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Studies on Heated Protein Quality Using Homoarginine Method

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Abstract

To determine the quality of heated protein, *in vitro* method, including lysine, lysinoalanine, and fructose-lysine as well as homoarginine by guanidination of lysine, was assessed using heated casein with or without glucose. *In vivo* methods such as PER, digestibility and BV were also tried on homoarginine, lysinoalanine, fructoselysine, and lysine. The nonreactive lysine for guanidination was hardly digestive, while the non heat damaged lysine side chains in the protein were accessible for guanidination as well as for the digestion. A linear correlation(r=0.80) was obtained between PER and digestibility of the analysed lysine. Digestibility of homoarginine was higher than that of true protein. However, in the guanidinated heated casein with glucose, digestibility of homoarginine was significantly reduced. It is suggested that the homoarginine method may mislead to over- or underestimation of the damaged protein quality.

Key words: homoarginine, protein digestibility, lysine bioavailability, protein efficiency ratio

INTRODUCTION

Due to some problems of N-balance measurement technique, Hagemeister and Erbersdobler(1) proposed a new labeling method by transforming lysine to homoarginine, to distinguish the exogenous(food) from the endogenous proteins. This is achieved by the reaction of the lysine side chains in the dietary protein with methylisourea, thus adding a guanidino group to the ε-amino group of lysine(2). Up to 99% of lysine in a protein can become guanidinated if proper conditions are provided(3). The main advantage of the homoarginine labeling is that homoarginine does not appear in endogenous proteins because it is not utilized for protein synthesis(4). Moreover, it is almost completely absorbed up to the ileum(5). An additional advantage might be that homoarginine, contrary to other chemical derivatized amino acid side chains(6), is said to be partially transformed into lysine and urea by arginase in the liver(7,8) thus preventing lysine deficiency (9). Stevens and Bush(9), Tews et al.(10), and Schmitz (11), however, reported that the growth of rats was greatly impaired by this treatment, which demonstrated that there was no sufficient retransforming of homoarginine into lysine. Stevens and Bush(9) and Tews et al.(10) also reported that supplement of lysine improved modestly the impaired food intake and the growth when rats were fed a lysine limited diet containing homoarginine.

Hagemeister et al.(5) claimed that the homoarginine measurement technique is not reliable if cross-linking or derivatization in heat treated protein blocked the accessibility of homoarginine to the ε-amino group.

The objectives of the work reported in the present paper were: 1) whether the digestibility of protein is impaired by guanidination, 2) to examine whether the homoarginine labeled part of the tested proteins were digested, and what would happen with the availability of the nontransformed lysine, namely if it was cross-linked or derivatized by heat treatment. These results should provide the answer for a question of what extent the homoarginine method can be used if the lysine groups are partly blocked by crosslinking or derivatizating in heat treated protein.

MATERIALS AND METHODS

Heat treatment of the caseins

Casein(14.7% N of dry matter) was used for the control diet. Parts of this casein were heated in an autoclave at 134°C for 3h. Another portion of the ca-

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sein, weighing 1300g, was mixed with 600g of glucose monohydrate and suspended in 3.6L tap water. The casein-glucose-solution was poured into stainless steel trays to a depth of about 1cm and then baked in an air stream at 65°C for 68h. The baked material was ground to a particle size of less than 0.5mm[2].

Homoarginine labeling of the untreated and the heat treated caseins

Half of the 3 different caseins(untreated casein, with or without glucose heat treated casein) were guanidinated with 0.4M *o*-methylisourea(MIU) according to the procedure of Schmitz et al.(4).

Animals and diets

In all experiments, rats of the Wistar strain(supplied by Zentralinstitut für Versuchstierzucht, Hannover, Germany), aged 3 weeks and weighing about 60g, were used and fed *ad libitum*.

During a 3 day adjustment period the animals were fed a untreated casein based diet. The protein content (N×6.25) in the rations amounted to 10%, but without the N arising from guanidination(Table 1).

Experiments were carried out in a temperature controlled room(12h light-dark cycle, 25°C, 55% relative humidity). All experimental procedures described were approved by the Animal Care and Animal Ethics Commitee of the Kiel University in Schleswig-Holstein, Germany, according to established guidelines for the care and use of laboratory animals(12).

