

ISSN (print) 1454-7406
ISSN (electronic) 2393-4603

**UNIVERSITATEA DE ȘTIINȚE AGRICOLE
ȘI MEDICINĂ VETERINARĂ
"ION IONESCU DE LA BRAD" DIN IAȘI**
University of Applied Life Science and Environment



**SCIENTIFIC PAPERS
VETERINARY MEDICINE**

***LUCRĂRI ȘTIINȚIFICE
SERIA MEDICINĂ VETERINARĂ***

**VOLUME 63
NO. 4**

EDITURA "ION IONESCU DE LA BRAD"



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Anatomical peculiarities of the broad-snouted caiman's skull and vertebral column bones (*Caiman latirostris*)

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Abstract

It was noticed that the caiman skull is diaspid, presenting two temporal fossae, one superior (dorsal) and one inferior (lateral). The cranial cavity is reduced compared to the development of the skull, with the orbits and nostrils having a lateral orientation. The occipital has a single articular condyle for the atlas. In front of the first vertebra, atlas, there is an undeveloped proatlas vertebra. The cervical vertebrae have developed transverse processes. There is also a ventral crest on the body of the cervical vertebrae. The thoracic vertebrae have detached transverse processes, which grow towards the end of the region, serving alone for the articulation with the ribs. The sacral region is short, and consist in two vertebrae which articulate with the ilium. The caudal vertebrae bear relatively long neural and haemal spines, forming a ventral arch for the blood vessels.

Keywords: caiman, peculiarities, skull and vertebral column bones

Introduction

The broad-snouted caiman (*Caiman latirostris*) is a crocodylian reptile American of medium size (for their group), generally 1,5-2 m long, part of the *Reptilia Class, Crocodylia Order*. This order includes big, predatory and semi-aquatic reptiles, crocodiles (*Fam. Crocodylidae*), alligators and the caimans (*Fam. Alligatoridae*) and the gavials (*Fam. Gavialidae*). Found in eastern and central South America, including southeastern Brazil, northern Argentina, Uruguay, Paraguay and Bolivia. It is found mostly in freshwater marshes, swamps, and mangroves, usually in still or very slow-moving waters (4,5, 6).

The aim of the study was the highlight of the specific characters of the skull and vertebral column bones in broad-snouted caiman, having many morphological peculiarities, the knowledge of them being important zoologically, but also clinically, for those animals which are kept in captivity, in zoo parks and can present various pathologies.

Materials and methods

The study was conducted on a corp of a broad-snouted caiman (*Caiman latirostris*), that belonged to Bârlad Zoo. It was a male caiman, 24 years old. For the analysis, the bones were prepared by boiling and cleaning. By using classical anatomical methods there were noticed the particularities of the skull and vertebral column bones. Each bone was examined by observing both its dimensions, the development of certain processes and any difference regarding other species of

reptilians. For illustration, they were photographed with the Olympus camera, and they were processed in Adobe Photoshop.

Results and discussions

The dorsal surface of the skull is sculptured with pits, grooves, and ridges. Sculpturing is also present, to a smaller extent, on the lateral and ventral aspect of mandibular surfaces. The oral part of the rostrum is formed by the premaxillae. Located dorsally on the premaxillae is the large opening for the nostrils. Another opening on the premaxillae, is the incisive foramen. The largest bones of the rostrum are the maxillae. The nasals are short and relatively narrow bones that are placed dorsomedially on the rostrum and reach the external naris posteriorly.

The frontal constituting the majority of the medial orbital margins. Anteriorly, the frontal forms an elongated pointed process that is wedged between the prefrontals and nasals. Medially, the orbits are separated from each other by the cartilaginous interorbital septum.

The portion of the skull above and posterior to the eyes is comprised of the postorbitals, squamosals, parietal, and most of the frontal. The posteromedial part of the cranial table is formed by the parietal, which is another relatively large bone.

The postorbitals form the anterolateral portions of the cranial table. Ventrally, the postorbitals send descending, pillar-like processes that form significant portions of the postorbital bars. The postorbital bars are also formed by the ascending processes of the zygomatic ventrolaterally and by the ectopterygoids ventromedially. The squamosals make up the posterolateral parts of the cranial table (fig. 1).



Fig. 1 Broad-snouted caiman skull in dorsal aspect:

- 1 – Incisive foramen; 2 – Premaxilla; 3 – Maxilla; 4 – Nasal; 5 – Prefrontal; 6 – Frontal; 7 – Orbit; 8 – Postorbital bar; 9 – Jugal/Zygomatic; 10 – Quadratojugal; 11 – Quadrate; 12 – Inferior temporal fossa; 13 – Superior temporal fossa; 14 – Postorbital; 15 – Squamosal; 16 – Parietal; 17 – Proatlas; 18 – Atlas.



Fig. 2 Broad-snouted caiman skull in ventral aspect:

- 1 – Premaxilla; 2 – Incisive foramen; 3 – Maxilla; 4 – Neurovascular foraminas; 5 – Palatine; 6 – Ectopterygoid; 7 – Jugal/Zygomatic; 8 – Chonae; 9 – Quadratojugal; 10 – Quadrate; 11 – Pterygoid; 12 – Secondary chonae; 13 – Atlas.

The caiman skull is diapsid, presenting two temporal fossae, one superior (dorsal) and one inferior (lateral). In caimans, the supratemporal fossae usually close with ontogeny or they are small. The zygomatic (jugals) bones are elongated and form the majority of the ventral margins of both the orbits and laterotemporal fossae (2, 8, 9,10).



Fig. 3 Broad-snouted caiman skull with mandible - in occipital view:
1 – *Supraoccipital*; **2** – *Exoccipital*; **3** – *Paracondylar process*; **4** – *Proatlas*; **5** – *Atlas*; **6** – *Ectopterygoid*; **7** – *Pterygoid*; **8** – *Quadratojugal*; **9** – *Quadratojugal*; **10** – *Retroarticular process*; **11** – *Mandibular adductor fossae*; **12** – *Basioccipital*.



Fig. 4 Broad-snouted caiman mandible - dorsal aspect:
1 – *Dentary*; **2** – *Splenial*; **3** – *Coronoid*; **4** – *Surangular*; **5** – *Angular*; **6** – *Mandibular adductor fossae*; **7** – *Glenoid fossae*; **8** – *Retroarticular process*.

The quadratojugals are located at the posterolateral portions of the skull, contacting the jugals anteriorly and anterolaterally, and quadrates medially. The quadratojugals contribute to the laterotemporal fossae's posterior margins. The quadrates are large, found at the posterolateral parts of the skull. The distal or posterior portions of the quadrates bear the quadrate condyles that articulate with the mandibles (fig. 1).

On the ventral surface, the hard palate consists in premaxilla, separated almost entirely by the incisive foramen, together with the wide palatine processes from the maxilla and palatine bones. The alveolar margins bear numerous small openings called neurovascular foramina (8,9). The dorsal surfaces of the palatines together with those of the pterygoids form the nasopharyngeal duct. The large oval openings ventrally on the skull are the suborbital fenestrae. The pterygoids are large, wing-like elements that contact the palatines anteriorly and ectopterygoids laterally (fig. 2).

Posteromedially on the pterygoids are the secondary choanae that are fully enclosed by the pterygoids. The ectopterygoids are robust bones that contact the maxillae and zygomatic dorsally and the pterygoids posteroventrally.

The aboral surface of the skull consists in supraoccipital plate dorsal, in sagittal plan, a wide exoccipital, that bears two paracondylar process and a small basioccipital. The basioccipital is located medioventrally on the occiput and forms the semispherical occipital condyle (fig.3). The foramen magnum is relative large at the center of the occiput (8,10).

