

Veterinaria



ONCOLOGY OF THE SUGAR GLIDER

Stans

SURVEILLANCE OF FMD AND PPR DISEASES IN ALGERIA BY GIS AND WEB GIS

Dahmani, Khelifi-Touhani, RL Boufeniza, A Boufeniza, Chourak, Ouchene

EFFECTS NEW SYNTHES XANTHENE-3-ONES ON P. AERUGINOSA

Smajović, Veljović, Čelebić

MEAT QUALITY OF DIFFERENT COLOURED QUAILS

A Güngören, G Güngören, Şimşek, Yılmaz, Bahşi, Aslan

HEMATOLOGICAL AND IONIC PARAMETERS IN HOLSTEIN DAIRY COWS

M Mekroud, Khelifi-Touhami, Titi, Arzour-Lakehal, A Mekroud, Ouchene

FORENSIC MEANING OF CORE TEMPERATURE

E Dervišević, L Dervišević, Ajanović, Bešić, Salihbegović, Mujarić, M Dervišević

EFFICACY OF WHOLE CELLS VACCINE AGAINST EDWARDSIELLOSIS IN STRIPED CATFISH

Dwinanti, Utami, Tanbiyaskur, Rarassari, Prayogo, Mukti

GROSS BETA COUNTING IN RADIATION

Gradašćević, Karaman, Tepavčević, Mujić

SOYBEAN MEAL SUBSTITUTION IN QUAILS' FEED

Bensalah, Agabou

RECTOVAGINAL FISTULA AND ATRESIA ANI IN A KITTEN

Gezer, Buse Erdoğan, Yılmaz, Günay Uçmak, Baykal, Kırşan

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Content

Review Article

- 135**
ONCOLOGY OF THE SUGAR GLIDER (PETAURUS BREVICEPS): A COMPREHENSIVE REVIEW

Jelle Stans

Research Article

- 143**
FMD AND PPR DISEASES MONITORING AND SURVEILLANCE IN ALGERIA: APPLICATION OF GEOGRAPHIC INFORMATION SYSTEM

Ali Dahmani, Nadjet-Amina Kheliti-Touhani, Larbi-Redouane Boufeniza, Amira Boufeniza, Asma Chourak, Nassim Ouchene

- 155**
THE EFFECTS OF NEWLY SYNTHESIZED XANTHENE-3-ONES ON PSEUDOMONAS AERUGINOSA – EXPERIMENTAL STUDY ON RATS

Alisa Smajović, Elma Veljović, Mirza Čelebičić

- 165**
QUALITY PROPERTIES AND FATTY ACIDS COMPOSITION OF BREAST MEAT FROM JAPANESE QUAILS WITH DIFFERENT VARIETIES GROWN UNDER WARM CLIMATE

Alper Güngören, Gülşah Güngören, Ü. Gülcihan Şimşek, Ökkeş Yılmaz, Muammer Bahşi, Sultan Aslan

- 177**
HEMATOLOGICAL PROFILE IN HOLSTEIN DAIRY COWS ACCORDING TO THE DIFFERENT PHYSIOLOGICAL STAGE

Meriem Mekroud, Nadjet-Amina Khelifi-Touhami, Amal Titi, Nedjouda Arzour-Lakehal, Abdeslam Mekroud, Nassim Ouchene

- 187**
FORENSIC MEANING OF CORE TEMPERATURE - AN INDICATOR FOR ASSESSING THE SEVERITY OF HEATSTROKE IN AN ANIMAL MODEL

Emina Dervišević, Lejla Dervišević, Zuriša Ajanović, Aida Bešić, Adis Salihbegović, Ekrema Mujarić, Muamer Dervišević

- 197**
EFFICACY OF AUTOGENOUS FORMALIN KILLED WHOLE CELLS VACCINE TO AGAINST EDWARDSIELLOSIS IN STRIPED CATFISH (*Pangasionodon hypophthalmus*)

Sefti Heza Dwinanti, Nabella Aryani Utami, Tanbiyaskur, Madyasta Anggana Rarassari, Sugeng Prayogo, Retno Cahya Mukti

- 205**
GROSS BETA COUNTING METHOD IN RADIATION PROTECTION SYSTEM OF ANIMAL PRODUCTION CYCLE

Nedžad Gradašević, Nejra Karaman, Lana Tepavčević, Nedim Mujić

- 215**
PARTIAL REPLACEMENT OF SOYBEAN MEAL BY BROAD BEANS OR FIELD PEAS IN JAPANESE QUAILS' DIET

Amel Bensalah, Amir Agabou

- 227**
A CASE OF SIMULTANEOUSLY DETECTED RECTOVAGINAL FISTULA AND ATRESIA ANI IN A KITTEN

Bahar Gezer, Rabia Buse Erdoğan, Merve Yılmaz, Zeynep Günay Uçmak, Aslıhan Baykal, İsmail Kırşan

Professional Paper

- 233**
ANALYZING THE DISTRIBUTION OF SLAUGHTERED CATTLE BREED IN EDIRNE SLAUGHTERHOUSE

Sevinç Arap, Nursen Öztürk, Halil Güneş



*Obrazovanje -
put u budućnost*

REVIEW ARTICLE

ONCOLOGY OF THE SUGAR GLIDER (*PETAURUS BREVICEPS*): A COMPREHENSIVE REVIEW

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ABSTRACT

Sugar gliders (*Petaurus breviceps*) are increasingly being kept as pets. Despite their popularity, relatively little is known about the oncology of this species. To summarize the current body of knowledge, a literature search was performed to identify published cases. Only a small number of cases was identified that described cancer in sugar gliders. The identified case reports described several tumour types, ranging from mammary (adeno) carcinoma to haemangiosarcoma. In the majority of cases, no metastases were reported. In several cases, the primary tumour was removed using a surgical approach. Pain management also was a cornerstone of the treatment. The prognosis was often poor, with only two animals out of eight being alive at the time of publication. It is clear that further research is required to get more insight into the epidemiology and characteristics of cancer in this species.

Keywords: Cancer, cases, epidemiology, sugar glider, treatment

INTRODUCTION

The sugar glider (*Petaurus breviceps*) is a small, nocturnal marsupial originally from New Guinea and Australia (Campbell et al., 2019; Varriale et al., 2019). These animals are kept as an exotic pet in several countries such as Italy (Varriale et al., 2019) and the United States (Campbell et al., 2019). Therefore, several studies have investigated important aspects of husbandry such as nutrition (Dierenfeld, 2009) and behavioural aspects (Mallick et al., 1994). Work has also been published on medical aspects such as common emergencies (Lennox, 2007; McLaughlin and Strunk, 2016), common procedures (Johnson-Delaney, 2006), surgical procedures (Morges et al., 2009; Miwa and Sladky, 2016) and diagnostic approaches (Evans and Souza, 2010). Cusack and colleagues (2016) published a case of bilateral hydronephrosis, while Johnson-Delaney and Lennox (2017) investigated reproductive disorders. Other conditions, where case reports or series were published, include pulmonary hyalinosi (Sokol

et al., 2017), zoonoses (Pignon and Mayer, 2011), toxoplasmosis (Barrows, 2006) and cancer (Gentz et al., 2003; Sokol et al., 2017; Son et al., 2021). Several cases of neoplasia in sugar gliders have been described. Gentz and colleagues (2003) described 33 cases of neoplastic lesions of different histological types. In a more recent retrospective study, three cases of cancer in gliders were identified (Sokol et al., 2017). These studies did, however, not describe the case characteristics, such as symptoms and treatment in detail. Relevant details have been described in single case reports (Churgin et al., 2015; Chen et al., 2018). To this date, no review has been published. Therefore, this paper aims to provide a comprehensive review of the current knowledge of cancer in the sugar glider.

SEARCH STRATEGY AND LITERATURE INCLUSION

The Medline database (through PubMed) was searched using the keywords “Sugar glider*” (103 results) and “Petaurus” (85 results). Additionally, Google Scholar was searched using “Sugar glider tumor” (724 results), “Sugar glider cancer” (1970 results), “Petaurus tumor” (520 results) and “Petaurus cancer” (453 results). The titles of the publications in the search results were screened for potential eligibility for inclusion. The abstracts of these selected papers were read and the papers were included if they (1) described cancer in at least one sugar glider, (2) they mentioned at least basic information about the animal, (3) mentioned at least basic information about the disease and (4) mentioned at least basic information about the treatment and outcome. Relevant information was then extracted from the publication.

IDENTIFIED LITERATURE

Eight single case reports were identified that met the inclusion criteria (Hough et al., 1992; Marrow et al., 2010; Keller et al., 2014; Rivas et al., 2014; Churgin et al., 2015; Lindemann et al., 2016; Chen et al., 2018; Son et al., 2021). The extracted data is shown in Table 1.

