Electrophoresis of serum and muscle proteins of pigs fed lysine.

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Electrophoretic analyses of muscle proteins have been made by a number of authors. In an electrophorogramme of an extract of rabbit striated muscle Jacob (1) differentiated three groups of components: the first contained mainly three fractions, n, m, l; the second, k, j; the third, mainly h. The author determined also their per cent ratio.

According to Kotter, Lenz and Prändl (2), the fraction subdivisions, n, m, l, given by Jacob correspond to Myogen n. Waber, or l to Myogen A n. Baranowski, and n,m, to Myogen Baranowski, and they relate to Myosin; h represents myoalbumin. The complex n, m, l contains many important enzymes.

Salobir (3) studied, by paper electrophoresis, pig skeletal muscles in normal conditions and differentiated three groups: 1, the fastest mobile group of myoalbumins; 2, myosin group; 3, the slowly mobile group of myogens, in which myoglobulin is found. This division would correspond to groups III, II, I, given by Jacob.

Götze (4) carried out fundamental electrophoretic studies of original meat extract and of a chemically regrouped meat extract with a view to the isolation of myoglobin, in different types of animals: horses, cows, sows, fattening pigs, sheep, lambs. Cellulose acetate membrane foils of 20 x 100 mm were used by him as medium carriers.

The present note is part of a dissertation on the problem of the effect of dietary synthetic lysine on the biochemical and technological characteristics of pork, and is a continuation of the investigations, part of which was reported at the 15th European Meeting of Meat Research Workers in Helsinki.

E X P E R I M E N T A L

MATERIALS

Two groups of 20 Large White pigs each were created for the experiments. They were equalized by the method of analogies in sex, age and live weight and were fed according to two different patterns, in which the level of lysine was equalized.

The first group, the control, received balanced standard feed mixes according to the formulae of feed industry, from birth till slaughter.

The second - the experimental - group after reaching a live weight of 50 kg received simple feed mixes (maize, barley, wheat bran) with 2,5 g of imported synthetic lysine added per kg of feed.

After reaching a live weight suitable for the production of pasteurized canned meats (110 kg) both groups were slaughtered. Meat from a muscle from each animal of both the control and the experimental groups was taken for the investigations.

METHODS

The work was carried out according to J.J. Scheidigger's method, modified by Dr. Bogdanov. Its essence consists in the following: 1% agar gel is used, in which there is veronal and sodium veronal buffer only for the preparation of the agar plates on which the substance investigated is applied on the start line. Dr. Bogdanov has replaced the buffer in the cells by 1% aqueous solution of carboxymethyl cellulose.

Electrophoretic separation is performed only on the agar layer where the intimate processes of the substance under investigation take place.

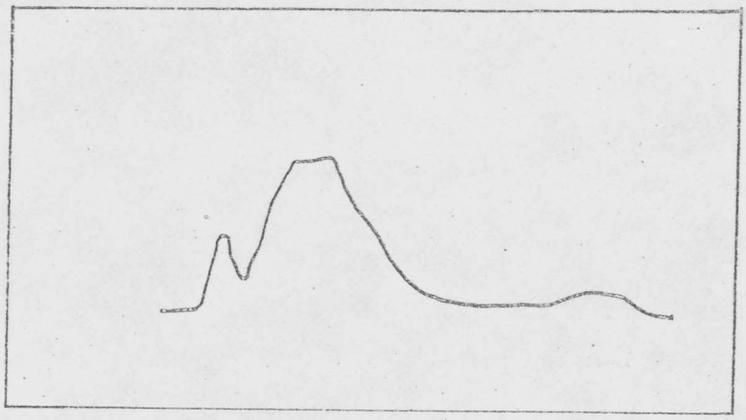
Preparation of extract. 2,5 g of the sample, pre-comminuted muscle, is weighed and ground in a mortar at a low temperature with a phosphate buffer in the ratio of 1:2 for 1 hour. The mass obtained is centrifuged and then filtered. The filtrate is ready for pipetting on the start line of the agar plates. The phosphate buffer is prepared from KH_2PO_4 and Na_2PO_4. On the agar buffer poured in advance on the glass plates, the start line is determined. A hole with a groove is cut and some of the meat extract is pipetted. The plates prepared in this way are put into a vat in which there is a buffer, 1% solution of carboxymethyl cellulose. The buffer has a pH of 8,2. The electrophoresis is performed in 4 hours at a voltage of 250-280 V.

