost i iskoristivost bjelančevina, što je u svezi s poboljšanom iskoristivosti dušika i smanjenjem izlučivanja dušika.

Ključne riječi: pšenica, ječam, kukuruz, amino kiseline, bjelančevine, stvarna probavljivost, biološka vrijednost, neto iskoristivost bjelančevina