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REVIEW ARTICLE

A SYSTEMATIC REVIEW OF EFFECT OF CALVING SEASON AND PARITY AS NON-GENETIC FACTORS ON MILK PRODUCTION TRAITS OF CATTLE

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ABSTRACT

This study was carried out to systematically review the effect of calving season and parity on milk production traits. This systematic review was conducted in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Google Scholar, PubMed, ScienceDirect, and Web of Science databases were searched systematically using 'non-genetic factors/season of calving /parity', 'milk production traits', and 'cattle' as keywords. The results indicated that fifteen (n = 15) articles were used from the ninety-four (n = 94) articles identified and screened. The results of this systematic review indicated that 8 out of 10 articles found milk yield to be significantly affected by parity, whereas 7 out of 13 articles indicated that calving season did not significantly affect milk yield. Out of 4 articles, 3 of them showed that parity had a significant effect on fat. Parity had a significant effect on lactose and protein, 1 of these articles investigated and found significant effect of parity and calving season on somatic cell score (SCS). Out of 13 articles, 5, 4, and 2 of these articles investigated fat, protein, and lactose, respectively. The results showed that 3 articles on fat, 4 on protein and 2 on lactose were significantly affected by calving season. In conclusion, parity and calving season had a great influence on fat, protein, lactose, and SCS. Milk yield was not affected by calving season, however, it was affected by parity. Thus, calving season and parity can be used to improve milk production traits.

Keywords: Fat, milk yield, protein, lactose, SCS

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INTRODUCTION

Non-genetic factors are those effects that are not part of the genetic make-up of an animal (Nyamushamba et al., 2014). Hussain et al. (2015) stated that evaluation of the influence of non-genetic factors on milk production traits was important for formulating breeding improvement programme for dairy cattle (Hussain et al., 2015). Milk consumption is very high per person per year worldwide, and the consumption is bound to go up due to increased urbanization and wages (Hoka et al., 2019). Thus, factors that affect milk production must be identified and addressed if milk production is to keep pace with the increasing demand. However, studies conducted on the effect of calving season and parity draw different conclusions. According to Bolacali and Öztürk (2018), non-genetic factors such as parity and calving season affect the cattle milk production traits, while Fouda et al. (2017) stated that non-genetic factors such as parity and calving season did not affect the cattle milk production traits. Based on our knowledge, there is no systematic review on the effect of calving season and parity on milk production traits of cattle. The objective of this study was to systematically review the articles on the effect of calving season and parity on milk production traits of cattle and to provide information on the influence that calving season and parity have on milk production traits of cattle. Hence, the current systematic review is needed to combine their results as to give a summarized conclusion for the possibility of these non-genetic factors to be used in selection for improvement of milk production traits. This systematic review will help researchers and dairy cattle farmers to know the milk production traits of dairy cattle that are influenced by parity and calving season.

MATERIAL AND METHODS

Eligibility criteria

It is necessary to identify the Population, Exposure, and Outcomes (PEO) components of the research question to undertake a systematic review, as explained by Bettany-Saltikov (2010). The population was defined as "Cattle", with an exposure of "non-genetic factors" and outcomes of "Milk production traits". A preliminary search of the PEO components on PubMed, Google Scholar, Web of Science, and ScienceDirect databases was conducted before deciding to carry out the study.

Search strategy for identification of relevant studies

The results of this systematic review were presented according to the recommendations proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009). The search of published studies was independently conducted by both authors in the databases of Google Scholar, PubMed, Science Direct, and Web of Science from 10 August to 20 October 2023. The following keywords: 'non-genetic factors/season of calving/parity', 'milk production traits', and 'cattle' were used when performing publication search.

Inclusion criteria

Titles and abstracts found using the search strategy were screened manually to identify studies that were potentially relevant. Studies were considered for inclusion in this systematic review provided that they included effect of parity and/or season of calving on milk production traits of cattle.

Exclusion criteria

Articles that had the missing keywords and the ones that were talking about different species other than cattle were excluded. Duplicate studies were also excluded.

Data extraction

The study content and data were extracted independently by both authors and an agreement was reached concerning all key items. The following data were extracted from the selected studies: first author's name, year of publication, geographical location, cattle breed, sample size, studied milk production traits and non-genetic factors.

RESULTS

Searched results

Process that was followed when selecting studies to be included in this systematic review is shown in Figure 1. Publications that were identified in the search engines were ninety-four (n = 94); after screening, a total of fifteen (n = 15) studies were included in this systematic review.

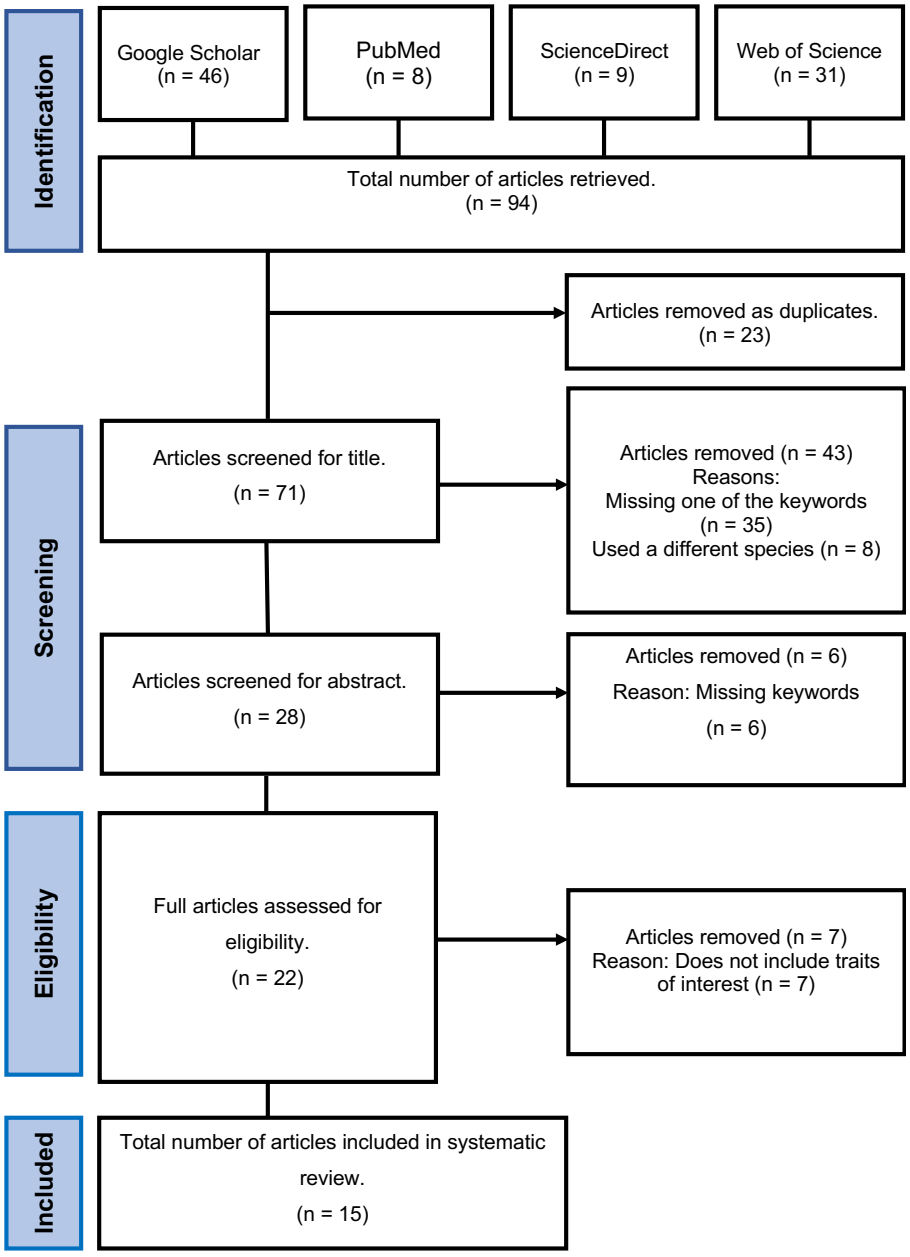

Figure 1
Flowchart of the process of study selection

Characteristics of included studies

The characteristics of the fifteen (n = 15) included articles are presented in Table 1. Out of 15 included studies, most of them used Holstein cattle breed with 26.67% (n = 4), followed by Jersey cattle breed (Mostert et al., 2001; Nyamushamba et al., 2014; Beneberu et al., 2020) with 20% (n = 3), then Sahiwal cattle breed (Verma et al., 2016; Pandey et al., 2019) with 13.33% (n = 2). All the remaining cattle breeds (Ayrshire, Naks, Deoni, Simmental, Friesian, Red Dane, HF×Deoni crossbred) were only investigated in one article (n = 1) with 6.67%. A total number of 148051 animals were used within the reviewed articles, ranging from 18 to 116073.

Table 1 Characterization of included studies

Author	Year	Country	Breed	Sample size	Milk production traits	Non-genetic factors
Amimo et al	2007	Kenya	Ayrshire	1955	Milk yield	Parity
Barsila	2019	Nepal	Naks	18	Milk yield, fat, protein, lactose	Parity, season of calving
Beneberu et al	2020	Ethiopia	Jersey	2912	Milk yield	Parity, season of calving
Bhutkar et al	2014	India	Deoni	114	Milk yield	Season of calving
Bolacali and Öztürk	2018	Turkey	Simmental	706	Milk yield	Season of calving
Boujenane	2021	Morocco	Holstein	6343	Milk yield, fat, protein, lactose, SCS	Parity, season of calving
Fouda et al	2017	Egypt	Holstein	1575	Milk yield	Parity, season of calving
Hoka et al	2019	Kenya	Friesian	20	Milk yield	Parity
Hussain et al	2015	India	Tharparkar	230	Milk yield	Season of calving
Jónás et al	2016	Turkey	Holstein	4891	Milk yield, fat, protein, lactose	Parity, season of calving
Mostert et al	2001	South Africa	Holstein, Jersey	116073	Milk yield, fat, protein	Season of calving
Nyamushamba et al	2014	Zimbabwe	Red Dane, Jersey	12307	Milk yield	Parity, season of calving
Pandey et al	2019	India	Sahiwal	392	Milk yield	Season of calving
Verma et al	2016	India	Sahiwal	259	Milk yield, fat	Parity, season of calving
Wondifraw et al	2013	India	HF×Deoni crossbred	256	Milk yield	Parity, season of calving

SCS – Somatic cell score, HF – Holstein Friesian

Publication by country

The countries of origin for included studies are shown in Figure 2. The findings of this systematic review indicated that the included studies were published from 9 different countries worldwide. India was ranking first with 5 articles out of 15 included articles (Wondifraw et al., 2013; Bhutkar et al., 2014; Hussain et al., 2015; Verma et al., 2016; Pandey et al., 2019), followed by Kenya (Amimo et al., 2007; Hoka et al., 2019) and Turkey (Jónás et al., 2016; Bolacali and **Öztürk**, 2018) with 2 articles each. The remaining 6 countries (Egypt, Ethiopia, Morocco, Nepal, South Africa, Zimbabwe) had 1 article each out of 15 included articles.

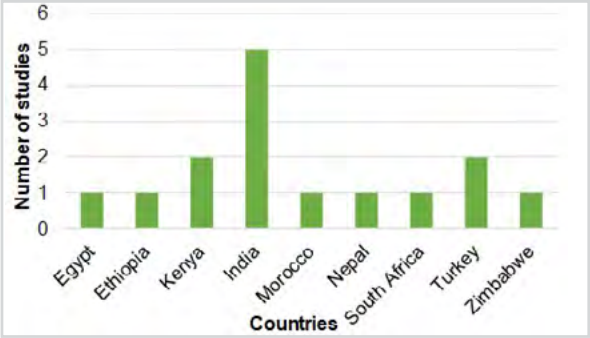

Figure 2 Publication by country

Publication by year

Figure 3 below displays the publication by year of included studies (n = 15) in the current systematic review. The results showed that the studies included were published from 2001 to 2021. Most of the studies were published in 2019 with 3 out of 15 included articles (Barsila, 2019; Hoka et al., 2019; Pandey et al., 2019), followed by 2014 (Bhutkar et al., 2014; Nyamushamba et al., 2014) and 2016 (Jónás et al., 2016; Verma et al., 2016) that had 2 published studies each out of 15 included articles. The other 8 different years (2001, 2007, 2013, 2015, 2017, 2018, 2020, 2021) had 1 publication each out of 15.

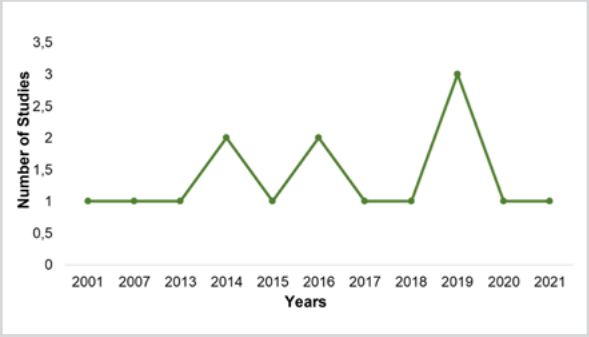

Figure 3 Publication by year

Investigated parities

Table 2 indicates the parities that were investigated in the included articles. Out of 15 selected studies, about 10 studies investigated the effect of parity on milk production traits. These studies all used parity number 2, followed by 3 different parities (1, 3, 4) that were investigated by 9 studies out of 10 each; then 2 parities (5, 6) were used by 6 articles out of 10 each. The results also indicated that parity 7 was looked into by 3 studies out of 10, and parities from 8 to 10 were all investigated by 1 article out of 10 articles.

Table 2 Investigated parities

Author	Breed	N	Parity
Amimo et al. (2007)	Ayrshire	1955	1-7
Barsila (2019)	Naks	18	2,4,6
Beneberu et al. (2020)	Jersey	2912	1-6
Boujenane (2021)	Holstein	6343	1-3
Fouda et al. (2017)	Holstein	1575	1-4
Hoka et al. (2019)	Friesian	20	1-4
Jónás et al. (2016)	Holstein	4891	1-5
Nyamushamba et al. (2014)	Red Dane, Jersey	12307	1-7
Verma et al. (2016)	Sahiwal	259	1-6
Wondifraw et al. (2013)	HF×Deoni crossbred	256	1-10

Investigated calving seasons

The investigated calving seasons in the studies included in this systematic review are presented on Table 3. Out of the 15 reviewed articles, 13 of them explored the calving season as one of their non-genetic factors. All the 13 studies researched about summer as one of the calving seasons, followed by 12 studies out of 13 that included winter in their studies, then autumn that was investigated by 11 articles out of 13; lastly, 9 out of 13 studies looked into spring as one of the calving seasons.

Table 3 Investigated calving seasons

Author	Breed	N	Calving season
Barsila (2019)	Naks	18	Summer, Autumn
Beneberu et al. (2020)	Jersey	2912	Summer, Autumn, Winter
Bhutkar et al. (2014)	Deoni	114	Summer, Autumn, Winter, Spring
Bolacali and Öztürk (2018)	Simmental	706	Summer, Autumn, Winter, Spring
Boujenane (2021)	Holstein	6343	Summer, Winter
Fouda et al. (2017)	Holstein	1575	Summer, Winter
Hussain et al. (2015)	Tharparkar	230	Summer, Autumn, Winter, Spring
Jónás et al. (2016)	Holstein	4891	Summer, Autumn, Winter, Spring
Mostert et al. (2001)	Holstein, Jersey	116073	Summer, Autumn, Winter, Spring
Nyamushamba et al. (2014)	Red Dane, Jersey	12307	Summer, Autumn, Winter, Spring
Pandey et al. (2019)	Sahiwal	392	Summer, Autumn, Winter, Spring
Verma et al. (2016)	Sahiwal	259	Summer, Autumn, Winter, Spring
Wondifraw et al. (2013)	HF×Deoni crossbred	256	Summer, Autumn, Winter, Spring

Distribution of articles by non-genetic factor

The distribution of the studies included in this systematic review by non-genetic factors is displayed in Table 4 below. Only 53.3% (n = 8) studies investigated both parity and calving season, whereas 13.33% (n = 2) focused only on parity (Amimo et al., 2007; Hoka et al., 2019) and 33.33% (n = 5) only focused on calving season (Mostert et al., 2001; Bhutkar et al., 2014; Hussain et al., 2015; Bolacali and Öztürk., 2018; Pandey et al., 2019).

Table 4 Distribution of articles by non-genetic factor

Author	Breed	Sample size	Non-genetic factors
Amimo et al. (2007)	Ayrshire	1955	Parity
Barsila (2019)	Naks	18	Parity, season of calving
Beneberu et al. (2020)	Jersey	2912	Parity, season of calving
Bhutkar et al. (2014)	Deoni	114	Season of calving
Bolacali and Öztürk (2018)	Simmental	706	Season of calving
Boujenane (2021)	Holstein	6343	Parity, season of calving
Fouda et al. (2017)	Holstein	1575	Parity, season of calving
Hoka et al. (2019)	Friesian	20	Parity
Hussain et al. (2015)	Tharparkar	230	Season of calving
Jónás et al. (2016)	Holstein	4891	Parity, season of calving
Mostert et al. (2001)	Holstein, Jersey	116073	Season of calving
Nyamushamba et al. (2014)	Red Dane, Jersey	12307	Parity, season of calving
Pandey et al. (2019)	Sahiwal	392	Season of calving
Verma et al. (2016)	Sahiwal	259	Parity, season of calving
Wondifraw et al. (2013)	HF×Deoni crossbred	256	Parity, season of calving

Distribution of articles by milk production traits

Table 5 highlighted the distribution of the included studies by milk production traits. This systematic review only took into consideration 5 different milk production traits, namely: milk yield, fat, protein, lactose, and SCS. All the studies (100%) included in this systematic review investigated milk yield. Out of 15 reviewed studies, only 33.33% (n = 5) of them studied fat, followed by 26.67% (n = 4) that investigated protein (Mostert et al., 2001; Jónás et al., 2016; Barsila., 2019; Boujenane., 2021), with 20% (n = 3) of them studying lactose (Jónás et al., 2016; Barsila., 2019; Boujenane., 2021) and 6.67% (n = 1) SCS (Boujenane., 2021).

Table 5 Distribution of articles by milk production traits

Author	Breed	Sample size	Milk production traits
Amimo et al. (2007)	Ayrshire	1955	Milk yield
Barsila (2019)	Naks	18	Milk yield, fat, protein, lactose
Beneberu et al. (2020)	Jersey	2912	Milk yield
Bhutkar et al. (2014)	Deoni	114	Milk yield
Bolacali and **Öztürk** (2018)	Simmental	706	Milk yield
Boujenane. (2021)	Holstein	6343	Milk yield, fat, protein, lactose, SCS
Fouda et al. (2017)	Holstein	1575	Milk yield
Hoka et al. (2019)	Friesian	20	Milk yield
Hussain et al. (2015)	Tharparkar	230	Milk yield
Jónás et al. (2016)	Holstein	4891	Milk yield, fat, protein, lactose
Mostert et al. (2001)	Holstein, Jersey	116073	Milk yield, fat, protein
Nyamushamba et al. (2014)	Red Dane, Jersey	12307	Milk yield
Pandey et al. (2019)	Sahiwal	392	Milk yield
Verma et al. (2016)	Sahiwal	259	Milk yield, fat
Wondifraw et al. (2013)	HF×Deoni crossbred	256	Milk yield

SCS: Somatic count score.

Effect of parity on milk production traits

Effect of parity on milk production traits findings of the selected studies are shown in Table 4. Out of 15 reviewed articles, 10 of them studied the effect of parity on milk production traits. These articles investigated milk yield and the results showed that parity on 8 articles out of 10 included articles, had a significant effect on milk yield, whereas 2 out of 10 articles showed that parity had no significant effect on milk yield. Out of the 10 reviewed papers, only 4 of them considered fat, and the findings showed that 3 articles discovered that parity had a significant effect on fat, whereas 1 showed that parity had no significant effect on fat. The results also indicated that 3 articles showed parity had significant effect on protein (Jónás et al., 2016; Barsila., 2019; Boujenane., 2021), lactose (Jónás et al., 2016; Barsila., 2019; Boujenane., 2021), and only 1 article showed the significance on SCS (Boujenane., 2021).

Table 6 Effect of parity on milk production traits

Author	Breed	Parity	Milk production traits	Sign
Amimo et al. (2007)	Ayrshire	1-7	Milk yield	*
Barsila (2019)	Naks	2,4,6	Milk yield	*
			Fat	*
			Protein	*
			Lactose	*
Beneberu et al. (2020)	Jersey	1-6	Milk yield	*

Author	Breed	Parity	Milk production traits	Sign
Boujenane (2021)	Holstein	1-3	Milk yield	*
			Fat	*
			Protein	*
			Lactose	*
			SCS	*
Fouda et al. (2017)	Holstein	1-4	Milk yield	ns
Hoka et al. (2019)	Friesian	1-4	Milk yield	*
Jónás et al. (2016)	Holstein	1-5	Milk yield	*
			Fat	*
			Protein	*
			Lactose	*
Nyamushamba et al. (2014)	Red Dane, Jersey	1-7	Milk yield	*
Verma et al. (2016)	Sahiwal	1-6	Milk yield	ns
			Fat	ns
Wondifraw et al. (2013)	HF×Deoni cross-bred	1-10	Milk yield	*

Sign – Significant, * – Significant at $p<0.05$, ns – Non-significant

Effect of calving season on milk production traits

Table 5 indicates the findings of the effect of calving season on milk production traits of the reviewed articles. Only 13 out of 15 studies included in this systematic review indicated the effect of calving season on milk production traits of interest. The results showed that 7 out of 13 studies found that calving season had no significant effect on milk yield, whereas 6 out of 13 articles showed that calving season had significant influence on milk yield. A total of 3 out of 13 studies discovered that calving season had a significant influence on fat, whereas 2 out of 13 studies indicated that there was no significant effect. Calving season on 4 studies out of 13 showed significant effect on protein. About 2 out of 13 articles highlighted that season of calving affected lactose significantly, whereas 1 article discovered that it did not significantly affect lactose. Only 1 out of 13 articles indicated that season used for calving significantly affected SCS.

Table 7 Effect of calving season on milk production traits

Author	Breed	Calving season	Milk production traits	Sign
Barsila (2019)	Naks	Summer, Autumn	Milk yield	*
			Fat	*
			Protein	*
			Lactose	*
Beneberu et al. (2020)	Jersey	Summer, Autumn, Winter	Milk yield	ns
Bhutkar et al. (2014)	Deoni	Summer, Autumn, Winter, Spring	Milk yield	ns
Bolacali and Öztürk (2018)	Simmental	Summer, Autumn, Winter, Spring	Milk yield	ns

Author	Breed	Calving season	Milk production traits	Sign
Boujenane (2021)	Holstein	Summer, Winter	Milk yield	*
			Fat	ns
			Protein	*
			Lactose	ns
			SCS	*
Fouda et al. (2017)	Holstein	Summer, Winter	Milk yield	ns
Hussain et al. (2015)	Tharparkar	Summer, Autumn, Winter, Spring	Milk yield	ns
Jónás et al. (2016)	Holstein	Summer, Autumn, Winter, Spring	Milk yield	*
			Fat	*
			Protein	*
			Lactose	*
Mostert et al. (2001)	Holstein, Jersey	Summer, Autumn, Winter, Spring	Milk yield	*
			Fat	*
			Protein	*
Nyamushamba et al. (2014)	Red Dane, Jersey	Summer, Autumn, Winter, Spring	Milk yield	*
Pandey et al. (2019)	Sahiwal	Summer, Autumn, Winter, Spring	Milk yield	ns
Verma et al. (2016)	Sahiwal	Summer, Autumn, Winter, Spring	Milk yield	ns
			Fat	ns
Wondifraw et al. (2013)	HF×Deoni crossbred	Summer, Autumn, Winter, Spring	Milk yield	*

Sign – Significant, * – Significant at $p<0.05$, ns – Non-significant

DISCUSSION AND CONCLUSION

It is necessary to optimize the factors affecting milk production traits to enhance productivity of dairy cattle (Fouda et al., 2017). Hence, the objective of this systematic review was to provide information on the effect of parity and season of calving on milk yield, fat, protein, lactose, and SCS of cattle. Knowledge of non-genetic factors such as parity and calving season and their influence on cattle milk productive performance is important in the formulation of management and selection decisions (Beneberu et al., 2020). The results of this systematic review indicated that 10 out of 15 studies investigated the influence of parity on milk production traits, whereas 13 out of 15 included studies researched about the effect of calving season on milk production traits. Majority of the reviewed articles discovered that parity affected milk yield, fat, protein, lactose and SCS. The findings of this systematic review indicated that parity had an influence on the investigated milk production traits. Majority of the studies found that calving season had no influence on milk yield, however, it affected fat, protein, lactose, and SCS. There are no comparisons of the other systematic review findings due to this systematic review being the first one reporting on the effect of non-genetic factors (parity, calving season) on milk production traits of cattle based on the knowledge that we have. The findings of this systematic review imply that parity and calving season can be used to improve milk production traits such

as fat, protein, lactose, and SCS. Furthermore, parity can be used to improve milk yield, whereas calving season cannot be used to improve milk yield. The contribution of this systematic review to the body of knowledge is that parity and calving season can be used in the selection for increased milk production traits, reproductive efficiency, formulation of management and in making selection decisions. The limitation of this systematic review is that few (less than 34%) of the reviewed articles investigated on milk production traits such as fat, protein, lactose, and SCS and the difference between results of articles indicating the effect of calving season on milk yield was low. Hence, it is highly recommended that more studies need to be conducted on the effect of parity and season of calving on milk production traits such as milk yield, fat, protein, lactose, and SCS.

The current systematic review concludes that parity affects milk yield, fat, protein, lactose and SCS of cattle. Calving season affects fat, protein, lactose, and SCS but not milk yield of cattle. Parity and calving season might be used as potential factors for the improvement of milk production traits of cattle.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – TCM, TLT; Design – TCM, TLT; Supervision – TLT; Resources –TCM, TLT; Materials – TCM; Data Collection and Processing – TCM, TLT; Interpretation – TCM; Literature Search – TCM, TLT; Writing Manuscript – TCM; Critical Review – TLT. Both authors approved the final manuscript.

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SISTEMATSKI PREGLED EFEKATA TELIDBENE SEZONE I PARITETA KAO NEGENETSKIH FAKTORA NA KARAKTERISTIKE PROIZVODNJE MLIJEKA KOD GOVEDA

SAŽETAK

Ovo istraživanje je izvedeno sa ciljem sistematskog pregleda literature o efektu telidbene sezone i pariteta na karakteristike proizvodnje mlijeka. Ovaj sistematski pregled je obavljen prema Preferentnim izvještajnim karakteristikama za sistematski pregled i meta-analize (PRISMA). Baze podataka Google Scholar, PubMed, ScienceDirect i Web of Science su sistematski pretražene koristeći kao ključne riječi "negenetske faktore/telidbenu sezonu/paritet", "karakteristike proizvodnje mlijeka" i "goveda". Prema rezultatima, u istraživanje je uključeno 15 (n = 15) radova od identificiranih i pregledanih devedeset i četiri (n = 94). Rezultati ovog sistematskog pregleda su pokazali da je u 8 od 10 članaka dokazano da je mliječnost krava signifikantno povezana sa paritetom, dok je u 7 od 13 članaka dokazano da telidbena sezona nije signifikantno utjecala na mliječnost. 3 od 4 članka su dokazala da paritet ima signifikantan efekat na sadržaj masnoće. Paritet je signifikantno utjecao na sadržaj laktoze i proteina. U jednom od članaka je istraživan i dokazan signifikantan efekat pariteta i telidbene sezone na broj somatskih stanica (SCS). Od 13 članaka, u 5 je istraživan sadržaj masnoće, u 4 proteina, a u 2 laktoze. Rezultati 3 članka o sadržaju masti, 4 o sadržaju proteina i 2 o sadržaju laktoze su pokazali da je na iste signifikantno utjecala telidbena sezona. U zaključku, paritet i telidbena sezona imaju veliki utjecaj na sadržaj masnoće, proteina, laktoze i SCS. Mliječnost krava nije bila pod utjecajem telidbene sezone, ali je bila pod utjecajem pariteta. Na ovaj način se telidbena sezona i paritet mogu koristiti za poboljšanje karakteristika proizvodnje mlijeka.

Ključne riječi: Laktoza, masnoća, mliječnost, protein, SCS

RESEARCH ARTICLE

RADIOLOGIC INDICES OF CARDIAC MENSURATION IN THE AFRICAN GRASSCUTTER (*THRYONOMYS SWINDERIANUS*)

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ABSTRACT

This research aimed to establish reference radiographic indices for future cardio-thoracic appraisals in the African grasscutter. Twenty healthy animals consisting of equal number of juveniles and adults were used. Forty thoracic projections consisting of dorsoventral (DV), ventrodorsal (VD), right lateral (RtL) and left lateral (LeL) views were acquired. The evaluated parameters included thoracic and cardiac diameters on the DV/VD radiographs, and cardiac long and short axes on the RtL/LeL radiographs. Cardiac indices generated were cardio-thoracic ratio (CTR), vertebral heart score (VHS) and cardiac axis ratio (CAR). The reference values of CTR on VD and DV were 0.52 ± 0.02 and 0.50 ± 0.02, respectively. The difference was not significant ($P > 0.05$). The mean radiographic values of CTR for juvenile and adults on DV were 0.48 ± 0.04 and 0.52 ±.0.01, respectively. The reference values of VHS on RtL and LeL were 8.70 ± 0.23 and 8.60 ± 0.21, respectively. The difference was not significant ($P > 0.05$). The mean values of VHS for juveniles and adults on RtL were 8.20 ± 0.30 and 9.20 ± 0.12, respectively. The difference was not significant ($P > 0.05$). These indices are relevant in future diagnosis of cardiac diseases in the African grasscutter.

Keywords: African grasscutter, cardiac axis ratio, cardio-thoracic ratio, radiology, vertebral heart score

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INTRODUCTION

In veterinary diagnostic radiology, thoracic projections are usually obtained and evaluated for patients presenting cardio-thoracic manifestations. In such investigations, the heart size and other thoracic organ dimensions are determined. Previously, clinicians read thoracic radiographs based on subjective or personal opinion of structures assessed, oftentimes resulting in high error margin. The advent of measurement methods in radio-diagnostics has proved more sensitive and accurate, eliminating the high error margin associated with subjective interpretation (Ukaha et al., 2002; Hansson, 2004). These radiographic measurements generate indices that depict the size of the cardiac silhouette. These indices are usually achieved by measurements made on four key thoracic radiographic projections, namely, dorsoventral (DV), ventrodorsal (VD), right lateral (RtL), and left lateral (LeL) projections. Dennis et al. (2010) opined that increased diagnostic yield and accuracy of cardio-thoracic radiographic appraisals is achieved by the use of these four thoracic radiographic projections. Some of the radiographic indices that depict the size of cardiac silhouette include the cardio-clavicular ratio (Gardner et al., 2007), cardiac long axis-thoracic height ratio (Diniz et al., 2013), vertebral heart score (VHS) (Luciani et al., 2019), vertebral left atrial size (Puccinelli et al., 2021), cardio-thoracic ratio (CTR) (Sak and Pazvant, 2021), aortico-cardiac ratio (Ukaha et al., 2002) and cardiac axis ratio (CAR) (Ukaha et al., 2002). However, CTR, VHS and CAR are of interest in the present study.

