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SUMMARY

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BIODIVERSITY OF THE THORACIC LIMB SKELETON IN COYPU

Anca ȘEICARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Veterinary Medicine, 105, Spaiul Independenței, District 5, Bucharest, Romania

Corresponding author email: ancaseicaru@gmail.com

Abstract

The paper presents the thoracic limb skeleton of the coypu as compared to the rabbit. The scapular girdle is represented by the scapula and the clavicle, bones that are well developed in this species. The acromion is short and tuberous. The ratio between the infraspinous and supraspinous fossae is 2/1. The deltoid tuberosity of the humerus appears evident. Contrary to existing literature, which describes the absence of the supratrochlear foramen, we identified it in two of the three investigated corpses. Between the bones of the forearm a wide interosseous space is present. The thoracic basipodium is represented by 9 carpian bones because a central bone is interposed between the proximal row and the distal row. The following bones appear in the latero-medial line of the proximal row: pisiform, scaphoid, triquetral, lunate. The latero-medial line of the distal row contains the following bones: hamate, capitate, trapezoid and trapezium.

Key words: *coypu, rabbit, thoracic limb skeleton.*

INTRODUCTION

The coypu belongs to the *Animalia* Kingdom, *Vertebrata* Phylum, *Mammalia* Class, *Myocastoridae* Family, *coypus* Species.
This study aims to bring an addition to the existing data in the literature. The coypu, which is a semi-aquatic mammal, is used for its fur, but also for human consumption (Hrițcu et al., 2000).
The rabbit belongs to the *Animalia* Kingdom, *Vertebrata* Phylum, *Mammalia* Class, *Lagomorpha* Order, *Lepodidae* Family (Coțofan et al., 1982; Harcourt-Brown, 2002)
It is also used for its fur and for consumption (Coțofan et al., 1982).
The morpho-topographic knowledge of the bones of the anterior limb enables the veterinarian to properly identify the species (Coțofan et al., 2003; Brewe et al., 1994; Gheție et al., 1971).

MATERIALS AND METHODS

The study material was represented by three adult coypu and three rabbits corpses. The working methods were:

- Maceration of the limbs in water at room temperature, in order to remove the soft structures (muscles);
- Boiling, as an alternative method of processing, was performed in water with common detergents.

At the end of the maceration process, the bones were washed with water jet and subjected to the bleaching process with 11% hydrogen peroxide solution; after bleaching the bones were washed and dried.
For the removal of decomposed organic substances during maceration, direct cleaning with the scalpel, scissors and forceps were used.
After completion of the bone cleansing process, the anatomical features were described in detail, with the bones being photographed.
The homologation of the formations was made according to Nomina Anatomica Veterinaria 2017.

RESULTS AND DISCUSSIONS

In **coypu**, the scapular girdle includes the scapula and the clavicle that are well developed. The extremities of the clavicle merge with the sternum through a fibrous strucure.
The scapula is very wide, has a transverse orientation, and an acromion with a recurved extremity.
The para-acromion is short and tuberous (Figure 1).

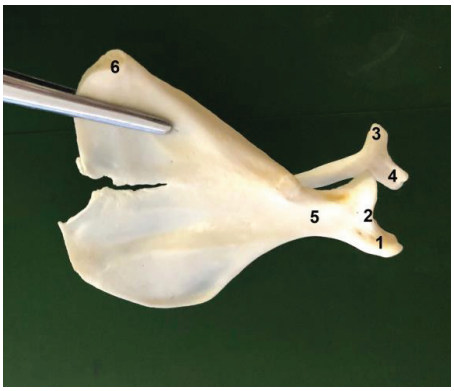

Figure 1. Scapula of the coypu - medial view (original):
1 - supraglenoid tuberosity; 2 - glenoid cavity; 3 - paraacromion; 4 - acromion; 5 - neck of scapula; 6 - thoracic angle

The ratio of the supraspinous and infraspinous fossae is 2: 1. The medial face of the subscapular fossa is shallow, showing muscle insertion lines. The cervical angle of the scapula is obtuse, and the thoracic angle is slightly elongated.

Figure 2. Scapula of the coypu - medial view (left) and front view (right) (original):
1 - subscapular fossa; 2 - supraspinous fossa; 3 - infraspinous fossa; 4 - scapular spine; 5 - glenoid cavity; 6 - supraglenoid tuberosity; 7- paraacromion; 8 - acromion

The great axis of the glenoid cavity is oval with sagittal orientation. On the dorsal edge of the scapula there is a suprascapular cartilage. The cervical edge appears convex-concave, and the caudal edge is thick with numerous muscle insertion lines (Figure 2).

The humerus has a prismatic body, and the articular head exceeds in height the non-divided humeral tuberosity. Its deltoid tuberosity is well developed (Figure 3).
The humerus groove is wide, deep, while the extensor crest is evident. The tubercle for the ters major muscle is elongated dorso-ventrally.

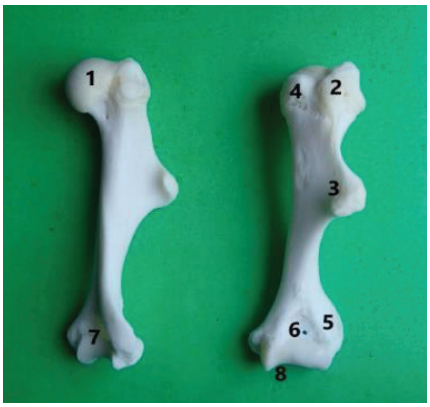

Figure 3. Humerus in coypu (original):
1 - humeral head; 2 - olecanian fossa; 3 - small tubercle; 4 - large tubercle; 5 - deltoid tuberosity; 6 - the lateral epicondyle crest; 7 - supratrochlear foramen; 8 - humeral trochlea

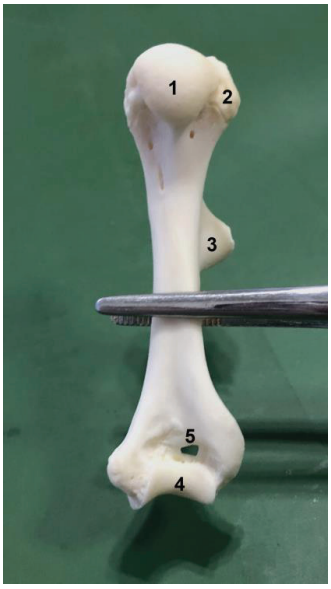

Figure 4. Humerus in coypu- cranial view (original):
1-humeral head; 2-small tubercle; 3-deltoid tuberosity; 4-distal humeral joint; 5-supratrochlear foramen

The distal humeral joint is formed by a trochlea, and the olecranon fossa has the axis disposed transversally (Figure 4). In two out of the total of three investigated corpses, the supratrochlear foramen was present, though reduced in size, while the absence of the epitrochlear foramen was noticed, contrary to the literature, which claims the absence of the both.

The radius presents well-developed medial and lateral tuberosities. The proximal extremity of the radius presents a medial-laterally elongated glenoid cavity. The body of this bone is strongly twisted in the shape of the "S" letter.

The distal extremity of the radius in coypu displays a deep and slightly wavy glenoid cavity. The ulna presents a well developed olecranon with a non incised tuberosity (Figure 5).

Figure 5. Radius and ulna in coypu - lateral view (original):
1 - olecranon; 2 - olecranon tuberosity; 3 - cranial articulation surface; 4 - articulary cavity;
5 - interosseous space of the forearm

The semilunar incision is well outlined and very deep in its middle third. The body of the ulna is more developed than that of the radius; this particularity is different as compared to the rabbit.

Between the forearm bones in coypu there is a large interosseous space.

On the edge of the proximal extremity of the ulna there is a radial incisura which serves for the articulation of the radius (Figure 6).

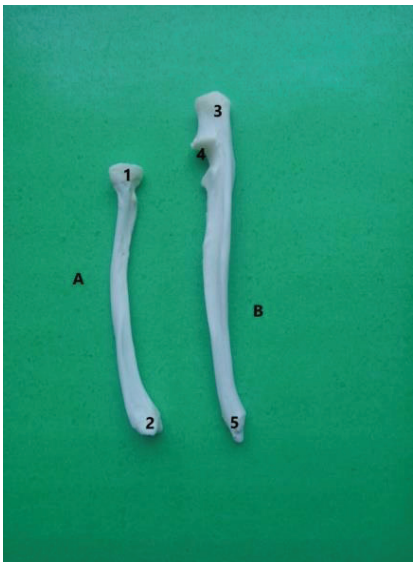

Figure 6. Zeugopodium of the anterior limb in coypu (original):
1 - proximal extremity of radius; 2 - distal extremity of radius; 3 - olecanon; 4 - semilunar notch; 5 - distal extremity of ulna; A - radius; B - ulna

The carpal bones in the proximal row are represented by the pisiform, pyramidal, scafo-ulnar and facoid bones. The distal row is formed by the unciform, capitate, trapezoidal and trapezium bones. Between the two rows of carpal bones there is the central bone.

The pyramidal bone has a glenoid cavity, and the scafo-ulnar bone has a glenoid cavity and a condylum. The distal extremity of the first row of carpal bones has the aspect of a flat-condyloid joint surface. The articular surface of the distal extremity from the bones in the second row, presents three glenoid cavities: one cavity for the articulation with the proximal extremity of the fourth metacarpal, a second one for proximal extremity of the third metacarpal, and the third is common for the metacarpals of fingers II and III.

In coypu there are 5 metacarpal bones with a dorso-palmar flattened shape. The first finger presents a proximal phalanx and a distal phalanx. The rest of the fingers (II, III, IV, V) are made up of three phalanx. The distal phalanx presents

a prismatic form, with a flexor tubercle on the ventral face, flanked axially and abaxially by a wide and deep intertubercular groove.

Rabbits, as compared to nutria, show a series of particularities due to the specific use of the thoracic limbs. The scapula appears triangular and elongated dorso-ventrally. On its lateral side, the scapular spine ends with a long acromion and a well-developed para-acromion, as opposed to the coypu where this structure was smaller.

The ratio between the infraspinous fossa and the supraspinous fossa is 2/1. The cranial edge of the scapula is thin, with a convex dorsal half and a scapular notch on its ventral half (Figure 7). Dorsally to the glenoid cavity, there is a well-developed supraglenoid tuberosity, sharp and curved medially.

Figure 7. Scapula in rabbit - lateral view (left) and medial view (right) (original):
1 - infraspinous fossa; 2 - supraspinous fossa; 3-scapular spine; 4 - paraacromion; 5 - acromion; 6 -glenoid cavity; 7 - supraglenoid cavity; 8 - subscapular fossa

The caudal angle of the scapula is thickened and tuberous, and the dorsal edge of this bone features a suprascapular cartilage.

The cranial angle of the scapula is slight and rounded.

The ventral angle appears thickened, and on the medial and lateral sides of the scapular neck vascular grooves can be observed.

The clavicle of this species is narrow, compared to the coypu where it appears well-developed.

The humerus is a long bone which, laterally to its articular head, exhibits the greater tubercle where an articular surface for the clavicle is found. The bicipital groove is slightly deep.

The cranial side of its body appears plane and widened proximally, while the lateral side displays a light torsion groove. Caudally, the torsion groove is delimited by the ridge of the lateral epicondyle until its distal extremity where this ridge becomes thick and rough.

The distal extremity of the humerus has tall, sharp and equally sized trochlear lips, while the olecranon fossa appears deep (Figure 8). The presence of the supratrochlear hole was not noted in any of the bodies examined, even though it is described in the literature.

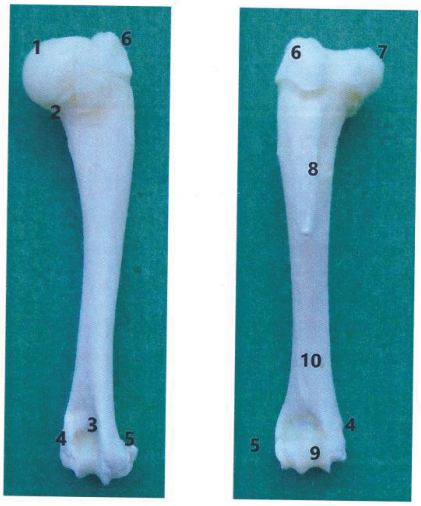

Figure 8. Humerus in rabbit - lateral view (left) and medial view (right) (original):
1-humeral head; 2-neck of humerus; 3-olecranon fossa; 4-medial epicondyle; 5-lateral epicondyle; 6-greather tubercle; 7-small tubercle; 8-deltoid tuberosity; 9-humeral trochlea; 10-brachial groove

The forearm bones of this species appear curved cranially and they are joined through an interosseous ligament. The proximal extremity of the radius has two diarthrodial articular surfaces and two glenoid cavities. Distally, the radius has a wavy carpal surface (Figure 9).

Concerning the proximal extremity of the ulna, the olecranon appears oriented cranially, with a wide semilunar notch. It has two diarthrodial articular surfaces. The ulna has a distal joint with the carpal bones from the first row (Figure 10).

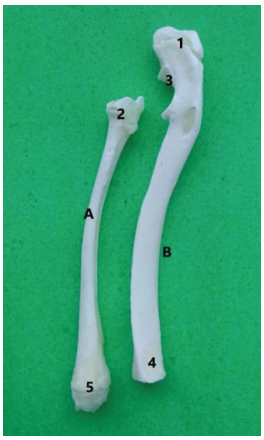

Figure 9. Radius and ulna in rabbit (original):
A - radius; B - ulna;
1 - olecranon; 2 - proximal extremity of radius;
3 - semilunar notch; 4 - distal extremity of radius;
5 - distal extremity of ulna

Figure 10. Radius and ulna in rabbit - cranial view (original):
1 - olecranon; 2 - olecranon tuber; 3 - radial articular surface; 4 - interosseous space of forearm (proximal and distal); 5 - distal extremity of ulna;
6 - distal extremity of radius

The basipodium of the rabbit features, between the two rows of carpal bones, a central bone. The pisiform is elongated and has two articular surfaces for the pyramidal bone and for the distal extremity of the ulna. The pyramidal bone has articular surfaces for the semilunar, the pisiform, the ulna as well as a distal extension to articulate with the V metacarpal. The semilunar bone has a dorsal convex articular surface and a palmar concave one. The scaphoid bone has an articular area for the semilunar bone represented by two elongated articular surfaces. The unciform is the most developed bone in the distal row. The capitate has proximal articular surfaces for the semilunar and the scaphoid. The trapezoid bone has an articular surface for the trapeze and for the I metacarpal.

Metacarpal I is reduced in size compared to the other 4 bones in the same segment. Each metacarpal has, on its proximal extremity, a plane and wide articular surface for the corresponding carpal bone in the distal row. The body of the metacarpals is slightly cylindrical. The distal extremity of metacarpal I has a depression on its medial side.

In the house rabbit, the first phalange is a long bone, whose proximal extremity is fitted with two glenoid cavities separated by a medial groove. Under the proximal articular surface, on the palmar side, two tubercles can be observed, disposed laterally and medially. At the distal extremity, the first phalange has two condyloid articular surfaces separated by a median groove. The proximal extremity of the II phalange has two articular cavities separated through an elongated ridge with dorso-palmar orientation. The distal extremity of phalanx II presents a trochlear surface for articulation. The distal phalange or the II phalanx (the claw) has two proximal articular cavities separated by a reduced ridge. There are two great sesamoids per each finger, and they articulate with the proximal extremity of the first phalange with a proximally oriented tip. The small sesamoid completes the proximal articular surface of the third phalange.

CONCLUSIONS

The clavicle of the rabbit is a narrow bone unlike in coypu where it is long and well-developed.
In coypu, the ratio of the supraspinous and infraspinous fossae of the scapula is 2/1.
The acromion is flanked by a short and tuberous para-acromion.
The deltoid tuberosity appears exaggeratedly developed, approachable transcutaneous.
Contrary to the literature data, where it is described as being absent, in two of the three

investigated body corpses, the supratrochlear hole is evident at the distal extremity of the humerus.

In all the examined rabbit bodies the supratrochlear hole was not found, even though it is mentioned in the literature.

The distal phalanx presents a prismatic form, with a flexor tubercle on the ventral face, flanked axially and abaxially by a wide and deep intertubercular groove.

Between the forearm bones in coypu, a large interosseous space was noticed, which differentiates this species from leporids, where the interosseous space does not appear due to the two bones being articulated through an interosseous ligament along their entire length.

Both in coypu and in the rabbit, the thoracic basipodium is formed of 9 carpal bones due to the central bone which is located between the two rows of carpal bones.

REFERENCES

Brewer N.R., Cruise L.J. (1994). Anatomy and Physiology. In: Manning PJ, Ringler DH, Newcomer CE, editors. The biology of the laboratory rabbit. San Diego. Academic Press; p. 63-71.

Coțofan V., Hrițcu Valentina, Negrea S., Cura P., Cozariuc I. (1982). Caractere morfologice diferențiale ale scheletului axial la nutrie comparativ cu unele mamifere domestice. Lucr. Sem. Șt. „Metode noi de sporire a producției la animale“, Iași.

Coțofan V., Cocoș Lucia, Cura P., Hrițcu Valentina, Negrea A. (1982). Caractere morfologice diferențiale ale oaselor memebrului toracic la nutri, comparativ cu unele mamifere domestice. Lucr. Sem. Șt. „Metode noi de sporire a producției la animale”, Iași.

Coțofan V., Predoi G. (2003). Anatomia topografică a animalelor domestice. Editura Bic All, București.

Harcourt-Brown F. (2002) – Textbook of rabbit medicine. Oxford, Butterworth-Heinemann.

Hrițcu Valentina, Coțofan V. (2000). Anatomia animalelor de blană: nutria, dihorul. Editura Ion Ionescu de la Brad, Iași.

Gheție V., Hillebrand A. (1971). Anatomia animalelor domestice, Vol. I. Editura Academiei Republicii Socialiste România.

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COMPARATIVE STUDY ON THE BIOLOGICAL FACTORS OF INFLUENCE ON THE SPERM QUALITY IN DIFFERENT BREED ROOSTERS

Lucica SIMA[1], Rosalie BĂLĂCEANU[2]*, Nicolae DOJANĂ[2]

[1]ROMVAC COMPANY, 7 Soseaua Centurii, Ilfov County, 729913, Romania
[2]Department of Physiology, Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independentei, Bucharest, 050097, Romania, Phone +040723352253

*Corresponding author email: rosaliebalaceanu@fmvb.ro, phone +40 722 159 699, fax +4021 318 04 98

Abstract

In this experiment, the influences of the breed, age, light intensity, and foot disorders on Cornish, Sussex and Plymouth Rock rooster sperm properties were monitored. The ejaculate volume, sperm density, motility and anomalies, dead spermatozoa, as well as the fecundity (calculated as hatching rate of the eggs provided by the hens inseminated by these roosters) were analyzed. Research has revealed different effects according to breed, age and light intensity on the volume of ejaculate, sperm density and motility. Ejaculate volume was higher in Cornish while density, motility, sperm anomalies and fecundity were higher in Sussex and Plymouth Rock. Age decreased significantly the ejaculated volume, and increased sperm anomalies and dead sperm percentages but didn't modify density and motility percentages of Cornish roosters. The intensity of light improved the biological characteristics of the sperm: ejaculated volume, density and fecundity increased significantly in Cornish but not in Sussex and Plymouth Rock. In Cornish roosters, the frequency of leg disorders diminished the ejaculated volume, sperm density and egg fecundity and increased the percent of the sperm anomalies and dead sperm.

Key words*: sperm properties, fecundity, breeds, age, light, roosters.*

INTRODUCTION

According to Khaki et al. (2009), Lee et al. (2012) and Barkhordari et al. (2013) one of the major issues on breeding in farm animals is infertility, and approximately 30% of the problems are related to the males (Okoro et al., 2016). The specialized literature presents a broad approach to the main factors influencing the quality of the semen in birds, which are: 1. specific factors: genetic factors (breed, line), male traits (waist, age, health); 2. non-specific factors: the composition and quality of the food, and technology of exploitation. If the appraisals extend to fecundity (and even to hatchability), then to the factors above it can add other factors such as those related to incubation technology as well as those related to the physiological conditions of the inseminated females. Roosters with high semen producing capacity are often few and they degenerate due to changes in factors such as age, poor nutrition, unfavourable climatic conditions, and poor management (Okoro et al., 2016). The specialized literature presents a broad approach to the field, and, although in principle, things are known, the specialized publications are still supported by numerous works in that field. It is therefore very important to know the proportion of abnormal spermatozoa in a semen sample in order to determine the best semen characteristics for optimum fertility (Alkan et al., 2002). Our study focused on the effects of breed, age, light intensity and health status on the main characteristics of sperm in roosters.

MATERIALS AND METHODS

Research has been carried on three rooster breed: White Chornish, Sussex and Plymouth Rock, 30, 42 and 57 weeks aged, raised in the industrial system. The animals benefited from a program of 14.5 hours light from 5:30 to 20:00. The sex ratio was 1/4.5 in Cornish roosters, 1/5.5 in Sussex roosters and 1/6 in Plymouth Rock roosters. Cornish roosters were fed on 220 g of forage per capita and per day while

Sussex and Plymouth Rock chickens were fed *ad libitum*. Sperm sampling was performed according to the method described by Bunaciu et al. (1979). The semen was collected between 9:00 and 10:30. The ejaculate samples were collected in glass tubes which were kept at 37°C in suitable containers until sperm analysis. The volume of the ejaculate was measured using graduated glass micropipettes with a volume of 1.0 mL.

Sperm motility was determined by manual method. Viability smears were prepared on glass slides using 1% Eosin-Y and 10% Nigrosin and air dried. For each sample, at least 200 intact sperm cells were evaluated for percentage vitality estimation. Sperm density was determined after fixing the samples with 95% ethyl alcohol solution and counting sperm using a Türk count chamber and a Potain pipette for sample dilution. The percentage of sperm with abnormalities was manually determined by examination of the Eosin Nigrosin stained smears. To determine the effect of foot disorders (pododermartitis), characteristic of the Cornish breed, Cornish males who were diagnosed with such pathologies were used to obtain sperm samples.

Fertility was determined on the basis of the results obtained by incubation in the industrial system. In this sense, the percentage of eggs fertilized at the first mirage (after 6 days of incubation) was determined for each breed. The data were statistically analysed by ANOVA using a general linear model procedure of SAS (2002). The calculated descriptive statistics were mean and standard error of mean (SEM). Statistic comparison was performed between the experimentally different breed groups, and between the health and the affected Cornish group (with pododermatitis). The significance level for null hypothesis rejection was stated at $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 presents the main characteristics of sperm as well as the fertility of 30-week-old rooster breeds: white Cornish, Sussex and Plymouth Rock. At this age, the differences between breeds are partly no significant statistically (for sperm density and percentage of dead sperm), but significant for ejaculate volume (higher in the Cornish breed, $P < 0.002$), sperm motility (higher in the Rock roosters, $P < 0.011$), the percentage of abnormal sperm (higher in Cornish, $P < 0.031$) and fertility (higher in Sussex and Rock, $P < 0.030$). The volume of the ejaculate correlates on the other hand with the size of the breed, the Cornish breed being from this point of view with the highest size. Higher rates of fertility in the Sussex and Plymouth Rock breeds may be correlated with sperm density, higher in these breeds than in Cornish, and also with sperm motility.

Studies on the properties of sperm in different rooster breeds (Cornish, Sussex, Minirock, Leghorn) were also performed by Bunaciu et al. (1978) and Bunaciu et al. (1979). Bunaciu et al. (1978) found a correlation between the percentage of abnormal sperm and motility,
which shows that if the percentage of abnormal sperm is higher, the motility is lower.

Table 1. Morphological sperm properties and fecundty in Cornish, Sussex and Plymouth Rock rooster breeds, 30 week aged

Breed	Ejaculate volume (mL)	Sperm density ($x10^6$/mL)	Sperm motility (%)	Sperm anomalies (%)	Dead sperm (%)	Fecundity (%)
Cornish	0.19± 0.04	2.16 ± 0.10	88.7 ± 2.2	2.59 ± 0.20	0.64 ± 0.09	79.9 ± 7.7
Sussex	0.17 ± 0.02	2.82 ± 0.21	92.5 ± 5.5	2.83 ± 0.21	0.48 ± 0.10	84.0 ± 9.1
Plymouth Rock	0.12 ± 0.01	2.88 ± 0.22	92.2 ± 9.3	3.26 ± 0.12	0.61 ± 0.21	83.3 ± 3.2
P value	0.002	0.06	0.011	0.031	0.18	0.030

Note: data were calculated as mean ± standard error of mean

P values were calculated based on HSD (*honest significant difference)* Tukey test. Differences were considered significants for P<0.05. Fecundity was calculated as number of embryonic eggs and number of incubated eggs ratio

Evolution of Cornish rooster sperm properties according to age is presented in Table 2. According to the data from Table 2, ejaculate volume, sperm density, sperm anomalies and dead sperm percentages decreased significantly ($P < 0.05$) form 30 to 57 week of age. Fecundity percentage decreased significantly also ($P = 0.004$). The fact guides for the reformation of these roosters before or at the age of 57 weeks. Sperm motility percentage and sperm anomalies percentage remained unchanged statistically ($P = 0.19$ and $P = 0.09$, respectively), which would plead for the possibility of extending the exploitation of these breeds in industrial conditions at least up to the age of 57 weeks of life. Decreasing by about 10 percent the fertility of this breed from 30 to 57 weeks can ultimately orient the breeder's interests. Calculated phenotypic correlation between sperm motility on the one hand and the occurrence of defective sperm cells on the other was found negative by Jarinkovičová et al. (2012), as well as the correlation between sperm motility and sperm numbers, and between sperm motility and ejaculate volume. Changes in sperm properties in Cornish roosters have been described in connection with the season by Elagib et al. (2012): summer season caused a significant drop in semen volume in one year old and sperm concentration in two years old roosters. Summer also caused an increase in percent of dead sperm.

Effects of light intensity on the sperm features in different breed roosters are presented in Table 3.