The largest bones of the mandibles are the dentaries. The posterodorsal portions of the mandibles are formed by the surangular bones, while the angulars form the posteroventral. Together with the posterior portions of the dentaries, the anterior of the surangulars and angulars surround the external mandibular fenestrae, the largest openings of the mandibles. Attached medially on both the surangulars and angulars are the articulars bones. Anteriorly on the articulars are the concave fossae (glenoid fossae) that articulate with the condyles of the quadrates. The articulars develop the long retroarticular processes that project posterodorsally. The smallest bones of the mandibles are the coronoids (fig.4).

The number of vertebrae is similar across all living crocodylians and usually comprises 9 cervical, 12 thoracic, 5 lumbar, 2 sacral, and depending on the species between 35 and 45 caudal. The vertebrae are procoelous type, the vertebral body is concave anteriorly (vertebral fossa), while caudal aspect is spheroidal convex (articular head) that articulates with the concave anterior of the succeeding vertebra. All vertebrae in living crocodylians are procoelous, except for the atlas, second sacral, and first caudal vertebra (8,10). The first cervical vertebra comprises the atlas-proatlas complex which articulates with the only one occipital condyle. The proatlas has a triangular pyramid appearance, attached to the dorsal aspect of the atlas. The second sacral vertebra is biconcave, by having concave anterior and posterior ends. The posterior articular head on the first sacral vertebra is weakly developed. The first caudal vertebra is biconvex (fig.11), by having semi-spheroidal convex articular heads both cranial and caudal.

Axis bears the odontoid process, not very prominent, with a flat dorsal surface, which articulates with the atlas.

Projecting laterally from the neural arches of the cervical and first two thoracic vertebrae are the diapophyseal processes, and projecting laterally from the body are the parapophyseal processes which articulates with the cervical ribs (ventral bony blade), for the cervical region, or with the true ribs, in case of the first 2 thoracic vertebrae. Between the diapophyseal processes and the parapophyseal processes there is a large space corresponding to the transverse foramen in mammals (fig.5). In the cervical region, the articular processes are wide and detached, the spinous process grow in height, from C4 to C9 and also, anteroventrally on the body of all cervical (except the atlas) and first three or four thoracic vertebrae are short and thin ventral crest (1, 3, 4, 7).



Fig. 5 Cervical vertebra of broad-snouted caiman - caudal view:

1 – Vertebral body; **2** – Parapophyseal process; **3** – Diapophyseal (transvers) process; **4** – Ventral bony blade/ cervical rib; **5** – Caudal articular process; **6** – Cranial articular process **7** – Spinous process; **8** – Vertebral foramen; **9** – Ventral crest.

The thoracic vertebrae have transverse processes that grow in length towards the middle of the region and then decrease towards the lumbar region and provide articular surfaces for both the head and the tubercle of the ribs (fig. 6, 7, 8).

The lumbar vertebrae have transverse processes with a constant length, not very detached, together with a constant height of the spinous processes and wide articular processes, with flat surfaces (fig. 9).

The sacrum consist only in two vertebrae, the first one being more developed than the second one. The fusion between the vertebrae take place at the level of the bodies and arches, the spinous and transverse processes remain distinct. The transverse processes are robust, short and strong, articulated with ilium through a wide lateral surface.



Fig. 6: The dorsal aspect of the cervical vertebrae

The thoracic vertebrae have transverse processes that grow in length towards the middle of the region and then decrease towards the lumbar region and provide articular surfaces for both the head and the tubercle of the ribs.



Fig. 7 Second thoracic vertebrae of broad-snouted caiman - caudal view:

1 — Vertebral body; 2 — Parapophyseal process; 3 — Diapophyseal (transvers) process; 4 — Rib; 5 — Costal cartilage; 6 — Caudal articular process 7 —Cranial articular process; 8 — Spinous process; 9 — Vertebral foramen; 10 — Ventral crest.



Fig. 8 Fifth thoracic vertebra of broad-snouted caiman - caudal view:

1 — Vertebral body; 2 — Head of rib; 3 — Costal tubercle; 4 — Diapophyseal (transvers) process; 5 — Costal cartilage; 6 — Caudal articular process 7 — Spinous process; 8 — Vertebral foramen; 9 — Ventral crest.

The lumbar vertebrae have transverse processes with a constant length, not very detached, together with a constant height of the spinous processes and wide articular processes, with flat surfaces.

The sacrum consist only in two vertebrae, the first one being more developed than the second one. The fusion between the vertebrae take place at the level of the bodies and arches, the spinous and transverse processes remain distinct. The transverse processes are robust, short and strong, articulated with ilium through a wide lateral surface (fig. 10).

The first caudal vertebrae resemble more a lumbar vertebra, with short and flat transverse processes, a wide detached spinous process and well developed articular processes. The body has convex articular surface in both sides (fig. 11). Attached posteroventrally on most caudal vertebrae are the hemal processes with hemal arches placed between two adjacent vertebrae. Posteriorly, toward the tip of the tail region, the caudal vertebrae gradually decrease their dorsoventral heights and lose their transverse, articular and spinous processes, their bodies become more elongated and the posterior convex surface are also getting less prominent, until the small and elongated body are all that remain of the last caudal vertebrae (fig. 12, 13).



Fig. 9 Lumbar vertebra of broad-snouted caiman - caudal view:

1 – Vertebral body; 2 – Transvers process;
3 – Caudal articular process; 4 – Cranial articular process; 5 – Spinous process.

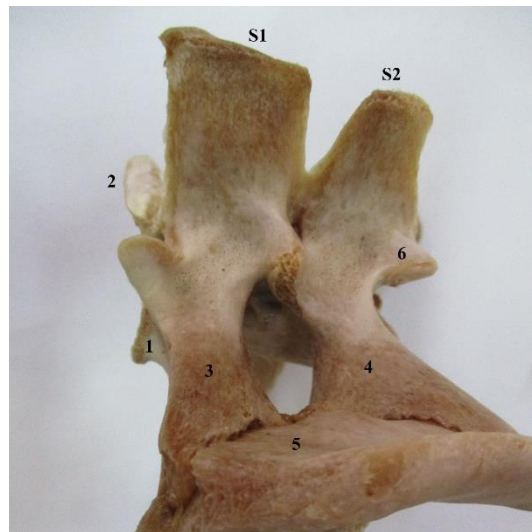


Fig.10 sacral vertebrae in broad-snouted caiman – lateral view:

1 – Vertebral body; 2 – Cranial articular process; 3 – Transvers process of S1; 4 – Transvers process of S2; 5 – Ilium; 6 – Caudal articular process

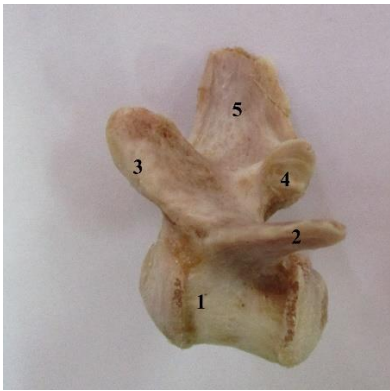


Fig. 11 First caudal vertebrae in broad-snouted caiman – lateral view:

1 – Vertebral body; 2 – Transvers process;
3 – Cranial articular process; 4 – Caudal articular process; 5 – Spinous process.

