



Quantification of Maillard reaction products in animal feed

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Abstract

Individual Maillard reaction products (MRPs), namely furosine, which is formed from Amadori product of lysine during acid hydrolysis, as well as *N*- ϵ -(carboxymethyl)lysine (CML), pyrroline and the arginine derivative MG-H1 (methylglyoxal-derived hydroimidazolone 1) were quantified in 78 samples of animal feed, belonging to 17 different feed types. The concentrations of the MRPs were dependent on the heat treatment during processing. Within similar feed types, significant differences in concentrations could be observed. MRPs can be suitable indicators to evaluate the impact of technological processing on the nutritional quality of animal feed.

Keywords Maillard reaction · Glycation · Cattle feed · Pyrroline · *N*- ϵ -(carboxymethyl)lysine (CML) · Methylglyoxal-derived hydroimidazolone 1 (MG-H1) · Furosine

Introduction

Heat treatment during processing is applied to preserve animal feed, but also to improve the digestibility of feed components in general. Among the chemical reactions occurring during heat treatment, the Maillard reaction (also referred to as “glycation”) between amino compounds and reducing carbohydrates is of outstanding importance [1]. With respect to animal feed, conclusions concerning the impact of the Maillard reaction on the final product are mainly based on parameters such as “reactive lysine” [2] or digestibility [3, 4]. At present, however, there is merely a small amount of information available about the amount of individual glycation compounds in various animal feeds [5–7]. Our laboratory recently has found considerable variations in the amount of free MRPs in commercial milk, most probably due to the nutritional uptake of glycated proteins during feeding of cows [7]. Feed which was exposed to the Maillard reaction is often described as “heat-damaged” [3] and as nutritionally unavailable [8]. However, recent studies have shown that

microorganisms are able to utilize certain MRPs [9, 10]. It is, therefore, conceivable that the Maillard reaction does not always have to be regarded as protein damaging. To obtain an overview about the extent of the Maillard reaction and to draw conclusions concerning an impact of processing on the nutritional value, the MRPs *N*- ϵ -2-furoylmethyl-L-lysine (furosine), which is formed from Amadori product of lysine during acid hydrolysis, as well as *N*- ϵ -(carboxymethyl)lysine (CML), 2-amino-6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-hexanoic acid (pyrroline) and the arginine derivative *N*- δ -(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) were quantified in samples of animal feed for the first time.

Materials and methods

Samples of animal feed

A total of 78 samples of 17 different feedstuffs from ten different precursor materials, including alfalfa, sugar beet, corn, barley, pea, stillage (wheat), rapeseed, grass and mixture of grass and sugar beet, were analysed (see Table 1). These samples of feedstuffs were provided by the educational and research farm of the department for animal husbandry and feeding (Saxon State Office for Environment, Agriculture and Geology).

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Table 1 Concentrations of CML, MG-HI, pyrroline and furosine in animal feed in mg/100 g protein

