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https://dx.doi.org/10.4314/nvj.v42i4.3 **ORIGINAL ARTICLE**

Effects of Four Different Methods of Skeletal Processing on The Guinea Fowl (*Numida Meleagridis*).

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SUMMARY

The process of skeletal processing which involves soft tissues removal, bone cleaning, articulation and labelling is a fundamental step in achieving gross anatomical and archeological studies in museum display of skeletal specimens. It also helps to further highlight the functional anatomy of bones. Several methods of bone preparation have been practiced so as to achieve desired quality bone specimens in the shortest possible time with limited resources. To this end, this study was carried out on 16 (8 males and 8 females) helmeted guinea fowl (*Numida meleagridis*) a representative of the avian species using four different bone preparation methods (Burial, cold maceration, chemical and insect larvae) at 31^{o}c to determine the most suitable in this species. Dissection to remove feathers, skin and internal organs was performed prior to each method. Burial in soil took 14 days for complete bone recovery, turned the bones uniformly light brown while producing an indelible putrefying smell with no evidence of cracks on the bones. Cold maceration also took 14 days for complete bone recovery, however, the bones turned whitish, producing a strong putrid smell with no cracks on the bones observed. Chemical method using 3 concentrations of sodium hydroxide (NaOH) (2%, 3% and 5%) took approximately 10 hours, 8 hours and 4 hours respectively for complete cream coloured bone recovery with no odour but cracks were conspicuous on the bones with increasing concentration of sodium hydroxide. Use of insect larvae took approximately 4 months to produce non-uniform brown-coloured bones articulated via the ligaments having an unpleasant odour with no cracks. Considering the pros and cons of the effects associated with each method, this study concludes that the use of insect larvae was most suitable for a non-urgent bone recovery while the use of NaOH at 3% concentration was suitable for urgent bone recovery of the helmeted guinea fowl.

Keywords: Burial, Helmeted guinea fowl, Insect larvae, Maceration, Sodium Hydroxide.

INTRODUCTION:

The Guinea fowl (*Numidia meleagridis*) sometimes called the pet speckled hen is a member of the family Numididae and order Galliformes (Wanmi *et al.,* 2018). They are indigenous to Africa but has been introduced to various countries around the world and are among the oldest gallinaceous birds (Abdul-Rahman *et al.,* 2019). Guinea fowls are native to grasslands and woodlands in Africa and occupy all habitats except dense forest and treeless deserts (Moreki and Radikara, 2013). There are over 50 million semi-domesticated guinea fowls in Nigeria which constitute about 25 percent of the entire population of domestic poultry (Ikani and Dafwang, 2014). Its production has become commercially viable in places like Europe and America (Issaka and Yeboah, 2016).

Bony structures of the body also known as the skeleton support, protect and forecast the body structure of an organism (Frandson *et al*., 2010). It can be defined as a hard framework of the body which supports soft structures (Abdulrahman and Yusuf 2021) and is responsible for the maintenance of the shape, flexibility as well as locomotion of the body (Hall, 2015). Gross anatomical, archeological studies and museum display of skeletal specimens can only be possible by adequate processing of skeleton which involves soft tissues removal, bone cleaning, articulation of bones and labelling (Boyle, 2010). Skeletons must be properly prepared, so that quality specimens can be obtained, avoiding damaged or modified bones which may impair or influence the morphological analysis of the original features and the resulting taxonomic identification (Brito de Oliveira 2018). Various known methods have been used in achieving this in large and small animals (Ajayi *et al*., 2016).

Despite the number of research into the Guinea fowl such as the cloacal bursa (Onyeanusi *et al*, 1993) respiratory system (Ibe *et al.,* 2008) and Os coxae (Lavanya *et al.,* 2017), to mention a few; information on the effects of various methods of skeletal processing has not been documented. Therefore, this study was aimed at comparing the suitability of skeletal processing methods such as Maceration, Burial, Chemical (NaOH) and Larvae on the guinea fowl.

MATERIALS AND METHODS:

Study design

Sixteen (16) Guinea fowl (*Numidia meleagridis*) 8 females and 8 males with an average weight of 3.0 kg were purchased in Ilorin, Kwara State, Nigeria and housed in the Department of Anatomy Laboratory, Faculty of Veterinary Medicine, University of Ilorin, Nigeria being fed with grains and watered *ad libitum* prior to commencement of study. The birds were divided into 4 groups containing

each sex for each method of bone preparation. Euthanasia was achieved by severing the jugular vein. Four different methods were used to clean the bones namely: Cold water maceration, Burial, Chemical (sodium hydroxide) and insect larvae. The birds were dissected using a surgical blade to remove feathers, skin, thoracic, abdominal and pelvic contents while the muscles were teased away leaving the bones with minimal soft tissue attachments.

Cold maceration

The birds were put into different plastics buckets containing water enough to submerge the bones. The plastic buckets were then covered air tight and placed under the sun for days after which the water was drained and the bones recovered and dried. The number of days it took for complete maceration was noted and recorded.

Burial

Each defleshed carcass was wrapped in a sack and buried for days, after which the bones were dug out, washed and sundried. The number of days it took for complete bone recovery was noted.

Chemical (Sodium Hydroxide, NaOH)

Solutions of Sodium hydroxide with concentrations of 2%, 3% and 5% were prepared in different containers. The defleshed carcasses were then submerged in them and placed under sunlight for some minutes while checked to avoid over digestion. The bones were then washed with running water and dried at room temperature.

Insect Larvae

The defleshed carcass were placed in a bowl (after being sprinkled with water) and allowed to stay outdoors for 3 days to allow for house flies to lay their eggs. They were then kept indoors at room temperature for days to allow for larva appearance, growth and feeding on the soft tissues.