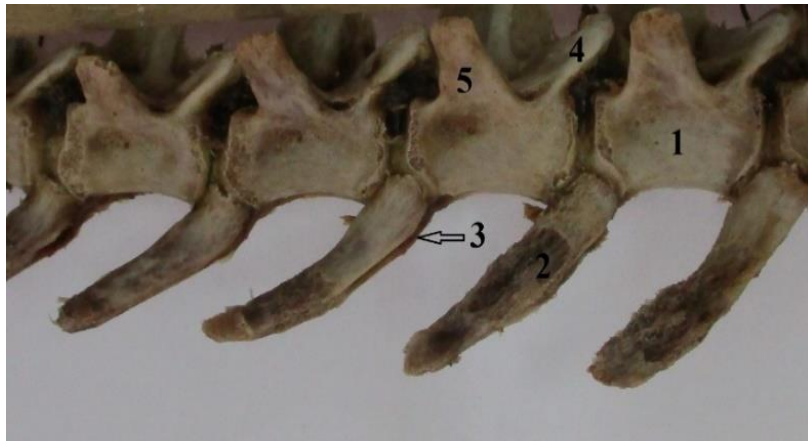


Fig. 12 Caudal vertebrae in broad-snouted caiman – ventro-lateral view:

1 – Vertebral body; 2 – Hemal process; 3 – Hemal arc; 4 – Cranial articular process; 5 – Transverse process.

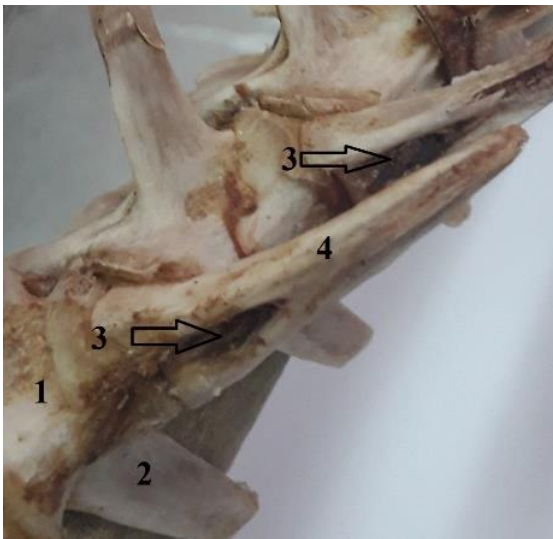


Fig. 13 Caudal vertebrae in broad-snouted caiman – ventro- view:

1 – Vertebral body; 2 – Transverse process; 3 – Hemal arc; 4 Hemal process.

Conclusions

1. The caiman skull is diaspid, presenting two temporal fossae, one superior (dorsal) and one inferior (lateral).
2. The vertebrae are procoelous type, the vertebral body is concave anteriorly (vertebral fossa), while caudal aspect is spheroidal convex (articular head) that articulates with the concave anterior of the succeeding vertebrae.
3. Most caudal vertebrae have attached hemal processes with hemal arches placed between two adjacent vertebrae.

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Anatomical peculiarities of the broad-snouted caiman's limb bones (*Caiman latirostris*)

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Abstract

The aim of the study was the highlight of the specific characters of the limb bones in broad-snouted caiman, and for that, the investigations were conducted on the bones which were collected from a dead broad-snouted caiman that belonged to Bârlad Zoo. It was a male caiman, 24 years old. For the analysis, the bones were prepared by boiling and cleaning. It was noticed the particular conformation of the girdles, in case of the thoracic one, the scapula and the coracoid bone being almost similar developed, and both of them form the glenoid cavity to joint with humerus. It exist also an elongated interclavicular bone. The humerus is curved, ulna is more massive than the radius, between them existing a large radio-ulnar arcade, these aspects being similar to birds. There are 5 carpal bones, 3 in the proximal row, and 2 in the distal one, the radial carpal bone being merged with the intermediate one, resulting the most developed carpal bone. The thoracic metapodium includes 5 metacarpal bones, the 5th one being more reduced. The ilium is dorsally placed to ischium, the two bones participating to form the acetabular cavity (foramen). The pubis, more reduced, is cranially placed to ischium with which it articulates. The femur is very curved, and caudo-medially is highlighted the 4th trochanter. The fibula is well developed, but the tibia is more massive, and between them is a large interosseous space. The pelvic basipodium includes the calcaneum and astragalus, well developed, in the proximal row and two bones in the distal row. The 5th metatarsal bone is rudimentary, the others being well developed. The acropodium includes 5 digits for the thoracic limb and 4 in the pelvic one, the number of the phalanges having one more than the number of the digit, except the 5th digit of the thoracic limb. The conformation of the limb caiman bones is generally similar to other crocodiles.

Keywords: peculiarities, limb bones, caiman

Introduction

The appendicular skeleton, thoracic and pelvic one, has a common structure in vertebrates which includes 4 major parts: *the girdle* (zonoskeleton) (*Cingulum*), the *stylopodium*, the *zeugopodium* and the *autopodium*. The last one is divided in 3 subsegments: *basipodium*, *metapodium* and *acropodium*. Generally, the girdles include 3 bones: scapula, coracoid bone and the clavicle for the thoracic girdle and ilium, ischium and pubis for the pelvic one. The thoracic stylopodium is represented by the humerus and the pelvic one by the femur and patella. The zeugopodium includes two bones: radius and ulna in case of the thoracic limb and tibia and fibula in the pelvic one. The thoracic basipodium is formed by the carpal bones and the pelvic one by the tarsal bones. The metapodium includes the metacarpal (thoracic limb) and metatarsal bones (pelvic limb) and the acropodial bones are represented by the phalanges and the sesamoid bones (1,8). Major different characters in the structure of the limb skeleton can be observed between the vertebrate classes, but sometimes significant characters can be found until the genus level or as interspecific peculiarities in a genus species. The most significant differences can be observed for the acropodium, these segments being far for the trunk, and as consequences undergoes important changes in relation to the living conditions. These peculiarities are closely correlated with the locomotion and ground touch type of the animals, and as results, some bones can disappear or are rudimentary, other merge etc.

The caimans are small-sized crocodiles, the broad-snouted caiman being a South American crocodylian (Brazil, Bolivia, Argentina, Uruguay, Paraguay) of medium size (11) (for their group), generally 1,5-2 m long, part of the *Reptilia Class, Crocodylia Order*. This order includes big, predatory and semi-aquatic reptiles, namely the real crocodiles (*Fam. Crocodylidae*), alligators and the caimans (*Fam. Alligatoridae*) and the gavials (*Fam. Gavialidae*) (5, 13, 14). Besides this order, *Reptilia* includes also *Chelonia Order* (tortoises), *Sauria Order* (lizards) and *Ophidia Order* (snakes) (4, 7). These are species adapted both to the aquatic and terrestrial environment. The locomotion, in case of many species is easier in the aquatic environment, because they possess short legs, except for the snakes which have no legs, and that makes the terrestrial locomotion heavier. Anyway, there are many morphological peculiarities in this group, the knowledge of them being important zoologically, but also clinically, many species making part from pets, but also for those animals which are kept in captivity in zoo parks and can present various pathologies.

Materials and methods

The studies were carried out on a broad-snouted caiman (*Caiman latirostris*), which was purchased from Bârlad Zoo after his death. It was a male caiman, 24 years old, 1.5 m long.

After the dissection, the limb bones were collected and prepared for examination. They were boiled and cleaned of debris and then systematically analyzed and described using the reference terms from N.A.V. (*Nomina Anatomica Veterinaria*).

Results and discussions

Thoracic girdle includes the scapula (*Scapula*) and the coracoid (*Coracoideum*) bone, similarly developed, both of them participating with an articular surface to form a large glenoid cavity for the humeral head. The scapula and the coracoid bone are flat bones, the scapula being ventro-cranially oriented and the coracoid ventro-caudally. The lateral face is slightly convex, the medial one slightly concave, the scapula having a relatively constant width until the distal third where exist a neck, the caudal notch being deeper. The coracoid bone is a little shorter than the scapula, but wider ventrally where it presents a rounded border and, on the cranial border a deep and large notch. Near the jointing side with scapula it presents a foramen, described as coracoidal foramen (*Foramen coracoideus*) (6, 10) (fig. 1., 2.).