Feed	Type/degree of processing	No of sam- ples	CML/mg/100 g protein			MG-HI/mg/100 g protein			Pyrroline/mg/100 g protein			Furosine/mg/100 g protein						
			Min	Max	ϕ	Median	Min	Max	ϕ	Median	Min	Max	ϕ	Median	Min	Max	ϕ	Median
Alfalfa	Fresh	2	3.7	19.8	11.8	11.8	0.3	0.9	0.6	0.6	0.2	0.6	0.4	0.4	11	20	15	15
	Silage	1	3.3	3.3	3.3	3.3	0.2	0.2	0.2	0.2	0.5	0.5	0.5	0.5	14	14	14	14
	Hay	1	21.2	21.2	21.2	21.2	1.8	1.8	1.8	1.8	6.9	6.9	6.9	6.9	29	29	29	29
Sugar beet	Dried	8	8.7	25.8	18.0	18.8	1.5	4.5	3.1	2.8	10.1	30.0	16.4	14.9	189	342	235	219
	Pressed pulp, silage	2	19.9	21.2	20.6	20.6	0.8	1.0	0.9	0.9	0.2	0.3	0.3	0.3	43	51	47	47
Corn	Pellet of dried pulp	7	32.6	86.8	47.5	43.7	121.9	181.8	157.3	157.8	20.2	548.8	425.0	518.6	70	394	250	225
	Silage	8	1.3	13.9	6.6	5.0	5×n.d.	1.1	0.3	0.0	0.2	3.2	0.9	0.4	6	113	55	52
Barley	Dried	7	1.2	5.8	2.6	1.9	2.0	17.3	8.0	8.5	2.8	11.8	7.8	10.6	28	122	71	57
	Dried	8	0.9	9.2	2.3	1.4	0.1	12.9	1.8	0.2	0.1	2.1	0.5	0.3	7	44	25	24
Pea	Raw grain	1	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4	0.1	0.1	0.1	0.1	18	18	18	18
	Grain silage	1	5.9	5.9	5.9	5.9	1.9	1.9	1.9	1.9	1.2	1.2	1.2	1.2	470	470	470	470
Stillage (wheat)	Toasted ^a grain	5 ^a	21.4	28.5	25.1	26.8	11.0	27.5	19.6	17.6	79.7	228.3	169.3	185.4	660	957	797	803
	From distillation	2	31.4	31.5	31.5	31.5	12.6	16.4	14.5	14.5	5.7	7.0	6.4	6.4	205	257	231	231
Rapeseed	Meal	7	2.7	14.8	10.1	9.8	10.0	18.0	13.5	11.7	29.5	54.3	41.4	41.0	74	179	102	89
Grass	Silage	8	2.8	18.1	6.7	4.2	1×n.d.	0.9	0.5	0.4	0.2	3.4	0.8	0.4	14	64	34	31
High-performance feed	Pellets (mixture)	7	10.8	25.8	15.4	13.8	27.0	47.7	36.8	36.7	27.3	77.0	45.5	36.4	37	231	145	125
Sugar beet and grass	Silage	3	4.9	7.6	5.9	5.1	n.d.	n.d.	n.d.	n.d.	0.2	0.2	0.2	0.2	n.d.	n.d.	n.d.	n.d.

^aDifferent temperatures of toasting: 75/77/80/85/90 °C

Reagents

LC–MS grade methanol and acetonitrile were obtained from Fisher Chemical (Loughborough, UK). Pepsin, pronase E, sulfuric acid and creatinine were from Merck (Darmstadt, Germany), and CML and [$^2\text{H}_2$]CML were obtained from PolyPeptide Group (Strasbourg, France). 2-Amino-2-(hydroxymethyl)propane-1, 1-diole-hydrochloride (TRIS–HCl) was purchased from Serva Feinbiochemica (Heidelberg, Germany). Nonafluoropentanoic acid (NFPA), hydrochloric acid, prolidase, leucine aminopeptidase and *n*-heptane were from Sigma-Aldrich (Seelze, Germany). Lithium citrate buffer, lithium citrate/borate buffer, lithium hydroxide and ninhydrin were purchased from Sykam (Fürstenfeldbruck, Germany). Wieninger's catalyst was obtained from Honeywell (Seelze, Germany). Boric acid was purchased from Carl Roth (Karlsruhe, Germany) and sodium hydroxide from Grüssing (Filssum, Germany). Water was distilled (twice) before analysis.

Pyrraline [11] and MG-H1 [12] were synthesized according to the specified literature. The synthesis of [$^{13}\text{C}_6$, $^{15}\text{N}_2$]pyrraline and [$^{13}\text{C}_6$]MG-H1 was performed in the same manner but using [$^{13}\text{C}_6$, $^{15}\text{N}_2$]lysine (pyrraline) and [$^{13}\text{C}_6$]arginine (MG-H1) instead of the unlabeled amino acids.

Analysis of MRPs

The MRPs CML, MG-H1 and pyrraline were analysed after enzymatic digestion via LC–MS according to the literature [7]. Furosine was quantified after acid hydrolysis with 6 N HCl as reported in literature [13] with separation at a cation exchange resin column and post-column derivatization with ninhydrin [14]. All measurements were performed in duplicates.