Table 2. Sperm morphology and fecundity in Cornish roosters according to age

Age (weeks)	Ejaculate volume (mL)	Sperm density ($x10^6$/mL)	Sperm motility (%)	Sperm anomalies (%)	Dead sperm (%)	Fecundity (%)
30	0.19± 0.02	2.42 ± 0.11	89.2 ± 6.6	2.32 ± 0.04	0.87 ± 0.09	79.9 ± 6.0
42	0.21 ± 0.02	3.16 ± 0.06	88.72 ± 6.5	2.22 ± 0.04	1.11 ± 0.05	74.4 ± 5.4
57	0.15 ± 0.00	2.68 ± 0.06	91.3 ± 5.6	3.76 ± 0.03	1.91 ± 0.00	70.0 ± 5.5
P value	0.002	0.02	0.19	0.09	0.01	0.004

Note: data were calculated as mean ± standard error of mean

P values were calculated based on HSD (*honest significant difference)* Tukey test. Differences were considered significants for $P<0.05$. Fecundity was calculated as number of embryonic eggs and number of incubated eggs ratio

Table 3. Effect of light iensity on the sperm features in different breed roosters

Ligh intensity (W/m^2)	Ejaculate volume (mL)			Sperm density ($x10^6$/mL)			Fecundity (%)		
	Cornish	Sussex	Plymouth Rock	Cornish	Sussex	Plymouth Rock	Cornish	Sussex	Plymouth Rock
2.2	0.190	0.178	0.173	2.56	2.76	2.65	78.8	84.2k	84.5
6.3	0.231	0.188	0.193	2.54	2.78	2.65	80.3	86.6	86.0
8.4	0.230	0.182	0.184	2.64	2.90	2.78	86.9	87.6	88.9
P value	0.020	0.061	0.052	0.020	0.043	0.034	0.001	0.010	0.06

P values were calculated based on HSD (*honest significant difference)* Tukey test. Differences were considered significants for $P<0.05$. Fecundity was calculated as number of embryonic eggs and number of incubated eggs ratio

The increase of the light intensity caused the volume of the ejaculate to increase in all the three rooster breeds under observation. However, these increases were significant only in the Cornish breed ($P = 0.020$), being encoded at a difference of 0.04 mL, representing a percentage of 21.05%. But sperm density increased significantly in the entire three rooster breeds. These increasing were of 0.11 x 10^6/mL in Cornish, 0.14 x 10^6/mL in Sussex and 0.13 x 10^6/mL in Rock roosters. Improvement of these traits of sperm morphology was positively reflected in fertility rates, which increased significantly in all three

rooster breeds. According to Dzoma (2010), fertility may be influenced by rooster and/or hen factors. Rooster fertility is influenced by age, stage of the breeding season, frequency of ejaculation, sperm supply, disease and nutrition. Hen fertility is influenced by reproductive age, feed energy levels, disease and efficiency in sperm storage. The vitamin content of the food, as well as the structure of the diet are factors that influence the quality of the sperm in the roosters (Danikowski et al., 2002).

Analysis of foot disorders in Cornish roosters led to the identification of up to 17% roosters with pododermatitis caused by contact with acid litter. These conditions

were accompanied by a weight loss below the average of healthy roosters by 24.5%. The morphological analysis of the sperm of these roosters led to the identification of a significant decrease of the ejaculate volume up to 22%, a decrease of the spermatogenesis reflected in the decrease of sperm density and an increase of sperm anomalies and dead sperm percent. The fecundity decreased up to 31% compared with health roosters of the same age. Bunaciu *et al.* (1987) conducted extensive studies on the effects of foot conditions on sperm quality in roosters. The authors drew attention to the economic importance of their treatment or prevention in the industrial raising complexes.

CONCLUSIONS

The morphological and functional characteristics of sperm in roosters are under the influence of biological and environmental factors, including age, breed, light, and claw disorders. Foot disorders can cause a significant decrease in the morphological characteristics of the sperm in Cornish roosters and the increase of the light intensity can have positive effects on spermatogenesis and fecundity.

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REFERENCES

Alkan, S., Baran, A., Ozdas, O. B. and Evecen, M. (2002). Morphological defects in turkey semen. Turkish Journal of Veterinary & Animal Science 26:1087-1092.

Barkhordari, A., Hetmatimoghaddam, S., Ajebali, M.A., Khalili, T. A. and Noorani, M. (2013). Effect of Zinc Oxide nanoparticle on viability of human spermatozoa. Iran Journal of Reproductive Medicine 11:767-771.

Bunaciu, P., Ştefănescu,M., Dinu Viorica, Gomoiu,V., (1978). Folosirea raţională a cocoşilor din rasa Cornish pentru însămânţări artificiale (The rational use of the Cornish roosters for artificial insemination). Revista Creşterea Animalelor 6, p.15-18.

Bunaciu, P., Ştefănescu, M., Dinu Viorica, Bunaciu Maria, Matei, Gh., Gomoiu, V. (1979). Cercetări privind influenţa regimului de utilizare a cocoşilor asupra procesului de spermatogeneză (Research on the influence of the use regime of roosters on the process of spermatogenesis). Revista Creşterea Animalelor, 4; p.29-32.

Bunaciu, Maria, Bunaciu, P., Dinischiotu Anca (1987). Tipurile de anormalitate spermatică în corelaţie cu fecunditatea la două linii de cocoşi. Lucrările Ştiinţifice ale I.C.P.P.A.M. Balotești, vol.V, p.139-145.

Danikowski, S., Sallmann, H.P., Halle, I. Flachowsky, G. Influence of high levels of vitamin E on semen parameters of roosters. Journal of Animal Physiology and Animal Nutr. 86 (11-12), 2002, Pages 376-382.

Dzoma, B.M.(2010). Some factors affecting fertility and hatchability in the fanned ostrich: A review. Journal of Animal and Veterinary Advances. Volume 9, Issue 2, Pages 229-239.

Khaki, A., Fathiazard, F. Nouri, M., Khaki, A. A., Khamenehhi, H.J. and Hamadeh, M. (2009). Evaluation of androgenic activity of *Allium cepa* on spermatogenesis in the rat. Folia Morphologica 68:45-51.

Lee, B., Jung J. and Kim, H. (2012). Assessment of red onion on antioxidant activity in rat. Food and hemical Toxicology 50:3912-3919.

Okoro V.M. O., Anayochukwu Mbajiorgu C., Mbajiorgu E.F.(2016). Semen quality characteristics of Koekoek breeder roosters influenced by supplemental inclusion levels of onion and garlic mixture at 35-41 weeks of age. R. Bras. Zootec., 45(8):433-440.

Jarinkovičová, L., MácHal, L., MácHal, J., Filipčík, R., Tumová, E., Horský, R. (2012). Relationship of ejaculate quality and selected biochemical parameters of blood in cockerels of three laying lines. Czech Journal of Animal Science Open Access Volume 57, Issue 8, Pages 370-376.

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MONITORING *CARASSIUS GIBELIO* MUSCLE TISSUE QUALITY UNDER BLACK CUMIN OIL FORTIFICATION AND TYPICAL COLD STORAGE

Ştefania Mariana RAITA, Mara GEORGESCU*

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Veterinary Medicine College of Bucharest, 011464, 59 Mărăşti Blvd, District 1, Romania

*Corresponding author e-mail: dr_georgescu_mara@yahoo.com

Abstract

On the background of the growing need for natural alternatives to fresh fish preservatives, the present study is focused on the assessment of Nigella sativa seed oil effect over the freshness and morphological structure of Carassius gibelio (Prussian carp) muscle tissue, during typical cold storage. The monitoring involved storage of fish samples at regular temperature conditions, recommended for the food industry (0 ± 1°C, on ice). The Carassius gibelio fish samples were divided into three groups: control group (C), without NSSO; test group 1, fortified with 0.1% v/w NSSO (T1) and test group 2, fortified with 0.6% v/w NSSO (T2). Histological and freshness monitoring reveal that treatment groups T1-T2 keep normal morphological structure of fish muscle throughout the monitoring period, while showing slightly improved freshness indicators (pH and TVB-N), compared to control samples. This study strengthens the proposal for NSSO as a natural enhancer of fresh fish quality and shelf life.

Key words*: Carassius gibelio, NSSO, freshness indicators.*

Introduction

Carassius gibelio is known as the Gibel carp, or the Silver Prussian carp and belongs to the *Cyprinidae* family, being pretty common for freshwater ecosystems, mostly in Europe, Russia, Korea and north-east China.

In Romania, Prussian carp fishing is the most practiced amateur fishing, while for consumption, it is known as the most preferred local fresh water fish (Tăpăloagă D, 2017; 2018).

As fish and fishery products are on the top listed food preferences, even for the sensitive categories of consumers (EUMOFA, 2018; Romania Insider, 2019), these are highly perishable food commodities (Sulieman H.M.A., 2012), which require shelf life extension solutions.

Current research literature is rich in proposals of natural preservation methods, among which is *Nigella sativa* seed oil. *Nigella sativa* has recognized *in vitro* (Bakal S.N., 2017) and *in vivo* (Rafati S., 2014) antimicrobial effect against various microorganisms and was proposed as antibacterial solution for various types of commodities, such as cheeses (Georgescu M. et al., 2018a) and fresh fish (Ozpolat and Duman, 2017).

Considering our promising previous results indicating *Nigella sativa* as an efficient antimicrobial solution for other food commodities (Georgescu M. et al., 2018b; Georgescu M et al., 2019), this paper is focused on assessment of *Nigella sativa* seed oil effect over the freshness and morphological structure of *Carassius gibelio* (Prussian carp) muscle tissue, during typical cold storage.

MATERIALS AND METHODS

The conducted research presented in this article is focused on assessment of *Black cumin seed oil (Nigella sativa seed oil - NSSO)* fortified *Carassius gibelio* (Prussian carp) muscle tissue quality, during the storage period of six days. The monitoring involved storage at regular temperature conditions, ideally applied in the food industry (0 ± 1°C, on ice), for fresh Prussian carp whole fish samples, supplemented with various amounts of black cumin seed oil (0.1 and 0.6% v/w).

Sample preparation

Carassius gibelio whole fish weighing 50-80 g/fish (Figure 1) was caught in July 2019, from marsh located close to Bucharest, "Chiroiu 4 Marsh" (Chiroiu-Pământeni commune,

Ialomița county). Fish were transported to laboratory in Bucharest, in ice boxes. Sample preparation included gutting, removal of head and gills, and washing. The *Carassius gibelio* individual fish were divided into two groups: control group (C), without NSSO and test group (T), fortified with *Nigella sativa* seed oil (NSSO). The test group (T) was further divided into 2 groups, depending on the type of NSSO fortification: group T1 - fortified with 0.1% v/w NSSO and group T2, fortified with 0.6% v/w NSSO. All groups of samples were stored on ice in trays which were introduced in a domestic, commercial refrigerator, set at 3°C (at ideal storage conditions) throughout the monitoring period. Each group included three gutted, head-less and gill-less whole fish, one for each set of analysis.

Figure 1. *Carassius gibelio* whole fish, cought from "Chiroiu 4 Marsh" (Chiroiu-Pământeni commune, Ialomița county)

Nigella sativa cold pressed seed oil (NSSO), marketed under the name "Black caraway oil (Ulei de Negrilică)", was purchased from a Romanian company, Carmita Classic, Alba Iulia (Alba County, Romania) (Figure 2).

Figure 2. Black cumin oil -"Black caraway oil (Ulei de Negrilică)", Carmita Classic, Alba Iulia (Alba County, Romania)

NSSO was displayed to the surface of *Carassius gibelio* whole fish samples in appropriate volume/weight using a micropipette, followed by mildly massaging the oil onto each sample using a gloved hand, according to the method described by Ozpolat E. and Duman M. (2017).

Treatment groups were packed in plastic bags without using vacuum (using high barrier nylon polyethylene bags) (T2 - T4), or were covered in ice (control group, C and T1 group) and stored at designated temperatures until analysis (Figure 3).

Freshness indicators analysis

The pH of samples was measured according to an AOAC method (Association of Official Analytical Chemists, 1990). A total of 10g of sample (from each treatment group) was homogenized with 90 ml deionized water and the pH was measured with a digital pH meter (Fisher Scientific Accumet Basic).

Total volatile basic nitrogen (TVB-N, mg/100g fish flesh) was determined by direct distillation of fish after addition of magnesium oxide (MgO), according to the Romanian Standard for determination of total volatile basic nitrogen in meat (SR 9065-7:2007).

Figure 3. *Carassius gibelio* whole fish sample preparation: sample weighing (left); Treatment groups packed in high barrier nylon polyethylene bags) (right)

Histological analysis

Fish muscle samples were prepared into 1-2 cm diameter sections, immediately fixed in buffered formalin and posteriorly embedded in paraffin. Once fixed, a dehydration was performed by increase of alcohol degree (70, 80, 96, 98°), followed by immersion in xylene (twice) and two baths in paraffin, each sample remained 1 hour in each solution. Automatic processing took 5 hours. Histological sections of 5 µm in thickness, transverse and vertical, were obtained and subsequently stained with haematoxylin-eosin (HE) to evaluate the morphology patterns of the muscle fibers. To stain, a deparaffinization was carried out using a xylene immersion for three times (20, 15 and 10 minutes, respectively) and the tissue was rehydrated by decreasing of the alcohol degree, 100° (3 min), 96° (1 min), 80° (1 min) and 70° (1 min), followed by immersion in distilled water (3 min).

Data analysis

The study design included three batches of *Carassius gibelio* whole fish samples: control group (without NSSO), on ice, and placed at refrigerator (group C) and test groups T1-T2. Both test groups were fortified with NSSO: T1 - fortified with 0.1% v/w NSSO and group T2, fortified with 0.6% v/w NSSO. Test groups were subjected to the same storage conditions as the control group. The three batches of samples were considered the treatments, which were analyzed at days 1, 3 and 6 of storage. For the freshness indicators, variance analysis of data was performed by One way analysis of variance (ANOVA) using SAS (ANOVA version 9.1). The threshold of significance level between treatments was $p < 0.05$.

RESULTS AND DISCUSSIONS

TVB-N content

The values of TVB-N at study start-up were higher than those described by recent publications, for *Cyprinidae* fish, as Pramod K (2019) found an initial value of 4.57 mg/100g, compared to our study, which indicated values ranging from 8.22 to 8.87 mg/100 g. As expected, the TVB-N values followed a rising shift throughout the monitoring period (Figure 4). However, the total volatile basic nitrogen (TVB-N) limit values considered by scientific literature and current available legislation, ranging from 25 mg/100g to 30 mg/100 g (Oehlenschlager J, 1992; Regulation no. 2074/2005), were not reached by any of the treatment or control groups of samples (fig 4).

In terms of differences noted between the rising trends of TVB-N content of control samples,

and treatment samples, there is an obvious difference between T2 and control (C), as revealed in figure 4. Control group samples reached a value of 21.73 mg/100g at day 6 of refrigerated storage on ice, while T2 samples revealed a lower maximum value, of 18.36 mg/100g. However, no statistical significance is associated with this difference ($p > 0.05$).

For treatment 1, *Carassius gibelio* whole fish samples enriched with 0.1 % v/w NSSO, the TVB-N rising shift pattern is inconstant, as on day 3, the indicated value is slightly higher (13.32 mg/100g) than for control group samples (12.8 mg/100g), against expectations, while on day 6, the TVB-N content of T1 group samples is only slightly lower (20.24 mg/100g) than for control group samples (21.73 for control group samples).

Our study reveals that NSSO in the designated concentrations did not significantly influence the TVB-N pattern of fluctuation during cold storage, for *Carassius gibelio* whole fish samples.

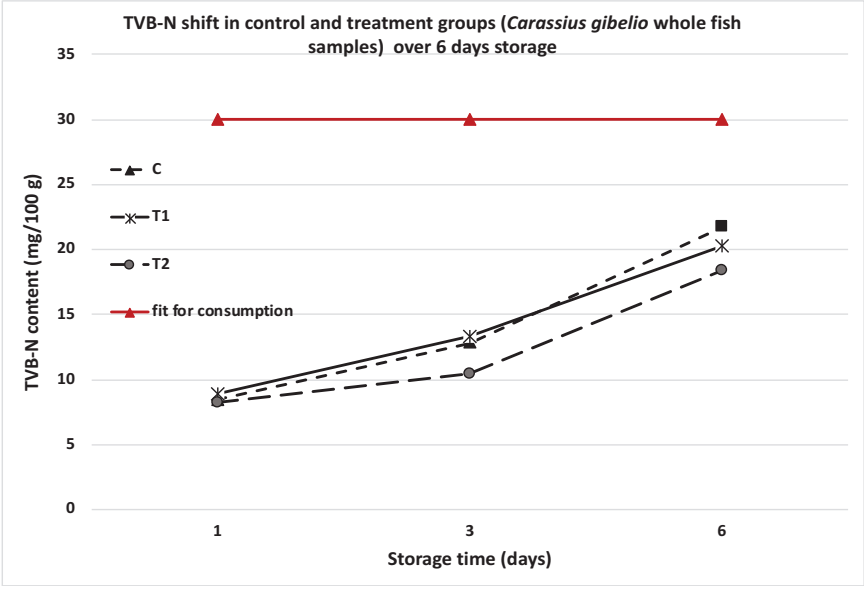

Figure 4. Results of TVB-N content in control and treatment *Carassius gibelio* whole fish samples

pH trend

The initial values of the pH for all considered samples ranged between 6.18 and 6.22 and were higher than pH values communicated by other scientific studies for *Carassius gibelio* fresh muscle samples, who report values ranging from 5.55, to 6.07, with an average of 5.77± 0.13 (Zhang Z et al., 2016). However, the values revealed in our study were much lower than those indicated for similar species, such as Crucian carp, during similar storage on ice, for which the reported initial pH value averaged 7.06 ± 0.04 (Kaifeng L et al., 2015). As there currently are no regulated maximum admitted limits of fresh fish pH, we are considering the lowest maximum admitted limit (7.0) available in international guides and regulations, considered as the threshold limit for condemnation (Goncalves A.A., 2017). None of the samples in our study exceeds this limit and therefore can be considered fit for consumption from this point of view.

Post-mortem raise in fish muscle pH follows quickly, soon after the initial drop induced by la lactic acid accumulation, and is due to accumulation of ammonia and trimethylamine, which are associated with spoilage. Our results reveal a difference in pH trend between the treatment groups, as lower pH values are seen for T1 and T2 groups of samples. There is also a positive connection between the NSSO concentration of enrichment and pH rising trend, as lower values were seen for T2 group of samples (6.22-6.49, with an average of

6.33), compared to T1 group of samples (6.18-6.54, with an average of 6.34) (Figure 5). However, no statistical significance ($p > 0.05$) was indicated for the positive correlation between the NSSO concentration and the beneficial effect on the pH of the fish muscle.

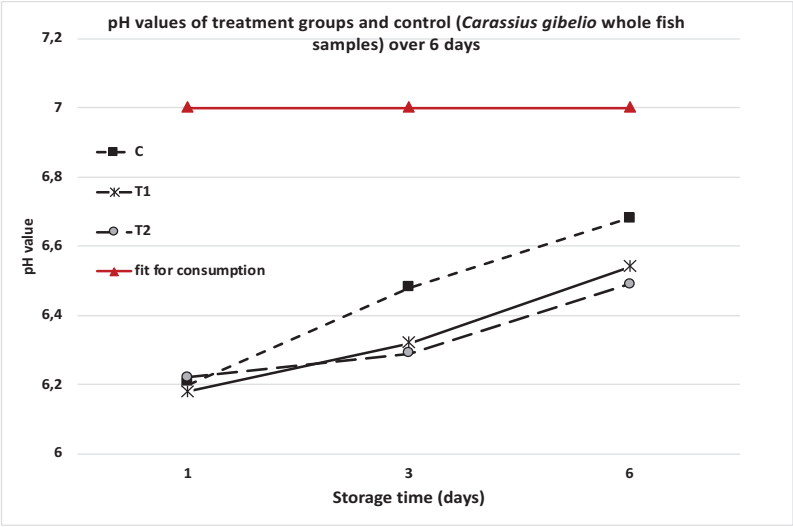

Figure 5. Results of pH trend in control and treatment *Carassius gibelio* whole fish samples

Histological analysis results

Fish

The organization of *Carassius gibelio* muscle tissue exhibited a typical morphological pattern found in fish. The muscular tissue from *Carassius gibelio* samples is composed of skeletal striated muscle fibers of fusiform shape with numerous nuclei arranged peripherally, the intermuscular connective tissue is poorly represented. (Figures 6-14).

Following histological evaluations no significant differences were observed between the samples collected on the first, the third and the sixth day of storage.

In all examined samples (9) the integrity of the muscle cells and the presence of numerous blood capillaries were observed.

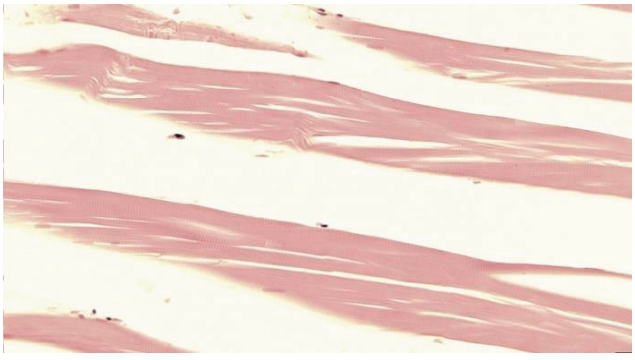

Figure 6. Histological assessment of *Carassius gibelio* whole fish samples at day 1 – Control group (Longitudinal section. Ob. 40X, Col. HE)

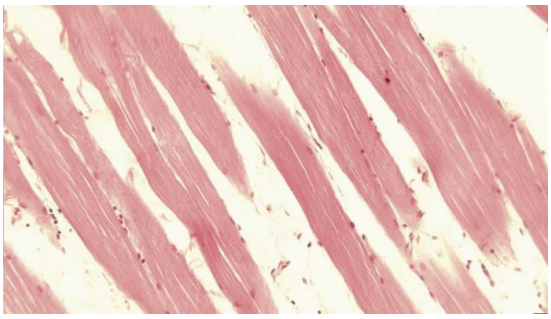

Figure 7. Histological assessment of *Carassius gibelio* whole fish samples
at day 1 – T1 group, 0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

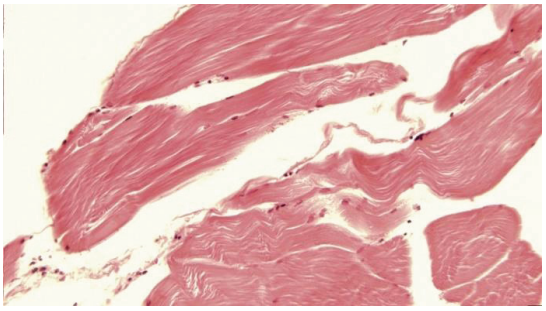

Figure 8. Histological assessment of *Carassius gibelio* whole fish samples
at day 1 – T2 group 0.6% v/w NSSO (Longitudinal section and transverse. Ob. 40X, Col. HE)

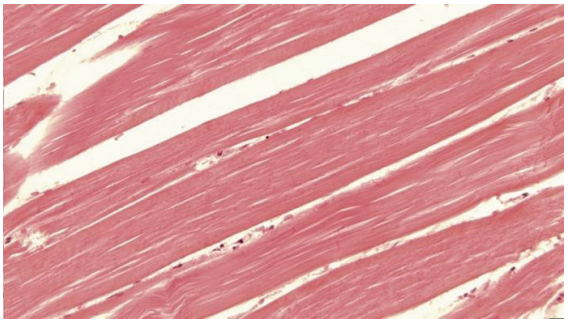

Figure 9. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – Control
(Longitudinal section. Ob. 40X, Col. HE)

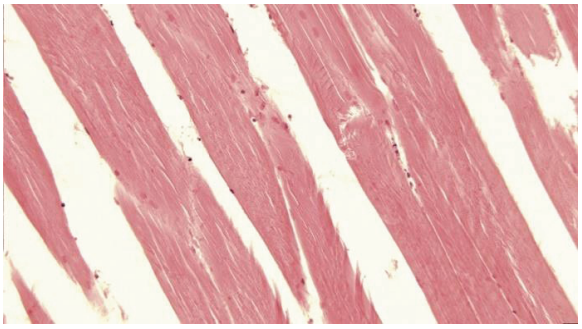

Figure 10. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – T1,
0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

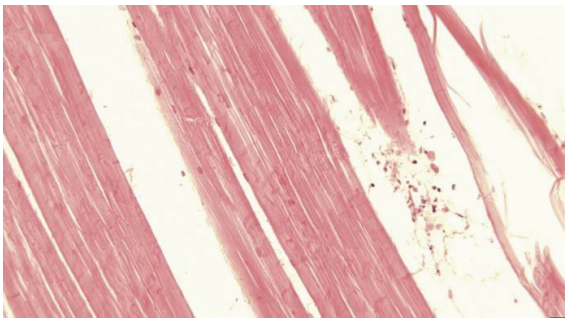

Figure 11. Histological assessment of *Carassius gibelio* whole fish samples at day 3 – T2, 0.6% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

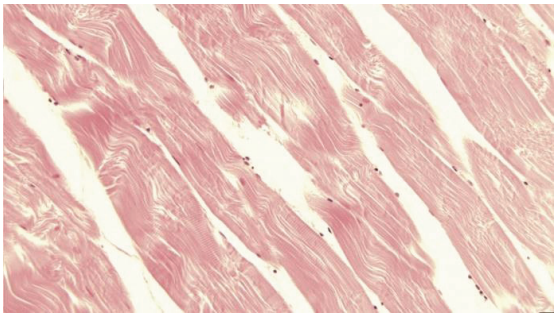

Figure 12. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – Control group (Longitudinal section. Ob. 40X, Col. HE)

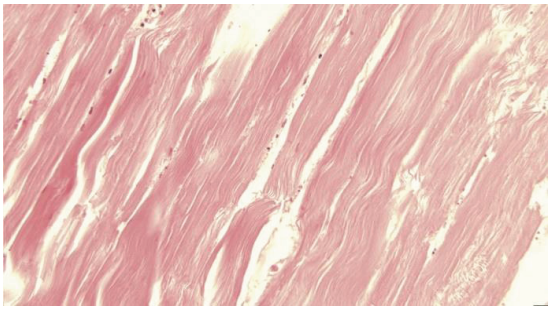

Figure 13. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – T1, 0.1% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

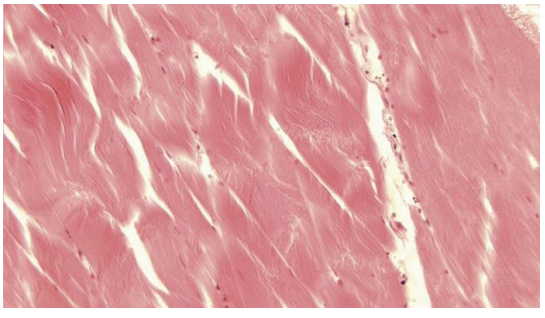

Figure 14. Histological assessment of *Carassius gibelio* whole fish samples at day 6 – T2, 0.6% v/w NSSO (Longitudinal section. Ob. 40X, Col. HE)

CONCLUSIONS

Our results reveal that treatment groups T1-T2 keep normal morphological structure of fish muscle throughout the monitoring period, while showing slightly improved freshness indicators (pH and TVB-N), compared to control samples. This study strengthens the proposal for NSSO as a natural enhancer of fresh fish quality and shelf life.