The CTR is the ratio of the maximum diameter of the cardiac silhouette to the thoracic diameter, obtained on a VD or DV radiographic projection. It is used to determine the difference between normal and enlarged cardiac silhouette. Therefore, it is beneficial in diagnosis of heart diseases (Baisan et al., 2016). The study of Hemingway et al. (1998) showed striking evidence that CTR of healthy animals, within the range considered normal in clinical practice, can predict coronary heart disease mortality, independent of established coronary heart disease risk factors such as blood pressure. It provides an accurate measure of the left ventricular mass, which can be extrapolated to myocardial ischaemia or infarction and pericardial effusion (Baisan et al., 2016). Monitoring CTR of any animal diagnosed of cardiovascular disease is very important to the clinician, as it helps in predicting the severity and progress of the condition (Oguntoyinbo et al., 2016).

The VHS is an easy and objective radiographic method routinely employed to establish normal heart size (Buchanan, 2000; Sleeper and Buchanan, 2001) and monitor changes in heart size due to therapy or cardio-thoracic disease (Sleeper and Buchanan, 2001). It usually serves as a reference in the diagnosis of cardiomegaly (Luciani et al., 2019). It is the summation of the number of thoracic vertebral bodies scaled by the radiographic values of the short and long cardiac axes. It is obtained by placing the vernier caliper of a defined cardiac axis over the thoracic vertebrae, parallel to the vertebral canal, and counting caudally, from the cranial extremity of the body of the 4th thoracic vertebrae, to the nearest 0.1-0.5 vertebra. Unlike the CTR that can be obtained on either the VD or DV projections only, VHS can be obtained from any of the four key thoracic radiographic projections (Luciani et al., 2019; Dias et al., 2021). A strong positive correlation has been recorded between CTR and VHS in capuchin monkeys (Rocha-Neto et al., 2015) and dogs (Baisan et al., 2016). This suggests that both indices are valid methods that can be used in the discrimination between normal heart size and cardiomegaly or microcardia in these species. The CAR, an index that measures the ratio of the short axis to the long axis of the heart on a lateral view, has been established for the male and female as well as for puppy and adult Nigerian indigenous dog (Ukaha et al., 2002). It is not influenced by respiratory phases, unlike CTR, thus, must not be taken at full inspiration (Ukaha et al., 2002).

Thoracic radiography is a less expensive, less technical, and widely acceptable imaging modality for the non-invasive evaluation of thoracic organs and diagnosis of cardio-pathologies in veterinary clinical practice, when compared with a more

advanced procedure such as echocardiography. It has been widely applied in dogs, with information available for various local and exotic breeds. Unfortunately, those results have limitations and cannot be applied to other breeds of dogs or species of animals, because of conformational differences in body shapes of animals (Gulanber et al., 2005). Therefore, reference radiographic values ought to be established and published specifically for every animal. Over the years, thoracic radiography has been used to establish normal heart size and diagnose cardiac anomalies in rodents such as African hedgehogs (Black et al., 2011), agouti (Diniz et al., 2013; De Moura et al., 2015), laboratory mice (Ulum and Noviana, 2018), pet rats (Dias et al., 2021) and guinea pigs (De Silva et al., 2022). However, to the best of the authors' knowledge, this is the first time this diagnostic image modality is applied to establish cardiac indices in the African grasscutter (Thryonomys swinderianus).

Results of this study will serve as normal radiologic reference values of cardiac mensuration in the African grasscutter, necessary for the diagnosis of cardiovascular anomalies in the rodent. This is necessary as this large wild rodent, the fourth largest in the world, is currently bred and domesticated as an integral component of the mini- livestock industry in Nigeria and elsewhere (Ibe et al., 2023). Literature has already documented reports of clinical signs of heart failure which occurred sporadically in captive colonies of African grasscutters derived from in-bred male and female litter mates (Rahman et al., 2015). Consequently, the specific objectives of the present study were to establish and compare reference values of CTR in juvenile and adult African grasscutters from measured CD and TD obtained on VD and DV projections of plain radiographs; establish and compare reference values of VHS and CAR in juvenile and adult African grasscutters from measured SA and LA of the heart obtained on RtL and LeL projections of plain radiographs; compare the established reference values with those of other members of the order Rodentia and class Mammalia in available literature.

MATERIAL AND METHODS

Ethical approval

The experimental procedure has the approval of the Research Ethics Committee, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria. The approval reference number is MOUAU/CVM/REC/202214. Management of the experimental animals was as stipulated in the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council, USA (National Academic Press, Washington, DC: http://www.nap.edu).

Experimental animals and management

Twenty African grasscutters (*Thryonomys swinderianus*) of known ages were used for the study. Ten of the animals were purchased as weaners at the age of one month and one week from an organized grasscutter farm in Ibadan, Nigeria. The remaining ten were purchased as adults between the ages of 15 and 17 months, from an organized grasscutter farm in Umuahia, Nigeria. The animals were transported in well ventilated wooden cages to the animal holding of the Radiology Unit in the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. Here, they were transferred into, and kept singly, in standard laboratory cages, specific for housing large rodents like African grasscutters.

The puppies were managed for three months to attain the juvenile age (four months). During this period, the adults were also acclimatized for two weeks. All animals were fed with cane grass, corncob, husk and entire maize plant, potato, yam and paw-paw peels, foraged legumes such as *Centrosema macrocarpum* and *Calopogonium caeruleum*. These plants were supplemented with concentrates (Chikun Finisher®). Drinking water was provided *ad libitum*. They were clinically examined and ascertained to be apparently healthy, based on the absence of prior clinical conditions documented by the grasscutter farm, and the clinical examinations. On a daily basis, the cages were cleaned and the litters were replaced with fresh feed.

Anesthetic protocol

The animals were restrained physically for weighing, using a well-ventilated metal cage of known weight. The animal's weight was obtained by subtracting the weight of the cage from the combined weights of the animal and the restraint cage. Animals' weights were obtained using a baby scale. Thereafter, the animals were sedated with Xylazine hydrochloride (XYL-M2®, Belgium; 2.0mg/Kg/IM). Five minutes later, they were anaesthesized with ketamine hydrochloride (Jawa Ketamine®, Swiss Parentrals India; 8.0mg/Kg/IM). This was to reduce experimental stress and ensure compliance of the animals as well as safety of the radiologist. Vital signs were monitored using a stethoscope (Classic II Infant 28′ Stethoscope, Littmann 3 M, USA) and rectal thermometer (Electro-therm TC100A, Cooper Instruments, Virginia, USA).

Radiographic exposures

The animals were carefully placed on the radiographic table so that the radiographs were centered and collimated to the thorax (Figure 1). Forelimb- ties and sandbags were employed to achieve maximum collimation. Blue-sensitive radiographic films (BEGOOD® Medical X-ray Films China, 24 x 30 cm; 100NIF), loaded in the dark room, were exposed at full inspiration using a mobile X-ray machine (Dean Dynamax 40; GEC Medical Equipment Group Ltd, England; 44kVp; 10 mAs; 90 cm focus film-distance; 0 cm object film-distance). Only one radiologist performed all exposures in order to reduce exposure bias and variation. The VD (Figure 1), DV, RtL and LeL projections of the thorax were obtained from each animal. Exposed films were routinely processed in a dark room and studied using an X-ray viewer. Images of the radiographs were obtained with a digital camera (Casio Exilim EX-H5, USA; 12.1 Megapixel).

Figure 1 An adult African grasscutter positioned with forelimb-ties and sandbags for VD radiographic projection of the thorax

Landmarks for radiographic measurements

The thoracic indices obtained on the VD and DV projections were the CD and TD, from which the CTR was computed for each animal. Furthermore, the LA and SA were thoracic indices obtained on the RtL and LeL projections, for the computation of VHS and CAR for each animal. The indices were measured with a digital vernier caliper (British Whitworth tools, UK; sensitivity: 0.01mm). Landmarks for the measurements include:

1. Cardiac diameter (CD): summation of two transverse lines (a + b), each measured from the mid-sagittal to the widest lateral margin of the heart (Figure 2).
2. Thoracic diameter (TD): a transverse distance between the costal surfaces of the shafts of two corresponding ribs at the level of the apex of the right diaphragmatic dome (Figure 2: c).
3. Cardio-thoracic ratio (CTR):
   CD ÷ TD
4. Short axis (SA): the widest distance, perpendicular to the long axis, between the cranial and caudal borders of the heart (Figure 3: a).

5. Long axis (LA): a distance from the base of the heart, at the level of tracheal bifurcation (carina), to its apex (Figure 3: b)
6. Vertebral heart score (VHS): summation of the number of thoracic vertebral bodies scaled by the radiographic values of SA and LA, obtained by placing the vernier caliper representing the value of SA and LA over the thoracic vertebrae, parallel to the vertebral canal, and counting caudally, from the cranial extremity of the body of the 4th thoracic vertebrae to the nearest 0.5 vertebra (Figure 3: $a^{/} + b^{/}$).
7. Cardiac axis ratio (CAR): SA ÷ LA

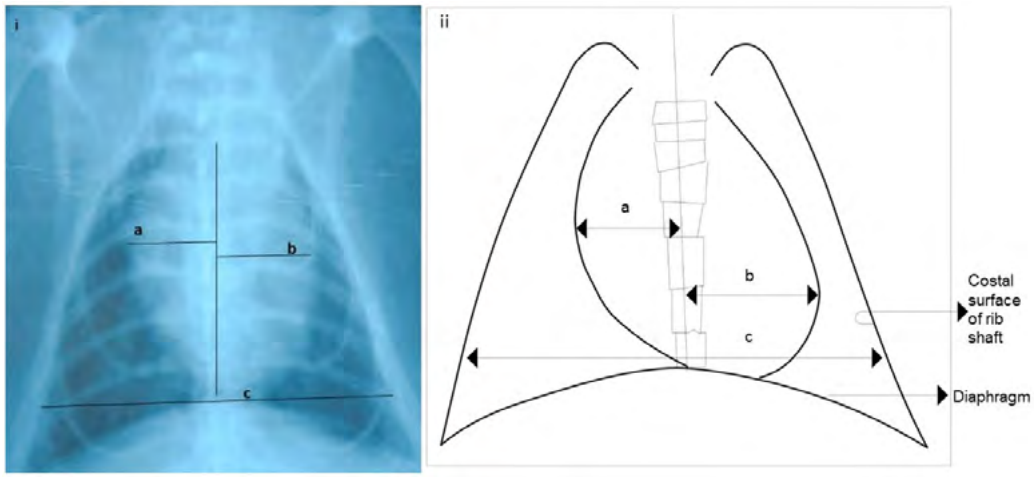

Figure 2 Landmark for radiographic measurement of CD (a + b) and TD (c) in the African grasscutter

(i) Plain radiograph (VD Projection) of the adult African grasscutter; (ii) Schematic representation of the radiograph

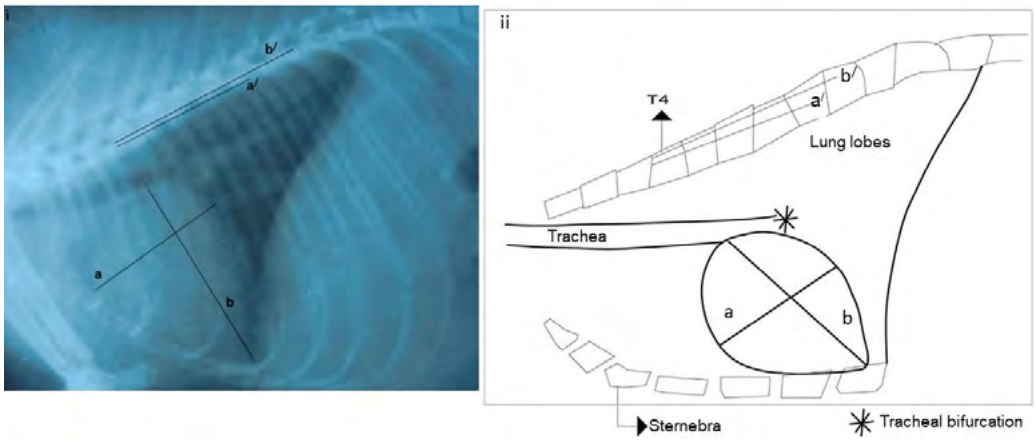

Figure 3 Landmark for radiographic measurement of SA (a), LA (b) and VHS (number of vertebral body units represented by $a^{/} + b^{/}$) in the African grasscutter

(i) Plain radiograph (LeL Projection) of the juvenile African grasscutter; (ii) Schematic representation of the radiograph

Statistical analysis

The data obtained were analyzed using a commercially available statistical software for Windows operation system, GraphPad 5.0 (GraphPad Software Inc, San Diego, CA, USA). The mean radiographic values ± Standard Error of Mean (SEM) of all the measured indices and ratios from animals in each age group were obtained and represented in tables and graphs. The mean radiographic values of CD, TD, SA and LA were compared between opposite radiographic projections in each of the two age groups using two-tailed t-test. The mean radiographic values of the CTR, VHS and CAR of the juveniles and adults were also compared using the two-tailed t-test. In each of the two age groups, Pearson correlation coefficient was calculated to evaluate the association between each of CTR, VHS and CAR with nose-rump length. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

The mean body weight and nose-rump length of the adult African grasscutters used for this study were 2.59 ± 3.37 kg (95% range: 1.95-3.85 kg) and 462.7 ± 16.22 mm (95% range: 407.6-500.2 mm), respectively. Similarly, the mean body weight and nose-rump length of the juveniles were 0.78 ± 0.14 kg (95% range: 0.23-1.00 kg) and 260.2 ± 16.49 mm (95% range: 200.1-293.9 mm), respectively. Irrespective of the age, each animal presented a cardiac silhouette located between the 3rd and 5th intercostal space in the middle mediastinum.

Reference values of CTR, VHS and CAR in the African grasscutter

Table 1 shows the reference values of some cardiac mensuration (CTR, VHS and CAR) in the African grasscutter, obtained from opposite radiographic projections, irrespective of age. There was no significant difference in the radiographic values of any of the indices between opposite radiographic projections ($P > 0.05$).

Table 1 Reference Values of CTR, VHS and CAR in the African grasscutter

	CTR		VHS		CAR	
	VD	**DV**	**RtL**	**LeL**	**RtL**	**LeL**
Minimum	0.42	0.39	7.00	7.00	0.67	0.67
Maximum	0.64	0.61	9.50	9.50	0.73	0.81
Mean (± SEM)	0.52 ± 0.02[a]	0.50 ± 0.02[a]	8.70 ± 0.23[b]	8.60 ± 0.21[b]	0.70 ± 0.01[c]	0.71 ± 0.02[c]

[a-c]Mean (± SEM) values of a radiographic thoracic index with the same alphabet superscript are not statistically different between opposite radiographic projections ($P > 0.05$)

Comparison of CTR between juvenile and adult African grasscutters

Table 2 shows the mean radiographic values of the CD, TD and CTR obtained on the VD and DV projections, in each of the two age groups. There was no significant difference in the radiographic values of CD between the VD and DV projections in any of the two age groups ($P > 0.05$). Similar findings were made for the TD and CTR. Figure 4 is a chart that compared the mean radiographic values of CTR between juvenile and adult age groups, obtained on the DV and VD projections. The result did not show any significant difference in mean radiographic values obtained on either the DV or VD projections ($P > 0.05$). There was a significant negative linear correlation between the radiographic values of CTR and nose-rump length in the juveniles ($R^2 = 0.91$; $P < 0.05$). A regression formula was deduced from the graph (Figure 5), as $y = -0.0021x + 1.0297$; y being the CTR (dependent

variable) and x being the nose-rump length. There was no significant linear correlation between the radiographic values of CTR and nose-rump lengths in the adult (R^2: 0.46; $P > 0.05$).

Table 2 Cardiac diameter (CD), thoracic diameter (TD) and cardio-thoracic ratio (CTR) in juvenile and adult African grasscutter (*Thryonomys swinderianus*)

	JUVENILES		ADULTS	
	VD	**DV**	**VD**	**DV**
Radiographic Indices				
CD (mm)	21.64 ± 1.04[a]	21.67 ± 1.59[a]	35.23 ±1.46[b]	35.87 ±52.09[b]
TD (mm)	44.72 ± 3.96[c]	47.25 ± 4.37[c]	64.80 ±43.31[d]	68.78 ±83.95[d]
CTR	0.49 ± 0.04[e]	0.48 ± 0.04[e]	0.54 ±.0.01[f]	0.52 ±.0.01[f]

[a-f]Mean (± SEM) values of a radiographic thoracic index in an age group with the same alphabet superscript are not statistically different ($P > 0.05$)

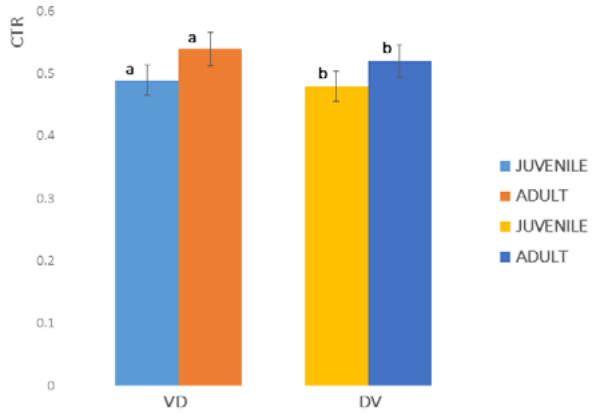

Figure 4 Comparison of mean values of CTR between juvenile and adult African grasscutters in the VD and DV radiographic views.

Mean values of CTR with the same letter superscript in each radiographic view are not statistically significant ($P > 0.05$)

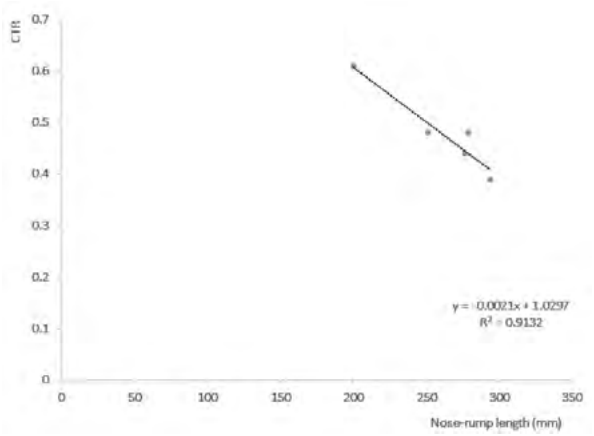

Figure 5 Negative linear relationship between CTR and nose-rump length in juvenile African grasscutter ($P < 0.05$)

Comparison of VHS between juvenile and adult African grasscutters

The mean radiographic values of the VHS obtained on the RtL and LeL projections in the juvenile were 8.20 ± 0.30 and 8.20 ± 0.30, respectively. There was no significant difference in these values ($P > 0.05$). Similarly, the mean radiographic values of the VHS obtained on the RtL and LeL projections in the adults were 9.00 ± 0.16 and 9.20 ± 0.12, respectively, with no significant difference ($P > 0.05$). Furthermore, there was no significant difference in the radiographic values of VHS between the juvenile and adult age groups obtained in either of the opposite projections ($P > 0.05$). Lastly, there was no significant correlation

between the VHS and nose-rump length in both the juvenile and adult age groups ($P > 0.05$).

Comparison of CAR between juvenile and adult African grasscutters

Table 3 shows the mean radiographic values of the SA, LA and CAR obtained on the RtL and LeL projections in each of the two age groups. There was no significant difference in the radiographic values of SA between the RtL and LeL projections in any of the two age groups ($P > 0.05$). Similar findings were made for the LA and CAR. Figure 6 is a chart that compared the mean radiographic values of CAR between juvenile and adult age groups, obtained on the RtL and LeL projections. The result did not show any statistical difference in mean radiographic values obtained on either the RtL or LeL projections ($P > 0.05$). There was a significant negative linear correlation between the radiographic values of CAR and nose-rump length in the adults (R^2: 0.89; $P < 0.05$). A regression formula was deduced from the graph (Figure 7), as $y = -0.0014x + 1.3536$; y being the CAR (dependent variable) and x being the nose-rump length. There was no significant linear correlation between the values of CAR and nose-rump lengths in the juvenile (R^2: 0.68; $P > 0.05$).

Table 3 Short axis (SA), long axis (LA) and cardiac axis ratio (CAR) in the juvenile and adult African grasscutter (*Thryonomys swinderianus*)

	JUVENILE		ADULT	
	LeL	**RtL**	**LeL**	**RtL**
Radiographic Indices				
SA	20.13 ± 1.18^{a}	20.68 ± 1.41^{a}	33.72 ± 2.4^{b}	33.54 ± 1.81^{b}
LA	29.31 ± 92.50^{c}	27.68 ± 2.68^{c}	46.53 ± 2.60^{d}	47.37 ± 2.60^{d}
CAR	0.70 ± 0.02^{e}	0.68 ± 0.01^{e}	0.73 ± 0.02^{f}	0.71 ± 0.01^{f}

[a-f]Mean (± SEM) values of a radiographic thoracic index in an age group with the same alphabet superscript are not statistically different ($P > 0.05$)

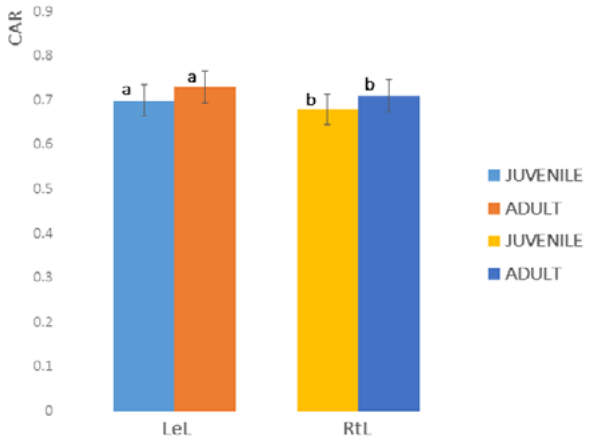

Figure 6 Comparison of mean values of CAR of juvenile and adult African grasscutters in the LeL and RtL radiographic views.

Mean values of CAR with the same letter superscript in each radiographic view is not statistically significant ($P > 0.05$)

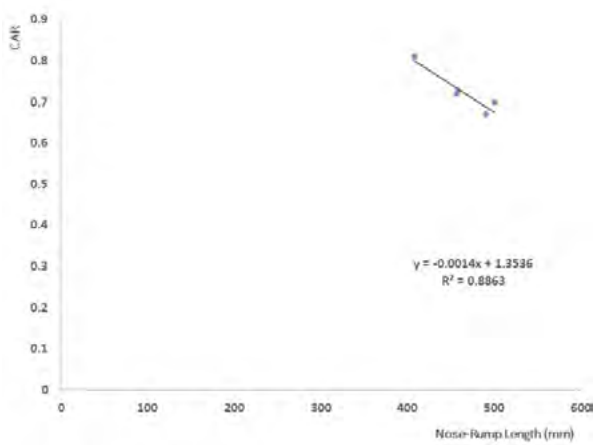

Figure 7 Negative linear relationship between CAR and nose-rump length in adult African grasscutter ($P < 0.05$)

DISCUSSION AND CONCLUSION

The establishment of radiographic reference values for the size of cardiac silhouette of any animal is a prerequisite for accurate radiographic diagnosis of cardiac disease in veterinary clinical practice. Studies have proved that the relationship between these radiographic values and variables such as age, breed and sex vary with animal species and even breeds of the same species. Unfortunately, the African grasscutter, a rodent of the *Thryonomyidae* family has only two recognized species (the second being *Thryonomys gregorianus*) and no classified breeds. To the best of our literature search, this is the first radiographic reference thoracic values available for this genus of rodents. Thus, this discussion is constrained to compare the findings with those of other rodents and mammals only. Furthermore, the sample size may be a limitation of the present study. However, a compilation of anatomical studies on the African grasscutter by Ibe et al. (2023) revealed that the rodent was nearly homogenous in morphotype. Moreover, the wide range of body weight in both juvenile and adult subjects in the present study infers that a higher sample size may not yield strikingly different values. It is also pertinent to emphasis that absolute caution was employed by the radiologist to ensure exposures were taken at full inspiration. This was to exclude bias due to change of cardiac axis with varied distention of the diaphragm.

The present study has reported a reference value of CTR in the African grasscutter, measuring an average of 0.52 on the VD and 0.50 on the DV projections. This is comparable to the values of 0.53 on VD, and 0.50 on DV projections of Nigerian indigenous dogs reported by Ukaha et al. (2021a). It is also comparable to the values of 0.51 and 0.52 obtained for male and female agouti, respectively, by De Moura et al. (2015) and values of 0.55 and 0.52 obtained for male and female tufted capuchin monkeys, respectively, by Rocha-Neto et al. (2015). It is, however, less than the value of 0.56 obtained for bats by Gardner et al. (2007). Dimopouloset al. (2013) opined that CTR of more than 0.55 indicated cardiomegaly in humans. Unfortunately, there is no reference value of CTR in African grasscutters with confirmed state of cardiomegaly or microcardia.

The values of CTR obtained on the VD and DV views of the African grasscutter from this study did not differ significantly, irrespective of age. Sak and Pazvant (2021) made similar observation in the Persian cats. There are divergent views as to the preferred radiographic projection for cardiac mensuration; Buchanan (2000) advocates the preferred use of DV projections in dogs because cardiac contours are more consistent and the heart is magnified in VD radiographs due to the varied distance between the heart and the radiographic cassette. Conversely, Van Den Broek and Darke (1987) preferred VD to DV views for determination of CTR in cats. The present study and that of Sak and Pazvant (2021) have shown that either view can be applied for the establishment of CTR in animals.

The significant negative correlation obtained between CTR and nose-rump length in the juvenile African grasscutter from this study implies that the value of CTR decreased with an increase in nose-rump length at approximately the same rate in the juvenile African grasscutters. The regression formula generated can be used to estimate CTR in juvenile African grasscutters of known nose-rump length. This is beneficial in clinical practice where there is no access to an X-ray machine for the radiographic evaluation of CTR.

This study has also presented reference values of VHS in juvenile and adult African grasscutters, without any significant difference between the two age groups. Similarly, Sleeper and Buchanan (2001) reported that the difference in VHS between young and adult dogs were not significant. Likewise, Black et al. (2011) observed that there was no significant difference in VHS between younger and older African hedgehogs. This implies that VHS does not depend on age of the animal, at least in the dogs, African hedgehogs and African grasscutter; thus, this clinical standard used in the determination of heart size in the adult African grasscutter can also be used in the juvenile.

The reference value of VHS for the African grasscutter from this study (8.7) is higher than the values of 7.5, 7.7, 8.1 or 8.2 established for adult cats, irrespective of breed (Litster and Buchanan, 2000), pet rats (Dias et al., 2021), agoutis (De Moura et al., 2015) and African hedgehogs (Black et al., 2011) respectively. It is lower than the values of 9.3, 9.4, 9.8, 10.1, 10.3 or 10.5 established for the tufted capuchin (Rocha-Neto et al., 2015), common marmosets (Wagner and Kirberger, 2005), Nigerian indigenous dog (Ukaha et al., 2021b), west African dwarf goats (Ukaha et al., 2013), dachshund dog (Birks et al., 2017) and Australian cattle dog (Luciani et al., 2019), respectively. This implies that the normal heart size in the adult African grasscutter is bigger than that of the rat and cat, but smaller than that of the above named non-human primates, small ruminant and breeds of dog. In the present study, there was no significant difference in the radiographic values of VHS between opposite projections in both the juvenile and adult African grasscutters. Ukaha et al. (2013), Ulum and Noviana (2018), Puccinelli et al. (2021) Sak and Pazvant (2021) and De Silva et al. (2022) made similar observation in the adult west African dwarf goats, laboratory mice, Chihuahua dog, Persian cats and guinea pigs, respectively. Conversely, Birks et al. (2017) and Luciani *et al*. (2021) observed that the VHS was significantly higher on the RtL than the LeL projections in the dachshund and Australian cattle dog breeds, respectively. These differences imply that significant breed and species variations exist in VHS of opposite radiographic projections. Thus, reference values of VHS must be intrinsic to the breed and/or species in view, and should not be extrapolated to other breeds or species. Furthermore, Dias et al. (2021) emphasized that VHS within normal limit of a named breed or species did not preclude an actual cardiac disease, mainly in cases where heart enlargement is not part of the process, as the animal can develop concentric cardiac hypertrophy without dilation. Further examination of the cardiac borders is advisable in such cases.

The values of CAR in the juvenile and adult African grasscutter from the present study did not differ significantly, neither was there any significant difference between the CAR of opposite projections in any of the two age groups. In their study, Ukaha et al. (2002) also observed that CAR between puppies and adult Nigerian indigenous dogs did not differ significantly. The authors also observed that CAR of opposite projections did not differ significantly in both the puppies and adult Nigerian indigenous dog. However, Ukaha et al. (2002) attributed the consistent (though, non-significant) higher value on the LeL projection to the higher value of LA on the RtL projection due to the pulling effect of the cardiophrenic ligament positioned on the left cardiac silhouette. This may also explain the reason CAR was also slightly higher on the LeL projection in the African grasscutter.

The significant negative correlation obtained between CAR and nose-rump length in the adult African grasscutter from this study implies that the value of CAR decreased with an increase in nose-rump length at approximately the same rate in the adult African grasscutters. The regression formula generated can be used to estimate CAR in adult African grasscutters of known nose-rump length. This is also beneficial in clinical practice where there is no access to an X-ray machine for the radiographic evaluation of CAR.

Reference values of CTR, VHS and CAR on radiographic projections of clinically healthy juvenile and adult African grasscutters have been provided in this study. These values will be beneficial to clinicians as objective tools to evaluate cardiac size in the juvenile and adult African grasscutters. They will also be beneficial for research purposes. There is a need for future studies to determine same values in African grasscutters with confirmed cases of microcardia or cardiomegaly.

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CONTRIBUTIONS

Concept - CSI, ROU; Design - CSI, ROU, NUN, KTJ, COU, YET, CJO, CU; Supervision - CSI; Resources - CSI, ROU; Materials - CSI; Data Collection and Processing - CSI, ROU, NUN, KTJ, COU, YET, CJO, CU; Interpretation - CSI, ROU, NUN, KTJ, COU, YET, CJO, CU; Literature Search - CSI, ROU, NUN, KTJ, COU, YET, CJO, CU; Writing Manuscripts - CSI, ROU, NUN, KTJ, COU, YET, CJO, CU; Critical Review - CSI, ROU, NUN; All the authors approved the final manuscript.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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RADIOLOŠKI INDEKSI KARDIJALNIH MJERENJA KOD AFRIČKOGA DIVOVSKOG TRSTIČNOG ŠTAKORA (*THRYONOMYS SWINDERIANUS*)

SAŽETAK

Cilj ovog istraživanja je odrediti referentne radiografske indekse za kardiotorakalnu evaluaciju kod afričkoga divovskog trstičnog štakora. U istraživanje je uključeno dvadeset zdravih životinja istoga broja mladih i odraslih jedinki. Četrdeset torakalnih projekcija koje se sastoje od dorzoventralnih (DV), ventrodorzalnih (VD), desnih lateralnih (RtL) i lijevih lateralnih (LeL) snimaka je dobijeno. Evaluirani parametri su uključivali torakalne i kardijalne dijametre na DV/VD snimcima te kardijalne duge i kratke osovine na RtL/LeL snimcima. Kardijalni indeksi koji su generirani uključivali su: kardio-torakalni omjer (CTR), vertebralni srčani skor (VHS) i omjer kardijalne osovine (CAR). Referentne vrijednosti za CTR na VD i DV su iznosile 0.52 ± 0.02 i 0.50 ± 0.02. Razlika nije bila signifikantna ($P > 0.05$). Srednje radiografske vrijednosti CTR-a za mlade i odrasle jedinke za DV su iznosile 0.48 ± 0.04 i 0.52 ±.0.01. Referentne vrijednosti za VHS i RtL i LeL su iznosile 8.70 ± 0.23 i 8.60 ± 0.21. Razlika nije bila signifikantna ($P > 0.05$). Srednje vrijednosti za VHS za mlade i odrasle jedinke za RtL su iznosile 8.20 ± 0.30 i 9.20 ± 0.12, Razlika nije bila signifikantna ($P > 0.05$). Ovi indeksi su relevantni u budućem dijagnosticiranju kardijalnih oboljenja kod afričkoga divivskog trstičnog štakora u budućnosti.

