## **Proceedings of the**



# 4-7 july 2000 - Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910

(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

## PLA M. and DALLE ZOTTE A.

## HARMONISATION OF CRITERIA AND METHODS USED IN RABBIT MEAT RESEARCH (Round Table)

Volume A, pages 539-545

### HARMONISATION OF CRITERIA AND METHODS USED IN RABBIT MEAT RESEARCH

PLA M.<sup>(1)</sup> and DALLE ZOTTE A.<sup>(2)</sup>

<sup>(1)</sup> Department of Animal Science, University Polytechnic of Valencia, 46071-Valencia, Spain <sup>(2)</sup>Department of Animal Science, University of Padova. Agripolis, 35020 - Legnaro (PD), Italy

#### **INTRODUCTION**

The first proposal for harmonisation of the criteria used in the dissection of rabbit carcasses was developed by Blasco *et al.* (1992), and was revised in 1994 in Kaposvar (Hungary). The document was then reported by Blasco and Ouhayoun (1996) and finally was approved by the Commission of Harmonisation of the WRSA.

Furthermore, the Commission of Harmonisation has a paper with the aim to standardise the rabbit meat criteria *sensu stricto* (Ouhayoun and Dalle Zotte, 1996). This paper concerned a list of techniques and analysis that have been subsequently used for rabbit meat studies. Nevertheless, a description of rabbit meat sample collection and preparation procedures is lacking. However, more specific and, consequently, different sampling methods could be used in particular studies.

#### 1. SLAUGHTERING, HOT CARCASS MEASUREMENTS AND CHILLING

#### 1.1. Transport to the slaughterhouse

At the end of the experimental period, rabbits should be transported to the slaughterhouse, usually near the farm, avoiding the stress that can affect meat quality. Otherwise, *it is recommended* to indicate average temperature, duration of the transport from the farm to the slaughterhouse and, where necessary, the resting period before slaughter. It is recommended to indicate if the rabbits were slaughtered in other facilities. In order to determine the dressing percentage, the liveweight should be recorded at the slaughterhouse. If transport conditions are particularly stressful, the transport losses could be detected by weighing the animals before the transportation and then just before the slaughter (assuming that animals are immediately slaughtered upon arrival).

#### 1.2. Fasting

If fasting has been practised *it is recommended* to specify the type of fasting and its duration.

#### 1.3. <u>Slaughter conditions</u>

*It is recommended* that an adequate description of the type of stunning: electrical (voltage, frequency and duration), neck hit or other practises, used to kill rabbits and which prevent suffering be provided. Slaughterhouse temperature during carcass processing and time between the slaughter and the beginning of chilling should be indicated.

#### 1.4. Hot Carcass Weight

Hot carcass weight (HCW) is the weight of the carcass 30 min after slaughter. As Blasco and Ouhayoun (1996) indicated, the carcass does not include blood, skin, distal parts of the tail, fore and hind legs, gastrointestinal and urogenital tracts. HCW includes head, liver, kidneys and the thorax and neck organs (oesophagus, trachea, lungs, thymus and heart). The hindleg section in the middle of the tarsus permits the hanging of the carcass for further processing; however, *it is recommended* to cut the hindlegs between the distal epiphyses of the tibia and the *tarsus-calcaneus*.

#### 1.5. Chilled Carcass

The Chilled Carcass (CC) is the carcass prepared as indicated above, chilled for 24 hours in a ventilated cold room ( $0^{\circ}$ -  $4^{\circ}$ C). The washing of carcass with water should be avoided. *It is recommended* to hang the carcass during chilling and to provide adequate ventilation.

Weight of the carcass after chilling is the Chilled Carcass Weight (CCW), while Drip Loss Percentage (DLP) is the difference between hot and chilled carcass weights expressed as percentage of HCW. *It is recommended* to indicate the exact temperature of the cold room, the chilling time and the air speed if the aim of the experiment is to study the chilling effect, because these three factors have an important influence on the rates of evaporation and on the drip loss.

