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BIOCHEMICAL DISTINCTION OF *DIAPHRAGMA PARS LUMBALIS* FROM OTHER BEEF MUSCLES.

J. BOUSSET and B.L. DUMONT

Laboratoire de recherches sur la viande de l'INRA,
F 78350 Jouy en Josas, France.

SUMMARY

The biochemical composition of the muscle *Diaphragma pars lumbalis* (D) ((frequence of each of the five isoenzymes of the LDH (ISO1, ISO2, ISO3, ISO4, ISO5), ratio of heart to muscle form of LDH (H-M), amount of haem iron (Fe), total nitrogen (NT), soluble nitrogen (NS), sarcoplasmic proteic nitrogen (Nps) and non proteic nitrogen (Npn)) has been studied comparatively to that of 26 other muscles of the beef carcass in order to propose a simple and accurate method for distinguishing D from other cuts of beef.

Analysis were made on 270 samples from ten beef carcasses chosen to represent a set of the carcasses existing in the French market.

The composition of D was very different of that of the other muscles, specially as the following ratios were concerned : Fe/NS, Fe/ISO5, ISO5/ISO1, Fe/Npn, Fe/Nps, H/M.

To distinguish D from other types of beef muscles it is suggested to use the value of the ratio :

$$\frac{ISO5 \times NS}{Fe}$$

which value was found to be 0.278 + 0.098 for D compared to 2.463 ± 1.134 for the set of the 26 other muscles.

INTRODUCTION

The carcass of meat animals comprises about two hundred paired but not strictly symmetrical muscles. These muscles, of different size and weight, perform the various functions involved in locomotion and maintenance of the posture of the live animals. Identification of the individual intact muscles is easily achieved after dissection due to their relative size and their specific shape, well described in texbooks of muscular anatomy (e.g. for beef BROWN *et al.* 1978). On the cross-section of individual muscles - presented as meat cuts - it is also feasible to identify their anatomical origin from the appearance of the meat grain resulting from the perimysium traits (DUMONT, 1986). It is also well known that muscle composition varies greatly from one muscle to another (LAWRIE, 1985) and such variation has been explored in beef for some of the major chemical components (e.g. for nitrogen content - BOUSSET and DUMONT 1984 or for haem iron - BOUSSET and DUMONT 1985). Some recent studies have shown that it was possible - namely in beef (TALMANT *et al.* 1986) - to characterize biochemically the contractile and metabolic type of muscles. But up to now the relationships between components have not been studied thoroughly enough to discriminate individual muscles on the basis of their chemical composition. So that it is not yet possible, only from some chemical determinations, to find out the anatomical location any piece of meat is coming from. The chemical distinction of muscles would be of some interest in various instances, as well for economic reasons (for the meat trade) as for regulations. This is the case, for instance, of the muscle *Diaphragma* which must be differentiated from other skeletal muscles of cattle for customs inspection in the EEC. The present work reports the results obtained in a comparison of the chemical composition of Diaphragma and other major skeletal muscles of the beef carcass to developp a method for the detection of bovine diaphragma muscle.

MATERIALS AND METHODS

The animals used in this study (N=10) have been chose to represent a very large sample of the different types of cattle found in the French market of beef, as age, carcass weight and carcass conformation are concerned. The average carcass weight was 316.2 + 66.9 k the age (estimated from teeth evolution according BRAZAL *et al.* (1971) was 51.2 + 23.7 months and the conformation score (according DUMONT *et al.* (1975) wa 9.1 + 3.3 (in a scale from 1 to 16). The sample of animals comprised six females (cows and heifer), three steers and one bull.

The animals were killed at the abattoir of the Meat Research Departement at the INRA Center of Theix and carcasses were chilled in order to avoid any cold shortening. 24 h *post mortem* carcasses were disssected and the muscles were then stored at 0°C up to their sampling, made 3 or 4 days *post mortem*. On each muscle location one slice 5 cm depth, was taken and completely trimmed of external fat and epimysium. The sample was minced and carefully mixed and the following chemical determinations were made for a first group (I) of 27 muscles :

* haem iron according to HORNSEY (1956) (Fe),
* total amount of nitrogen (NT), water soluble nitrogen (NS), sarcoplasmic proteic nitrogen (Nps), non proteic nitrogen (Npn), according the method described by BOUSSET (1980), all the determinations of nitrogen being made by the Kjeldahl's method).
* determination of the five isoenzymes of lacticodeshydrogenase (LDH) (ISO1, ISO2, ISO3, ISO4, ISO5), after separation by acrylamide gel electrophoresis of one extract of muscle by ClK 0.15 M.

The ratio of heart to muscle forms of the LDH (H-M) was determined by calculation from the known tetrameric composition of each isoenzyme and the relative value of each one.