Ključne riječi: Afrički divovski trstični štakor, kardio-torakalni omjer, omjer kardijalne osovine, radiologija, vertebralni srčani skor

RESEARCH ARTICLE

THREE-DIMENSIONAL MORPHOLOGICAL VARIATION AND ALLOMETRIC ANALYSIS IN DOG SCAPULA

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ABSTRACT

In canines, the scapula is a flat bone that serves to connect the thoracic limb with the trunk and the clavicle, which is located above the shoulder joint. In this study, computer tomography was employed to create models of the scapulae from 25 dogs. There are 12 breeds with different age, sex and weight. 562 semi landmarks were placed on the scapulae. Applying Procrustes analysis, variance in shapes of different breeds and ages of dogs were classified. Also, with the 3D Slicer Program extension called Dense correspondence analysis, we could show the shape variation with colorimetric measurement on a mean templated scapula. Lastly, using allometric analysis, this research also tried to point out if there is a shape change with the size change based on different breeds in dogs. In this study conducted on dogs, it was seen that PC1 explained the highest shape variation, explaining 25.4% of the total variation. It was seen that dogs were separated according to their size in PC1 values. While the PC1 value was high in small breed dogs, this value was generally negative in dogs with larger body size. While the scapula was wider in small breed dogs, the scapula in large breeds was thin and long in shape. The effect of size on shape was statistically significant. Geometric morphometrics is a valuable analytical approach for identifying the differences in animal species and their dimorphic features. This study has highlighted the effectiveness of geometric morphometrics in distinguishing shape variations and allometric differences among various breeds and age groups of dogs.

Keywords: Carnivore, shape analysis, veterinary anatomy

INTRODUCTION

The dog exhibits an unparalleled range of skeletal diversity, unlike any other mammal. Dogs can span an extensive spectrum in terms of weight and height (Dyce et al., 1987; Hourdebaigt, 2003). Beyond variations in size, dogs can also display differences in the length and shape of their bones. This remarkable array of dog breeds, each with its unique shape, has been meticulously crafted through artificial selection processes. These practices have led to the establishment of distinctive physical traits within hundreds of unique breed populations. Scapula, also called the shoulder blade, is the largest flat bone located on lateral sides of the body, where the last cervical part of the columna vertebralis meets the costas (König, 2009). The scapula is a bone that connects the forelimb to the trunk via muscular connections. Positioned at the proximal end, the scapulae play a vital role in shaping the shoulder joint (Bahadır and Yıldız, 2012).

It features a prominent ridge or spine, *spina scapulae*, running down its lateral surface, which is called *facies lateralis*, effectively dividing this side into two distinct regions: *fossa supraspinata* and *fossa infraspinata* (König, 2009). Towards the end of this spine, there's a bony ledge known as the *acromion*. In dogs, acromion ends with *processus hamatus*. As the scapula extends towards its end, it tapers at the neck and forms a shallow articular socket called *cavitas glenoidalis* (Demiraslan and Dayan, 2021). This glenoid cavity combines with the head of the humerus to create the shoulder joint. The ridge on the craniodorsal side of the *cavitas glenoidalis* is referred as the *tuberculum supraglenoidale,* while the ridge on the caudoventral side is known as the *tuberculum infraglenoidale* (Bahadır and Yıldız, 2012). Notably, the *tuberculum infraglenoidale* is unique to dogs and cats. The medial surface of the scapula, *facies costalis,* is flat and relatively smooth (Demiraslan and Dayan, 2021).

Geometric morphometrics is a field that uses geometric principles and quantitative methods to analyze and compare the variations in size and shape of biological structures in animals (Adams et al., 2016) as well as in humans (Ajanović et al., 2023). It focuses on anatomical landmarks. Procrustes analysis is a foundational technique in geometric morphometrics (Slice et al., 2007). It aligns, scales, and superimposes shapes to eliminate differences in position, orientation, and size, allowing for the direct comparison of shapes (Mitteroecker and Gunz, 2009). Principal Component Analysis (PCA) is often used to analyze shape variations (Boz et al., 2023). It reduces complex shape data into a smaller set of variables (principal components) that capture the most significant variations in shape. In shape analysis, landmarks are specific locations on an animal's body or structure that hold anatomical or biological importance (Jashari et al., 2022). These landmarks are used to capture the shape information accurately. Landmarks can be 2D points (e.g., on photographs) or 3D points (in three-dimensional space) (Gurbuz et al., 2022; Hadžiomerović et al., 2023; Gundemir et al., 2023(a); Gundemir et al., 2023(b); Szara et al., 2023).

In osteology, taxonomy covers the systematic classification and grouping of skeletal remains according to their anatomical and morphological traits. This involves identifying and organizing bones from different species, breeds or individuals into distinct groups or categories. The goals of taxonomy in osteology include species identification, understanding individual variation, comparative anatomy, functional morphology, evolutionary studies, and forensic analysis. Being the largest flat bones in the body, scapulae still have limited reference stories of gender and breed analysis. Recent studies have given the scapulae more credit than before. Hence, investigating the allometry in the scapulae can help researchers understand how different bones grow and change in size relative to the overall size of a dog and also to estimate the age and sex of individuals based on the size and proportions of their skeletal parts. This information can also help to understand and classify the evolutionary relationships and species identification of skeletal remains.

MATERIALS AND METHODS

Specimens

For this research, computed tomography scans of the thoracic area from 24 dogs were employed. It's noteworthy that the subjects included in the imaging process did not exhibit any orthopedic issues. The dogs used in this study were given under in the **Table 1** with their gender, average age and weight.

Table 1 Dogs used in the study, their average age and average weight

Dogs	Female	Male	Age	Weight (kg)
GOLDEN RETRIEVER (n:5)	2	3	10.6	32.1
COCKER (n:4)	5	0	10.75	14.15
ROTTWEILER (n:4)	1	3	9	42.32
BULLDOG (n:2)	0	2	5	28.5
PEKINGESE (n:2)	1	1	10	5.75
BEAGLE (n:1)	1	0	13	15
PITBULL (n:1)	1	0	7	31
JACK RUSSELL (n:1)	0	1	5	11
DOGO ARGENTINO (n:1)	0	1	11	39
CAVALIER KING CHARLES (n:1)	1	0	8	10.8
CHIHUAHUA (n:1)	0	1	10	5
INGILIZ SETTER (n:1)	0	1	13	18

Computed tomography and modelling

For the acquisition of computed tomography scans, Siemens (Somatom Scope vc30b) was utilized at the Animal Hospital, Faculty of Veterinary Medicine, Istanbul University-Cerraphasa. Uniform scanning parameters, including a 0.6 mm slice thickness, 110 kV, and 28 mA, were applied to all samples, resulting in a total scanning time of approximately 14 seconds. Following the completion of the scanning process, the images were transferred to a computer, and subsequent segmentation was carried out. The 3D Slicer program (version 5.4.0) was used to create three-dimensional models of the scapulae, after removing the soft tissues from the bone.

In the Slicer program version 5.4.0, the PseudoLM Generator module in the GeoMorph extension was utilized to generate pseudo-landmarks. A source landmarks template was established using this plug in, employing a spacing tolerance of 3%. The 'Original Geometry' option was selected to derive a sampling pattern based on the model's geometry. The initial number of sampled points in the template was set to 15. Subsequently, a template mesh was generated using the 'Generate Template' function. A 'Project points to surface' operation was executed. Following this, an enforce spatial sampling rate was applied to eliminate samples with a point-to-point distance lower than the spatial sampling rate. As a result of these operations, a total of 562 pseudo-landmarks were obtained. The resulting draft of pseudo-landmarks was saved for application to other samples.

In the analysis, the ALPACA tool was used for the efficient transfer of landmarks from a draft pseudo-landmark to the target 3D model. A batch processing approach was implemented to apply the draft pseudo-landmark across 24 samples, using the "Single Template (ALPACA)" option. The

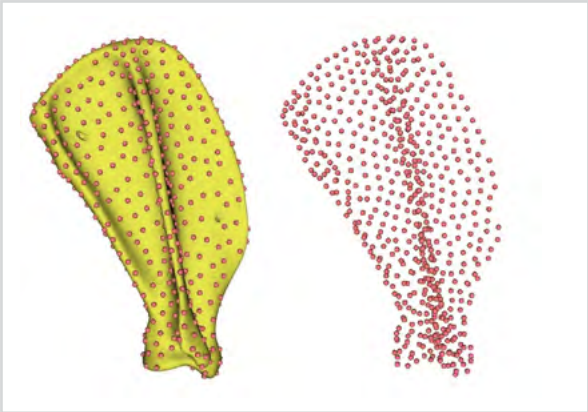

Figure 1 Semi-landmarks

identical mesh model served as both the source and the target, with the draft pseudo-landmark prepared for that specific sample, acting as the source landmark. The process concluded with the execution of Run-auto landmarking, resulting in the recording of 562 semi-landmark sets, each documented separately for all samples (Figure 1).

Shape analysis

Principal component analysis (PCA) was applied to reveal the shape variations of all scapulae. PCA changes were obtained in 3D from Slicer (version 5.4.0). Procrustes distances were obtained for all samples. R Studio (version 4.3.2) and Past (version 4.03) statistics program were used for statistical analysis.

3D Slicer Extension called "DeCa", A Dense Correspondence Analysis, was also utilized for colorimetric visualization on shape variation. (R) First, using the panel in DeCa, Rigid Alignment of Models, we aligned all the Scapula models of the specimens with the selected reference model. This step is mandatory because the results of aligned models and landmark files which we will use in the shape variation analysis will be saved in the data folder. Using the "Generate Mean" tab, after choosing a reference model, a mean template of the group is created with rigidly aligned models and landmarks. Additionally, the toolkit provides the visualization of heatmaps illustrating average and individual differences in shape.

Regression Analysis and allometry

After the centroid size and procrustes distance were obtained for each sample, multivariate regression was performed with these values. It was investigated whether allometry existed in the dog scapula and the statistical significance of the results.

RESULTS

PCA test was conducted to reveal the shape variations in the scapulae. As a result of PCA, a total of 24 PCs were obtained. PC1, which explains the highest variation in shape, accounts for 25.4%, while PC2 explains 14.7%. PC3 explains 9.7%. The scatter plot of PC1 and PC2 for the scapula is provided in Figure 2.

Golden Retrievers, Cockers and most of the big breed dogs are in the negative space of PC1. Pekingeses have the highest PC1 scores in all dog breeds, which makes them have a high rate in shape variation. Jack Russel, which is also a small breed of dog, also has positive PC1 score. As seen in the Table, Cockers have low rate in shape variation with negative PC1 and PC2. Rottweilers are also scattered in the negative PC1 space. Rottweilers are in the negative space of PC1, scattered around all the axes in the PC2 chart.

In breed base, smaller breeds seem to have higher PC1, when bigger breeds tend to have lower PC1 scores. PC2, on the other hand, isn't dependent on the breed size. We can see PC2 scores change in individual differences.

In the mean 3D mesh comparisons of maximum and minimum percentage of PC1, in maximum mean shape of the scapulae is wider and more sharp-edged (Figure 3). Increased PC1 explains anteriorly located margo cranialis though decreasing PC1 results in more oval margo dorsalis. Angle of the caudal border becomes more pronounced when the PC1 increases, in the mean template of the minimum percentage of PC1, angulus caudalis was not clear.

Spina scpaulae get elevated with the increase in PC1 mean, while the processus hamatus part

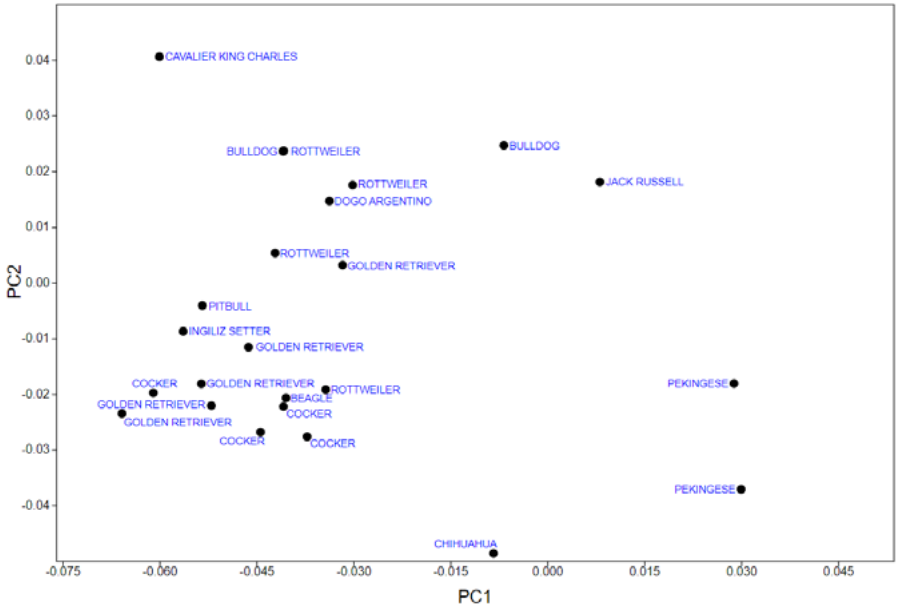

Figure 2 Principal component analysis scatter plot comparing scapula morphology

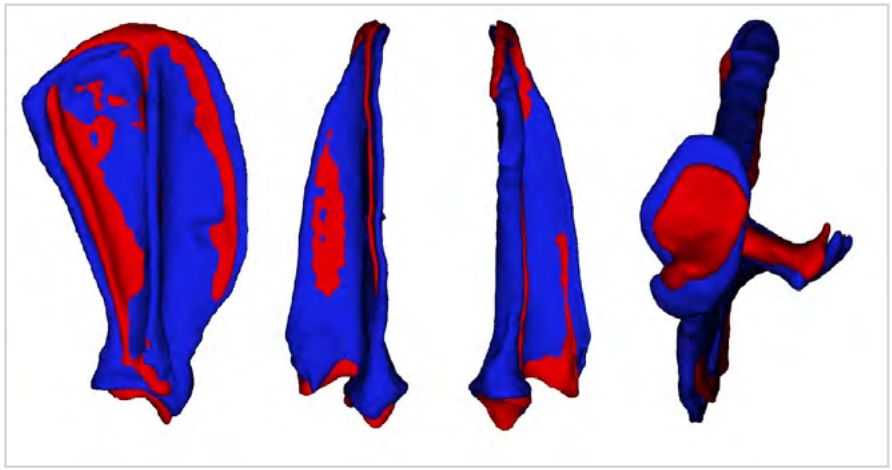

Figure 3 The negative and positive values of PC1 (red is negative limit, blue is positive limit)

in acromion gets more prominent/ more hook-looking as the rate of PC1 decreases. Cavitas glenoidalis in shape shows very distinct difference within maximum and minimum PC1 percentages. Decreased PC1 has smaller and squared formation, while an increase results in more elliptical and bigger cavitas glenoidalis. As seen in cavitas glenoidalis, maximum PC1 mean mesh has bigger, more evident and craniodorsally located tuberculum, but minimum has smaller, more hooked and ventrally located tuberculum.

In the mean mesh models of maximum and minimum percentages of PC2, both models have similar width and length (Figure 4). Margo dorsalis appears more rounded at increase. Angulus cranialis and specially angulus caudalis appear sharper and steeper in decrease of PC2. Acromion part of the maximum PC2 mesh is more elevated than minimum; meanwhile processus hamatus is located ventrally, is longer and more prominent in minimum PC2 mesh.

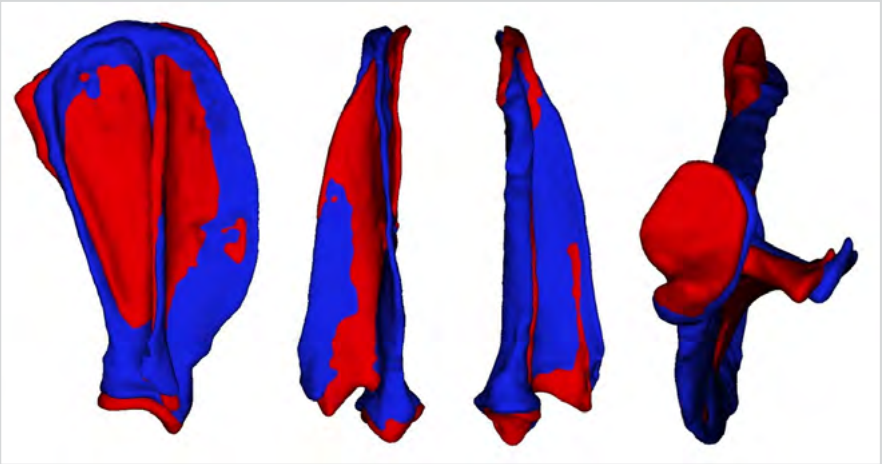

Figure 4 The negative and positive values of PC2 (red is negative limit, blue is positive limit)

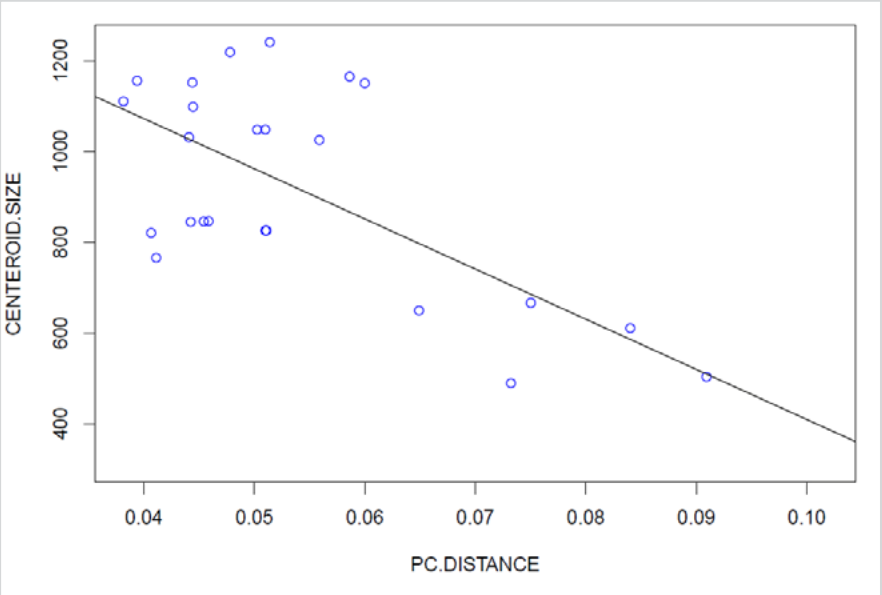

Figure 5 Allometry for scapula

In this chart (Figure 5), we analyzed the correlation of centroid size on procrustes distance, and it resulted in negative correlation. It is seen that specimens with low centroid size have higher procrustes distance scores mean, while most of our group consisting of big breed dogs with higher centroid sizes tend to have lower PC distance. As a result, the effect of size on shape was statistically significant.

Result of negative correlation can be explained with the number of big breed dogs compared to our total number of dogs. It is seen that they create the majority of the specimens. Because average procrustes distance is mostly based on larger dogs with high centroid size scores, small breed dogs such as Pekingese and Chihuahuas that we used in our analysis have high procrustes distance scores.

In DeCa analysis, colorimetric results indicate shape variations (Figure 6). Numbers are shown in milimetric scale between 16.7-1.4 mm. Ventral and dorsal part of the scapulae have the highest rate in shape variation. As seen in the PCA, tuberculum supraglenoidale and cavitas glenoidalis at the ventral section have a variation reaching up to 16.7 mm. Margo dorsalis part of the scapulae has a high rate of variation. As we reach the central part of the scapulae, the variation rate goes down to 1.4 mm between the breeds. Margo cranialis has a higher

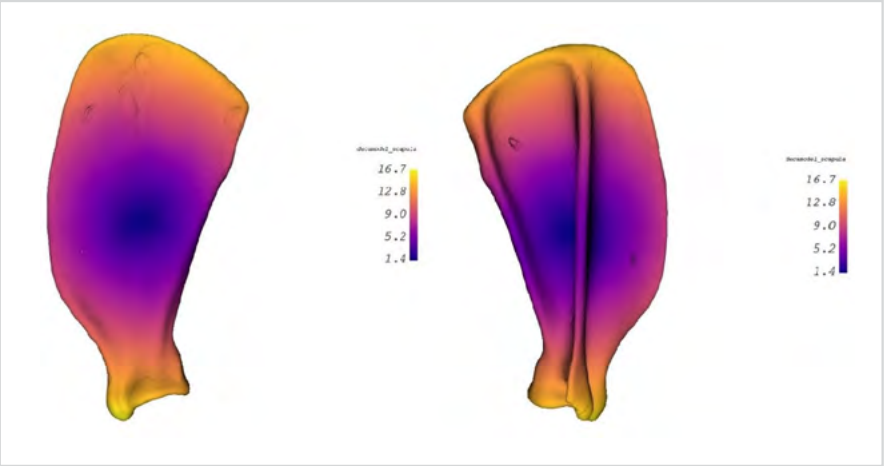

Figure 6 Results of DeCa

variation than margo caudalis, which is painted in deeper purple indicating smaller milimetric change in the shape of the bone.

DISCUSSION AND CONCLUSIONS

Geometric morphometrics is a valuable analytical approach for identifying the differences in animal species and their dimorphic features. This study has highlighted the effectiveness of geometric morphometrics in distinguishing shape variations and allometric differences among various breeds of dogs. In this study conducted on dogs, it was seen that PC1 explained the highest shape variation, explaining 25.4% of the total variation. It was seen that dogs were separated according to their size in PC1 values. While the PC1 value was high in small breed dogs, this value was generally negative in dogs with larger body size. While the scapula was wider in small breed dogs, the scapula in large breeds was thin and long in shape.

In a study conducted on a total of 36 cat scapulae, research focused on the utility of linear measurements taken over the bones for gender determination, and the relationship among the cat weights was observed (Oktay et al., 2023). According to the obtained results, it was observed that linear measurements, when evaluated both in terms of gender and the scapula length and width, showed variations in the calculated area measurements according to weight. In other words, changes in the bone area could be observed along with weight variations. The linear measurement changes in this article can be used to explain gender differences. Additionally, in various studies, it has been observed that these linear measurement methods can vary in explaining gender differences and also different breed types of animals. Therefore, a method other than the 3D landmarking system, as in our study, can define variations related to breed using linear measurements.

Oktay's (2023) study on cats investigated the effect of weight gain on scapula measurements. He said that in this study conducted on cats, the relationship between linear measurement results and weight was statistically significant ($p < 0.05$). In this study conducted on dogs, it was seen that size had an effect on shape. Size affected shape statistically. In these studies conducted on 2 different carnivore species, it was seen that the shape or measurements of the scapula were related to the size of the animal. In future studies, different hypotheses can be developed using different carnivore species, and research can be conducted on this subject.

In recent years, shape analysis studies have attracted attention in the field of veterinary anatomy. One of the most important operations in these analyses is landmark operations. These operations are performed manually through different software. However, with the developing technology in recent years, there are softwares that automatically perform landmark operations (Maga et al., 2017). In this study, landmarks were automatically placed on the scapula. Thanks to computer-aided landmark

placement processes, large data was processed in a short time. Additionally, the accuracy of manual marking varies from person to person (Porto et al., 2021). It is thought that automatic landmark operations would have an important place in geometric morphometry.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – CNG, MK, NM; Design – B**Ç,** MTT; Supervision – EO, MK; Resources –CNG, NM, MTT; Materials – MTT; Data Collection and Processing – NM, EO; Interpretation – CNG, NM; Literature Search – MTT, EO; Writing Manuscript – NM, EO, MK; Critical Review – MK.

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TRODIMENZIONALNA MORFOLOŠKA VARIJACIJA I ALOMETRIJSKA ANALIZA SKAPULE KOD PASA

SAŽETAK

Skapula kod pasa je pljosnata kost koja služi za povezivanje torakalnih ekstremiteta sa trupom i klavikulom smještenom iznad ramenog zgloba. U našem istraživanju je korištena kompjuterizirana tomografija kako bi se kreirali modeli skapula 25 pasa. Uključeno je 12 pasmina različite starosti, spola i težine. Na skapule su postavljena 562 semi-orijentira. Primjenom Procrustes analize varijacije oblika, klasificirane su različite pasmine i starosne dobi. Korištenjem ekstenzije programa 3D Slicer Program zvane Denzna korespondentna analiza, bili smo u mogućnosti procijeniti varijacije oblika kolorimetrijskim mjerenjem skapule označene kao srednji uzorak. Na kraju smo koristeći alometrijsku analizu u ovom istraživanju pokušali dokazati da li promjenu veličine kod različitih pasmina prati i promjena oblika. U istraživanju provedenom na psima je vidljivo da je PC1 objasnio najveću varijaciju oblika i dao objašnjenje za 25.4% ukupnih varijacija. Vidljivo je da su psi kategorizirani prema veličinama u PC1 vrijednostima.

Dok je PC1 vrijednost bila visoka kod malih pasmina, kod velikih pasmina je generalno bila negativna. Dok je kod malih pasmina skapula bila šira, kod velikih pasmina je bila tanka i izdužena. Učinak veličine na oblik je bio statistički signifikantan. Geometrijska morfometrija predstavlja dragocjen analitički pristup za identifikaciju razlika kod životinjskih vrsta i njihovih dimorfnih karakteristika. Ovo istraživanje je naglasilo učinkovitost geometrijske morfometrije u razlikovanju varijacije oblika i alometrijskih analiza među različitim pasminama i starosnim skupinama pasa.

Ključne riječi: Analiza oblika, karnivor, veterinarska anatomija

RESEARCH ARTICLE

HISTOPATHOLOGICAL PULP RESPONSE OF TEETH CAPPED WITH CALCIUM ALUMINATE CEMENT AND BIODENTINE: EXPERIMENTAL STUDY ON RODENTS

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ABSTRACT

This study aimed to evaluate the pulpal response after pulp capping using experimental nanostructured calcium aluminate cement (ALBO-CA) and tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France). Class I cavities were prepared on 72 teeth (36 first upper molars and 36-second upper molars) in 18 Wistar strain rats. The dental pulp was capped in 36 teeth with experimental ALBO-CA (group A) and 36 teeth with Biodentine (group B). All cavities were restored with glass ionomer. After 28 days animals were sacrificed and the following were analyzed: the inflammatory response of the pulp, the presence of bacteria, and the formation of a dentinal bridge. After 28 days, bacteria were not present. Mann-Whitney U test did not show a statistically significant difference in the inflammatory response of the pulp between the groups ($U = 640.00$; $Z = 0.105$; $p = 0.916$). The chi-square test did not show a statistically significant difference in the formation of the dentinal bridge between the examined groups (Chi-square = 1,443; $p = 0,230$). ALBO-CA and Biodentine had similar effects on inflammation, pulp response, and formation of dental bridges in rats.

Keywords: ALBO-CA, pulp capping, rats, tricalcium silicate

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INTRODUCTION

Cement materials in dentistry have been developed to imitate the lost dentine tissue, to mimic biological features as much as possible, and to display bioactive characteristics (Ilić et al., 2019).This is not always simple because of dentine specificity, namely, its close contact with the pulp. In that sense, the local bioactivity of these cement materials is important to induce mineralization within the adjacent dentine (Ilić et al., 2019).

Calcium hydroxide (CH) became recognized as a valuable capping material. The initial effect of CH applied to the exposed pulp is the development of superficial necrosis. Namely, a chemical injury is provoked by hydroxyl ions leading to the formation of a zone of firm necrosis over the vital tissue. The necrosis causes slight irritation and stimulates the pulp to regenerate. Thereafter, the repair process occurs, including migration and proliferation of mesenchymal and endothelial pulp cells as well as collagen formation. The presence of Ca ions stimulates precipitation of $CaCO_3$ contributing to the mineralization initiation (Dammashke, 2008). Shortcomings of CH-based materials include poor mechanical characteristics, inability to preserve high pH values at the site of administration for a certain period, the possibility of primary tooth resorption, dissolution after one year and degradation during acid etching or tooth flexure, poor marginal seal with composite/amalgam restoration and weakening of the root during apexogenesis in a long-term therapy (Saad, 1988). Indeed, long-term use of CH capping may cause progressive calcification of the root canal space.

New generations of cementitious materials began to be produced to overcome the disadvantages of CH. A significant improvement in this field occurred in 1993 when Torabinejad introduced a novel material mineral trioxide aggregate (MTA) that is based on calcium silicate (CS) particles. The main advantage of CS in comparison to CH is its better mechanical and bioactive characteristics. MTA also has some drawbacks such as a long setting time, high cost, and potential for discoloration (Parirokh and Torabinejad, 2017; Jafari, 2017). Biodentine is tricalcium silicate cement. Contrary MTA(Mineral Trioxide Aggregate), it contains zirconium oxide as an X-ray contrast agent. The addition of 15% calcium carbonate to its composition improved the microstructure and facilitated the handling of cement. Biodentine has several advantages which include good sealing ability, adequate compressive strength, short setting time, biocompatibility, bioactivity, and biomineralization properties. Biodentine is mechanically stronger, less soluble, and gives a tighter seal. These features make Biodentine a suitable direct pulp-capping material (Deshmukh et al., 2018).

There is far less data in the scientific literature on materials based on calcium aluminates (Chang et al., 2014; Woodmansey et al., 2015; Janković et al., 2018; Walsh et al., 2018; Paraš et al., 2019; Čolović et al., 2019; Janković et al., 2020). The Endobinder calcium aluminate material has been successfully used for the repair of bony defects (Čolović et al., 2019). It confirmed biocompatibility during subcutaneous implantation in rats (Janković et al., 2018). According to Chang et al. (2014), given optimal pressure resistance, short setting time, and a high degree of biocompatibility, tricalcium aluminum cement may be a suitable material for vital pulp therapy. However, a small number of studies have considered this issue, and in doing so, calcium aluminate cements exhibited a beneficial effect on the reparative abilities of the pulp (Chang et al., 2014; Woodmansey et al., 2015; Walsh et al., 2018).

ALBO-CA is newly synthesized nanomaterial based on calcium aluminate. The particle size of nanomaterials is similar to the size of biological molecules and structures, which gives these materials a great advantage in tissue engineering and regenerative medicine. A nanostructured calcium aluminate cement ALBO-CA was synthesized by a special method, a combination of the hydrothermal salt gel method and the method of self-combustion waves. Nanoparticles improve particle activity and shorten the curing time to 10-15 minutes (Janković et al., 2018). It is expected that newly synthesized nanomaterials

based on calcium aluminates will have a beneficial effect on the reparative abilities of the pulp. This study aimed to evaluate the pulpal response after pulp capping using experimental nanostructured calcium aluminate ALBO-CA and tricalcium silicate Biodentine (Septodont, Saint Maur des Fosses, France).

