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A CHARACTERISTIC OF KERATIN-UREA GRANULATES *)

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Key words: the keratin-urea granulats, dialysis, cystine, aminoacid composition, proteolytic enzymes, pepsin, trypsin.

The keratin-urea granulate obtained by means of heating feathers with urea was analysed. The rate of release of urea during dialysis depends on the physical condition of the granulate. The preparation contains up to 20% of soluble protein fraction homogenous in terms of molecular weight and which shows an increased content of cystine as compared with the aminoacid composition of total keratin. Keratin present in the granulate is susceptible to the action of proteolytic enzymes. Both pepsin and trypsin cause 50% proteolysis in vitro.

Urea is one of the main sources of extraprotein nitrogen used for the production of feed for ruminants. Many solutions have been developed with relation to the introduction of urea to ruminant feed [2, 3]. Despite its numerous positive features, urea has a number of disadventages for the feeding proces, e.g., a deterioration of flavour and too rapid solubility in the water medium. The latter property leads to an immediate amonolysis which in turn results in decreased utilization of the nitrogen contained in urea for aminoacid and then protein synthesis by the bacterial flora of the rumen.

Research has been carried out for several years on the process of preparation of keratin-urea granulates having optimum properties of a urea preparation used for feeding ruminants. Keratin in feathers is a rich source of protein nitrogen which so far has been utilized only in a very limited degree in the feeding process.

In their former report, the authors of this paper have presented

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a characteristics of a feather keratin preparation [1] obtained on the basis of a patented method of solubilizing keratin by means of dimethylsufoxide [6]. The present paper presents some properties of keratin-urea preparations obtained from hen feathers. These preparations were made with a view to use them as an admixture to ruminant feed.

MATERIAL AND METHODS

The method of preparing keratin-urea granulates has been discussed in detail in the authors' previous publication [7]. Granulates which differed in the time of heating the feathers with urea were used for the tests. These preparations obtained on a large-laboratory scale, were parallely used for feeding purposes as components of ruminant feed. Protein in the granulates was determined by the quantitative Lowry method [4]. The method was calibrated using bovine serum albumin. The particular protein samples were prepared for determinations in a solution of 0.5 n NaOH.

The aminoacid composition of granlates was determined after hydrolysis of the samples in a solution of 6NHCl at 100°C for 24 hrs. After evaporation of the acid, the dry residue was dissolved in an appropriate buffer and the proportional aminoacid composition was determined using a Jeol aminoacid analyser.

In order to determine the susceptibility of granulates to the action of proteolytic enzymes a pepsin preparation made by "Bacutil" was used of the activity of 4000 units/g together with 150-200 unit/g of trypsin obtained from the same firm. 0.5% and 0.05% enzyme solutions were prepared: pepsin in 0.1 n HCl and trypsin in 0.1 N $NaHCO_3$. 10 ml of suspensions of the preparations tested, previously prepared in a solution of 0.1 N HCl or 0.1 N $NaHCO_3$ respectively ,were mixed with 0.5 ml of an enzymatic preparation and placed in a thermostat (37°C for 24 hrs). After digestion the samples were centrifugated. The supernatant was rejected, the sediment dissolved in 20 ml of 10% NaOH and the content of non-digested protein in the sediment was determined.

GEL-FILTRATION

Columns 1.0×75 cm filled with Sephadex G-150 and equilibrated with 10 mM sodium acetate, pH 2.0 were used for the tests. The protein content was determined in 1 ml samples by determination of absorbance at 280 nm.

RESULTS

The analysed keratin-urea contained 58-60% water, 33-43% urea and 10-20% keratin. The content of particular components in the granulate depended on the method of their preparation.

The stability of urea with keratin protein depends on the physico-chemical condition of the preparation. Analytical tests were carried out for wet keratin-urea preparations, dried ones (at 38°C for 24 hrs) as well as initial preparations and those dispersed in a homogenizer. 100 mg samples in 10 ml water were placed in a dialysation bag and dialysed in running water for 24 hrs. In addition, one sample of the wet initial preparation was mixed with urease solution in order to increase the effectiveness of the urea removal.

