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Improvment of dietary grain protein utilization in order to reduce environmental impact from pollution

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ABSTRACT

Proteins varie in its amino acid composition and this one is never ideal or sufficient for the animal. That's why the diets are, very often, supplemented by some synthetic amino acids. Two different cereals produced in Slovenia are tested here (wheat Rezka and barley Gotic) supplemented or not by one (Lys) or two (Lys+Met) amino acids to obtain a better utilisation in the view of a weight gain superior , and the decrease of the nitrogen excreted. The results obtained by nitrogen balance studies with laboratory rats, show that these two cereals have a CP, TD and BV inferior to in literature cited. The PER gives some very good values for the weight gain in the presence of synthetic amino acids (2,23 g/1g protein for wheat and 2,4 g/1g protein for barley against 1,75g and 1,7 g without supplementation). Moreover the excretion of nitrogen shows a real decrease (34,4% for wheat and 35,4% for barley) for both third groups in comparison with the control group.
Keywords: animal nutrition, protein, aminoacids, nitrogen balance, cereals, wheat, barley, rat

ZUSAMMENFASSUNG

Bessere Anwendung der Proteine im Futtergetreide im Interesse einer verminderten Umweltverschmutzung

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Die Proteine unterscheiden sich aufgrund ihrer Aminosäuren-Zusammensetzung voneinander. Sie sind für die Tiere weder ideal noch ausreichend. Deshalb wird das Futter mit synthetischen Aminosäuren ergänzt. In diesem Versuch wurden in Slowenien zwei Getreidesorten getestet (Weizen Recka und Roggen Gotic). Diese wurden mit ein (Lysin) bzw. zwei (Lysin und Methionin) Aminosäuren ergänzt mit dem Ziel, eine höhere Gewichtszunahme zu erreichen bzw. die N-Ausscheidung zu verbessern. Die erhaltenen Ergebnisse weisen darauf hin, dass in den beiden Getreidesorten die Werte für CP, TD und BV niedriger sind als in der Fachliteratur angegeben. In Anwesenheit von synthetischen Aminonsäuren brachte PER ein gutes Ergebnis hinsichtlich der Gewichtszunahme (beim Weizen wurden pro Gramm Protein 2,23 g, beim Roggen 2,4 g Zunahme erreicht gegenüber den Gewichtszunahmen ohne Ergänzung von 1,75 bzw. 1,7). Weiterhin war eine reale Verringerung der Nitrogenausscheidung (bei Weizen

34,4%, bei Roggen 35,4%) in der zweiten und dritten Versuchsgruppe gegenüber der ersten (Kontrollgruppe) festzustellen.
(Schlüsselwörter: Tierfütterung, Proteine, Aminosäuren, N-Gleichgewicht, Getreide)

INTRODUCTION

From the economical point of view, modern animal production gives a special emphasis to the wellbeing of animals. In ration formula the objective is for use knowledge about nutrients, feedstuffs and animals in the development of nutritionally adequate rations that will be eaten in sufficient amounts to provide the level of production desired at a reasonable cost. Protein is a critical nutrient particulary for young rapidly growing animals. Optimal use of protein is a must in any practical feeding system, because protein supplements are usually much more expensive than energy feeds and wasteful usage increases the cost of production in almost all instances.

Synthetic amino acids are used in feed manufacture to improve protein quality. Improving the cereal protein utilisation by additional limiting essential amino acids feeding of animals, results also in possibilities to lower total crude protein level in the diet and enable lower excretion of remaining nitrogen with a prevention of environmental nitrogen load.

That is why it is very important to test different diets (based on cereals) and their influence on the nitrogen balance, protein utilisation and production when they are supplemented with some limiting components. In fact, in this case, the supplementation of the diets of animals (rats) with limiting amino acids could enhance the quality of the dietary protein.

Two different Slovenian grain samples (wheat Rezka and barley Gotic), with addition of one or two synthetic essential amino-acids will be tested. The influence of the supplementation of these diets on the nitrogen balance and on protein utilisation (positive effects expected) will be analysed.