MATERIAL AND METHODS

Approval of the ethics committee

The study was conducted in the vivarium of the Faculty of Natural Sciences and Mathematics in Banja Luka, after obtaining the consent of the Ethics Committee of the University Clinical Center in Banja Luka, number 01-9-192.2/15, Bosnia and Herzegovina.

Tested materials

Experimental nanostructured ALBO-CA based on calcium aluminate (CA) was compared with tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France).

ALBO-CA ($CaO \bullet Al_2O_3 + CaCO_3 + Bi_2O_3$) was obtained by mixing $CaCO_3$, Bi_2O_3, and $BaSO_4$ with a calcium aluminate phase in a ratio of 2: 2: 1. The mixture was finally mixed with distilled water in a ratio of powder/water 2: 1, to achieve the consistency of cement paste.

BiodentineTR is composed of a highly purified C3S powder prepared synthetically from a mixture of powder constituents: SiO_2-16.9 %, CaO-62.9 %, and ZrO_2-5 %. C_2S and C_3S particles form 70 wt% of the above mixture's dehydrated powder. Biodentine does not contain $CaSO_4$, aluminate, or alumina ferrite. The liquid component is distilled water with the addition of $CaCl_2$ (Jafari and Jafari, 2017; Singh et al. 2014).

Resin-modified glass ionomer Fuji VIII (GC Corporation, Tokyo, Japan) was used for the restoration of all cavities, the composition of which is represented: powder: silicate glass and liquid: polyacrylic acid.

Design of the study

The study included 18 laboratory rats of the Wistar strain and 72 teeth, i.e. 36 first upper molars and 36 second upper molars. The animals were 10-11 weeks old and weighed an average of 190-280 g. During the experiment, all rats were provided with free access to food and water, a 12-hour shift of light and darkness, air temperature of 20-23°C, while the humidity was 60% ± 10%. Before the dental procedure, rats were anesthetized by induction of general anesthesia (Ketamine Hydrochloride Injection USP Rotexmedica-Germany at a dose of 50 mg/kg body weight). The dose of anesthetic was determined according to the body weight of the rats, which was measured for each rat individually. Immediately before the start of work, all teeth were mechanically cleaned of soft deposits with a soft brush and toothpaste and then disinfected with chlorhexidine digluconate (0.1% Chlorhexamed-Fluid, GlaxoSmithK, Buhl, Germany). Due to difficult access and reduced visibility of rat molars, only the first and second upper molars of rats were included in the experiment. A magnifying glass (4.5 ×, Zeiss, Oberkochen, Germany) was used for the same reasons. Rats were divided into two experimental groups, A and B, with 9 rats in each group.

* In group A, ALBO-CA was applied to the opening pulp of the first and second upper molar on the right and left sides.

* In group B, the tricalcium silicate cement Biodentine (Septodont, Saint Maur des Fosses, France) was used in the same way as ALBO-CA.

Class I cavity preparations on the occlusal surface of non-carious first and second upper molars were done with a technical micromotor and a sterile ISO 008 round diamond drill bit, with continuous water cooling, until the depth of the cavity was approximately half the size of the drill bit. After cavity preparation in one rat, a drill bit was replaced. Dental pulp was exposed with a sterile sharp endodontic explorer (DG16, Dental USA, Mc Henry, IL, USA), pulpal blood was removed with sterile cotton balls, and the cavity was washed with saline to remove possible blood residues and

dentin dust. After that, the tested materials (ALBO-CA and Biodentine) were applied to the pulp. The cavities were restored with glass ionomer cement (GC Fuji VIII, GC Corporation, Tokyo, Japan).

According to the study plan, the animals were sacrificed by intravenous injection of pentobarbital after 28 days of observation.

After separating the upper jaws with a surgical scalpel and scissors (decapitation), they were stored in 10% neutral buffered formalin, and the material was delivered to the Laboratory of the Department of Pathology of the Clinical Center in Banja Luka and prepared for pathohistological analysis.

Pathohistological analysis

Preparation of the material was started by decalcification of the jawbones in EDTA (ethylene-diamino-tetracycline-acetate) for 3 hours, after which the pulp sections were molded into the paraffin blocks. Cutting was performed with a microtome (every 4μm). Cross-section staining was performed by the hematoxylin-eosin method. For these purposes, we used a light microscope (Celestron Labs CB2000CF Compound Binocular Microscope).

The following were analyzed: the absence of bacteria, the presence of bacteria in 1/3 of the tooth pulp, the presence of bacteria in 2/3 of the tooth pulp, and the diffuse presence of bacteria in the pulp.

Furthermore, the inflammatory response of the pulp was evaluated, using the modified criteria of Accorinte et al. (2008): the absence of inflammation, i.e. none or a few scattered inflammatory cells, the presence of a low number of polymorphonuclear leukocytes, the presence of a high number of polymorphonuclear leukocytes and pulp necrosis.

The formation of the dentinal bridge at the end of the observation period was also taken into account as one of the possible indicators of the success of the therapy. Dentine bridge was quantified using the modified criteria of Accorinte et al. (2008) as formed and not formed.

Statistical Data Analysis

The Man-Whitney U test and the Chi square test were used for statistical analysis of the obtained results of direct pulp capping in rats of the Wistar strain.

RESULTS

The results of the histological analysis are shown in Tables 1 and 2, and Figures 1 and 2.

Analysis of the inflammatory response of the pulp of the teeth of rats in group A, which ALBO-CA was applied to, indicated the absence of inflammation in 63.9% of cases, the presence of a low number of polymorphonuclear leukocytes in 250% of cases, the presence of a high number of polymorphonuclear leukocytes in 8.3% of cases, and pulp necrosis in 2.8% of cases (Table 1).

After direct pulp capping of rat teeth with Biodentine in group B, inflammation was completely absent in 61.1% of cases, in 30.6% of cases the presence of a low number of polymorphonuclear leukocytes was noted, while in 8.3% of cases, the presence of a high number of polymorphonuclear leukocytes was noted. Pulp necrosis was absent (Table 1).

It is clear from Table 1 that both examined materials, experimental ALBO-CA (group A) and tricalcium silicate cement Biodentine (group B), gave the largest percentage of the absence of the inflammatory response of the pulp. In group B in which Biodentine was applied to the pulp of rat teeth, a slightly higher presence of a small number of polymorphonuclear leukocytes was observed, but the Mann-Whitney U test did not show a statistically significant difference in the inflammatory response of the pulp between the groups ($U = 640.00$; $Z = 0.105$; $p = 0.916$). Bacteria were not present in any of the samples.

Analysis of the formation of a dentinal bridge on the teeth of rats after 28 days shows that both examined materials in a higher percentage led to the formation of the dentinal bridge (Table 2, Figure 1 and 2). ALBO-CA material had a higher frequency of dentinal bridge formation (66.7%), while dentinal bridge formation was absent in 33.3% of cases.

After the application of Biodentine (group B), dentinal bridge formation occurred in 52.8%, while the same was absent in 47.2% of cases. The Chi-square test did not show a statistically significant difference in the formation of the dentinal bridge between the examined groups (Chi-square = 1,443; p = 0,230).

Table 1 Inflammatory response of rat tooth pulp after application of ALBO-CA and Biodentine

			Inflammatory pulp response				Total
			Absence of inflammation	Low no. polymor-phonuclear Le	High no. polymor-phonuclear Le	Necrosis	
Group	A	N	23	9	3	1	36
		%	63.9%	25.0%	8.3%	2.8%	100.0%
	B	N	22	11	3	0	36
		%	61.1%	30.6%	8.3%	0.0%	100.0%
Total		N	45	20	6	1	72
		%	62.5%	27.8%	8.3%	1.4%	100.0%

Table 2 Dentinal bridge formation of rat tooth pulp after application of ALBO-CA and Biodentine

			Dentine bridge		Total
			Formed	Not formed	
Group	A	N	24	12	36
		%	66.7%	33.3%	100.0%
	B	N	19	17	36
		%	52.8%	47.2%	100.0%
Total		N	43	29	72
		%	59.7%	40.3%	100.0%

Figure 1 Longitudinal section of dental pulp section, odontoblast layer with fully formed dentinal bridge with dentinal tubules continuous with surrounding dentin after direct pulp capping ALBO-CA, HE 400x

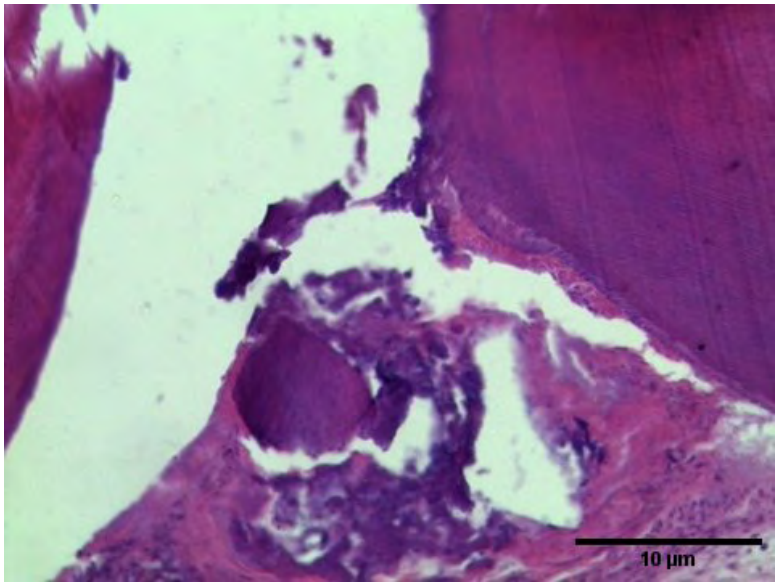

Figure 2 Section of the tooth, part of the pulp and dentin with a perforation of the pulp chamber of the tooth covering an incompletely formed dentin bridge (fibrin and calcifications of the dentin island) can be seen after directly covering the pulp with Biodentine HE 400x

DISCUSSION AND CONCLUSION

Pulp capping materials protect the vital pulp tissue threatened by caries or trauma. Most of the available pulp capping materials have numerous advantages and disadvantages (Da Rosa et al., 2018; El-Mal et al., 2019; Al-Sherbiny et al., 2020). Therefore, the studies on new pulp capping materials are still very current.

Recently, a new calcium aluminate-based material called ALBO-CA has been synthesized. According to our knowledge, there are only a few *in vivo* studies on the use of calcium aluminate cement in vital pulp therapy and pulpotomy procedures (Woodmansey et al., 2015; Walsh et al., 2018). Therefore, this study compared the efficacy of ALBO-CA and Biodentine as direct pulp capping materials. We selected Biodentine for comparison due to its so far excellent results as a pulp-capping agent.

Both animal and human teeth are usedto demonstrate the effects of pulp-capping materials onvital pulp tissue (Negm et al. 2017). Rats have significant advantages in terms of ethical and

economic reasons. The advantages of rats are their economic cost and good adaptation to life in laboratory conditions (Katica and Delibegović, 2019; Katica et al., 2020). In the last half a century, several studies have been published that have used rat molars to assess the response of pulp tissue to a direct pulp capping procedure or pulpotomy (Kramer et al., 2014; Kim et al., 2016).

According to the results of this study, both the examined materials, the newly synthesized ALBO-CA and Biodentine gave the absence of the inflammatory response of the pulp in most cases, indicating the biocompatibility of these materials. After the application of ALBO-CA, the inflammation of the pulp tissue was absent in a slightly higher number of cases, and a weaker presence of a small number of polymorphonuclear leukocytes was noted, but this difference was not statistically significant.

Good bond strength with the tooth also ensures lower micropermeability, which, in addition to working in aseptic conditions, resulted in complete absence of bacteria in all tested samples.

In the available literature, dealing with the problem of antimicrobial activity of calcium aluminate cement (Souza et al., 2013; Silva et al., 2014; Radović et al., 2019), calcium aluminates mainly exhibited a comparable antimicrobial effect with MTA, while in the study of Radović et al., the ALBO-CA material even exhibited a slightly greater antibacterial effect against E. coli compared to MTA. The antibacterial properties of Biodentine are attributed to its high pH, which is achieved by the action of hydroxyl ions on the surrounding tissue (Bhavana et al., 2015).

In this study, both examined materials after an observation period of 28 days, in a higher percentage led to the formation of a dentinal bridge. An interesting earlier study by Carmo et al. (2018), demonstrated the ability of several formulations of calcium aluminate cement (CACb) and Biodentine to form apatite crystals on their surface, after contact with PBS or deionized water for 14 days. Cement surfaces were analyzed using SEM, EDS-X, and FTIR.

The percentage of formed dentinal bridges was slightly higher after direct coating of the pulp with nanostructured calcium aluminate ALBO-CA, but without a statistically significant difference.

Many studies have evaluated the capacity of Biodentine in direct closure of exposed pulp in animals (Popović-Bajuć et al., 2014; Kim et al., 2016; El-Din et al., 2020; Souza et al., 2021) and humans (Jayanandan et. al., 2021). They reported good results for direct pulp capping with Biodentine.

In a study by Jayanandan et. al. (2021), performed was pulp capping with calcium hydroxide, mineral trioxide aggregate (MTA), Biodentine and EndoSequence root repair material (ERRM). 15 orthodontic patients requiring the extraction of four premolars (60 teeth in total) were included in the study. After closing the pulp, the teeth were extracted after 8 weeks. We analyzed the extracted teeth with cone beam computed tomography (CBCT) and histological sections to determine the quality of the dentinal bridge and pulp response. A dentine bridge was formed in 9/15 cases of teeth treated with MTA and ERRM (60%). In contrast, only 6/15 calcium hydroxide cases and 7/15 Biodentine cases showed complete bridging (40–46%). These differences were not statistically significant ($p > 0.05$), which is somewhat in line with our results because in this study Biodentine showed a lower quality of the dentine bridge in comparison to MTA and ERRM, although without a significant difference. There was also a slightly more pronounced inflammatory response of the pulp tissue after the application of Biodentine, as in our study, with the fact that here the MTA and ERRM groups differed significantly from the calcium hydroxide and Biodentine groups.

However, in the available literature, in most studies Biodentine showed either a similar effect to the materials with which it was compared in direct pulp coverage or, contrary to our knowledge, a more favorable therapeutic effect.

De Souza et al. (2021) evaluated the pulpal responses to mineral trioxide aggregate (MTA Angelus) and Biodentine, focusing on mineralized barrier

formation and inflammatory and degenerative events in 80 male Wistar rats. Lower first molars were mechanically exposed, covered with either MTA or Biodentine, and restored with silver amalgam. Teeth covered with gutta-percha and restored with silver amalgam served as a positive control, while untreated teeth served as a negative control. Pulp responses were evaluated after 14 or 21 days. Biodentinr and MTA showed satisfactory results, showing a milder inflammation response ($p<0.0001$) and a more pronounced formation of mineralized barriers ($p<0.0001$) compared to teeth covered with gutta-percha. Biodentine has shown favorable properties in vital pulp therapy, similar to MTA.

Biodentine was shown to be more successful after direct pulp capping of rabbit incisors compared to TheraCal LC in a study by Kayad et al. (2023). It had better results in terms of thickness and continuity of the dentin bridge, while both Biodentine and TheraCal LC had a similar inflammatory effect on the pulp.

Studies examining the dentinogenic potential of materials in which the basic component is calcium aluminate have shown good results. The direct pulp capping results for calcium aluminate obtained by this study can be compared with the results of the author Janković (2018) in which calcium aluminate cements showed a higher ability to form dentinal bridges compared to experimental calcium silicate cements and commercial MTA.

The findings of the authors Josipović (2020) and Radović (2019) also confirmed that the newly synthesized calcium aluminate-based material ALBO-CA was biocompatible, as well as having had the ability to induce calcified tissue after 30 days on sheep teeth after direct pulp coating. The authors attribute this finding to the ALBO-CA synthesis process, which results in higher reactivity of the material particles.

Several studies have considered the use of calcium aluminate cement in the pulpotomy procedure (Kramer et al., 2014; Woodmansey et al., 2015) and apicotomy procedures (Walsh et al., 2018). On that occasion, confirmed was the similar dentinogenic potential of calcium aluminate cement with MTA (Kramer et al., 2014; Walsh et al., 2018), similar effects on inflammation, pulp reaction, periodontal ligament, cement formation, and apical tissue healing in dogs (Walsh et al., 2018). The only exception is in the study by Woodmansey et al. (2015), where inflammation in samples with calcium aluminate cement Quick-Set was somewhat more pronounced compared to MTA, contrary to our findings, which the authors attributed to differences in the chemical composition of the material.

By the limitations of this study, ALBO-CA and Biodentine had similar effects on inflammation, pulp response, and formation of dental bridges in rats. That can be considered a reasonable therapeutic result. One limitation of this study was that pulp capping was performed on healthy, non-carious rat teeth. It is recommended to continue *in vivo* the research of nanostructured calcium aluminate cement on caries lesions on animal and human teeth.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception – OJ; Design – OJ, RA; Supervision - TA; Materials – OJ, IĐ; Data Collection and/or Processing – OJ, JK, JL, KV; Analysis and/or Interpretation – OJ, RA, JK, KV, IĐ; Literature Search – OJ, RA, TA, JL, KV; Writing Manuscript – OJ; Critical Review – TA, JL.

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PATOHISTOLOŠKI ODGOVOR ZUBNE PULPE NAKON PREKRIVANJA KALCIJ ALUMINATNIM CEMENTOM I BIODENTINOM: EKSPERIMENTALNO ISTRAŽIVANJE NA GLODARIMA

SAŽETAK

Cilj istraživanja jeste evaluacija odgovora zubne pulpe na prekrivanje eksperimentalnim nanostrukturnim kalcij aluminatnim cementom (ALBO-CA) i biodentinom, trikalcij silikatnim cementom (Septodont, Saint Maur des Fosses, Francuska). Na 72 zuba kod 18 Wistar štakora su pripremljeni kaviteti 1. klase (36 prvih gornjih molara i 36 drugih gornjih molara). Kod 36 zuba, zubna pulpa je prekrivena s eksperimentalnim ALBO-CA (grupa A), a kod 36 zuba sa biodentinom (grupa B). Svi kaviteti su ispunjeni sa staklenim jonomerima. Životinje su nakon 28 dana žrtvovane nakon čega su analizirani upalni odgovor zubne pulpe, prisustvo bakterija i stvaranje dentinskog mosta. Nakon 28 dana nije uočeno prisustvo bakterija. Mann-Whitney U test nije dokazao statistički signifikantnu razliku u upalnom odgovoru zubne pulpe između grupa ($U = 640.00$; $Z = 0.105$; $p = 0.916$). Chi-square test nije dokazao statistički signifikantnu razliku u formiranju dentinskog mosta između ispitivanih grupa (Chi-square = 1.443; p = 0,230). ALBO-CA i biodentine su imali slično djelovanje na upalu, odgovor zubne pulpe i formiranje dentinskih mostova kod štakora.

Ključne riječi: ALBO-CA, prekrivanje zubne pulpe, štakori, trikalcij silikat

RESEARCH ARTICLE

MORPHOMETRIC STUDY OF NEUROCRANIUM IN DIFFERENT MALE CHICKEN BREEDS RAISED IN TÜRKIYE

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ABSTRACT

In the poultry industry, male chicks serve various purposes, depending on the specific production system and market demands, mostly for meat production. In the systems where male chicks are raised for meat production, they may be slaughtered when about six to nine weeks old. The aim of this study was to assess the male neurocranium of different chicken breeds through linear morphometric parameters. The morphometric study was undertaken on 70 skulls of the chicken breeds: 12 Ataks (AT), 12 Sasso (SS), 12 Lohmann Brown (LB), 12 Broiler (BR) and 10 Leghorn (LG) raised in Türkiye. In total, eight linear measurements were determined in accordance with the anatomical structure of the chicken skulls, and two skull indices were calculated. The data revealed that the length and width of the neurocranium of five laying hen breeds are quite similar and longerthan for the Broiler breed (BR). The longest and widest skull belong to the (SS) breed with 43.01±4.05mm and 29.12±2.61mm, respectively. The smallest skull belongs to (BR) breed with length and width of 32.07±3.38mm and 22.44±2.44mm, respectively. The cranial length (Cl) in the (AT) breed is statistically different from the (BR) breed, $p<0.001$ and (LG) breed, $p<0.05$. The maximum width of the cranium in all breeds is statistically different, $p<0.001$ from the (BR) breed, and the (SS) is statistically different from the (BR), $p<0.001$ and (LG) breed, $p<0.01$. In conclusion, the data from this study can serve for further research in the similar fields and for the comparative studies on galliform species.

Keywords: Cranium, chicken breed, linear parameters, male chicken

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INTRODUCTION

The domestic chicken (*Gallus gallus domesticus*) has been selectively bred for many years, and hundreds of breeds have been developed and crossed with other breeds. In Türkiye, some of these breeds are bred mainly for egg production, like Leghorn, Lohman Brown meat production, like Broiler as well as double-purpose breeds, for eggs and meat, like Sasso breed (Yıldırım and Kaya, 2017; Tutkun et al., 2018).

In the poultry industry, male chicks, also known as cockerels, serve various purposes, depending on the specific production system and market demands, mostly for meat production, but also for breeding programs, pet food, by-products, research and education.

In the systems where male chicks are raised for meat production, they may be slaughtered when about six to nine weeks old, depending on the growth rate and market demands, but usually after eight weeks of age.

The bones of the head skeleton consist of the thin plates, which are extensively pneumatised (Baumel, 1993; Bahadir, 2002; Feduccia, 1975). This is facilitated by the fusion of these bones in the relatively early stages of growth (Plateau and Foth, 2021). The boundaries between the bones of the avian neurocranium are almost indistinguishable (Koch, 1973; Baumel, 1993). The avian skull consists of two parts, the cranium (neurocranium) and the facial skeleton (viscerocranium). The skull of an avian has several adaptations to be light and aerodynamic to facilitate flight, or to ensure food intake and its swallowing (King AS, McLelland, 1975; Nickel et al., 1977; König et al., 2016).

Chicken neurocranium is the structure very well ossified, which protects the brain and makes connection with the vertebral column. Especially, the neurocranium structures have a great importance in taxonomy, evolutionary science and the comparative anatomy studies (Marugán -Lobon J, Buscalioni, 2009). Different morphological and morphometric studies are made on the avian head skeleton (Markos et al., 2024; Sridevi et al., 2020; Gündemir et al., 2020b; Verdiglione and Rizzi, 2018; Ilgun et al., 2016; Degrange and Picasso, 2010; Acosta Hospitaleche, 2009; Acosta Hospitaleche et al., 2009; Acosta Hospitaleche and Tambussi, 2006; Cakir, 2001). Other studies are focused on evolutionary and functional avian anatomy (Tokita et al., 2017; Marugán-Lobón and Buscalioni, 2006; Gussekloo et al., 2001). Additional studies are focused on the comparation and diversity (Pecsics, 2023; Zusi, 1993), or the detection of the sexual dimporphism, even if the birds do not show such clear sexual dimorphism as mammals (Szara et al., 2022a; Szara et al., 2022b; Pazvant et al., 2022; Gündemir et al., 2020a; Verdiglione and Rizzi, 2018; Rathert et al., 2017; Dillon, 2014).

Craniometry performed through different linear measurements of the head skeleton is a method that is commonly used in taxonomic studies of vertebrates, comparative anatomy, zooarchaeology, etc. (Jashari et al., 2022; Duro et al., 2021; Gündemir, 2019; Avdić et al., 2013; Bärmann et al., 2013).

For very long times, chickens have been used as a model organism for the study of the vertebrate development. The comparative morphology and morphometry of the juvenile avian skulls are poorly known, and the literature is limited. Ever since the different studies wereundertaken, their morphological variability and morphometry has never been quantified in detail for different chicken breeds.

The aim of this study was to assess the male neurocranium of different chicken breeds through linear morphometric parameters.

MATERIALS AND METHODS

Samples

The morphometrical study was undertaken on 70 skulls of six different chicken breeds: 12 Ataks (AT), 12 Sasso (SS), 12 Lohmann Brown (LB), 12 Broiler (BR) and 10 Leghorn (LG), raised in Türkiye. The skulls of two-month old male chicken

were received in the slaughterhouse after they had been slaughtered. Samples with pathological morphological disorders were excluded from the study. All collected skull samples were sent to the Animal Anatomy lab and were subjected to maceration to remove the skin, muscles and soft tissues. The skulls were then boiled for 30 minutes and soaked in 35% hydrogen peroxide for 10 minutes to remove fatty, soft tissues and the splanchnocranium parts, and, finally, the clean neurocraniums were allowed to dry for 10 days at room temperature.

The eight linear measurements were determined in accordance with the anatomical structure of the chicken skulls, as described by Baumel (1993), Ino et al. (2008), Gusselkoo et al. (2001), Hall et al. (2009), Onar et al. (1997), Singh et al. (2015) and also the Nomina Anatomica Avium (NAA) (Baumel et al., 1993). The measurement points defined on the chicken neurocranium are shown in Figure 1 and 2. All linear measurements were taken in millimeters with a digital calliper (±0.2 mm). The photographs of the samples were made with a Samsung photo camera NX210 20.3 MP. The linear measurements are:

1. Cranial length (Cl): Length between Prominentia cerebellaris and the middle point of Frontonasal suture.
2. Maximum width of neurocranium (Mwn): Width between the bases of the Postorbital processes.
3. Maximum width of the neurocranium base (Mwnb): Width between the lateral edges of the Paraoccipital processes.
4. Nuchal surface height (Nsh): Height between the ventral margin of Foramen magnum and Crista nuchalis transversus in the midline.
5. Foramen magnum height (Fmh): Height between the middle of dorsal and ventral margins of Foramen magnum.
6. Foramen magnum width (Fmw): Maximum width of Foramen magnum.
7. Occipital condyle height of (Och): Height between the middle of dorsal and ventral margins of occipital condyle.
8. Occipital condyle width (Ocw): Maximum width of occipital condyle.

From these measurements were calculated also the cranial and Foramen magnum index according to the formulas:

Cranial index (CrI)= Maximum width of neurocranium x 100 /Cranial length

Foramen magnum index (FmI)= Foramen magnum height x 100 /Foramen magnum width

Statistical analysis

The statistical analysis was performed using the SPSS 22 package program, which calculated the mean values, standard deviations, minimum, maximum and P values for all measurements. ANOVA was used for comparison between groups.

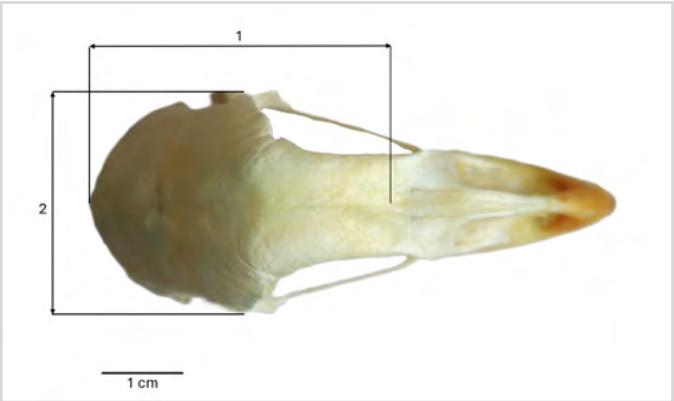

Figure 1 Linear measurements on the chicken skull (dorsal surface). Cranial length (Cl), 2. Maximum width of neurocranium (Mwn)

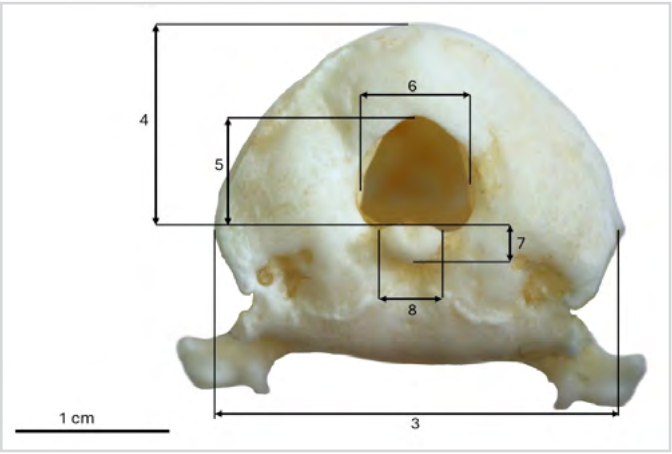

Figure 2 Linear measurements on the chicken skull (nuchal surface). Maximum width of the neurocranium base (Mwnb), 4. Nuchal surface height (Nsh), 5. Foramen magnum height (Fmh), 6. Foramen magnum width (Fmw), 7. Occipital condyle height of (Och), 8. Occipital condyle width (Ocw)

RESULTS

The linear measurements of the neurocranium for six breeds of male chicken are presented in Table 1 as mean, standard deviation and minimum and maximum values.

Table 1 Morphometric linear parameters in male skulls of different breeds of chicken

Measure-ments	Data	AT	LB	SD	SS	BR	LG
(mm)	**N**	**12**	**12**	**12**	**12**	**12**	**10**
	Min	38.21	40.86	35.19	39.04	26.62	36.49
Cl	Max	45.95	44.56	44.55	53.79	39.63	39.56
	Mean	41.88	42.26	39.21	43.01	32.07	38.11
	Stand. dev	2.39	1.20	2.61	4.05	3.38	1.05
	Min	26.30	26.87	26.31	26.80	17.86	23.29
Mwn	Max	28.54	28.87	29.22	35.89	27.28	28.81
	Mean	27.29	27.80	27.48	29.12	22.44	26.46
	Stand. dev	0.69	0.62	0.93	2.61	2.44	1.51
	Min	26.40	27.17	25.50	26.51	24.14	25.83
Mwnb	Max	28.18	28.81	29.30	34.99	28.75	28.12
	Mean	27.55	27.77	27.22	28.68	25.75	26.85
	Stand. dev	0.58	0.51	1.22	2.47	1.34	0.70
	Min	10.80	12.66	13.01	12.47	10.11	11.40
Nsh	Max	13.16	14.23	13.96	29.23	14.46	13.30
	Mean	11.78	13.40	13.36	15.36	11.83	12.34
	Stand. dev	0.64	0.48	0.30	4.69	1.19	0.66
	Min	6.86	6.96	6.88	7.44	6.17	3.58
Fmh	Max	8.03	8.34	8.53	8.99	8.24	7.43
	Mean	7.43	7.51	7.52	8.11	7.01	6.23
	Stand. dev	0.42	0.40	0.53	0.44	0.59	1.06

	Min	7.85	8.18	7.19	7.76	6.99	5.85
Fmw	Max	9.92	10.38	9.03	9.23	8.53	8.14
	Mean	8.80	8.87	8.60	8.71	7.61	7.42
	Stand. dev	0.52	0.64	0.57	0.45	0.43	0.62
	Min	2.41	2.71	2.20	2.82	2.07	2.63
Och	Max	3.52	3.51	3.22	3.46	3.93	4.05
	Mean	3.02	2.93	2.84	3.05	2.85	3.05
	Stand. dev	0.30	0.24	0.30	0.18	0.51	0.43
	Min	3.37	3.63	3.57	3.85	4.10	3.67
Ocw	Max	4.82	4.64	4.74	5.66	5.29	4.23
	Mean	4.14	4.19	4.19	4.44	4.79	3.93
	Stand. dev	0.42	0.26	0.36	0.46	0.31	0.17

The data show that the length and width of the neurocranium of five laying hen breeds are quite similar and longer for the broiler breed (BR).

