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POST MORTEM INCREASE OF CREATININE IN PORK MEAT

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It has been indicated that the increase of creatinine content in m. *long. dorsi* of pigs in first 24 h after slaughter amounts to approx. 0.1 μmol/g, and in the course of later cold storage of meat samples or meat deproteinized acid extracts to 0.05 μmol/g/day. In watery meat of a low $pH_{45\ min}$, the increase of creatinine is significantly higher than normal meat. The reaction of dehydration of creatine to creatinine can be inhibited by storing meat samples in a freezer or as water-meat homogenates in a cold stores.

INTRODUCTION

The reaction of dehydrating creatine to creatinine in vivo, in the system, has a non-enzymatic character and is rather irreversible [5], whereas in vitro it is at least partly reversible and depends on the acid-base equilibrium [2, 4]. Both the acid reaction of meat and the basic reaction of fresh urine lead during their cold storage to changes in the content of creatine and creatinine [8, 3]. The total content of the two components, creatine and creatinine in serum does not change during the storage of serum: it remains constant for several days in a cold store (4°C) or even several months in a freezer (-15°C), according to the author's own observation.

The concentration of creatine in meat after slaughter averages 40 μmol/g and the concentration of creatinine — approx. 1 μmol/g. During the storage of meat the content of creatine decreases and that of creatinine increases [3, 12]. These changes can interfere with the variations of these two components caused by other factors, as for example feeding program. Therefore it is important to have an effective method to stop transformation of creatine into creatinine during storage of the analytical samples.

The purpose of the present study was to assess the increase of creatinine in pork meat taking place during storage after slaughter and to investigate the possibilities of storing the experimental material (samples) in an unchanged condition. A comparison was also made of the results of the determinations of creatine and creatinine from the samples taken 45 min and 48 h after slaughter in the meat of various pH.

MATERIAL AND METHODS

Meat samples were taken from m. *long. dorsi* of pigs 5 and 45 min after slaughter from one half of the carcass, and 24 h and 48 h after slaughter from the another half of the same carcass. The first samples (5 min after slaughter) were taken immediately after bleeding out of the pigs, the 45 min samples were taken after scalding, cleaning and cutting the carcasses into halves. The next day, after cutting up, five lumbar vertebrae were excised and a sample (slice), was cut out for analysis 24 h after slaughter. The rest of a carcass was kept in a cold store; 48 h after slaughter the last sample was taken in the similar way and prepared for analysis according to the previously described procedure [7].

6g meat samples were homogenized with 44 ml water (1 min), protein was precipitated by means of 10 ml 20% TCA, the samples filtered off through filter paper. 0.05 ml filtrate was taken for a determination of creatine and 2 ml for a determination of creatinine. Creatine was determined by the diacetyl method using alpha naphthol [1] and creatinine — by the picrate method [13].

The content of creatinine was determined: 1) in samples taken from m. long. dorsi 5 and 45 min, 24 and 48 h after slaughter, 2) in samples ground 48 h after slaughter and kept for 2, 4, 6 and 8 days respectively in a cold store (4°C), 3) in acid filtrates obtained after the precipitation of protein kept in a cold store for 1, 2, 4, 6 and 12 days, 4) in water-meat homogenates kept for 2 days in a cold store, 5) in meat samples kept in a freezer (-15°C) for 3 months and 6) in acid deproteinized filtrates and water-meat homogenates kept for 24 h at 37°C. Samples from 10 pigs were taken for each variant. Results of creatine and creatinine determinations were taken from 4 experiments two of which (I and III) were made on material from control stations [12], one (II) on pigs fed with doses having different protein levels [9] and one (IV) on pigs coming from industrial fattening (slaughtered when weighing approx. 110 kg) were also analysed. In all pigs, creatinine was determined 45 min and 48 h after slaughter and creatine 48 h after slaughter. In two of these tests creatine was determined also 45 min after slaughter.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that the rate of creatinine increase in m. long. dorsi on the first day after slaughter amounts in the average to approx. 0.1 μmol per g, and on the next day it was half lower. The results obtained concern changes in the content of creatinine in pork meat in the course of the post-slaughter procedure. The highest growth of creatinine on the first day after slaughter may be caused by the higher temperature of the muscles in the carcass during the first hours after slaughter, i.e. by the effect of temperature on the speed of the dehydration reaction of creatine to creatinine [4].