Two case series (Gentz et al., 2003; Sokol et al., 2017) described several cases retrospectively, but did not meet the inclusion criteria because they did not report any information on the treatment pursued. No other case series were identified. Additionally, no studies investigating any risk factors for neoplastic disease in sugar gliders were identified. Finally, no prospective trials were identified that assessed potential treatment strategies.

CASE CHARACTERISTICS AND TREATMENT

The ages of the animals at presentation ranged from 4 (Hough et al., 1992) to 15 years (Lindemann et al., 2016), while the average age was 8.5 years. Three out of the eight animals were male. Little additional consistent information about the animals was mentioned.

Two cases of mammary (adeno)carcinoma were reported (Keller et al., 2014; Churgin et al., 2015). Other types of cancer ranged from lymphosarcoma (Hough et al., 1992) to hemangiosarcoma (Rivas et al., 2014). In the majority of cases, no metastases were reported. Symptoms were very variable and depended on the type and location of a tumour. Likewise, the treatment also varied significantly according to the specific case. However, surgical removal of the primary tumour was performed in several cases (Keller et al., 2014; Rivas et al., 2014; Chen et al., 2018). In several cases, antibiotic treatments were also initiated. It is also clear that pain management is very important.

Unfortunately, in five cases (Hough et al., 1992; Marrow et al., 2010; Keller et al., 2014; Churgin et al., 2015; Lindemann et al., 2016), the animal was eventually euthanized at the time the case was published. In one further case, the animal died (Son et al., 2021). In only two cases, the glider was alive without signs of cancer (Rivas et al., 2014; Chen et al., 2018).

Table 1 Extracted data from the included case reports

Ref.	Age	Sex	Cancer type	Metastasis	Symptoms	Treatment	Outcome
Hough et al., 1992	4 yrs.	M	Cutaneous lymphosarcoma	Lymph nodes Sinusoids of the spleen	Initial Suppurating ulcer right hind leg Small vesicles around pinnae Swollen digits Day 7 Reduced swelling digits Areas with vesicles increased in size Day 18 Extended limb ulceration Most digits swollen Nodules on trunk skin	Initial (for diagnosis of dermatitis / infection) Lincomycin (30 mg / day) Metacresolsulphonic acid (topical) Day 7 (for diagnosis of dermatitis / infection) Debridement of ulcer Amoxycillin (30 mg / day) Topical gentamicin Power containing zinc bacitracin (250 U/g) Neomycin sulphate 5 mg/g Plymixin B sulphate 5000 U/g Day 18 (for diagnosis of dermatitis / infection) Metronidazol (80 mg/kg oral / day)	Euthanized 5 days after day 18 treatment
Marrow et al., 2010	10 yrs.	M	Paracloacal transitional cell carcinoma with squamous differentiation	None identified	Initial No clinical signs Pericloacal mass 5 months Self-mutilation cloacal skin Tenesmus Reduced food intake Large, firm mass Abdominal distention	Initial Enrofloxacin (5 mg/kg orally every 12 hours for 10 days)	Euthanized 5 months after initial presentation
Keller et al., 2014	9 yrs.	F	Mammary carcinoma	None identified	Initial Subcutaneous mass right caudal abdomen Days after surgery at day 5 Self-mutilation of tail	Initial Meloxicam (0.5mg/kg oral, once a day) Day 5 Surgical excision 150 Gy of strontium-90 radiation therapy	Euthanized within 14 days after the surgery

Ref.	Age	Sex	Cancer type	Metastasis	Symptoms	Treatment	Outcome
Rivas et al., 2014	11 yrs.	F	Dermal hemangiosarcoma	None identified	Initial No overt clinical signs Lesion on right patagonium Day 9 Increased size of lesion	Initial (for diagnosis of infected wound) Meloxicam (0.2 mg/kg, orally, every 24 hours, for 10 days) Trimethoprim-sulfamethoxazole (15 mg/kg, orally, every 12 hours) Day 9 Surgical excision Post-operative care Enrofloxacin (10 mg/kg, orally, every 24 hours for 7 days) until day 28 after presentation Meloxicam (0.2 mg/kg, orally, every 24 hours for 4 days) until day 28 after presentation	No local or systemic sign of disease after 1 year
Churgin et al., 2015	9 yrs.	F	Mammary adenocarcinoma	Sublumbar Pouch lymph node Axillary lymph nodes Lung	Initial Lethargy Partial anorexia Mass within the pouch 6 months Weight loss Growth of pouch mass 7 months Inability to bear weight on right rear leg Growth of pouch mass	No specific treatment	Euthanized 7 months after initial presentation
Lindemann et al., 2016	15 yrs.	F	Adrenocortical carcinoma Hepatocellular carcinoma	None identified	Initial Diarrhea Weight loss Mass in midabdomen Circling to the right Impaired vision 72 hours Lethargy Circling to the right Impaired vision	Fluid therapy 40 mL/kg subcutaneously 2.5% dextrose Vitamin B complex 0.05 mL subcutaneously Amoxicillin-clavulanic acid 12.5 mg/kg orally, every 12 hours for 10 days Prednisolone 0.2 mg/kg, orally every 12 hours	Euthanized 72 hours after presentation

Ref.	Age	Sex	Cancer type	Metastasis	Symptoms	Treatment	Outcome
Chen et al., 2018	4 yrs.	M	Paracloacal gland carcinoma	None identified	Initial Self-mutilation cloacal region Vocalisation & straining during elimination Hyperemic & swollen pericloacal region Day 178 Swelling Vocalisation during elimination Cloacal bleeding Reduced activity Reduced appetite Day 217 Right side cloaca larger than left Mass below skin Day 229 Swelling right side cloaca Intensified straining during elimination with blood Four weeks after day 229 Vocalisation during elimination	Initial Correction of husbandry Enrofloxacin 5mg/kg orally, twice a day Metronidazole 25mg/kg orally, twice a day Meloxicam 0.2mg/kg orally, once a day (42 days) Nystatin 2000 IU/kg orally, twice a day for 10 days E-collar (one month) Day 178 E-collar Tramadol 2mg/kg orally, twice a day (5 weeks) Meloxicam 0.2mg/kg orally, once a day (5 weeks) Sulfamethoxazole/trimethoprim 15mg/kg orally, twice a day (5 weeks) Metronidazole 25mg/kg orally, twice a day (5 weeks) Metoclopramide 0.1mg/kg orally, twice a day (5 weeks) Sucralfate 10mg/kg orally, twice a day (5 weeks) Day 217 Chloramphenicol 25mg/kg orally, twice a day (1 week) Day 229 Surgical removal of glands Ciprofloxacin 10mg/kg orally, twice a day Meloxicam 0.2mg/kg orally, once a day Metronidazole 25mg/kg orally, twice a day Tramadol 2mg/kg orally, twice a day Lactulose 0.5mg/kg orally, twice a day (7 days) E-collar	Alive 515 days following surgery
Nguyen et al., 2021	6 yrs.	F	Disseminated histiocytic sarcoma	Urinary bladder Medial iliac lymph nodes Pericloacal mass Liver Spleen Small intestine Uterus Left ovary	Initial Swelling at pouch	None mentioned	Died 8 days after initial presentation

DISCUSSION AND CONCLUSION

A relatively small number of case reports was identified about cancer in sugar gliders. This means that there is a clear lack of external and internal validity in the current body of literature.

There was a wide range of ages at presentation. However, with an average of 8.5 years, it could be stated that it seems that age is a potential risk factor for cancer in gliders. There was a relative equal distribution between males and females. However, based on these limited cases, it is very difficult to make sound conclusions about potential risk factors and epidemiology of the condition.

There were several different histological types of cancer described in the case reports. Previous case series have reported more cases and could provide a better overview of the most common types of cancer that occur in sugar gliders (Gentz et al., 2003; Sokol et al., 2017). In most cases, there were no metastases reported. However, it is not clear which mechanisms play a role in metastasizing in this species and which types may be predisposed to metastasize.

Several treatment strategies were utilized depending, amongst others, on the type and location of the primary tumour. Based on the current results, it does not seem feasible yet to develop standardized, evidence-based treatment guidelines for specific cancer types. It is, however, clear that surgical excision is an important component of several treatment

schedules. Unfortunately, most of the animals were euthanized or died, while only two animals were alive at the time of publication of their case. This confirms that further research is required to assess and establish therapeutic strategies.

Further research could start with a better reporting of cases. An important role could be played by communities of owners. These communities are very active in discussing health problems with their pets. They could be approached to assist in the recording of cases. Additionally, retrospective studies could be conducted of cases already seen in veterinary practices. Finally, prospective clinical trials should be conducted to establish effective and save therapeutic strategies.

Relatively few case reports were identified regarding cancer in sugar gliders. There was a large variation in the age at which the animals were diagnosed and in the tumour types that were described. In several of the published cases, surgical removal of the primary tumour was performed. This was often complemented with pain management. Unfortunately, prognosis was very poor, since only two out of eight animals were alive at the time of publication. Further research is required to get more insight into the epidemiology and characteristics of cancer in this species.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest.