Two groups of muscles were considered. The first group (I) included 27 muscles. For most of them the samples were taken in the middle part of the muscle : *vastus lateralis* (number 7 in Fig.1), *tensor fasciae latae* (8), *gluteus medius* (9), *triceps brachii caput laterale* (30), *rectus femoris* (36), *supraspinatus* (42), *adductor* (49), *infraspinatus* (57), *transversus abdominis* (61), *rectus abdominis* (62), *semispinalis capitis* (67), *serratus ventralis pars cervicis* (72); for the others, the sample was taken at specific places : *semitendinosus* (middle (4), 1/3d cranial part (5) 1/3d caudal part (6)), *psoas major* (4th lumbar vertebra (12), *longissimus dorsi* ((3d lumbar vertebra (15), 10/11th thoracic vertebra (18)), *pectoralis profundus* (middle (31), 1/3d cranial part (32)), *semimembranosus* (middle (33), 1/3d cranial part (34), 1/3d caudal part (35)), *gluteobiceps* (middle (1), 1/3d caudal part (3)).

The second group (II) included all the muscles of the group (I) and twelve other muscles considered at the middle part of the muscle (*latissimus dorsi* (19), *pectineus* (39), *gracilis* (40), *gastrocnemius caput mediale* (48), *subscapularis* (60), *iliacus* (63), *obliquus internus abdominis* (65), *splenius* (68), *vastus internus* (69), *gastrocnemius caput laterale* (70), or at specific location (*gluteobiceps* (1/3d cranial part (2)) *cutaneus trunci* (at the level of the navel (28)).

The statistical treatement comprised calculations of

- mean and standard deviation of the different traits for each muscle ;
- mean and standard deviation of each trait for the whole muscles of group (I), except the muscle *diaphragma pars lumbalis* ; this set of data based on 260 determinations is proposed to be considered as representative of the "beef" composition (B) ;
- comparisons of means of ratios of the most discriminating variates, according Student's test ;
- analysis of the variation within muscles of the

group (I) by the multivariate method of centered data (LEFEBVRE 1976).

RESULTS AND DISCUSSION

Table 1 gives the average value of the different traits for muscle *Diaphragma* and for the whole other muscles of the group (I), which may be considered as "usual" or "normal beef muscles. Comparatively to B the *diaphragma* can be distinguished by various chemical characteristics,

- some being more important in D, like Fe, ISO1, H-M ratio,
- others being less important (NS, Npn or Nps).

Table 1 - Composition of Diaphragma and other beef muscles

Trait	Diaphragma n=10	Other beef muscles n=260
Fe µg/g	31.85	18.25
NT g/100g	3.01	3.43
NS g/100g	0.63	0.82
Npn g/100g	0.30	0.39
Nps g/100g	0.33	0.44
ISO1 %	29.93	8.42
ISO2 %	20.67	11.84
ISO3 %	25.00	18.35
ISO4 %	10.43	12.11
ISO5 %	13.97	49.28
H/M %	1.57	0.45
NS/NT %	0.21	0.24
Npn/NS %	0.48	0.47
Npn/NT %	0.10	0.11
Fe/ISO5 %	2.62	0.40
ISO5/ISO1 %	0.48	13.55
Fe/NT %	10.58	5.35
Fe/NS/NT %	153.40	77.04
Fe/Npn %	105.80	47.89
Fe/Nps %	103.65	43.38
Fe/NS %	50.99	22.62

When one considers the ratios between the values of the different characters which are opposite such as Fe/NS, ISO5/ISO1, Fe/ISO5, H-M, Fe/Npn, Fe/Nps, the distance between D and B muscles is by far more important.

The general distinction between D and the whole muscles is also found from the individual comparisons by the t test of Student of the mean of D and that of each of the 26 other muscles.

Table 2 gives the t value of the significant differences observed between D and other muscles.

It thus appears that the differences between D and each of the 26 other muscles are very highly significant as a whole.

The multivariate analysis of centered data on the group (I) showed that most of the variation existing in the population is explained (for 67.6 p 100) along the first axis by the opposition existing between the variates Fe and ISO5. It is then clear that these two traits are major variates and may easily separate D from the different muscles.

As an example Fig. 1 shows the location of the mean of each of the 39 muscles studied in group (II).

It is clear taht D is discriminated -more or less strongly- from usual beef muscles both by a higher value of Fe and a lower percentage of ISO5.

These two characteristics have the same general significance for the metabolic orientation of the muscle *diaphragma* which metabolic type is a highly oxydative