#### 2. MEASURES ON CHILLED CARCASS

#### 2.1. Reference carcass

The Reference Carcass (RC) is the CC that includes the dissectable fat deposits and it excludes the head, liver, kidneys and the thorax and neck organs (oesophagus, trachea, lungs, thymus and heart).

RC should be weighted (RCW) and measured: Dorsal Length (DL) Thigh Length (TL) and Lumbar Circumference (LCL) are the linear measurements *recommended* by the WRSA.

Carcass colour is expressed as L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup> parameters (CIE, 1976) measured 24 h *post mortem* on clean carcass surface. *It is recommended* to measure carcass colour at the level of the lumbar region, and on the m. *Biceps femoris* (BF) surface, using a CR-200 or CR-300 Minolta Chromameter. The illumining should be D65 and measurements should be taken using an 8- $mm^2$  aperture with a zero degrees incidence angle.

Subjective or objective perirenal fat colour and consistency should be measured 24 h post mortem.

#### 2.2. <u>RC composition</u>

Fat deposits should be dissected from RC as follows:

Interscapular fat

Perirenal fat (between the 7<sup>th</sup> thoracic and 7<sup>th</sup> lumbar vertebrae)

*It is recommended* to indicate if other fat deposits on abdominal wall or on inguinal region were dissected.

After fat dissection, RC should be cut between the 7<sup>th</sup> and 8<sup>th</sup> thoracic vertebrae and between the 6<sup>th</sup> and 7<sup>th</sup> lumbar vertebrae. Forelegs should be separated from fore part including *scapulae* and insertion and superficial thoracic muscles. Four joints will be obtained: Forelegs Thoracic cage Loin

#### 2.3. Hindleg dissection

Hind part

For further processing, hindleg should be separated by cutting, anteriorly, around the inguinal region, and posteriorly including *os coxae* (Blasco and Ouhayoun, 1996).

#### 3. MEAT QUALITY

For meat quality measurements, the following muscles or meat portions should be considered:

- a) The whole *Longissimus dorsi* (LD) muscle (from the 8<sup>th</sup> thoracic to the 7<sup>th</sup> lumbar vertebrae) or the *Longissimus lumborum* (LL) muscle (from the 1<sup>st</sup> to the 7<sup>th</sup> lumbar vertebrae), accurately dissected without fat and subcutaneous fascia.
- b) The BF muscle.
- c) The loin joint.
- d) The hindleg, separated as above mentioned.

#### 3.1. <u>pHu</u>

Muscle pHu is the pH measured 24 h *post mortem*. The average carcass pHu should be estimated by measuring pH on LD (1 cm from the *processus spinosus* at the level of the 5<sup>th</sup> lumbar vertebra) and BF muscles. The pHu should be measured after incision of muscle aponevrosis using a thin electrode equipped with temperature probe. Because of the moderate thickness of BF muscle, *it is recommended* to avoid excessive penetration of the electrode in the muscle sample.

#### 3.2. <u>Meat colour</u>

Instrumental meat colour (L\* a\* b\*) should be measured immediately after carcass division. *It is recommended* to perform this measurement on a transversal section of the LL muscle, at the level of the  $7^{\text{th}}$  lumbar vertebra section of the muscle, as indicated above.

#### 3.3. Water Holding Capacity

Water Holding Capacity (WHC) is usually estimated using different methods, according to the process which meat will be subjected. For meat of other animal species, the reference methods for WHC determination are drip loss and cooking loss (Honikel, 1998). In case of small animals, such as rabbits, it is reasonable to substitute drip loss with a classic method, such as the filter paper method (Grau and Hamm, 1957).

The WHC should be determined on a meat sample collected from the CC 24 hours *post mortem*. Otherwise, the elapsed time and the refrigerating conditions should be indicated. If the WHC is determined using the cooking-loss method, *it is recommended* to use the whole hindleg or loin joint, and not the dissected LD, in order to avoid an excessive laceration of the muscular tissue and the consequent cellular fluid loss. For a rabbit weighing between 2.5-3 kg, the following cooking methods are recommended:

- 1. Samples are kept in a water bath at 75°C for 2h. The meat cooked in this way may be used for Warner-Bratzler shear force measurement.
- 2. Samples are kept in electric oven (200°C) until 85°C of internal temperature is reached. The meat cooked in this way may be used for sensory evaluation.