Analysis of crude protein content

The total protein content was determined by Kjeldahl method [15] using Wieninger's catalyst. For the calculation of the protein content, the factor 6.25 was used.

Results and discussion

Overview on Maillard reaction products in animal feed

Overall, 78 samples of 17 different feedstuffs from ten different precursor materials were analysed for CML, MG-H1, pyrraline and furosine. The concentrations of these MRPs are shown in Table 1.

For fresh and humid (silage) feed samples (according to Table 1), low concentrations of MG-H1 and pyrraline

were quantified, with pyrraline ranging from not detectable (below 0.03 mg/100 g protein) up to 1.2 mg/100 g protein and MG-H1 up to 1.9 mg/100 g protein (both in pea silage). In these feedstuffs, furosine was determined in higher concentration, ranging from not quantifiable (below 3 mg/100 g protein) up to 470 mg/100 g protein (pea silage). Furthermore, CML was found in relatively high quantities up to 21 mg/100 g protein (silage of sugar beet pulp).

The drying of feed at mild temperatures (e.g. in the case of dried alfalfa or dried corn) has a small effect on the concentrations of MG-H1 and pyrraline (MG-H1 up to 17 mg/100 g protein in dried corn and pyrraline up to 30 mg/100 g protein in dried alfalfa). The concentrations of furosine in dried feed varied between 7 mg/100 g protein (dried barley) and 342 mg/100 g protein (dried alfalfa), and the concentrations of CML ranged between 0.9 mg/100 g protein (dried barley) and 26 mg/100 g protein (dried alfalfa).

In comparison to the fresh and slightly processed feed (silage), a high content of CML (up to 86 mg/100 g protein in dried sugar beet pulp), MG-H1 (up to 182 mg/100 g protein in dried sugar beet pulp), pyrraline (549 mg/100 g protein in dried sugar beet pulp) and furosine (up to 957 mg/100 g protein in toasted peas) could be quantified in highly heated (toasted) and/or pelletized feeds.

It should be noted that the concentrations of the MRPs varied widely between different samples of the same type of feed. For example, in pelletized sugar beet pulp, the amount of CML was between 33 and 87 mg/100 g protein, MG-H1 between 122 and 182 mg/100 g protein, pyrraline between 22 and 549 mg/100 g protein and furosine ranged from 70 to 394 mg/100 g protein. In addition, it is important to mention that the analysis of individual MRPs (e.g. CML or furosine) is not sufficient to estimate the blockage of amino acids such as lysine in different feeds, because the concentrations of individual MRPs do not correlate with each other. Each feed should be considered individually with regard to the blockage of different amino acids during processing.

Conclusions

The investigation showed that humid and minimally processed animal feeds contain lower concentrations of MRPs than highly processed feeds. The daily intake of MRPs can differ greatly depending on the feed used. In addition, it was observed that the concentrations of the individual MRPs can also vary considerably within the same feed types and therefore an individual evaluation of the intake of MRPs must be made for individual feedstuffs and cannot apply to processing degree of feed (e.g. fresh feed, silage, dried feed, pelletized feed). Furthermore, it was found that a determination of a particular MRP is not sufficient to assess the

modification of proteins. An analysis of different MRPs is recommended for a more accurate evaluation of the protein modification via the Maillard reaction. However, the concentrations in animal feed are similar to the concentrations in food so the daily intake of MRPs via feed might be substantially higher (due to the daily feed intake) than the intake calculated for humans [16].

Investigations from Schwarzenbolz et al. indicated that certain MRPs can be resorbed in the digestive tract of cattle [7]. The bioavailability of the MRPs analysed in the feed (also depending on the individual feed) and whether or to what extent resorption occurs in the digestive tract of cattle needs to be examined in further studies. In addition, it should be investigated if a (partial) degradation of the MRPs might occur during the intestinal passage (e.g. during ruminal digestion) and if MRPs can be utilized by the microbiota in the digestive tract of cattle.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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