The other linear measurements show less differences between the breeds. The longest and widest skull belong to the (SS) breed with 43.01±4.05mm and 29.12±2.61mm, respectively. The smallest skull belongs to (BR) breed with the length and width of 32.07±3.38mm and 22.44±2.44mm, respectively.

Table 2 shows the ANOVA results of comparisons of the neurocranium linear parameters between the chicken breeds.

Table 2 ANOVA results of linear morphometric parameters in male skulls of different chicken breeds

Measurements	Breeds	LB	SD	SS	BR	LG
	AT	0.9994	0.1652	0.9103	p<0.001	p<0.05
Cl	**LB**		0.07778	0.9838	p<0.001	p<0.001
	SD			p<0.05	p<0.001	0.9332
	SS				p<0.001	p<0.001
	BR					p<0.001
Mwn	**AT**	0.9743	0.9998	0.09256	p<0.001	0.8585
	LB		0.9969	0.3929	p<0.001	0.4312
	SD			0.1693	p<0.001	0.7185
	SS				p<0.001	p<0.01
Mwnb	**BR**					p<0.001
	AT	0.9985	0.9901	0.318	p<0.05	0.8275
	LB		0.9096	0.5658	p<0.01	0.5966
	SD			0.09458	0.09228	0.988
Nsh	**SS**				p<0.001	p<0.05
	BR					0.3963
	AT	0.3901	0.4238	p<0.01	1	0.9884

	LB		1	0.1986	0.425	0.8275
Fmh	**SD**			0.1775	0.4599	0.853
	SS				p<0.01	p<0.05
	BR					0.9924
	AT	0.9996	0.9994	0.07374	0.5081	p<0.001
Fmw	**LB**		1	0.1524	0.3171	p<0.001
	SD			0.159	0.3064	p<0.001
	SS				p<0.001	p<0.001
	BR					p<0.05
Och	**AT**	0.9996	0.9427	0.9982	p<0.001	p<0.001
	LB		0.8234	0.9763	p<0.001	p<0.001
	SD			0.9966	p<0.001	p<0.001
	SS				p<0.001	p<0.001
Ocw	**BR**					0.9697
	AT	0.984	0.7756	0.9999	0.8077	1
Cl	**LB**		0.9877	0.9468	0.9921	0.9566
	SD			0.6452	1	0.6906
	SS				0.683	1
	BR					0.7254
Mwn	**AT**	0.9997	0.9993	0.3326	p<0.001	0.7299
	LB		1	0.5118	p<0.01	0.5493
	SD			0.5431	p<0.01	0.5195
	SS				0.147	p<0.05
	BR					p<0.001

The cranial length (Cl) in the (AT) breed is statistically different from (BR) breed, $p<0.001$ and (LG) breed, $p<0.05$, but the (SS) breed is statistically different from the (LB) breed, $p<0.001$, (BR) breed, $p<0.001$ and (LG) breed, $p<0.001$.

The maximum width of the cranium of all breeds is statistically different, $p<0.001$ from the (BR) breed, and the (SS) is statistically different from the (BR), $p< 0.001$ and (LG) breed, $p<0.01$.

No statistical differences are shown in the Occipital condyle height of (Och).

The parameters for the BR and LG breeds are the most statistically different from all other breeds in this study.

Based on the cranial index (Table 3), the (AT) and (LB) breeds have the longest skulls compared to the (SD), (BR) and (LG) breeds, which were more quadrate in shape.

Table 3 Skull indices

Indices	**Breeds**					
	AT	**LB**	**SD**	**SS**	**BR**	**LG**
Cranial Index	65.14	65.79	70.08	67.71	69.97	69.43
Foramen magnum Index	84.43	84.66	87.38	93.15	92.11	83.94

From the data of the Foramen magnum index, the (SS) and (BR) breed have Foramen magnum more circular than the others, while the (LG) breed had the shortest parameters of Foramen magnum, and its shape was more triangular.

DISCUSSION AND CONCLUSION

The chicken neurocranium is small, compact and very well-ossified structure with the very smooth external surface (Baumel, 1993; Nickel et al., 1977; König et al., 2016; Süzer et al., 2018). Based on the study of Plateau and Foth (2021), the neurocranium of the chicken is fully mature in the first months of their life, which is demonstrated by the invisibility of the sutures between the bones, especially in the nuchal and frontal areas.

For decades, chickens have been used as a model organism for the study of vertebrate development, but their morphological and morphometric variability has never been quantified, and the skull anatomy of the breeds in comparison to fowl has never been described (Davey and Tickle, 2007).

The linear parameters based on well- defined structures of the chicken neurocranium have great importance in assessing the skull dimensions, their shape and type in order to compare it among the breeds, or to compare it with other avian species.

The results of this study present some crucial linear parameters of the neurocranium in six different breeds of male chicken which can help to assess the skull dimensions, predict the growth dynamic and also be used in the taxonomic studies.

In general, the shape and size of the male chicken neurocranium measured in this study show that the larger skull belongs to the double production-breeds like Sasso (SS) and the smallest, the Broiler breed, and this is significantly affected bythe genotype also in all studied linear parameters (Verdiglione and Rizzi, 2018).

There is not much information in the literature about the morphometrical parameters of the poultry species skulls in general, and the comparisons with other poultry species are limited.

The study shows full ossification skulls with the invisible sutures in all six chicken breeds.

Foramen magnum, in almost a triangular shape was positioned in the centre of the nuchal surface, and its width ranged from 27.64% in LG to 31.95% in AT, LB and 29.53% in BR. This demonstrates a big difference between laying hens and broilers, which means the opening for the spinal cord is larger in egg-producing chicken in compare to the broilers. The data from this study on male chicken are a bit different in comparison with those of *Amazona aestiva* species, which represents 22.6% of the skull maximum caudal width, and of *Diopsittaca nobilis* species with 20.2% (Souza et al., 2017).

Based on the measurements of the length and maximum width of the neurocranium of the male chickens for the egg-producing part of the study, which ranged from 26.46 mm to 29.12 mm, we can say that the data are quite comparable with Padovana chicken weighing 1798 g, and the skull weight was 26.3 mm (Verdiglione and Rizzi, 2018). This is potentially another argument in favor of full ossifications of the skull in the age of about eight to ninth weeks. These data can support the similarities among the avian neurocraniums, mostly in domesticated species, which can be explained by the domestication process (Stange at al., 2018).

In conclusion, the data from this study can serve for further research in the similar fields and for the comparative studies on galliform species.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest to declare.

CONTRIBUTIONS

Concept – SD, NM; Design – SD, BU, BC; Supervision – SD, BCC; Resources –BU, NM, BC; Materials – AK, BC; Data Collection and Processing – BU, NM; Analysis and Interpretation – BCC, BU, NM; Literature Search – BC, BCC, AK; Writing Manuscript – SD, BCC; Critical Review – BU, NM, BC.

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MORFOMETRIJSKA STUDIJA NEUROKRANIJUMA KOD RAZLIČITIH SOJEVA MUŠKIH PILIĆA UZGOJENIH U TURSKOJ

SAŽETAK

U peradarstvu se muški pilići koriste u različite svrhe u ovisnosti od specifičnog sistema proizvodnje i zahtjeva tržišta, uglavnom za proizvodnju mesa. U sistemima u kojima se muški pilići uzgajaju zbog proizvodnje mesa, pilići se mogu klati u uzrastu od šest do devet sedmica. Cilj našeg istraživanja je procijeniti neurokranijume muških pilića različitih sojeva korištenjem linearnih morfometrijskih parametara. Proveli smo morfometrijsko istraživanje na 70 lubanja različitih sojeva pilića: 12 Ataka (AT), 12 Sasso (SS), 12 Lohmann Brown (LB), 12 Broiler (BR) i 10 Leghorn (LG) pilića uzgojenih u Turskoj. Obavljeno je ukupno osam linearnih mjerenja prema anatomskoj strukturi pilećih lubanja, pri čemu su izračunata po dva lubanjska indeksa. Podaci su pokazali da su dužina i širina neurokranijuma kod pet vrsta pilića dosta slični međusobno i da su veći nego kod soja Broiler (BR). Najduža i najšira lubanja pripadaju soju SS i iznose 43.01±4.05 mm, odnosno 29.12±2.61 mm. Najmanja lubanja pripada soju BR sa dužinom i širinom od 32.07±3.38 mm, odnosno 22.44±2.44 mm. Kranijalna dužina (Cl) kod soja AT se statistički signifikantno razlikuje u odnosu na soj BR, $p<0.001$ i soj LG, $p<0.05$. Maksimalna širina kranijuma kod svih sojeva se statistički signifikantno razlikuje, $p<0.001$, u odnosu na soj BR sa statističkom signifikantnošću od $p<0.001$ za sojeve SS i BR i $p<0.01$ za LG. Možemo zaključiti da podaci iz ovog istraživanja mogu biti korišteni za daljnja istraživanja u sličnim poljima, kao i za komparativne studije galiformnih vrsta.

Ključne riječi: Kranijum, linearni parametri, muški pilići, vrsta pilića

RESEARCH ARTICLE

GROSS ANATOMY AND HISTOLOGY OF THE URINARY SYSTEM OF THE SPUR-WINGED GOOSE (*PLECTROPTERUS GAMBENSIS*)

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ABSTRACT

This research aimed to investigate the gross anatomy and histology of the urinary system of the Spur-winged goose. A total of 10 matured healthy Spur-winged goose, 5 males and 5 females were used for this study. These birds are collected from a commercial poultry farm in Damaturu, Yobe State, Nigeria and transported to the Gross and Histology Postgraduate Research Laboratory of the Department of Veterinary Anatomy, University of Maiduguri, Nigeria. The birds were acclimatized for 10 days, before euthanized. The abdominal cavity of the birds was exteriorized to grossly observe the kidneys, ureters and cloaca before harvested for microscopic study. Grossly, the kidney appeared brownish, dorso-laterally flattened with three distinct lobes. The ureter appears as a tubular vessel emanating from the kidneys and enters the cloaca at the dorsomedial aspect. The cloaca was observed as a common organ that connects the urinary, reproductive and digestive system. Histologically, the kidney parenchyma was divided into renal cortex and medulla, containing the central vein and renal corpuscles respectively. The lamina epithelia of the ureter were lined with a pseudostratified columnar epithelium containing loose connective tissues and lymphoid cells. The mucosa of the cloaca possesses a short and slender villi projection. Currently, there is no baseline information regarding the anatomic characteristics of the urinary system of the Spur-winged goose. Therefore, this present study serves as firsthand information on the anatomy of the urinary system of the Spur-winged goose.

Keywords: Avian, cloaca, kidney, ureter, waterfowl

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INTRODUCTION

Geese are among water birds of the family Anatidae. The Anatidae family is a biological family of birds that includes geese, swans and ducks (Makram, 2018). It has many subfamilies, those related to geese and swans are Plectropterinae, Anserinae, and Tadorninae respectively (Johnsgard, 2010). Geese are regarded as an important species economically due to their resistance to most avian diseases, ability to tolerate a wide range of adverse conditions, and large egg size (Jalaludeen et al., 2004; Patki et al., 2012). Generally, geese are herbivorous, and are monogamous breeders. There are more than 50 wild hybrids from wild species that undertake annual migrations (Makram, 2018). They are excellent producers of eggs and meat from very low-quality waste products, and therefore large expenses are not needed for their management (Dekaet al., 2015).

The Spur-winged goose (*Plectropterus gambensis*) is considered members of the aquatic bird family (Anatidae), which is classified as one of the 10 subfamilies (Plectropterinae). Africa is the native home of this species where it's found in a number of African countries like South Africa, Tanzania, and Algeria (Daniels, 2008), but reported to be extinct in Egypt (Makram, 2018). They are also among the groups of large African waterbirds (CRC, 2008). The characteristic features of the Spur-winged goose include a body covered with black feathers, white patches around the eyes, face and wings respectively. They have long pinkish legs adapted for swimming. The male can be easily distinguished from female, by the presence of a large red facial patch that extends back from the red bill and presence of a knob at the base of the upper mandible (Ogilvie and Young, 2004). Generally, Spur-winged goose prefer quiet stretches of riverbanks and wetlands for breeding, and are also among the migratory birds designated to be conserved by the Conservation of African-Eurasian Migratory Water birds (AEWA) (Warwik, 2002).

The urinary system of birds comprises paired kidneys, ureters and a cloaca (Mobiniand Abdollahi, 2016). The kidneys are reddish brown, elongated and flattened (Singh et al., 2023). Consequently, each ureter opens into the urodeum of the cloaca, serving as the passage for urine to exit the body (Alabdallah, 2022). The cloaca is a common organ that connects the urinary, digestive, and reproductive system in birds, which consists of three compartments; coprodeum, urodeum and proctodeum respectively (Joshi and Meshram, 2018). The renal system of birds is considered very special among the vertebrate species (Echols, 2006), due to the presence of two types of nephrons (Bacha and Bacha, 2012), absence of renal pelvis (Dhyaa et al., 2014), and urinary bladder (PourhajiMotab and Rasuli, 2021) except in Ostrich and Rheas due to separate storage of urine and feces (Al-Ajeely and Mohammed, 2012). Presently, there are very few valuable reports peculiar to morphologic characteristics of the urinary tract of waterfowl species, despite the numerous existing literatures on the general anatomy of avian urinary systems. The present study was aimed at investigating the gross anatomy and histology of the urinary system of the Spur-winged goose with emphasis on the kidneys, ureters and cloaca respectively.

MATERIALS AND METHODS

Ethical consideration

This research work was approved by the University of Maiduguri Animal Use and Ethics Committee (AUEC). The research approval code (AUP-R004/2023) was received from the University of Maiduguri Animal Use and Ethics Committee (AUEC).

Animals

This study was conducted on 10 matured healthy Spur-winged goose, 5 males and 5 females. These birds were collected from a poultry commercial farm and transported to the Gross and Histology Postgraduate Research Laboratory of the Department of Veterinary Anatomy, University of Maiduguri, Nigeria. The birds were allowed to acclimatize for 10 days in a special wooden

cage. Commercial poultry diet and water was provided ad libitum. According to a technique previously described by Majama et al. (2016), the body weight of the geese was recorded using an electronic balance just prior to euthanasia. Euthanasia was achieved using ketamine (25mg/kg) and xylazine (5mg/kg) administered through the wing vein of matured geese, as described by Durrani et al. (2008).

Gross study

The birds were placed on a dorsal recumbency on an examination table. The abdomen was carefully dissected with a sterile scissors and scalpel blade. Blood, fat, and other tissues attached to the abdominal structures were gently removed to exteriorize the urinary tract of the bird. Photographs of the organs at different anatomical planes were taken using a Nikon D90 digital camera.

Histological study

According to Winsor (1994), about 0.5-centimeter square of each of the kidney, ureter and cloaca tissues were collected immediately after gross examination. These tissues were fixed in 10% formalin for 48 hours, dehydrated with graded levels of ethyl alcohol (70%, 90%, and 100%), and embedded in paraffin wax to form tissue blocks. The blocks were then sectioned at 4µm thickness, placed on glass slides and stained with hematoxylin and eosin (H&E). Thereafter the slides were viewed using DB2-180M digital biological microscope at different magnifications (×100, and ×400), and relevant photomicrographs of the sections were taken using Scope image version 9.0 (AD 2.0) software.

RESULTS

The gross study of the kidney of the Spur-winged goose showed the kidney appears as a brownish-flattened organ located intra-abdominally, occupying the synsacral fossa (Figure 1). The kidney is characterized by the presence of cranial, middle and caudal lobes, respectively. The ureter was observed as a tubular organ emanating from the kidneys and entering the cloaca at the dorsomedial aspect. The cloaca was observed as a common organ that connects the urinary, reproductive and digestive system (Figure 2).

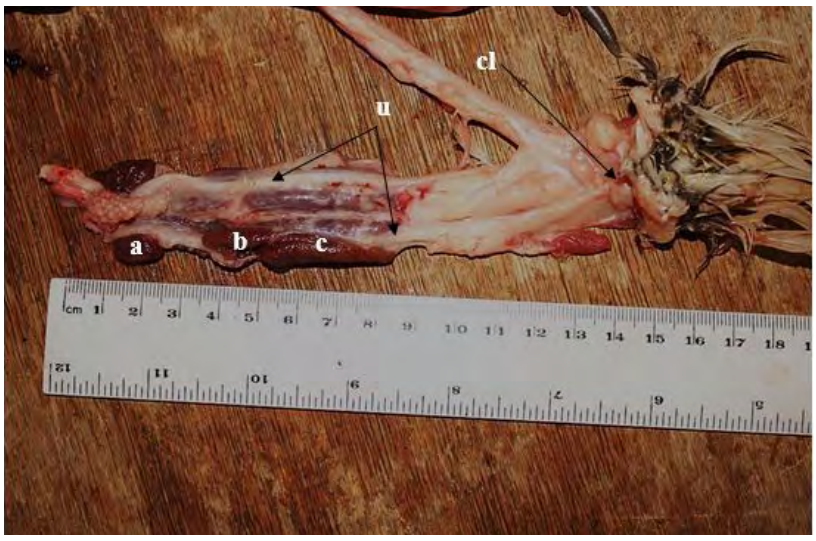

Figure 1 A Photograph showing the gross appearance of some organs related to the urinary system within the abdominal cavity of the Spur-winged goose. (a) Heart, (b) Lungs, (c) Ovary, (d) Kidney and (e) Intestine

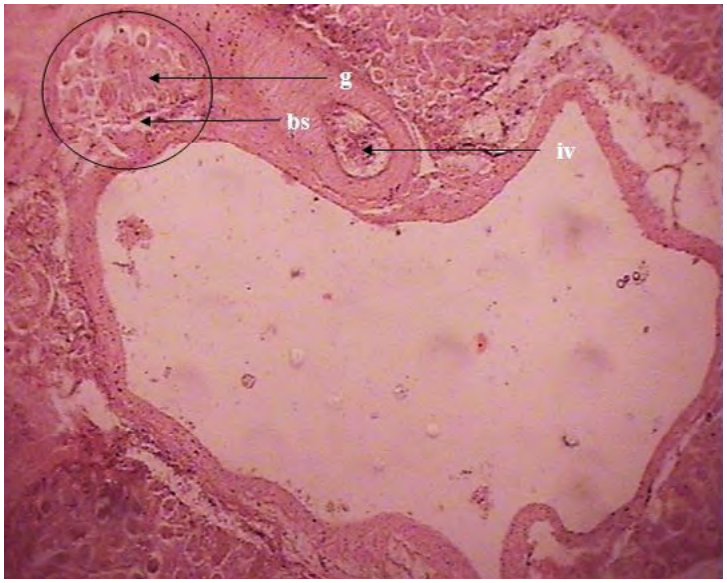

Figure 2 A Photograph showing the dorsal view of the kidney with distinct (a) cranial lobe, (b) middle lobe, (c) caudal lobes, (u) ureters, and (cl) cloaca

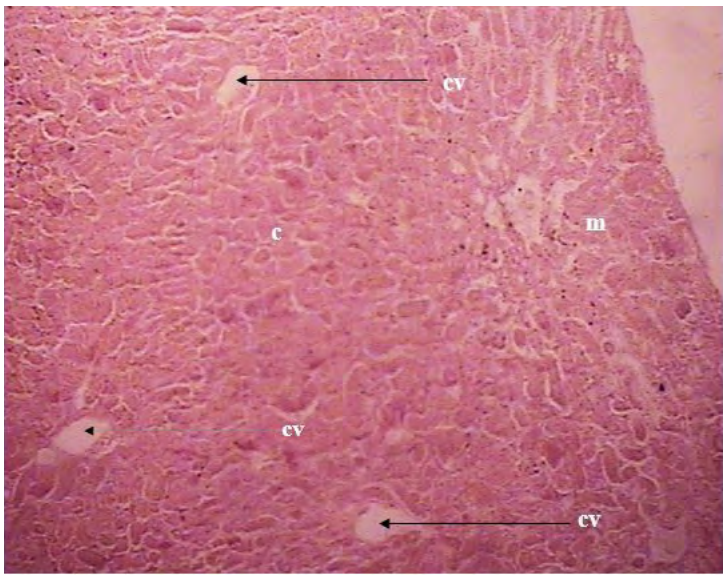

Figure 3 Photomicrograph of the kidney parenchyma: showing the (cv) central vein, (c) cortex, (m) medulla. H&E stain 100x

The histological section of the kidney showed a cortical and medullary region, and a central vein seen at the cortical region (Figure 3). The renal corpuscle consists of an outer Bowman's capsule, separated from the glomerulus by a centrally located space (Bowman's space). Intralobular vein is also visible (Figure 4). The glomerulus consists of tightly packed mesangial cells, surrounded by glomerular capillaries and podocytes (Figure 6). The kidney parenchyma consists of the proximal and distal tubules with an intralobular vein (Figure 7). The histological sections of the ureter showed the mucosa distinct layers; stratified transitional epithelial cells at the lamina epithelialis, the lamina propria was seen containing loose connective and lymphoid tissues, and the tunica muscularis was also seen (Figure 8). The tunica musculari swas seen having smooth muscle fibers arranged in outer longitudinal, middle circular and inner longitudinal muscle fibers at the urodeum. The cloacal mucosa was characterized with pseudostratified epithelium possessing projecting villi. The lamina propria possesses loose connective tissues (Figure 9).

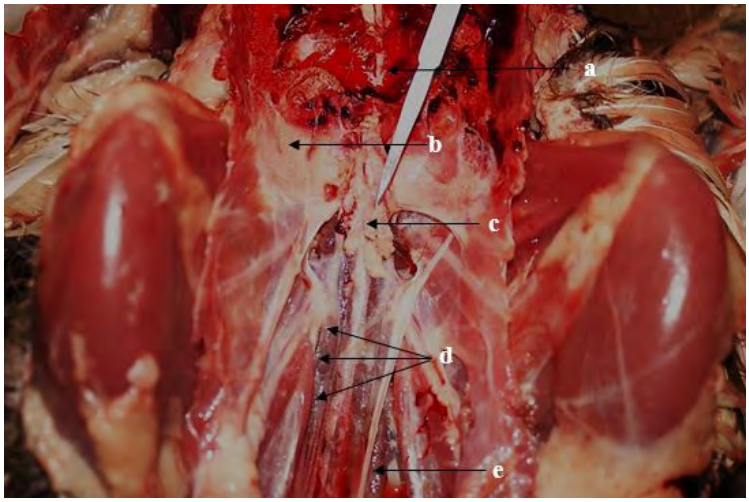

Figure 4 Photomicrograph of the kidney parenchyma showing: (circle) renal corpuscle, (g) glomerulus, (bs) Bowman's space and (iv) intralobular vein H&E stain 100x

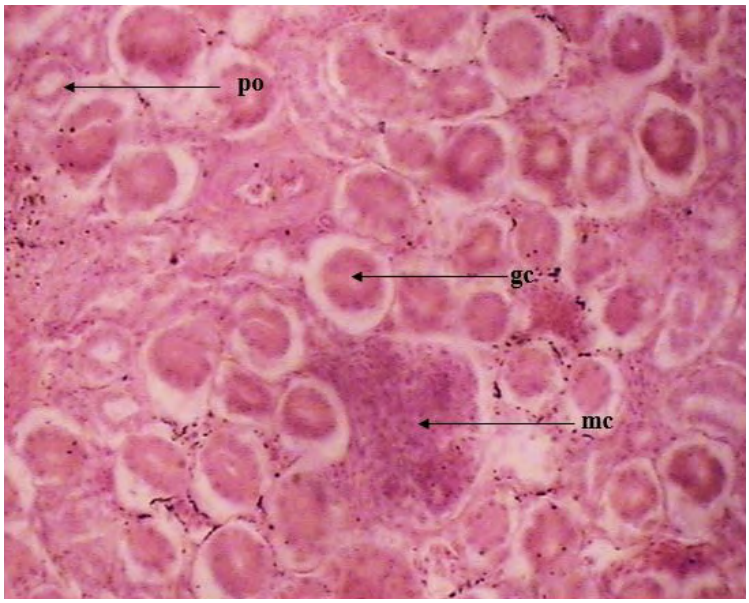

Figure 5 Photomicrograph of the glomerulus and Bowman's capsule showing: (gc) renal glomerular capillaries, (mc) mesangial cells, (bs) Bowman's space, and (pbs) parietal layer of Bowman's space. H&E stain 100x

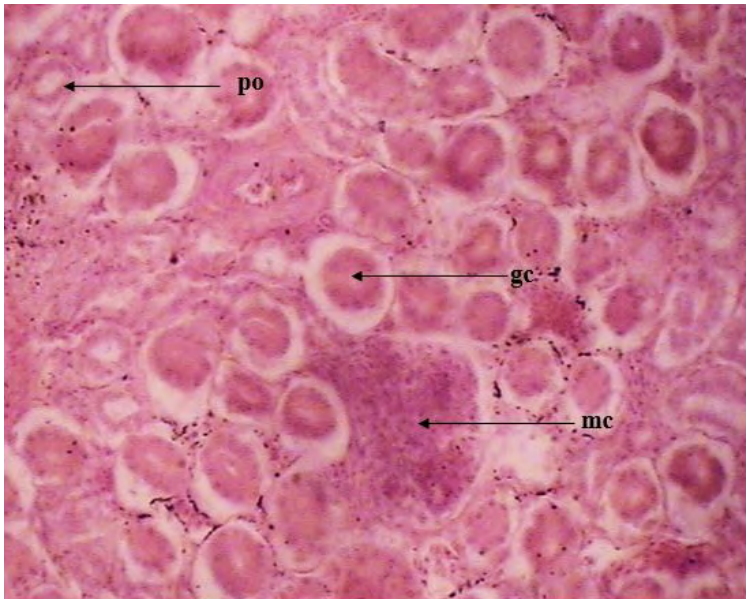

Figure 6 Photomicrograph of the glomerulus showing: (gc) glomerular capillaries, (mc) mesangial cells and (po) podocytes H&E stain 400x

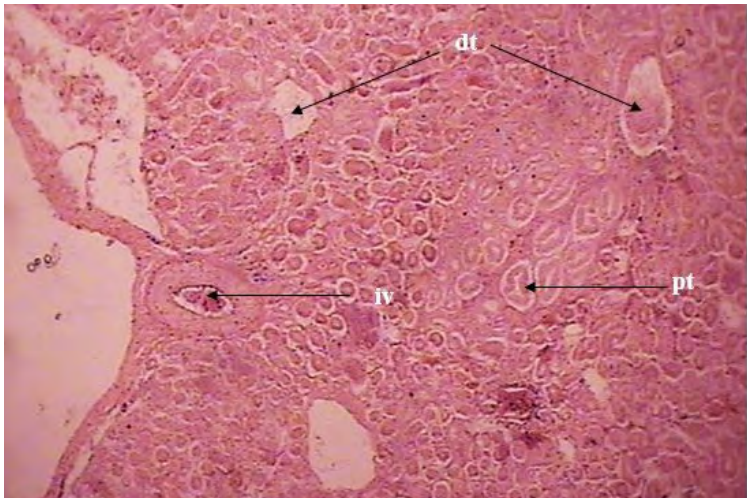

Figure 7 Photomicrograph of kidney parenchyma showing: (dt) distal tubules, (pt) proximal tubule, and (iv) intralobular vein H&E stain 100x**.**

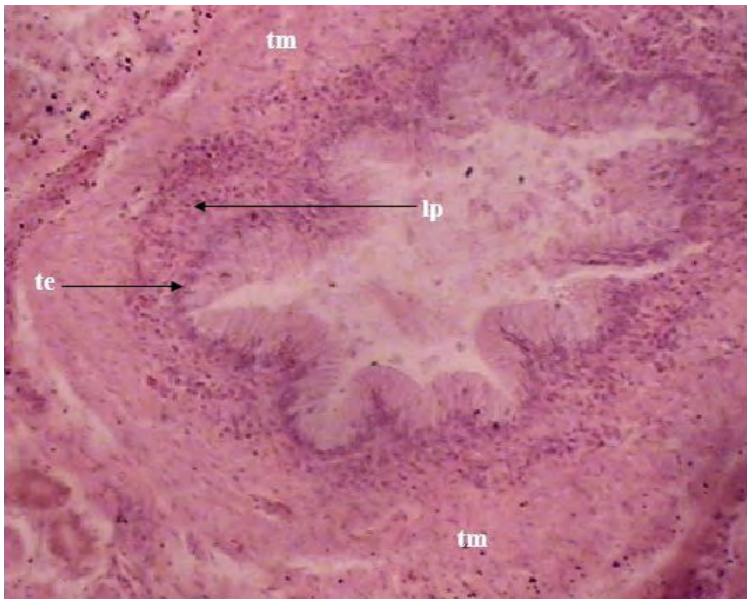

Figure 8 Photomicrograph of the ureter showing: (te) transitional epithelium, (lp) lamina propria, and (tm) tunica muscularis. H&E stain 400x

Figure 9 Photomicrograph of the cloaca showing: (pc) pseudostratified columnar epithelium, (lp) lamina propria and (ln) lymphoid nodules. H&E stain 400x

DISCUSSION AND CONCLUSION

The avian kidney is considered a vital organ of excretion involved in the removal of excess water and metabolic waste from the system (Mobini and Abdollahi, 2016). This present study reveals that the kidneys of Spur-winged goose were positioned retroperitoneally, located between the synsacrum and the iliac fossa as observed by Singh et al. (2023) in Pratapdhan bird, Pourhaji Motab and Rasuli (2021) in Pheasant, and Dhyaa et al. (2012) in harrier, chicken, and Mallared. In most species of birds, the kidneys were reported as dorso-laterally flattened and reddish brown in colour. This report was similar to the present study and that of Khadim and Daoud (2014) in barn owls, Reshag et al. (2016) in Great flamingo and Singh et al. (2021) in Guinea fowl. In contrast, Dhyaa et al. (2014) reported grayish colouration of the kidneys in Mallard ducks, brownish red to dark red in adult pigeon (Al-Ajeely and Mohammed, 2012), pink to brownish in canary (McLelland, 1990). This could be attributed to the amount of blood supply to the kidneys and ecological variation among avian species. The kidney was incompletely divided into cranial, middle and caudal lobes, with the caudal lobe being the largest, which concur to that of other avian species reported by Abdul-Gahaffor et al. (2012) in racing pigeon, Batah (2012) in coot bird, and Singh et al. (2021) in Guinea fowl.

The ureter connects the kidney to the cloaca, serving as the passage for metabolic waste and urine to exit the body (Alabdallah, 2022). The ureter was seen grossly as a tubular organ running from the medial aspect of the kidney to the dorso-medial aspect of the cloaca where it enters as the urodeum. This finding is similar to most avian ureters as reported by Al-Ageely and Mohammed, (2012) in racing pigeon, Sreeranjini et al. (2010) in Japanese quails, Kumar et al. (2018) in the Aseel and Rhode breed of poultry.

The cloaca is considered a significant region as outer opening to the urinary, digestive and genital organs that functions as a site for thermoregulation in birds (Hoffman et al., 2007). The three compartments, namely, the coprodeum, urodeum and proctodeum are found fused and positioned cranially to caudally, which are similar to most avian species as reported by Mohammed (2017) in Turkey and Joshi and Meshram (2018) in White leghorn fowl. The coprodeum appears as the largest of the three compartments of the cloaca, as reported by Gumus et al. (2004) and Oliveria (1996) in domestic fowl, and Mohammed (2017) in turkey.