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ONKOLOGIJA MALOG ZLATNOG LETAŠA (*PETAURUS BREVICEPS*): SVEOBUHVAATNI PREGLED

SAŽETAK

Mali zlatni letaši (*Petaurus breviceps*) se sve češće drže kao kućni ljubimci. Uprkos njihovoj popularnosti, malo je poznato o onkologiji ove vrste. Kako bi se napravio presjek postojećih saznanja na ovu temu, izvršen je pregled literature sa ciljem identifikacije objavljenih slučajeva, a koji su kod malih zlatnih letaša pronađeni u malom broju. Identificirani prikazi slučajeva su opisali nekoliko tipova tumora, od mamarnog (adeno) karcinoma do hemangiosarkoma. U većini slučajeva nisu opisane metastaze. U nekim slučajevima, primarni tumor je otklonjen operativnim putem. Terapija bola je predstavljala osnovu terapije. Prognoza je često bila loša, pri čemu su u doba publikacije samo dvije životinje od njih osam bile žive. Jasno je da su potrebna daljnja istraživanja kako bi se stekao bolji uvid u epidemiologiju i karakteristike karcinoma ove vrste.

Ključne riječi: Karcinom, slučajevi, epidemiologija, mali zlatni letaš, terapija

RESEARCH ARTICLE

FMD AND PPR DISEASES MONITORING AND SURVEILLANCE IN ALGERIA: APPLICATION OF GEOGRAPHIC INFORMATION SYSTEM

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ABSTRACT

The study aimed to describe the evaluation and implementation of spatial databases that are directly related to animal health in GIS and web-GIS by visualization of the spatio-temporal distribution of animal diseases such as peste des petits ruminants (PPR) and foot-and-mouth disease (FMD) in Algeria. A methodology has been adapted based on the classical steps of GIS and performed using freely available Qgis 3.10. Such methodology can be largely applied to different types of diseases. We have also created a model of website-based «VETALGIS» (Veterinary Algerian GIS) in order to digitalize the veterinary sector and minimize the problem of the lack of data, organize data and facilitate access, which will improve networking and communication between institutions responsible for livestock disease management. GIS spatial analysis techniques have proven to be a useful tool that can support the decision-making process in planning, implementing and monitoring FMD and PPR control strategies in endemic and high-risk areas.

Keywords: Geographical Information System (GIS), peste des petit ruminants, foot and mouth disease

INTRODUCTION

The livestock sector is one of the most important agricultural sectors, particularly in developing countries. However, the subsistence of pastoral livestock is limited by the frequent presence of trade-sensitive diseases that can affect the economic aspect of any country. This can make their potential for infection a further challenge to control. Some of those diseases are PPR and FMD (Paton et al., 2009; Knight-Jones and Rushton, 2013; Gitonga, 2015; Amaral et al., 2016).

In recent years, new and modern tools have proven essential for monitoring and surveillance of these diseases (Mfmf et al., 2018).

The geographic information system (GIS) is one of those tools which has been used for a wide variety of purposes in different fields, including veterinary sciences, especially epidemiology (Hay, 2000, Rinaldi et al., 2006).

The study aimed to describe steps in the evaluation and implementation of a spatial database directly related to animal health using GIS/web-GIS for the visualization of the spatio-temporal distribution of animal diseases, such as FMD and PPR in Algeria. It also aimed to show the utilisation of GIS/Web GIS techniques in the planning and implementation of disease monitoring and control programs in endemic and high-risk PPR and FMD areas in Algeria.

MATERIAL AND METHODS

Study area

The selection of areas to be included in the study was made intentionally and on the basis of available financial resources and the following criteria: (1) The area is classified as an endemic or high-risk area for PPR on the basis of Director of Veterinary Services (DVS) recordings and official disease reports (Elsawalhy et al., 2010; Gitao et al., 2014; FAO and OIE, 2015); (2) The inhabitants are pastoralists with high sheep and goat populations (KNBS, 2009); (3) Is an important route for small ruminant stocks to neighbouring countries (Aklilu, 2008). Study has been divided into two scales: the small scale and the large scale. The large scale represents entire country of Algeria (Figure 1), while the daïra of Ksar El Boukhari was selected for the small scale (Gitonga, 2015).

Administrative data

Data was collected from the Ministry of Agriculture and Rural Development (MADR) of the People's Republic of Algeria (<http://madrp.gov.dz/>). These records include information such as animal population and disease outbreaks, which are linked to geographical data by being captured at a particular address or region (hence, the usefulness of relational databases). In this study, the detailed information contained in the reports was structured

in the databases and divided into two scales (large and small scale).

Large scale

In order to investigate the distribution characteristics of FMD and PPR in Algeria, the data on FMD and PPR cases from different years (2014-2015-2016-2018 for FMD and 2011-2012-2013-2016-2018 for PPR) were collected from the DVS of the Ministry of Agriculture of Algeria.

Small scale

Number of affected animals in the daïra of Ksar El Boukhari in 2019 was collected from the Agriculture Directorate of media province, Ksar El Bukhari subdivision.

Survey data

Data were collected for a specific purpose, often in a single procedure. Updating the data requires significant effort and can only be performed occasionally. One of the main difficulties with the survey data is that information quickly becomes obsolete, especially in countries with rapidly growing development and populations (Vinodhkumar et al., 2016). In the present study, data were represented by geographical coordinates (x,y) derived from the addresses obtained from the Agriculture Directorate of media province, Ksar El Bukhari subdivision.

Internet data

Data on FMD and PPR cases reported in the last few years were collected from the World component and transformed from its existing form into a form that can be used by GIS. Data for use in GIS may be available in a variety of formats:

In this case, the vector layer "DZ Admin" was downloaded from the National Institute for Mapping and Remote Sensing (INCT) (<http://www.inct.mdn.dz/webinctsim/telechargement.php>). The second vector layer is in the form of a point, contains the information collected on the ground and/or acquired from the Minister of the MADR concerning the fields of information shown.