After dialysis, the dry matter content determined was gravimetrically. The results are shown in Fig. 1.

The smallest losses of weight were observed in the case of wet, initial keratin-urea granulate and the highest in the dispersed and dried granulate.

The heating of feathers with concentrated urea causes a partial solubilization of the keratin. The effect of the time of heating on the solubilization level of keratin in keratin-urea granulates is shown in Fig. 2. Samples of particular keratin-urea granulates of identical weight were homogenized in a constant volume of water in a glass homogenizer of the type Potter-Elvehjem and after centrifugation of the sediment the content of soluble protein and of protein remaining in the sediment was determined. The results show that solubilization of keratin is small and it

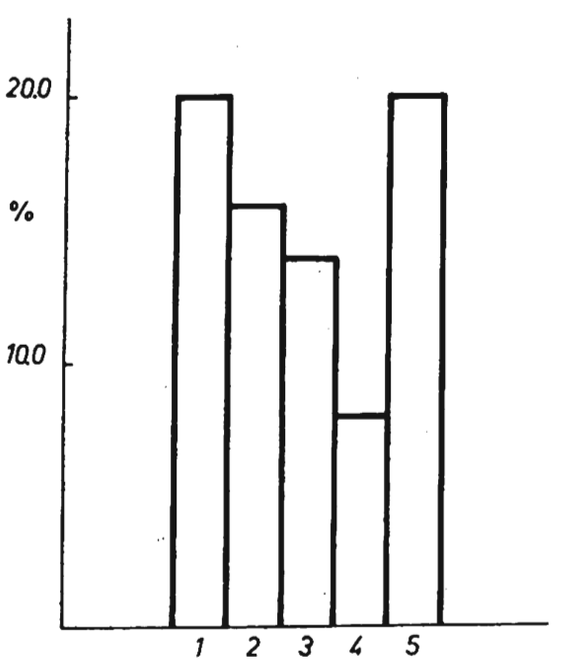

Fig. 1. Dry matter content in the keratin-urea preparation after 24 hrs dialysis: 1 — initial wet preparation, 2 — initial dried preparation, 3 — desintegrated wet preparation, 4 — desintegrated dried preparation, 5 — initial wet preparation with urease added