Histologically, the kidney parenchyma is simply divided into the renal medulla and renal cortex which are not markedly divided. The cortex makes up a vast section of the kidney, enclosing the central vein and the medulla, making up a small portion similar to most avian kidneys hence coincides with the report of Nabipour et al. (2009). This finding also agrees with that of Al-Ajeely and Mohammed (2012) in racing pigeons, and Guo et al. (2014) in Ningdu yellow chicken. The proximal and distal convoluted tubules are lined with a simple columnar epithelium (Bacha and Bacha, 2012), except for the presence of brush border lining the surface of the epithelium in the proximal tubule, which is similar in other avian species, as reported by Nabipour et al. (2009) and Dhyaa et al. (2014). In contrast, a simple cuboidal epithelium was reported in racing pigeon (Al-Ajeely and Mohammed, 2012), and in coot bird (Batah, 2012). The renal corpuscles comprised of the Bowman's capsule, which is separated from a centrally located glomerulus by a space (Bowman's space). The glomeruli are lined externally with the parietal cells containing mesangial cells, podocytes, and capillaries. This agrees with the findings of Batah (2012), who reported that the renal cortex of coot bird was composed of large and small renal corpuscles, each containing a Bowman's capsule and glomerulus.

Histologically, the mucosa of the ureter is lined with stratified transitional epithelium, similarly agreeing with the findings of Al-Ajeely and Mohammed (2012) in racing pigeon. The muscularis layer consisted of inner circular and outer longitudinal muscle fibers and the adventitia as the last layer seen in most avian ureters, as reported by Mirabella et al. (2007) in duck, and Oliaii and Mobini (2017) in Japanese quails.

Histologically, the cloacal mucosa of the urodeum possesses tall villi lining the surface of the epithelium. These findings tally with Oliveira et al. (2004), who reported the same in ratite birds,except that there are abundant mucosal glands and the villi are thicker and pyramidal in nature. In contrast, Joshi and Meshram (2018), reported that the tunica mucosa of white leghorn fowl containeds short and flat villi. This could be as a result of species variation. The villi were lined with columnar cells, the lamina propria possessed a loose connective tissues and lymphoid cells which agrees with similar features reported by Verma (2000) and Joshi and Meshram (2018) in chicken and white leghorn fowl, respectively.

The Spur-winged goose has particular anatomical characteristics that contribute to its distinguishing look and functioning. The urinary system lacks a bladder, therefore, urine is eliminated via the urodeum of the cloaca. Currently, not much information has been documented on anatomical characteristics of urinary system of Spur-winged goose. Therefore, this present study serves as resourceful information on the gross anatomy and histology of the urinary system of the Spur-winged goose.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Concept and Design–YBM; Supervision and Fundings–YBM; Materials and Data collection and/or processing–IDK; Literature review and Analysis and/or interpretation of the data–MMK; Writing and Critical review–AMW.

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ANATOMIJA I HISTOLOGIJA URINARNOG SISTEMA OSTRUGASTE GUSKE (PLECTROPTERUS GAMBENSIS)

SAŽETAK

Cilj istraživanja je ispitati anatomiju i histologiju urinarnog sistema Ostrugaste guske. U istraživanje je uključeno ukupno 10 zrelih zdravih jedinki Ostrugaste guske, od čega 5 mužjaka i 5 ženki. Ptice su nabavljene sa komercijalne peradarske farme u Damaturu, u državi Yobe u Nigeriji i prevezene u Postdiplomski istraživački laboratorij za anatomiju u histologiju Katedre za veterinarsku anatomiju Sveučilišta Maiduguri u Nigeriji. Ptice su aklimatizirane u trajanju od 10 dana prije nego što su eutanizirane. Potom je izvršena eksteriorizacija abdominalne šupljine gusaka zbog makroskopskog pregleda bubrega, uretera i kloake, prije pripreme za mikroskopski pregled. Bubrezi su makroskopski izgledali smeđkasti, dorzolateralno spljošteni sa tri jasno uočljiva lobusa. Ureteri su izgledali poput cjevastih šupljina koje se odvajaju od bubrega i ulaze u kloaku sa dorzomedijalne strane. Kloaka se doimala kao zajednički organ koji povezuje urinarni, reproduktivni i digestivni sistem. Histološki, bubrežni parenhim je podijeljen na bubrežnu koru i srž koje sadrže i centralnu venu i renalne kosrpuskule. Ureteralna Lamina epithelialis je obložena sa pseudostratificiranim cilindričnim epitelom koji sadrži rijetko vezivno tkivo i limfoidne stanice. Kloakalna sluznica posjeduje kratke i tanke vile. Trenutno ne postoje osnovne informacije o anatomskoj građi urinarnog sistema Ostrugaste guske. Stoga naše istraživanje služi kao osnova za poznavanje anatomije urinarnog sistema ove vrste.

Ključne riječi: Bubreg, ptičija, kloaka, ureter, vodene ptice

SHORT COMMUNICATION

FIRST FINDINGS OF YELLOW-NECKED MOUSE (*APODEMUS FLAVICOLLIS* (MELCHIOR, 1834)) IN THE NEUM AREA IN BOSNIA AND HERZEGOVINA

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ABSTRACT

The first documented surveys of the populations of small mammals in the coastal area of the sea in Bosnia and Herzegovina in recent history were carried out from September, 25-28, 2023. A total of 50 Sherman LFG traps were set in two locations: Lastva and Tanko Sedlo on the Klek peninsula. In total, four individuals of the species *Apodemus flavicollis* (Melchior, 1834) and two individuals of the species *Apodemus sylvaticus* (Linnaeus, 1758) were caught, which represents the first finding of this species in the Neum area. The absence of the expected species *Apodemus epimelas* (Nehring, 1902) is a surprise considering the habitat type.

Keywords: *Apodemus flavicollis*, Bosnia and Herzegovina**,** Neum, small mammals

INTRODUCTION

The species *Apodemus flavicollis* (Melchior, 1834) is a relatively common species in Bosnia and Herzegovina with numerous findings, except in the southernmost part – south from Mostar region. Species' southern border distribution is Dinara Mountain, stretching eastward across the mountain chain of Cincar, Vran, Čvrsnica, Prenj/Velež, Crvanj, Zelengora, Bjelašnica (Gacko), all the way down to Orjen Mountain, bordering with Montenegro. South of that line, the species was never recorded (Figure 1; Petrov, 1992).

South of this natural border is a domination of karst environment, with the influence of Mediterranean climate within the Mediterranean-Dinaric floristic zone – more precisely, a submediterranean area

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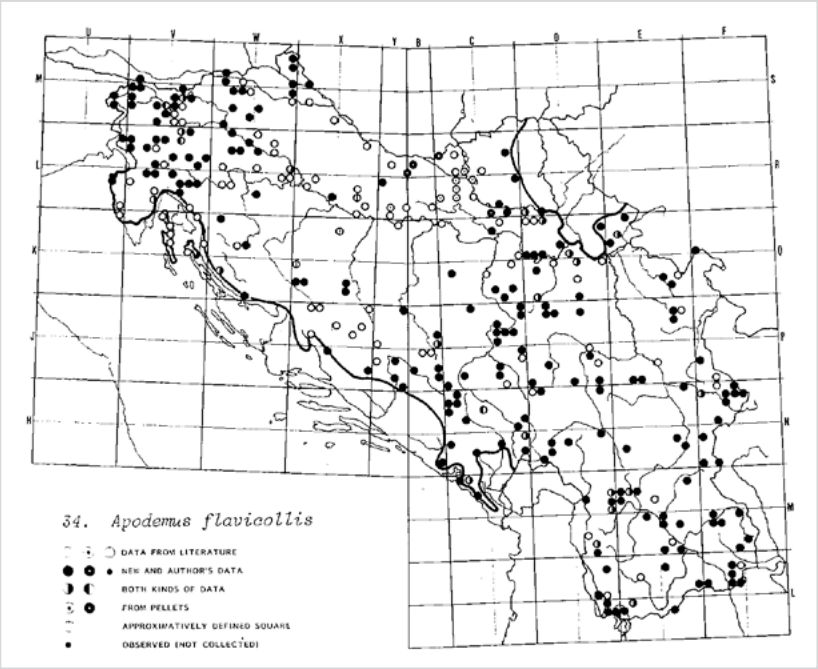

Figure 1 Distribution range of Apodemus flavicollis (Melchior, 1834) in Yugoslavia (Petrov, 1992)

which is characterized by average altitude of 750 m, vegetation period from 230-260 days per year and with unfavourable ratio between precipitation and evapotranspiration (0,66; Stevanović et al. 1983). This area is a niche of wester n broad-toothed field mouse species *Apodemus epimelas* (Nehring, 1902), which favours the climatic and other conditions in the area.

According to Petrov, 1992, species *Apodemus flavicollis* (Melchior, 1834) distribution is absent along the Adriatic coast, except on the islands of Cres and Rab. Species generally prefer forest habitats but can also been found in mixed forest-bushy or bushy vegetation with lots of seeds. Species can be found along the human settlements but, generally, it is mostly absent due to the presence of domestic predatory animals (cat).

The aim of this article is to present the first ever finding on *Apodemus flavicollis* (Melchior, 1834) species south of Mostar area in Bosnia and Herzegovina and along the Adriatic coast, in the environment of more expected, *Apodemus epimelas* (Nehring, 1902) species.

MATERIAL AND METHODS

The field research was performed from 25-28 September 2023 on the Klek peninsula, along the Adriatic coastline in Bosnia and Herzegovina. The research area is dominated by bushy maquis scrubland and rocky vegetation with unique and quite rich flora species. Latest field research in the area revealed 245 plant taxa, of which as many as 16 taxa are new for Bosnia and Herzegovina (UNEP-GEF, 2021).

We use 50 Sherman LFG (Large, folding, galvanized) traps which we put in 2 transect line fields with 25 traps each:

- Transect line 1 – the area of Lastva – along the existing macadam road to Opuće;
- Transect line 2 – the area of Tanko Sedlo, in bushy vegetation.

The transect line 1 in the area of Lastva was 256 m long, making 10,1 m of average coverage per trap, while the transect line 2 in the area of Tanko Sedlo was 216 m long , making 8,64 m of average coverage per trap. Both coverage areas were satisfying, keeping in mind water and food scarcity in the karstic environment (Figure 2, Figure 3).

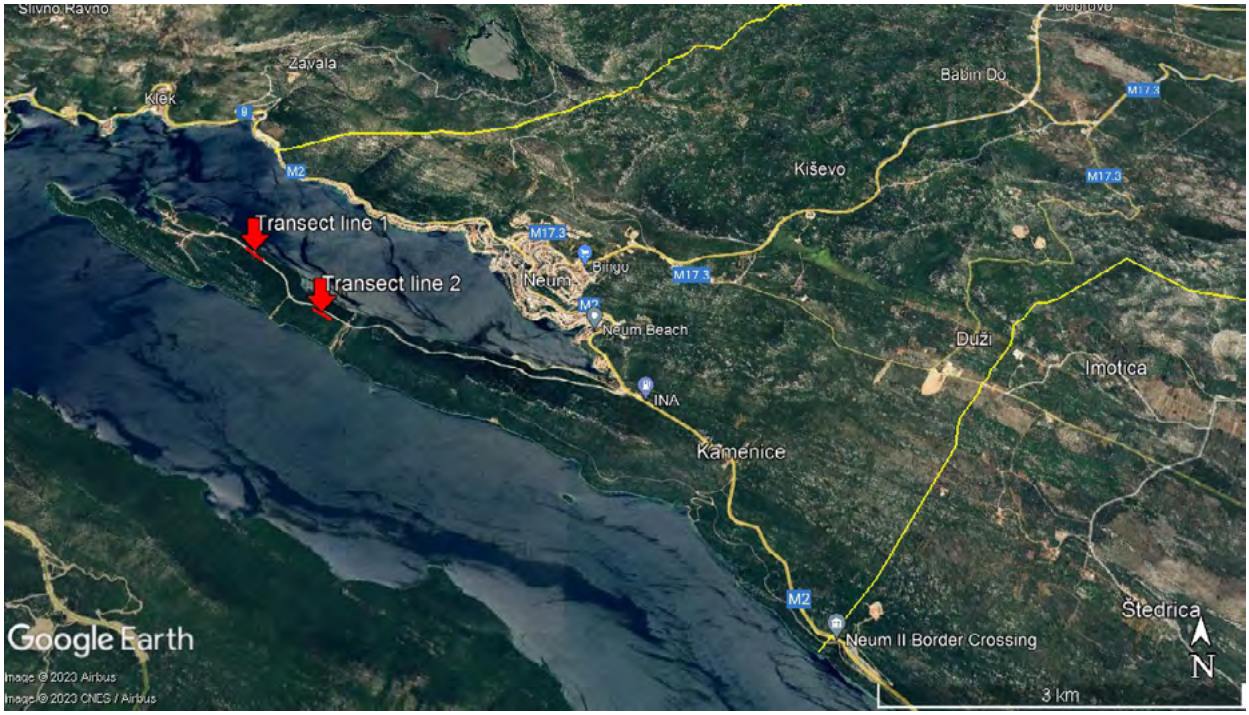

Figure 2 Position of transect lines (red) in Neum municipality (Google Earth)

The used baits were a combination of corn flips with peanut aroma and vegetable (carrot) and fruit (apple) pieces in order to prevent dehydration. The traps were checked twice a day (morning and evening) and were replenished with fresh baits, if necessary. In order to prevent re-catch, the team marked each caught individual with a waterproof marker on the ventral side before release. By doing this, we prevent damaging or harming the caught animals.

Other used survey equipment includes:

- GPS Garmin eTrex 30,
- CANON EOS 1100D DSLR Camera with EF-S 18-55mm f/3.5-5.6 IS II lens,
- Camera of Samsung Galaxy A52 cell phone.

As an identification guide, we use "Field Guide to European Mammals" (Twisk et al, 2019), which is currently most up-to date field guide in Europe.

Figure 3A) Putting traps on Lastva location **B)** Trapping site on Lastva location

Figure 4 Caught individual of Apodemus flavicollis (Melchior, 1834)

RESULTS

In total, six small mammal individuals were caught. Caught individuals belong to the two species:

- *Apodemus sylvaticus* (Linnaeus, 1758) with two individuals,
- *Apodemus flavicollis* (Melchior, 1834) with four individuals.

Both *Apodemus sylvaticus* (Linnaeus, 1758) were caught in Tanko Sedlo with one specimen of *Apodemus flavicollis* (Melchior, 1834), while three *Apodemus flavicollis* (Melchior, 1834) were caught on the Lastva location (Figure 4).

Team did not take any individual measurements. No species were harmed physically during trapping, species detection and release. All individuals survive trapping successfully, and they were released in nature after taking pictures.

DISCUSSION AND CONCLUSION

Based upon the literature data, both species were historically not present in the Neum area (Petrov, 1992; Mitchell-Jones et al, 1998). Reason for this may be a lack of scientific field research on small-mammals in this specific area in the past, or researchers did not publish their findings. However, species *Apodemus sylvaticus* (Linnaeus, 1758) was recorded numerous times in the past in the nearby areas of Neretva Delta, Pelješac peninsula and the area near Slano (Petrov, 1992), which proves that the presence of *Apodemus sylvaticus* (Linnaeus, 1758) is historically confirmed and expected.

On the other hand, the presence of species *Apodemus flavicollis* (Melchior, 1834) was not recorded historically, even in the nearby locations (Petrov, 1992; Mitchell-Jones et al, 1998). The closest confirmed location is Orjen Mountain in Bosnia and Herzegovina. It is worth to mention that species was historically absent from the Adriatic coast from the Novigradsko Sea in the Republic of Croatia to nearby Tivat in Montenegro (Figure 1, Petrov, 1992).

That makes the finding presented in this paper the first documented finding of species *Apodemus flavicollis* (Melchior, 1834) on the Adriatic Sea shore. Major surprise was the absence of highly expected *Apodemus epimelas* (Nehring, 1902) species (Petrov, 1992; Mitchell-Jones et al, 1998), but further and more detailed field research is needed to prove its presence or absence. The future research must cover the wider area on the Klek peninsula in different seasons in order to gain a clear insight into the composition of the populations of small mammals along the Adriatic Sea coast in Bosnia and Herzegovina.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – AA; Design – AA, SKA; Supervision

– SKA; Resources - AA; Materials – AA; Data Collection and/or Processing – AA, SKA, MH; Analysis and/or Interpretation –AA, SKA, MH; Literature Search – SKA; Writing Manuscript – AA; Critical Review – AA.

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PRVI PRONALAZAK ŽUTOGRLOG MIŠA (APODEMUS FLAVICOLLIS (MELCHIOR, 1834) U PODRUČJU NEUMA U BOSNI I HERCEGOVINI

SAŽETAK

Prvo dokumentovano istraživanje populacije malih sisara u obalnom području Bosne i Hercegovine u novije vrijeme je provedeno u periodu od 25. - 28. septembra 2023. godine. Postavljeno je ukupno 50 Sherman LFG zamki na dvije lokacije, Lastva i Tanko Sedlo na poluostrvu Klek. Ukupno su uhvaćene četiri jedinke vrste Apodemus flavicollis (Melchior, 1834) i dvije jedinke vrste Apodemus sylvaticus (Linnaeus, 1758), što predstavlja prvi pronalazak ovih vrsta u neumskom području. Odsustvo očekivane vrste Apodemus epimelas (Nehring, 1902) predstavlja iznenađenje, uzimajući u obzir tip i tip staništa, dok pojava vrste Apodemus flavicollis (Melchior, 1834) predstavlja jedinstveno znanstveno otkriće obzirom da ova vrsta nije dokumentovana u području duž Jadranske obale, od Novigradskog mora u Republici Hrvatskoj do Tivta u Crnoj Gori i područja južno od grada Mostara u Bosni i Hercegovini.

Ključne riječi: *Apodemus flavicollis*, Bosna i Hercegovina, mali sisari, Neum

CASE REPORT

MANAGEMENT OF IDIOPATHIC STRINGHALT IN A 14-YEAR-OLD THOROUGHBRED GELDING IN NIGERIA: A CASE REPORT

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ABSTRACT

Stringhalt is characterised by delayed protraction and excessive flexion of the hock during progression and may affect one or both pelvic limbs. No instances of stringhalt in Nigeria have been reported. Stringhalt, a rare neuromuscular disorder in horses, underscores the importance of documenting cases to enhance collective understanding. The present case aimed to describe the successful management of idiopathic stringhalt in a 14-year-old Thoroughbred gelding. The 500-kg gelding in a stable of 20 horses exhibited sudden abnormal movements in the right hind leg, noticed three days prior. The horse displayed normal vital parameters, exhibiting a distinct gait with moderate hyperflexion in the affected hind limb during walking and trotting. Based on the history and clinical examination, the diagnosis was unilateral idiopathic stringhalt, grade III.The horse received intravenous phenytoin sodium (15 mg/kg) and intramuscular multivitamin and amino acids injection (15 ml/horse) for five days, leading to the complete resolution of clinical signs and the resumption of normal activity. Prompt diagnosis and treatment are crucial for managing idiopathic stringhalt in horses. The successful outcome with phenytoin sodium and multivitamin supplementation highlights their efficacy. Further studies on stringhalt's aetiology in Nigeria are warranted.

Keywords: Aberrant gait, hind limb, horse**,** hyperflexion, stable

INTRODUCTION

Stringhalt, also known as equine reflex hypertonia, is a condition in horses where one or both hind limbs exhibit excessive and prolonged flexion during movement (Furr et al., 2011). It is a long-standing horse disease that Kendall first described in Australia in 1884 (Araujo et al., 2008).

There are two primary classifications of stringhalt: Australian stringhalt, associated with plants, and classical stringhalt, as identified by Duque et al. (2014). According to Martens (2019), classical

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stringhalt typically manifests in isolated instances and primarily affects one limb. The precise cause of this condition remains elusive, leading to its designation as idiopathic stringhalt. However, potential causes, such as trauma to the dorsal metatarsal region, thalamus abnormalities, and reduced nerve conduction in the hind limbs, have been documented (Crabill et al., 1994).

Australian stringhalt is a type of stringhalt linked to plants, specifically caused by consuming various related weeds like *Taraxacum officinale* (European dandelion), *Hypochaeris radicata* (Australian dandelion), or Malva (mallow). Australian stringhalt, as the name suggests, is predominantly found in Australia, but occurrences have been recorded in New Zealand, North America, Brazil, Chile, Japan, and Europe (Martens, 2019). The clinical signs of Australian stringhalt usually diminish with time, when the horse is no longer exposed to the weed (Brockman, 2017).

The most common signs of the condition are involuntary, exaggerated upward movement of the hind limb, kicking upwards towards the belly, hopping or jerking, incoordination, dragging hind hooves, muscle atrophy of the lower hind limb, and the inability to stand up without assistance (El-Hage et al., 2019; Valberg and Baird, 2022).

The diagnosis of stringhalt relies on observing clinical signs and ruling out other neurological and orthopedic abnormalities. This process may involve techniques such as electromyography (EMG), nerve conduction velocity (NCV), and diagnostic imaging(El -Hage et al., 2019). When intoxication is suspected, relocating the horse to a different paddock, providing an alternative source of feed and water, and identifying and collecting samples of the suspected toxic plants from the horse's environment for analysis may be sufficient, and many cases seem to recover spontaneously. In cases of classical stringhalt, the most successful results have been achieved through tenectomy of the lateral extensor of the digit, involving the removal of a portion of the muscle. Improvement may not be noticeable until 2–3 weeks after surgery, and not all cases show a positive response, which is expected due to the distal axonopathy nature of the condition. Alternative treatment approaches include administering high doses of thiamine and phenytoin (Valberg and Baird, 2022).

To the best of the authors' knowledge, there is no documented case of stringhalt in Nigeria. Stringhalt is a relatively rare neuromuscular disorder in horses, making it essential to document and share individual cases to contribute to the collective understanding of the condition. The present case aimed to describe the successful management of idiopathic stringhalt in a 14-year-old Thoroughbred gelding.

Case report

History

In a stable of 20 horses, a 14-year-old Thoroughbred gelding weighing 500 kg was presented to the Veterinary Teaching Hospital, University of Jos, Nigeria, with a complaint of sudden aberrant movement in the right hind leg. The condition was noticed three days before the presentation. There was no history of recent trauma or previous medical problems. The patient, as well as the remaining 19 horses in the stable, were fed concentrates and hay of the same source two times a day in their stalls.

Clinical examination and diagnosis

All vital parameters were within the normal range. On lameness examination, the horse showed a typical goose-stepping gait and moderate hyperflexion of the afflicted hind limb, which was more evident when the horse first walked off and continued as the horse trotted. The affected pelvic limb was held in an adducted position and ascended rapidly and steeply towards the abdomen (Figure 1). There was no pain upon applying a hoof tester to the sole of the hoof.

Based on the absence of a history of ingestion of any toxic weed, recognisable clinical indications present at both walk and trot, and the moderate hyperflexion of the right hindlimb noted when walking and trotting, the clinical diagnosis of

Figure 1
Hyperflexion of the right hind limb while being walked

unilateral idiopathic stringhalt grade III was made. The grading was done according to the grading scheme for the categorization of stringhalt-affected horses adapted from Huntington et al. (1989) and Domange et al. (2010).

Treatment and outcome

Phenytoin sodium injection (Dilantan®, Sterimax Inc., Ontario, Canada) was administered intravenously at 15 mg/kg for 5 days. Multivitamin and amino acids injection (Introvit®, InterchemieWerken 'De Adelaar' B.V., Netherlands) was administered intramuscularly at 15 ml/horse for five days. Upon completion of treatment, all clinical signs subsided, and the gelding returned to normal activity.

DISCUSSION AND CONCLUSION

Stringhalt can impact horses of any breed, and it may manifest in either one or both hind legs. The present case highlights the clinical diagnosis and successful management of unilateral idiopathic stringhalt, a condition characterised by hyperflexion of the hind limb during movement, particularly when walking or trotting, in a 14-year-old Thoroughbred gelding.

Stringhalt can be diagnosed through clinical signs (El-Hage et al., 2019). The clinical signs observed in the present case are consistent with other cases of stringhalt in different countries (Araujo et al., 2008; Kachwaha et al., 2012; Duque et al., 2014). Stringhalt is the condition most often confused with shivers (Baird et al., 2006). The affected right hind limb in the present case was held in an adducted position and ascended rapidly and steeply towards the abdomen, which differentiates it from a shiver. Shiver is rather held in an abducted position, away from the body (Draper et al., 2015).

Since stringhalt in a horse has two major categories (Duque et al., 2014), the stringhalt in the present case was categorised as idiopathic stringhalt because there was no indication of plant toxication, as the patient and the other horses in the stable were fed hay from reliable sources. In line with Martens (2019), idiopathic stringhalt, typically manifesting as an isolated and unilateral condition, was also observed in the present case.

The case was successfully treated with phenytoin and multivitamin injections. Phenytoin has been used to successfully treat stringhalt in some cases (Huntington et al., 1991; Takahashi et al., 2002; Furr et al., 2011). According to anecdotal evidence, phenytoin appears to be the consistently most effective medication when given to horses with stringhalt, (El-Hage et al., 2019). Phenytoin acts as a sodium channel blocker and is thought to regulate tetanic muscle contractions and

abnormal nerve activity (El-Hage et al., 2019). It has been said that vitamin B1 (thiamine) and vitamin E (tocopherol), which are both in the multivitamin injection used in this study, can help treat stringhalt, though these reports are only anecdotal (Huntington et al., 1989; Armengou et al., 2010; Domange et al., 2010). Vitamin B1 plays a crucial role as a cofactor in neuronal cell metabolism, neurotransmitter production, and myelin synthesis. On the other hand, vitamin E functions as an antioxidant, presumably employed to decrease oxidative damage to the distal axons of the long nerves (Armengou et al., 2010).

The absence of additional sophisticated diagnostic procedures, including electromyography, nerve conduction velocity study, and diagnostic imaging, poses a limitation in this case report. These methods could have provided valuable insights into the underlying cause or pathophysiology of the stringhalt condition in this specific horse. Unfortunately, our facilities were constrained by the limited availability and accessibility of these advanced diagnostic tools.

This case report emphasises the importance of prompt diagnosis and appropriate treatment in managing idiopathic stringhalt in horses. The successful outcome observed in this case supports the use of phenytoin sodium and multivitamin supplementation as a viable treatment option for similar cases. As this is the first documented case of stringhalt in Nigeria, further studies are necessary to clarify stringhalt aetiology in Nigeria.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ERE; Design – OOA; Supervision – WPM, DOO; Resources –ME, WPM; Materials – DOO; Data Collection and Processing – OOA, KSW; Analysis and Interpretation – SI; Literature Search – OOA, ERE; Writing Manuscript – OOA, WPM; Critical Review – WPM, ME, ERE

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TRETMAN IDIOPATSKOG "PIJETLOVOG HODA" KOD ČETRNAESTOGODIŠNJEG PUNOKRVNOG KASTRATA U NIGERIJI: PRIKAZ SLUČAJA

SAŽETAK

"Pijetlov hod" (engl. Stringhalt) je karakteriziran zakašnjelom protrakcijom i pojačanom fleksijom skočnog zgloba pri hodu, s tim da može zahvatiti jedan ili oba pelvična ekstremiteta. Dosad u Nigeriji nije bilo prikaza slučaja "pijetlovog hoda". "Pijetlov hod" kao rijetko neuromišićno oboljenje kod konja naglašava važnost dokumentiranja slučajeva sa ciljem produbljivanja kolektivnog znanja. Cilj prikaza slučaja jeste opisati uspješan tretman idiopatskog "pijetlovog hoda" kod četrnaestogodišnjeg punokrvnog kastrata. Kod kastrata teškog 500 kg koji potječe iz štale sa 20 konja je prije tri dana došlo do iznenadne pojave abnormalnih pokreta stražnje desne noge. Konj je imao normalne vitalne parametre, a hod mu je bio karakteriziran umjerenom hiperfleksijom stražnje noge u hodu i kasu. Na temelju istorije bolesti i kliničkog pregleda je postavljena dijagnoza unilateralnog idiopatskog "pijetlovog hoda", III stupanj. Konj je tretiran intravenskim fenitoin-natrijem (15 mg/kg) i intramuskularnim injekcijama multivitamina i aminokiselina (15 ml/konj) u trajanju od pet dana, što je dovelo do potpunog povlačenja kliničkih znakova i nastavka normalne aktivnosti. Brzo dijagnosticiranje i terapija su od presudnog značaja za liječenje idiopatskog "pijetlovog hoda" kod konja. Povoljan ishod postignut sa fenitoin-natrijem i multivitaminskim preparatima naglašava njihovu učinkovitost. Potrebna su daljnja istraživanja etiologije "pijetlovog hoda" u Nigeriji.

Ključne riječi: Aberantni hod, hiperfleksija, konj, stražnje noge, štala

CASE REPORT

MYASTHENIA GRAVIS IN A TOY POODLE DOG: A CASE REPORT

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ABSTRACT

Myasthenia gravis (MG) is an immune-mediated, neuromuscular disorder primarily characterized by muscle weakness and excessive fatigue. Three forms of myasthenia gravis have been described in dogs: focal, generalized, and acute fulminating form. A six-year-old, male toy poodle presented with weakness, intermittent paresis, voice change, and inability to jump or climb the stairs for seven days. Clinical examination revealed ataxia, muscle weakness, and ambulatory tetraparesis. Forelimb hopping revealed poor follow-through, and wheelbarrow was abnormal. The withdrawal reflex was reduced. Complete blood count showed thrombocytopenia, a slight increase in mean platelet volume (MPV), and decreased level of plateletcrit (PCT). Biochemistry analysis was unremarkable. An acetylcholine receptor (AChR) antibody test was performed due to the suspicion of myasthenia gravis. The test detected circulating antibodies to the acetylcholine receptor and positive results confirmed the suspected diagnosis. Acetylcholinesterase (AChE) inhibitors are usually the first line of therapy. Pyridostigmine bromide is the most commonly used AChE inhibitor, and it was the drug of choice in this case (2,5 mg/kg PO q 12h). The dose of pyridostigmine bromide was reduced by 50% after nine months, and the therapy was discontinued after one year due to the test results in the reference values, and the absence of clinical signs.

Key words: Dog, muscle, myasthenia gravis, pyridostigmine bromide, tetraparesis

INTRODUCTION

Myasthenia gravis (MG) is a neuromuscular disorder primarily characterized by muscle weakness and excessive fatigue due to abnormal transmission of the message between the nerves and the muscles (Shelton, 2002). Previous classification has included both - acquired and congenital MG. However, in recent years, the term myasthenia gravis solely refers to an acquired autoimmune disease with autoantibodies against the neuromuscular junction of skeletal muscle, while the term "congenital myasthenic syndromes"

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has replaced the term "congenital myasthenia gravis" (Shelton, 2016). There are three forms of myasthenia gravis in dogs: focal, generalized and acute fulminating form (Penderis and Martin-Vaquero, 2016). Clinical signs and findings vary from patient to patient, but often include: focal or generalised muscle weakness (appendicular, facial, pharyngeal and/or laryngeal), regurgitation, dyspnea and aspiration pneumonia in dogs with megaesophagus, voice changes, neurologic deficits (Dewey et al., 1997). The main test to diagnose myasthenia gravis is AChR antibodies test, which detects AChR autoantibody concentration using radioimmunoassay (Mignan et al., 2020). Treatment begins with anticholinesterase drugs and, if muscle strength has not returned to normal, immunosuppressive treatment should be initiated (Platt and Shelton, 2014). Here, we report the first recorded case of myasthenia gravis in a dog from Bosnia and Herzegovina.