N-Balance trials

After an adjusting period of 7 days, 40 female rats were divided into 7 homogenous groups. Six groups contained 6 rats and one group contained 4 rats in order to control the endogenous N losses. Animal distribution was based on the bodyweight and were then placed into individual balance cages. The balance period consisted of a 5 day preliminary period and a 6 day collection period. True protein digestibility(TD), protein efficiency ratio(PER) and biological value(BV) were measured.

Analytical methods

Diets, the feces and urine samples of the N-balance trials were analyzed for total nitrogen using the Kjeldahl method. For lysine-, lysinoalanine-(LAL ; an example for crosslinking which occurs mainly in proteins heated under alkaline conditions by the reaction of the ε-amino group of lysine with dehydroalanine) and homoarginine-balance the food as well as the feces and urine were hydrolyzed in 6M HCl(final concentration), and for the determination of furosine(the most abundant amino acid derivative in human nutrition which is found after the reaction of lysine with glucose) in 7.8M HCl and measured with an amino acid analyser(Liqumat III, Kontron, Germany) as described by Erbersdobler and Hupe(13).

Statistical methods

Results are means±SEM. Statistical tests were performed by analysis of variance using the VMS SAS (SAS Institute INC., CARY, N.C. : 27512 USA) and regression analysis using the Statgraphics(r)(statistical package VS 3.0, 1988). The pooled estimate of variance was employed to calculate any significant differences according to Scheffé-test($p<0.05$) in comparing individual groups.

Table 1. Composition of the experimental diets for the N-balance trials (g/kg)

Composition	Experimental groups					
	1	2	3	4	5	6
Casein[1]	120					
Casein, guanidinated[1]		120				
Heat damaged casein[1]			118			
Heat damaged casein, guanidinated[1]				118		
Casein+glucose heat damaged[1]					159	
Casein+glucose heat damaged, guanidinated[1]						128
Basal diet[2]	880	880	882	882	841	872

[1]The protein content(N×6.25) in the rations amounted to 10%, but without the N arising from guanidination
[2]Altromin C1004 Eiweissarm II(Altromin, Lage, Germany)

RESULTS

Homoarginine labeling of the untreated and the heat treated caseins

The analyzed data of the tested proteins are shown in Table 2. The extent of conversion of lysine to homoarginine was 94.3% in the untreated casein. Meanwhile, only 83.5% of the lysine in the heat damaged casein and 57.4% of the lysine in the heat damaged casein+glucose mixture were transformed to homoarginine. The results show that, presumably due to the crosslinking or derivatisation, both types of heat damage exhibit an inferior accessibility to the ε-amino group of lysine.

The heat damaged casein contained 2.4g LAL/kg protein and the heat damaged casein+glucose mixture contained 99.8g FL/kg protein. The amount of FL in the guanidinated heated casein+glucose mixture was highly reduced under the alkaline condition(pH 10.5) for guanidination, while the amount of LAL in the heat damaged guanidinated casein was slightly raised.

N-balance trials

The results are shown in Table 3. Food intake, PER, and BV were significantly reduced in the diets containing homoarginine instead of lysine in the guanidinated caseins. In the heated proteins food intake, PER, and BV were also significantly reduced. The results suggest that the residual lysine in these caseins was not efficiently utilized in all cases and homoarginine was not enough retransformed to lysine for obtaining

Table 2. Conversion(%) of lysine to homoarginine in three guanidinated protein sources, content of analysed lysine (AL), lysinoalanine(LAL), fructoselysine(FL) and homoarginine(HA) of untreated, heated casein, untreated and guanidinated casein, respectively

Proteins	Conversion[1]	AL	LAL	FL	HA
	%	g/kg protein			
Casein	–	88.0	–	–	–
Guanidinated casein	94.3	5.0	–	–	128.0
Heat damaged casein[2]	–	82.0	2.4	–	–
Heat damaged casein, guanidinated	83.5	16.0	2.9	–	104.0
Casein+glucose heat damaged[3]	–	58.0	–	99 8	–
Casein+glucose heat damaged, guanidinated	57.4	23.0	–	39.3	40.0

After treatment these protein sources were mixed well and analyzed as a single determination
[1]Conversion(%) based on the molar basis of the transformation of lysine into homoarginine
[2]Autoclaving of casein at 134°C for 3h
[3]Baking of a mixture of 1.3kg casein plus 600g with 3.6L tap water

