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AMINO ACID PROFILE OF TURKEY HENS FOR THE DETECTION OF MEAT FRAUD WITH AMINO ACID SOLUTIONS

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INTRODUCTION:
Meat is a high-quality food with an appropriate price. Manipulations of poultry meat appeared already for a long time. In a past contribution [1], the BBC refers about additions of water to chicken meat up to 50 % of the original weight, as well as additions of cattle or pork protein hydrolysates. The weight manipulation of fresh meat is forbidden in Europe [2] and the addition to meat products is regulated in Germany [3]. Generally, the addition of water can be proved by the determination of the water to protein ratio (Feder number). However as soon as protein hydrolysates are added along with water, this becomes difficult, as under such circumstances the Feder number might not change. Looking for an alternative method, we were interested if it is possible to detect the injection of protein hydrolysates into turkey meat by quantification of the free amino acids content (AAC) of muscle. As it is known from mammalian muscle, that quite dramatic changes can occur within the protein matrix of muscle during the first days *post mortem* due the action of ions and endogenous proteases (maturation), a systematic investigation of the AAC at different time points from 0 h to 99 h after slaughter was performed. The AAC is enhanced by the release of amino acids from proteins by proteases, but also reduced by the depletion reactions towards ketogenic and glucogenic metabolites. Further on, the different conditions of the meat production (e. g. gender, age, race, feeding and animal farming) can lead to different AAC [4-6] of the animals at the time of slaughter.

MATERIAL AND METHODS:
Turkey hens (B.U.T. big 6, average weight 10320 g, average age 112 days, at least in duplicate) from Germany were used. Breast meat samples were stored at -80 °C before use. The homogenisation (12 000 rpm at -20 °C, 0.025 M EDTA / 0.100 M Tris buffer) was done with 2.0 g turkey meat. The amino acid contents were determined with cation exchange chromatography (3 µm beads, pH-range from 2.9 to 10.4, flow rate 180 µl/min, membraPure GmbH, Berlin, Germany) with an external standard solution which was processed in the same way as the samples.
One gram of pig gelatin protein was hydrolysed in 8 ml 6 M HCl at 150 °C for 1 h and diluted to a final concentration of 0.5 M amino acids in water. Pieces of 5 g breast muscles were injected with 5 ml of gelatin protein hydrolysate and worked up equally as the unmodified meat samples.
Statistical analysis was calculated with a Student´s t-test using Excel (Microsoft Office Professional Plus 2010, Microsoft Corporation, Redmond, USA).

RESULTS AND DISCUSSION:
The free AAC from slaughterhouse sampling showed only low differences from one to the other sampling time, shown for two exemplary amino acids:

Tab. 1: Free AAC from turkey breast muscle stored over 99 h at 4 °C.

Amino Acid	**Free Amino Acid Content (average in mg / 100 g)**							
	0 h	**1 h**	**3 h**	**6 h**	**27 h**	**51 h**	**75 h**	**99 h**
Glycine	1.08±0.13	1.14±0.09	1.39±0.12	1.21±0.13	1.47±0.08	1.36±0.14	1.29±0.15	1.61±0.38
L-Valine	1.05±0.09	1.09±0.08	1.32±0.11	1.39±0.31	1.45±0.39	1.48±0.13	1.70±0.24	1.66±0.27

The AAC of most of the proteinogenic amino acids were fluctuating within the first six hours (data not shown). The depletion reactions are energy-depending and can therefore only last until the energy is consumed. On the other hand the hydrolysis reactions performed by proteases are not depending on energy supply. As these are competing reactions, the AAC is only steadily rising when the in meat stored energy is used up. Nevertheless, the differences in the AAC over the whole 99 h were small but highly significant ($p < 0{,}001$). The total AAC of all proteinogenic amino acids is increased from about 26 mg / 100 g at 0 h to about 43 mg / 100 g at 99 h.

As a next step protein hydrolysate from pig gelatin was added to the samples:

Tab. 2: Free AAC from turkey breast muscle with and without the addition of gelatin protein hydrolysate.

Amino Acid	Free Amino Acid Content (average in mg / 100 g)		
	Without hydrolysate	With hydrolysate	Difference
Glycine	1.35±0.39	74.44±1.39	73.09
L-Valine	1.36±0.37	18.22±1.74	16.86

In order to obtain an unambiguous proof, the changes by the addition of hydrolysates must be significant and obvious higher than the differences because of the maturation process or different production conditions. Both requirements were fulfilled for the exemplary amino acids (each with $p << 0.001$). With an amount in gelatin for glycine of 21 % this was to be expected, however, this also applied for L-valine with an amount of 2.4 %. This is not surprising, as the manipulations are expected in a range of about 10 % of the weight in order to be economically relevant. This means an addition of at least 2 g of amino acids per 100 g, while the total free AAC is about 0.1 g per 100 g.

CONCLUSION:

Only amino acids with low natural variations (maturation, production conditions) are suitable for the proof of the addition of protein hydrolysates by the determination of the free AAC in fresh meat from turkey hens´. The addition of gelatin protein hydrolysate led to significant changes even of those amino acids added only in low amounts. Therefore, the proof of the addition of this hydrolysate by the determination of the free AAC was possible.

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