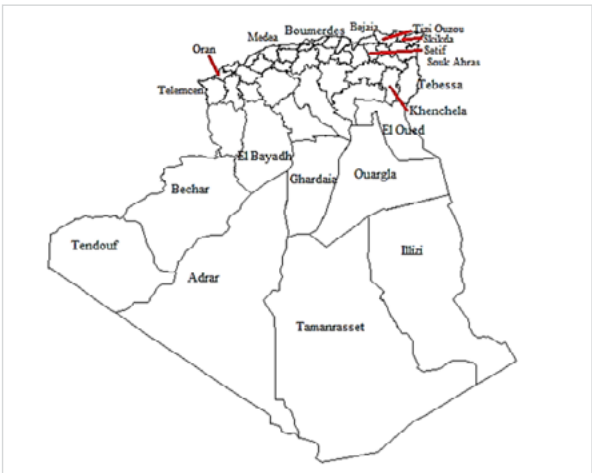


Figure 1 Presentation of study area

RESULTS

Large scale

PPR results Goat PPR

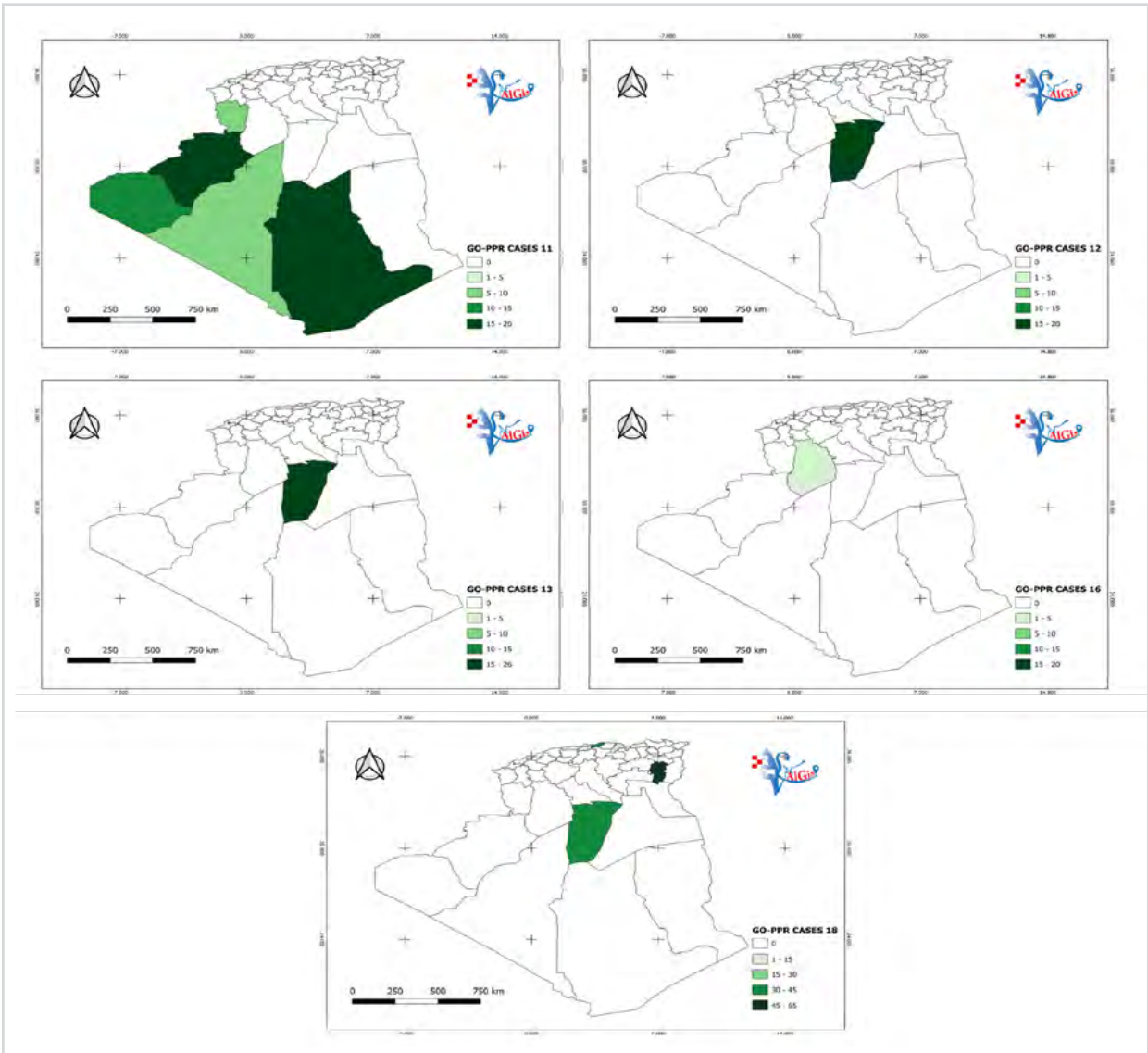


Figure 2 PPR in goats in Algeria

The maps (Figure 2) show that goat PPR cases in Algeria were concentrated in the Saharian states (Tamanrasset, Bechar, Tindouf, Adrar) in 2011, while some cases have been reported in Ghardaia

in 2012 and 2013, as well as Beyedh in 2016. For 2018, we have recorded the highest level of cases in Khenchela, Ghardaia, Bejaia and Boumerdas.

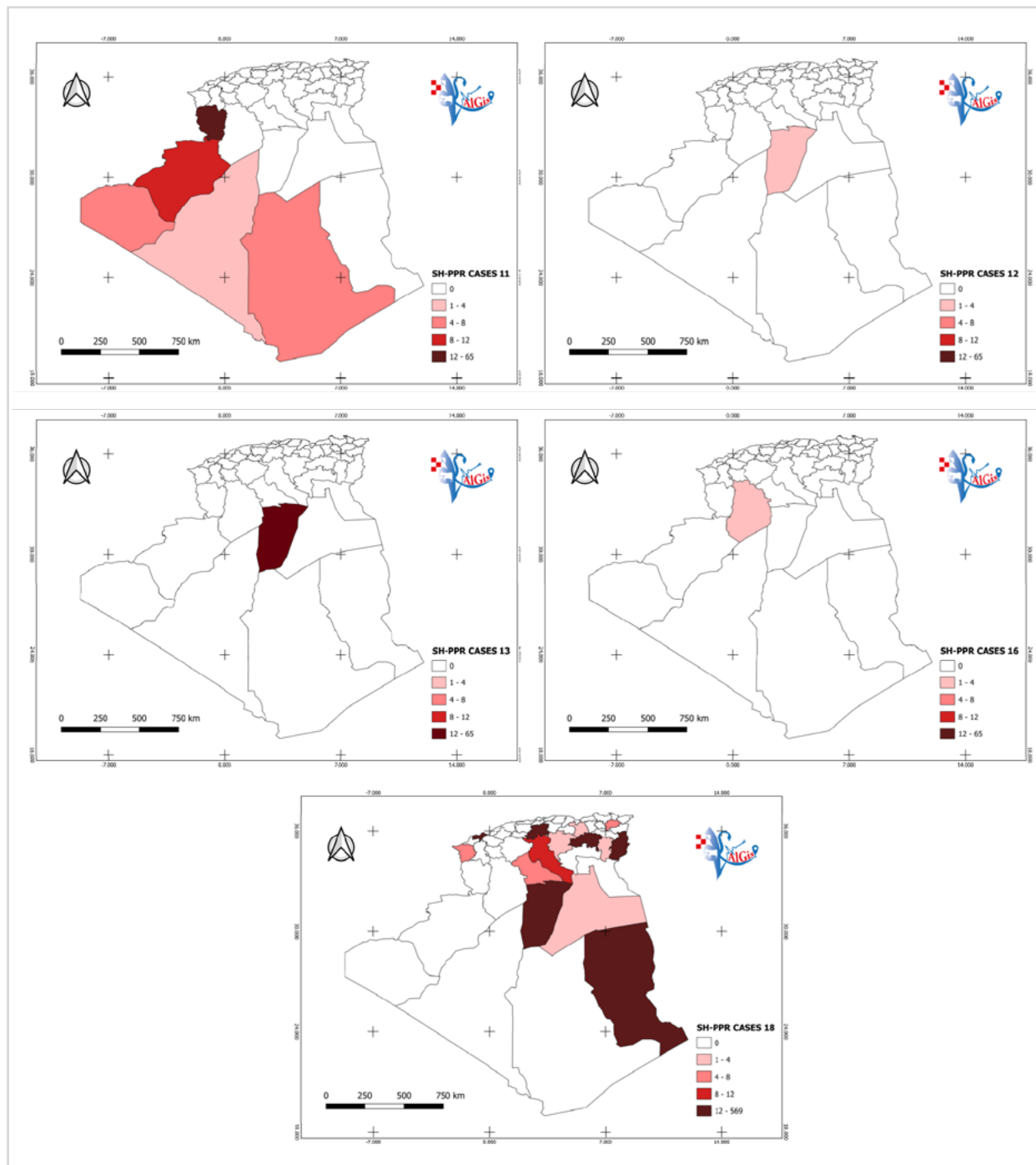


Figure 3 Distribution of PPR cases in sheep in Algeria

Sheep PPR

The figure 3 shows a random distribution of cases over the years (2011, 2012, 2013, 2016 and 2018, respectively). The highest number of cases was reported in 2018 in Illizi, Ghardaia, Tebessa, Khenchela, Medea and Oran.

Cattle FMD

Cattle FMD cases were recorded in the northern and interior states in 2014 while we record just one case in El Oued in 2015. In the years 2017 and 2018 cases were recorded from different states of the coast, the interior and the north of Sahara. The highest number of cases was reported in Setif (Figure 4).