It exist also an interclavicle (*Interclavicula*) (9), which is elongated, but placed cranially to the sternal cartilages (9).

The humerus (*Humerus*) has a relatively twisted and curved diaphysis and wide extremities. The proximal articular surface is an elongated head, and the distal one includes two wide condyles (a trochlea) above of them, caudally, existing a superficial olecranon fossa. Laterally, can be observed a prominent deltoid crest that has a nipple aspect (fig. 3., 4.).

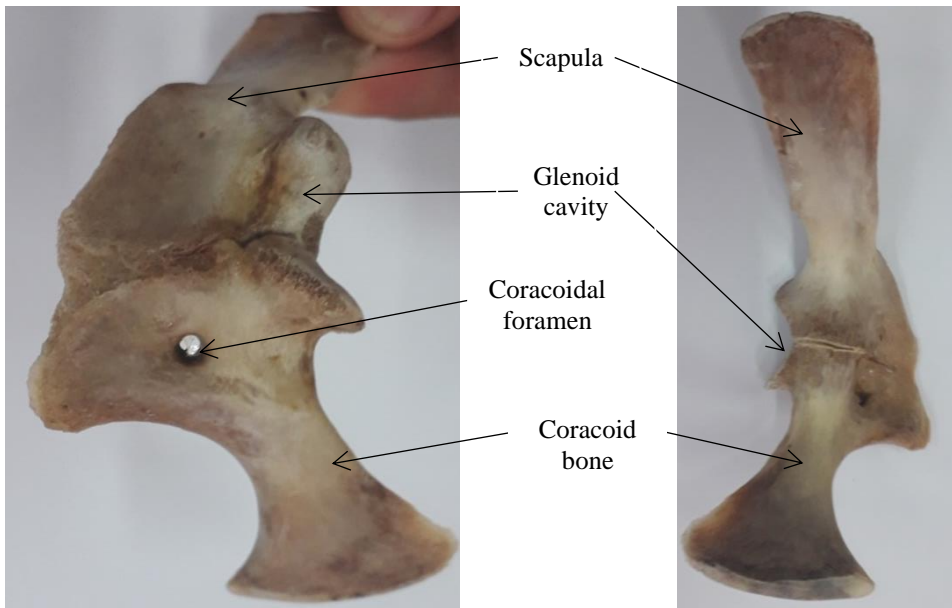


Fig. 1. Scapula and coracoid bone of caiman; caudo-lateral aspect

Fig. 2. Scapula and coracoid bone of caiman; medial aspect

At the level of the zeugopodium can be observed that the ulna (*Ulna*) is more developed than the radius (*Radius*), the two bones being in contact at the extremities and between them exist a large radio-ulnar arcade, as in birds. The body of the ulna is curved and the olecranon is reduced. The radius is short, his diaphysis is more straight and thinner. The articular surfaces of the two bones have a wavy aspect, convex-concave and elongated transversally (fig. 5.).

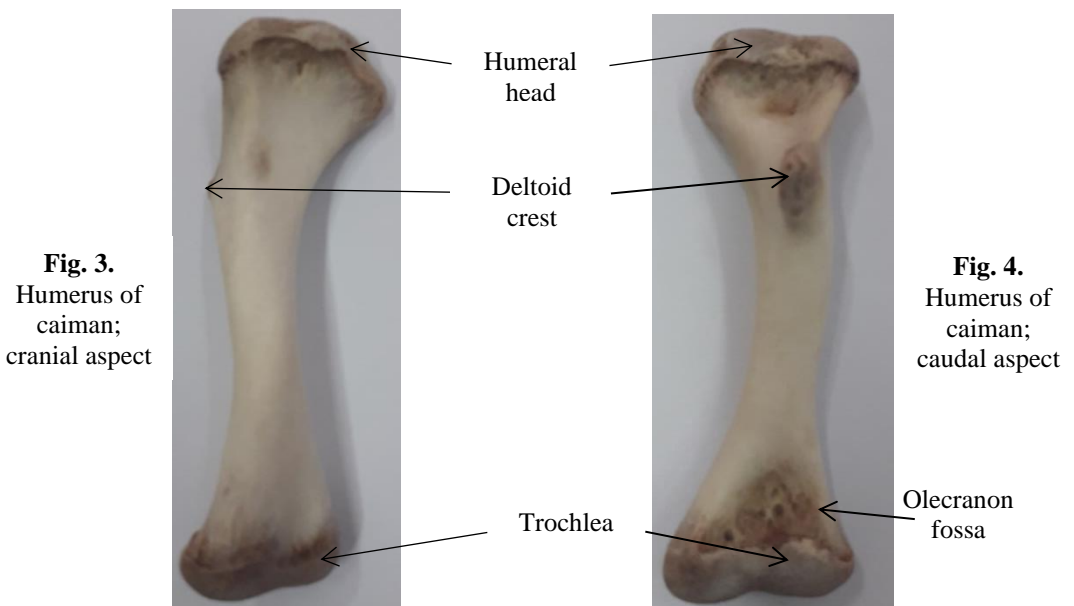


Fig. 3. Humerus of caiman; cranial aspect

Fig. 4. Humerus of caiman; caudal aspect

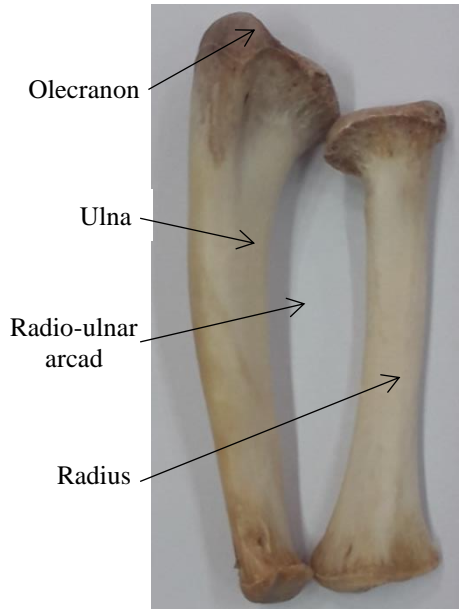


Fig. 5. Radius and ulna of caiman, cranial aspect

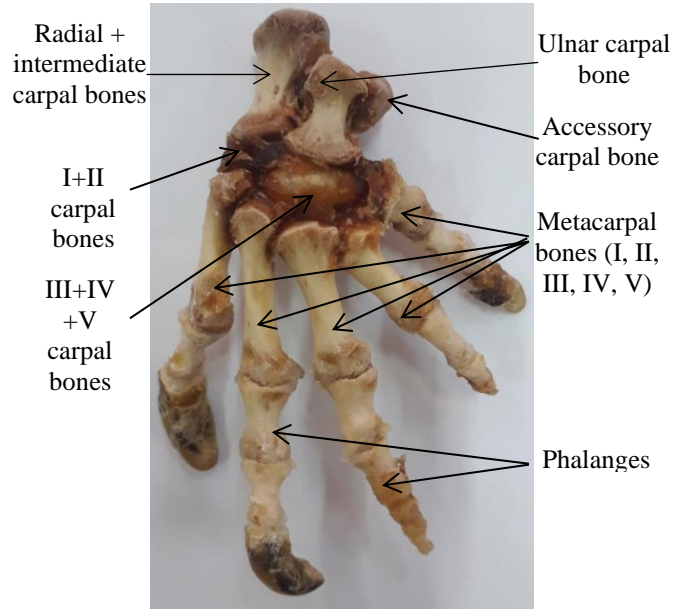


Fig. 6. Carpal, metacarpal bones and the phalanges in caiman, dorsal aspect

The thoracic basipodium includes the two rows of carpal bones: the proximal and the distal one. The proximal row comprises the accessory carpal bone (IV) (*Os pisiforme*), the ulnar carpal bone (III) (*Os triquetrum*), and the radial carpal bone (I) (*Os scaphoideum*) merged with the intermediate carpal bone (II) (*Os lunatum*), the two bones forming the most developed carpal bone (*Os scapholunatum*). The dorsal faces of the ulnar and radial+intermediate carpal bones have a semilunar aspect because of the lateral notches (fig. 6.). The distal row includes two bones, flattened, having rounded faces (except the proximal and the distal one), a medial bone, more reduced which represents the merged I and II carpal bones (9), and a lateral one, bigger, representing the III, IV and V carpal bones (9) which are also merged. Between the ulnar carpal bone and lateral carpal bone from the distal row exist a cartilage that represents the central carpal bone (10).