Table 3. Food intake(FI), protein efficiency ratio(PER), biological value(BV) and true digestibility of protein(TD) and digestibility of homoarginine(HAD), analysed lysine(ALD), lysinoalanine(LALD) and fructoselysine(FLD) of casein and heated casein, untreated and guanidinated respectively[1]

Proteins	FI	PER	BV	TD	HAD	ALD	LALD	FLD
	g/d		%	%	%			
Casein	12.9±0.7[a]	2.8±0.4[a]	67.3±5.7[a]	96.0±0.7[a]	–	98.2±0.7[a]	–	–
Guanidinated casein	9 8±2.5[b]	0.9±0.4[b]	46.9±3.6[b]	91.0±4.9[b]	98.8±0.6[a]	79.8±5.6*[b]	–	–
Heat damaged casein[2]	12.9±0.6[a]	2.2±0.5[c]	62.7±3.5[c]	90.3±2.9[b]	–	92.9±1.4[c]	64.3±3.8[a]	–
Heat damaged casein, guanidinated	7.4±0.8[c]	0.0±0.0[d]	45.4±9.0[b]	89.6±3.0[b]	96.0±1.0[b]	38.1±19.4[d]	56.6±3.6[b]	–
Casein+glucose heat damaged[3]	13.5±0.7[a]	1.6±0.5[a]	47.5±8.3[b]	92.5±1.0[b]	–	90.8±1.3[a]	–	98.5±0.8[a]
Casein+glucose heat damaged, guanidinated	10.8±1.8[b]	0.9±0.4[b]	42.9±5.5[b]	83.8±2.9[c]	74.0±1 7[c]	65.7±4.7[c]	–	96.4±2.9[a]

[1]Results are the means±SEM for 6 rats(*4 rats) per group
Means in a column with different superscript letters are significantly different($p<0.05$) according to ANOVA followed by Scheffé range test
[2]Autoclaving of casein at 134°C(2.1 bar) for 3h
[3]Baking of a mixture of 1 3kg casein plus 600g glucose with 3.6L tap water

sufficient growth. The true digestibilities of the heat damaged caseins and the guanidinated caseins were generally reduced. But the homoarginine showed a high digestibility in the guanidinated caseins with exception of the guanidinated heated casein+glucose mixture. In the guanidinated heated casein+glucose mixture, 26% of ingested homoarginine were excreted in feces. The digestibilities of LAL and FL of the heat damaged guanidinated caseins were slightly reduced.

DISCUSSION

Apparently, in both types, the damaged lysine gives almost no access to the transformation to homoarginine(Table 2), as was found earlier by Hagemeister et al.(5) and Schmitz et al.(4). The guanidinated caseins fed rats consumed significantly less food than the non-guanidinated caseins fed rats. This can be explained not only by an lysine deficit but by an lysine imbalance, as was demonstrated by Peng et al.(14) and Tews et al.(10,15). In the guanidinated caseins, the biological value(BV) and PER were reduced(Table 3). This reduction was greater in the heat damaged caseins although contents in residual lysine(16 or 23g/kg protein, respectively) were higher than those of guanidinated non heated casein(5g/kg protein). This suggests that the residual lysine in the guanidinated heated caseins was hardly available while the 0.5g residual lysine in the guanidinated untreated casein was obviously utilized. This was also demonstrated by the low digestibility of analyzed lysine in the heat damaged caseins. Moughan et al.(16) found no significant correlation($r=-0.48$) between *in vivo* lysine digestibility in rats and the available lysine values measured by the fluro dinitrobenzene method. Nair et al.(17), however, reported a best fitting correlation between the PER of heat treated dried skim milk samples in rats and the available lysine values measured by the homoarginine method.

In this present paper, PER was well correlated($r=0.80$; $p<0.001$) to the digestibility of analysed lysine which represents the residual lysine. This indicates that the sites in the protein molecule, which were not involved in crosslinking or derivatisation(as measured by a high degree of guanidination) were digested. The parts, however, in which lysine was either not or only poorly accessible for guanidination, exhibited a reduced digestibility. As a consequence to this, the amino acid availability in heat damaged protein appears to be highly dependent on the amount. The distribution of the crosslinks and derivatives over the whole protein as was discussed by Erbersdobler(18).