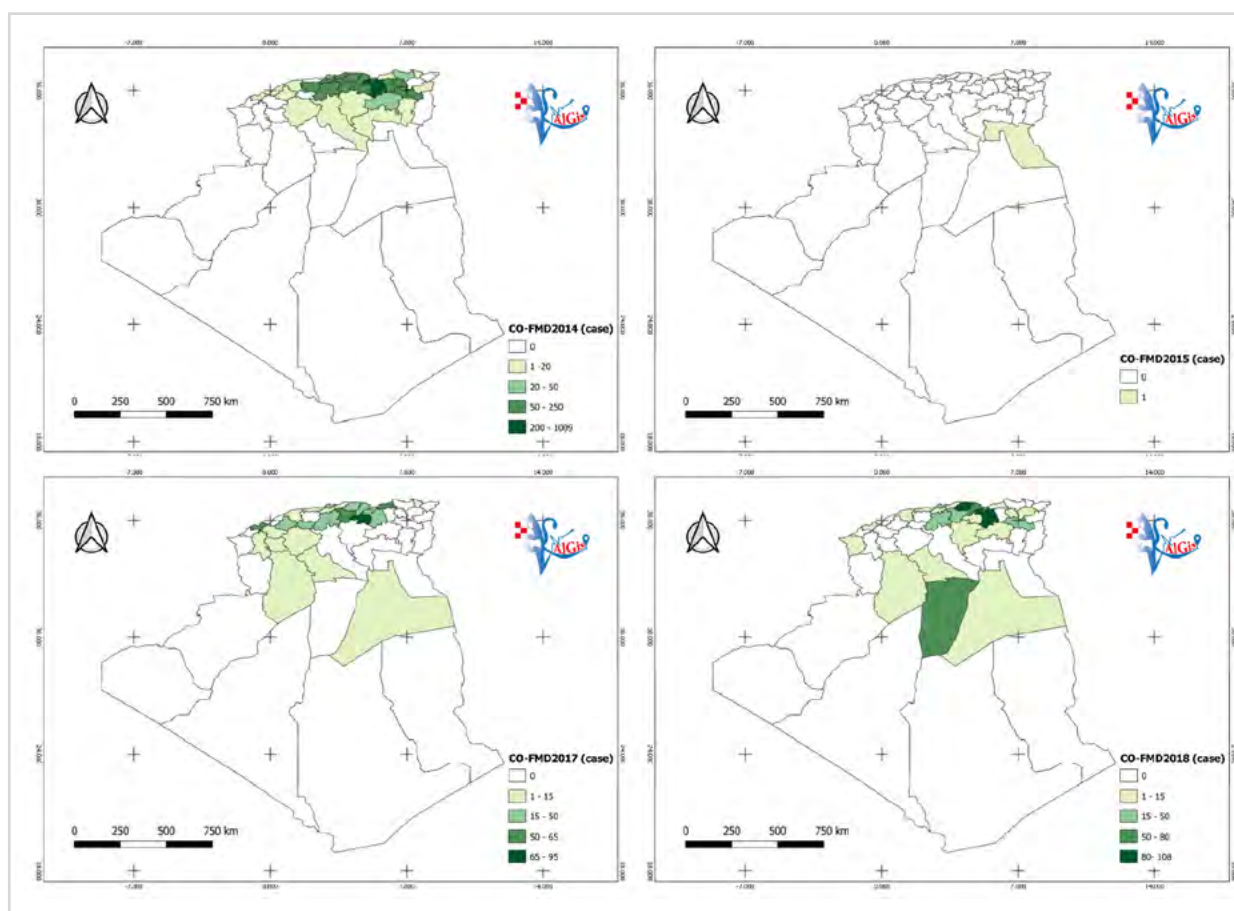


Figure 4 Distribution of FMD cases in cattle in Algeria

Ovine FMD

No sheep FMD cases were registered in 2014, while the highest number of cases was recorded

in El Oued in 2015. In the years 2017 and 2018, cases were recorded from different states of the interior and Sahara (Figure 5).

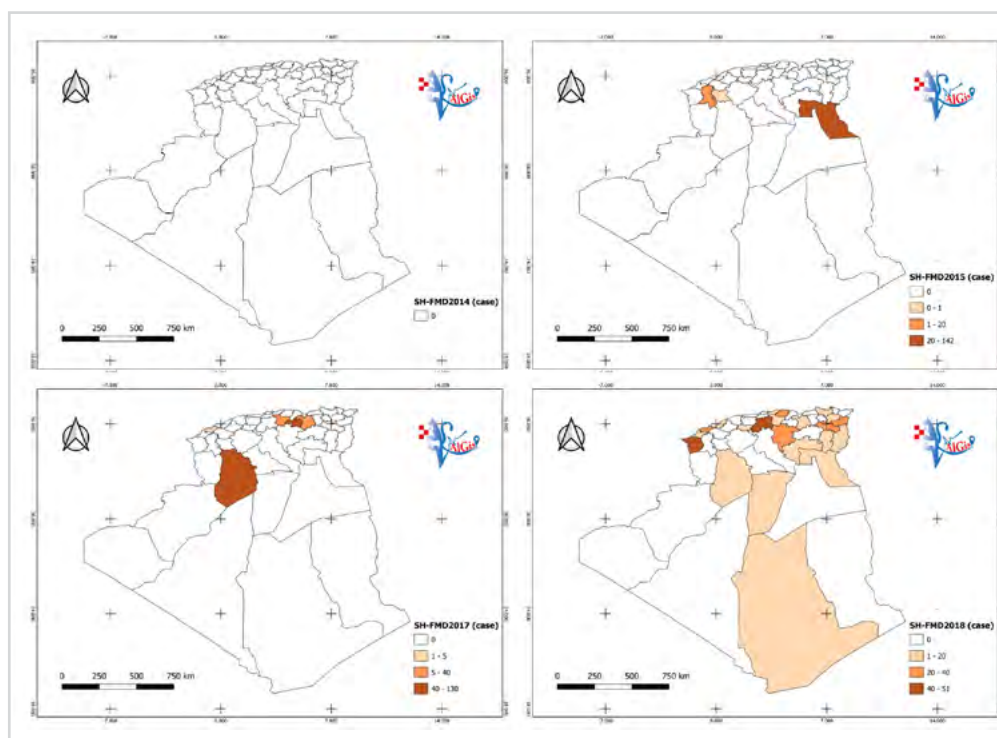


Figure 5 Distribution of sheep FMD in 2014, 2015, 2017 and 2018

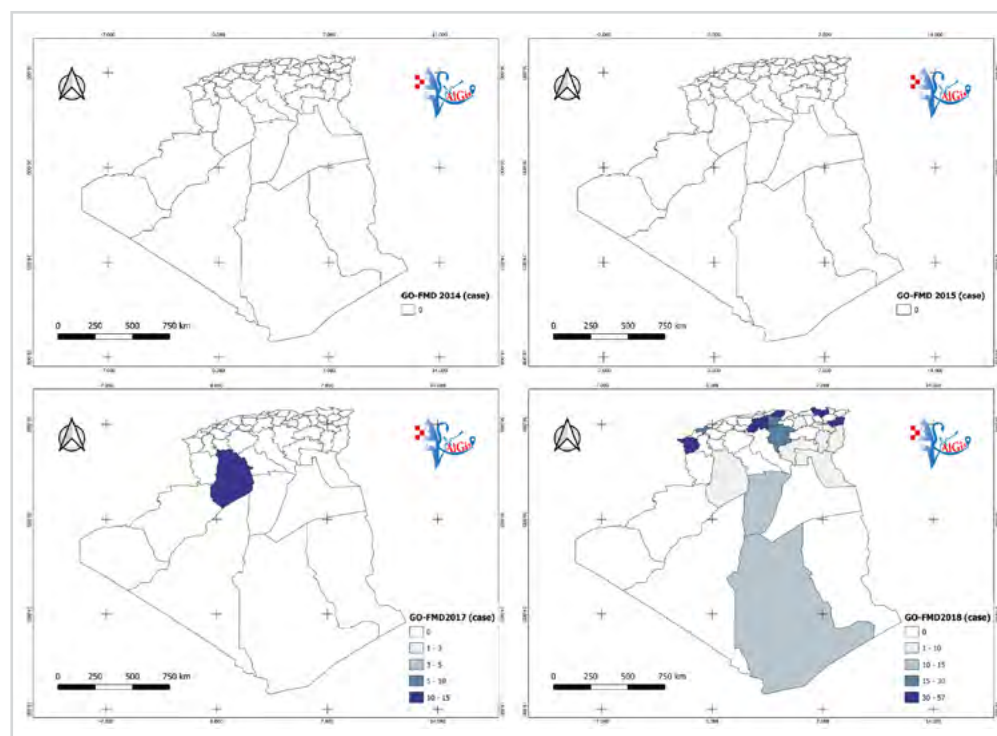


Figure 6 Distribution of goat FMD in 2014, 2015, 2017 and 2018

Goat FMD

Case of goat FMD was not registered in neither 2014 nor 2015. In the year 2017, several cases were registered in Beyedh, and the highest number of cases were recorded in 2018 in Souk Ahras, Skikda, Medea, Tizi Ouzou and Tlemcen (Figure 6).

Small scale

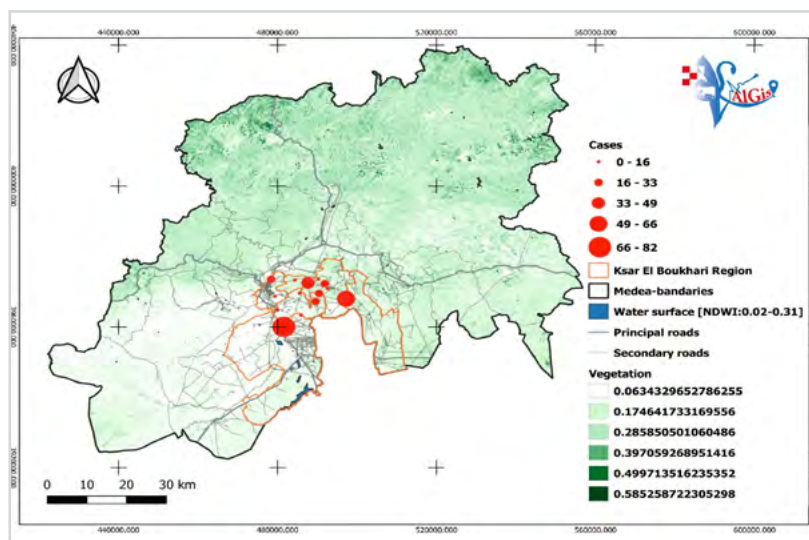


Figure 7 Medea's map showing FMD outbreaks declared in January and February, 2019