The thoracic metapodium presents 5 metacarpal bones (*Ossa metacarpalia I-V*). The 5th metacarpal bone is reduced, short, the most developed being the 2nd (the longest) and the 3rd (the thickest) metacarpal bones. The bones make joint proximally, in the rest existing a large space between them (fig. 6.).

The acropodium represents the skeleton of the digits (I-V) (*Ossa digitorum manus*). Each finger has a phalanx more than his number, except the 5th one that has three phalanges. The last phalanx is reduced in contrast with the size of the claw and has a conical shape with a blunt apex (fig. 6.).

The pelvic girdle comprises the ilium (*Os ilium*), ischium (*Os ischii*) and pubis (*Os pubis*) that don't weld. The ilium is the most developed being thicker, dorsally placed to the ischium, having the dorsal border slightly rounded and caudally becomes narrower and

has a terminal prominence. On the lateral face presents a crest that dorsally delimits a fossa which finishes in the acetabular foramen (*Foramen acetabuli*). The medial face makes joint with the sacral vertebra the sacro-iliac joint contact being a strong one. The ischium, ventrally placed to ilium and acetabular foramen, has a ventro-caudal direction. It's more elongated, thinner and narrower, the extremities (proximal and distal one) being wider and is medially curved. On the cranial border, closely to the acetabular foramen presents an articular surface for pubis. Between ilium and ischium results the acetabulum which is perforated, being transformed into the acetabular foramen (fig. 7.). The pubis is more reduced, cranially placed to ischium in a ventro-cranial position. Near the articular surface for ischium the body is narrower, forming the neck, then becomes wider ventrally where presents a rounded border with a cartilage. The lateral face is slightly convex, the medial one being slightly concave (Fig. 8.).

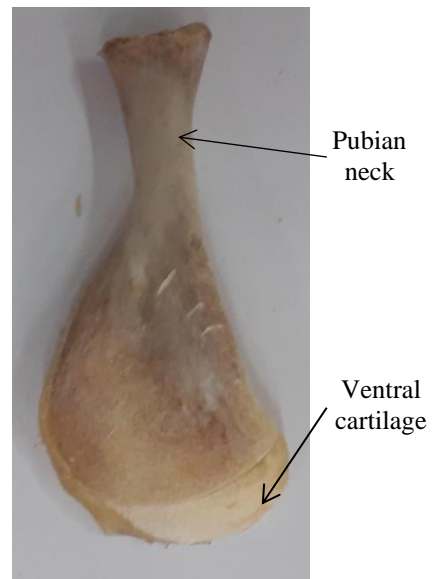
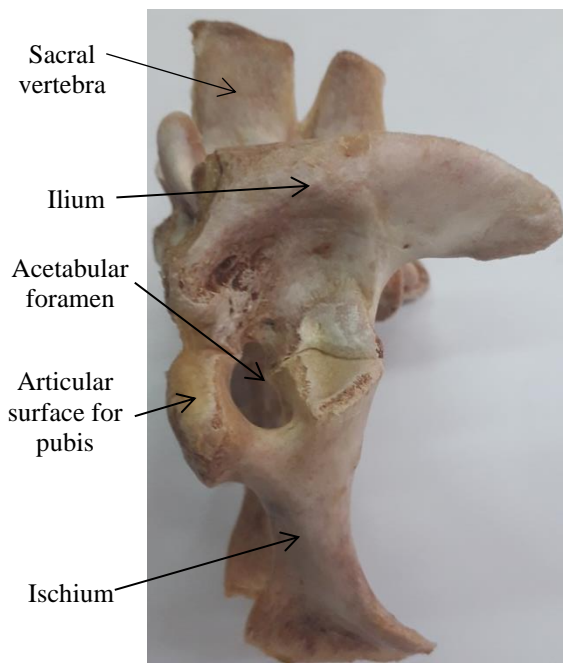


Fig. 7. Ilium and ischium of caiman, lateral aspect **Fig. 8.** Pubis of caiman, lateral aspect

The femur bone (*Os femoris*), as the humerus, has a twisted and more curved body that reflects the complex and combined movements of the limbs at crocodiles, at the proximal extremity having an elongated articular head and distally, the articular surface comprises a large trochlea cranially that continues with two condyles caudally. In the proximal third, caudo-medially can be observed a prominence described in crocodiles as the 4th trochanter (*Trochanter quartum*) (2, 12) (fig. 9., 10.).

The pelvic zeugopodium comprises the tibia (*Tibia*) and fibula (*Fibula*), between them existing, as in the case of the thoracic zeugopodium, a large interosseous space. Tibia is more massive than the fibula, being thicker proximally and more flattened distally. The proximal articular surface has a triangular shape, is almost planiform and continuous,

slightly convex, and the distal one is very convex having a condylar aspect coming in contact with the astragalus. The fibula, well developed, has a slightly flattened body, wider at the extremities. His articular surfaces, proximal and the distal one, are convex, elongated, as the condyles.

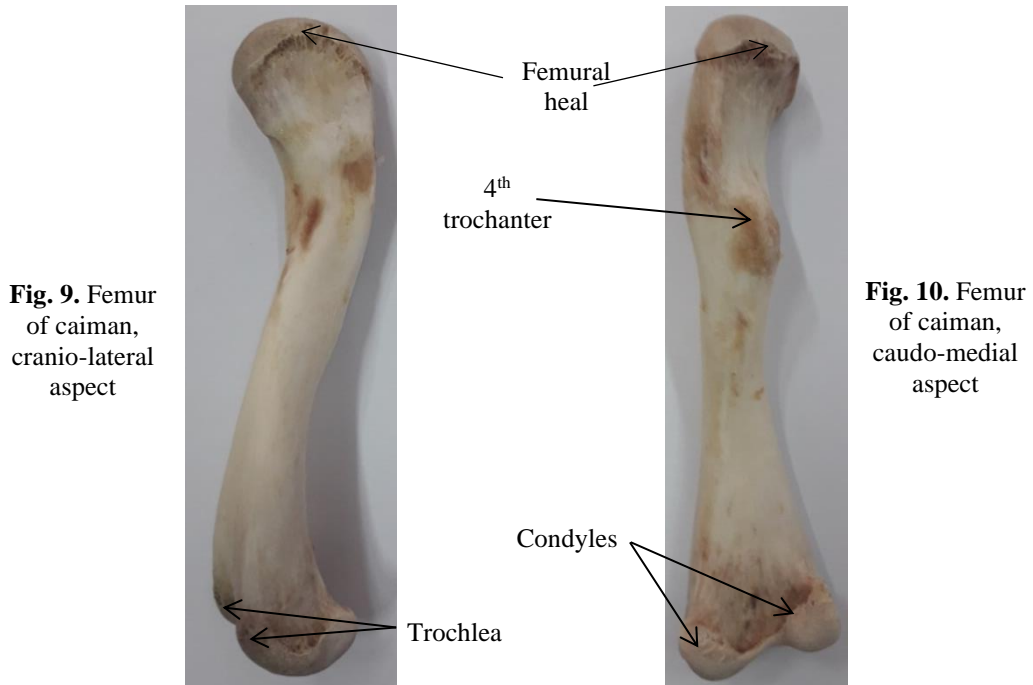


Fig. 9. Femur of caiman, cranio-lateral aspect

Fig. 10. Femur of caiman, caudo-medial aspect

The tarsus comprises four bones: the calcaneum (*Calcaneus*) and the astragalus (*Talus*) in the proximal row and the cuboid (*Os cuboideum*) and a cuneiform (*Os cuneiforme*) bone in the distal row. The calcaneus is slightly elongated latero-plantary, excavated proximally and laterally and presents a concave proximal articular surface for fibula. The talus is relatively spherical, has two concave proximal articular surfaces, one deep for tibia and the other, more superficial for fibula. The bones from the distal row have similar sizes, being slightly flattened proximo-distally, the others faces being rounded (fig 11., 12.).