Case description

A six-year-old, male toy poodle presented with weakness and intermittent paresis, initially in the hind limbs and then evolving to the forelimbs for seven days. The owner also reported that voice change has been noticed, as well as the inability to jump or climb the stairs. The history, physical examination and neurological evaluation were followed by spine radiography and blood sampling from the cephalic vein into an IDEXX EDTA KE/1.3 microtube and an L-heparin LH/1.3 microtube for hematology and biochemistry blood tests. Hematological blood analysis was performed using a ProCyte® Hematology Analyzer, (Idexx Laboratories Inc., REF: 98-70000-01), and blood biochemistry using a Catalyst One® Chemistry Analyzer, (Idexx Laboratories Inc., REF: 89-92525-00). Potential differential diagnoses included metabolic disorders, lesion in the C6-T2 spinal cord segment, myasthenia gravis, or acute polyradiculoneuritis. Due to the suspicion of myasthenia gravis, an AChR antibody test (indirect fluorescent antibody test (IFAT) method) was performed in an accredited Laboklin laboratory. IFA test is useful for the diagnosis of autoimmune diseases, including myasthenia gravis. In this test, cells grown in culture and fixed to a glass slide are permeable to antibody. These cells are exposed to serum from a patient suspected of AChR antibodies and then to a fluorescent antibody. After an incubation and wash, the cells can be examined for fluorescence by fluorescence microscopy. Visible fluorescence demonstrates the presence of AChR antibodies in the patient's serum (Im et al., 2019).

At clinical examination, the patient was responsive. According to WSAVA recommendations (2013), the body condition score was 5/9. Its body temperature, and heart and respiratory rates were within reference ranges. The peripheral lymph nodes were unremarkable. The neurological evaluation confirmed ataxia, muscle weakness, and ambulatory tetraparesis. Forelimb hopping revealed poor follow-through and wheelbarrow was abnormal. The withdrawal reflex of all limbs was reduced. The blood analysis revealed thrombocytopenia (PLT 50 K/µl, normal range 148-484 K/µl), a slight increase in MPV (13,4 Fl, normal range 8,7-13,2 fL), and decreased level of PCT (0,07%, normal range 0,14-0,46%). The rest of the parameters for cell blood count were in the normal range. Biochemistry analysis (Chem 17 CLIP) was unremarkable, and no abnormalities were found in electrolytes (Lyte 4 CLIP). Spine radiography did not show any changes or damages. Oral cobalamin (BOSNALIJEK a 1000 mcg – 1 tablet twice a day) and propentofylline (Canergy a 100mg – ¼ tablet twice a day) therapy was prescribed before the diagnosis of MG was confirmed. The AChR antibody test detected circulating antibodies to the acetylcholine receptor, and positive results confirmed the diagnosis of myasthenia gravis (cell line TE671: 800, reference values < 400; cell homogenate: 600, reference values < 300). Time elapsed from the onset of clinical signs until confirmation of diagnosis is four weeks. At the control examination, before the start of the therapy with pyridostigmine bromide (Mestinon a 60 mg – 2,5 mg/kg PO q 12h), a significant improvement was observed. The patient could climb the stairs although muscle

weakness and hind limb ambulatory paresis were still noticeable. Daily oral treatment with pyridostigmine bromide further improved the condition. After nine months, the clinical signs of the disease have completely disappeared. The AChR antibody test was repeated, and the AChR antibody titre was not elevated any more (cell line TE671: <50; cell homogenate: <50). The dose of pyridostigmine bromide was reduced by 50%, and the test has been repeated again after three months (cell line TE671: <50; cell homogenate: <50). Due to the results in the reference values and the absence of clinical signs, Mestinon therapy was discontinued two months ago (Table 1). The dog has been in follow-up for two months, and there has not been recurrence of the clinical signs during this period.

Table 1 Acetylcholinreceptor-antibodies test results and pyridostigmine dosage

PARAMETER	1ST TEST (December, 2022)	2ND TEST (September, 2023)	3RD TEST (December, 2023)	REFERENCE VALUES (circulating antibodies to the Acetylcholinreceptor)
Cell line TE671	800.00	<50	<50	< 400
Cell homogenate	600.00	<50	<50	<300
Pyridostigmine dosage	2.5 mg/kg q 12h	1.25 mg/kg q 12h	Discontinued	

DISCUSSION AND CONCLUSION

Myasthenia gravis is an immune-mediated, neuromuscular disorder with autoantibodies against neuromuscular junction of sceletal muscle, which results in impaired neuromuscular transmission. As a result of autoantibody mediated destruction, this disease is manifested clinically as muscular weakness (Shelton, 2016; Dewey et al, 1997). Three forms of myasthenia gravis have been described in dogs: focal, generalized, and acute fulminating (Dewey et al., 1997). Focal myasthenia gravis includes 26% to 43% of all cases, and the only clinical signs of this form are regurgitation, megaoesophagus, and/or dysphagia (Platt and Shelton, 2014). Generalized form includes dogs with appendicular muscle weakness, as was found in this case, and it includes 57% to 64% cases of myasthenia gravis. This form is characterized by different degrees of muscular weakness, and 90% of dogs with generalized form have megaesophagus (Khorzan et al., 2011). In this case, megaesophagus was excluded due to the absence of clinical signs in the form of dysphagia and regurgitation, as well as due to the radiographs. Acute fulminating form presents severe and rapidly progressing form of myasthenia gravis which is associated with a rapid onset of paralysis and megaesophagus (Penderis and Martin-Vaquero, 2016; Platt and Shelton, 2014). German Shepherd Dogs, Golden Retrievers, Labrador Retrievers, Akitas, terrier group, Scottish Terriers, German Shorthaired Pointers, and Chihuahuas appear to be predisposed to acquired myasthenia gravis (Platt and Shelton, 2014; Shelton et al., 1997). The Poodle is not considered to be predisposed, but there is a recorded case report of myasthenia gravis in this breed (Richardson, 2011). In dogs, just like in humans and cats, a bimodal age of onset has been presented. It usually occurs in young dogs between 4 months and 4 years, and in older dogs between 9 and 13 years of age, but in this case, the dog was 6 years old. Myasthenia gravis should be considered

in every animal with megaesophagus, dysphagia, and/or muscular weakness. A complete blood count and biochemistry panel should be performed to rule out other causes of generalized weakness (Khorzad et al., 2011). Muscle damage may lead to an increased value of the muscle enzyme creatine kinase (CK) (Penderis and Martin-Vaquero, 2016). The "gold standard" for the diagnosis is positive testing by measurement of AChR autoantibody concentration using radioimmunoassay (Khorzad et al., 2011). The treatment is based on response and severity of the disease, but AChE inhibitors are usually the first line of therapy. The mechanism of action of these drugs is to inhibit hydrolysis of acetylcholine at the neuromuscular junction, which prolongs the action of acetylcholine (Khorzad et al., 2011). Pyridostigmine bromide is the most commonly used AChE inhibitor. It is given at a dose of 0,5-3,0 mg/kg q8-12h (Platt and Shelton, 2014). If muscle strength has not been returned to normal, immunosupresive therapy should be considered (0,5 mg/kg orally q24h) (Platt and Shelton, 2014). In this case, the initial dose was 2,5 mg/kg. Given that the response to the therapy was adequate, and given that the chances of remaining in remission are greater in dogs that only received AChE inhibitors (Khorzad et al., 2011), corticosteroids were not used.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ALJ, DČ, TM; Supervision – DČ; Data Collection and/or Processing – ALJ, DČ; Analysis and/or Interpretation – ALJ, DČ, TM; Literature Search – ALJ; Writing Manuscript – ALJ, DČ, TM; Critical Review – DČ.

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MIASTENIJA GRAVIS KOD TOY PUDLE: PRIKAZ SLUČAJA

SAŽETAK

Miastenija gravis predstavlja autoimuno neuromišićno oboljenje karakterizirano prvenstveno mišićnom slabošću i izraženim zamaranjem. Opisana su tri oblika miastenije gravis pasa: fokalni, generalizirani i akutni fulminantni oblik. Šest godina star mužjak toy pudle doveden je sa znakovima slabosti, intermitentne pareze, promjene glasa, te nemogućnosti skakanja i penjanja uz stepenice, izraženih tokom posljednjih sedam dana. Kliničkim pregledom utvrđena je ataksija, mišićna slabost i ambulatorna tetrapareza. Proba poskakivanja prednjih ekstremiteta je bila oslabljena, proba kolica odsutna. Refleks povlačenja bio je oslabljen. Hematološkom analizom krvi utvrđena je trombocitopenija, blago povišene vrijednosti srednjeg volumena trombocita i snižena vrijednost trombokrita. Biohemijskim panelom nisu utvrđene abnormalnosti. Zbog sumnje na miasteniju gravis rađen je test za dokazivanje antitijela na acetilholinske receptore. Testom su detektovana cirkulirajuća antitijela na acetilholinske receptore, a pozitivnim rezultatom testa dijagnoza je potvrđena. Kada je u pitanju terapija, inhibitori acetilholinesteraze su prvi izbor, a najčešće korišteni je piridostigmin bromid koji je i u ovom slučaju propisan u dozi od 2,5 mg/kg na svakih 12h. Nakon devet mjeseci doza piridostigmin bromida snižena je za 50%, a terapija je prekinuta nakon godinu dana zahvaljujući rezultatima testova u referentnim vrijednostima, uz odsustvo kliničkih znakova.

Ključne riječi: Miastenija gravis, mišić, pas, piridostigmin bromid, tetrapareza

PROFESSIONAL PAPER

PRILOG POZNAVANJU PROIZVODNJE BRČANSKE ŠUNKE NA KLASIČAN NAČIN

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SAŽETAK

Značaj suhomesnatih proizvoda u prehrani je veoma veliki, a ujedno spadaju u mesne prerađevine koje su se najranije počele proizvoditi. Glavni razlog je taj što je process proizvodnje relativno jednostavan, rok trajanja je dug, rad je prikladan, a nutritivna vrijednost je visoka.

U Bosni i Hercegovini goveđa šunka je visokovrijedan i popularan suhomesnati proizvod, a uvjeti i tradicija proizvodnje razlikuju se u pojedinim regijama Bosne i Hercegovine pa je razumljivo da će se gotov proizvod razlikovati po kvaliteti i senzorskim svojstvima. Za dobivanje kvalitetne goveđe šunke potrebno je izvršiti odabir goveda. Za proizvodnju "Brčanske šunke" koristi se meso I kategorije (but) i meso II kategorije (leđa-rozbratna). Karakterističnost "Brčanske šunke" je proces konzerviranja, u kojem se dodaju sol, šećer i voda bez nitrita, nitrata i drugih dodataka.

Time dobivamo željena organoleptička i mikrobiološka svojstva koja zadovoljavaju potrebe potrošača.

Istraživanjem je obuhvaćen tehnološki proces kroz različite faze proizvodnje „Brčanske šunke". Cilj je dodatno animirati masovniju proizvodnju tradicionalnih suhomesnatih proizvoda koje odlikuje visok nutritivni i higijenski kvalitet. Time bi se stanovništvu omogućila primjerenija i sigurnija prehrana, što će dovesti do brže obnove i revitalizacije proizvodnih i prerađivačkih kapaciteta, a ruralnim kućanstvima postizanje održivosti u vidu samozapošljavanja.

Ključne riječi: Brčko, hrana, meso, šunka

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UVOD

Pod pojmom suhomesnati proizvodi podrazumijevamo dijelove životinjskog mesa tretirane soljenjem ili salamurenjem, dimljenjem ili sušenjem na vazduhu. Prilikom stavljanja u promet, suhomesnati proizvodi, najčešće nose naziv po onom dijelu tijela životinje odakle taj dio mesa potiče (Vujović, 2008).

Značaj suhomesnatih proizvoda u ishrani je veoma veliki, jer se od rane jeseni do kasnog proljeća nalaze na našem tržištu kao jedna od veoma cijenjenih proteinskih komponenti. Suhomesnati proizvodi spadaju u mesne prerađevine koje su se najranije počele proizvoditi. Osnovni razlog za to je relativno jednostavan proces proizvodnje, duga održivost, jednostavno manipulisanje, kao i visoka hranljiva vrijednost (Puljić, 2022).

Goveđa šunka je visokovrijedan i popularan suhomesnati proizvod u Bosni i Hercegovini (BiH), a sve više nalazi mjesto i u inostranstvu. Proizvodi se različitim tehnološkim varijantama. Uvjeti i tradicija proizvodnje razlikuju se u pojedinim regijama BiH pa je razumljivo da će se gotov proizvod razlikovati po kvaliteti i senzorskim svojstvima. Iako se proizvodnja odvija u nekoliko regija BiH, ukupna količina goveđeg pršuta proizvedena svake godine ne zadovoljava u potpunosti potrebe BiH. Zato nastojimo povećati proizvodnju goveđe šunke na industrijski način, jer za ovaj proizvod vlada veliki interes na domaćem i inozemnom tržištu.

Meso, građa i sastav

Meso u širem smislu riječi čine jestivi dijelovi tijela stoke za klanje, peradi, kunića i divljači u neprerađenom i prerađenom obliku, a neobuhvata tjelesna tkiva i organe riba, rakova, školjki, žaba i puževa. U užem smislu riječi meso čini skeletna (poprečno–prugasta) muskulatura s uraštenim vezivnim i masnim tkivom, hrskavicama, mastima, limfnim čvorovima, te limfnim i krvnim žilama i nervima. Sporedni proizvodi dobiju se klanjem stoke, s tim da postoje jestivi sporedni proizvodi (koriste se za ishranu ljudi i ubrajaju se u meso u širem smislu) i nejestivi sporedni proizvodi (koriste se samo za tehničke svrhe ili su neupotrebljivi) (Živković, 2001; Ritz et al., 1986; Oluški, 1966).

U jestive sporedne proizvode spadaju mozak, jezik, srce, pluća, grudna žlijezda, jetra, slezena, bubrezi, testisi, želudac teladi i svinja, predželuci goveda, teladi i ovaca (burag i kapura), tanka crijeva ovaca, dio debelog crijeva svinja, goveda, teladi, ovaca i janjadi, glava (ošurena ili odstranjene kože), donji dijelovi nogu (odvojeni u skočnom i koljenom zglobu), repni dio kičme sa muskulaturom, potkožno masno tkivo svinja (slanina) i naslage masnog tkiva u trbušnoj dupljii na crijevima (salo i oporci svinja i loj goveda, ovaca i koza), koža svinja bez dlaka, koža glave i nogu teladi (ošurena ili bez dlaka), gubice goveda, krv i kosti (Živković, 2001).

Skeletna muskulatura je po hranjivoj vrijednosti najvrijedniji sastojak mesa i čini meso u najužem smislu riječi, a sastoji se od poprečno-prugastog mišićnog tkiva. Ono je građeno od kontraktilnih mišićnih vlakana (ćelija) obavijenih tankom vezivno-tkivnom ovojnicom ili endomysium. Ova vlakna su međusobno povezana u snopiće koje obavija nešto deblji sloj vezivnog tkiva ili perimysium. Određeni broj snopića je povezan još debljom vezivno-tkivnom ovojnicom ili epimysium u mišić, a više mišića koji čine anatomsku i funkcionalnu cjelinu obavija deblja ovojnica ili fascia (Hamill i Botinestean, 2016; Ritz i sar., 1986).

Veličina (dužina i promjer) mišićnih vlakana i snopića, kao i mišića varira ovisno o anatomskoj regiji i funkciji koju obavljaju. Te razlike u strukturi i sastavu mišićnog i intersticijalnog vezivnog tkiva po anatomskim regijama su osnov kategorizacije dijelova mesa na trupu po kvalitetu. Meso je, čak i kad potiče od iste životinjske vrste, suviše varijabilnog i heterogenog sastava da bi moglo da posluži kao strogo standardna sirovina za dobijanje sasvim precizno definisanihi standardnih proizvoda, namijenjenih savremenim tržištima (Hamill i Botinestean, 2016).

Klasifikacija mesa

Tehnološke operacije hlađenja, smrzavanja, usitnjavanja, salamurenja, soljenja te drugi načini

pripremanja mesa i sirovina za proizvode od mesa, izbor različitih crijeva u koje će kobasice biti punjene, a naročito velike varijacije u režimima hladnog ili toplog dimljenja, barenja i kuhanja, sušenja, fermentacije, hlađenja i pakovanja itd. Komplikuju proizvodnju prerađevina od mesa i njihov finalni kvalitet do te mjere da je teško dati njihovu logičnu i tehnološki opravdanu klasifikaciju (Smajić, 2014).

Nema sumnje da se u klasifikaciju proizvoda od mesa, sa gledišta tehnologije, moraju uzeti u obzir dva važna kriterijuma: sirovinski sastav i karakter tehnološkog procesa. Podjela, koja bi se zasnivala na dosljednoj primjeni oba ova važna kriterijuma, bila bi isuviše komplikovana, dok je uzimanje u obzir samo jednog od njih nedovoljno. Podjela proizvoda od mesa, koja se na ovom mjestu daje, zasniva se prvenstveno na tehnološkom osnovu za većinu proizvoda od mesa te je na prvo mjesto stavljena razlika između sirovih i termički obrađivanih proizvoda od mesa (Ganić i sar., 2012; Milosavljević, 2004).

Kad se općenito govori o goveđoj šunki treba imati na umu činjenicu da je to trajni suhomesnati proizvod, a pravi se od dijelova junećeg buta kao što su šol, ruža i frikando, zatim od slabinske i leđne muskulature (ramstek) koji može da se soli isuši u cijelom komadu.

Od dijelova buta se sijeku komadi mesa dužine 20-60 cm i debljine oko 10 cm. Meso prije siječenja mora biti svježe i ohlađeno u dubini od +4 do +7°C. Za soljenje se koristi kuhinjska so. Na 100 kg mesa potrebno je oko 8 kg soli. Soljenje se vrši tako što se u komade mesa dobro utrlja sol. Slaže se u posude, a prilikom slaganja može da se pomalo do soli. Soljenje traje tri nedelje. Poslije toga meso se ispere u hladnoj void radi rasoljavanja. Komadi mesa se vezuju kanapom provučenim na krajevima i kače u pušnice. Cijeđenje traje oko 24 sata. Zatim se pristupa dimljenju koje traje od 7 do 10 dana. Dimi se sa bukovim drvetom i piljevinom, a temperatura u pušnici treba da bude do 20°C. Kada se dobije poželjna boja i procijeni da je dimljenje završeno, pršut se stavlja na zrenje sa prirodnom ventilacijom i temperaturom oko 15°C. Zrenjetraje od 15 do 30 dana, zavisno od debljine komada mesa. Čuva se u tamnim prostorijama, sa blagom cirkulacijom vazduha, na temperaturi 10-15°C (Listrat i sar., 2016).

I u proizvodnji "Brčanske šunke", korištene su može se reći "tradicionalne" metode koje su svojstvene za ovu vrstu suhomesnatog proizvoda.

Potreba za ispitivanjima provedenim u ovom radu, leži u motivisanju masovnije proizvodnje tradicionalnih suhomesnatih proizvoda koje odlikuje visok nutritivni i higijenski kvalitet. To bi omogućilo pravilniju i sigurniju ishranu stanovništva, a što bi generisalo bržu obnovu i revitalizaciju proizvođačko-prerađivačkih kapaciteta, kao i održivost seoskih domaćinstava u vidu samozapošljavanja.Upravoovaj rad se i bavi o jednom trajnom suhomesnatom proizvodu tj. Goveđoj "Brčanskoj šunki".

MATERIJAL I METODE

Istraživanja definisana u ovom radu vršena su u periodu od tri godine u pogonu Kompanije za proizvodnju mesa i suho mesnatih proizvoda "Soko", vlasništvo gospodina Husnije Ibranovića iz Brke, Brčko Distrikt, BiH i laboratoriju Zavoda za higijenu i tehnologiju namirnica Univerziteta u Sarajevu – Veterinarskog fakulteta.

Sirovo meso za proizvodnju "Brčanske šunke" poticalo je od mesa krava zaklanih u komunalnoj klaonici u Gornjem Rahiću, koja je udaljena od pogona za proizvodnju suhomesnatih proizvoda 6 kilometara. Nakon klanja životinje i primarne obrade, meso je skladišteno na hladnom režimu od +4^{O}C. Narednog dana, nakon hlađenja, butna muskulatura je iskoštena i oblikovana u komade odgovarajuće veličine. Za labaratorijska ispitivanja (organoleptička, mikrobiološka, fizikalno-hemijska) uzorcisirovog mesa (oblikovani komadi) i finalnog proizvoda "Brčanske šunke", su kontinuirano uzimani iz proizvodnog pogona tri godine. Dostavljanje uzoraka u labaratorij Zavoda obavljeno je rashladnim uređajem na uobičajeni način.

Proces proizvodnje "Brčanske šunke"

U toku tehnološkog procesa proizvodnje "Brčanske šunke" provjerena je opravdanost različitih postupaka konzerviranja natrijevim hloridom (suhi i vlažni postupak) i utjecaj na kvalitet, higijensku ispravnost i održivost finalnog proizvoda. Istraživanjem su obuhvaćene sljedeće grupe uzoraka:

1. sirovo oblikovano meso – inicijalni uzorak
2. uzorci mesa konzervirani suhim postupkom do 10 dana
3. uzorci mesa konzervirani vlažnim postupkom do 10 dana
4. uzorci mesa nakon spiranja i cijeđenja
5. uzorci mesa nakon dimljenja do 10 dana
6. uzorci "Brčanske šunke" nakon zrenja do 10 dana

Kodnavedenihgrupauzorakasirovogoblikovanog mesa iuzoraka u toku tehnološkog procesa proizvodnje, kao I finalnih proizvoda "Brčanske šunke", nakon proizvodnje i skladištenja na različitim temperaturnim režimima, obavljene su sljedeće pretrage:

1. organoleptičko ispitivanje sirovog oblikovanog mesa i "Brčanske šunke" u toku proizvodnje, zrenja (fermentacije) i skladištenja,
2. mikrobiološko ispitivanje sirovog oblikovanog mesa i "Brčanske šunke" u toku proizvodnje, zrenja (fermentacije) i skladištenja, kao i mikrobiološki status radnih površina, pribora, uređaja, radne opreme i ruku radnika u pogonu za proizvodnju pršuta,
3. fizikalno-hemijsko ispitivanje sirovog oblikovanog mesa i "Brčanske šunke" u toku proizvodnje, zrenja (fermentacije) iskladištenja,
4. iskoristivost (randman) polaznog (inicijalnog) sirovog mesa, kao i kaliranje proizvoda u različitim tehnološkim fazama proizvodnje.

Tehnološki process proizvodnje "Brčanske šunke" se odvija u nekoliko faza (Šema1).

Da bi se dobila kvalitetna goveđa šunka potrebno je izvršiti odabir goveda. Koriste se starija goveda svih kategorija (Živković, 2001; Smajić, 1987; Čaušević i sar., 1986; Oluški, 1973). Zaizradu "Brčanske šunke" korištene su starije krave dobi od 10 do 12,13 godina i tjele snete žine od 400 do 600 kg. Priprema životinje za klanje, primarna obrada trupa i hlađenje rađeni su u komunalnoj klaonici u skladu s odredbama Pravilnika o organizaciji službenih kontrola proizvoda životinjskog porijekla namijenjenih ishrani ljudi (Službeni glasnik BiH", 2012). Poslije hlađenja (do temperature u dubini mesa do $+4^{O}C$) vrši se iskoštavanje, a potom se pristupatzv. oblikovanjukomada mesarezanjem.

Naime, za izradu "Brčanske šunke" koristi se meso I kategorije (but) i meso II kategorije (leđa-rozbratna). Od buta se koristi: šol – m. gracillis, m. abductor, m. adductor, m. pektineus; ruža – m. quadriceps (m. vastus lateralis et medialis, m. rectus femoris); frikando – m. semitendineus, m. semi membranaceus, biceps femoris i rozbratna – odnosno ramstek (leđa i slabine) - m. longisimus dorsi. Oblikovani komadi mesa su prosječne težine do 1000 g, dužine oko 27 cm i obima oko 26 cm (Čaušević i sar., 1986).

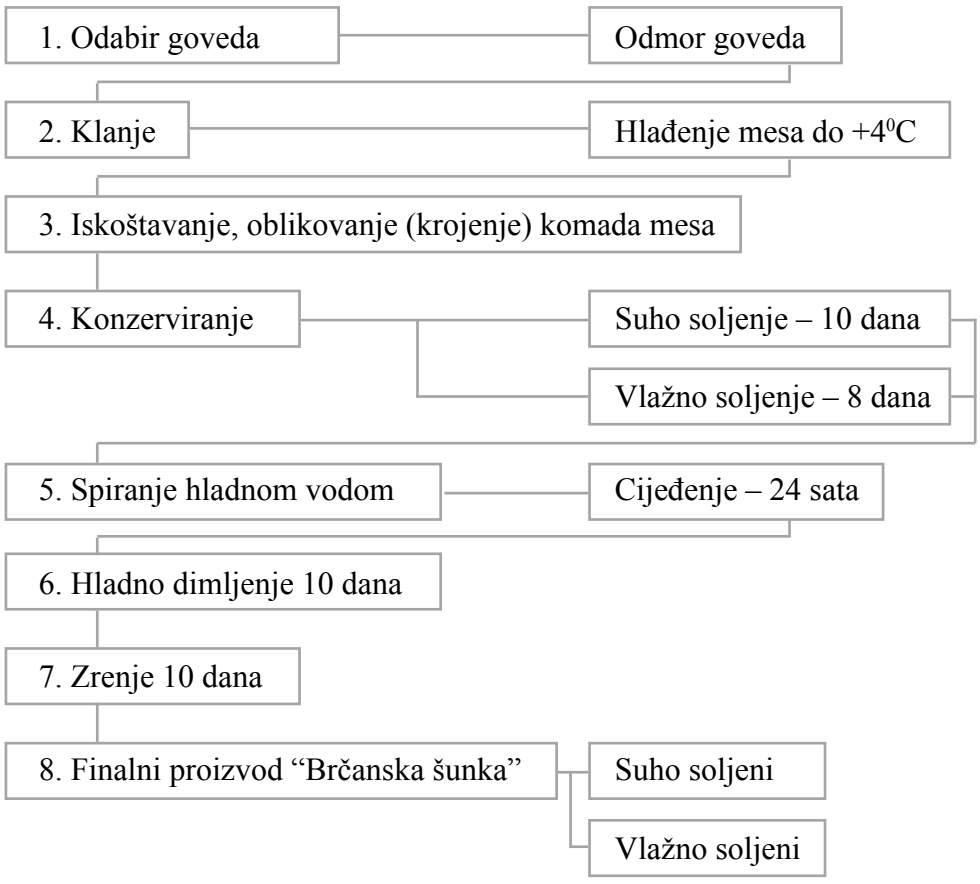
U izradi "Brčanske šunke" korištena su dva postupka konzerviranja: suho i vlažno soljenje.

Kod suhog soljenja pripremljeni komadi mesa se dobro utrljavaju sa suhom smjesom soli i šećera. Na 100 kg mesa koristi se 8 kg soli i 200g šećera, bez nitrata i nitrita i slaže u plastičnu burad koja se zatvaraju poklopcem. Soljenje traje 10 dana. Potom se vrši ispiranje komada mesa hladnom vodom, a zatim kačenje na kuke i cijeđenje. Cijeđenje traje 24 h, a potom se meso vješa na drvene motke u razmakuod 10 do 15 cm istavlja u pušnicu.

Proces hladnog dimljenja (suhom bukovom piljevinom) traje 10 dana, a nekada i duže ovisno o debljini iskrojenih komada mesa. Na otvore kroz koje ulazi dim u pušnicu stavljaju se hladne tj. Pokvašene lanene plahte koje imaju za cilj da zadrže što više čestica dima. One se mijenjaju tri

puta dnevno. Sa završenim dimljenjem meso se prebacuje na zrenje koje traje 10 dana u tamnoj prostoriji sa blagom cirkulacijom i temperaturom od 10 do 15°C. Zrenje ili u našem kraju uvriježen termin "odmor mesa" je bitno jer u ovo jfazi "Brčanska šunka" dobija svoj specifičan izgled, ukus i aromu. Sa završenim zrenjem vrši se skladištenje finalnog proizvoda (Čaušević i sar., 1986).

Kod vlažnog soljenja meso se potapa u smjesu vode, soli i šećera, također, bez nitrata i nitrita i to na 100 kg mesa dodaje se 7 kg soli, 0,5 kg šećera i vode, toliko da za 10 cm prekrije meso. Temperatura ove smjese mora biti od 6 do 10°C. Salamura (so+šećer) se izlije u plastičnu burad, a zatim se slaže komad po komad prethodno oblikovanog mesa. Sa završenim slaganjem mesa bure se zatvara poklopcem i drži u prostoriji temperature od 10°C. Proces vlažnog soljenja traje do 8 dana. Po isteku tog vremena komadi mesa se vade iz salamure i daljnja obrada je identična kao kod suhog soljenja (Smajić, 2014; Milosavljević, 2004).



Šema1 Faze u toku tehnološkog procesa proizvodnje "Brčanske šunke"

Sheme 1 Stages in technological process of "*Brčko prosciutto"* production

REZULTATI

Rezultati istraživanja prikazani suTabelama od 1 do 5. Inicijalni hemijski sastav mesa prikazan je u Tabeli 1.

Tabela 1 Fizikalno-hemijski parametri sirovog mesa pripremljenog za tehnološki proces prerade

Table 1 Physico-chemical parameters of raw meat prepared for technological processing

Vrsta uzorka	Broj uzoraka	Voda %	Mast %	Pepeo %	Protein %	pH
Butna muskulatura	10	74.01	4.05	0.92	21.01	5.56

U suho soljenim uzorcima mesa, odmah nakon faze soljenja srednja vrijednost sadržaja vode je bila 68.44%, NaCl 3.60%, ukupnog šećera 0.21%, a pH 5.52, odnosno u vlažno soljenim uzorcima vode 73.76%, NaCl 3.10%, ukupnog šećera 0.22%, a pH 5.53. Nakon faze zrenja u suho, odnosno vlažno soljenim uzorcima sadržaj vode se smanjio i srednja vrijednost je bila 50.19%, odnosno 56.84%, a NaCl se povećaona 5.52%, odnosno 4.43%, dok je sadrža jukupnih šećera zadržao istu vrijednost kod oba uzorka, odnosno 0.20%, a pH se u oba slučaja neznatno smanjio na 5.36 odnosno 5.31 (Tabela 2).

Tabela 2 Fizikalno-hemijski parametri u mesu i pršutu tokom tehnološkog procesa prerade

Table 2 Physico-chemical parameters of meat and prosciutto during technological processing

Faze tehnološkog procesa prerade	Način soljenja	pH	Voda %	NaCl %	Ukupni šećer %
Inicijalna vrijednost	-	5.56	74.01	-	-
Faza soljenja	Suho	5.52	68.44	3.60	0.21
	Vlažno	5.53	73.76	3.10	0.22
Faza cijeđenja	Suho	5.48	65.42	4.33	0.20
	Vlažno	5.47	72.28	3.92	0.22
Faza dimljenja	Suho	5.42	51.15	5.10	0.19
	Vlažno	5.40	57.85	4.20	0.19
Faza zrenja	Suho	5.36	50.19	5.52	0.20
	Vlažno	5.31	56.84	4.43	0.20

Tabela3 Zastupljenost mikroorganizama na radnim površinama, priboru i rukama radnika u proizvodnom pogonu

Table 3 Presence of microorganisms on working areas, tools and workers' hands during processing

Bris površina	Broj uzoraka	Aerobne mezofilne bakterije /cm^2	Psihrofilne bakterije /cm^2	Halofilne bakterije /cm^2
Radni stol	10	1200	940	650
Nož	10	20	15	Negativne
Kecelja radnika	10	250	115	85
Ruke radnika	10	110	94	47
Kada za soljenje	10	430	560	930
Motka za kačenje mesa	10	9000	780	920
Sirovo meso	10	540	220	97
Folija za vakuumiranje	10	160	83	Negativne

Napomena: salmonele, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli* te *Proteus* vrste nisu izolirane u ispitivanim uzorcima.