REFERENCES

Bakal S.N., Bereswill S., Heimesaat M.M. (2017). Finding Novel Antibiotic Substances from Medicinal Plants - Antimicrobial Properties of *Nigella sativa* Directed against Multidrug-resistant Bacteria. Eur J Microbiol Immunol (Bp). 16;7(1):92-98. 2017. http://doi.org/10.1556/1886.2017.00001.

EUMOFA (2018). The EU fish market – 2018 Edition. European Market Observatory for Fisheries and Aquaculture Products. European Commision.

Georgescu M., Dobrea M., Tăpăloagă D, Raita Ș., and Dobrea V. (2018a). Functional evaluation of *Nigella sativa* seed oil effect on pathogen enriched artisan cheese. Journal of Biotechnology 280. S54. ISSN: 0168-1656. DOI:10.1016/j.jbiotec.2018.06.173.

Georgescu M., Raita Ș.M., Tăpăloagă D. (2019). Total nitrogen, water-soluble nitrogen and free amino acids profile during ripening of soft cheese enriched with *Nigella sativa* seed oil, The EuroBiotech Journal 3 (2), 90-9. DOI: https://doi.org/10.2478/ebtj-2019-0010.

Georgescu M., Savu C., Neagu I., Georgescu D. (2014). Coliform bacteria contamination profiling of raw milk cheeses using statistical assessment of type-linked correlations. AgroLife Scientific Journal. 3: 39-44.

Georgescu M., Tăpăloagă P.R., Tăpăloagă D., Furnaris F., Ginghină O., Negrei C., Giuglea C., Bălălău C., Ștefănescu E., Popescu I.A., Georgescu D. (2018b). Evaluation of antimicrobial potential of *Nigella sativa* oil in a model food matrix. Farmacia, 2018; 66(6): 1028- 1036.

Goncalves A.A. (2017). The pH of the seafood meat: a problem that deserves to be clarified. MOJ Food Process Technol. 2017;4(1):25-26. DOI: 10.15406/mojfpt.2017.04.00081.

Kaifeng Li., Yulong B., Yongkang L., Huixing S. et al. (2012). Formation of Biogenic Amines in Crucian Carp (Carassius auratus) during Storage in Ice and at 4°C. Journal of food protection. 75. 2228-33. 10.4315/0362-028X.JFP-12-143.

Kaifeng Li., Yongkang L., Huixing S. (2015) Postmortem Changes of Crucian Carp (*Carassius auratus*) During Storage in Ice, International Journal of Food Properties, 18:1, 205-212, DOI: 10.1080/10942912.2011.614986.

Oehlenschlager, J. (1992). Evaluation of some well-established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In Quality Assurance in the Fish Industry, Huss, H.H. (editor). Elsevier Science Publishers B. V., Netherlands, 339-351, (1992).

Ozpolat E. and Duman M. (2017). Effect of black cumin oil (*Nigella sativa L.)* on fresh fish (*Barbus grypus*) fillets during storage at 2 ± 1 °C. Food Sci. Technol (Campinas) vol.37 no.1 Campinas Jan./Mar. 2017. http://dx.doi.org/10.1590/1678-457x.09516.

Pramod K. Prabhakar, Prem P. Srivastav & Sant S. Pathak (2019). Kinetics of Total Volatile Basic Nitrogen and Trimethylamine Formation in Stored Rohu (*Labeo rohita*) Fish, Journal of Aquatic Food Product Technology, 28:5, 452-464, DOI: 10.1080/10498850.2019.1604598.

Rafati S., Niakan M., Naseri M. (2014). Anti-microbial effect of *Nigella sativa* seed extract against staphylococcal skin Infection. Medical Journal of the Islamic Republic of Iran. 2014; 28:42.

Regulation (EC) No 2074/2005, laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organization of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.

Romania Insider (2019). Romania's fish market expected to reach EUR 380 mln this year. Available at https://www.romania-insider.com/romania-fish-market-estimation-2019. Accessed 20.06.2019.

Sulieman H.M.A., Bari L.O.A. and Hafiz M.A. (2012). Determination Of Quality And Shelf Life Of Three Marine Fishes (Coral Trout, Greasy Grouper And Red Mouthed Bream) Based On Total Volatile Nitrogen Test (TVN). International Journal of Livestock Research. Vol:2 (2) May 2012, pg.227-235.

Tăpăloagă D., Tăpăloagă P.R., Ilie L.I., Georgescu M., Georgescu D.A. (2018). From conventional to organic agriculture - Romanian past and future perspectives -, Scientific Papers-Series D-Animal Science, Accession Number: WOS:000448065500043, ISSN: 2285-5750, eISSN: 2393-2260,Volume: 61,Issue: 1,Pages: 239-244, Published: 2018.

Tăpăloagă D., Tăpăloagă P.R. (2017). Study regarding animal organic farmin Romania - current status and trends, Scientific Papers. Series D. Animal Science. Vol. LX, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285- 5750.

Zhang Z., Tian X., Dapeng L.I. (2016). Tissue pH and gut ecomorphology in six freshwater teleosts occupying different trophic levels. Turk J Zool. (2016) 40: 713-719. doi:10.3906/zoo-1511-5.

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THE INFLUENCE OF PARASITIC DISEASE ON IMMUNITY CELLULAR SYSTEM IN ONE HEALTH APPROACH

Ana-Maria COMAN[1], Emilia BALINT[2], Dragos-Constantin COMAN[3]✉, Nicolae MANOLESCU[4]

[1]Institute of Oncology *Prof. Dr. Alex. Trestioreanu*, 252 Sos. Fundeni, District 2, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, District 5, Bucharest, Romania
[3]Sanitary Veterinary and Food Safety Department, 11 Street Corlatesti, Prahova, Romania
[4]Romanian Academy, 125 Calea Victoriei, District 1, Bucharest, Romania

Coresponding author email: dragospus@yahoo.com

Abstract

Wanting to study a special topic in the field of comparative oncology, the component part of the new global medical concept One Health, we proposed to develop the cytomorphological basis of adenopathies caused by the chronic parasitic infestations with Fasciola hepatica, Echinococcus granulossus or Dicrocelium lanceolatum.
Our research have unfolded over 4 years, on a number of 267 animals: 82 horse, 147 sheep, 16 swine and 22 cattle, which presented, at post-mortem examination, one or more adenopathies, alongside the characteristic lesions of a chronic parasitic disease.
From these animals we harvested the lymph nodes with lesions and we have done smears to examine the populations of the cellular system of immunity.
Through this screening we succeeded to make a real "movie" of cytomorphological lesions, transposing, step by step, the transformations of lymphnodal cell populations which are persistently stimulated by the hyperantigenity induced by the chronic parasitism, from the stage of chronic lymphadenitis, to the malignant prelymphoma.

Key words*: adenopathies, One Health approach, parasitic zoonoses.*

INTRODUCTION

Although the *One Health* approach has, over time, been promoted in many academic organizations, scientific publications and international professional associations, at present, the trends indicate an increase of them due to unifying vision, protective to "primary prophylaxis of life" through the elaboration and implementation of policies, research programs and laws regarding the reduction of human and animal diseases, ensuring a healthy food and responsible management of natural resources (Stroud C. et al, 2016; Manlove K.R.J.G. et al., 2011; Zinsstag J. et al., 2011).

One of the major concerns of the concept, alongside of antibiotic resistance, are the zoonotic diseases that cause incalculable damage through losses of human and animal lives (Didă I.C., Duca I., 2002; Dima C. et al, 2008). This diseases are considered "high priority" for the system of diseases surveillance, being performed numerous research, surveillance and control activities (United Nations, 2017; Pachauri R.K. et al, 2014; Kuenzli E., 2016; FAO, 2017; UNEP, 2017; Dobre G., 2016). Also, the zoonoses are the main concern of the factors involved in food safety policies for animal origin (Dima C. et al., 2008).

In present are more than 200 zoonotic diseases and it is estimated that 75% of emerging pathogens are of zoonotic origin, especially bacteria, fungi and parasites (Dima C. et al., 2008). From the data published by WHO and the UN through FAO, more than 100 zoonotic infectious agents are represented by different types of parasites (Didă I.C., Duca I., 2002).

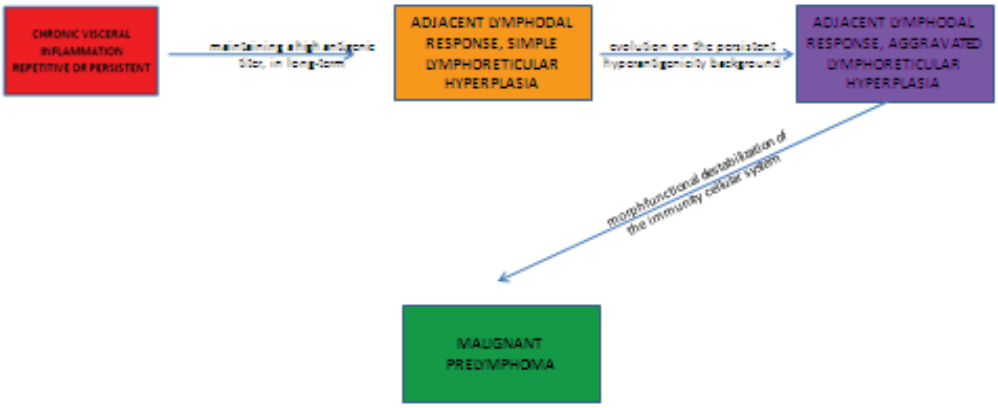
Thus, the present study complements the current measures of eradication, control and monitoring of zoonotic parasitic diseases by demonstrating that these parasites can open a path to the century illness, *the cancer*.

MATERIALS AND METHODS

The presented results refer to the data obtained on 267 slaughtered animals, including: 82 horse, 147 sheep, 16 swine and 22 cattle. The anatomo-pathological examination of the animal carcasses revealed the presence of characteristic lesions of each parasitosis, respectively: (i) chronic hepatitis, hypertrophic or atrophic hepatic cirrhosis - to the sheep fasciolosis; (ii) chronic bronchopneumonia and/or chronic hepatitis - to the swine and the bovine echinococcosis; (iii) chronic hepatitis and cholangitis – to the cattle dicroceliosis. Along to the visceral lesions, we have detected, in adjacent or satellite lymph nodes, changes in macroscopic (i) highlighted color, (ii) consistency or (iii) volume, that could be considered to represent the "key" of our hypothesis. From these lymph nodes, there were made smears by uniformly stretching of the lymph nodal “juice” and their staining using the May-Grunwald Giemsa (MGG) technique.

RESULTS AND DISCUTIONS

The examination of the smears gave us the possibility to confirm the evolutionary transformation stages of the lymph nodes cell populations as a result of the persistent hyperantigenicity induced by the chronic parasitosis (Scheme 1).



Scheme 1. The stages of transformation from persistent chronic lymphadenitis to malignant lymphoma

Thus, we have succeeded to achieve a true "movie" from chronic parasitic lymphadenitis, going on with simple lymphoreticular hyperplasia, then, with aggravated lymphoreticular hyperplasia and, finally, with malignant pre-lymphoma. In the animal series, the quantitative and qualitative values of lymphonodal cellularity are relatively similar in the "quasi" normality status being dominated by the combination of antigenically exposed lymphocytes, together with virgin lymphocytes (Figure 1).

The chronic inflammation can take three forms, depending on the species, respectively: (i) the dominance of the virgin lymphocyte population; (ii) the dominance of the antigenically exposed lymphocyte population; (iii) the presence, in equal relative numbers, of both the virgin and antigenically exposed lymphocytes (Figure 2).

Simple lymphoreticular hyperplasia is characterized by the dominant presence of antigenically exposed lymphocytes together with the reticular, dendritic, histiocytic and lymphoblastic cells, without cell atypia (Figure 3).

The cellularity of the aggravated lymphoreticular hyperplasia varies depending the anatomical area of limph node which is involved in the immune process. Thus, we found 3 distinct morphocytological forms in which was predominant one of cellular form: (i) lymphoblastic, (ii) histio-macrophage-dendritic or (iii) reticular. The cells atypia are not present and the number of blast cells is less than 20% (Figure 4).

The status of malignant pre-lymphoma also takes one of the three forms of previous status, the difference consisting of the increased number of blast cells, up to 40% and appearance of the cells atypia (Figure 5, Figure 6).

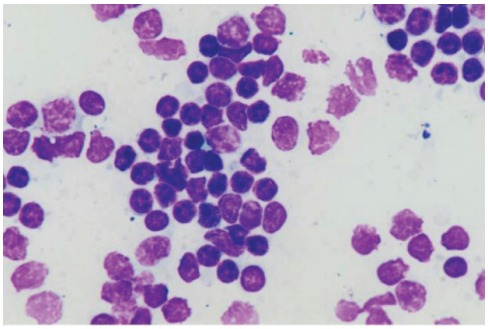


Figure 1. Sheep lymph node, "quasi" normality status, combination of antigenically exposed lymphocytes and virgin lymphocytes. MGG X 1000

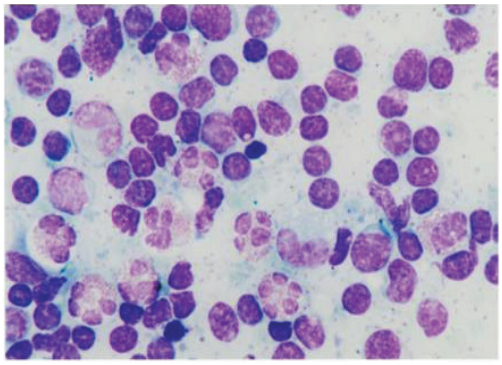


Figure 2. Sheep lymph node, chronic lymphadenitis of parasitic origin, numerous eosinophilic granulocytes, adult lymphocytes, and rare prolymphocyte. MGG X 1000

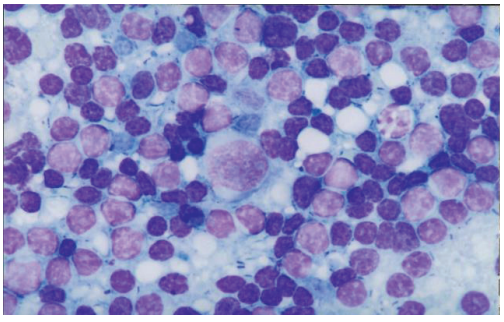


Figure 3. Swine lymph node, simple lymphoreticular hyperplasia, numerous blast cells along reticular cells and adult lymphocytes; blast coefficient is 6-7%. MGG X 1000

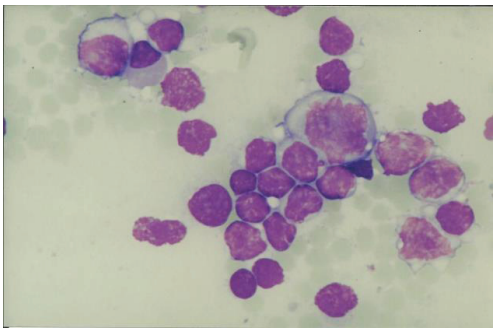


Figure 4. Bovine lymph node, aggravated lymphocytic hyperplasia, numerous lymphoblastic cells; blast coefficient is 20%. MGG X 1000

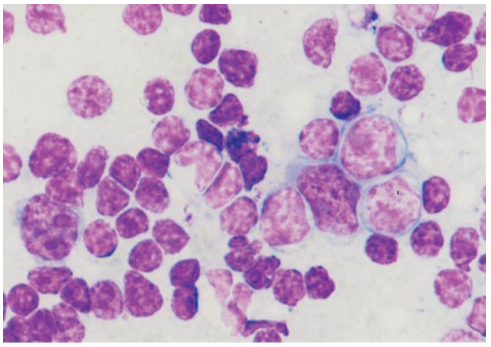


Figure 5. Sheep lymph node, the onset phase of the malignant pre-lymphoma, large number of atypical lymphopoetic series blast cells. MGG X 1000

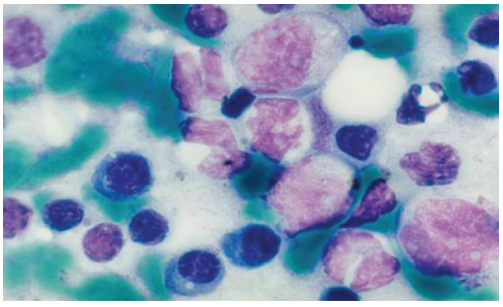


Figure 6. Horse lymph node, malignant pre-lymphoma characterized by the presence of malignant lymphoblastic cell with obvious atypia. MGG X 2000

CONCLUSIONS

The study demonstrates that the presence of a chronic visceral inflammation - in our case, parasitic infestation, inducing a persistent hyperantigenity, may determinate irreversible changes in immune cell populations, thereby causing the development of a lymph node neoplasm.

The results bring in discussion, the capital importance of anatomopathological and laboratory examinations in slaughter house, to ensure salubrious carcasses, starting from the desideratum that man's health is inseparably linked to animal health and also, to the health of the living environment.

REFERENCES

Didă I.C., Duca I. (2002). *Parasitic diseases, the epidemiological risk factor*. Scientia Parasitologica, vol. 2: 13-16.

Dima C., Niculiță P., Popa M., Miteluț A. (2008). *Monitoring and prevention stategies of zoonotic diseases using traceability and HACCP systems*. Scientific works, U.S.A.M.V.B., Series A, LI.

Dobre Gheorghe (2016). *Bolile emergente*. Lumea apicolă, 49: 22-25.

Food and Agriculture Organization of the United Nations (2017). *Cross-sectoral issues, biosecurity*. http://www.fao.org/biodiversity/cross-sectoral-issues/biosecurity/en (accessed October 4, 2017).

Kuenzli, E. (2016). *Antibiotic resistance and international travel: Causes and consequences*. Travel Medicine Infectious Diseases 14(6):595-598.

Manlove, K. R., Walker J. G., Craft M.E., Huyvaert K. P., Joseph M. B., Miller R. S., Nol P., Patyk K. A., O'Brien D., Walsh D. P., Cross P. C. (2016). *"One Health" or three? Publication silos among the One Health disciplines*. PLoS Biology 14(4):e1002448.

Pachauri, R. K., Allen M. R., Barros V. R., Broome J., Cramer W., Christ R., Church J. A., Clarke L., Dahe Q., Dasgupta P. (2014). *Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change.* Geneva, Switzerland: Intergovernmental Panel on Climate Change.

Stroud, C., Kaplan B., Logan J. E., Gray G. C. (2016). *One Health training, research, and outreach in North America*. Infection Ecology & Epidemiology 6:33680.

United Nations (UN) (2017). *The Sustainable Development Goals report 2017.* New York.

United Nations Environment Programme (UNEP) (2017). *Towards a pollution-free planet background report.* Nairobi, Kenya.

Zinsstag, J., Schelling E., Waltner-Toews D., Tanner M. (2011). *From "one medicine" to "One Health" and systemic approaches to health and well-being*. Preventive Veterinary Medicine 101(3-4):148-156.

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EPIDEMIOLOGICAL STUDY OF THE HYPERPLASIA LYMPH NODES INCIDENCE FROM SLAUGHTER PIGS

Dragos-Constantin COMAN[1], Emilia BALINT[2], Nicolae MANOLESCU[3]

[1]Sanitary Veterinary and Food Safety Department, 11 Corlatesti Street, Prahova, Romania, dragospus@yahoo.com
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, Bucharest, Romania, emilia_balint@yahoo.com
[3]Romanian Academy, 125 Calea Victoriei, Bucharest, Romania, manolescunicolae@yahoo.com

Corresponding author email: dragospus@yahoo.com

Abstract

It is know that cellular immunity system has an overwhelming implication in pathology, therefore, if it has persistent stimulate, for a long period of time, the cellularity of it can undergo a marked changes, from the blast cells proliferation until the appearance of atypia and cellular monstrosity, thus foreshadowing the installation of the worst plague of our century, cancer.
Thus, chronic lymph node hyperplasies represent an anatomo-clinical manifestations of the borderline between benign and malignant, both histologic and clinic, involving, in the same time, the lymphocytic and reticulo-histiocytic cell lines. This chronic hyperplasia status may have a short-term evolution, ending with healing, but, in the same time, it may have a multi-relapse, wich, if is not treated, it will became a true malignant lymphoma.
Starting from these lesions, in this paper, we realised an epidemiological study, to the slaughtered animals from swine species, regarding the incidence of chronic lymph node hyperplasia, the precursor of malignant lymphoma, reported to the pathology and location of adenopathies.

Key words*: limph node, hiperantigenity, swine.*

INTRODUCTION

The food is the most important factor who influencing the organism in different senses, and this interdependence is directly conditioned by the sanitation and trophico-biological quality. (Stănescu et. al., 1998; Rusen et al., 2007; Tudor 2005).
Thus, one of the important branches of veterinary medicine is represented by the insurance of a safe animal origin food, which doesn't present a risk to the consumer (Tudor et al., 2009).
The increase of the population and, implicitly, the demand for food, supplemented by the particularities of the life standard, determinated an increase of culinary preferences oriented towards meat and meat products (Kearney, 2010; Modlinska and Wojciech, 2018;
Raasadhika et al., 2018; Brian A. et al., 2014), which determinated the elaboration of strict legislative norms regarding the consumption of animal origin foods, the illnesses animals being a real challenge for the specialists from public health, researchers, microbiologists and hygienists (Regulation (CE) No 178/2002, Regulation (CE) nr. 2073/2005, Regulation (CE) nr. 853/2004).
To assesse the health animals status of the slaughtered swines, for 4 years period, the authors carried out an epidemiological study about the incidence of lympho nodes hyperplasia, according to different parameters such as visceral lesion picture or localization of adenopathies.

MATERIALS AND METHODS

The researches of the present study were conducted over for a 4 years, within an authorized slaughterhouse in Prahova county, on a number of 149 animals of the swine species that, to the post-mortem examination of animal carcasses revealed chronic anatomical-pathological changes in the lung, liver, cardiac, splenic, etc. Also, during the investigations we

have observed changes like shape, size, color, consistency, to the level of the adjacent or satellite lymph nodes of these tissues or organs, but also adenopathies that were not accompanied by visceral changes, which determinated us to focus on the microscopic study of these hematopoietic structures, by making smears of lympho nodal juice, using the May-Grunwald Giemsa staining method.

RESULTS AND DISCUSSIONS

Based one the cytomorphological results, we detected 4 categories of chronic reactivity status of limph nodes of, respectively: chronic inflammation, benign lymphoreticular hyperplasia, aggravated lymphoreticular hyperplasia and malignant pre-lymphoma. Thus, following of these results, we realized an epidemiological study on the incidence of lymphonodal morphological statuses depending on the chronic pathology detected and the topographic location of the adenopathies.

1) The epidemiological study related to the clinical pathology detected

The visceral pathology revealed, following the post-mortem examination of the swine carcasses, comprises a wide range of pathological disorder such as: fibrous pericarditis, cardiac dilatation due to functional impairment, chronic bronchopneumonia, hepatomegaly, splenomegaly, gastritis, hydatidosis, enteritis, etc.

The incidence of visceral pathology to the animals with benign lymphoreticular hyperplasia in the affected lymph nodes is marked by the chronic bronchopneumonia (41 cases), representing 72% from total - Chart 1.

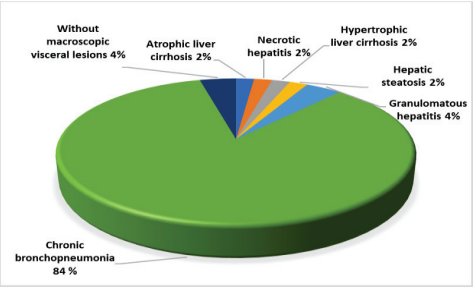

Chart 1. The incidence of visceral pathology to the swine with lymphonodal status of benign lymphoreticular hyperplasia

As with the previous status, the aggravated hyperplasia is dominated by the incidence of pulmonary pathology, respectively chronic bronchopneumonia 58.7% (37 cases) - Chart 2.

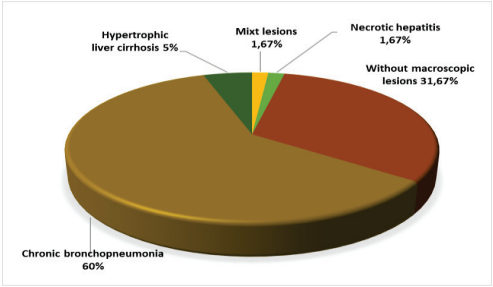

Chart 2. The incidence of visceral pathology to the swine with lymphonodal status of aggravate lymphoreticular hyperplasia

The visceral lesions detected in the case of lymphonodal status of malignant pre-lymphoma are bronchopneumonia 48% (11 cases), splenomegaly 18% (4 cases), hydatidosis - 8% (2 cases: pulmonary - 1 case, hepatic - 1 case), hepatomegaly 4% (1 case) and fibrous pericarditis 4% (1 case). Also there were 4 cases (20%) without macroscopic visceral lesions, but with single or multiple lymphadenopathy, which, from cytomorphological point of view, presented lesions of the malignant pre lymphoma - Chart 3.