Nitrogen balance studies in biological experiments with different animals (measuring amounts of ingested and by feces and urine excreted N) can give the answer what amounts of ingested or absorbed amino acids are utilized for a new protein synthesis in the body. The systems of ranking dietary proteins after this method are: Biological Value (BV), Net Protein Value (NPV) and Net Protein Utilisation (NPU). (*McLaughlan* and *Campbell*, 1969).

The literature can give, after numerous experiments, an ideal protein or an ideal menu for one animal, for example, the two different propositions for the ideal protein for the pig (*Cole*, 1978; *Fuller et al*., 1979).

The best solution to create imbalance is to use specially designed mixtures. These mixtures can be tested for the growing of animals (*Pant*,1972). The test of some mixtures which contain 1 or 2 more limiting amino acids represent a real basis to study the influence of the supplementation on the growth or on the utilisation of the compounds of the diets by the rats and later on by the farm livestock.

It is important to study the food protein quality because a tremendous variety of feedstuffs is used for animal feeding troughout the world. The variety in a given location will depend, in fact, on the local products grown or harvested and the class and species of animal involved. 85-90% of the nitrogenous compounds in the seed is in form of proteins, but the proteins, their solubility and amino acids content vary from cereal to cereal.

Wheat and barley are two of the most important cereals used for feeding. A lot of differences exist between the both. Barley is know to be low in lysine and will normally show a positive response to a lysine supplement, but it is important to note that a considerable variation in protein quality from one barley lot to another is observed (*Bengtsson* and *Eggum*, 1969; *Schiller*, 1971). The TD of total N, lysine and methionine are, respectively, 80.2%, 78.3% and 86.4% in rats experiments with barley (*Poppe*, 1969). Finally with high lysine barley the BV, in rats experiments, is increased by 34.8% (*Jorgensen et al*., 1997). Wheat protein quality is inferior to that in barley, due to a very low lysine and threonine contents, but the TD is higher than in barley. With rats *Nerhing* and *Bock* (1961) obtained a value of 69% for BV.

MATERIALS AND METHODS

The diets are prepared in the laboratory. A sample is chosen (wheat Rezka or barley Gotic) and represents the higher amount in the diet. Chemical analyses of the different cereal sample are realised, moreover the analyses of the amino acids composition of both was also realised. Some other components are added to the test diets in determined quantity:

- sample (87,52%; 2625,6g for 3 kg), cereal here.
- cellulose (3%; 90g)
- sunflower oil (4%; 120 g)
- premix (4%; 120g)
- $CaCO_3$ (1,35%; 40,5g)
- NaCl (0,13%; 3,9g)

The components were mixed and divided into 3 portions, the first for the control group of rats, and the two others for the two different test groups. In the two last diets one or two synthetic amino acids were added to test their influence on the nitrogen balance.For the experiment with wheat Rezka:

- 4,1g of Lys are added (diet 2)
- 4,1g Lys and 5,2g of Met are added (diet 3)

For the experiment with barley Gotic:

- 3,0g of Lys are added (diet 2)
- 3,0g of Lys and 5,3g of Met are added (diet 3)

The amino acids are added after the known amino acid composition of tested proteins to cover 75% of the optimal requirements of the animals (NRC, 1995).

The rats used are from the WISTAR (Novo Mesto) strain, at the start of the experiment they were 42 days old. 12 rats divided in three groups of four were used in each experiment.

The room during all the experience had to be practically at the same temperature (about 21°C) and with special moisture conditions (about 60%). The light was regulated by an automatic timeswitch during all the experiment to obtain the same conditions every day (12 hours light regime).

Twelve cages were used, one per a rat. These metabolic cages permit to collect the urine and the feces during all the experiment, normally without any contamination between the urine and feces. In these cages food and water were always available.

Nitrogen balance experiments were performed after the mode of work developed in the laboratory of Institute for Nutrition (*Orešnik et al*., 1981,1982,1984).

Some tries for determination of crude protein and nitrogen concentration were also performed with wheat flour (BCR) to see if the results are in accordance with the certificate value of the reference materials.