U sirovom mesu početne prosječne vrijednosti aerobnih mezotermnih bakterija bile su 5.0x10^3/g, psihrotrofa 0.3x10^2/g, halofilnih bakterija 0.5x10^2/g, enterobakterija 0.6x10^2/g i mikro koka 0.2x10^2/g. Najveća prosječna vrijednost aerobnih mezofilnih bakterija u uzorcima suho mesnatog mesa utvrđena nakon cijeđenja bila je u rasponu od 7,1 x103/g, odnosno 7.5 x10^3/g, manje količin eutvrđene su u konačnom proizvodu nakon zrenja, u rasponu od 5.3 x10^3/g, odnosno 5.5 x10^3/g. Maksimalni prosječni broj psihrotrofnih bakterija u konačnom proizvodu kretao se od 1.8 x10^2/g do 2.0 x10^2/g (suhi ili mokro suhomesnati proizvod), a minimalni broj bakterija u suhomesnatim proizvodima od 0.5 x10^2/g do 0.7 x10^2/g. Najveći broj halofilnih bakterija nađen je u konačnom proizvodu nakon zrenja, u rasponu od 5.9 x10^2/g do 6.1 x10^2/g (suhi ili mokrislani proizvod). Najmanji broj halofilnih bakterija utvrđen je u mesu nakon soljenja i srednja vrijednost se kretala od 1.0 x10^2/g do 1,2 x10^2/g. Entero bakterije i mikro koke nakon soljenja i u daljnjem toku prerade nisu izolovane (Tabela 4).

Tabela 4 Dinamika mikroorganizama u mesu tokom tehnološkog procesa proizvodnje

Table 4 Dynamics of microorganisms in meat during technological processing

Vrsta uzorka	Način soljenja	Aerobne mezofilne bakterije u 1g	Psihrofilne bakterije u 1 g	Halofilne bakterije u 1 g	Entero-bakterija u 1g	Mikrokoke u 1g
Sirovo meso	-	5.0x10^3	0.3x10^2	0.5x x10^2	0.6 x10^2	0.2 x10^2
Soljeno meso	Suho	6.0 x10^3	0.7 x10^2	1.2 x10^2	negativne	negativne
	vlažno	6.5 x10^3	0.5 x10^2	1.0 x10^2	negativne	negativne

Vrsta uzorka	Način soljenja	Aerobne mezofilne bakterije u 1g	Psihrofilne bakterije u 1 g	Halofilne bakterije u 1 g	Entero-bakterija u 1g	Mikrokoke u 1g
Meso nakon cijeđenja	Suho	7.1 x10^3	1.1 x10^2	3.3 x10^2	negativne	negativne
	vlažno	7.5 x10^3	1.0 x10^2	3.0 x10^2	negativne	negativne
Meso nakon dimljenja	Suho	5.8 x10^3	1.7 x10^2	4.2 x10^2	negativne	negativne
	vlažno	6.3 x10^3	1.6 x10^2	4.0 x10^2	negativne	negativne
Meso nakon zrenja	Suho	5.3 x10^3	1.8 x10^2	5.9 x10^2	negativne	negativne
	vlažno	5.5 x10^3	2.0 x10^2	6.1 x10^2	negativne	negativne

Napomena: salmonele, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli* te *Proteus* vrste nisu izolirane u ispitivanim uzorcima.

"Brčanska šunka" proizvedena od sušenog mesa ima ocjenu senzorske ocjene 20,00 bodova (prosjek), što pripada posebnoj ocjeni, a "Brčanska šunka" od sušenog mesa ima ocjenu senzorske ocjene od 19.83 boda (prosjek), što pripada do prvog razreda. Površina uzorka je suha, čista i tamnosmeđe boje. Rubovi su uredno obrubljeni bez ureza, mišićno tkivo na presjeku je smeđe boje, sredina presjeka je crvenkasto smeđa, a prisutne su tačkice masnog tkiva (mramoriranje) krem boje. Konzistencija je čvrsta i elastična. Miris je ugodan, prihvatljiv, blago aromatičan, kao i okus (pun okus). Senzorska ocjena "Brčanske šunke", pripremljene od suhe slanine i pripremljene za čuvanje, iznosila je 20.00 bodova (prosjek), što pripada posebnoj kategoriji, dok je mokraslanina dobila 19.63 boda (prosjek) i pripada kategoriji I (Tabela 5).

Tabela 5 Organoleptička ocjena suho odnosno vlažno soljenog finalnog proizvoda "Brčanske šunke" pripremljene za skladištenje

Table 5 Organoleptic evaluation of dry-and moist-salinated final product "*Brčko prosciutto*" prepared for storing

Finalni proizvod- dani	Vrsta soljenja	Organoleptičkaocjena						Poeni	Klasa
		I	II	III	IV	V	VI		
1	Suho	3.00	6.00	2.00	3.00	4.00	2.00	20.00	Extra
	vlažno	2.89	5.92	1.97	2.95	3.96	1.93	19.63	I

Legenda:

I - Spoljni izgled	- bodovanje od 0-3	Ekstra klasa	20 bodova
II - Sastavi izgled presjeka	- bodovanje od 0-6	I klasa	od 18.00 – 19.99
III - Konzistencija	- bodovanje od 0-2	II klasa	od 16.00 – 17.99
IV - Boja	- bodovanje od 0-3	III klasa	od 14,00-15,99
V - Ukus imiris	- bodovanje od 0-4	Van klase	manje od 14,00 bodova
VI - Prihvatljivost proizvoda	- bodovanje od 0-2		

DISKUSIJA I ZAKLJUČAK

Sastav mesa kao početne komponente u proizvodnji "Brčanske šunke" je u svom sastavu približan vrijednostima iz literature (Smajić, 2014; Rašeta, 1981). Nešto veći procenat proteina je iz razloga što se za proizvodnju "Brčanske šunke" biraju starija mršava goveda (slabe uhranjenosti).

Kao što se vidi iz tabela 1 i 2, promjena mase suho, odnosno vlažno soljenih uzoraka mesa po fazama tehnološkog procesa prerade imala je očekivani trend. Kod suho, odnosno vlažno soljenih uzoraka mesa nakon faze soljenja došlo je do povećanja mase za 1.06, odnosno 9.49%, nakon faze cijeđenja povećanje je bilo 0.93, odnosno 8.03%, a da bi nakon faze dimljenja kalo iznosio 38.57, odnosno 42.35%. Sa završenom fazom zrenja kalo je iznosio 40.39, odnosno 43.04%. Što će reći, da je randman za suho, odnosno vlažno soljene uzorke iznosio 59.61, odnosno 56.59%. Navedene vrijednosti kala su slične navedenim vrijednostima drugih autora (Vial i sar., 2021; Pegg i Honikel, 2014; Barat i Taldra, 2011; Milosavljević, 2004; Čaušević i sar., 1986; Ritz i sar., 1986; Rašeta, 1981). Razlike u kaliranju između suho i vlažno soljenih uzoraka mesa i finalnih proizvoda dovode se u vezu sa različitim osmotskim pritiskom, a samim tim i različitom otpuštanju vode iz mesnog supstrata.

Kod ispitivanih mikro ambijenata, odnosno radnih površina, najveći broj aerobnih mezofilnih bakterija utvrđen je na motkama za kačenje mesa i srednja vrijednost je iznosila 9000/cm^2**,** na površini radnog stola 1200/cm^2, a najmanji na površini ruku radnika 110/cm^2 i površini noža 20/cm^2**.** Psihrofilne bakterije su utvrđene u najvećem broju na površini radnog stola 940/cm^2 i površini motki za kačenje mesa 780/cm^2. U manjem broju ispitivane bakterije su utvrđene na površini noža 15/cm^2 i površini folije za vakumiranje 83/cm^2. Halofilne bakterije su utvrđene u najvećem broju na površini kade za soljenje 930/cm^2 i površini motke za kačenje mesa 920/cm^2, a nisu izolirane na površini noža i folije za vakuumiranje. Analiza drugih radnih površina, pribora, uređaja i ruku radnika u toku proizvodnje "Brčanske šunke" ukazuje na smanjenu kontaminiranost navedenim skupinama mikroorganizama. Ovi rezultati ukazuju na potrebnu povećanu pažnju u održavanju mikroklimatskih uslova proizvodnje, korištenje i adekvatnu primjenu dezinfekcionih sredstava u sanaciji radnih površina kao i održavanje lične higijene samih radnika (Živković, 2001; Tadić i sar., 1987).

U proizvodnji suhomesnatih proizvoda posebna pažnja se poklanja inicijalnoj mikroflori sirovina, jer se u toku tehnoloških faza prerade pod raznim uvjetima razmnožavaju preživjeli mikroorganizmi. Srednja vrijednost ukupnog broja mikroorganizama kod sirovog mesa kretala se od 0.2x10^2/g za mikrokoke, 0.3 x10^2/g za psihrofilne bakterije, 0,5x10^2/g za halofilne bakterije, 0.6x10^2/g za enterobakterije, do 5.0 x10^3/g za aerobne mezofilne bakterije. Utvrđene skupine bakterija su u tolerantnim vrijednostima tako da je sirovo meso sa mikrobiološkog aspekta predstavljalo povoljnu sirovinu za tehnološki proces proizvodnje "Brčanske šunke".

Nakon soljenja (suho i vlažno), 10. dan u ispitivanim uzorcima mikroorganizmi su pokazivali neznatno povećanje tako da je srednja vrijednost halofilnih bakterija povećana na 1.0, odnosno 1.2x10^2/g, psihrofilnih bakterija na 0.7, odnosno 0.5 x10^2/g, a aerobnih mezofilnih bakterija na 6.0, odnosno 6.5x10^3/g. Najveća srednja vrijednost aerobnih mezofilnih bakterija u uzorcima suho, odnosno vlažno soljenog mesa je utvrđena nakon cijeđenja i iznosila je 7.1, odnosno 7.5 x10^3/g.

Po fazama tehnološkog procesa proizvodnje suho, odnosno vlažno soljenog mesa i finalnog proizvoda, aerobne mezofilne bakterije su utvrđene u najvećem broju u mesu nakon cijeđenja od 7.1, odnosno 7.5x10^3/g, a u manjem broju su utvrđene u finalnom proizvodu nakon zrenja i srednja vrijednost se kretala od 5.3, odnosno 5.5 x10^3/g. Najveći broj psihrofilnih bakterija utvrđen je u finalnom proizvodu i kretao se od 1.8, odnosno 2.0 x10^2/g, a najmanji broj u mesu nakon soljenja i iznosio je 0.5, odnosno 0.7 x10^2/g. Halofilne bakterije u najvećem broju su utvrđene u finalnom proizvodu nakon zrenja i kretale su se od 5.9,

odnosno 6.1 x10^2/g, a najmanji broj u mesu nakon soljenja i kretao se od 1.0, odnosno 1.2 x10^2/g. Enterobakterije i mikrokoke nakon soljenja i u daljnjem toku prerade nisu izolirane.

Inicijalni broj mikroorganizama (aerobne mezofilne bakterije, psihrofilne i halofilne) utvrđen u sirovom mesu, zadržao se tokom tehnoloških faza prerade na nivou iste logaritamske potencije, što se može dovesti u vezu sa bakteriostatskim efektima NaCl-a i pojedinih sastojaka dima (fenoli, formaldehidi i sl.), odnosno baktericidnim na enterobakterije i mikrokoke. Navedene bakterijske skupine su utvrđene jedino u sirovom mesu u broju 0.2, odnosno 0.6x10^2/g, što je znatno niže od vrijednosti koje su utvrdili drugi autori (Barat i Taldra, 2011). U ispitivanim uzorcima tokom tehnoloških faza prerade, kao i finalnom proizvodu nisu utvrđene salmonela, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, E. coli kao ni proteus vrste, što se sa mikrobiološkog aspekta ocjenjuje veoma povoljno, a finalni proizvod proglašava mikrobiološki ispravnim jer zadovoljava sve kriterije Pravilnika o mikrobiološkim kriterijumima za hranu. ("Službeni glasnik BiH", 2013).

Organoleptička ocjena "Brčanske šunke" proizvedene od suho, odnosno vlažno soljenog mesa vršena je prema prihvaćenim standardima (Listrat i sar., 2016; Ritzet et al., 1986), metodom poentiranja sa maksimalnih 20 bodova. "Brčanska šunka" proizvedena od suho soljenog mesa organoleptičkom ocjenom poentiran je sa 20.00 bodova i svrstan je u ekstra klasu, a od vlažno soljenog mesa poentiran je sa 19.83 boda i svrstan je u I klasu. Na osnovu organoleptičkih ocjena te mikrobioloških i fizikalno hemijskih pretraga finalni proizvodi odgovaraju odredbama Pravilnika o ustinjenom mesu, poluproizvodima i proizvodima od mesa ("Službeni glasnik BiH", 2013) i Pravilnika o mikrobiološkim kriterijumima za hranu ("Službeni glasnik BiH", 2013).

Zbog specifičnih organoleptičkih i fizikalno-hemijskih svojstava, kao i izrazitom prepoznatljivošću na tržištu, odnosno tradicionalnu udomaćenost u ovom kraju prednost dati suho soljenoj formi "Brčanske šunke" (koja je i dobila veći broj bodova u odnosu na vlažnu formu) te je kao takvu i kandidovati kao reprezenta posavskog kraja.

Motivirati masovniju proizvodnju tradicionalnih suhomesnatih proizvoda koje odlikuje visok nutritivni i higijenski kvalitet bi omogućilo pravilniju i sigurniju ishranu stanovništva, a što bi generiralo bržu obnovu i revitalizaciju proizvođačko-prerađivačkih kapaciteta. U tom smislu bi trebalo izvršiti doedukacije postojećih individualnih proizvođača u smislu sinhronizacije tehnološkog procesa proizvodnje, uvesti vakuumiranje proizvoda što bi doprinijelo podizanju cjelokupnog kvaliteta i dalo pečat prepoznatljivom originalnom proizvodu ovog kraja uz naglasak da je riječ o prirodnom proizvodu. Također, bilo po poželjno i institucionalno, ali i finansijski stajati iza ovakvih proizvođača, jer osim što se bave proizvodnjom održavaju trend tradicionalne proizvodnje zdrave hrane.

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PRODUCTION OF BRČKO PROSCIUTTO USING A STANDARD METHOD ADDENDUM TO THE KNOWLEDGE OF PRODUCTION OF BRČKO PROSCIUTTO USING A STANDARD METHOD

ABSTRACT

Dried meat has been considered of great importance in human nutrition, also being the earliest processed meat to be manufactured, the main reasons being a relatively simple production process, long shelf life, facilitated labor and high nutritional values.

In Bosnia and Herzegovina, a beef prosciutto has been a valuable and popular dried meat product, while the conditions and manufacturing traditions differ countrywide, with the final products of variable quality and organoleptic features. Livestock selection is a prerequisite for the production of high-quality beef prosciutto. I category (Round) and II category (Rib) meat is used for the production of Brčko prosciutto. Brčko prosciutto has a specific conservation method characterized by the use of salt, sugar and nitrite- and nitrate-free water, with no other additives. In this way, the consumer-desirable organoleptic and microbiological features are achieved.

We researched a technological process through different stages of manufacturing of Brčko prosciutto. The aim is to additionally enhance a mass manufacturing of the traditional dried meat products characterized by high nutritional and hygienic quality.

Keywords: Brčko, food, ham, meat

PROFESSIONAL PAPER

VETERINARSKA PERSPEKTIVA U OČUVANJU VRSTA SA CRVENIH LISTA FAUNE U BOSNI I HERCEGOVINI

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SAŽETAK

U radu je naglašena neophodna uloga doktora veterinarske medicine u očuvanju životne sredine i divljih životinja. Veterinarska perspektiva ne samo da poboljšava razumijevanje, već i doprinosi efikasnom upravljanju divljim životinjama i njihovoj dobrobiti uz proaktivnu integraciju postojeće tehnologije unutar veterinarske prakse. Analizirajući izazove u provođenju mjera očuvanja, uz posebno istaknutu potrebu zakonodavnih poboljšanja, uspostavljanje neophodnih institucija, izgradnju kapaciteta, finansijsku podršku i sveobuhvatno planiranje su zapravo zaključci rada koji nude rješavanje problema u vezi sa Nacionalnom Crvenom listom i mjerama očuvanja okoliša u Federaciji Bosne i Hercegovine (FBiH).

Keywords: Crvena lista, konzervacija, velike zvijeri, *Spalax leucodon*

UVOD

Doktori veterinarske medicine bi trebali posjedovati dovoljno razvijenu svijest i biti među prvim u ešalonu koji štite zdravlje divljih životinja i doprinose ukupnom očuvanju biološkog diverziteta, (Aguirre et al., 2009; Mainka, 2001). Sistematičnim radom bi trebali doprinijeti efikasnijem upravljanju divljim životinjama, posebno s aspekta dobrobiti žitvotinja (Lanfranchi et al., 2003). Upravljanje vrstama i ekosistemima treba da podrazumijeva "One Health" princip (Cunningham et al., 2017).

Da bi se veterinarska medicina efikasno povezala sa zaštitom ugroženih vrsta Crvenom listom (Škrijelj et al., 2013), nužno je da doktori veterinarske medicine pored stručno-operativnog rada, integriraju svoje znanje u širu perspektivu očuvanja biodiverziteta. Oni mogu imati ključnu ulogu u praćenju i održavanju zdravlja populacija divljih životinja, vršiti zdravstvene procjene ugroženih vrsta ispitujući pojedince na bolesti, povrede i ukupnu procjenu zdravlja populacije, kao i biti uključeni u rehabilitaciju ozlijeđenih životinja ili napuštenih mladih životinja. Doktori veterinarske medicine, također, igraju ulogu u podizanju svijesti javnosti, sa već uspostavljenom nacionalnom mrežom veterinarskih organizacija, o važnosti očuvanja biodiverziteta

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i ulozi zdravlja divljih životinja u stabilnosti ekosistema. Sposobnosti reagovanja u hitnim slučajevima, poboljšane već postojećim mrežama veterinarskih stanica, ustanova i organizacija širom svijeta, osiguravaju brzu podršku tokom kriza (Mainka, 2001).

Ova podrška podrazumijeva tri nivoa. Logističko-resursni nivo bi podrazumijevao skup svih materijalno-tehničkih i ljudskih resursa uključenih u veterinarske organizacije, a posebno u podršci upravljanju djelatnosti sa fokusom na upravljanje zoološkim vrtovima, prihvatilištima i vivarijumima u sklopu obrazovnih i istraživačkih institucija. Drugi nivo jeste pružanje habitata u kriznim situacijama za opstanak određenih vrsta. Treći nivo podrške jeste u praktičnim konzervacijskim akcijama gdje je doktor veterinarske medicine nasušna potreba ekologa ili populacionog biologa. Bez doktora veterinarske medicine pokušaji konzervacije na individualnom ili u populacijskom smislu su u najmanju ruku neozbiljni za mnoge životinjske skupine. Imali smo intenciju ukazati na distinkciju između dvije oblasti, iako su one skoro neodvojive jedna od druge. Međutim, nužno je uzeti u obzir njihove definicije i suštinske razlike. Upravljanje vrstama sa crvenih lista je direktna zaštita biodiverziteta kroz zaštitu od izumiranja pojedinih vrsta, bilo to na lokalnom, regionalnom ili globalnom nivou. S obzirom na obuhvat djelovanja ili planiranja konzervacije vrsta, tu su uvijek pravne, ekonomske, sociološke, političke i mnoge druge implikacije koje se moraju uzeti u obzir i zato je neophodno imati multidisciplinarni pristup koji podrazumijeva zajedničko djelovanje mnogih struka koje su adekvatne da riješe potencijalne probleme i iznjedre najbolje moguće prakse upravljanja biodiverzitetom.

Doktor veterinarske medicine trebao bi biti svjestan svoje pozicije u neminovno složenom sistemu upravljanja resursima i zaštite životne sredine i da ga to ne ograničava ili isključuje od participiranja u aktivnostima i funkcijama za koja su potrebna druga znanja i vještine kao npr. iz domena političkih znanosti ili ekonomije. Moramo promijeniti svijest i znati da doktori veterinarske medicine nisu samo primarno involvirani u kurativi domestifikovanih životinja, već mogu biti itekako konstruktivni, posebno kroz aktivno participiranje u kreiranju novih i implementiranju postojećih međunarodnih strategija i konvencija, kao što su CBD, Bernska konvencija, Ramsar, Cites i druge (Mainka, 2001). Definitivno, samo aktivno učešće u oblikovanju ovih politika osigurava uključivanje veterinarske perspektive i struke u procese donošenja odluka.

Crvene liste: Koliko su zaštićene vrste, zaista zaštićene?

Crvena lista ugroženih vrsta IUCN-a (The International Union for Conservation of Nature) jeste najobimniji izvor informacija u svijetu o globalnom statusu zaštite vrsta životinja, gljiva i biljaka i njihovoj povezanosti s uslovima života. IUCN Crvena lista je kritičan indikator zdravlja svjetskog biodiverziteta. Daleko više od liste vrsta i njihovog statusa, to je moćno sredstvo za informisanje i pokretanje akcija za očuvanje biodiverziteta i promjenu politika, što je ključno za zaštitu prirodnih resursa koji su nam potrebni da preživimo. Ista nam pruža informacije o rasponu, veličini populacije, staništu i ekologiji, korištenju i/ili trgovini, prijetnjama i akcijama očuvanja koje će pomoći u donošenju potrebnih odluka o očuvanju biodiverziteta. Iako Crvena lista sama po sebi ne predstavlja obavezujući dokument i prioritetnu listu, ona se često koristi pri kreiranju lokalnih, nacionalnih i međunarodnih politika i aktivnosti (ref. IUCN ECARO. 2019).

Nacionalna Crvena lista za zaštitu vrsta u Bosni i Hercegovini (BiH) podrazumijeva Vodič za reviziju i usklađivanje i povezana je s IUCN-ovom Regionalnom kancelarijom za Istočnu Evropu i Centralnu Aziju, s adresom u Beogradu.

Crvene liste flore i faune predstavljaju bitan alat za procjenu biodiverziteta i prepoznavanje vrsta koje su izložene riziku od opadanja brojnosti ili izumiranja (Bachman et al., 2019). Međutim, javljaju se izazovi u prevođenju ovih lista u efikasne mjere zaštite, što je naglašeno neadekvatnostima u zakonodavstvu, planovima upravljanja i definisanim odgovornostima (Rodrigues et al., 2006).

Problem nedostataka odgovarajućih koraka, mjera, planova upravljanja i tačno definiranih nadležnosti za zaštitu ugroženih vrsta i njihovih staništa navedenih u Crvenoj listi dovodi do neadekvatne zakonske regulative i nedostatka sredstava za provođenje zaštite i monitoringa (Rodrigues et al., 2006). Bitan problem je upravljanje pojedinim zaštićenim vrstama na osnovu entitetskih propisa o lovstvu, gdje su pojedine zaštićene vrste definirane kao divljač čiji je lov dozvoljen (Kunovac et al., 2017). U ovakvim okolnostima imamo i prisutan pokušaj stvaranja zatvorenih sistema upravljanja omalovažavanjem drugih mišljenja i onemogućavanja utjecaja ostalih interesnih grupa "stakeholdera" u planiranju zaštite i upravljanja pojedinim vrstama, nazivajući ih "laicima i neupućenima" (Kunovac, 2023).

Kontinuirana procjena statusa vrsta i ekosistema omogućava informirane odluke, implementaciju ciljanih mjera očuvanja te doprinosi dugoročnom zdravlju i održivosti ekosistema (Stem et al., 2005).

Sistematsko rješavanje prijetnji i uvezivanje postojećih kapaciteta je jedino rješenje za cjelokupno zdravlje i održivost ekosistema. Ove mjere trebaju uključivati i podizanje svijesti javnosti i uključivanje lokalne zajednice, jer nedostatak podrške i ravnodušnost javnosti često predstavljaju izazove u implementaciji učinkovitih strategija zaštite vrsta (Gann et al., 2019).

Tema očuvanja vrsta u BiH je složena, posebno uzimajući u obzir komplicirano državno uređenje. Pretpostavka je da postoji nesrazmjer između stvarnog broja radnji koje ugrožavaju zaštićene vrste i njihova staništa i broja prijavljenih ili otkrivenih slučajeva, što rezultira nedovoljno poznatom statistikom (Lelo, 2017). Ovo stanje proizilazi iz nedostatka svijesti u institucijama i javnosti o značaju i posljedicama ovakvog djelovanja na ekosisteme. Također, institucije zadužene za zaštitu prirode suočavaju se s ograničenjima kapaciteta (Lelo, 2017).

Kaznena politika se smatra neadekvatnom, a slučaj krivolova risa iz 2014. godine jasno pokazuje poteškoće u primjeni postojećih zakona (Lelo, 2017). Većina dostupnih podataka i analiza dolazi iz nevladinog sektora, što ukazuje na nedostatke u praćenju i izvještavanju, posebno na državnom nivou. Tako da možemo navesti primjer slijepog kučeta (*Spalax Leucodon*), strogo zaštićenog u susjedstvu (NN 144/2013), ali navedenog sa nepostojanjem dovoljno podataka u FBiH Crvenoj listi kako bi dobio legislativnu zaštitu (Škrijelj et al., 2013).

Problem dodatno komplicira nedostatak direktne nadležnosti državnog nivoa u sektoru zaštite prirode, što rezultira velikim brojem nadležnih institucija, nejasnoćama u nadležnostima, problemima u razmjeni informacija i značajnim nedostatkom osoblja. Iako postoje međunarodne obaveze koje obezbjeđuju određeni napredak, izazovi i dalje ostaju.

Rješenje problema zahtijeva podizanje svijesti i aktivno uključivanje javnosti u zaštitu zaštićenih vrsta. Važno je podsticati javnost da prijavi i prati nezakonite radnje koje ugrožavaju ove vrste i njihova staništa, a kao pozitivnu stranu treba istaći deklarativnu spremnost i obavezu zemlje da ispuni međunarodne sporazume.

Veterinarska perspektiva naročito postaje značajna u rješavanju problema nadležnosti i legislative državnog nivoa jer omogućava Ministarstvu vanjske trgovine i ekonomskih odnosa BiH propisivanje i provođenje zaštite faune kroz zakonski okvir i nadležnost u oblasti veterinarstva preko zakona koji reguliraju ovu oblast, a u direktnoj su nadležnosti ministarstva (Zakon o dobrobiti životinja i Zakon o veterinarstvu BiH).

Kada je riječ o nacionalnim crvenim listama flore i faune, ključnu ulogu u provođenju i praćenju mjera očuvanja imaju planovi upravljanja vrstama i osnivanje Federalnog zavoda za zaštitu okoliša. Planovi upravljanja vrstama pružaju smjernice o konkretnim koracima koji su potrebni kako bi se dugoročno sačuvali ugroženi organizmi uz harmoničan suživot sa svim korisnicima prostora (Jurić, 2019).

Federalni zavod za zaštitu okoliša, s druge strane, trebao bi omogućiti aktivno učešće svih ključnih sudionika povezanih s pitanjem očuvanja vrsta.

U saradnji sa njima, zajedno se razrađuju ključni problemi, ciljevi, prioriteti, mjere i aktivnosti. Iako je Zakonom o zaštiti prirode iz 2013. godine u FBiH ("Sl. novine FBiH" 15/21) predviđeno osnivanje Federalnog zavoda za zaštitu prirode, ta institucija još uvijek nije uspostavljena pa Federalno ministarstvo okoliša i turizma trenutno obavlja sve poslove u vezi s tim, uprkos ograničenim ljudskim kapacitetima.

U saradnji sa stručnjacima, naučnim institucijama i lokalnim zajednicama, Federalni zavod za zaštitu okoliša treba pridonijeti formulisanju učinkovitih planova upravljanja vrstama kako bi se dugoročno očuvala biološka raznolikost u FBiH. Sistematski pristup koji uključuje Federalni zavod za zaštitu okoliša, kao i druge relevantne institucije, posebno u saradnji s akademskom zajednicom, ključan je za uspješnu implementaciju programa očuvanja i osiguranje dugoročne zaštite zaštićenih vrsta i njihovih staništa.

ZAKLJUČAK

U skladu sa navednim, a u cilju rješavanja navedenih problema vezanih za nacionalnu crvenu listu nameću se odgovarajući zaključci:

- Nužnost usklađivanja, unapređivanja i revidiranja postojećeg Zakone iz oblasti okoliša i veterinarstva sa državnom nadležnošću, s posebnim osvrtom na propise o lovstvu kako bi se očuvale zaštićene vrste.
- Uspostavljanje Federalnog zavoda za zaštitu prirode te osiguravanje usklađenosti sa Zakonom o zaštiti prirode iz 2013. godine.
- Permanentne aktivnosti na poboljšanju kapaciteta i koordinaciji budućeg Federalnog zavoda za zaštitu prirode i Federalnog ministarstva okoliša i turizma.
- Ojačavanje saradnje sa relevantnim sudionicima, tj. članicama Univerziteta u Sarajevu: Prirodno-matematičkim, Poljoprivredno-prehrambenim, Šumarskim i Veterinarskim fakultetom, kao i Zemaljskim muzejom BiH u Sarajevu.
- Nužnost obezbjeđivanja dodatne finansijske podrške za neophodne mjere konzervacije te razvijanje i implementiranje sveobuhvatnih planova konzervacije za ugrožene vrste.

Navedeni zaključci bi imali za cilj rješavanje velikog broja izazova u vezi sa crvenom listom i mjerama očuvanja u FBiH, kao i način rješavanja nadležnosti na državnom nivou. Kroz zakonodavne reforme, uspostavu ključnih institucija, izgradnju kapaciteta, finansijsku podršku i sveobuhvatno planiranje, program teži poboljšanju konzervacijskih napora i promocija održive zaštite biološke raznolikosti, a istovremeno omogućava aktivno uključivanje veterinarske struke u zaštitu biodiverziteta.

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VETERINARY PERSPECTIVE ON THE CONSERVATION OF RED LIST SPECIES OF THE FAUNA IN BOSNIA AND HERZEGOVINA

ABSTRACT

In our research, we emphasize the indispensable role of veterinarians in conserving endangered species. It highlights the need for legislative improvements, establishment of necessary institutions, capacity building, financial support, and comprehensive planning to address challenges related to the national Red List and environmental conservation in BiH. The involvement of veterinary medicine in species conservation is crucial for effective management and protection of wildlife populations. Integrating enhanced networks of veterinary clinics, institutions, and organizations strengthens the infrastructure for conservation efforts. We conclude that legal system improvements, establishment of the Federal Institute for Nature Protection, enhancing collaboration with relevant stakeholders, and securing financial support for conservation efforts all create potentials for problem solving in relation to the national Red List in order to preserve the environment in the Federation of Bosnia and Herzegovina (FBiH).

Key words: conservation, Red list, *Spalax leucodon*

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