Homoarginine in the guanidinated proteins was highly digested. The reduced digestibility of homoarginine in the guanidinated heated casein+glucose mixture (Table 3) was possibly caused by an interference to uptake by fructoselysine(FL). It is well known that the rates of absorption and metabolism of protein-bound FL in the small intestine tend to be extremely low(19-21). Schuttert et al.(22) found that homoarginine was not metabolized by incubating with the small intestinal digesta of the rats. A FL inhibition effect on the transport of several amino acids was found earlier by Erbersdobler et al.(21) in experiments with everted intestinal gut segments of rats. From these conditions, it was suggested that the large amounts of FL interfered with the uptake of homoarginine in the small intestine and considerable parts of the homoarginine were transported together with FL into the large intestine. While FL was almost completely decomposed by bacteria in the large intestine (21) homoarginine was excreted as more intact form.

These facts may lead to wrong conclusions such as an overestimation or underestimation of the quality of the heat treated proteins if the homoarginine method is applied uncritically in the heat damaged protein.

Because the existence of FL is highly dependent on pH(23), the amount of FL in the guanidinated heated casein+glucose mixture was strongly reduced. For this reason, it was not possible to use FL as a second indicator of the digestion of the heat damaged protein.

The BV was not reduced in the guanidinated proteins as much as expected. As mentioned above, the lysine which was not retransformed to HA was partially utilizable as is shown by the digestibility of analysed lysine. However, the homoarginine was retransformed into lysine by arginase at a slow rate, and occurs possibly only after an adoptation period of several days as reported by Ryan et al.(7), Prior et al.(8) and Stevens and Bush(9).

About 20% of the ingested homoarginine was found in the urine, which is considerably higher than the 4 % found by Schmitz et al.(4). This leads to the suggestion that the arginase activity for retransforming homoarginine back to lysine is not as high as pre-

dicted. This is confirmed by the low performance(BV and PER) in the rats on the guanidinated proteins. This conclusion is supported by the work of Stevens and Bush(9) and Tews et al.(10). In the non guanidinated heat treated proteins, the digestibility of LAL is in accord with results of Finot(24) and Karayiannis et al.(25). The digestibility of FL is 98.5% in this study. This value is a little higher than that of Finot et al.(26).

Digestibility of LAL and FL of the guanidinated heat treated proteins were slightly reduced.

In conclusion, the present study has shown that the parts in the protein in which the lysine side chains were not heat damaged, are accessible for the guanidination as well as for the digestion. The non reactive residual lysine for guanidination in the heat-treated caseins was hardly available. This non-available residual lysine represents that the lysine moieties are blocked by initial Maillard reaction or crosslinkings, which are partially liberated by the acid hydrolysis before analzying amino acid.

The homoarginine digestibility was significantly reduced, especially in the guanidinated heated casein + glucose mixture. It is suggested that the homoarginine method may mislead to over- or underestimation of the heat damaged protein quality.