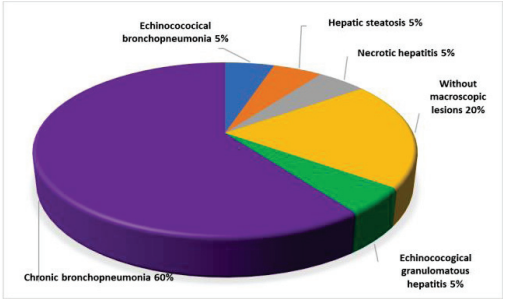

Chart 3. The incidence of visceral pathology to the swine with lymphonodal status of pre-lymphoma

2) The epidemiological study related to the location of the adenopathies

The location of the adenopathies, after the post-mortem examination of the slaughtered swine, both in the visceras and in the carcass, revealed that the trachea-bronchial lymph nodes were the most affected, respectively 120 in cases, as

shown in table no. 1, where it is observed that in all the statuses of lymph node disorders they occupied the first place. The second localization as an incidence was found in the mediastinal lymph nodes, being present in 53 cases. Other locations of adenopathies can be found in the above Table 1.

Table 1. Localization of chronic lymph node lesions according to the cytomorphological statuses

	M	T	La	R	C	S	P	Mz	Pp
Benign lymphoreticular hyperplasia status	19	43	1	3	-	-	5	-	-
Aggravated lymphoreticular hyperplasia status	15	46	1	-	2	2	1	12	-
Malignant pre-lymphoma status	3	14	-	-	1	3	1	1	1
Multiple, mixed lymph node lesions	16	17	2	-	-	2	-	1	-
TOTAL	53	120	4	3	3	7	7	14	1

*M-mediastinal, T- tracheobronchial, La-lomboaortic, R-renal, C-celiac, S-splenic, P-portal, Mz-mezenteric, Pp-popliteu

CONCLUSIONS

The incidence of visceral pathology in slaughtered pigs was dominated by chronic bronchopneumonia, in all 4 lymph node reactive statuses;

The most affected lymph nodes were in the respiratory sphere, respectively the tracheobronchial lymph nodes;

The need to implement a normative act regarding the consumption of carcasses and organs from animals with pre-neoplastic or neoplastic pathology, given that their metabolism is profoundly affected by the presence of this systemic disease.

REFERENCES

Stănescu Vasile (1998). *Hygiene and Food Control*. Publishing House Tomorrow's Romania Foundation.

Rusen Gabriela, Tudor Laurenţiu, Paul Pişcoi (2007). *Slaughtering animals to obtain red meat*. Publishing House Agricola.

Tudor Laurenţiu (2005). *Veterinary health control and technology of animal products*. Publishing House Printech.

Tudor Laurenţiu, Ciocârlie Nicoleta, Ilie Ionel Lucian, Ceauşi Constantin (2009). *General technologies in the food industry*. Publishing House Printech.

Kearney John (2010). *Food consumption trends and drivers*, Philos Trans R Soc Lond B Biol Sci., 365(1554): 2793–2807.

Modlinska Klaudia, Pisula Wojciech (2018). *Selected Psychological Aspects of Meat Consumption. A Short Review*. Nutrients, 10(9): 1301.

Sharma Rasadhika, Nguyen Trung Thanh, Ulrike Grote (2018). Changing Consumption Patterns Drivers and the Environmental Impact, Sustainability, MDPI, Open Access Journal, vol. 10(11), 1-19.

Brian A., Keating Mario Herrero, Peter S. Carberry, John Gardner, Martin B. Cole (2014). *Food wedges: Framing the global food demand and supply challenge towards 2050*. Global food Security, 3(3-4): 125-132.

Regulation (CE) nr. 2073/2005.

Regulation (CE) nr. 178/2002.

Regulation (CE) nr. 853/2004.

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CRITICALLY ILL PATIENTS- INCIDENTS IN THE POST ANESTHESIA PERIOD

Ruxandra COSTEA, Ruxandra TUDOR, Andra DEGAN, Roxana TURCU, Cătălin IVAȘCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: costearux@gmail.com

Abstract

Critically ill and emergency patients often need anesthesia and analgesia for examination, clinical procedures or surgery. Anesthesia must be performed based on advanced planning. Anesthesia for this group of patients is unpredictable and with rapid consequences. Critically ill patients are prone to have a low ability to maintain homeostasis and tissue oxygenation. Good outcomes relay on rapid identification, diagnosis and stabilisation. This study covers a general approach for anesthesia in emergency and critically ill patients and the major complications that can occur in the recovery period. Various aspects will be taken into consideration related with the anesthetized critical ill patient, anesthesia protocols and the most common complications.

Key words*: anesthesia, recovery, emergency, complications.*

INTRODUCTION

Anesthesia influences directly the additional physiological dysfunctions that may worsen patient status. Pre-anesthetic physical evaluation for the emergency or critically ill patients may be difficult and should be undertaken by a trained clinician in order to have a complete image about the patient. Critically ill patients are likely to be unstable and complex, thereby requiring intensive care and monitoring (Costea et al., 2017). Supportive care with individualized plans should be developed for every patient based on recognition of the individual needs and risks in order to make anesthesia a safe and reversible procedure for the critically ill patient. (Carter, 2015).

MATERIALS AND METHODS

This study summarises the most common complications related with the anesthetized critical ill patient, for 320 cases (canine, feline). The period analysed in this study was 2016-2018 and covers the first 72 hours after anesthesia. The data, collected from the Faculty of Veterinary Medicine of Bucharest - Clinical Department, has been statistically processed and interpreted.

Pre-anesthetic evaluation for emergency patients follows a structured **ABCD** algorithm (airway, breathing, circulation, disability-patient's consciousness level and any neurological deficits are established) that allows rapid assessment (Tudor, 2018).

History (including known medical conditions, previous anesthesia, age, breed, behavior), physical examination, type of procedure (risk of pain, hemorrhage, limited monitoring access, risk of hypothermia), type of anesthesia (sedation, general anesthesia, loco-regional anesthesia) should be evaluated (Kirby, 2017).

Time for diagnosis and planning can be very limited, which increases the patient's anesthetic risk.

Based on the preanesthetic physical evaluation patients were classified according to American Society of Anesthesiologists scoring system (ASA physical status scoring system is used to grade patient's anesthesic risk-Table 1).

Common preanesthetic dosages should always be carefully checked and administrated in combination, adjusted to the patient's clinical condition. Induction was made with propofol 2.5-4.5 mg/kg for rapid airway control-endotracheal intubation (Figure 1) and anesthesia maintained with volatile anesthetic agent (isoflurane).

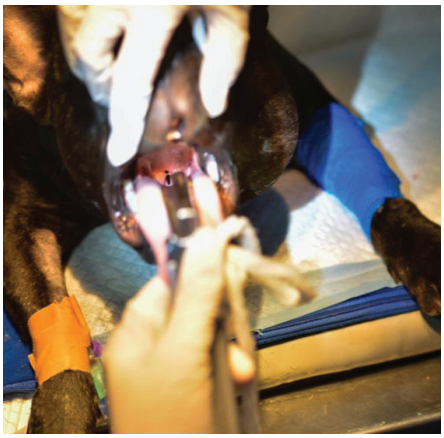

Figure 1. Endo-tracheal intubation for an emergency patient

Table 1. ASA physical status scoring system is used to grade patients their anesthesia risk

	ASA category	Description
ASA 1	Normal healthy patients	- no significant disease; general anesthesia with minimal risk
ASA 2	Patients with mild systemic disease	-localized infection, compensated disease
ASA 3	Patients with severe systemic disease	- severe systemic disease (ex. anemia, moderate hypovolemia, other morbidity influencing anesthesia)
ASA 4	Patients with severe systemic disease that is a threat to life	- ex. uremia, toxemia, severe dehydration, hypovolemia, anemia, cardiac decompensation
ASA 5	Moribund patients (life threatening disease, not expected to survive 24 hours +/- procedure)	- ex. extreme shock, terminal malignancy, infection, severe trauma
E	Emergency	–emergency

In order to decrease the amount of anesthetics required during the procedure, local analgesia protocols were used pre-emptively, before the procedures (blocks with lidocaine 1-2 mg/kg). In a multimodal approach, local anesthesia and analgesia will decrease pain and the need for use of systemic drugs, providing regional anesthesia by reversibly blocking the transmission of nociceptive signals from nerve endings to the central nervous system (CNS). Techniques were choosen according to individual risks, pain intensity and procedure's duration. The duration of the procedures for emergency patients under anesthesia, varied from 20-130 minutes, with a mean of 46 minutes.

RESULTS AND DISCUSSIONS

Recovery from anesthesia is a challenging task for critically ill patients and can be affected by medication over dosage (antagonise if possible), previous neurological injuries, ischemia, embolism, myocardic disturbance or severe hypoxia or hypothermia.

Continued monitoring of the critically ill patient during anesthesia is vital and accurate record keeping is essential.

The use of noninvasive and/or invasive monitoring techniques helped for organs and systems dysfunctions:

- heart rate, pulse (frequency, quality);
- mucous membranes' color;
- capillary refill time;
- pulse (rate & quality);
- rate and pattern of respiration;
- pulse oximetry;
- temperature;
- urinary output;
- continuous ECG;
- indirect blood pressure monitoring (oscillometry, Doppler).

Complications during the recovery time of the critically ill patient's, requires a proper anesthetic management, accurate and a continue monitoring during and after the procedures.

The most common problems encountered were hypoxemia, hypoventilation, hypotension and hypothermia.

Respiratory monitoring during recovery time from anesthesia involved the inability of the patient to maintain correct gas exchanges: transport of O_2 from air to the tissue cells (oxygenation) can be impaired or transport of CO_2 from cells to outside (ventilation) can be also impaired. Complications recorded:

- Hipoxemia -11%;
- Hypoventilation- 13%;
- Hyperventilation- 24%;
- Upper airway obstruction- laryngeal oedema, laryngospasm- 3.75%.

Cardiovascular monitoring after emergency procedures under general anesthesia, required measurements for macrovascular and microvascular parameters (Figure 2).

Macrovascular parameters-measured before the tissue beds

- mucous membrane and capillary refill time
- heart rate/rhythm/contractility
- pulse
- arterial blood pressure, central venous pressure
- urinary output

Microvascular parameters-measured after blood flows trough the tissue beds

- Lactate,Base excess, central venous oxygen saturation

Figure 2. Cardioavascular monitoring

Common complications recorded:

- bradycardia (15%);
- tahycardia (17%);
- hypotension (58%);
- hypertension (12%);
- acidosis (5.5%).

These complications can be connected to profound CNS depression due to high doses or high concentration of anesthetics, hypoxia or hypothermia in the recovery period, acid-base abnormalities, decompensate shock, high levels of K, Ca (Costea, 2017).

For tachycardic patients heart efficiency is decreasing and oxygen needs are increasing releated to pain, fever, SIRS, sepsis, hypovolemia (Blades, 2017).

Hypotension was the most commonly reported complication (58%). Hypertension (SAP>160 mmHg) in the recovery period (12%) was related to fever, pain, patients with renal disorders, endocrine pathology, CNS disorders, arterial thromboembolism.

Cardiac arrest occured in 5.2% of the critical cases (Figure 3) in the recovery period and was recorded in cases involving:

- Head trauma;
- Respiratory distress;
- Cardiac disease;
- Sepsis;
- Severe metabolic disease;
- Prolonged seizures.

Common causes of cardiovascular emergencies were represented by respiratory failure, acid-base and electrolyte imbalance, hypothermia, air embolism and cardiac disease (Kutcher, 2016).

Temperature monitoring in the recovery period of anesthesia for criticially ill patients is extremly important (Brodeur, 2017).

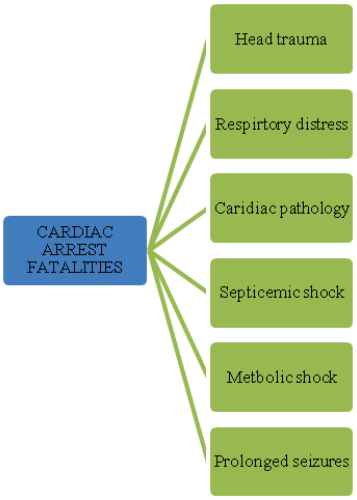

Figure 3. Most common cases of cardiac arrest fatalities in the recovery period of anesthesia

Figure 4. Monitoring for critically ill patients

Most common complication is hypothermia (79% of cases hypothermia versus 5% hyperthermia) and determines:

- peripheral vasoconstriction;
- arrhythmias;
- decreased cerebral flow;
- decreased ventilation;
- lowers the effect of analgesics.

Hyperthermia after anesthesia determines high risk to develop tachycardia, arrythmias, tachypnea or coagulopathy related with:

- ✓ thermoregulations problems;
- ✓ medication (opioids in cats);
- ✓ iatrogenic (extreme warming of the patient);
- ✓ *malignant hyperthermia* (disorder with genetic predisposition)-1 case.

CONCLUSIONS

Preanesthetic examination and an appropriate protocol selection with a multi-modal approach should assure any critically patient's analgesia and a safe recovery from anesthesia.

Regular monitoring is absolutely necessary for any emergency potential critically ill patient. Good outcome are based on knowledge preparation, rapid diagnosis and treatment of any emergency situation.

Causes of cardiovascular emergencies were represented by respiratory failure, acid-base and electrolyte imbalance, hypothermia, air embolism and cardiac disease The most common respiratory complication recorded during the recovery time of the critically ill patient's was hyperventilation.

Any critically ill patient should be considered a potential hypotensive patient.

Cardiac arrest occured in 5.2% of the critical cases in the recovery period.

Regarding temperature monitoring during the recovery time of the critically ill hypothermia was more frequent than hyperthermia and involved many other clinical complications.

REFERENCES

Blades Golubovic, S., & Rossmeisl Jr, J. H. (2017). Status epilepticus in dogs and cats, part 2: treatment, monitoring, and prognosis. *Journal of veterinary emergency and critical care*, *27*(3), 288-300.

Brodeur, A., Wright, A., & Cortes, Y. (2017). Hypothermia and targeted temperature management in cats and dogs. *Journal of veterinary emergency and critical care*, *27*(2), 151-163.

Carter, J. E., & Warne, L. N. (2015). The big question in veterinary anaesthesia. *Australasian Anaesthesia*, (2015), 195.

Costea, R., Degan, A., & Tudor, R. (2017). Crystalloids/colloids ratio for fluid resuscitation during anesthesia. *Scientific Works. Series C. Veterinary Medicine*, *63*(1), 65-66.

Costea, R. (2017). Consideraţii privind anestezia şi terapia intensivă pentru pacienţii cu patologie intracraniană. *Practica Veterinara. ro*.

Kirby, R., & Linklater, A. (2017). *Monitoring and Intervention for the Critically Ill Small Animal*. Iowa USA: john Wiley and Sons, INC.

Kutcher, M. E., Forsythe, R. M., & Tisherman, S. A. (2016). Emergency preservation and resuscitation for cardiac arrest from trauma. *International Journal of Surgery*, *33*, 209-212.

Tudor, R., Degan, A., Costea, R., & Predoi, G. (2018). Postoperative analgesic management of geriatric dogs that underwent soft tissue surgery. *Scientific Works. Series C. Veterinary Medicine*, *64*(2), 82-87

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CONTRIBUTIONS TO THE DIAGNOSIS OF NEOPLASIA IN THE HEAD REGION IN DOGS

Roxana DASCALU, Bogdan SICOE, Larisa SCHÜSZLER, Cristian ZAHA, Daniel BUMB, Cornel IGNA

Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, 119 Calea Aradului, 300645, Timisoara, Romania

Corresponding author email: ignacornel@gmail.com

Abstract

The purpose of this study was to establish some correlations between clinical signs and location of neoplasia - establishing the most commonly encountered clinical signs and the type of tumor most commonly involved in cerebral compression in dogs (primary or secondary neoplasia). All 20 canine patients, with suspicion for neoplasia in different locations in the head region following clinical and neurologic examination, were examined using a CT scanner, as well as cytology/histopathological examination. Meningioma was the most frequent diagnosed intracranial primary central nervous system tumor. Neoplasia in the head region are invasive, most of which have lead to cerebral compression. Carcinoma, originating in the mammary gland, spread by hematogenous path, has lead, in a large percentage, to brain metastases.

Key words*: neoplasia, head, dog.*

INTRODUCTION

Although in several epidemiological studies it has been reported that primary brain tumors do not show an increased frequency in dogs and cats, with the use of advanced imaging techniques (CT and MRI), they appear to be an important part of the pathology of the two species (Henry & Higginbotham, 2010; Morris & Dobson, 2001).

In veterinary medicine, in most cases, neoplasms are detected at an advanced stage, which is why cancer is one of the most common causes of exitus (Cooley et al., 2003; Lord et al., 2007; North & Banks, 2009).

MATERIALS AND METHODS

The research was carried out in the Imaging Diagnostics Laboratory and the Surgery Clinic of the Faculty of Veterinary Medicine in Timişoara, investigating 20 clinical canine patients, who, following clinical and neurological examination, were suspected of neoplastic disease in different segments of the head region.

Patients underwent CT scaning (Siemens Somatom Definition AS), under general anesthesia with 2% xylazine (Narcoxyl - MSD Animal Health)/acepromazine 2% (Aceprom - KEPRO B.V. NETHERLANDS) - Propofol 1% (Fresenius Kabi).

The obtained data were evaluated using multiplanar reconstruction technique (MPR) using 1 mm sections, and for an easier visualization of the lesions 3D reconstruction of the evaluated region using the volumetric rendering technique (VRT) was used. In patients with suspected neoplastic disease of the nasal cavities based on conventional CT imaging, we intended to identify the changes that were reported to be pathognomonic (Forrest, 2018; Schwarz & Saunders, 2011; Wisner & Zwingenberger, 2015): osteolysis of the turbinates and the bones forming the nasal cavities cavities, extension into the frontal sinuses, presence of soft-tissue mass in the nasal cavity, and lysis of the cribriform plate with extension into the cranial vault.

Regarding the patients in which brain involvement was noted on conventional CT, presence of the following aspects was investigated: mass component, lesion distribution (single / multiple), lysis of the cranial vault, deviation of falx cerebri and compression/deformation of the cerebral

ventricles, hydrocephalus. Postcontrast CT was performed at 1-3 minutes after intravenous administration of iodine contrast medium (Iopamiro 370-Bracco) at a dose of 2 ml/kg.
Contrast/density enhancement (uniform, non-uniform, in the center/periphery of the lesion) was then described.

RESULTS AND DISCUSSIONS

Out of the total of 20 cases with neoplasms, 12 had cerebral compression. Cerebral compression was due to an intracranial primary central nervous system tumor (Figure 1) in 16.66% of the cases, with the remaining 83.34% being the result of metastatic disease by various mechanisms. These secondary lesions were mostly the result of direct expansion of neoplasms in the nasal cavity (33.33%) and disemination of primary mammary gland neoplasms (25%).

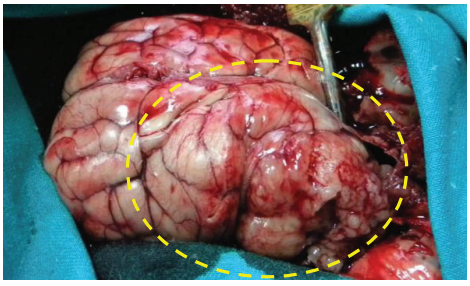

Figure 1. Meningioma and endotheliomatous fibromatosis localized in the frontal lobe of the brain - macroscopic aspects - extra axial mass with right frontal lobe compression

Cerebral compression occured in a small proportion by extension from the pituitary gland, the skull (Figure 11) and the external ear (in similar proportions of 8.33%). Other authors have also reported that the majority of secondary brain tumors in dogs are the result of local extension of nasal tumors, which represent 30% of CNS metastases (Henry & Higginbotham 2010).

Tumors localized in the nasal cavities

The most common symptoms in these cases were dispneea (62.5%), epistaxis (50%) and exophthalmos (50%). Swelling in the naso-frontal region and chronic serous-muco-purulent discharge were observed in similar proportions of 37.5%.
Examination of the nasal cavities revealed lysis of the turbinates and the nasal septum, as well as replacement of these structures with fatty-like tissue mass, which in some cases resulted in lysis of the cribriform plate and infiltration / compression of the cerebral parenchyma (Figure 2).

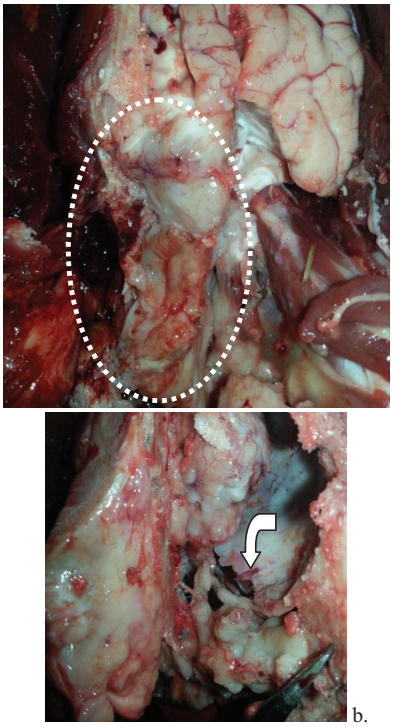

Figure 2. Bilateral nasal tumor with frontal sinus invasion, osteolysis of the cribriform plate (b) and infiltration of the frontal lobes of the cerebral hemispheres (a)

The most commonly diagnosed tumors originating in this site were adenocarcinoma (40% - two cases) and squamous cell carcinoma (40% - two cases). In a smaller proportion, undifferentiated carcinoma was identified (Figure 3) (20% - one case).
The results are similar to the data in the literature, according to which adenocarcinoma is the most common intranasal tumor in dogs (Henry & Higginbotham, 2010), and squamous cell carcinoma is a frequently diagnosed tumor (Morris & Dobson, 2001).

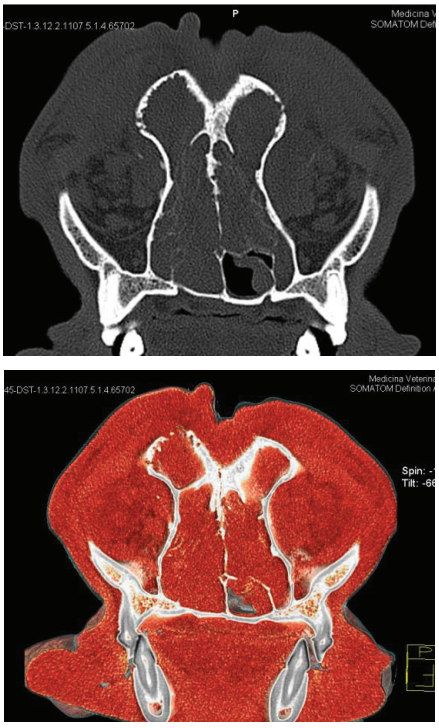

Figure 3. Undifferentiated carcinoma with bilateral localition in the nasal cavities

Tumors localized in the oral cavity (5 cases)
The most commonly associated clinical signs were the presence of an ulcerative mass (Figure 4), located in the caudal portion of the hard palate mucosa, anorexia and halitosis (60%).

Figure 4. Oral tumor: lesion with ulcerated surface

Out of the total five cases studied, in only four was a histopathological diagnosis of certainty established. Tumor types diagnosed in equal proportions (25% - one case) were malignant melanoma, squamous cell carcinoma (Figure 5), plasmocytoma, and a metastasis secondary to a vaginal sarcoma.

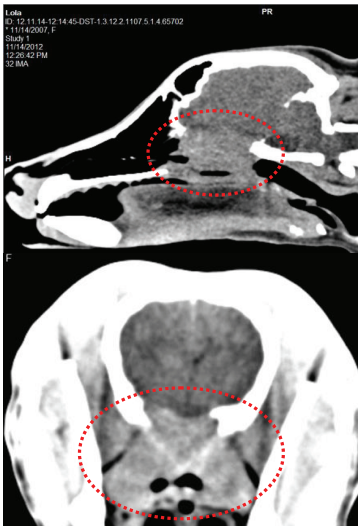

Figure 5. SCC (squamous-cell carcinoma) originating in the nasal cavity which resulted in extension in the orbit and cranial vault: cerebral compression through direct extension

The most common malignant tumors of the mandible and maxilla in dogs are, in decreasing order, malignant melanoma, squamous cell carcinoma (SCC) and fibrosarcoma (FSA) (Henry & Higginbotham, 2010; North & Banks, 2009). Squamous cell carcinoma (SCC) is the most common malignant tumor of the oral cavity in dogs, accounting for approximately 20-30% of all oral malignant tumors according to some authors (Morris & Dobson, 2001; North & Banks, 2009).
Malignant melanoma accounts for approximately 30-40% of all malignant tumors of the oral cavity in dogs (Morris & Dobson, 2001).
Extramedullary Plasmacytoma (EMP) has been reported in association with multiple myeloma. EMP is characterized by neoplastic cell infiltration into soft tissues without bone marrow involvement (Henry & Higginbotham, 2010).
In dogs, this condition appears to originate from the cutaneous or mucosal tissues, with more frequent localization in the oral cavity and the skin of the head and digits (Henry & Higginbotham, 2010). Oral form of plasmocytoma may be associated with periodontal disease (Henry & Higginbotham, 2010).

Tumors located in the orbit/zygomatic region (1 case)

The only case studied with a mass originating in the orbit was diagnosed as being a malignant giant celltumor/malignant fibrous histiocytoma (histopathological examination) (Figure 6).