Formulas

- Apparent digestibility (AD, %) =(N intake-N feces)/N intake
- True digestibility (TD, %) = (N intake- (N feces-N endogenous))/N intake
- Endogenous N feces (MNF, mg) =15,2x (average body weight/0,075).
- Endogenous N urine (ENU, mg) =0,081x (average body weight)+3,01
- Biological value (BV, %) =[N intake-(N feces-MNF)-(N urine-ENU)]/(N intake-(N feces-MNF).
- Net protein utilisation (NPU, %) =(BVxTD)/100.
- Protein efficiency ratio (PER, g) =Gain in body weight(g)/Protein consumed (CP).

Statistical studies

Data were analysed by teh Analysis of Variance (ANOVA) procedure (SAS, 1990) from SAS® software (Realese 6.11), taking into consideration the grain (wheat of barley) and type of diet (control, lysin supplemented amd lysin and metionin supplemented) as the main effect. Comparisons of different kinds of treatment were done by Tukey test. If not explained otherwise, then the least significant difference (5%) was used to separate treatment means.

RESULTS

It is important to note that the wheat has some superior values in the crude protein than the barley. The addition of Lys only increases the CP of the wheat diet but not really the value for barley. On the contrary the addition of Lys and Met increases the CP in both diets.

Table 1

Nitrogen (N) and crude protein (CP) contents in test diets

		N (mg/100g)	CP (g/100g)
Wheat (1)	1 (Control)	165	10,34
	2 (Con+Lys)	175	10,91
	3 (Con+Lys+Met)	177	11,07
Barley (2)	1 (Control)	138	8,65
	2 (Con+Lys)	139	8,68
	3 (Con+Lys+Met)	144	9,01

1. Tabelle: Gehalt von Nitrogen (N) und Rohprotein in Fütterungsversuchen

Weizen(1), Gerste(2)

Table 2

Average weight of rats, daily gain and consumed quantity of test diet

GROUP (1)		AVERAGE WEIGHT start of experiment g (2)	AVERAGE WEIGHT end of experiment g (3)	AVERAGE DAILY GAIN g (4)	CONSUMED TEST DIET g/day (%) (5)
WHEAT (6)	1 (Control)	99,0	111,3	2,45	13,49
	2 (C+Lys)	112,9	128,0	3,00	15,62
	3 (C+Lys+Met)	116,5	136,2	3,93	15,96
BARLEY (7)	1 (Control)	113,5	126,8	2,66	17,75
	2 (C+Lys)	106,8	120,7	2,78	16,61
	3 (C+Lys+Met)	120,0	141,1	4,21	19,22

2. Tabelle: Durchschnittsgewicht, tägliche Zunahme und Futterverbrauch von Ratten

Gruppe(1), Durchschnittsgewicht zu Versuchsbeginn(2), Durchschnittsgewicht zu Versuchsende(3), durchschnittl. Tageszunahme(4), Futterverbrauch(5), Weizen(6), Gerste(7)

The average weight of animals after the five days adaptation period were already different due to the different form of diet proposed to the different groups. At the end of the experiment the weight of the rats was again really different and the differences between the weights are now more important between the three different groups of each experiment. For the weight gain the addition of the synthetic amino acid was favourable in both experiments. But in both experiments, and particularly in the barley experiment, the addition of Met and Lys to the diet seems to be more important and the difference in weight gain between the group 2 and 3 was very high. Finally the weight gain difference between the group 1 and 3 represents 60,4% in wheat and 58,3% in barley experiments. The amount of wheat diet consumed by the rats increased regularly but the difference between the group 2 and 3 is not really important compared to the difference in weight gain. Barley diet consumed group 1 more than the group 2 but its weight gain was inferior. A big difference in the consumption in the group 3 can be noted, for this group the difference in the weight gain follows the same sense as in wheat experiment.

The consumed amounts of nitrogen increased normally between the group 1 and the two others due to the increasing of the amount of N in the diet after addition of the synthetic amino acid and to higher diet consumption. The excreted amounts of nitrogen in urine increased, but irregularly, between the different groups. On the contrary and in general (exception for the wheat group 2) the amount of N excreted by the feces is lower in the groups 2 and 3 than in the group 1. Finally it can be noted that for wheat experiments higher amount of N was excreted with urine and on the contrary with the feces in barley groups.