The metatarsal bones (*Ossa metatarsalia I-V*) are similar with the metacarpal bones, but more elongated. There are four metatarsal bones well developed (I-IV), the 5th one being rudimentary (fig. 12.).

The pelvic acropodium (*Ossa digitorum pedis*) includes 4 digits (I-IV), each one having a one more phalanx than his number (the I digit has 2 phalanges, the IV digit has 5 phalanges) (fig. 12.).

In relation with the legs morphology of the crocodiles, the most of the bones are short.

It can be observed that the autopodium, particularly the acropodium is well developed, to support the body weight, but also in relation with the locomotion, especially

for the aquatic environment in correlation with the interdigital membrane that exist in case of the pelvic limbs.

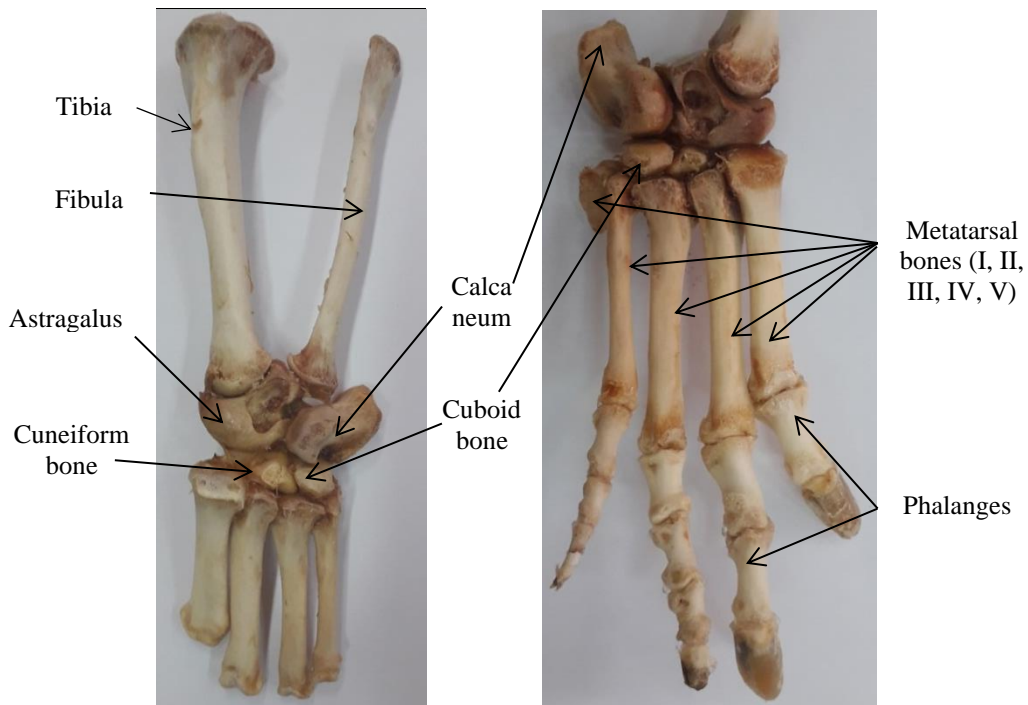


Fig. 11. Tibia, fibula, tarsal and metatarsal bones of caiman. dorsal aspect

Fig. 12. Pelvic autopodium in caiman. dorsal aspect

Conclusions

1. The bones of the caimans, particularly the broad-snouted caiman, have a similar conformation with that of the other crocodiles, especially from the caiman group, fewer characteristics being proper.

2. In relation with other vertebrate it can be noted the similarities with the birds, especially in case of the stilopodium and zeugopodium bones.

3. Because of the combined movements, the most of the bones are curved, especially in case of the stilopodium and zeugopodium and also, the articular surfaces are elongated.

4. The bones of the girdles are relatively massive and strong and the autopodium is well developed.

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Semen traits in cornish roosters during the process of physiological ageing

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Abstract

In this paper, we determined the evolution of ejaculate volume, sperm concentration, motility and viability along with the evolution of the activity of some enzymes (TGO, alkaline phosphatase, ATPase and lactic dehydrogenase) in seminal plasma of Cornish roosters at 30, 42, 57 and 63 weeks of age. The monitored parameters showed a specific evolution that characterizes the physiological ageing process in Cornish roosters. Thus, the volume of ejaculate showed a significant decrease ($P = 0.011$) from 0.39 to 0.32 microliters. The number of sperm per ejaculate decreased from 0.86 to 0.29x10⁹ ($P = 0.002$). Motility decreased from 69% to 41% ($P = 0.001$) and sperm viability decreased from 89 to 74% ($P = 0.00$). The percentage of abnormal spermatozoa decreased not significantly, from 96 to 94%, some types of abnormalities increasing and others decreasing in frequency during the monitoring period. The activity of the monitored enzymes characterized the decrease of the anaerobic metabolism of spermatozoa and the increase of the membrane permeability of the spermatozoa in the physiological ageing process.

Keywords: rooster, semen feature, ageing process

Introduction

Modern technologies of breeding and exploitation of hens face, among other things, a number of reproductive problems. These are generated by a multitude of factors: environmental, nutritional, technological, caused by biological material, etc. As for the biological material, these problems are caused by either females or males. In the case of males, one of the most common and chronic problems is that of fertility. The issue of cock fertility is also supported by many factors, among which age is one of the most relevant. In most breeds of hens, the ageing speed of the males is not synchronized with the period of technological exploitation of the hens, the roosters usually ahead of the hens. Cornish inbred chicken strains are used as male parental genetic lines to produce broiler hens. Genetic categories of Cornish inbred strains are subjected to high selection pressure. Cornish breed is particular due to the side effects of a high selection pressure for growth rate, conformation and constitution. They have led to a decrease in the reproductive performance of males of this breed. Knowing these ageing effects upon the sperm traits, especially in older roosters, about to be removed from selection programs, can be a support for specialists in preventing the decline in reproductive performance of this breed. The present paper aims to determine the evolution of sperm properties (ejaculated volume, density, motility, viability, and spermatozoon abnormalities) and enzymatic chemical composition of the semen plasma in Cornish roosters during their technological exploitation.

Material and method

The research was carried out on industrially raised Cornish roosters in order to obtain male parents for tetralinear hybrids of meat (broiler). A number of 20 roosters aged 20 weeks with an average weight of 2.24 kg were accommodated in individual boxes of 0.7 x 0.7 m, connected to the automatic watering system of the hall. The feeding was done manually with a commercial feed recipe containing as main ingredients by %: wheat 40.2, barley 31.4, oats 10.6, soybean meal 5.5,

grass meal 2.2, fish meal 5.5, dicalcium phosphate 0.7, limestone 0.9, vitamin and mineral premix 0.6. The roosters benefited of artificial lighting, after a light program, of 16.5 light hours (from 5:30 to 22) from 23 to 63 weeks of age. Experimental analyses were performed at the ages of 30, 42, 57 and 63 weeks.