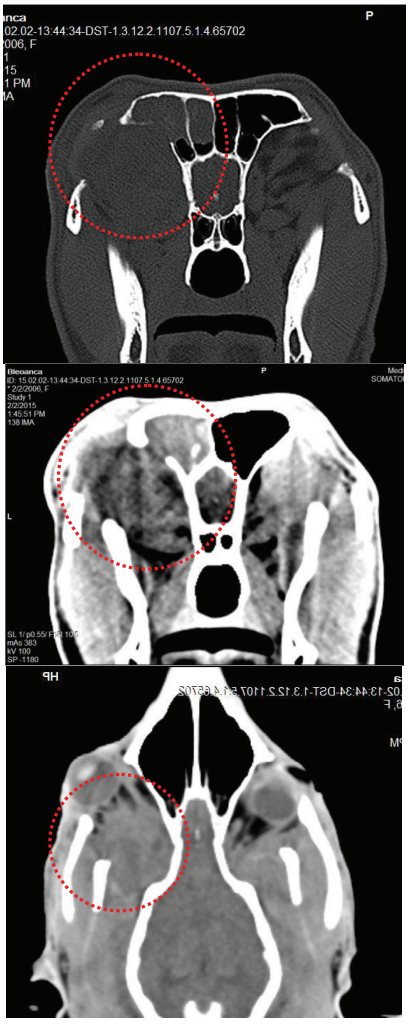

Figure 6. Tumor with orbital localization with extension into the frontal sinus and nasal cavity

Tumors located in the ear (2 cases)

The identified types of tumors were adenocarcinoma (1 case - 50%) (Figure 7) and high-grade malignant sarcoma (1 case - 50%).

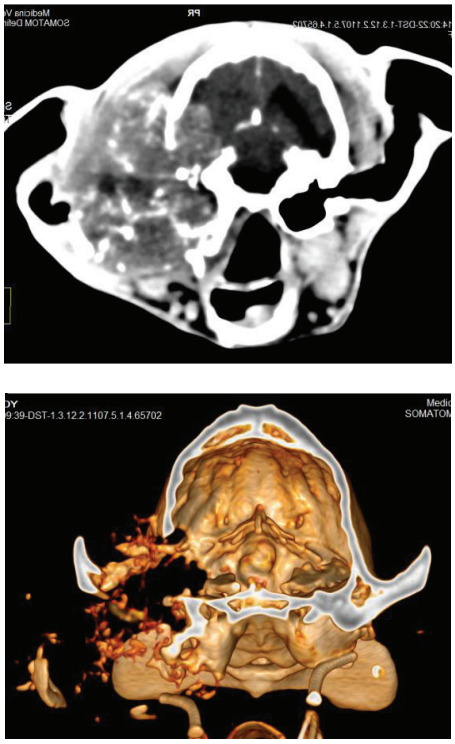

Figure 7. Adenocarcinoma originating in the external ear: osteolysis of the cranial vault - the temporal bone especially (squamous part, petrous portion, tympanic bulla); soft tissue mass showing scattered bone remnants; cerebral compression through direct extension

In the literature (Ayres & Liptak, 2012; Morris & Dobson, 2001), it has been reported that adenocarcinoma of the ceruminous glands, alongside with squamous cell carcinoma and carcinoma of unknown origin, are the most common malignant tumors encountered at this level. Carcinoma of the ceruminous glands and other carcinomas from the ear canal are locally invasive tumors, resulting in destruction of the cartilage and deep bone that are adjacent to the ear (Morris & Dobson, 2001).

Adenocarcinomas originating in the ear are also extremely destructive, leading to tympanic bulla osteolysis and erosion/lysis of the petrous and squamous parts of the temporal bone (Wisner & Zwingenberger, 2015), severe changes seen also in the dog with adenocarcinoma included in our study. The proportion in which squamous cell carcinoma (SCC) produces invasion and lysis of adjacent bone tissue reported in the literature varies between

70% (Morris & Dobson, 2001) and 77% of cases (Evans & Shofer, 1988; North & Banks, 2009).

Intracranial central nervous system tumor

The observed macroscopic aspects varied from the presence of extraaxial lesions with brain compression (Figure 1) or intraaxial infiltration.

The lesions inside the cranial vault were located either in meninges (Figure 1) or in the parenchyma (Figure 8), or were the result of extension from other regions (the cranial vault) (Figure 11). These lesions were either tissue masses that contained necrotic portions or well-defined nodules highly vascularized.

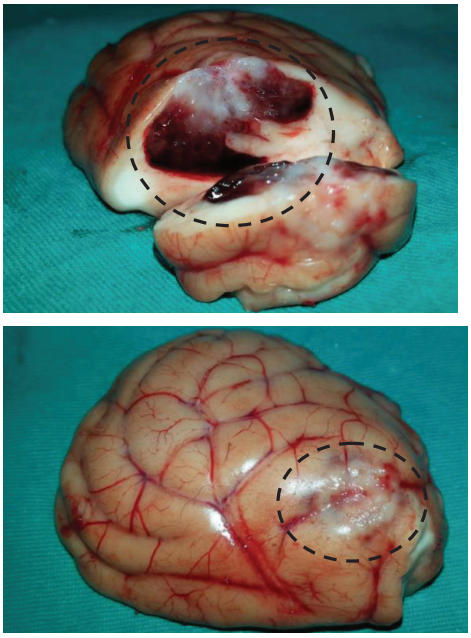

Figure 8. Metastasis in the left frontal lobe secondary to a mesenchymal malignant sarcoma originating in the mammary gland

Both intracranial primary tumors were meningiomas (Figure 9) (2 cases - 100%).

The results are in accordance with data in the literature that state that meningioma is the most common cerebral tumor in dogs, accounting for approximately 40% (Vandevelde et al., 2012) - 45% of primary intracranial neoplasms (Henry & Higginbotham, 2010).

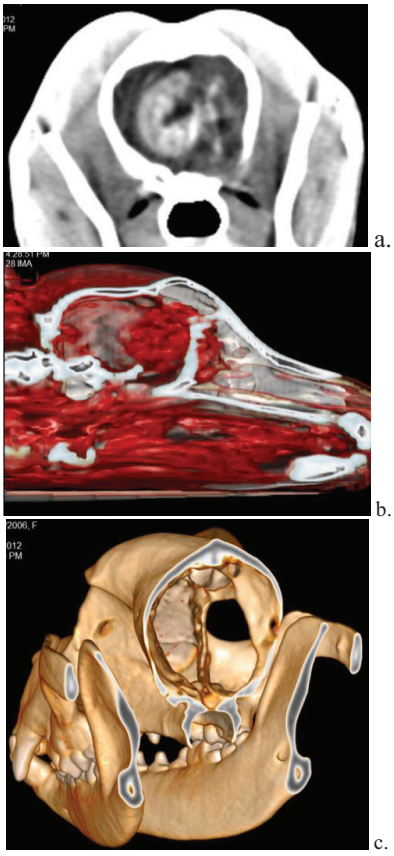

Figure 9. Fibromatous and endotheliomatous meningioma located in the right frontal lobe of the cerebral hemisphere; a. MPR postcontrast: Significant contrast enhancement, but uneven, the mass presents hypodense areas; b. and c. VRT: osteolysis of the cranial vault

Metastases resulting from haematogenic dissemination (3 cases) were secondary to mammary carcinoma (66.67%) (Figure 10) and in one case to mammary sarcoma (33.33%). In the three cases with secondary brain tumors that were studied, where the spread to the central nervous system (CNS) was done by haematogenic dissemination (originated in the mammary gland), pulmonary metastases were identified in two situations (66.66% - 2 cases).

The most common metastatic sites for mammary carcinomas are the lymph nodes and lungs. Metastasis in the bone, liver or brain is rare in animals compared to humans (Lana et al., 2007). According to other authors (Henry & Higginbotham, 2010) the presence of pulmonary metastases associated with those in the brain occurs in 80% of the cases.

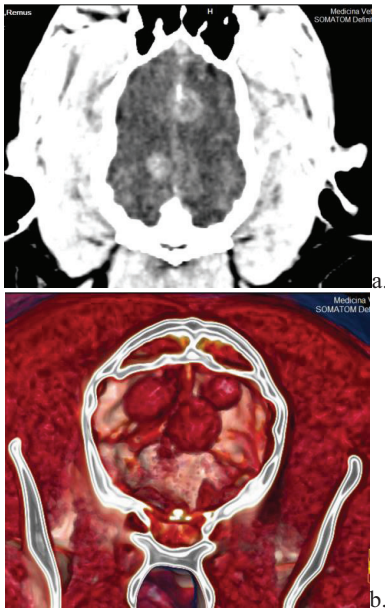

Figure 10. Cerebral metastases secondary to a mammary carcinoma: a. MPR; b VRT

Metastatic tumor types that have been reported in dogs as having a haematogenous CNS dissemination include transitional cell carcinoma, hemangiosarcoma (HSA), mammary carcinoma (Lecouteur & Withrow, 2007; North & Banks, 2009), adenocarcinoma, melanoma, lymphoma, undifferentiated sarcoma (Henry & Higginbotham, 2010) and prostatic and lung carcinomas (Lecouteur & Withrow, 2007; North & Banks 2009). Hemangiosarcomas (HSA) are the most common secondary cerebral tumors in dogs (29%) (Vandevelde et al., 2012) - (51%) (Henry & Higginbotham, 2010), followed by pituitary gland tumors (25%), lymphosarcoma (12%) and carcinomas (12%) (Henry & Higginbotham, 2010; Vandevelde et al., 2012).
Also in human medicine, brain metastases occupy the first position as frequency (approximately 50%) of intracranial tumors (Gorgan, 2012).
Tumor types that are reported to be responsible for occurrence of metastatic lesions are bronchial-lung cancer in men and breast cancer in women (Gorgan, 2012).
Tumors localized in the cranial vault (1 case)
The only case in the study originating in the bones of the cranial vault was diagnosed with chondrosarcoma (Figure 11).

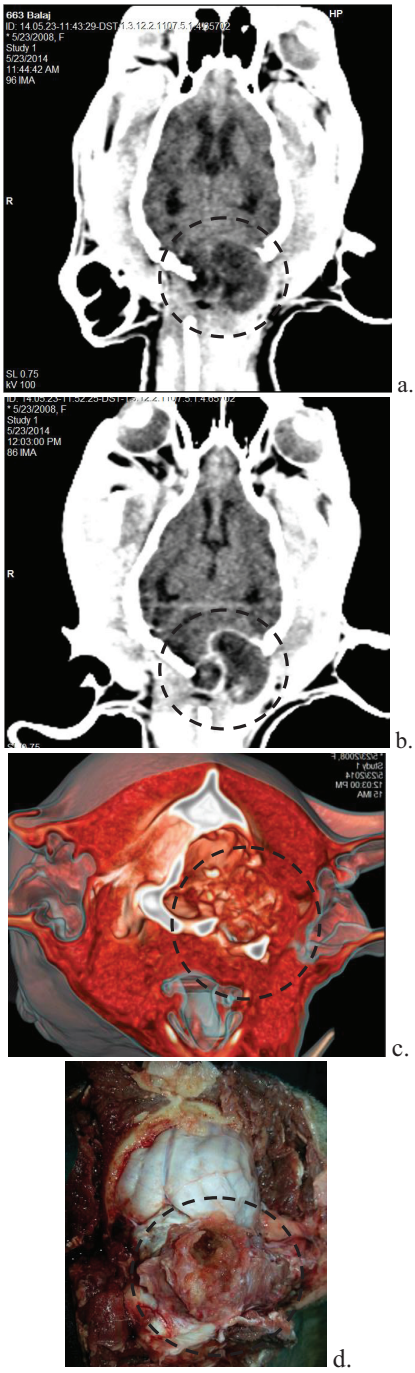

Figure 11. Tumor of the cranial vault extending within the cranial vault and direct metastasis in the brain (chondrosarcoma) (663/23.05.2014): a. MPR precontrast; b. MPR postcontrast; c. 3-D VRT reconstruction (occipital, atlas and temporal bone osteolysis); d. macroscopic appearance

Chondrosarcoma represents the second primary bone tumor as frequency encountered in dogs, representing approximately 5-10% of the total primary bone tumors (Morris & Dobson, 2001). Compared to OSA, chondrosarcoma (CSA) occurs in younger patients (average age of 6-7 years) and usually affects medium to large breeds (Henry & Higginbotham, 2010). The bone tissue of the skull, and the temporal bone may be affected by primary bone tumors such as osteosarcomas and chondrosarcomas, and less frequently by benign tumors such as osteomas (Schwarz & Saunders, 2011; Withrow et al., 2013).

This information corresponds to the changes presented by the animal studied, which was a 6 year old medium-sized mix-breed dog.

In the cases which presented primary and secondary tumors with cerebral compression (direct or indirect), convulsions were the most common clinical sign (12 cases), representing 50% of the cases (6 cases). It has been associated with cerebral cortex compression - frontal lobes. Ataxia was present in 25% of the cases, and circling, tetraplegia and abnormal head positions (tilt) were detected in similar proportions of 16.66%. The results obtained in this study are consistent with data reported in the literature, according to which the most common clinical signs associated with brain tumors are: convulsions (45%), circling (23%), ataxia (21%) and head tilt (13%) (Henry & Higginbotham, 2010). Convulsions are the most common clinical signs associated with these tumors, and are often one of the first signs of a tumor in the cerebral cortex (Morris & Dobson, 2001; Bagley et al., 1999; Snyder et al., 2006; North & Banks, 2009). Brain neoplasms should be considered as a differential diagnosis in cases where the first episode of seizures occurred after the age of 4 years (Henry & Higginbotham, 2010). Convulsions are one of the most common clinical signs associated with these tumors, and are often one of the first signs of a tumor in the cerebral cortex (Morris & Dobson, 2001; Bagley et al., 1999; Snyder et al., 2006; North & Banks, 2009).

This was also noticed in the present study except for one case (24/10.01.2014), in which the first episode of seizures occurred at the age of 2.5 years and was the result of a frontal lobe compression secondary to enlargement of an undifferentiated carcinoma in the nasal cavity.

Performing CT or MRI investigations is of primary importance in patients with neurological deficit (Horner's syndrome or peripheral vestibular syndrome) in order to assess the degree of invasion in adjacent structures (Ayres & Liptak, 2012).

CONCLUSIONS

Meningioma was the most frequently diagnosed intracranial primary central nervous system tumor. Neoplasia in the head region are invasive, most of which have lead to cerebral compression (60%). Carcinoma, originating in the mammary gland, spread by hematogenous path, has lead, in a large percentage (66,67%), to brain metastases.

Convulsions (50%) and ataxia (25%) were clinical signs most commonly associated with primary or secondary tumors with cerebral compression.

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REFERENCES

Ayres, S. A., Liptak, J. M. (2012). Head and neck tumors. In: Kudnig, S.T., Séguin, B. (Ed.) *Veterinary Surgical Oncology,* (pp. 81-118), Oxford (United Kingdom): Wiley-Blackwell.

Bagley, R. S., Gavin, P. R., Moore, M. P. (1999). Clinical signs associated with brain tumors in dogs: 97 cases (1992– 1997). Journal of the American Veterinary Medical Association, 215:818–819.

Cooley, D. M., Schlittier, D. L., Glickman, L. T. (2003). Exceptional longevity in pet dogs is accompanied by cancer resistance and delayed onset of major diseases. Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 58:1078–1084.

Evans, S. M., Shofer, F. (1988). Canine oral nontonsillar squamous cell carcinoma. Prognostic factors for recurrence and survival following orthovoltage radiation therapy. Veterinary Radiology and Ultrasound, 29:133–137.

Forrest, L. (2018). The Cranial Nasal Cavities: Canine and Feline In: Thrall, D.E. (Ed.) *Textbook of*

veterinary diagnostic radiology, 7rd ed. (pp. 183-203) St. Louis, Missouri: Saunders Elsevier.

Gorgan, M. R. (2012). Neurochirurgie, notiţe de curs. Bucureşti.

Henry, C., Higginbotham, M. L. (2010). *Cancer management in small animal practice*. St. Louis. Missouri: Saunders Elsevier.

Lana, S. E., Rutteman, G. R., Withrow, S. J. (2007).Tumours of the mammary gland. In: Withrow S.J., Vail D. M. (Ed.) *Small Animal Clinical Oncology*. 4th ed. (pp. 619–636). St. Louis. Missouri: Saunders Elsevier.

Lecouteur, R. A., Withrow, S. J. (2007). Tumours of the nervous system. In: Withrow S.J., Vail D.M. (Ed.) *Small Animal Clinical Oncology*, 4th ed. (pp. 659–685). St. Louis. Missouri: Saunders Elsevier.

Lord, L. K., Yaissie, J. E., Marin, L. (2007). Results of a web-based health survey of retired racing greyhounds. Journal of Veterinary Internal Medicine, 21:1243–125.

Morris, J., Dobson, J. (2001). *Small Animal Oncology* (pp. 90, 94–121, 192–193). Oxford: Blackwell Science Ltd.

North, S., Banks, T. (2009). *Small Animal Oncology*. Edinburgh: Saunders Elsevier.

Schwarz, T., Saunders, J. (2011). Veterinary Computed Tomography, (pp. 93–170, 185–197). West Sussex, UK: Wiley-Blackwell.

Snyder, J. M., Shofer, F. S., Van Winkle, T. J. (2006). Canine intracranial primary neoplasia: 173 cases (1986–2003). Journal of Veterinary Internal Medicine, 20:669–675.

Vandevelde, M., Higgins, R. J., Oevermann, A. (2012). *Veterinary neuropathology: essentials of theory and practice* (pp. 129-156). Oxford (United Kingdom): John Wiley & Sons, Ltd

Withrow, S. J., Vail, D. M., Page, R. L. (2013). *Small Animal Clinical Oncology,* 5th ed. (pp. 54, 98, 111–130, 310–311, 386, 392–394, 466, 583). St. Louis, Missouri, USA: Elsevier Saunders.

Wisner, E., Zwingenberger, A. (2015). *Atlas of Small Animal CT and MRI* (pp. 155-277). Iowa, USA: Wiley-Blackwell.

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COMPARATIVE ASPECTS REGARDING THE PRESENCE OF BEHAVIORAL CHANGES OF AGGRESSIVE TYPE IN SEROPOSITIVE *TOXOPLASMA GONDII* INDIVIDUALS - HUMANS AND CATS

Valentina SIMION, Cristina FERNOAGA, Mario CODREANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67

Correspondent author: valusha_2006@yahoo.com

Abstract

The aggressive behavior encountered in the feline patient, represents a major problem in the approach and care handling for this patient, hard quest for a complete clinical and neurological evaluation, also in applying some investigation methods and running a full internal and external set of analysis: blood workouts, ultrasounds, X-rays, MRIs. In this study, we searched for triggers, causes and effects that give the aggressive state of the feline patient and we found that toxoplasmosis, hyperthyroidism, epilepsy, dental pain, arthritis, discomfort from coinfections with other bacteria, viruses, parasites, trauma, lack of sensitive reactions and cognitive response malfunctioning in geriatrics, could all contribute for this behavior. In this comparative study of human, feline and other mammals with latent infection with T. gondii, we found common aggression traits presented in some psychiatry studies linked to the human latent infection, as well as in feline and other mammals brain studies with toxoplasmosis confirmed with different laboratory technics.

Key words*: aggressivity, serology, test panel, coinfections.*

INTRODUCTION

There are different types of feline aggressivity: Among cats (eg 2 males, mating of fighting for territory); in fear or defensive (danger: a person, animal, object or sound based); for territory (other cats passing by, or even dogs or humans by selection, new cat, change of territory, new human in the family); playful type (could easily turn into a fight with deep marks and broken house objects); redirected type (most dangerous, has one target - animal or human- but if the cat cannot reach it, it will attack the next thing coming, other cat or human); by over-petting (when it is too much, one should better stop the petting- the cat will let you know); induced by pain or other stimuli (inflammatory muscles, osteoarthritis, frustration, closed environment for too long, otitis, dental or fight injuries); The maternal type (protective mothers will attack- contact in the first days of litter should be avoided); Idiopathic type (explained after thorough medical exams, clinical history of the pet – they easily attack, bite the owner, the hyperexcitability status is high for long periods of time – very important to establish the difference between redirected and idiopathic aggression features, for it is decreases the comfort of both cat and owner for sharing the same environment – many of them end up being euthanized by request; The hunting type is easily understandable, even between the kittens in their play time.

Common features of aggression found in humans and cats

Based on studies performed on humans and cats, it is a proven fact that there are common traits of expressed aggressive behavior, witch in time these types of aggression got classified more extensively, but we can speak about psychiatric disorders like: impulsivity, violence, chronic depression, self-inflicted or suicidal tendencies. Gregg & Siegel (2001) studied aggressive behavior on criminals and thieves, first class with a predator aggressive features, second with defensive reactions. They studied the neurological pathways of aggression and found that medial and lateral hypothalamus, the amygdala and the central grey periapeduct activity are responsible for this behavior. Amygdala is known to be the center for processing information regarding sensitive, visceral, interceptive, cognitive and

emotional stimuli, and the role of being able to change the animal's behavior via hypothalamus and PAG accordingly. Berenreiterova et al. (2011) has a study on rats infected with *T. gondii*, that suggests that the tropism of the parasite's cysts is distributed mainly in amygdala, hypothalamus, olfactive area, sensitive and motor areas, but the localization was not restricted just for these areas, so it is not preferential.

***Toxoplasma gondii*. Short general characterization**

Antropozoonotic parasitic agent, a unique protozoa member of *Toxoplasmatinae* Subfam., *Sarcocystidae* Fam., *Eimeria* Subord., *Eucocidia* Order, *Coccidia* subclass, *Sporozoa class*, *Apicomplexa* Phillum. *T. gondii* is a 5 µm parasite, it infects nearly one third of the global population of individuals at this moment, 80% of the French population, 60% of American population as studies suggest. In our country there are no actual per country screening or other registered information about the prevalence of *T. gondii,* except some local and the ones found among pregnant women region surveys (44%) (Jaroslav et al., 2014). And for the cats we are trying to have a more elaborate picture of this influential parasite.

Infection pathway of *T. gondii* in the intestinal wall and the immune response of the host

When parasites take over the intestinal wall. they break the tight bound between the enterocytes. This parasite uses different strategies to infest the enterocytes, followed by strong reactions like the release of nitric oxide, some cytotoxic molecules causing neuropathic damage. Enterocytes then release chemokines and cytokines attracting leukocytes, macrophages and dendritic cells (activating self-immunity and their microbiocidal direct function). Interleukins like IL-12 activate CD4 immune adaptative response.

T lymphocytes with NK cells and NKT, in the presence of IL-15, release IFN γ activating the defense mechanism for expelling the parasite out of the enterocytes. B cells are activated also for releasing antibodies that could cross the epithelial barrier by transcytosis and reach the lumen. Excepting the bactericidal factor, if IFNγ is uncontrollable in its release, it could damage the integrity of intestinal epithelium. The intraepithelial lymphoid cells are cytotoxic for the infected enterocytes and may produce TGF - β limiting the production of IFN-γ (Schulthess, J. et al., 2008).

Immune response in neurotransmitted *T. gondii*

T. gondii acute phase activates a powerful response with the release of IFN γ, IL-12 and CD8+ T lymphcells. The production of IFNγ is most essential for stopping *T. gondii* tachizoytes replication, it controls the infection in CNS and prevents toxoplasmic encephalitis, easily transforming them in cysts without letting them spread. For triggering this pathway, *T. gondii* has a gene called profiline, inducing the expression of IL-12 (Plattner et al., 2008). Same IFNγ maintains the low number of tachizoytes and prevents the reactivation of the cysts in the tissues at the time of infestation. IFNγ has its own secondary clinical depression side effect when used as treatment for antitumor purpose, it degrades the tryptophan, the main precursor for serotonin, the one responsible for the depressive state. *T. gondii* infestation upon IFN's secretion could affect indirectly some cognitive functions. Graeff's work says that the serotonin level could be having a role in the initiation of the fear behavior, but this is not always proven fact, for the animals or humans. Dynamic of antibodies response. The medium dynamics of different isotypes, but their level can differ from patient to patient and used serological technique. IgM can be detected even years after initial detection (Figure 1) (Robert-Gangneux, Florence, and Marie-Laure Dardé, 2012).

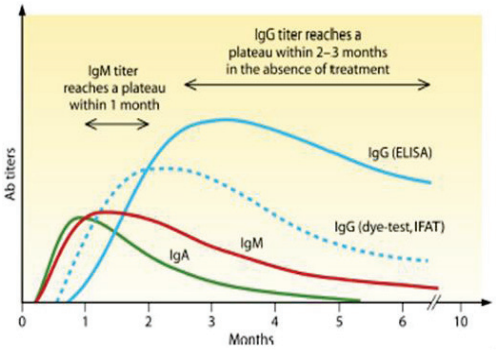

Figure 1. *T. gondii* antibody response by Robert-Gangneux, Florence, and Marie-Laure Dardé, 2012

MATERIALS AND METHODS

For the in-house analysis in the Veterinary medical laboratory of Faculty of Veterinary Medicine, sampling and processing of feline samples, a fresh sample was worked on, without refrigeration, almost instantly.

For testing and detection of IgG antibodies of *T. gondii* in domestic cats either serum, plasma or blood was used, samples being processed by specialized personnel, schooled in the authorized laboratory and according to protocols and operational procedures.

The period taken into account for testing IgG antibodies for *Toxoplasma gondii* was between 01.01.2015 - 13.05.2018.

The total number of processed samples for qualitative detection of IgG antibodies against *T. gondii* was of 544 felines, presented in the clinic, for different affections: neurology and coordination with paralysis or limping, cardiology, ophthalmology, stomatology, or kept under surveillance and treatment for general, internal or metabolically diseases.

Toxoplasma/Chlamidophyla determinations are based on antigen-antibodies reactions.

Having as principle an immunoenzymatically method, **IMMUNOCOMB** is a plastic device, resembling a comb, on which **Toxo/Chlamidophyla** is fixed.

