Lacunae area/fragment area ratio as a marker in distinguishing between terrestrial animal *vs.* sea mammals*

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Introduction

- The use of microscopic methods in association with computer image analysis to identify the origin of animal materials in animal meals has been proposed.
- Image processing, integrated with morphometric measurements, can provide accurate and reliable results and can be a very useful aid to the analyst in the characterization, analysis and control of feedstuffs.

Objective

Aim of this study was to evaluate the potential of image analysis measurements in combination with the microscopic examination technique for the detection of constituents of animal origin in feedstuffs in distinguishing between land mammals *vs*. sea mammals.

Materials & Method

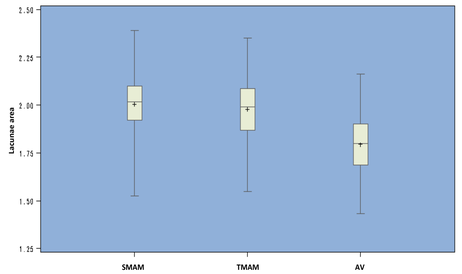
- For this purpose, pure samples containing poultry (AV) terrestrial mammalian (TMAM) and sea mammals (SMAM) material (Sources: Walloon Agricultural Research Centre, Belgium,; VSA, University of Milan and SAFEED-PAP Project) were analysed.
- Sediment fractions of each sample were observed with a compound 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 772 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 30 geometric variables plus the lacunae area/fragment area ratio were obtained.
- Data were analysed by ANOVA (GLM procedure) and by LDA procedure of SAS statistic software.

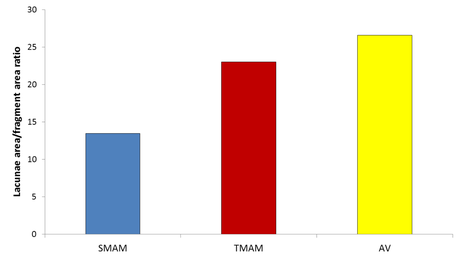
Results

- Results obtained in the present study indicated that even though most of variables measured were significantly (P<0.001) different between TMAM and SMAM vs. poultry in term of mean, no differences between TMAM and SMAM have been detected (lacunae area only shown).
- However, when lacunae area/fragment area ratio was considered some differences have been observed. SMAM material have shown the lowest lacunae area/fragment area ratio (13.47) compared to the TMAM and AV ones (23.03 and 26.58, respectively).
- In both, measured variables and lacunae area/fragment area ratio, porpoise was the main source of misclassification.

Conclusions

- Data here presented indicate that some of the variables/descriptors provided by image analysis related to lacunae dimensions and features have some potential in distinguishing poultry material from mammal's material, but not in classifying AV and TMAM from SMAM.
- By contrast, lacunae area/fragment area ratio appears promising for distinguishing between terrestrial animal vs. sea mammals.





Lacunae area box plots (on the left) and variables and lacunae area/fragment area ratio (on the right) in the different animal class.

Results of LDA for different varibles

Variables/descriptors	% correct classification AV vs MAM	% correct SMAM vs TMAM	Variables/descriptors	% correct classification AV vs MAM	% correct SMAM vs TMAM
Area	70	29	**Size (width)**	71	71
Aspect	66	43	**Perimeter2**	59	71
Area/Box	59	71	**Perimeter convex**	60	71
Box X/Y	54	57	**Perimeter ellipse**	62	43
Axis major	55	29	**Perimeter ratio**	56	71
Axis minor	72	57	**Area Polygon**	70	29
Diameter max	54	43	**Box Width**	54	43
Diameter min	72	57	**Box Height**	56	57
Diameter mean	70	71	**Feret (min)**	71	71
Radius max	53	43	**Feret (max)**	53	43
Radius min	70	43	**Feret (mean)**	60	57
Perimeter	60	71	**Form factor**	60	71
Radius Ratio	67	57	**Roundness**	64	71
Roundness	63	71	**Convex area**	70	43
Size (length)	53	43	**Solidity**	64	86

Acknowledgment / Contacts

*This study has been developed in the frame of the Cost Action FA0802 Feed for Health (www.feedforhealth.org).

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