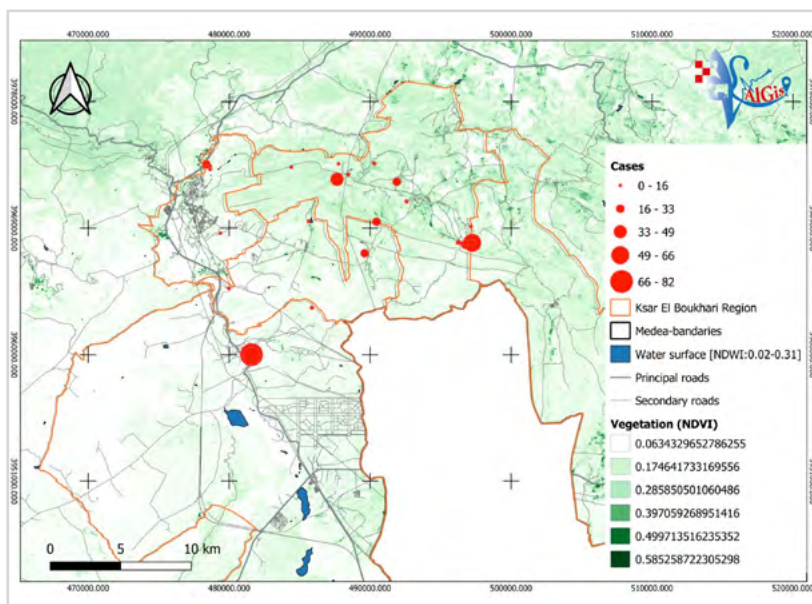


Figure 8 FMD outbreaks in Ksar El Boukhari region declared in January and February, 2019 The FMD outbreaks were declared in Saneg, Mfatha, and Ksar El Boukhari (Figure 7, 8)

E/ Web map results

Figure 9 shows the map completed and exported in the web and accessibility to the outbreak information by a click.

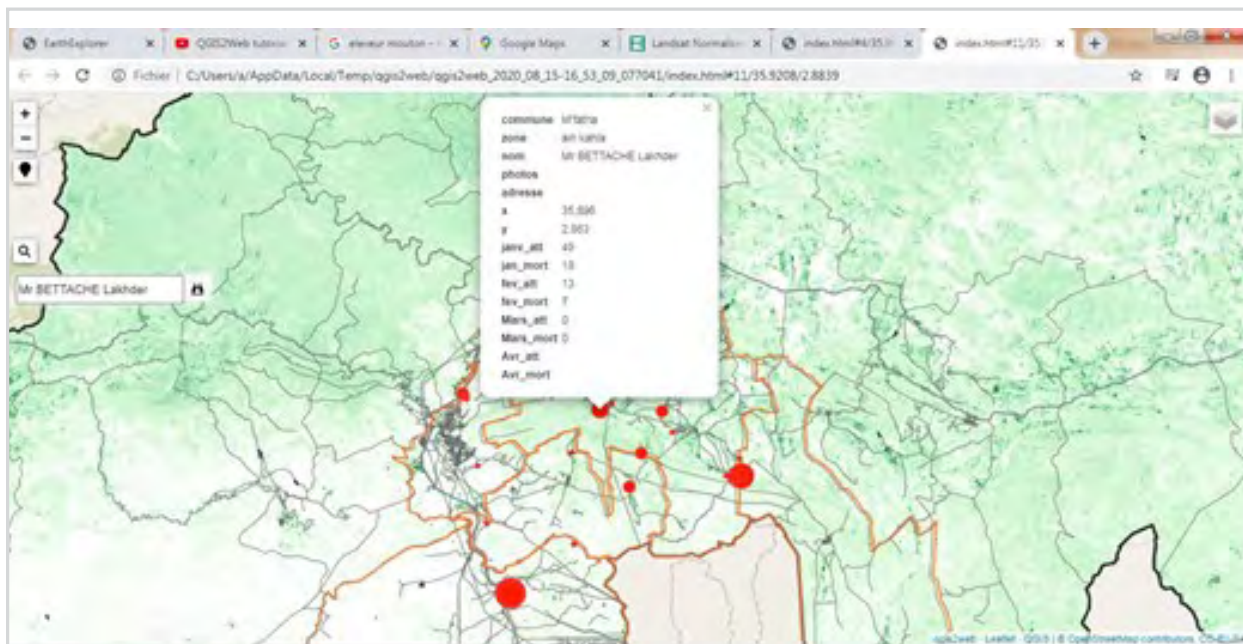


Figure 9 Medea's web map exported by the plugin QGIS2Web

DISCUSSION AND CONCLUSION

Small-scale results revealed that most FMD cases were located along the main road, pasture and communal water points. Therefore, it can be estimated that the directional distribution of the disease may be related to animal transport from breeding areas, shared pastures and watering points. It is also well known that biological, ecological and meteorological factors can influence the emergence of infectious diseases.

Algeria is a vast country, and there are obvious climatic differences between the different regions. Thus, the weather conditions may also influence the transmission of FMD virus and PPR virus. The specific factors that explain this directional transmission pattern need to be further investigated (He et al., 2015; Ma et al., 2017).

The lack of information on notifiable diseases in any country has been largely attributed to the lack of notification of these diseases; this has led to a failure of the already weak surveillance and reporting structure (Catley et al, 2012), which

wasthe case in the present study.

The VETALGIS digital platform is a website that allows easy and rapid access to available information on livestock diseases.

PPR and FMD outbreaks and cases were reported by the Ministry of Agriculture of the People's Republic of Algeria. As shown in the results, the number of FMD outbreaks reported in 2018 was lower than the previous years, which is a reminder that more effective measures have to be implemented to prevent future outbreaks. The number of PPR cases was high compared to previous years, which means that there were some gaps at the level of the prevention system.

In fact, this study has revealed a great amount of information, particularly on the distribution and contribution of different species to disease transmission, between and within farms. But, it is difficult to determine to what extent these findings can be generalized to other situations, with different animals, livestock systems, livestock and human networks, climates and pre-existing levels of immunity.

The three main limitations constraining the livestock disease control efforts in Algeria are the lack of inclusion and correlation when planning livestock disease control programs, underreporting, and the lack of veterinary services and a frail livestock identification system.

The first difficulty faced was due to underreporting of the PPR and FMD epidemics at the county level. This affected the information available in the offices of the DVS. Reports were only available for a few years (2014-2015-2016-2018 for FMD and 2011- 2012-2013-2016-2018 for PPR). Due to these limitations, maps of the spatial and temporal distribution of PPR and FMD outbreaks in Algeria could not be well generated, showing heterogeneity in the analysis. The other challenge was to identify the areas to be included in the study, without official outbreak reports.

Accordingly, the following recommendations are made:

The appropriate government agency should develop and support the application of GIS in veterinary science to prevent and reduce the spread of disease and to evaluate its economic impact;

The clinical veterinarians and their staff should know how to use GIS in the office and in the field;

It is strongly recommended that GIS tools and their applications be used to produce maps for slaughter site selection in urban areas of Algeria;

The policies for livestock disease control should not be applied as general interventions, but need to be based on the ecological, social, cultural and economic context of the target communities;

It is strongly recommended that the government strengthens prevention and control measures for the sensitive areas, and that the export of animals and livestock products be strictly limited;

Veterinary surveillance can reinforce by several actions implemented in the region, such as the geo-localization of holdings and the individual identification of all animals in the region;

One of the main problems in obtaining the status of 'free zone with vaccination' is the maintenance of this health status, due to the permanent surveillance procedures required;

As eradication of FMD is not feasible in many endemic countries, hence it is necessary to assess the cost-effectiveness of control if the incidence of the disease can be reduced by continuous mass vaccination. There is a need to explore opportunities to share GIS applications and innovations specifically focused on veterinary science;

Enforcement of transportation restrictions on animals from both FMD-affected and high-risk areas is deemed an imperative.

The application of GIS in veterinary science is progressing rapidly, and it is necessary for every veterinarian to understand the basics of GIS to support and advance its use in veterinary science. For this purpose, the potential of GIS applications in this field is recognized to be enormous. However, the community of users of GIS in veterinary science is rather small compared to the other sectors;

Due to the transboundary nature of FMD and PPR, a single country in an endemic area is unable to control and progressively eradicate PPR unless its neighboring countries share a similar objective. The Global Strategy also recommends the establishment of regional roadmaps for PPR. These strategies aim to reduce the PPR burden in endemic countries while developing strategies to prevent the PPR introduction into countries free of the disease;

The following recommendations should be taken into account, as PPR control strategies in pastoral areas of Algeria should be adapted to specific geographical regions taking into account prevalent diseases of small ruminants, existing disease control practices, the socio-economic status of communities and access to veterinary services. PPR annual vaccination program should also include CCPP vaccination of goats as well as target animals aged 6 to 12 months. Policy makers should

adopt the use of GIS and post-vaccine serological surveys to monitor the effectiveness and coverage of PPR vaccination campaigns in pastoral areas of Algeria;

For endemic areas, it is necessary to examine the current impact of FMD and then to evaluate possible control measures, their effectiveness and cost. A costbenefit analysis can then assess the impact over time; when control measures are in place, benefits and costs will vary between the groups over the long term of a control program. More investigators need to adopt the use of GIS spatial analysis techniques in the design, impact monitoring and development of control strategies for infectious livestock diseases; Other studies should be conducted using GIS techniques to monitor the evolution and distribution of animal diseases in Algeria, detect the different problems, and solve them later in order to preserve the public health and livestock resources in Algeria.