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REFERENCES

1. Hagemeister, H. and Erbersdobler, H. · Chemical labelling of dietary protein by transformation of lysine to homoarginine ; a new technique to follow intestinal digestion and absorption. *Proceeding of the Nutrition Society*, **44**, 133A (1985)
2. Mauron, J. and Bujard, E. : Guanidination, an alternative approach to the determination of available lysine in food. Paper presented at 6th International Congress of Nutrition, Livingstone, Edingburgh(1964)
3. Maga, J. A. : Measurement of available lysine using the guanidination reaction. *J. Food Science*, **46**, 132(1981)
4. Schmitz, M., Hagemeister, H. and Erbersdobler, H. : Homoarginine labeling is suitable for determination of protein absorption in Miniature Pigs. *J. Nutr.*, **121**, 1575(1991)
5. Hagemeister, H., Schmitz, M. and Erbersdobler, H. : Reliability and limitation of the homoarginine method for evaluation of protein digestibility in the pig. In "*Milk proteins*" Barth, C. A. and Schlimme, E (eds.), Steinkopff Verlag, Darmstadt, p.68(1989)
6. Carpenter, K. J. : The use of ileal content analysis to assess the digestibility of amino acids. In "*Protein in human nutrition*" Porter, E and Rolls, P.(eds.), Academic Press, London, p.343(1973)
7. Ryan, W. L., Barak, A. J. and Johnson, R. J. · Lysine, homocitrulline, and homoarginine metabolism by the isolated perfused rat liver. *Arch. Bioch. Biophy.*, **123**, 294(1968)
8. Prior, R. L., Milner, J. A. and Visek, W. J. : Urea, citrate and orotate excretions in growing rats fed amino acid deficient diets. *J. Nutr.*, **105**, 141(1975)
9. Stevens, C. M. and Bush, J. A. : New synthesis of α-amino-ε-guanidino-n-carproic acid(Homoarginine) and its possible conversion *in vivo* into lysine. *J. Biol. Chem.*, **183**, 139(1959)
10. Tews, J. K., Repa, J. J., Lichy, R. and Harper, A. E. : Food choices and meal patterns of rats selecting from amino acid diets containing homoarginine. *Nutrition Report International*, **36**, 989(1987)
11. Schmitz, M : Möglichkeit und grenzen der homoarginin-markierungsmetode zur verdaulichkeitsmessung von proten beim schwein. *Ph. D. Dissertation*, Institut für Physiologie und Biocheme der Ernährung der Bundesanstalt für milchforschung in Kiel(1988)
12. Tierschutzgesetz 7833-3, BGBL, Bonn. Germany, p.1319 (1986)
13. Erbersdobler, H. F. and Hupe, A. · Determination of lysine damage and calculation of lysine bioavailability in several processed foods. *Z. Ernahrungswiss.*, **30**, 46(1991)
14. Peng, Y., Tews. J K. and Harper, A. E. : Amino acid imbalance, protein intake, and changes in the rat brain and plasma amino acid. *Am. J. Phys.*, **222**, 314(1972)
15. Tews, J K., Greenwood, J., Pratt, O. E. and Harper, A. E. · Dietary amino acid analogues and transport of lysine or valine across the blood-brain barrier in rats. *J. Nutr.*, **118**, 756(1988)
16. Moughan, P. J., Schrama, J., Skilton, G. A. and Smith, W. C. · *In-vitro* determination of nitrogen digestibility and lysine availability in meat and bone meals and comparison with *in-vivo* ileal digestibility estimates. *J Sci. Food Agric.*, **47**, 281(1989)
17. Nair, B M., Laser, A., Burvall, A. and Asp, N. G. : Gas chromatographics determination of available lysine. *Food Chemistry*, **3**, 283(1978)
18. Erbersdobler, H. F. : Factors influencing uptake and utilization of macronutrients. In "*Nutrient availability · Chemical and biological aspects*" Southgate, D. A. T., Johnson, I. T. and Fenwick, G. R (eds.), Royal Society of Chemistry, Cambridge, p.330(1989)
19. Ford, J. E. and Shorrock, C. : Metabolism of heat damaged proteins in the rat. *British J. Nutr.*, **26**, 311(1971)
20. Erbersdobler, H. F. · Über Untersuchungen zur intestinalen Resorption von Epsilon-Fructoselysine. *Z. Tierphyiol. Tierernährung und Futtermittelkunde*, **28**, 171(1971)
21. Erbersdobler, H. F., Brandt, A., Scharrer, E. and Von Wangenheim, B. : Transport and metabolism studies with fructoseamino acids. *Prog. Fd. Nutr. Sci.*, **5**, 257(1981)
22. Schuttert, G., Moughan, P J. and Jackson, F. : *In vitro* determination of the extent of hydrolysis of homoarginine by arginase in the small intestine of the growing rat. *J.*

Agric. Food Chem., **39**, 511(1991)
23. Görnhart, L. : Die nicht enzymatische Bräunung von Lebensmitteln II. *Fette, Seifen, Anstrichmittel*, **57**, 429(1955)
24. Finot, P. A. : Lysinoalanine in food proteins. *Nutrition Abstracts and Reviews*, **53**, 67(1983)
25. Karayiannis, N. I, MacGregor, J. T. and Bjeldanes, L. F. : Lysinoalanine formation in alkali-treated proteins and model peptides. *Fd. Cosmet.Toxicol.*, **17**, 585(1979)
26. Finot, P. A., Bujard, E., Mottu, F. and Mauron, J. : Availability of the true Schiff's bases between lysine and lactose in milk. In "*Protein crosslinking, nutritional and medical consequences*" Friedman, M.(eds.), Plenum Press, New York, Vol. 86B, p.343(1977)

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