The ejaculates were obtained according to the method described by Bunaciu *et al.* (1978) and were collected in transparent glass graduated collection tubes; volumes were recorded directly in the tube immediately after collection at the lower margin of the semen meniscus and were expressed in μL . Sperm motility was assessed by a wet preparation technique using a Nihon Kohden optical microscope on a warmed plate. Motility was estimated by direct observation of spermatozoa in at least five fields, using 400x magnification and a lowered condenser to disperse the light. Motility was expressed here as the percentage of all spermatozoa showing progressive movements. Nonprogressive spermatozoa with other patterns of movement were not considered in this category. Sperm count was determined using a hemocytometer with a Nihon Kohden optical microscope. For this assessment, fresh semen samples were diluted (1:200) and fixed using neutral Hancock's solution (62.5 mL of 37% formaldehyde, 150 mL of 1% saline, 150 mL of sodium phosphate buffer, and 500 mL of double-distilled water) and a Potain pipette. The results were expressed as the number of spermatozoa per mL. Viability of the spermatozoa was evaluated by eosin-nigrosin staining (Merck, Darmstadt, Germany) according to Kondracki *et al.* (1968). The results were expressed as the percentage of all spermatozoa classed as viable. The spermatozoa abnormality frequency and types were also analyzed. For enzymatic assays, the sperm samples were diluted 1: 3, using an equal volume of 0,9% NaCl solution. The diluted samples were allowed to equilibrate for one hour at 4°C, then centrifuged at 2000 rpm in Janetzky centrifuge using glass centrifuge ampoules for one hour to separate sperm from seminal plasma. The two sperm components, seminal plasma and spermatozoa, were extracted into ampoules, separated by decantation and frozen at a temperature of -20°C for subsequent dosing. The enzymatic activity was determined according to the methods cited by Manta *et al.* (1966) thus: TGO activity was determined by the Rertman and Frankel's method and was expressed in units/L (one unite is the μg pyruvate liberated / mL / mg prot / 37°C; the activity of acid phosphatase from seminal plasma was determined by the Walter and Schutt method and expressed in units/L; one unit is the quantity of μg phosphate liberated / mL / mg prot / min / 60°C; ATPase activity was determined by the Zilversmith and Davis method and was expressed in units/L; one unit is the nmol phosphate liberated / mL / mg prot / min / 42°C; LDH activity was expressed in International Units (IU). The data obtained were centralized using the Excel 2010 program and the statistical processing was performed using the GraphPad program for Windows, version 8.0.2, GraphPad Software, Inc. The correlations of the age of the roosters and the sperm features were analysed by Pearson correlation coefficient. The significance between the groups was analysed by ANOVA and the the differences were considered significant for values of $P \leq 0.05$.

Results and discussions

The results regarding the evolution of the ejaculate volume according to the age of the roosters are presented in Table 1. The analysis of these data shows that the volume of ejaculate increased from the age of 30 to 42 weeks. From the age of 42 to 57 weeks, ejaculate values decreased significantly ($P = 0.013$). Expressed as a percentage and related to the value from 42 weeks, considered 100%, these decreased values were 71%. The evolution curve of this physiological parameter of sperm presents a particular relative bell shape with a peak located somewhere in the 42-week-old area.

Table 1

Item	Rooster age				Mean of period	P
	Week. 30	Week. 42	Week. 53	Week. 63		
Ejaculate volume	0.39 [#] ±0.04	0.44±0.10	0.35±0.019	0.23 [#] ±0.12	0.35±0.12	0.013
Spermatozoa number (x10 ⁹ / mL of semen)	2.22 ^a ±0.11	2.16±0.09	2.28±0.56	1.28 ^a ±0.32	1.98±0.87	0.011
Spermatozoa number (10 ⁹ / ejaculate)	0.86 ^c ±0.11	0.95 ^{b,c} ±0.09	0.79±0.56	0.29 ^b ±0.32	0.69±0.87	0.002
Sperm motility (%)	69.2±6.5	68.72±12.3	51.3 ^d ±7.9	41.3 ^d ±6.6	57.6±8.8	0.001
Sperm viability (%)	89.7±5.6	86.5±11.0	79.5 ^e ±6.5	74.4 ^e ±3.5	82.0±3.9	0,000
Normal spermatozoa (%)	96.3±12.5	96.0±6.9	95.7±8.5	94±4.5	96.2±6.9	0.065

Legend :

- values are expressed as mean ± standard error of mean;
- P value was calculated based on the HSD test, *honest significant difference*;
- values with the same exponent in the same row are significantly different.

Sperm density initially (at 30 weeks) showed values between 2.22 and 2.42 x10⁹ per mL. Subsequently, at the age of 42 weeks, the values showed an increase of 30.57%, with a statistically significant increase percentage (P = 0.011). By the age of reformation (53 weeks), spermatogenesis decreased, with sperm density decreasing to 1.28 x10⁹ / mL at 63 weeks.

Sperm motility presented a plateau on the age limits from 30 to 42 weeks. After the age of 42 weeks up to 63 weeks the breed lost off its sperm motility. In total, the breed showed a significant decrease in sperm motility during the monitored age (P < 0.001) of an amounts to 30.4%.

Sperm viability (defined as the percentage of sperm whose nucleus did not stain with eosin-nigrosin) had an average value of 74.4%. This biological parameter of sperm also showed a specific evolution during the monitored period, decreasing statistically significant (P < 0.01) during the monitored period, a value of of 25.2%.

Data regarding the volume of ejaculate in domestic bird species are revealed by the literature, respectively Bunaciu *et al.* (2009) as well as Bunaciu and Dojană (1982) and Dumitrescu (1978). Values for large roosters can reach as much as 1 mL according to the data from these authors. From the analysis of the Pearson correlation coefficient it results that the ejaculate volume in our monitored roosters correlates negatively with rooster age (r values showed oscillations from -0.52 to -0.81) but it remains significantly elevated until 63 weeks of age. Thus, we can say that the values of ejaculate volume in adult roosters are related to body weight and also influenced by age. Jarinkovičová *et al.* (2012) studied ejaculate samples from roosters from three breeding lines: Barred Plymouth Rock (BPR), Sussex Light (SU) and Rhode Island Red. Significant differences in ejaculate volume were reported by line: the highest ejaculate volume was reported at the BPR line (0.66 cm³) and the lowest at the SU line (0.46 cm³, P ≤ 0.01). Changes in ejaculate volume have been reported in connection with the intensity of male use in artificial insemination by Bunaciu *et al.* (1979), the method of sperm collection by Bunaciu *et al.* (1992), the introduction of various vitamin supplements in the diet, for example vitamin E revealed by Bălăceanu *et al.* (2019)

and Sima *et al.* (2019). In an experiment of Elagib *et al.* (2016) on Leghorn White (var. Bovans) roosters of two different ages (1 and 2 years) to study the effect of age and season on sperm characteristics, the authors found that there was a significant difference ($P < 0.05$) between two age groups in terms of sperm ejaculation volume: 0.22 ± 0.02 and 0.29 ± 0.25 mL, which is in line with the results we obtained on the Cornish monitored breed. On the other hand, the authors did not report any significant differences in spermatozoa concentration. The summer season caused a significant reduction in sperm volume by 8.7% in one-year-olds and in the spermatozoa concentration of 2-year-olds by 19.7%. Tabatabaei *et al.* (2010) conducted an experiment to study the characteristics of sperm in local Iranian broiler roosters (grandparents) aged between 26 and 45 weeks and found a decrease in sperm concentration as they age.