The serum, plasma or blood samples are place in the wells of the multicompartment developing plaque.

RESULTS AND DISCUSSIONS

For the purpose of determining the IgG Toxoplasma or Chlamydia titer, sample color intensity is compared on the corresponding dents of ImunoComb with the series of spots on its scale (included in the kit). The inferior spot represents *Toxoplasma* test. The middle spot represents *Chlamydia* test. Results for each disease is evaluated separately. Sample color intensity is compared with that of Positive Control (C+) included in the kit, for determining the titer. Positive Control for *Chlamydia* and *Toxoplasma* is calibrated at a 1:32 titer. The samples with a higher or equal color intensity with that of the positive control are considered positive.

RESULTS AND DISCUSSIONS

From the total of 544 domestic feline tested for IgG *Toxoplasma* antibodies detection, 258 were positive and 286 negative, test made during 01.01.2015-13.05.2018 in the Faculty of Veterinary Medicine of Bucharest Laboratory.

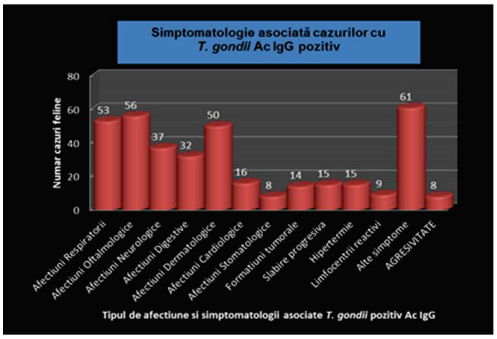

Figure 2. Main clinical signs in the presence of *T. gondii* IgG positive

In describing the symptomatology, for each category, we have extracted significant data in the moment of patient presentation, by clinicians, as follows (Figure 2):

Respiratory affections: cough, dyspnea, bronchitis, infections of the superior respiratory tract (nasal secretions, sneeze, blockage with ocular connections, often with feline infectious rhinotracheitis caused by type I Herpesvirus), broncho-pneumonia, interstitial pneumonia, infiltrations at broncho-alveolar level, pharyngitis, laryngitis, often associated with *Streptococcus, Pasteurella or Chlamydophila*.

Ophthalmological affections: *T.gondii* specific uveitis, severe secretions, anisocoria, iritis, corneal ulcerations, cataract, kerato-conjunctivitis, glaucoma, sinechia, iris vaso-dilatations, tapetum negrum hyperpigmentation, crystalin anterior chamber deposits, corioretinits, blepharitis.

Neurological affections: often have continued the ophthalmological tableau with mydriasis, photophobia, delayed attention, menace, other neuropathies were described as facial paralysis, balance loss, delayed proprioception on some limbs, grand mall type seizures, muscular tremor, exaggerated aggressivity or affection, depreciation alternated with excitability, affection of some cranial nerves, neuronal

parameters modifications, vestibular syndrome, spasms, dromomania, encephalitis.

Digestive affections: can be caused by the reaction of the digestive mucosa at the moment of parasite entry, or by association with Coronavirus or any other opportunist virus, *Helicobacter* dormand or ascarids, hematophague by egg ingestion, excessive licking.

Dermatological affection: pruriginous dermatitis, especially in areas where licking is impossible: itching, otitis, hypertrichosis (lateral, abdominal or on limbs), ulcerations, wounds, grating, eosinophilic complex, all being related to the activation phase of IgE given by the presence of *T. gondii*.

Cardiological affections: encountered in positive IgG antibodies for *T gondii* - arterial hypertensions, aortic, septum and left ventricle hypertrophia, congenital, cardiomyopathy - testing through SNAP pro-BNP is recommended.

Stomatological affections were gingivitis, some proliferative, stomatitis, plaque and halitosis, which appeared in associations with a *Calicivirus*, retrovirus FIV or FeLV.

Tumors: frequent in association with seropositivity were mammary tumors, abdominal formations, fribrosarcoma, squamocellular carcinoma, hepatic.

Progressive weight loss: manifested due to depreciated state by the parasitic infection, intestinal discomfort, pancreatitis, associated gastritis, biliary sludge, cholestasis, steatosis, angiocolitis, neurological or thyroid disfunctions.

Not rarely, there are mentioned falling from the balconies of the houses, blaming sexual instincts, birds chasing, exploring the surroundings, but what if the parasite impairs the equilibrium ways, the sight with its **ophthalmic deposits**, what if the topography of the cysts are affecting the decision making side of cognitive functions, amygdala, hypothalamic area could be affected by the cysts insertion, the inflammatory responses and lesions of the chemical imbalance they create.

Also, trauma and hormonal changes, such in **GnRH cells**, they might lose their original place once the **cysts** could trigger a mixt signal for secretion for **more androgenic** hormones and **less estrogens** and **progesterone**, the result might turn into an **aggressive behavior** (Figure 3).

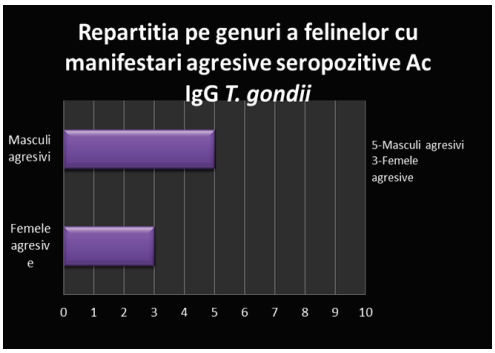

Figure 3. Gender distribution for positive IgG *T. gondii* with aggressive behavior

Because of such hormonal changes, the imbalance could cause **infertility**, **abortion** can be present in some cases, kittens can be severily immunodepressed at birth, therefore any viral agent could be fatal. *Herpesvirus* is active, they could go blind, especially if they are sold very early from their mother and the maternal immunity fails to be fully installed. From our analysis after serology testing of latent *T. gondii*, we conclude that from the point of age, cats were mostly manifesting symptoms between **11 months** and **2 years** of age. For the old ones, the number of testing itself drops, because they usually come for checking bigger issues like **tumors**, **degenerative processes**, or the situation where they stop coming. The youngest we've tested, and got a positive, at 2 months of age, also **positive** on *Coronavirus*, and 2-year-old ones, we can blame all this on a poor immunity settled. If the IgG antibodies are mainly transmitted from the mother, then the antigens for *Coronavirus* come from external grounds, an opportunistic virus that could be fatal.

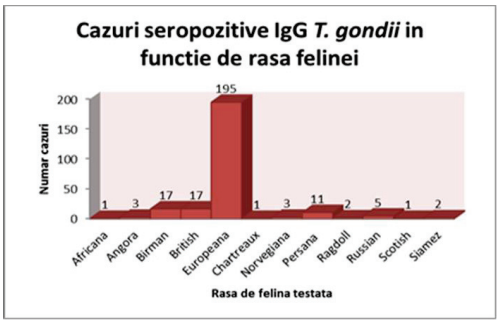

Figure 4. Breed distribution for *T. gondii* IgG

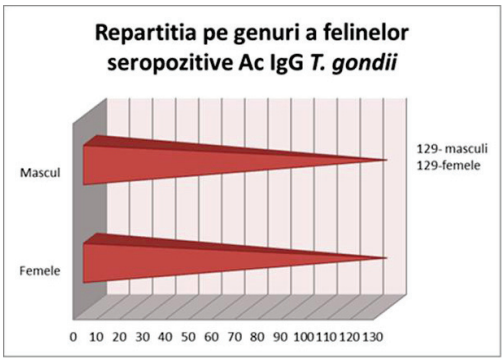

Figure 5. Gender distribution for *T. gondii* IgG

The 8 positive felines for latent *T. gondii* that manifested an aggressive behavior are the ones that the blood collection could be performed. Most definitely, the percentage is bigger if at least once in a cat's lifetime we test for these *T. gondii* antibodies, we recommend testing as soon as the cat gets into a household, for *T. gondii* is a zoonosis. These positive cats were very aggressive, some with epileptic crisis, anisocoria and ocular discharge, otitis, one was positive on latent *Coronavirus*, and some were said to be consuming fresh, uncooked cattle meat. On the biochemistry panel, a high Glucose was registered as a pattern of distress and may also from inflammation, also from a high level of glutamate changing the biochemistry of the nervous central system infested with the parasites.

For the serology testing we used immunochromatography tools same as mentioned for latent *T. gondii*, like **SNAP Combo FelV/FIV™** and also latent **IgG *Coronavirus*** fast test, with one possitive test of latent ***Calicivirus*** confirmed by the owner of one of the cats, we mentioned it for it could also decrease the immunity barrier of that cat, making room for other coinfections to manifest. For **FCoV, FPV** and **Giardia** and for **SNAP Giardia™ antigens** we worked with fresh **feces samples**, with same testing protocol described for any other **qualitative test**. For any enteropathy disorder we recommended this testing and we had a great prevalence of viral infections, the only problem was that we could **not test for *T. gondii* antigens** and neither the microscopical exams for feces, but this is part of a futured protocol of catching this parasite in action at the site, where it riches the enterocytes and then passing through all the barriers, masking itself in immunity cells. As it is mentioned, there is a great amount of 2 or more pathogens in association, the symptomatology is common for all of them and might not be tested at a first consult. The owners usually come either to soon, either too late when all internal organs are severely compromised: renal failure, hepatic damage, cardiopathies, pneumonia, etc. From the **negative cases (286)** for latent *T. gondii*, **4** of them were aggressive, but the cause for the aggressivity came from elsewhere, as it follows.

One **case of aggressive** feline tested **negative** for latent ***T. gondi***i and *Chlamydophila* spp., but had a caught based on the microbiology swab sample that grew positive for Streptococcus spp. and *Pasteurella* spp. Other pathogens were not asked for testing.

One case also **negative** for *T. gondii*, was a feline with renal lithiasis, discovered on the ultrasounds, assuming the pains were on a high level so the discomfort made the cat be aggressive idiopathically.

One case had a **positive *Criptococcus neoformans***, diagnosed by MV phd student Dobre Patricia from the Pathological Anathomy Dep., 2018, after extracting a CSF from the cat and analyzing it with cytology, the necropsic exam discovered multiple lesions in the cerebral tissue. We tested the fluid together and found a negative result for **FCoV IgG** and a **negative antigen for Rabies**.

The cat was euthanized for it was very dangerous to be around people, it attacked it's owners and everyone around. As a contamination way was supposed to have been pigeon feces from the balcony of the owner's house. And **the last case** was a fall from the balcony, **constipated** so again the disconfort factor present, both latent *T. gondii* and *FCoV* were **negative**, and with no other symptoms.

For the protocol to be complete in testing for *T. gondii,* we recommend testing in the laboratory for feces microscopy and **antigens, the antibodies, IgM, IgG** in the blood and if the symptoms persist, and neurological signs do not disappear**, PCR** from **CSF** is in order, to surprise any fragment of the parasite. If **MRI** with some contract substance could reveal the cysts, we could soon be able to understand better were they are located and maybe how

they affect the functions of our patients, and then treat accordingly.

CONCLUSIONS

The aggressive behavior remains a common subject for future study, both in veterinary neuropathies and human neuropsychiatry, and also a very important approach for the means of contention for investigating, applying a therapy and especially settling some living arrangements with its owner.

Research on damaged cerebral area and tissue, given by this parasite, had been shown to choose a special placing in the amygdala, hypothalamic, retrobulbar, areas responsible for processing the received signals, the response to them from different cortical areas, for cats or humans, and even for different other species naturally or artificially infected in the laboratory. The presence of *T. gondii* parasite disrupts neural mechanism, cognitive, visual, changes the brain biochemistry in the way that might affect the survival skills set of an individual, homeostasis and hormonal balance, involving decision making when to retreat, attack, defense, impulsivity or even self-harm. From our current study we can conclude that *T. gondii* has no gender affinity, that **common European race** is mostly predominant in testing numbers for positive latent *T. gondii,* the symptoms connect among themselves for all the epithelial surfaces, the respiratory with ocular and dermatology, where all the eosinophilic reactions take place, and the cycle is never interrupted without a proper treatment, we wonder if the toxoplasmic cat changes the symptoms when stressed, if a IgM reaction to the parasite reappears in the blood stream, or it changes biochemistry where the cysts are located. Co-infectious with viruses and bacteria could easily be found in immuno- depreciated by *T. gondii* cats, there might be a way to trigger a new wave of cysts replication, this is only a future idea to follow, but for sure it makes it easier for the new pathogen to insert. When in consult, after taking the patient's history, obvious symptomatology, vaccination status, temperature, dehydration status, dietary habits, plants in the house, other incidents or new cat, or any animal in the parameter, trauma, hormonal status, new changes in behavior, we recommend a full serology screening of the animal, especially when aggressive. After a full history panel of checking for latent and antigen forms of any viruses, parasites and bacteria available in the laboratory, if the symptoms are persistent and a certain disease might be present, it's mandatory that molecular biology should be next on the testing protocol, that is why we insist on the good communication between the veterinarian and the laboratory staff.

REFERENCES

Abdoli, Amir. (2014). *Toxoplasma, testosterone, and behavior manipulation: the role of parasite strain, host variations, and intensity of infection*. Frontiers in biology 9.2: 151-160.

Barnard, Christopher J. (2011). *Animal behaviour: mechanism, development, function and evolution*. Pearson Education

Berenreiterová, Miroslava, et al. (2011). *The distribution of Toxoplasma gondii cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis*. PloS one 6.12: e28925.

David, Clément N., et al. (2016). *GLT-1-dependent disruption of CNS glutamate homeostasis and neuronal function by the protozoan parasite Toxoplasma gondii. PLoS pathogens* 12.6 e1005643.

Fallahi, Shirzad, et al. (2017). *A literature review on maternal-fetal and reproductive disorders of Toxoplasma gondii infection*. Journal of gynecology obstetrics and human reproduction.

Gregg, Thomas R., and Allan Siegel. (2001). *Brain structures and neurotansmitters regulating aggression in cats: implications for human aggression*. Progress in Neuro-Psychopharmacology and Biological Psychiatry 25.1 (2001): 91-140.

Hetts, Suzanne (1999). Pet behavior protocols: What to say, what to do, when to refer. *AAHA press*, 1999.

Hunsperger, Robert W., and Verena M. Bucher. (1967). *Affective behaviour produced by electrical stimulation în the forebrain and brain stem of the cat*. Progress în Brain Research. Vol. 27. Elsevier, 103-127.

Joiner, Keith A., and David S. Roos (2002). *Secretory traffic in the eukaryotic parasite Toxoplasma gondii: less is more*. J cell Biol157.4: 557-563.

Julio-Pieper, Marcela, et al. (2011). *Exciting times beyond the brain: metabotropic glutamate receptors in peripheral and non-neural tissues*. Pharmacological reviews 63.1:35-58.

Mathai, Ashwin Jacob, et al. (2016). *Reciprocal moderation by Toxoplasma gondii seropositivity and blood phenylalanine–tyrosine ratio of their associations with trait aggression.* Pteridines 27.3-4: 77-85.

McConkey, Glenn A., et al. (2013). *Toxoplasma gondii infection and behavior–location, location,*

location? Journal of Experimental Biology 216,1 (2013): 113-119.

Mitra, Rupshi, Robert Morris Sapolsky, Ajai Vyas (2013). *Toxoplasma gondii infection induces dendritic retraction in basolateral amygdala accompanied by reduced corticosterone secretion.* Disease models & mechanisms 6.2: 516-520.

Oktenli, Cagatay, et al. (2004). *Transient hypogonadotrophic hypogonadism in males with acute toxoplasmosis: suppressive effect of interleukin-1â on the secretion of GnRH*. Human Reproduction 19.4: 859-866.

Prandovszky, Emese, et al. (2011). *The neurotropic parasite Toxoplasma gondii increases dopamine metabolism*. PloS One, 6.9: e23866.

Robert-Gangneux, Florence, and Marie-Laure Dardé (2012). *Epidemiology of and diagnostic strategies for toxoplasmosis.* Clinical microbiology reviews, 25, 2: 264-296.

Schulthess, J., et al. (2008). *Mucosal immunity in Toxoplasma gondii infection*. 389-395.

Mendez, Oscar A., Anita A. Koshy. (2017). *Toxoplasma gondii: Entry, association, and physiological influence on the central nervous system.* PLoS pathogens 13.7 (2017): e1006351.

Xiao, Jianchun, et al. (2014). *MicroRNA-132 dysregulation in Toxoplasma gondii infection has implications for dopamine signaling pathway*. Neuroscience 268: 128-138.

http://vetexpert-rf.ru/express-test/toxoplasmaAb/

https://phys.org/news/2017-12-toxoplasmosis-exploits-immune-cells-brain.html#jCp

https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006739

https://doi.org/10.1371/journal.ppat.1006351.g001

http://www.msunites.com/toxoplasma-gondii-parasite-and-neurological-disorders-research/

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EFFECT OF FEED SUPPLEMENTATION BY *MORINGA OLEIFERA* LEAVES MEAL ON QUAIL (*COTURNIX* SP.) PRODUCTION PERFORMANCES IN THE SUDANO-GUINEAN ZONE OF CAMEROON

François Djitie KOUATCHO[1*], Djanabou MOUSSA[1], Koné MAMADOU[2], Ndraouni FRIKI L[1], Bobga AOUDOU[1], Pidotcho GOLOMTA[1], Alexis TEGUIA[3]

[1]Department of Biological Sciences, Faculty of Sciences, University of Ngaoundéré, Cameroon
[2]UFR Environnent, Jean Lorougnon Guédé University, Daloa, Côte d'Ivoire
[3]Department of Animal Productions, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon

*Corresponding author email: franckdjitie@gmail.com

Abstract

The present study was carried out from April to May 2018 in order to evaluate the effect of feed supplementation by M. oleifera leaves meal (MOLM) on quail (Coturnix sp.) production performances in the sudano-guinean zone of Cameroon. For this purpose, 192 quails of 21 days old with similar live weight were divided into 12 batches of 16 birds (8 males and 8 females). To a feed containing 20.18% Crude Protein and 3013.78 kcal of M E, 0, 1, 2, or 3% of MOLM was added corresponding to treatments T0, T1, T2 and T3, respectively. Each of these experimental diets was randomly assigned to 3 batches in a completely randomized design with 4 treatments repeated 3 times each. Data were collected throughout the 5 weeks of trial on growth performances, carcass and some reproductive characteristics. At the 7 weeks of the trial, 2 males, 2 females per batch were sacrificed to evaluate carcass and some reproductive characteristics. The main results showed that regardless of sex, the significantly higher (224.13 ± 30.69 g) live weight was obtained with 2% of MOLM compared to the control (203.83 ± 16.67 g). The significantly higher (4.74 ± 0.93) feed conversion ratio was obtained with the T3 treatment compared to T2 (4.24 ± 0.80 g). Carcass yield of males ranged from 70 (T2) to 73% (T3) and from 67 (T2) to 69% (T0) in females. Only whole carcass yield, relative weights of the wishbones, neck and gizzard were significantly (P<0.05) affected by MOLM. First songs in males as well as the first egg were recorded at 6 weeks age with 3% MOLM, which also induced a higher (73.01%) egg laying rate than control batch (57.14%). Treatments supplemented with 2 and 3% MOLM increased testicular weight (4.63 ± 0.34g and 5.48 ± 0.46 g respectively) compared to the control (3.54 ± 0.73 g). It has been concluded that MOLM can be used up to 3% to improve growth performance of quail in the finishing phase. Also, a 3% supplementation could be used to improve reproductive performance.

***Key words**: growth, leaves, Moringa oleifera, quail.*

INTRODUCTION

Quail farming payed attention of Cameroonian farmers as a new way of diversifying sources of animal proteins, offering consumers new tastes and reinforcing the production of meat. Its low production cost associated with its small size, disease resistance, rapid growth and relatively short life cycle of up to 3 to 4 generations per year. Its high egg production from 250 to 350/female per year (Biagini, 2006; Sauveur, 1988), as well as the supposed therapeutic virtues that characterize these eggs (Tunsaringkarn et al., 2013) are the factors that favored his expansion in recent years. However, although the production of this species seems to interest some breeders, its production performance remains relatively low. This is due to the fact that its production standards are not mastered in our environment. According to surveys conducted by Katchouang et al. (2015), 60% of breeders in the Mfoundi department in Central region of Cameroon have not received formal training for their breeding. In addition, the same study shows that 86% of Yaoundé farmers would use chicken feed while the remaining 14% will prepare their own feed without any training. However, there are many unconventional food resources in Sudano Guinean zone such as *Moringa oleifera* (MO), which could provide important nutrient, vitamin and micronutrient inputs to quail by adding it to commonly used ingredients. Studies by various authors (David et al., 2012 and Olugbemi et al., 2010) have shown that *M. oleifera* leaves are rich in proteins, minerals, vitamins and amino acids such as arginine, lysine and methionine which are essential in poultry. According to

several studies, the incorporation of *M. oleifera* leaves meal (MOLM) into the diets of laying hens showed a significant improvement in productivity according to Kakengi et al. (2007) and a significant increase in the weight of eggs according to Paguia et al. (2014). In Japanese quail, the best level of production performance in Algeria was obtained with 0.2% supplementation of the staple diet with MOLM (Elkloub et al., 2015). Since quail farming and nutritional need are not mastered in the Sudano-Guinean zone of Cameroon, the present study aimed to assess the effect of feed supplementation with MOLM on growth performance, carcass characteristics, and reproduction in quail will be determined.

MATERIALS AND METHODS

Study area

The study was conducted in Ngaoundéré in the Sudano Guinean zone of Cameroon. The area is located between the 6th and 8th degrees north latitude and between the 10th and 16th degree east longitude with an average altitude of 1000 m. There is one rainy season of 8 months (April to November) and a dry season of 4 months (December to March). The rainfall is between 1500 and 1800 mm/year, the average annual humidity varies between 64.1% and 67.6% and the temperatures between 23 and 32°C. (Mbogning et al., 2011).

Materials and study management

M. oleifera leaves were cut early in the morning and then dried in the shade before being transformed into powder and kept until use.

A total of 192 quails of 21 days old and average weight of 64.25 ± 3.77 g were divided into 12 similar batches of 16 subjects (8 males and 8 females). They were housed in cages made of low-meshed planks and wire mesh at a density of 28 subjects/m^2. Four experimental diets (T0, T1, T2 and T3) formulated on the basis of the level (0, 1, 2 and 3%) of supplementation of the basic diet (average of 3000 Kcal Metabolizable Energy and 20% Crude Protein) with *MOLM* in the finishing phase.

The experimental died were formulated by adding 0, 1, 2 and 3% of the feed weight by MOLM corresponding to treatments T0, T1, T2 and T3, respectively.

Each of the 4 experimental diets was randomly assigned to 3 batches in a completely randomized design with 4 treatments repeated 3 times each. Water and feed were distributed *ad libitum* throughout the 5 weeks of the study. All birds benefited from similar management conditions.

Data collection

Experimental diets were weighed at the beginning of the week and distributed daily. Leftovers of each experimental unit were weighed every 7 days using an electronic scale of 5000 g range and 1g accuracy. Weekly feed intake was calculated as the difference between the amount of feed distributed during the week and leftovers of the same week.

At the beginning of the test and every 7 days after, quails were weighed on an empty stomach in the morning using an electronic scale of 2000 g range and 0.1g accuracy. The weekly weight gain was obtained by making the difference between two consecutive live weight.

Feed intake (FI) of each week was divided by weekly average weight gain of the same period to obtain the Feed Conversion Ratio (FCR).

At 07 weeks of age, 6 males and 6 females per treatment were slaughtered and characterized according to the method described by Genchev and Mihaylov (2008) for the evaluation of the carcass. The carcass, liver, heart, gizzard, head, thigh, chest, wing and leg weight data allowed us to calculate carcass yield and relative weight of carcass parts or organs all in relation to live body weight.

Statistical analysis

Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used according to the general linear model to compare the means of the different parameters. In case of differences between treatments, Duncan test was used at 5% significance level to separate means. IBM SPSS Statistics 21.0 software was used for analyzes.

RESULTS AND DISCUSSIONS

Average production performances

The effect of MOLM supplementation on average production performance at 7 weeks

(Table 1) shows that regardless of sex, feed intake (FI) was comparable between different treatments. Live weight significantly ($P < 0.05$) varied from 203.83 ± 16.67 g (T0) to 224.13 ± 30.69 g (T2) and in females, from 213.96 ± 18.62 g (T0) at 251.66 ± 6.79 g (T2). In females and regardless of sex, the significantly highest values were recorded with T2 treatment compared with T0 treatment who were otherwise comparable to T1 and T3 treatments. In males, significantly higher live weight was recorded with control and T2 compared to T3 treatment. The observations made for the mean weekly weight gain were the same as those for live weight. Regardless of gender, the feed conversion ratio significantly ($P < 0.05$) increased from 4.24 ± 0.80 g (T2) to 4.74 ± 0.93 g (T3), treatments supplemented with 1 and 3 % MOLM and the control group were otherwise comparable ($P > 0.05$). Supplementation of MOLM did not significantly affect feed conversion ratio of the different treatments in males.

In females however, feed conversion ratio significantly ($P < 0.05$) increased from 3.51 ± 0.06 g (T2) to 4.40 ± 0.56 g (T0). the values of feed conversion ratio for T0, T1, and T3 treatments in one hand and for T1, T2 and T3 on the other hand remained similar.