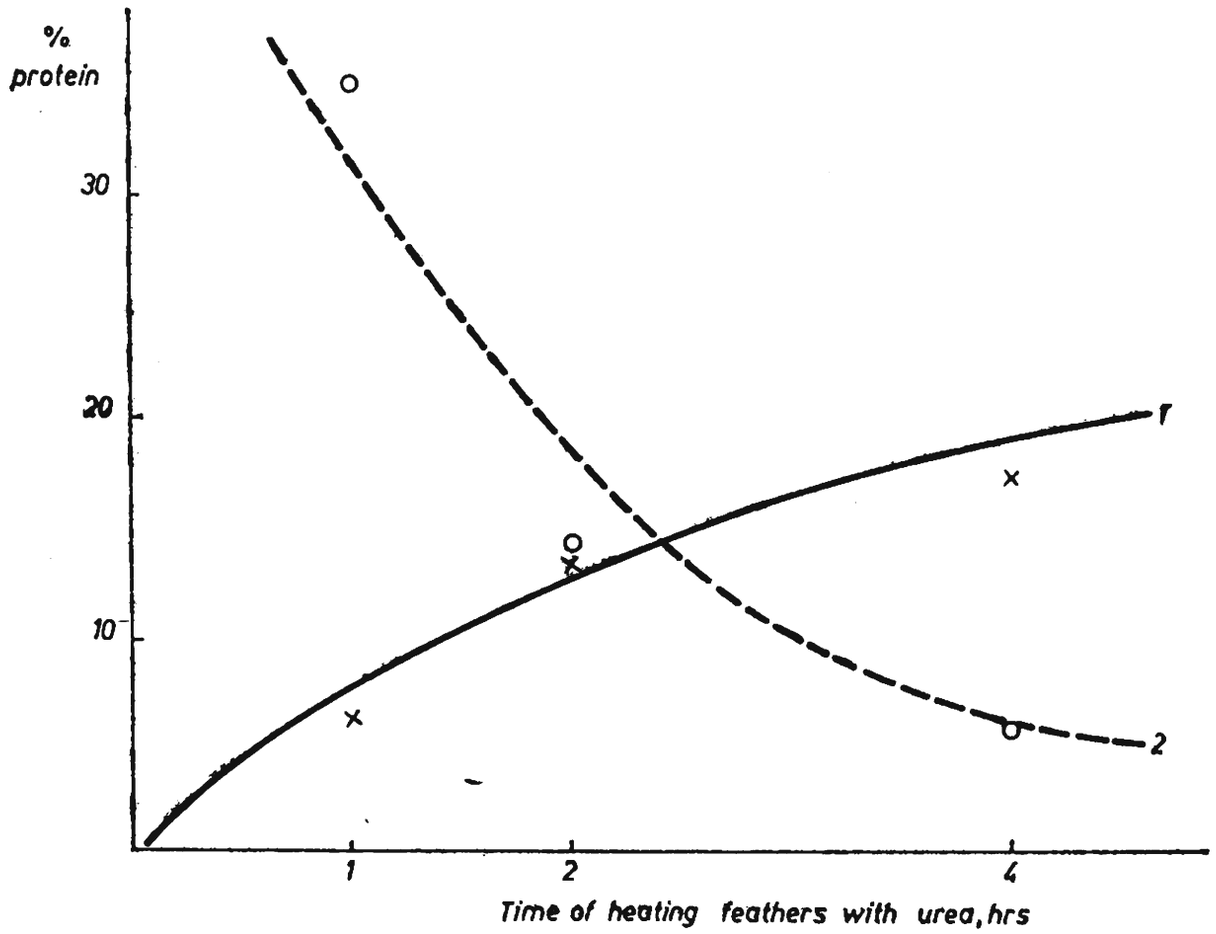

Fig. 2. Solubility of keratin as function of the time of heating feathers with urea: 1 — fraction soluble in urea, 2 — fraction insoluble in water

grows with the time of heating from 6% in a preparation heated during 1 hr to 16% in a preparation heated during 4 hr. The amount of soluble protein in concentrated urea solution depends also on the kind of the analysed keratin-urea preparation.

After removal of urea in particular preparations by means of dialysis, water insoluble protein is precipitated. The largest amount of protein is precipitated in a preparation heated for 1 and the smallest in a preparation heated for 4 hrs.

The soluble protein fraction obtained from a keratin-urea preparation was analysed by gel-filtration on a column with Sephadex G-150. One fraction of a relatively low molecular weight was obtained (Fig. 3).

The soluble protein fraction of a keratin-urea preparation differs from quantitative aminoacid composition of the total keratin of feathers by its increased content of cysteine and a smaller amount of serine and treonine. Table 1 presents the results of the determination of the amino-acid composition of the soluble proteins fraction.

The presence of the protein-soluble fraction in the keratin-urea preparation indicates the effect of heating with urea which modifies the structure of the keratin. In this connection, a test was made of the susceptibility of keratin-urea preparations to the action of proteolytic enzymes in vitro. The results of these experiments are collected in Table 2.