Motility is, along with the number of spermatozoa, one of the most important morphological parameters of sperm. Sperm motility generally reflects the viability of a spermatozoa population. The positive correlation between spermatozoa motility and fertilization capacity has been demonstrated in many species, although this correlation is not absolute. Spermatozoa motility is a functional measurement of the spermatozoa themselves. Important metabolic pathways are involved in regulating spermatozoa motility: calcium, the cAMP-dependent protein kinase pathway, kinases and phosphokinases, reactive oxygen species but also cell volume and plasma sperm osmolarity, as it was revealed by Pereira *et al.* (2017). One of the factors that significantly changes sperm motility in the artificial insemination industry is thinners and cryoprotectants, according to Bagoio and Capitan (2018). In a study on White Cornish roosters from 23 to 57 weeks of life, Sima *et al.* (2019) found that the percentage of sperm motility remained unchanged statistically ($P = 0.19$), which would argue for the possibility of expanding the exploitation of these breeds in industrial conditions at least until the age of 57 weeks of life. Technological conditions, vitamin deficiencies can be the cause of significant decreases in motility. This observation acquires concrete practical significance given that many breeders claim significant direct economic losses induced by the need to replace old roosters with younger roosters.

Along with motility, sperm viability is the basic parameter for assessing the fertilizing qualities of sperm. Like motility, sperm viability is influenced by a complex of factors. One paper that deals with the effect of age on sperm viability in roosters is that of Tabatabaei *et al.* (2010). The authors conducted a study on native broiler roosters (parents) aged 26, 34 and 43 weeks. The authors reported a decrease in sperm viability by about eight percent from 26 to 34 weeks of age and another eight percent from 34 to 43 weeks of life. No particular explanation was provided for this process other than the simple effect of the phenomenon of physiological ageing: "the exact reason for the decrease in sperm quality through ageing is not clear."

Surprisingly, the percentage of morphologically normal sperm showed no significant changes during the monitored period (Figure 1). However, it is important to remember the changes in the type of anomalies during the life of roosters. The evolution of the normal percentage of sperm in the ejaculate was revealed by Siudzinska and Lukaszewicz (2008) in a study performed on several cock breeds of different sizes: Green-Legged Partridge, Black Minorca, White Crested Black Polish, and Italian Partridge aged six months. The authors revealed values between 70.5 and 69.1%, which is in agreement with the results of our research on the Cornish breed. Only one breed, the Italian Partridge, which is a light-sized breed, had a much lower percentage, at 54.0%. Edens and Sefton (2009) also noted the absence of significant differences in sperm count and testicular histological structure related to the age of roosters from the age of 32 to 42 weeks. The results were confirmed by Bunaciu *et al.* (1987) on other rooster lines.

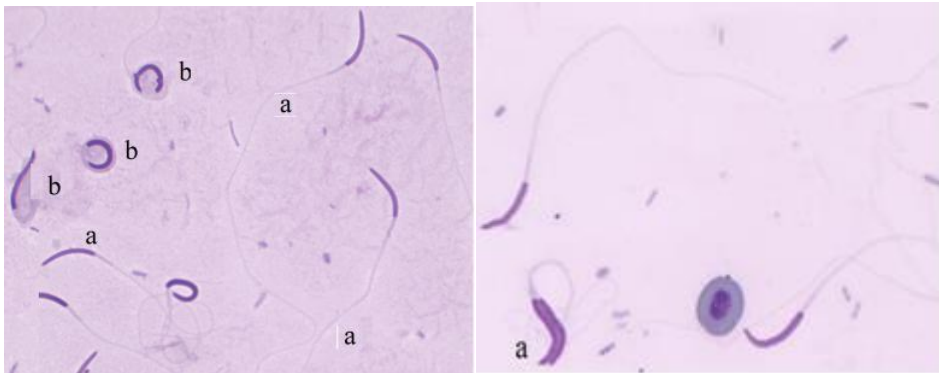


Figure 1 Sperm abnormalities in a 42-week-old Cornish rooster.

Left: a - normal spermatozoon; b - spermatozoon with protoplasmic drop (immatures); right: a - double-headed spermatozoon (eosin - nigrosin stain; ob. x90)

Morphological abnormalities of sperm were systematized into two broad categories: head and acrosome abnormalities (one category), and intermediate piece abnormalities and tail abnormalities (including end piece abnormalities, the second category.) The main head abnormalities were represented by: absence of head, double head, bent head, hook-shaped head, oversized head (thick, swollen, bumpy), undersized head, knotted head (Figure 1). The main anomalies of the acrosome were represented by: absence of acrosome, swollen acrosome, rounded acrosome and hook-shaped acrosome. It was found a total percentage of 2.8%. They were also the most common types of abnormalities from young to advanced ages. It was found that the percentage of sperm with head and acrosome abnormalities is negative and very intensely correlated with age, decreasing from young to advanced ages (r between -0.94 and -0.99). The presence of protoplasmic drop was also observed only until the age of 42 weeks. However, the percentage of sperm with a spiral tail increased from 0 to 1.5%.

Table 2

Evolution according to age of some semen enzymes in Cornish roosters

Enzyme	Age of the roosters				Mean on total period	r
	30 weeks	42 weeks	57 weeks	63 weeks		
TGO (U/L)	33.3 ^a ±2.2	34.2±2.2	26.0±3.0	18.1 ^a ±2.9	27,75±2,22	-0.88
Alkaline phosphatase (U/L)	1450.0±43.4	1540.0±16.9	1311.2 ^b ±2.0	111.31± ^b 13.0	1353.3±15,0	-0.82
ATP-ase (U/L)	57.6± 6.4	33.3±3.5	55.6 ^c ±4.3	50.4 ^c ±4.3	49.8±3.0	-0.11
Lactic dehydrogenase (IU)	1200±36	1065 ^d ±65	1190±76	954 ^d ±43	1102,2±32	-0,57

Note:

- values are expressed as mean ± standard error of mean;
- values with the same exponent in the same row are significantly different;
- r = Pearson's correlation coefficient.

For comparison, we specify the research conducted by Bunaciu et al. (1987, 1978) on three breeds of turkeys: White breed large type, White small type and Bronze breed: the results showed

that only in one of the breeds the percentage of dead and abnormal sperm increases as the males advance in the breeding season, respectively, while in the other two monitored breeds, the correlation with age was weak.

The seminal plasma activity of the investigated enzymes revealed a general tendency of inverse correlation with the age of the roosters (Table 2, negative r values for all the investigated enzymes). A close correlation with age showed TGO ($r = -0.88$) and LDH ($r = -0.57$). The source of plasma TGO can be considered to be sperm, which can lose intracellular enzyme by increasing the permeability of plasmalemma. Excessive decrease in LDH activity may be related to decreased intensity of aerobic carbohydrate metabolism, specific to the ageing process. According to Singer *et al.* (1980), alkaline phosphatase activity in semen showed an increasing trend with increasing sperm count, revealing increased membrane permeability of sperm, as in the case of TGO. According to Dumitru and Dinischiotu (1994), the activity of acid phosphatase seems to be correlated with the intensity of the sperm harvesting process since the activity of this enzyme was higher in Mini Rock roosters that were subjected to a more frequent sperm harvesting regime. On the other hand, as shown by the data presented by Dinischiotu *et al.* (1982), the evolution of ATP-ase activity in sperm is a function of sperm fertility, the authors proposing the determination of this enzyme as a test for assessing the fertilizing capacity of spermatozoa.

Conclusions

The ageing process in Cornish roosters is characterized by profound changes in the biological parameters of sperm. Ejaculate volume, sperm density, motility and viability decrease noticeably while the percentage of normal spermatozoa do not change significantly. However, at the age of 63 weeks, the sperm of these roosters still retain properties comparable to those of 53 weeks, which would make it possible to extend the period of keeping these roosters in the herd up to 63 weeks of life.

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