Table 2 - t values of differences between Diaphragma (D) and other beef muscles

Muscles compared to D	Traits: Fe / NS	ISO5 / ISO1	Fe / ISO5	H-M	Fe / Npn	Fe / Nps
1	-11.32	4.56	-5.55	-10.37	-16.23	-4.76
3	-13.08	6.61	-5.71	-11.92	-20.92	-5.32
4	-13.90	4.42	-5.92	-13.67	-22.89	-5.72
5	-13.06	4.09	-5.86	-13.47	-21.01	-5.46
6	-13.83	3.95	-5.96	-13.84	-21.39	-5.44
7	-11.95	3.54	-5.78	-14.11	-17.01	-4.82
8	-11.59	4.18	-5.69	-11.82	-18.56	-5.19
9	-13.43	4.07	-5.49	-11.60	-14.24	-5.26
12	-13.33	2.63	-5.52	-11.84	-14.55	-5.05
15	-12.80	3.96	-5.70	-12.47	-16.97	-5.61
18	-13.12	5.58	-5.75	-12.28	-18.19	-5.73
21	-10.73	4.58	-5.45	-11.20	-16.27	-4.49
30	-11.07	7.85	-5.35	- 9.35	-13.15	-4.48
31	-13.02	6.39	-5.64	-11.94	-18.83	-5.30
32	-11.23	5.62	-5.66	-12.33	-17.36	-4.57
33	-11.04	8.48	-5.76	-12.70	-20.99	-4.19
34	-12.72	6.53	-5.72	-12.35	-20.24	-5.26
35	-11.45	5.72	-5.67	-12.15	-18.42	-4.92
36	-12.60	5.53	-5.66	-12.60	-17.08	-4.94
42	- 9.51	13.60	-5.30	- 9.90	-12.02	-4.04
49	- 9.89	7.84	-5.59	-12.87	-16.27	-4.04
57	- 6.99	9.61	-5.08	- 8.90	- 9.04	-3.06
61	- 8.60	12.35	-4.82	- 7.65	- 9.73	-2.77
62	-11.69	11.91	-5.30	- 8.79	-17.86	-4.60
67	- 6.88	11.81	-4.93	- 8.61	- 9.78	-3.04
72	- 7.03	8.41	-4.79	- 6.34	-10.03	-3.21

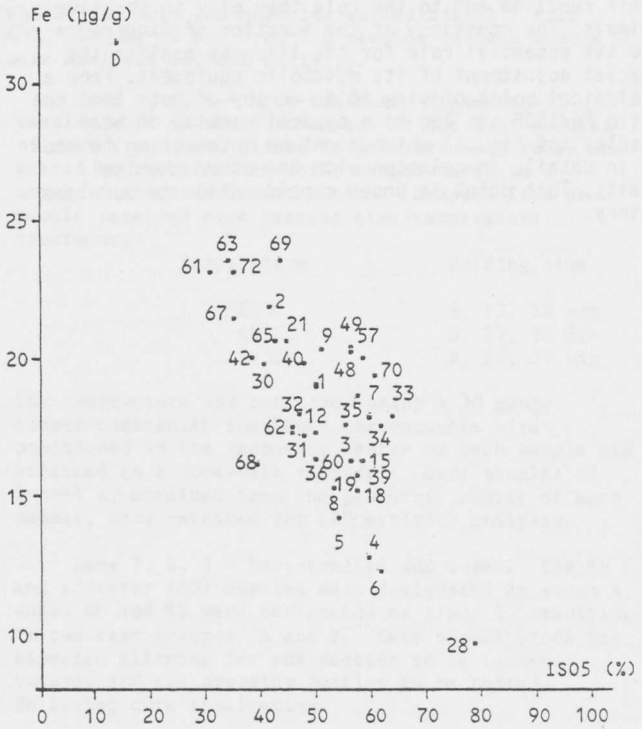

Fig.1 - Relationship between Fe and ISO5 means of the different beef muscles (see text for definition of the number of muscles)

one and poorly reductive compared to the set of the other 26 muscles.

In this situation any biochemical trait related to the oxydative metabolism of the muscle may be supposed to be of some interest to discriminate D.

In this field the recent proposals of GOTTESMAN and HAMM, suggesting both the use of the myoglobin content

and the β-hydroxyacyl-CoA-dehydrogenase (HADH) activity to indicate the presence of *diaphragma*, are based on the same principle.

The value of the Fe/ISO5 is quite of special interest to typify D muscle comparatively to other beef muscles. If we consider, in addition, another trait which is also very typical of D, like NS which is about 25 p cent lower in D than in other beef muscles, we may propose a very simple and accurate index for the differentiation between *diaphragma* and other skeletal muscles. This index (dd) is the ratio

$$dd = \frac{ISO5 \times NS}{Fe}$$

The value of dd was 0.278 ± 0.98 for D (with the range 0.114 - 0.442), and 2.463 ± 1.134 for the set of the 26 muscles (with a range of 0.747 - 6.787).

This index is based on determination of chemical components, reflecting the basic composition of the muscles, which may be prefered to any other traits such as those related to the enzyme activity, more or less disturbed *post mortem* according the conditions of storage. In addition the chemical determinations involved are relatively simple and known as being accurate.

The study has clearly shown that in the case of *diaphragma* it is possible to differentiate the muscle on the basis of its chemical composition. Further studies led to the conclusion that it is possible to differentiate many of the other musclesfrom each other.

Thus biochemical differentiation offers a useful mean to separate muscles when necessary. It makes also possible to study the origin of the variation existing between muscles, which may probably be attributed to their function and to the role they play in the live animals. The constancy of the function of *diaphragma* and its essential role for the life may explain the special adjustment of its metabolic equipment. From a biological point of view it is worthy of note that the ratio Fe/ISO5 has led to a typical ranking of beef muscles (cf Fig. 1) which might be interesting to study in detail, in relation with the other chemical traits. This point is under consideration in our laboratory.

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