Protein in keratin-urea granulates is subject to proteolysis in the pre-

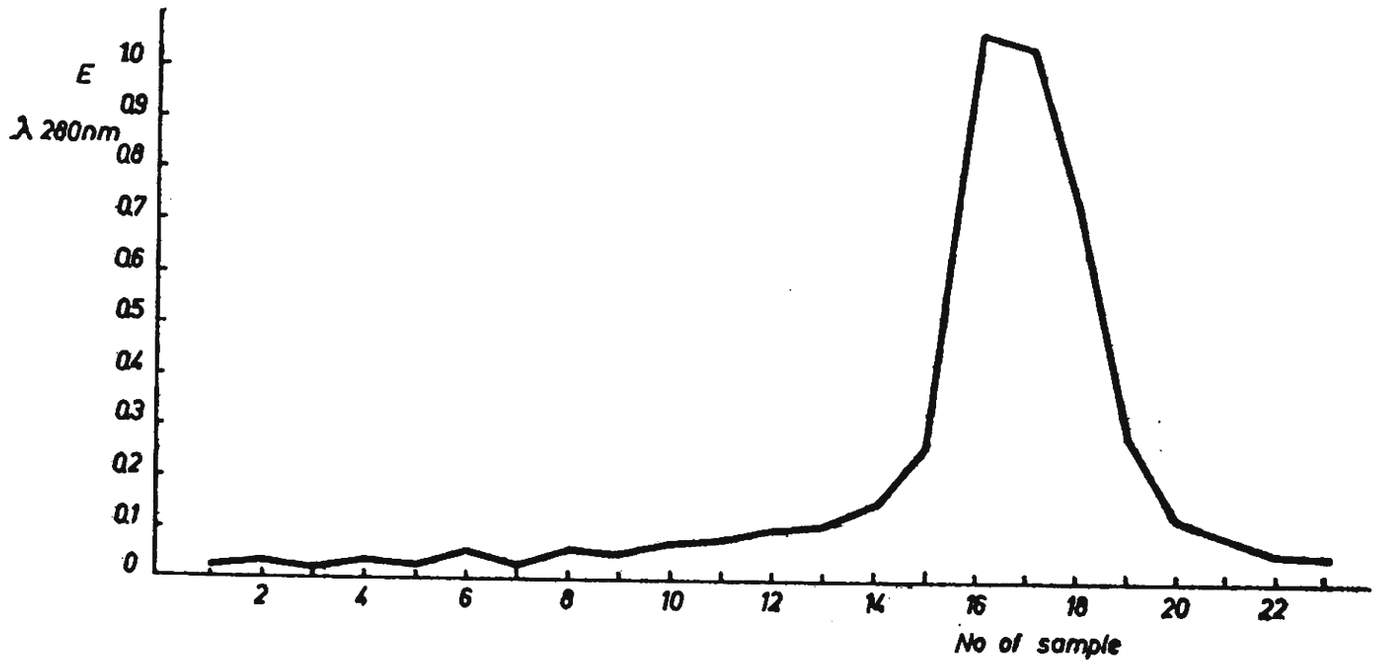

Fig. 3. Molecular filtration of a soluble keratin fraction on a column with Sephadex G-150

sence of both pepsin and trypsin. The degree of proteolysis depends on the concentration of the enzyme and only to a smaller extent on the presence or absence of urea in the incubation medium. Under the conditions of the experiment about 50% protein in the granulate was proteolysed.

Table 1. Aminoacid composition of soluble protein fraction of the kreatin-urea preparation

Aminoacid	Number of molecules/1000 res.	
	total protein	soluble fraction
Cysteic acid	11	40
Aspartic acid	58	54
Treonine	47	33
Serine	106	95
Glutamic acid	93	87
Proline	79	77
Glycine	146	142
Alanine	71	55
Valine	81	72
Isoleucine	47	50
Leucine	83	78
Tyrosine	17	17
Phenylalanine	37	37
Histidine	13	16
Lysine	14	14
Arginine	40	53
Methionine	trace	trace

Table 2. Proteolysis of the keratin-urea preparation *in vitro*

Type of sample	Pepsin			Trypsin		
	mg protein before incubation	mg protein after incubation	% digestion	mg protein before incubation	mg protein after incubation	% digestion
Keratin-urea preparation without dialysis	23.7	23.7	0	14.1	14.1	0
Keratin-urea preparation without dialysis with 0.05% enzyme added	23.7	18.0	24.0	14.1	10.5	26.5
Keratin-urea preparation without dialysis with 0.5% enzyme added	23.7	11.0	54.7	14.1	7.1	49.7
Keratin-urea preparation after dialysis	16.3	16.3	0	14.9	14.9	0
Keratin-urea preparation after dialysis with 0.05% enzyme added	16.3	13.2	19.5	14.9	9.7	35.0
Keratin-urea preparation after dialysis with 0.5% enzyme added	16.3	8.8	46.2	14.9	7.0	53.0

DISCUSSION

As an additive to ruminant feed, urea has been used since quite a long time in combination with various components [2, 3]. The new keratin-urea preparation obtained from hen feathers [7] described in a previous publication has been successfully used in feeding an experimental group of animals [5]. As shown by present experiment, the keratin-urea preparation releases urea during dialysis or under the influence of urease. The rate of this release depends on the form and physical properties of the granulate. The rate of the release of urea from the feed mix is of essential significance in the feeding of ruminants which utilize the nitrogen present in urea [2].