GIS represents a new technology in veterinary sciences, which provides significant added value in animal health, by studying, reporting information and modeling animal disease problems. As routine data are usually taken into low consideration for either epidemiological or management purposes in veterinary sciences, GIS can considerably increase the efficacy of communication to exploit those data. In the field of veterinary science, this methodology can be largely applied to different types of diseases. There are several situations in which it is suggested that GIS will play an increasingly important role in the future: the need to solve epidemiologically complex disease problems, and the need for rapid surveillance of highly contagious transboundary

diseases. However, applying sophisticated spatial techniques to poor-quality data will not create an insightful investigation. The people working with GIS should keep in mind that maps cannot be an alternative for better data collection and recording. The maps will never be better than the original input data. It's a mere translation of information in a language which is easy to understand.

Impact of FMD and PPR is high for some regions, but low for others. This impact varies according to the incidence of the disease and also the positive and negative impact of control measures, levels of access to veterinary services, animal health inputs and livestock markets.

GIS spatial analysis techniques have proven to be a useful tool that can support the decision-making process in planning, implementing and monitoring FMD and PPR control strategies in endemic and high-risk areas.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS:

Concept - DA, KTNA, BRL, ON, BA, CA; Design - DA, KTNA, BRL, ON, BA, CA; Supervision - KTNA, ON; Resources - DA, BA, CA; Materials - DA, BA, CA; Data Collection and/or Processing - DA, KTNA, BRL, ON; Analysis and/or Interpretation - DA, KTNA, BRL, ON; Literature Search - DA, BRL, BA, CA; Writing Manuscript - DA, KTNA, BRL; Critical Review - KTNA, ON.

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PRIMJENA GEOGRAFSKOG INFORMACIJSKOG SISTEMA (GIS) I WEB-GIS ZA NADGLEDANJE I PRAĆENJE SLINAVKE I ŠAPA I BOLESTI MALIH PREŽIVARA U ALŽIRU

SAŽETAK

Cilj istraživanja je evaluirati i implementirati prostorne baze podataka koje su izravno povezane sa zdravljem životinja u GIS-u i web-GIS-u vizualizacijom prostorno-vremenske distribucije bolesti životinja, kao što su kuga malih preživara (KMP) te slinavka i šap (SiŠ) u Alžiru. Usvojena je metodologija koja obuhvata klasične korake GIS-a, a primijenjena je korištenjem besplatno dostupnog Qgis 3.10. Ova metodologija je široko primjenjiva na različite tipove bolesti. Kreirali smo i model web stranice "VETALGIS" (Veterinarski alžirski GIS) sa ciljem digitalizacije veterinarskog sektora i minimiziranja problema nedostatka podataka, organizacije podataka i olakšavanja pristupa podacima, kako bi poboljšali razmjenu podataka i komunikaciju između institucija odgovornih za upravljanje stočnim bolestima. Tehnike prostorne analize GIS-a su dokazano sredstvo koje može olakšati donošenje odluka u planiranju, implementaciji i nadgledanju SiŠ i BMP, kao i u strategijama kontrole u endemskim i visokorizičnim područjima.

Ključne riječi: Bolesti malih preživara, geografski informacijski sistem (GIS), slinavka i šap

RESEARCH ARTICLE

THE EFFECTS OF NEWLY SYNTHESIZED XANTHENE-3-ONES ON *PSEUDOMONAS AERUGINOSA* – EXPERIMENTAL STUDY ON RATS

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A, Veljović E, Čelebičić M. 2023. The effects of newly synthesized xanthene-3-ones on *Pseudomonas aeruginosa* – Experimental study on rats. *Veterinaria*, 72(2), 155-164.

ABSTRACT

The need for new drugs that will have an antimicrobial effect is increasing every day. Xanthenes are cyclic, organic compounds of natural, semi-synthetic or synthetic origin, which in many studies have shown good antimicrobial, antioxidant, antiproliferative, antidiabetic, neuroprotective and many other biological effects and are interesting for further research. In the study, the antimicrobial activity of two newly synthesized xanthenic compounds was investigated: 2,6,7-trihydroxy-9-(2-hydroxy-5-bromophenyl)-3H-xanthene-3-one (Compound 1) and 2,6,7-trihydroxy-9-(3-bromophenyl)-3H-xanthene-3-one (Compound 2) to a wound infection caused by a bacterial strain *Pseudomonas aeruginosa* (ATCC 10145) on rats (n=36). The animals were divided into 6 groups. The first group was treated with Compound 1 in the concentration of 0,626 mg/g, the second group with Compound 1 in the concentration of 1 mg/g, the third with Compound 2 in the concentration of 0,626 mg/g and the fourth by Compound 2 in the concentration of 1 mg/g. The fifth and sixth groups were comparative (gentamicin) and control (vaseline). The Compounds were prepared in the form of a dermal preparation with the concentrations of 0,626 mg/g and 1 mg/g and were applied to the wound every day in the amount of 1 mg. Swabs of the wounds were taken on 1., 2., 3., and 7. day after infection to determine whether there has been a reduction in the number of bacteria. The results showed that both Compounds ultimately led to the subsidence of the infection, but a statistically significant difference was only observed between the groups that were treated with Compound 2 in the concentration of 0,626 mg/g compared to the control group.

Keywords: Antimicrobial effect, Xanthenes, *Pseudomonas aeruginosa*

INTRODUCTION

The problem of resistance in microorganisms to already existing antimicrobial drugs has long been alarming all over the world. Already known antibiotics, which have been in use for many years, but also some newer ones, are increasingly becoming ineffective in the fight against bacteria. Various studies indicate that inadequate prescribing and use of antibiotics (Davey et al., 2017) as well as the fact that the pharmaceutical industry is facing great challenges, economic and regulatory when it comes to the development of new drugs, contribute to this problem (Gross, 2013; Bartlett et al., 2013; Sengupta et al., 2013; Wright, 2014; Viswanathan, 2014; Read and Woods, 2014; Lushniak, 2014; Michael, et al., 2014). From a medical point of view, the result is that the therapeutic effect of antibiotics is disabled, that is, the patient's recovery is missing, and from an economic point of view, the costs of the patient's treatment increase due to the patient's longer stay in the hospital and longer recovery (Lushniak, 2014). The development of new drugs is a long-term and expensive process, and, considering the aforementioned resistance as well as the appearance of new pathogenic strains, there is a constant need to find new pharmacologically active compounds that will be used in human and animal therapy. In order to rationalize the time and money invested in the discovery and synthesis of new drugs, the attention is paid to the isolation and semi-synthetic production of compounds from natural materials so that often on the basis of knowledge obtained from the isolates, the synthesis of completely new compounds is also approached (Mishra and Tiwari, 2011).

One such group of compounds is xanthenes and their derivatives. It is a special group of tricyclic compounds that contain oxygen, characterized by a pyranheterocycle that contributes to their reactivity (Maia et al., 2021). Although they can also be found in nature (Imran et al., 2017; Robertson et al., 2019), a large number of xanthene compounds are of synthetic origin (Veljović et al., 2015; Kamat et al., 2021). Many toxicity tests show that xanthene

compounds have no toxic effect in the tested concentrations. So, in the study of Reddeman et al. (2019), it was determined that natural xanthene mangiferin, when administered orally to rats, does not show toxic effects up to a concentration of 2000 mg/kg bw/day, while in the study of Smajović et al. (2020), the toxic effect of synthesized xanthenes was investigated after parenteral administration, and no histopathological changes were observed in rats. Even at the molecular level, potential cytotoxicity and genotoxicity were not observed (Veljović et al., 2019).

Xanthene compounds show a very wide spectrum of biological activities. In a rat study that was performed by Epstein et al. (2014), neuroprotective action has been proven, while Manikandan et al. (2020), also in animal model, proved the antitumor activity of xanthene derivatives. Various *in vitro* studies show that xanthenes have an antitumor effect on human cell lines of various tumors, such as prostate, colon, lung and leukemia tumors (Giri et al., 2010). Anti-inflammatory (Banerjee et al., 2016), antiparasitic (Wu et al., 2005), antidiabetic (Ironi et al., 2016; Patarakijavanich et al., 2019), antioxidant (Veljović et al., 2015), and antifungal (Yunnikova et al., 2013) are just some of the pharmacological effects that xanthenes and their derivatives, whether of natural or synthetic origin, exhibit. What is of special interest for our study is the current knowledge about the antimicrobial activity of xanthene derivatives (Veljović 2018; Zukić 2018), and, especially, the tests of effects on *Pseudomonas aeruginosa*, where xanthene derivatives have shown good results (Amininasab et al., 2020a; Amininasab et al., 2020b).