Table 1. The increase of creatinine content in m. *long. dorsi post mortem* (n = 10)

Time post mortem	Creatinine content μmoles/g	Creatinine increase μmoles/g
	$\bar{x} \pm s$	$\bar{x} \pm s$
5 min.	0.743±0.054	
45 min.	0.775±0.055	0.032±0.032
24 hours	0.872±0.048	0.096±0.046
48 hours	0.920±0.069	0.049±0.034

n — number of pigs

The storage of ground meat samples in a cold store from the 2nd to the 8th day after slaughter caused a comparatively even growth of creatinine amounting to approx. 0.05 μmol/g/day (Table 2), i.e. equal to that achieved on the second day after slaughter (Table 1). Between the 8th and 10th day after slaughter, the growth of creatinine in the meat samples was higher; these samples had a smell of stale meat. Storage of deproteinized meat filtrates in a cold store caused a growth of creatinine averaging 0.05 μmol/g per day (Table 3). The growth of creatinine in

Table 2. Effect of the storage time of meat in a cold store (4°C) on the creatinine content

n	Time of storage days	Creatinine content, μmoles/g		Creatinine increase, μmoles/g	
		before storage $\bar{x}$ s	after storage $\bar{x}$ s	total $\bar{x}$ s	per day $\bar{x}$ s
10	2	1.018±0.073	1.150±0.056	0.097±0.031	0.049±0.011
10	4	0.959±0.054	1.101±0.072	0.142±0.034	0.035±0.008
10	6	1.037±0.130	1.313±0.128	0.276±0.028	0.046±0.009
10	8	1.037±0.130	1.591±0.340	0.554±0.229	0.069±0.028

n — number of pigs

filtrates was the same as in meat (Table 2). The growth of creatinine in filtrates was more even and the filtrates could be stored for a longer period as they did not deteriorate.

Table 3. Effect of the storage time of deproteinized meat extracts in a cold store (4 °C) on the creatinine content

n	Time of storage days	Creatinine content, μmoles/g		Creatinine increase, μmoles/g	
		before storage	after storage	total	per day
		$\bar{x}$ s	$\bar{x}$ s	$\bar{x}$ s	$\bar{x}$ s
10	1	1.033±0.117	1.081±0.106	0.050±0.013	0.050±0.013
10	2	1.052±0.090	1.138±0.086	0.086±0.011	0.042±0.005
10	4	0.799±0.222	1.050±0.151	0.258±0.080	0.056±0.011
10	6	1.026±0.113	1.358±0.094	0.341±0.056	0.057±0.009
10	12	0.856±0.082	1.485±0.091	0.629±0.106	0.052±0.009

n — number of pigs

The looking for a way of inhibiting the reaction of dehydration of creatine to creatinine resulted in two positive solutions. The samples of meat can be stored in a frozen condition (-15°C) at least during a period of 3 months without any changes in the creatinine content. Neither has the storage of water-meat homogenates in a cold store during two days any effect on the results achieved (Table 4). This method is highly practical as it is possible to prepare measured amount of water in homogenization flasks, to weigh meat samples in a fixed time, homogenize them, place them in a cold store and make all the determinations in one series on the following day. In case of a larger amount of analyses, it is more convenient to weigh meat samples in a preset period of time after slaughter, pack them in foil bags, freeze them and keep at -15°C.

At 37°C the growth of creatinine in acid filtrates amounted to approx. 1.5 μmol per day in terms of 1 g meat (Table 5) which means that it was

Table 4. Effect of meat samples preparation and time of storage on the creatinine content

n	Creatinine content in samples, μmoles/g			
	before storage $\bar{x}$ s	after storage of the water meat homogenates (+2°C, 2 days) $\bar{x}$ s	after storage of the meat samples in a frozen state (3 months) $\bar{x}$ s	difference $\bar{x}$ s
10	0.959±0.054	—	0.958±0.062	-0.001±0.012
10	1.055±0.076	1.062±0.078	—	0.007±0.011

ca 30 times higher than when the filtrates were stored in a cold store (Table 3). When storing water homogenates in a cold store, the growth of creatinine was analytically imperceptible (Table 4), but at 37°C it amounted to approx. 0.5 μmol/g/day (Table 5). This growth largely depended on pH. The homogenates which after a 24 h stay in a thermostat at 37°C had the lowest pH, showed the highest increase of creatinine (Table 5).

Table 5. Increase of creatinine content at 37°C, during 24 h, in water-meat homogenates and in deproteinized meat extracts

Samples	Creatinine content, μmoles/g				
	n	before storage $\bar{x}$	after storage $\bar{x}$	increase $\bar{x}$	pH*) $\bar{x}$
Water-meat homogenates	5	0.90	1.22	0.32	5.9
Water-meat homogenates	3	1.12	1.53	0.41	5.7
Water-meat homogenates	2	1.23	1.80	0.57	5.3
Deproteinized meat extracts	10	1.02	2.45	1.43	1.2

*) pH was measured after storage for 24 h at 37°C

The results of determinations of creatine and creatinine in pork meat 45 min and 48 h after sloughter showed that the content of creatine dropped and that of creatinine increased in the period between two determinations. However, the loss of creatine was higher than the growth of creatinine (Table 6). The determination of creatine in meat is less precise, observations of the reaction of dehydration of creatine to creatinine in meat shall be carried out only on the basic of a determinations of creatinine. The assessment of the effect of time, temperature and method of samples preparation were based therefore on the results of the determinations of creatinine (Table 1, 2, 3, 4 and 5). In the following tests, creatine was determined only once, 48 h after slaughter, when samples used for the determinations were taken from a larger average sample prepared from several *m. long. dorsi* (Table 7). Muscles were grouped according to pH range, related to the watery characteristics of meat.