Table 1. Quail average production performances basis on the experimental diets at 7 weeks of age

Trait	Production performances				
	Treatments	Total feed intake (g)	Live weight (g)	Weekly weight gain (g)	Total Feed Conversion Ratio
Female	T0		213.96±18.62^{a}	37.42±9.31^{a}	4.40±0.56^{b}
	T1		227.37±11.73^{ab}	40.78±2.93^{ab}	3.98±0.25^{ab}
	T2		251.66±6.79^{b}	46.85 ±1.69^{b}	3.51±0.06^{a}
	T3		234.90±18.53^{ab}	42.66±4.63^{ab}	3.93±0.43^{ab}
	Mean		231.97±19.00	41.93 ±4.75	3.95±0.46
Male	T0		193.70±6.35^{b}	32.36±1.85^{b}	5.05±0.45^{a}
	T1		187.40±4.27^{ab}	30.78±1.06^{ab}	5.27±0.30^{a}
	T2		196.59±5.88^{b}	33.06 ±1.47^{b}	4.98±0.12^{a}
	T3		184.04±1.86^{a}	29.94±0.46^{a}	5.55±0.11^{a}
	Mean		190.43±6.67	31.54 ±1.66	5.21±0.33
Mixed	T0	653.12±26.73^{a}	203.83±16.67^{a}	34.89±4.16^{a}	4.73±0.58^{b}
	T1	648.45±13.44^{a}	207.38±23.27^{a}	35.78 ±5.81^{a}	4.62±0.74^{ab}
	T2	658.70±11.90^{a}	224.13±30.69^{b}	39.97 ±7.67^{b}	4.24±0.80^{a}
	T3	665.79 ±20.52^{a}	209.47±30.24^{ab}	36.30±7.56^{ab}	4.74±0.93^{b}
	Mean	665.52±18.99	211.20±25.38	36.73±6.34	4.58±0.75

a, b : on the same column and for the same gender, the assigned values of the same letter are not significantly different (P > 0.05)

Live weight evolution and average weight gain

The evolution of the average live weight of quails (Figure 1) as a function of the supplementation rate of *MOLM* feed shows that at week 4, T2 treatment induced a live weight significantly higher (109.15 ± 17.81 g) than T3 treatment (101.4 ± 13.55 g).

At week 5, the control treatment was similar to T2 and T3 treatments and also induced significantly ($P < 0.05$) higher weights than the T1 treatment (134.8 ± 9.12 g).

By week 6 supplementation of feed by MOLM did not significantly ($P > 0.05$) affect body weight. At the end of the study, feed supplemented at 2% with MOLM induced a significantly ($P < 0.05$) higher live weight (224.13 ± 30.69 g) compared to the control treatment (203.83 ± 16.67 g).

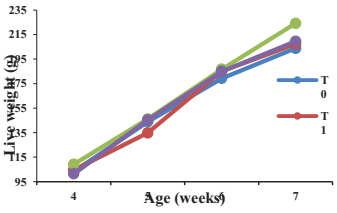

Figure 1. Evolution of weekly live weight regardless of gender based on experimental diets

Feed conversion ratio

Figure 2 shows the evolution of the Feed conversion ratio (FCR) as a function of the level of supplementation of *MOLM* in feed. Control treatment induced significantly higher ($P < 0.05$) feed conversion ratio at weeks 4 (2.75 ± 0.90 g) and 6 (5.39 ± 0.59 g) compared to the supplemented diets. At week 5, T1 induced the higher feed conversion ratio compared to other treatments which remained similar among them. At the end of the trial, the highest ($P < 0.05$) feed conversion ratio was obtained with T1 treatment (13.97 ± 8.03 g) compared to T2 treatment (7.85 ± 4.07 g) but remains comparable ($P > 0.05$) to T3 and T0 treatments.

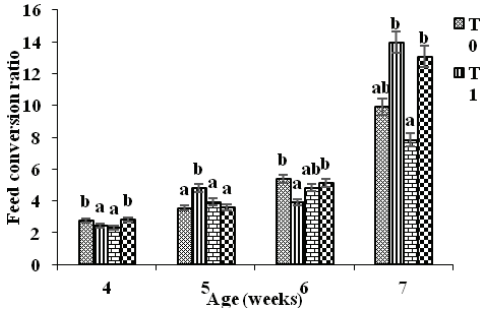

Figure 2. Weekly change in feed conversion ratio regardless of sex and based on experimental diets

Carcass yield and proportions of cuts parts

On the summarizes of the effect of *MOLM* supplementation on carcass yield of quail and the proportions (% BW) of its different cuts parts at 7 weeks (Table 2), it appears that the standard carcass yield, the proportions of thighs, wings, heads, legs and back were not significantly ($P > 0.05$) affected by the different levels of supplementation.

The highest whole carcass yields ($P < 0.05$) were obtained with diet supplemented at 1 and 2% (80.21 ± 4.23 g and 80.04 ± 3.58 g, respectively) with MOLM.

These values were also similar to that of the T3 treatment which was also similar to the control regardless of sex.

The significantly ($P < 0.05$) highest chest proportions were recorded at 1% of supplementation compared to the control and T3 treatments.

Chest proportions of T2 were otherwise similar ($P > 0.05$) to those of all other treatments. T3 treatment showed significantly higher ($P < 0.05$) neck proportions than other treatments.

Those of comparable T0 and T2 treatments did not significantly differ between them but were higher than values recorded with T1 treatment.

Table 2. Carcass yield and relative weight of different parts in relation to body weight (% BW) at 7 weeks old basis on the experimental diets

Characters (%BW)	Gender	Experimental diets			
		T0 (n=12)	T1(n=12)	T2(n=12)	T3(n=12)
Whole Carcass yield	Male	77.44±0.58^{a}	83.37±1.89^{b}	81.78 ±0.89^{b}	80.32±4.20^{ab}
	Female	74.90 ±0.94^{a}	77.0±3.36^{b}	78.32±4.72^{b}	75.03±3.90^{ab}
	Mean	76.17±1.56^{a}	80.21±4.23^{b}	80.04±3.58^{b}	77.68±4.63^{ab}
Standard carcass yield	Male	71.14 ±0.40^{a}	71.33±0.76^{a}	70.10±0.69^{a}	73.07±3.84^{a}
	Female	69.18±1.72^{a}	68.07±0.64^{a}	66.80±2.84^{a}	68.10±4.35^{a}
	Mean	70.17±1.56^{a}	69.70±1.89^{a}	68.44±2.59^{a}	70.56±4.59^{a}
Chest	Male	23.45±0.89^{a}	28.41±0.87^{b}	26.84 ±1.75^{ab}	25.41±1.39^{a}
	Female	23.60±1.99^{a}	25.99±1.35^{b}	24.12±1.05^{ab}	22.90±3.50^{a}
	Mean	23.52±1.39^{a}	27.20±1.68^{b}	25.49±1.97^{ab}	24.16±2.75^{a}
Thigh	Male	16.00±0.47^{a}	15.24±1.30^{a}	15.31±1.08^{a}	15.54±1.02^{a}
	Female	15.03±0.50^{a}	15.59±0.34^{a}	14.91±1.31^{a}	15.11±0.92^{a}
	Mean	15.52±0.69^{a}	14.41± 0.89^{a}	15.11±1.10^{a}	15.32±0.90^{a}
Wings	Male	6.14±0.23^{a}	6.52±0.42^{a}	6.18±0.30^{a}	6.26±0.42^{a}
	Female	7.02±1.12^{a}	5.51±0.42^{a}	6.22±0.75^{a}	5.97±0.35^{a}
	Mean	6.58±0.87^{a}	6.21±0.60^{a}	6.20±0.51^{a}	6.11±0.38^{a}
Neck	Male	6.13±0.64^{b}	3.74±0.40^{a}	5.22±0.40^{b}	6.81±0.48^{c}
	Female	5.82±1.19^{b}	4.21±0.37^{a}	5.07±0.66^{b}	7.66±0.86^{c}
	Mean	5.97±0.98^{b}	3.98±0.43^{a}	5.14±0.49^{b}	7.23±0.30^{c}
Back	Male	18.82±0.40^{a}	16.88±0.51^{a}	16.28±1.88^{a}	17.25±1.88^{a}
	Female	18.99±10.70^{a}	16.22±1.83^{a}	15.52±0.36^{a}	15.25±2.02^{a}
	Mean	18.91±6.78^{a}	16.55±1.25^{a}	15.90±1.28^{a}	16.60±2.28^{a}

a, b, c: on the same column and for the same gender, the assigned values of the same letter are not significantly different (P>0,05)

Relative weight of quail's organs

The effect of supplementing feed with *MOLM* feed on relative weight on some quail's internal organs at 7 weeks of age as presented in Table 3 show that the different experimental diets did not induced any significant variation on heart, liver and abdominal fat proportions.

The gizzard proportions of the control treatment were significantly the lowest compared to those of the T2 treatment and otherwise similar to other treatments. Despite the fact the Gonado-Somatic Index (GSI) of males of treatment T1 was the significantly lowest and that the values obtained with control, T2 and T3 were similar, we however noticed an increase of the GSI between T0, T1 and T2 treatments.

In females, GSI were not significantly affected ($P > 0.05$) by the level of feed supplementation by *MOLM.*

Table 3. Relative weight of some quail's organs at 7 weeks of age according to experimental diets

Characteristics (%BW)	Gender	Experimental diets			
		T0 (n=12)	T1(n=12)	T2(n=12)	T3(n=12)
Liver	Male	2,16±0,33^{a}	2,22±0,46^{a}	2,06±0,45^{a}	2,19±0,08^{a}
	Female	2,81±0,51^{a}	3,24±1,00^{a}	2,96±1,04^{a}	3,07±0,78^{a}
	Mean	2,48±0,52^{a}	2,73±0,89^{a}	2,51±0,86^{a}	2,63±0,51^{a}
Heart	Male	0,59±0,54^{a}	0,92±0,05^{a}	0,84±0,27^{a}	0,81±0,17^{a}
	Female	0,83±0,04^{a}	0,81±0,09^{a}	078±0,39^{a}	1,05±0,20^{a}
	Mean	0,84±0,48^{a}	0,87±0,08^{a}	0,80±0,04^{a}	0,86±0,12^{a}
Gizzard	Male	2,81±0,66^{a}	2,82±0,13^{ab}	3,83±1,09^{b}	3,65±1,40^{ab}
	Female	2,94±0,88^{a}	3,40±1,43^{ab}	4,59±0,23^{b}	3,90±1,30^{ab}
	Mean	2,87±0,70^{a}	3,11±0,96^{ab}	4,21±0,82^{b}	3,77±1,21^{ab}
Abdominal fat	Male	0,67±0,40^{a}	0,46±0,10^{a}	1,04±0,48^{a}	0,65±0,35^{a}
	Female	1,96±1,34^{a}	0,90±0,53^{a}	1,21±0,42^{a}	1,05±0,38^{a}
	Mean	1,31±1,10^{a}	0,68±0,41^{a}	1,12±0,41^{a}	0,84±0,39^{a}
Gonado somatic index	Male	1,84±0,34^{ab}	1,26±0,39^{a}	2,27±0,34^{b}	2,50±0,36^{b}
	Female	1,04±0,71^{a}	1,10±0,85^{a}	2,18±1,51^{a}	0,40±0,27^{a}

a, b: on the same column and for the same gender, the assigned values of the same letter are not significantly different (P>0,05)

DISCUSSIONS

The relative higher weekly total FI were obtained with T3 treatment compared to the control. These results are close to those of Paguia et al. (2014) who found that feed intake with levels of 0.20%, 0.40%, 0.60% and 0.80% supplementation of the diet by MOLM in laying hens was significantly higher than the FI of the control batches. This increase in consumption could be explained by some palatability of MOLM in these birds because of its richness in vitamins and nutrients (Paguia et al., 2014).

Overall, body weight has increased significantly with the supplementation level of MOLM in feed. at the end of the trial, T2 treatment induced high weights and the highest average weight gain regardless of the sex. These results are similar to those of Vali (2009) who obtained at 63 days, a live weight of 221.92 g but are higher than those of Bonos (2010), Abdel-Azeem and Abdel-Azeem (2010) who recorded at 6 weeks, weights of 172 g and 199 g respectively. This variability of the weight could be explained either by the age or by the orientation of some lines towards a precocity in the meat production (Cambell et al., 2003; Vali, 2009). The improvement in live weight and weight gain in our study with T2 treatment would mean a better assimilation of nutrients due to a level of supplementation of *MOLM* which seem to be optimal for the finishing period (Kakengi et al., 2003).

FCR increased overall over time. The high values of weekly FCR recorded during the last few weeks of growth, especially could be justified by the high feed consumption coupled with the low weight gains for the same period the highlighting the decrease in the bird's ability to convert the food at the end of growth periods (INRA 1989; Berrama et al., 2011).

Treatments T1 and T2 presented the highest whole carcass proportion compared to the control and could be due to an optimal level of MOLM in the diet. David et al. (2012) found that MOLM improves carcass yields at slaughter compared to the control in broilers, which is also the case in our trial for the whole

carcass and not for the standard. This increase in whole carcass yield could be due to the fact that *MOLM* is concentrated in nutrients in the raw form that also appears to reduce the activity of pathogenic bacteria and improves the digestibility of food, thus helping poultry to express its genetic potentials. Indeed, replacement of antibiotic growth promoters with MOLM by 0.1% has been beneficial on carcass growth and carcass yield of broilers David et al. (2012).

Regardless of gender, the significantly higher chest proportions were induced with T2 treatment. This result is contrary to the observations of Corrêa et al. (2008) who reported that the proportion of the chest increased significantly with the level of protein in the ration in female quail. Our results, however, corroborate those of Seyed-Alireza et al. (2011) who found by feeding Japanese quail with diet whose energy-protein ratio ranged from 107 to 138, significant effects on the proportions of the chest.

As with heart and abdominal fat, the proportions of the different batches were comparable to the control group, with slight increases with the supplementation level of MOLM. Similar trends were obtained by Bonos et al., 2010, although lower levels (1.66 to 1.99%), were recorded in Western highland of Cameroon by Djitie et al. (2015) and could then be explained either by the treatments and/or the environmental conditions amongst others. The T2 treatment induced the relative weight of the gizzards significantly higher compared to the control group, which is contrary to the relative weight of gizzard of *Coturnix japonica* at 07 weeks of age according to Attia et al. (2013). This result could be explained by the hyperactivity of the gizzard which is the crusher muscle of the digestive tract then lead to his development. However, Ologhobo et al. (2014) observed in their study that the relative weight of the gizzard decreased significantly by increasing the supplementation levels of the *MOLM* feed compared with control lots, which is not the trend obtained in our work. In addition, a difference between the ratio of gizzard/live weight was observed in favor of females in the control group compared with males. It may be related to increased consumption of the feed by females in preparation for oviposition (Sauveur, 1988). The significantly higher gonado somatic index recorded in male with T2 and T3 could be lead to a good development of testis and a precocity in the sexual maturity.

CONCLUSIONS

Diet supplemented with 2% powdered of *Moringa oleifera* leaves induced a significant increase in live weight and average weekly weight gain.

In general, diet supplemented with *M. oleifera* leaves meals significantly improved whole carcass yield, chest, neck and gizzard relative weight. T2 treatment is therefore the most appropriate for quail in the growth phase;

3% supplementation of diet with MOLM induced in males an increase in testicular weight and gonado-somatic index and in females a precocity at the entrance to reproduction and a high rate of spawning and can therefore be used in the reproduction phase.

REFERENCES

Abdel-Azeem et Abdel-Azeem F. (2010). The influence of different stocking density and sex on productive performance and some physiological traits of Japanese quail. *Egyptian Poultry Science Journal* 30, 203-227.

Attia Y. A., Abd El-Hamid A E, Ellakany H. F., Bovera F., Al-Harthi M. A., et Ghazaly S. A. (2013). Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. *Italian Journal of Animal Science, 12 (E37*): 222-229.

Berrama Z., Mefti H., Kaidi R., et Souames S. (2011). Caractérisation zootechnique et paramètres génétiques des performances de croissance de la caille japonaise *Coturnix japonica* élevée en Algérie. *Livestock Research for Rural Development*. Volume 23, Article #3.

Biagini F. (2006). Petits et mini-élevages dans le monde : principales espèces d'intérêt. Synthèse bibliographique. Biologie géosciences agro ressources et environnement productions animales en régions chaudes synthèse. Ecole Nationale Supérieure Agronomique de Montpellier Place Viala, 34060 Montpellier Cedex Master. 31 p.

Bonos E. M., Christaki E. V., et Florou-Paneri P. C. (2010). Performance and carcass characteristics of Japanese quail as affected by sex or mannan oligosaccharides and calcium propionate. *South African Journal of Animal Science,* 40(3): 173-181.

David, L. S., Vidanarachchi J. K., Samarasinghe K., Cyril H. W., et Dematawewa C. M. B. (2012). Effects of *Moringa* based Feed Additives on the Growth Performance and Carcass Quality of

Broiler Chicken. *Tropical Agricultural Research,* 24(1): 12– 20.

Djitie Kouatcho F., Kana J. R., Ngoula F., Nana N. F. C., et Teguia A. (2015). Effet du niveau de protéines brutes sur la croissance et la carcasse chez la caille (*Coturnix* sp) en phase de finition dans les Hautes Terres du Cameroun. *Livestock Research for Rural Development. Volume 27, Article #155.*

Elkloub M. E. L., Moustafa, R. F. H., Shata Mousa M. A. M., Hanan A. H., Alghonimy and Youssef S. F. (2015). Using moringa oleifera leaf meal on performance of japanese quail. *Egyptian poultry of Science, vol. 35,*14p

Genchev A., Mihaylova G., Ribarski S., Pavlov A., Kabakchiev M., (2008). Meat quality and composition in Japonese quails. *Trakia Journal of Science*, 6: 72-82.

INRA, (1989). L'alimentation des animaux monogastriques : Porcs, lapins, volailles. 2ème Edition. INRA, Paris, 282 p.

Kakengi A. M. V., Kaijage J. T., Sarwatt S. V., Mutayoba S. K., Shem M. N., Fujihara T. (2007). Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. *Livestock. Research. Rural Dev*., 9 (4). 446.

Katchouang A. S. N., Djitie F. K., Meutchieye F., Kana J. R., et Teguia A. (2015). Caractéristiques des élevages de caille (*Coturnix sp*) dans le département du Mfoundi, région du Centre, Cameroun. *Livestock Research for Rural Development, Volume 27, Article #77.* Retrieved October 9, 2018, from http://www.lrrd.org/lrrd27/4/katc27077.html.

Mbogning E., Tchoumboue J., Damasse F., Sobze M. S., et Canini A. (2011). Caractéristiques physico-chimiques des miels de la zone Soudano-guinéenne de L'Ouest et de l'Adamaoua. *Tropicultura* (3) 168-175.

Ologhobo A. D., Akangbe, E. I., Adejumo I. O., and Adeleye O. (2014). Effect of *Moringa oleifera* leaf meal as replacement for oxytetracycline on carcass characteristics of the diets of broiler chickens. *Annual Research and Review in Biology*, 4(2): 423-431.

Paguia H. M., Paguia R. Q., Balba C., et Flores R. C. (2014). Utilization and evaluation of *Moringa oleifera L*. As poultry feeds. *Apcbee Procedia,* 8: 343–347.

Sauveur R. (1988). Reproduction des volailles et production d'œufs. INRA Paris France, 450 p.

Seyed-Alireza S., Mehrdad I., Khosro G., Afshar M., Vahid R., Hasan F., Kamel A., Samad Zamanzad G. (2011). Effect of varying dietary energy to protein ratio on productive performance and carcass characteristics of japanese quail. *Animals biological research,* 2(1):149-155.

Tunsaringkarn T., Tungjaroenchai W., Siriwong W. (2013). Nutrient Benefits of Quail (*Coturnix coturnix japonica*) Eggs. *International Journal of Scientific and Research Publications,* 3(5): 1-8

Vali N. (2009). Growth, feed consumption and carcass composition of *Coturnix japonica, Coturnix ypsilophorus* and their reciprocal crosses. *Asian Journal of Poultry Science,* 3(4): 132-137.

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FLAME OR FURNACE AA (ATOMIC ABSORPTION) - WHICH TECHNIQUE SHOULD I USE IN MY LABORATORY? A BASIC OVERVIEW OF ELEMENTAL ANALYSIS

Florina RAICU, Constantin VLAGIOIU, Niculae TUDOR

Faculty of Veterinary Medicine, 105 Splaiul Independentei, Bucharest, Romania

Corresponding author email: stratoneflorina@yahoo.com

Abstract

If in a laboratory elemental analysis (heavy metals) on solutions is being performed, there are a couple of very efficient spectrometric techniques that can be used. Flame and Furnace Atomic Absorption (AA) is one of them. This article aims to highlight the applicability of both techniques (Flame Atomic Absorption Spectroscopy "FAAS" and the other one that is Graphite Furnace Atomic Absorption Spectroscopy "GFAAS") that are being used in the analysis of animal and non-animal food samples. For obtaining a relevant pieces of information in order to help the analyst, it is useful to make a parallel between the two AA techniques. This parallel is obtained by objectively answering to a series of questions to make sure that the chosen technique is the best from different point of views (type and number of samples, the type of the analyzed element, the time allocated for each analysis etc.) The defining elements include understanding the operating principles of each mentioned technique (FAAS and GFAAS), their strengths and their weaknesses (spectral interferences, background effects, matrix effects).

Key words*: applicability, FAAS, GFAAS, parallel, spectral interferences, background effects, matrix effects.*

INTRODUCTION

If in a laboratory elemental analysis (heavy metals) on solutions is being performed, there are a couple of very efficient spectrometric techniques that anyone could use. This article aims to draw a parallel between FAAS (Flame Atomic Absorbtion Spectroscopy) and the GFAAS (Graphite Furnace Atomic Absorption Spectrometry) techniques.

Picking the most effective tool for this job can sometimes be an intimidating task, especially because of the different levels of capabilities. In fact, every single technique may be able to perform a particular analysis at acceptable levels of accuracy and precision (Cordos et al., 1983).

However, by appraising the corresponding strengths and weaknesses of each of the techniques, particularly as they may apply to the practical and performance requirements of the analysis, it is possible to build a balanced basis for taking the best decision (Seely et al., 2009).

MATERIALS AND METHODS

Understanding how the methods work

In order to intelligently choose between these sure techniques - FAAS and GFAAS, for comparison purposes it is helpful to have a general understanding of basic principles and specialized details of each of the elemental analysis instruments (Walker, 2014).

Principles of analysis in spectroscopy

Spectroscopy is a name given to a category of preliminary techniques and procedures which surveys and appraise the effect of intake or emission of energy by chemical analysis of a sample exposed to qualitative and / or quantitative.

The spectroscopic analysis is planted on the synergy between electromagnetic waves and matter. The purpose of spectroscopy is to obtain information from a spectrum about the sample, information such as: internal format, configuration, content, dynamics (Lajunen, 2004).

Classification of spectroscopic methods

1. Atomic Spectroscopy:
   - Atomic Absorption Spectroscopy (AAS/OAS);
   - Atomic Emission Spectroscopy (AES/OES);
   - Atomic Fluorescence; Spectroscopy (AFS);
   - Roentgen Spectroscopy;
   - Electron Spectroscopy (XRS);
2. Molecular Spectroscopy/Mass spectroscopy (MS);
3. Spectroultraviolet-visible (UV/Vis);
4. Spectroscopy for ultrashort time;
5. Spectroelectrochemistry;
6. Laser Spectroscopy;
7. Astrospectroscopy (French et al., 1990).

Atomic spectrometry - principle

Atomic spectrometry is based on the changeover of the sample to level of free atoms by a process of atomization, which takes place in an atomization cell, followed by shelling with optical radiation, having a wavelength well-defined radiation that is emitted by a primary radiation source and sent to the atomization source.

The emission of spectral lines is caused by a passage from a higher energy level to lower energy one. The absorption is the reverse process. Spectral lines for which one is the ground state energy level are called resonance lines. From the analytical point of view, a great importance is represented by resonant lines arising from transitions between the ground state and the first excited level, which are generally the most intense.

The emission spectroscopy or atomic absorption analysis applies, most often, for metallic elements. Atomic absorption spectroscopy is based on absorption of radiations (Christian et al., 1970).

The atomization in Atomic Absorption Spectrometry

Atomic absorption spectrometry (AAS) is a part of the UV-VIS and optical methods. It relies on the measurement of the radiated power which is absorbed by a population of free atoms. Evaporating and atomizing techniques which imposed in AAS are: flame and electrothermal evaporation. In case of using the flame, we have flame atomic absorption spectrometry (FAAS) and for electrothermal evaporation, we speak about electrothermal evaporation atomic absorption spectrometry (ETV-AAS). Regarding the electrothermal evaporators, the graphite furnace is noticeable, being symbolized as GFAAS technique.

Flame Atomic Absorption Spectrometry (FAAS)

In FAAS method, either an air or a nitrous oxide mixed with acetylene flame is used, for evaporating the solvent and disband the sample into its fundamental atoms. When light from a hollow cathode lamp (selected based on the element to be determined) passes through the cloud of atoms, the destination atoms absorb the light from the lamp. A detector is measuring this, and the information is used to calculate the concentration of that element in the original sample.

If a flame is used, then the excitation temperature reached by a sample is limited to a maximum of approximately 2600°C (with the N2O/acetylene flame). For most of the elements, this is not a problem. Compounds of the alkali metals, for example, and most of the heavy metals such as lead or cadmium and transition metals like manganese or nickel are all atomized with good efficiency with either flame type, with typical FAAS detection limits in the sub-ppm range (French et al., 1990).

However, there are a number of disobedient elements like V, Zr, Mo and B which do not perform well with a flame source. This is because the maximum temperature reached, even with the N2O/acetylene flame, is insufficient to break down compounds of these elements. As a result, flame AAS sensitivity for these elements is not as good as other elemental analysis techniques (Smith et al., 1993).

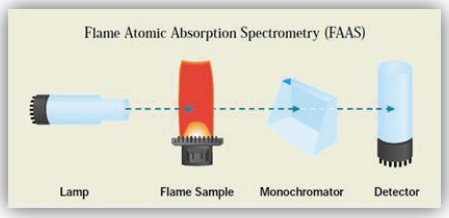

Figure 1. The components of the FAAS (French et al., 1990)

Strengths

- Facile usage;
- Very fast;
- Low cost;
- Relatively few interferences;
- Very compact instrument;
- Excellent performance;
- Robust interface.

Limitations

- Moderate detection limits;
- Element limitations;
- 1-10 elements per determination;
- No screening ability (Lajunen, 2004).