#### 3.4. Sensory properties

Sensory properties of the meat may include taste (salted, sweetness, bitter, acid), flavour (aniseed, grass, liver, sugar), texture (tenderness, juiciness, chewiness, fibrousness), and colour. They could be determined by a panel test of trained tasters. Off-flavours and off-odours derived from lipolysis of triacilglicerids and peroxidation of PUFA could be evaluated, too. For sensory evaluation, meat samples could be used fresh, stored at  $+4^{\circ}C$  for several days or at  $-20^{\circ}C$  for several months. Because storage temperature and time strongly affect meat sensory properties, it is fundamental to apply the same storage conditions on samples belonging to different experimental treatments. It is also recommended to indicate the exact temperature and time storage conditions.

Several sensory methods are available on literature (Ouhayoun and Dalle Zotte, 1996), most of them are officially accepted and their use is dependent on the aim of the experiment.

#### 3.5. Meat to bone ratio

Meat to bone ratio is a parameter of meat quality in small animals such as rabbits, because of its commercialisation with bones. According to Varewyck and Bouquet (1982) and Lukefahr *et al.* (1983), the meat to bone ratio of the hindleg is a good predictor of the meat to bone ratio of the carcass. In case of an accurately dissected hindleg, the prediction has an  $R^2 = 0.69$  (Hernández *et al.*, 1996). The dissected hindleg meat, that includes muscular, connective and adipose tissues, is a good representative of edible meat from the whole rabbit, and it may be used for chemical analysis.

#### 3.6. Meat sampling

After dissection, the meat should be stored at  $-20^{\circ}$ C until analysis. If different storage conditions are used, these should be specified. Storage time for samples belonging to different experimental treatments should not differ, due to the possible effects of this parameter on chemical composition and lipid oxidation. If other chemical composition characters need to be investigated (*e.g.*, fatty acid composition, cholesterol content, TBARS test, Total Volatile Nitrogen, and Myofibrillar Fragmentation Index), *it is recommended* to ground the whole portion just after dissection, and divide it in sub-samples according to the number of analyses planned.

#### 3.7. Chemical composition

For chemical analysis, meat samples could be used fresh or freeze-dried. In the former case, meat must be thawed (overnight at  $+4^{\circ}$ C) prior to chemical analysis.

#### a) Total lipids, protein, moisture and ash content

Official methods of analysis are available in the literature (AOAC, 1984) and specific references are reported in the previous paper (Ouhayoun and Dalle Zotte, 1996).

#### b) Fatty Acid (FA) composition

The FA determination can be performed on dissectable fat or on meat samples. The FA composition can be determined on fresh or freeze-dried samples (either raw or previously cooked). Because of the sample processing influence on the FA profile of the meat, *it is* strongly *recommended* to indicate the sample processing method used. The FA profile can be expressed in % of total FA or in mg/100g meat, using C17:0 or C19:0 as internal standard. Official methods of analysis are available in the literature (AOAC, 1984) and specific references are reported in the previous paper (Ouhayoun and Dalle Zotte, 1996).

#### c) Cholesterol content

The cholesterol content can be determined both on fresh (colorimetric method; Boehringer Mannheim Biochemia, 1995) and on freeze-dried meat (HPLC; Casiraghi *et al.*, 1994).

#### d) Lipid oxidation (TBA RS test)

High levels of unsaturated FA in meat lipids, such as in rabbit meat, may cause a reduction in their oxidative stability, decreasing the nutritive value and sensory quality of the meat and generating toxic lipid oxidation products. Erroneous meat sample collection and storage procedures can strongly affect the oxidative stability of lipids. For this reason, *it is recommended* to avoid heat, light and long storage, whenever these variables are not taken into account by the experimental protocol.

The thiobarbituric acid method (TBA RS), with its different variations (Tarladgis *et al.*, 1960; Sunderman *et al.*, 1995; Bergamo *et al.*, 1998), is the most widely used test for measuring the extent of lipid peroxidation in meat foods. As the TBA RS values are different in raw and in cooked meat, *it is recommended* to indicate if the test is performed on raw or on processed meat.