The correlation coefficients obtained between the rate of watery characteristics of meat and the content of creatinine 48 h after slaughter have shown that a higher content of creatinine is accompanied by a watery structure of meat [12]. Taking the limit pH values determined 45 min after slaughter (pH_1) (6) as a criterion of the watery structure of meat, the pigs (from two experiments) were divided into three groups and the mean values of creatine and creatinine were calculated (Table 7). The results presented point to the lowest content of creatinine and its

Table 6. Creatine and creatinine content in the porcine *long dorsi* muscle at 45 min and 48h *post mortem*

Experiment	n	Creatine content, μmoles/g			Creatinine content, μmoles/g		
		45 min $\bar{x}\pm s$	48 hours $\bar{x}\pm s$	difference $\bar{x}\pm s$	45 min $\bar{x}\pm s$	48 hours $\bar{x}\pm s$	difference $\bar{x}\pm s$
I	80	39.31±1.91	38.17±1.76	1.14±2.06	0.74±0.07	0.98±0.09	0.23±0.09
II	48	40.30±1.30	40.84±1.30	0.53±1.37	0.80±0.05	0.98±0.06	0.18±0.07

lowest growth in pigs with a pH_1 higher than 6.3. Similar results were obtained in each of the experiments made.

Table 7. Creatine and creatinine content in m. *long. dorsi* of pigs of various pH_1 ranges (pH_1 6.0) — PSE meat, (pH_1 = 6.0-6.3) — slightly PSE meat and pH_1 6.3 — normal meat

Items	Time after slaughter	Creatine and creatinine content, μmoles/g in the meat of pH:			Significance of differences
		6.0 $\bar{x}\pm s$	6.0-6.3 $\bar{x}\pm s$	6.3 $\bar{x}\pm s$	
Experiment III n = 80		n = 20	n = 20	n = 40	
Creatine	48 h	39.92 ±1.47	40.46 ±1.51	40.53 ±1.80	NS
Creatinine	45 min	0.867±0.071	0.780±0.068	0.784±0.060	x
Creatinine	48 h	1.116±0.085	1.048±0.080	0.983±0.077	xx
Creatinine increase		0.249±0.092	0.252±0.090	0.201±0.081	xx
Experiment IV n = 64		n = 6	n = 16	n = 42	
Creatine	48 h	41.28 ±1.20	41.06 ±1.33	41.50±1.45	NS
Creatinine	45 min	0.789±0.095	0.805±0.070	0.825±0.067	NS
Creatinine	48 h	1.146±0 102	1.112±0.093	1.046±0.088	xx
Creatinine increase		0.357±0.090	0.306±0.105	0.221±0.113	xx

In meat with a pH > 6.3, creatinine 45 min after slaughter was the highest in experiment III, and the lowest in experiment IV (Table 7). These results suggest that the content of creatinine in pork meat 45 min after slaughter and probably *in vivo* as well has no effect on the watery structure of meat; the differences observed in the content of creatinine in meat 48 h after slaughter are caused by post mortem changes. In watery meat the speed of dehydration of creatine to creatinine is higher. The content of creatinine in meat 48 h after slaughter seems to depend on two factors: pH and temperature.

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PRZYROST KREATYNINY W MIĘSIE ŚWIŃ *POST MORTEM*

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S t r e s z c z e n i e

Oznaczono zawartość kreatyniny w m. *long. dorsi* świń w czasie 5 i 45 min, 24 i 48 h po uboju i podczas składowania mięsa i jego wodnych homogenatów lub bezbiałkowych filtratów w chłodni oraz próbek mięsa w stanie zamrożenia. Wykazano, że przyrost kreatyniny pierwszego dnia po uboju wynosi ok. 0,1 μmola na g mięsa, a przy dalszym składowaniu w chłodni ok. 0,05 μmola na g na dzień (tab. 1 i 2). Podobny przyrost obserwowano przy przechowywaniu bezbiałkowych filtratów w chłodni (tab. 3). Zahamowanie reakcji odwodnienia kreatyny do kreatyniny na dłuższy okres — do 3 miesięcy uzyskano przechowując próbki mięsa w -15°C, a na krótszy okres — do 2 dni przechowując wodne homogenaty mięsa w chłodni (tab. 4). Podwyższona temperatura (37°C) powoduje znaczne przyspieszenie tej reakcji w wodnych homogenatach mięsa i jego bezbiałkowych filtratach (tab. 5).

Oznaczając zawartość kreatyny i kreatyniny w m. *long. dorsi* 45 min i 48 h po uboju obserwowano większy ubytek kreatyny niż przyrost kreatyniny (tab. 6). Śledzenie reakcji odwodnienia kreatyny do kreatyniny w mięsie na podstawie oznaczeń kreatyniny jest dokładniejsze. Przyrost kreatyniny w czasie od 45 min do 48 h po uboju i jej zawartość 48 h po uboju są istotnie wyższe w mięsie wodnistym (tab. 7).