After the definite time is over, the apparatus is switched off, the plates are removed from the vat and put into the solution for fixing. They are allowed to stay for 24 hours, then dried and stained with an especially prepared solution of CH_3COOH, CH_3COONa, glycerin, amido black 10-B. The plates are allowed to stay in the staining solution for 1 hour and then transfered into a washing solution for 10 min. They are dried and analysed using an automatic densitometer, Direktschreiber mit Integrator Typ 150 - DDR.

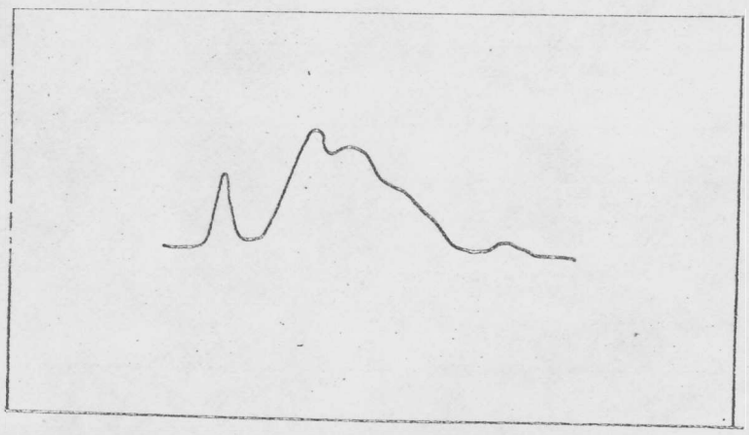
With the application of the modified method for muscle protein electrophoresis described, six fractions are obtained, four in front of the start line, and two behind it, designated by the author with the initials A, B, C, D, E and F. The recordings of the automatic densitometer were measured with the same apparatus and the data were processed mathematically. One record of an electro-

phorogramme of muscle protein foreach group is given below as an example.

Graphic Presentation of read off Electrophorogrammes:



Experimental Group



Control Group

In the following Table 1 are shown the mathematically processed data from the densitometer - a per cent ratio of the individual fractions.

Per cent ratio of individual fractions — Table 1

Group	A	B	C	D	E	F
Experimental	10,95 (± 3)	9,52 (±2,7)	27,14 (±3,2)	17,14 (±2,4)	19,05 (±2,3)	16,20 (±4,0)
Control	12,94 (±2,9)	17,91 (±3,1)	15,82 (±2,6)	34,53 (±4,8)	14,31 (±2,1)	4,43 (±0,65)

Note: The figures in brackets designate the standard deviation.

The protein fractions obtained in this way do not completely correspond (although six in number) in per cent ratios to those cited in literature (1, 2, 3, 4).

The electrophorogrammes from the plates have been photographed on slides which will be demonstrated to the participants in the Meeting. The percentages of the protein fractions given in Table 1 speak for significant differences between the per cent ratios of the individual fractions both before and behind the start line, in the experimental and the control groups.

The differences are the greatest in fractions C, D and F, while the remaining fractions have per cent ratios close to one another.

In the following Table 2, the travel rates of the individual muscle protein fractions in the experimental and the control group are shown.

Travel rate of individual fractions — Table 2

Group	A	B	C	D	E	F
	in front of start line				behind start line	
Experimental	2,7	1,5	1	0	1,3	4,2
Control	3,3	1,8	1,3	0	1,2	3,5

It is obvious from Table 2, that there are differences in the travel rates of the individual fractions in the experimental and the control groups.

Nevertheless, there are common principles in the travel rates of the fractions. So, in both groups fraction A is the fastest to travel (the travel rate being higher in the control group), and fraction D is the slowest. The differences are statistically reliable.

The investigations continue and for the time being the author is unable to give a final interpretation of the intimate mechanism of the occurrence of differences between the experimental and the control group.

The following conclusions can be made from the results obtained in the electrophoretic analyses of muscle proteins derived from pigs fed lysine or balanced feed mixes, respectively, i.e. the experimental and the control group -

1. In the modified method of electrophoresis described, six fractions are obtained (four in front of the start line and two

behind the start line).

2. Between the per cent ratios of the individual fractions in the experimental and the control groups there are statistically reliable differences.

3. Travel rate in the individual fractions is determined by a general regularity, fraction A being the fastest, and fraction D, the slowest, in both groups.

LITERATURE

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