Table 3

Average amounts of nitrogen and nitrogen excreted by feces and urine

GROUP (1)		CONSUMED NITROGEN mg/DAY (2)	EXCRETED N in FAECES mg/DAY (3)	EXCRETED in URINE mg/DAY (4)	N-BALANCE mg/DAY (5)
WHEAT (6)	1(Control)	224[a]	46	72	107[a]
	2 (C+Lys)	272[ab]	49	90	134[b]
	3 (C+Lys+Met)	282[b]	42	82	154[c]
BARLEY (7)	1(Control)	247	89	42	113
	2 (C+Lys)	231	73	44	114
	3 (C+Lys+Met)	277	80	54	144

Values with no equal superscritpts in the same column differ significantly. *(Die Werte mit ungleichen Indexen in der gleichen Spalte sind unterschiedlich signifikant.)*

3. Tabelle: Durchschnittliche Nitrogenaufnahme sowie Nitrogenausscheidung in Kot und Urin

Gruppe(1), Nitrogenverbrauch mg/Tag(2), Ausscheidung im Kot mg/Tag(3), Ausscheidung im Urin(4), N-Bilanz (5), Weizen(6), Gerste(7)

Table 4

Apparent digestibility (AD), true digestibility (TD), biological value (BV), net protein utilisation (NPU) and protein efficiency ratio (PER) of test diets protein

GROUP (1)		AD %	TD %	BV %	NPU %	PER g gain/1g prot
WHEAT (2)	1 (Control)	80,1	81,1	63,5	51,4[a]	1,75
	2 (C+Lys)	82,0	82,9	62,7	51,9[a]	1,76
	3 (C+Lys+Met)	83,7	84,6	67,8	57,4[b]	2,23
BARLEY (3)	1 (Control)	65,0[a]	66,1[a]	75,9	50,2	1,7
	2 (C+Lys)	67,8[ab]	68,9[ab]	75,5	52,0	1,9
	3 (C+Lys+Met)	71,2[b]	72,2[b]	75,7	54,6	2,4

Values with no equal superscripts in the same column differ significantly (P≤0,05). *(Die Werte mit ungleichen Indexen in der gleichen Spalte sind unterschiedlich signifikant.)*

4. Tabelle: Scheinbare Verdauung (AD),tatsächliche Verdauung (TD), biologischer Wert (BV), Netto-Proteinverbrauch (NPU) und Wirkungsgrad des verbrauchten Proteins im Versuchsfutter

Gruppe(1), Weizen(2), Gerste(3)

According to results presented in *Table 4* wheat protein was much better digestible than barley protein. In contrary the BV of wheat protein was lower than the BV of barley protein. NPU values and PER values obtained in control groups were similar in wheat and barley.

Supplementation the diets with Lys lightly improved the digestibility of both proteins and has not significantly influenced the BV, NPU and PER. In the group 3 (C+Lys+Met) higher NPU value was observed in the wheat diet and increased AD and TD and somewhat higher NPU values in barley diet.

Table 5

Statistical correlation among specific parameters obtained in the experiment

CORRELATION (1)				
FEED CONSUMPTION: (2) DAILY GAIN (5)	N CONSUMPTION: (3) DAILY GAIN	FEED+N CONSUMPTION: (4) DAILY GAIN	NPU: PER	NPU: DAILY GAIN
0,57	0,85	0,58	0,64	0,86

5. Tabelle: Statistische Korrelation der spezifische Parameter im Versuch

Korrelation(1), Futterverbrauch(2), Nitrogenverbrauch(3), Futter- und Nitrogen-verbrauch Tageszunahme(4), Gewichtszunahme (5)

Calculated statistically significant correlation coefficients give an explanation of relationships between in nitrogen balance studies measured parameters and animal growth. Amount of nitrogen consumed and NPU have the highest correlation to daily gain.