Photographs of the bones recovered from the various methods were taken as a whole noting the various effects.

Ethical approval:

Ethical approval was obtained from the ethics committee of the University of Ilorin, Nigeria.

RESULTS AND DISCUSSION

The male and female guinea fowls (*Numidia meleagridis*) used in this study had mean weights of 3kg and 2.5kg, respectively. The four methods of bone preparation used in this study showed significant pros and cons which determined the most appropriate for use in the guinea fowl as shown in Table I.

TABLE I: Comparison of results from the four methods of bone preparation employed

Parameters	**Maceration**	**Burial**	**Sodium hydroxide**			**Larvae**
			2%	3%	5%	
Time	7 days	14 days	9 hours	6 hours	3 hours	60 days
Colour change	white	Light brown	Cream	Cream	Cream	Brown
Odour	Strong putrid	Very strong putrid		Pleasant		Slightly unpleasant
Damaging effect	No effect	No effect	Cracks	More cracks	More cracks	No effect
Percentage of bone recovery						
Bone	Maceration	Burial	(2%) NaOH	(3%) NaOH	(5%) NaOH	Larvae
Skull	100	100	100	100	100	100
Cervical vertebrae	100	100	100	100	100	100
Costal bones	90	90	90	90	90	100
Thoracic vertebrae	100	100	100	100	100	100
Synsacrum	100	100	100	100	100	100
Caudal Vertebrae	100	100	100	100	100	100
Fore limb	100	100	100	100	100	100
Hind limb	100	100	100	100	100	100

There were different effects on the bones such as colour change, odour, time taken for complete bone recovery, and other relative changes shown by the four methods (Burial, Maceration, Sodium hydroxide, and larvae) of bone preparation used in this study.

Burial method took 14 days for complete bone recovery, turned the bones uniformly light brown while producing an indelible putrefying smell with no evidence of cracks on the bones. The number of days taken for bone recovery was similar to reports on the African giant rat by Onwuama *et al*., (2012) which also had similar weight as the guineafowl. We may safely deduce from this result that small animals with weights between 2-3kg may take 14 days at 31ºc for total bone recovery using this method. The indelible smell perceived from the bones could be as a result of micro-organism activity in combination with the soil's properties. This attribute together with the light brownish colouration of the bones is a definite disadvantage of this method as it persisted despite proper washing and drying. Its advantage lies in the fact that the bones remained intact with no evidence of cracks. Maceration (at 31℃) also took 14 days for complete bone recovery, however, the bones retained its whitish colouration, producing a strong putrid smell with no cracks on the bones observed. This method was similar to the burial method except that it produced whitish bones which is obviously an added advantage.

Chemical method using graded sodium hydroxide (NaOH) (2%, 3% and 5%) at 31℃ via a thermometer took approximately 10hours, 8hours and 4hours respectively for complete bone recovery. The bones appeared cream coloured with no odour but cracks were conspicuous on the bones with increasing concentration of the chemical. Sodium hydroxide (NaOH) produced a faster skeleton extraction at reducing time as concentration increased with no offensive odour. This was mainly due to the digesting ability of the chemical (NaOH) to disintegrate the muscles and ligaments converting them from solid to liquid (Shannon *et al*., 2007). Due to its alkalinity, NaOH in aqueous solution causes bond breakage in protein (Tugay *et al*., 2003) which is an essential part of muscles. It must be noted that higher temperature (due to sunlight exposure) played an important role in this process as a lower temperature may not achieve faster bone extraction even with an increased concentration of the chemical. Despite this seemingly time saving advantage, it was relatively expensive than the other methods used in this study and smaller semi-cartilaginous bones like the uncinate processes of the thorax were not recovered. Higher concentrations of NaOH extracted faster but digested smaller bones into bone halls thereby impacting a brownish colouration. This method is preferrable for very urgent bone extraction. Its effect produced in the guinea fowl was similar in the African giant rat (Onwuama *et al*., 2012).

Larvae method took approximately 4 months for complete bone recovery. It presented non-uniform brown-coloured bones articulated via the ligaments, produced an unpleasant odour but cracks were not observed. One of the greatest advantages of this method is its ability to leave the bones in its intact form articulating with each other since the larvae doesn't feed on the ligaments. Bones of the skull still maintained their positions making it easy to identify and study them. The uncinate processes of the ribs were visibly intact. Considering the relatively low cost, the ease of obtaining the larvae and ability to retain articulations of the recovered bones, this method readily becomes a viable alternative one. Its long cleaning period as well as the unpleasant odour of the recovered bones however remained a disadvantage. The unpleasant odour of this method was still not as pungent as that of burial and cold maceration.

The success of any bone preparation technique is determined by the time taken, the resources required, and the results obtained in relation to the intended purpose for which the preparation is required (Aggarwal *et al*., 2016). Taking this into consideration and based on the effects outlined above, the larvae method may be the most suitable for guinea fowl for a non-urgent procedure of bone cleaning while the chemical method of bone preparation using low concentration of Sodium hydroxide may be a suitable method for urgent bone cleaning of helmeted guinea fowl.

Plate 1 Helmeted Guinea fowl bones showing Uniform light brown colouration resulting from Burial method of bone preparation

Plate 2 Helmeted Guinea fowl bones showing whitish colouration resulting from cold water maceration

Plate3 Helmeted Guinea fowl bones showing Cream colouration resulting from NaOH(2%) method of preparation

Plate 4 Helmeted Guinea fowl bones showing cream colouration resulting from NaOH(3%) method of preparation

Plate 5a

Plate 5b

Helmeted Guinea fowl bones showing non-uniform brown colouration resulting from larvae method of bone preparation

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