Graphite Furnace Atomic Absorption Spectrometry (GFAAS)

This technique is approximately the same as flame AA technique. The difference is that the flame is replaced by a small graphite tube that is electrically heated, or cuvette, which is heated to a temperature up to 3000°C to generate the cloud of atoms.

The higher atom density and longer residence time in the tube improve furnace AAS detection limits by a factor of up to 1000x compared to flame AAS, down to the sub-ppb range. However, because of the temperature limitation and the use of graphite cuvettes, refractory element performance is still somewhat limited (Rains, 2008).

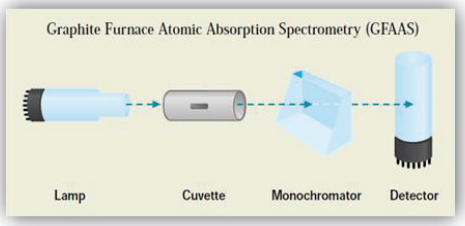

Figure 2. The components of the GFAAS (French et al., 1990)

Strengths

- Very good detection limits;
- Small sample size;
- Moderate price;
- Very compact instrument;
- Few spectral interferences.

Limitations

- Slower analysis time;
- Chemical interferences;
- Element limitations;
- 1-6 elements per determination;
- No screening ability;
- Limited dynamic range (Smith et al., 1993).

Interferences in AAS

This term represents all physical and chemical phenomena which act on the analyte's atoms, resulting in changing the signal, which would be obtained in their absence. If the sample and reference behave differently during measurement, interference will appear.

- Interferences in flame atomization

Spectral interferences in SAA: appear when the resonance emitted by the source line overlaps with an absorption line of another element in the flame, or over a molecular tape.

Physical: originated in the modification of the physical characteristics of the measuring solution (viscosity, surface tension, vapor pressure, temperature).

Chemicals: is the most important category of interference. Interference gets worse, as you increase the temperature difference between dissociation of the original compound, respectively, of the new one. Using the hot flames, as well as addition of salts of metals that form stable compounds with the interfering ion minimize these types of interference. (Cordos et al., 1983).

Interference of ionization: the atoms of analyte are partially ionized burning hot, which causes decreased absorption signal. In the analysis by FAAS working alkaline metal, most often, the flame temperature favors low temperature that increases atomization. Ionization effects can be reduced or eliminated by the addition of both the sample and the calibration standards, an item in excess ionized easier than the analyte.

- Interference in the graphite furnace atomization

Physical: volatility and viscosity affect pipetting solutions into the graphite tube. Surface tension affects the dispersion of the solution inside the oven and, consequently, the analytical response. If there are added surfactants (detergent or a surfactant such as Triton X), their behavior approach to the organic solutions.

Chemical (matrix effects) of the sample and the reagents used for the matrix can affect the dissolution of the analyte signal absorption, leading to a depression or an increase in the analytical response (Rains, 2008).
In GFAAS, chemical interferences due to the effects can cause the formation of stable or volatile compounds in the atomizer. Interference caused by the forming of volatile compounds can be avoided by modifying the matrix. This can be done by: choosing an appropriate temperature for drying-calcination cycle-atomization; adding a reagent in sample and calibration standards (the role of the matrix is to boost the volatility or to convert the analyte in a less volatile compound). In both situations, the change is made to the volatility of the analyte and the matrix to be adequately different to allow their separation in the ashing stage.
Studies regarding modifiers remain dominant as a research field in electrothermal atomization. Often, modifier consists of a mixture of substances, one that acts directly on the matrix and the other on the analyte. In some cases, using the organic modifiers, such as ascorbic acid, lactic acid, citric acid, EDTA, facilitates the reduction of the metal analyte and lowers the temperature of the atomization (Oliveira et al., 2017).

RESULTS AND DISCUSSIONS

It's implausible that a single technique is going to perfectly correspond all of a laboratory's needs. Unless you have lots of space, very skilled staff, and an extra budget, you'll probably have to make some compromises.
You should look in to the future, and try to anticipate what changes may occur in your analytical requirements. If possible, allow some room for system expansion.

Capital costs

Obviously, because there are a lot of instruments available from different manufacturers, and because so many options and configurations are possible, it is difficult to put absolute values on capital costs. A critical aspect is given by accessories or options, which may add considerably to the purchase price.
Practically, you can expect to pay from around $15-20,000 for a base configuration flame AA system. Speaking about the GF-AAS, the costs are at least double (Walker, 2014).

Operating costs

For GFAAS use, your reagents may also need to be enhanced from normal analytical grade to high-purity grade. You'll find major variations in the quantities of common supplies like argon, for instance, consumed during an analysis. GF-AAS only uses about 3.0 L/min of argon (Lajunen, 2004).

FAAS	acetylene/nitrous oxide gases (compressed air source) hollow cathode lamps reagents and standards power
GFAAS	argon gas hollow cathode lamps graphite tubes and cones reagents and standards power cooling water

Figure 3. Specific conditions for the operation of each technique (Lajunen, 2004)

Detection Limits

As principle, based on practice, GFAAS deliver the best detection limits from these two techniques (usually in the sub-ppb range), followed by FAAS (in the sub-ppm range) (Oliveira et al., 2017).

CONCLUSIONS

In order to make a parallel between the two AA techniques, anyone should objectively answer to a series of questions, such as:
Does this method have a *high applicability*?
What are *the sample types* to be analyzed?
What *elements* and *absorption ranges* are required?
How many components will be identified per each sample?
What is the *efficiency* and *precision requirements?*
Are *the analytical demands* likely to differ on a daily basis?
Are there *administrative issues* to take into consideration (e.g.: specific analysis protocol requirements)?
How many samples will be analyzed per day?

Who will be performing on the instrument?
What *level of operator's competence* is available?
How much *laboratory space* is available?
And (of course) what is *the available budget*?

	Flame AAS	GFAAS
Detection limits	Very good for some elements	Excellent for some elements
Sample throughput	10-15 secs per element	3-4 mins per element
Dynamic range	10^3	10^2
Precision		
short term	0.1-1.0%	0.5-5%
long term	2-beam 1-2% 1-beam < 10%	1-10% (tube lifetime)
Interferences		
Spectral	Very few	Very few
Chemical (matrix)	Many	Very many
Physical (matrix)	Some	Very few
Dissolved solids in solution	0.5-5%	> 20% (slurries)
Elements applicable to	68+	50+
Sample volumes required	Large	Very small
Semiquantitative analysis	No	No
Isotopic analysis	No	No
Ease of use	Very easy	Moderately easy
Method development	Easy	Difficult
Unattended operation	No	Yes
Capital costs	Low	Medium to high
Running costs	Low	Medium
Cost per elemental analysis		
High volume – few elements	Low	High
High volume – many elements	Medium	High

Figure 4. Summary of elemental analysis techniques (Lajunen, 2004)

REFERENCES

Christian G. D., Feldman F. Y. (1970). *Atomic Absorbtion Spectroscopy - Application in Agriculture, Biology and Medicine*, New York, USA: Wiley – Interscience Publishing House, 141-145.

Cordos, E., Manoliu, C. (1983). *The Absorbtion and Fluorescence Atomic Spectrometry.* Bucharest, RO: Tech. Buch. Publishing House, 98-104.

French W., Baxter D. C. (1990). *Spectrochimica Acta.* London: Elsevier Health Sciences.

Lajunen L. H. G. (2004). *Spectrochemical Analysis by Atomic Absorbtion and Emission.* Royal Society of Chemistry, 3(2), 78-84, 7(1), 276-285. doi: 10.1039/9781847551900.

Oliveira T. M., Augusto Peres J., Lurdes Felsner M., Cristiane Justi K. (2017). *Direct determination of Pb in raw milk by graphite furnace atomic absorption spectrometry (GF AAS) with electrothermal atomization sampling from slurries.* London: Elsevier Health Sciences. doi: 10.1016/j.foodchem.2017.02.143.

Rains T. C. (2008). *Chemical Interferences in Condensed Phase, in Flame Emission and Atomic Absorbtion Spectrometry*, Iowa, USA: Iowa State University Of Science and Technology, 1(3), 45-53.

Seely J. C., Hard G. C., Blankenship B. (2009). *Toxicology and Applied Pharmacology*, 5(3), 201-210.

Smith C. M. M., Nichol R., Littlejohn D. (1993). *J. Anal. Spectrom.* 8(5), 989-991.

Walker C. (2014). *Ecotoxicology: Effects of Pollutants on the Natural Environment.* Cleveland, Ohio: CRC Press Publishing House, 130-138.

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HISTOLOGICAL ASSESSMENT OF *CARASSIUS GIBELIO* MUSCLE TISSUE STRUCTURE UNDER BLACK CUMIN OIL FORTIFICATION AND STORAGE UNDER REGULAR AND ATYPICAL TEMPERATURES

Ștefania Mariana RAITA, Mara GEORGESCU*

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Veterinary Medicine College of Bucharest, 011464, 59 Mărăşti Blvd, District 1, Romania

*Corresponding author e-mail: dr_georgescu_mara@yahoo.com

Abstract

As Prussian carp (Carassius gibelio) is one of the most consumed local fish in Romania, the present study is focused on challenge testing of Nigella sativa fortified Carassius gibelio whole fish muscle sample morphological quality. The challenge testing involved exposure to atypical temperature conditions, other than those anticipated in the food industry, including stress temperature trial (exposure to storage temperature variations) for fresh Carassius gibelio whole fish, supplemented with 0.6% v/w Nigella sativa seed oil (NSSO). Histological evaluation of muscle structure was performed at day 3 of storage, for all samples. Histological assessment of dorsal skeletal fish muscle revealed no significant differences between the NSSO enriched challenged sample groups and the control group, after three days of storage. This study shows promising results for the possible use of Nigella sativa seed oil, as a natural solution for extending shelf life and enhancing the quality of cold-stored fresh Prussian carp.

Key words*: Carassius gibelio,NSSO, histological evaluation.*

INTRODUCTION

Prussian carp, *Carassius gibelio* is currently considered one of the most extensively spread fresh water species in Europe, Russia, Turkey and Asia. In Romania it is one of the most consumed species, being known as "the national fish", or "every Sunday Fisherman's fish" (Tăpăloagă D., 2017; 2018). Being one of the most recommended, nutritious and affordable food commodity, fish and fishery products must overcome their main disadvantage: the extremely high perishability (EUMOFA, 2018; Romania Insider, 2019; Sulieman H.M.A., 2012). As a consequence, extending fish shelf life without altering quality parameters, by using natural, inexpensive products, which are easily accepted by the consumers, remains a major research topic, as reflected by recent scientific literature (Jun M., 2019). Among various types of natural solutions, such as microbial-derived compounds (bacteriocins, reuterin, organic acids), algae and mushrooms, animal derived compounds, the plant-derived compounds are some of the most preferred: essential oils, plant extracts and natural wood smoke. Essential oils have antimicrobial and antioxidant properties which make them excellent candidates for shelf life extending in various perishable foods, such as fish and fishery products (Jun M., 2019).

There are numerous studies concerning the antimicrobial effects of *Nigella sativa in vitro* (Bakal S.N., 2017) and *in vivo* (Rafati S., 2014) against various spoilage and pathogenic microorganisms. *Nigella sativa* has been proposed as antibacterial solution for various types of commodities, such as cheeses (Georgescu M et al., 2018a) and fresh fish (Ozpolat and Duman, 2017).

Considering the antimicrobial and antioxidant potential of *Nigella sativa* seed oil (Georgescu M. et al., 2018b; Georgescu M et al., 2019), this study aimed to assess its influence on the histological structure of *Carassius gibelio* (Prussian carp) muscle tissue, subjected to various atypical cold storage temperatures and stress temperature treatment, during three days storage time.

MATERIALS AND METHODS

The present study is focused on challenge testing of *Nigella sativa* fortified *Carassius gibelio* (Prussian carp) muscle tissue quality. The challenge testing involved accelerated tests

at temperature conditions other than those anticipated in the food industry (5-10°C), including stress temperature trial (exposure to storage temperature variations) for fresh Prussian carp whole fish samples, supplemented with 0.6% v/w *Nigella sativa* (Black cumin) seed oil (NSSO).

Sample preparation

Carassius gibelio whole fish weighing 50-100 g/fish was caught early June 2019, from a designated Complex located 15 km away from Bucharest, called "Pescarium Corbeanca" (Corbeanca commune). Fish were transported to laboratory in Bucharest, in ice boxes. Sample preparation included gutting, removal of head and gills, and washing.

The *Carassius gibelio* individual fish were divided into two groups: control group (C), without NSSO and test group (T), fortified with 0.6% v/w NSSO. The test group (T) was further divided into 4 groups, subjected to different storage temperatures: on ice, and placed at refrigerator – group T1 (fresh ice was changed daily throughout the storage period), 0-4°C ± 1°C – group T2, 5-6°C ± 1°C – group T3 and stress temperature trial (STT) – group T4. The STT involved removing the treatment group samples from the refrigerator set to 6 ± 1°C, and keeping them at room temperature (21-24°C) for 5-10 minutes, daily, throughout the storage period. Group C (control) was stored on ice, and placed at refrigerator (in the same conditions as T1 group) throughout the storage period. Each group included two gutted, head-less and gill-less whole fish.

Nigella sativa cold pressed seed oil (NSSO), marketed under the name "Egyptian Black Cumin Oil (Ulei de Chimen Negru Egiptean)", was purchased from a Romanian company, Herbal Sana SRL, Bucharest (Figure 1). NSSO was displayed to the surface of *Carassius gibelio* whole fish samples in appropriate volume/weight using a micropipette, followed by mildly massaging the oil onto each sample using a gloved hand, according to the method described by Ozpolat E. and Duman M. (2017) (Figure 2).

Treatment groups were packed in plastic bags without using vacuum (using high barrier nylon polyethylene bags) (T2-T4), or were covered in ice (control group, C and T1 group) and stored at designated temperatures until analysis.

Figure 1. Black cumin oil - "Egyptian Black Cumin Oil (Ulei de Chimen Negru Egiptean)", Herbal Sana SRL, Bucharest

Figure 2. *Carassius gibelio* whole fish sample preparation: sample weighing (left); NSSO displaying onto the surface of *Carassius gibelio* whole fish samples in appropriate volume/weight using a micropipette (right)

Histological analysis

Fish muscle samples were prepared into 1-2 cm diameter sections, immediately fixed in buffered formalin and posteriorly embedded in paraffin. Once fixed, a dehydration was performed by increase of alcohol degree (70, 80, 96, 98), followed by immersion in xylene (twice) and two baths in paraffin, each sample remained 1 hour in each solution. Automatic processing took 5 hours. Histological sections of 5 μm in thickness, transverse and vertical, were obtained and subsequently stained with haematoxylin-eosin (HE) to evaluate the morphology patterns of the muscle fibers. To stain, a deparaffinization was carried out using a xylene immersion for three times (20, 15 and 10 minutes, respectively) and the tissue was rehydrated by decreasing of the alcohol degree, 100 (3 min), 96 (1 min), 80 (1 min) and 70 (1 min), followed by immersion in distilled water (3 min).

Data analysis

The study design included five batches of *Carassius gibelio* whole fish samples: control group (without NSSO), on ice, and placed at refrigerator (group C) and test groups T1-T4. All test groups (T1-T4), fortified with 0.6% v/w NSSO. Test groups were subjected to various temperatures: 3°C ± 1°C (T1), 5°C ± 1°C (T2), 7°C ± 1°C (T3) and stress temperature trial (STT) – group T4. The five batches of samples were considered the treatments, which were analyzed at day 3 of storage.

RESULTS AND DISCUSSION

For the control sample, the organization of *Carassius gibelio* dorsal skeletal muscle tissue exhibited a typical morphological pattern found in fish. Striated muscle from *Carassius gibelio* samples exhibited the typical morphologic pattern, multinucleated fibers with peripheral nuclei (Figure 3).

Histological evaluation of fish muscle revealed no significant differences between sample groups after three days of storage.

In all the examined samples, it is observed that the skeletal striated muscle fibers have an integral sarcolemma and the nuclei are disposed at the periphery having a flattened oval shape. The presence of numerous capillaries in the endomisium level is observed (Figures 4-7).

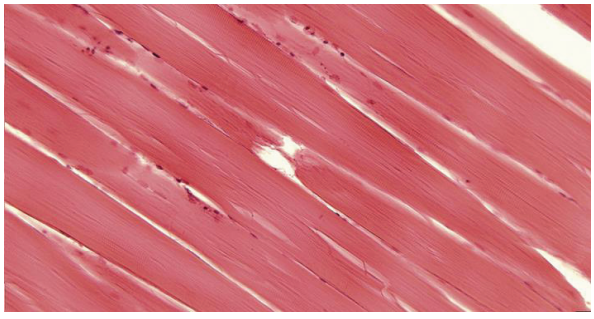

Figure 3. Muscle tissue organization in *Carassius gibelio* whole fish sample - **control sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

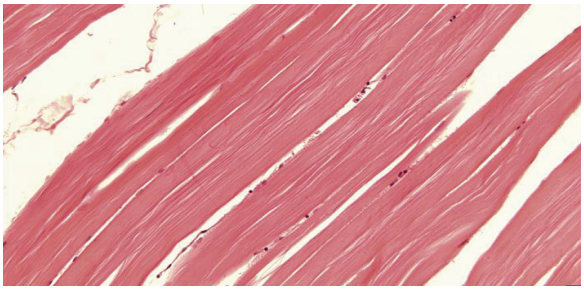

Figure 4. Muscle tissue organization in *Carassius gibelio* fillet -**T1 sample**
(Multinucleated fibers with peripheral nuclei and capillaries. Longitudinal section. Ob. 40 X, Col. HE)

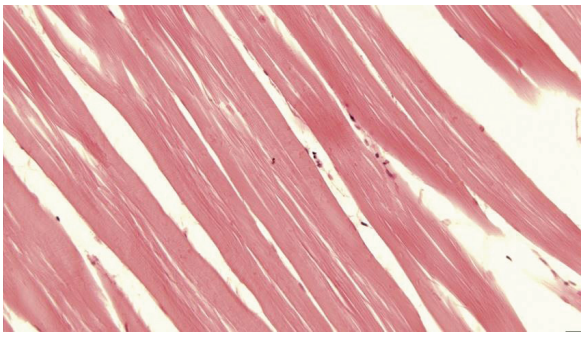

Figure 5. Muscle tissue organization in *Carassius gibelio* **whole** fish sample -**T2 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

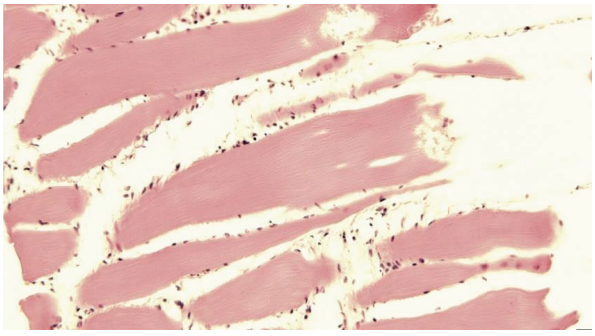

Figure 6. Muscle tissue organization in *Carassius gibelio* whole **fish** sample -**T3 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal and transverse section. Ob. 40 X, Col. HE)

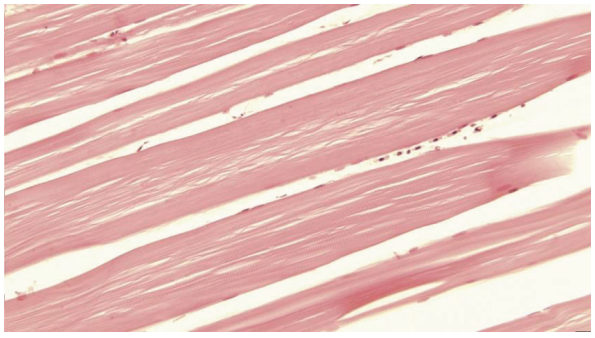

Figure 7. Muscle tissue organization in *Carassius gibelio* whole **fish** sample -**T4 sample**
(Multinucleated fibers with peripheral nuclei. Longitudinal section. Ob. 40 X, Col. HE)

Even if the recommended shelf-life for properly stored fresh fish is, according to FAO, as long as 16-21 days (adequately refrigerated on ice), the use-by date of most producers is between 3-4 days, while the sell-by date is as short as 1-2 days. Most studies indicate 15 days of adequately refrigerated storage as maximum time frame for good quality fresh fish, (Sulieman H.M.A et al., 2012). Considering the minimum sell-by period, of 1-2 days, our results of day 3 of storage were expected to reveal changes in the morphological structure of control samples and presumably differences for samples which were NSSO enriched. However, results indicated that all treatment groups maintained the normal morphological structure by day 3 of storage (including the ST group). Considering the literature data, the T2-T3 samples were expected to undergo certain degrees of morphological alterations of the muscle fiber, due to atypical temperature exposure, while the ST group sample (T4) was

expected to reveal significant alterations of the muscle tissue morphology. However, our results, which demonstrate maintaining of normal morphological structure in *Carassius gibelio* whole fish T1-T4 sample groups, suggest that *Nigella sativa* may be considered an efficient solution for prolonging the muscle quality and the shelf-life of fresh Prussian carp. Further research, involving larger study samples, could provide the statistical significance needed to support these findings. Also, sensory assessment of fresh and cooked NSSO enriched Prussian carp would be useful for selection of the most appropriate and efficient NSSO dosage.

CONCLUSIONS

The present study indicates that *Carassius gibelio* whole fish enriched with Nigella sativa seed oil and subjected to atypical and stress temperatures keep normal morphological structure of fish muscle throughout the storage period, despite the higher temperatures to which these treatments were subjected. These results suggest that fortification with NSSO might be associated with increased fish quality. NSSO fortification of *Carassius gibelio* whole fish exposed to stress temperature trial (STT), helps maintain the normal muscle tissue pattern morphology, with similar appearance to adequately stored NSSO-free fish. This study shows promising results for the possible use of NSSO as a natural solution for promoting longer shelf life and better quality for cold-stored *Carassius gibelio* whole fish.

REFERENCES

Bakal S.N., Bereswill S., Heimesaat M.M. (2017). Finding Novel Antibiotic Substances from Medicinal Plants - Antimicrobial Properties of *Nigella sativa* Directed against Multidrug-resistant Bacteria. Eur J Microbiol Immunol (Bp). 16;7(1):92-98. 2017. http://doi.org/10.1556/1886.2017.00001.

EUMOFA (2018). The EU fish market – 2018 Edition. European Market Observatory for Fisheries and Aquaculture Products. European Commision.

Georgescu M., Dobrea M., Tăpăloagă D, Raita Ş., and Dobrea V. (2018a). Functional evaluation of *Nigella sativa* seed oil effect on pathogen enriched artisan cheese. Journal of Biotechnology 280. S54. ISSN: 0168-1656. DOI:10.1016/j.jbiotec.2018.06.173.

Georgescu M., Raita Ş.M., Tăpăloagă D. (2019). Total nitrogen, water-soluble nitrogen and free amino acids profile during ripening of soft cheese enriched with *Nigella sativa* seed oil, The EuroBiotech Journal 3 (2), 90-9. DOI: https://doi.org/10.2478/ebtj-2019-0010.

Georgescu M., Savu C., Neagu I., Georgescu D. (2014). Coliform bacteria contamination profiling of raw milk cheeses using statistical assessment of type-linked correlations. AgroLife Scientific Journal. 3: 39-44.

Georgescu M., Tăpăloagă P.R., Tăpăloagă D., Furnaris F., Ginghină O., Negrei C., Giuglea C., Bălălău C., Ştefănescu E., Popescu I.A., Georgescu D., (2018b). Evaluation of antimicrobial potential of *Nigella sativa* oil in a model food matrix. Farmacia, 2018; 66(6): 1028- 1036.

Goncalves A.A. (2017). The PH of the seafood meat: a problem that deserves to be clarified. MOJ Food Process Technol. 2017;4(1):25-26. DOI: 10.15406/mojfpt.2017.04.00081.

Jun M, Xuan M., Jing X. (2019). Review on Natural Preservatives for Extending Fish Shelf Life. Foods 2019, 8, 490; doi:10.3390/foods8100490.

Kaifeng Li., Yulong B., Yongkang L., Huixing S. et al. (2012). Formation of Biogenic Amines in Crucian Carp (Carassius auratus) during Storage in Ice and at 4°C. Journal of food protection. 75. 2228-33. 10.4315/0362-028X.JFP-12-143.

Ozpolat E. and Duman M. (2017). Effect of black cumin oil (*Nigella sativa L.)* on fresh fish (*Barbus grypus*) fillets during storage at 2 ± 1 °C. Food Sci. Technol (Campinas) vol.37 no.1 Campinas Jan./Mar. 2017. http://dx.doi.org/10.1590/1678-457x.09516.

Rafati S., Niakan M., Naseri M. (2014). Anti-microbial effect of *Nigella sativa* seed extract against staphylococcal skin Infection. Medical Journal of the Islamic Republic of Iran. 2014; 28:42.

Romania insider (2019). Romania's fish market expected to reach EUR 380 mln this year. Available at https://www.romania-insider.com/romania-fish-market-estimation-2019. Accessed 20.06.2019.

Sulieman H.M.A., Bari L.O.A. and Hafiz M.A. (2012). Determination Of Quality And Shelf Life Of Three Marine Fishes (Coral Trout, Greasy Grouper And Red Mouthed Bream) Based On Total Volatile Nitrogen Test (TVN). International Journal of Livestock Research. Vol:2 (2) May 2012, pg.227-235.

Tăpăloagă D., Tapaloaga P.R., Ilie L.I., Georgescu M., Georgescu D.A. (2018). From conventional to organic agriculture - Romanian past and future perspectives -, Scientific Papers-Series D-Animal Science, Accession Number: WOS:000448065500043, ISSN: 2285-5750, eISSN: 2393-2260,Volume: 61,Issue: 1,Pages: 239-244, Published: 2018.

Tăpăloagă D., Tăpăloagă P.R. (2017). Study regarding animal organic farming in Romania - current status and trends, Scientific Papers. Series D. Animal Science. Vol. LX, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

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