



# Contribution of lacunae morpho-metric analysis in animal by-products characterization #



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## Introduction

- To enforce Regulation 2002/1774/EC, reliable analytical methods that identify the species of provenance of animal material are necessary;
- The official microscopic method for determining PAP has its strengths and limitations;
- Further development of the official reference method is therefore required.

## Aim

The aim of this study was evaluate the potential of image analysis measurements in combination with the official analytical method for the detection of constituents of animal origin in feedstuffs in distinguishing between land animals (i.e. poultry vs. mammals).

## Materials and methods

Reference samples containing poultry (**AV**) or mammalian (**MAM**) meat and bone meals (Agricultural Research Centre of Gembloux, Belgium, and SAFEED-PAP Project; VSA, University of Milan) were analysed. Sediment fractions of each samples were observed with a compound microscope (Olympus BX41, Germany) at several magnifications. Through a digital camera and an image analysis software (Image-for Plus 4.5.1, Media Cybernetics Inc., Silver Springs, USA), we obtained 823 bone fragment lacunae images at X40. Images have been processed and elaborated in order to obtain for each lacuna a monochrome mask on which several measurements were performed. On each lacuna 26 geometric variables were obtained. Data were analyzed by ANOVA (GLM procedure) and by PROC BOXPLOT procedure of SAS statistic software. In order to show the variability of the most discriminant variables we have performed graphic test (box-plot) for mean and median comparisons.

## Results

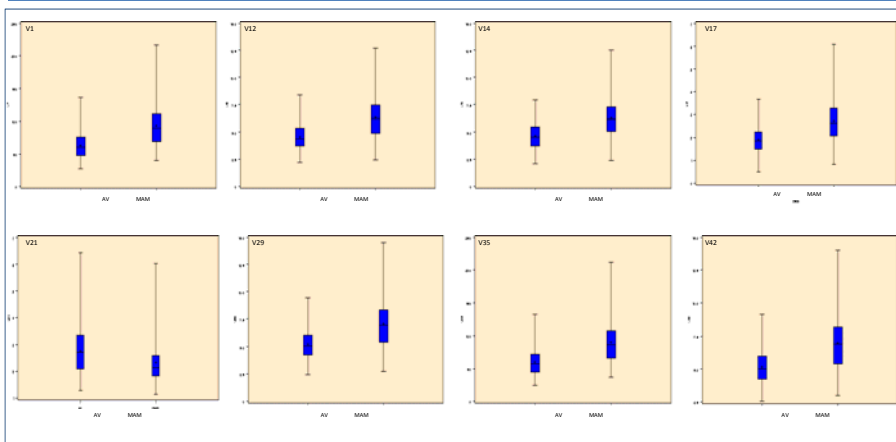
Overall mean of most discriminant lacunae variables

Variables/descriptors	Poultry	Mammalian	SEM
v1- Area, $\mu^2$	63.2	93.6	1.51
v12- Axis minor, $\mu$	4.58	6.33	0.08
v14- Diameter min, $\mu$	4.65	6.29	0.08
v17- Radius min, $\mu$	1.90	2.70	0.04
v21- Roundness	2.77	2.33	0.04
v29- Size width, $\mu$	5.22	7.06	0.09
v35- Area polygon, $\mu^2$	60.1	90.2	1.49
v42- Feret min, $\mu$	5.18	6.98	0.09

• Results obtained in the present study indicated that of 26 variables measured on lacunae, 23 were significantly ( $P < 0.001$ ) different between mammalian and poultry in term of overall mean.

• Graphic test (box-plot) for mean and median comparisons indicated that the most discriminant variables were:

- v1- Area
- v12- Axis minor
- v14- Diameter min
- v17- Radius min
- v21- Roundness
- v29- Size width
- v35- Area polygon
- v42- Feret min



## Conclusions

Data here presented indicate that some of the variables/descriptors provided by image analysis related to lacunae dimension and features appear promising for a reliable distinction between animal meal at the level of vertebrate classes, while for further characterisation, at higher taxonomic level, contribution of morphological variables deserve further investigation.

Keywords: meat and bone meal, bone lacunae, microscopic method, image analysis