Table 6

Excretion of nitrogen per g of daily gain.

GROUP (1)		Excreted N in feces and urine, mg (2)	Daily gain g (3)	Excreted N per g of gain mg (4)	Difference mg (5)	%
WHEAT (6)	(Control)	118	$2,45^{a}$	$48,2^{a}$		
1						
2	(Con+Lys)	139	$3,00^{b}$	$46,8^{a}$	-1,9	3,9
3	(Con+Lys+Met)	124	$3,93^{c}$	$33,4^{b}$	-16,6	34,4
BARLEY (7)	(Control)	131	$2,66^{a}$	49,2		
1						
2	(Con+Lys)	117	$2,78^{ab}$	42,1	-7,1	14,4
3	(Con+Lys+Met)	134	$4,21^{b}$	31,8	-17,4	35,4

Values with no equal superscripts in the same column differ significantly ($P \leq 0.05$). *(Die Werte mit ungleichen Indexen in der gleichen Spalte sind unterschiedlich signifikant.)*

6. Tabelle: Nitrogenausscheidung pro g täglicher Zunahme

Gruppe(1), Nitrogenausscheidung in Kot und Urin(2), Tageszunahme(3), Nitrogenausscheidung pro g Tageszunahme(4), Differenz(5), Weizen (6), Gerste(7)

It can be noted that the amino acid supplementation of the test diets lowered the amount of excreted nitrogen per g of daily gain.

DISCUSSION

Lang and *Schoen* (1952) give a biological value for wheat protein at 67% *Nerhing* and *Bock* (1961) find 69%. These values are superior than those obtained in the experiment with the Slovenian cereal. In the meantime obtained value of BV is superior than the value obtained by *Nerhing* and *Bock* (1961) - 60%.

For the TD, again, the values are inferior to the literature values, from 88,8% to 93,4% (*Nerhing* and *Bock*, 1961) against 81,4 to 84,6% for Slovenian samples.

For the barley samples the values are really different for the TD. The Slovenian samples give some results between 66,1 and 72,2%, *Wunsche* and *Bock* (1965) find a TD at 89,3%. From the same reference the BV of the experiment cereal (around 75,7%) is comparable with our results (74,8%).

Out of the results obtained in our experiments we can observe and confirm a generally accepted regularity: barley proteins have a lower digestibility than the wheat proteins but also higher BV.

Moreover the results show that the supplementation of this samples with synthetic amino acids improve the digestibility of the protein. In the same time the NPU seems to be the same between the two cereals.

It its well known that the BV gives not the best answer of the positive effect of added amino acids and moreover only the group 3 of wheat gives in our research a real better BV. *Glem-Hansen* and *Eggum* (1972) show that the content of non essential amino acids might affect BV. That is why BV is not only dependant on the first limiting amino acids, but on whole aminogram. So here not only lysine and methionine are limiting amino acids and more experiments are needed to find final solution. In fact it is possible to put out that the addition of lysine alone in wheat group 2 doesnt influence the protein utilisation, two amino acids together are more effective.

The PER seems to be the best method to study, the influence of the addition of amino acids to the diets on protein utilisation. This one increases regularly and it is a proof of a better utilisation of the diet's components. This result is really important in the view of practical feeding conditions. After the results obtained in our experiments the supplementation of wheat and barley based diets with lysine and methionine improved the daily weight gains of rats in average for 60,4% (wheat group 3) and for 58,3% (barley group 3).

The correlation between PER and NPU was statistically significant. In nitrogen balance experiments NPU gives a good estimation of protein utilisation. NPU and daily gain are close correlated. Beside this N consumption gives better explanation for animal growth than feed consumption alone.

Important part of this experiment was the reduction of animals nitrogen excretion after diet amino acid supplementation. Good results were obtained with both cereals. The two third groups of rats reduced theirs excretions by 34,4% for wheat and 35,4% for barley. The two Lys groups reduced the N excretion in smaller amounts than the Lys+Met groups. Better protein utilisation is connected with reduction of N excretion. This fact is important not only for animal nutrition but also for prevention of environmental nitrogen load.

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