#### e) Miofibrillar Fragmentation Index (MFI)

The MFI method estimates the intensity of meat tenderization and it is performed on meat samples at different *post-mortem* times (Olsson and Tornberg, 1992). The analysis can be made on fresh meat or alternatively on frozen meat, provided that at the start of freezing the *post-mortem* time is recorded. In the latter case, storage time of samples belonging to different experimental treatments should not differ.

#### f) Total Volatile Nitrogen (TVN)

The TVN method detects the levels of ammonia compounds and volatile amine (Gazzetta Ufficiale delle Comunità Europee, 1995). The TVN concentration in meat depends on the level of protein degradation. Based on the aim of the experiment, the meat sample can be raw, fresh, refrigerated, or frozen.

Acknowledgements: We are grateful to Prof. L. Maertens and Prof. S. Lukefahr by its useful comments.

#### REFERENCES

- AOAC. 1984. Official Methods of Analysis (14<sup>th</sup> Ed.). Association of Official Analytical Chemists, Arlington, VA.
- Bergamo P., Fedele E., Balestrieri M., Abrescia P., Ferrara L. 1998. Measurement of malondialdehyde levels in food by high-performance liquid chromatography with fluorometric detection. *J. Agric. Food Chem.* **46**, 2171-2176.
- Blasco A., Ouhayoun J., Masoero G. 1992. Status of rabbit meat and carcass: Criteria and terminology. *Options Mediterranéennes* **17**, 105-120.
- Blasco A., Ouhayoun J., 1996. Harmonisation of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Sci.* **4**(2), 93-99.
- Boehringer Mannheim Biochemia, 1995. Methods of Enzymatic BioAnalysis and Food Analysis using Test-Combinations. TC cholesterol (139050). *Boehringer Mannheim GmbH*, *Mannheim*, *Germany*.
- Casiraghi E., Lucisano M., Pompei C., Dellea C. 1994. Cholesterol determination in butter by high performance chromatography. *Milchwissenschaft* **49**(**4**), 194-196.
- Gazzetta Ufficiale delle Comunità Europee 1995. Determinazione della concentrazione di azoto basico volatile totale (ABVT) nei pesci e nei prodotti della pesca Metodica di riferimento. 95/149/CE. Allegato II, N.L. 97/85, 97/86, 97/87.
- Grau R., Hamm R. 1957. Uber das wasserbindungs vermogen des sagetiermuskels. II. Uber mitt. die bestimmung der wasserbindung des muskles. Z. Lebensem.-Unters. –Forsch. 105, 446.
- Hernández P., Pla M., Blasco A. 1996. Prediction of carcass composition in the rabbit. *Meat Sci.* **44(1-2)**, 75-83.
- Honikel K.O., 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* **49**(**4**), 447-457.
- Lukefahr S., Hohenboken W.D., Cheeke P.R., Patton N.M. 1983. Appraisal of nine genetic groups of rabbits for carcass and lean yield traits. *J. Anim. Sci.* **57**(**4**), 899-907.
- Olsson U., Tornberg E. 1992. The relationship between myofibril fragmentation and tenderness for beef meat. *Proc.* 38<sup>th</sup> ICoMST., Clermont-Fd, France, 399-402.

Ouhayoun J., Dalle Zotte A. 1996. Harmonisation of muscle and meat criteria in rabbit meat research. *World Rabbit Sci.* 4(4), 211-218.

- Sunderman F.W., Marzouk A., Hopfer S.M., Zaharia O., Reid M.C. 1995. Increased lipid peroxidation in tissues of nickel chloride-treated rats. *Annals of Clinical and Laboratory Science*, 15(3), 229-236.
- Tarladgis B.G., Watts B.M., Younathan M.T 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chems. Soc., **37**(1), 44-48.
- Varewyck H., Bouquet Y. 1982. Relations entre la composition tissulaire de la carcasse de lapins de boucherie et celle des principaux morceaux. *Ann. Zootech.* **31(3)**, 257-268.