Heating of feathers with concentrated urea solution applied in the preparation of the granulate causes formation of a soluble keratin fraction. The amount of this fraction after 4 hr heating does not exceed 20% of total keratin protein present in the preparation. Such a percentage of solubility may suggest the presence of feather keratin of a small fraction combined with the main keratin mass by hydrogen bridges. A prolongation of the time of heating keratin with urea causes that the keratin fraction becomes soluble in water, it is homogeneous in terms of molecular weight. This seems also to be confirmed by the aminoacid composition of the soluble fraction. One cannot exclude either a partial alkaline hydrolysis of keratin. A prolonged heating of feathers with concentrated urea causes its partial amonolysis to ammonia which leads to an alkalysation of the medium.

The thermal-urea modification of feather keratin increases its susceptibility to the action of proteolytic enzymes. As shown in one experiment, the level of proteolysis depends on the concentration of the enzyme and, under the conditions of the test, causes about 50% decomposition of keratin protein. This means that in addition to the soluble keratin fraction also a certain amount of the isoluble keratin fraction is proteolysed. This confirms the modification function of urea relative to keratin. Completely soluble fractions of feather keratin obtained in a different way very rapidly undergo enzymatic proteolysis in vitro [1].

Taking into account the nutritional importance of the keratin-urea granulate as an additive to ruminants feed, it may be assumed that in ruminants feeding, the susceptibility of a part of keratin to the action of proteolytic enzymes may contribute to a certain utilisation of nitrogen in keratin as well.

CONCLUSIONS

1. Keratin-urea granulates containing 80% of urea lose urea during dialysis depending on the degree of drying and desintegration of the granulate.

2. Heating of feathers with concentrated aquous urea solution causes a partial modification of keratin expressed by the formation of a soluble fraction increasing with the time of heating. Part of soluble protein is precipitated after the removal of urea.

3. Soluble feather keratin is a homogenous fraction in terms of the molecules weight and differs by its aminoacid composition from the aminoacid composition of total feather keratin.

4. Keratin urea granulates are susceptible to the action of proteolytic enzymes in vitro. Both in the presence of urea and after its removal, pepsin or trypsin cause a decomposition of about 50% of the keratin protein.

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Streszczenie

Otrzymane według wcześniej opisanej metody granulaty keratyno-mocznikowe poddano analizie niektórych właściwości. Stwierdzono, że trwałość połączeń mocznika z białkiem keratyny piór uzależniona jest od stanu fizykochemicznego. Największą retencję mocznika wykazują preparaty nierozdrobnionych wilgotnych granulatów (rys. 1). Wykazano również, że w granulatach, obok frakcji keratyny nierozpuszczalnej, występuje frakcja keratyny rozpuszczalnej. Ilość frakcji rozpuszczalnej zwiększa się wraz z wydłużaniem czasu ogrzewania piór z mocznikiem. Stopień rozpuszczalności frakcji keratynowej zależy od obecności mocznika (rys. 2). Rozpuszczalna frakcja keratyny stanowi jednorodny składnik o niskiej masie cząsteczkowej, określany na podstawie sączenia molekularnego (rys. 3). Stwierdzono różnice

w składzie aminokwasowym między keratyną niezmodyfikowaną a frakcją rozpuszczalną (tab. 1). Przeprowadzono badania podatności granulatów keratynowych na działanie pepsyny lub trypsyny in vitro z mocznikiem i po jego usunięciu. Wykazano, że białko w granulatach keratyno-mocznikowych podlega proteolizie zarówno w obecności pepsyny jak i trypsyny. Wielkość proteolizy zależy od stężenia enzymu i tylko w niewielkim stopniu od obecności lub braku mocznika w środowisku inkubacyjnym. Wyniki prezentuje tabela 2.