Pseudomonas aeruginosa is a gram-negative bacterium that is responsible for a wide range of serious acute and chronic diseases (Bitsori, 2012; Sousa and Pereira, 2014; Mayer-Hamblett, 2015; Winstanley et al., 2016; Lin et al., 2016; Newman, 2017; Diekema et al., 2019; Fabre et al., 2019; Montero et al., 2020). Also, open wounds in humans and animals are very susceptible to infection with this bacterium. Some studies even show that the wounds induced by *P. aeruginosa* are much bigger than some others caused by other

types of bacteria such as *Staphylococcus aureus*, which may be an indication that *P. aeruginosa* infection can be more detrimental to the wound healing process than when the infection is caused by another causative agents of the disease (Mendes et al., 2012). Antibiotics (e.g. beta lactams, fluoroquinolones, aminoglycosides), which are used in therapy alone, or in combination are becoming increasingly ineffective, bearing in mind that the rate of resistance of clinical isolates of *P. aeruginosa* has increased sharply in the last 5 decades (Zhang et al., 2015; Lynch et al., 2017), which is noticeable if the infection is caused by multidrug resistance (MDR) *P.aeruginosa* strains (Karlowsky et al., 2005; Nathwani et al., 2014; Ciofi Degli Atti et al., 2014; Potron et al., 2015; Carmeli et al., 2016).

The aim of this study is to examine the antibacterial effect of newly synthesized xanthenes: 2,6,7-trihydroxy-9-(2-hydroxy-5-bromophenyl)-3H-xanthene-3-one (COMPOUND 1) and 2,6,7-trihydroxy-9-(3-bromophenyl)-3H-xanthene-3-one (COMPOUND 2) on wound infection caused by bacterial strain *Pseudomonas aeruginosa* (ATCC 10145) so that they could eventually be used as potential antibiotics in human and veterinary medicine.

MATERIAL AND METHODS

Compounds

Tested compounds: 2,6,7-trihydroxy-9-(2-hydroxy-5-bromophenyl)-3H-xanthene-3-one (COMPOUND 1) i 2,6,7-trihydroxy-9-(3-bromophenyl)-3H-xanthene-3-one (COMPOUND 2) were synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sarajevo. The composition and structure of the tested compounds were confirmed by elemental analysis, infrared spectroscopy, nuclear magnetic resonance (¹H-NMR i ¹³C-NMR), mass spectrometry and crystallographic analysis (Veljović et al., 2015; Applova et al., 2017).

The dermal preparations the animals were treated with were prepared at the Department of Drug Formulation, University of Sarajevo - Faculty of

Pharmacy. The preparation of Compound 1 with the concentration of 0,626 mg/g was prepared by weighing a mass of 6,26 mg of Compound 1 and 10 g of vaseline on an analytical scale. Both ingredients were transferred to a mortar with a pestle and mixed until a uniform mass was obtained. In this way, 10 g of dermal preparation of Compound 1 with the concentration of 0,626 mg/g was prepared.

The same procedure was carried out for the other preparations, taking into account the type of compound and the concentration to be achieved.

Animals

As animal models in the study were used both gender rats, Wistar strain, aged 2-3 months, average body weight 180-250 g (n=36). The rats had free access to food and water during the experiment and a 12-hour shift of light and darkness. The air temperature ranged between 20 and 23°C, and humidity 60% ± 10%. The animals were divided into 6 groups depending on which treatment was used: the first group (n=6), Compound 1 in the concentration of 0,626 mg/g; the second group (n=6), Compound 1 in the concentration of 1mg/g; the third group (n=6) Compound 2 in the concentration of 0,626 mg/g; the fourth group (n=6), Compound 2 in the concentration of 1mg/g; the fifth group (n=3), comparative group with gentamicin ointment (GENTAMICIN BOSNALIJEK 1mg/g) and the sixth group (n=9), control group with vaseline (Vazelin Ph. Eur. 8.0., Semikem).

Preparation of inoculum

Bacterial strain *Pseudomonas aeruginosa* (ATCC 10145) was plated on agar, then left in a thermostat at 37°C for 24h. After the culture was developed, a part of the colonies from the Petri dish was captured by inoculation loop and transferred to a test tube with 0,9% NaCl. In order to obtain the desired concentration of 10⁶ CFU/ml, the turbidimetric method was used, in which the turbidity of the solution is monitored and the concentration is read on the turbidimeter.

Preparation of nutrient media

As a nutrient medium was used *Pseudomonas* Agar Base prepared according to the standard procedure. Petri dishes were previously sterilized for 15 minutes at 121°C in an autoclave, and then cooled to 45-50°C. The prepared medium was then poured into Petri dishes in a layer of 2-5mm. After they were completely cooled, the substrates were used for sowing.

Experimental procedure

At the beginning of the experimental procedure, all tested animals were acclimatized and then put under general anesthesia using 5 mg/kg xylazine hydrochloride 2% (2% Xylazin, CpPharma, Bergdorf, Germany) and 50 mg/kg ketamine hydrochloride (Ketamine HCl Injection USP, Rotexmedica, Germany) intramuscularly. After the hair covering was removed and the skin was disinfected with povidone-iodine solution (Povidonjod HF 10%, HEMOFARM d.o.o. Banja Luka), a 1-1,5 cm long incision was made on the back, taking care to make a full incision. The previously prepared inoculum was carefully instilled into the wound in a volume of 0,25 ml, after which the sterile gauze was placed on the wound, and the animals were returned to the cage. After 24 hours, the first swab of the wound was taken to determine whether an infection had developed.

After taking the swab, each animal was treated with 1g of the preparation, depending on the group it was in. Then the sterile gauze was placed on the wound, and the procedure of applying the preparation was repeated every day at the same time. The swabs were taken on the second, third and seventh day of the experiment. Then, they were seeded on a nutrient medium, left in a thermostat for 24 hours at a temperature of 37°C, after which the colonies were counted using the Koch method.

Statistical data processing

The statistical data processing was done in the program SPSS 24.0 (IBM Corp., 2016). Tests that are used were ANOVA and MIXANOVA, with

post hoc tests, and the difference at the $p < 0,05$ level was considered statistically significant.

Approval of the Ethics Committee

Approval for the experimental work was issued by the Ethics Committee of the Veterinary Faculty, University of Sarajevo, under number 01-02-18-12/18.

RESULTS

36 rats were utilized in the investigation and were split into two groups: the first ($n=18$) treated with Compound 1 in the concentrations of 0,626 mg/g and 1 mg/g, gentamicin and vaseline as comparative, i.e. control group (Table 1) and the second group ($n=18$) treated with Compound 2 in the concentrations of 0,626 mg/g and 1 mg/g, gentamicin and vaseline as comparative, i.e. control group (Table 2). As can be seen in both tables, the swab on Day 1 showed in all rats, except for two, the concentration of bacteria of 10^6 CFU/ml, which is a confirmation of the presence of infection. A decrease in the number of bacteria is observed after the 2nd day, while from the 3rd day, in some rats whose wounds were treated with Compound 1, the swabs were sterile, that is, in some rats whose wounds were treated with Compound 2, the wounds were sterile after the 7th day of infection.

Table 1 The number of bacteria in rats after the administration of Compound 1

	Compound 1 concentration 0,626 mg/g							Compound 1 concentration 1,0 mg/g							Gentamicin (comparative group)		Vaseline (control group)	
rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.day	10 ⁶	10 ⁵	10 ⁵	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶
2.day	10 ⁶	360	300	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁵	4176	S	10 ⁴	10 ⁶	10 ⁵	10 ⁶	10 ⁶	10 ⁵	10 ⁵	10 ⁵
3.day	10 ⁶	S	S	10 ⁴	10 ⁴	10 ⁵	10 ⁶	10 ³	28	S	126	10 ⁴	4	160	120	10 ⁴	10 ⁴	10 ⁴
7.day	10 ⁴	S	S	10 ²	10 ²	10 ³	412	568	S	S	20	292	S	136	20	6272	440	10 ³
S –sterile																		

Table 2 The number of bacteria in rats after the administration of Compound 2

	Compound 2 concentration 0,626 mg/g							Compound 2 concentration 1,0 mg/g							Gentamicin (comparative group)		Vaseline (control group)	
rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.day	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶
2.day	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁵	10 ⁶	10 ⁶	10 ⁵	10 ⁶	10 ⁵	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶
3.day	10 ⁵	464	10 ⁵	10 ⁵	10 ⁵	10 ⁵	2480	10 ⁴	10 ³	10 ⁵	1200	10 ⁵	380	10 ³	800	10 ⁵	10 ⁴	10 ⁴
7.day	1976	S	10 ³	5760	S	1712	S	9600	112	120	168	10 ³	S	256	S	10 ³	1560	1440
S –sterile																		

If we compare these two compounds, based on the statistical processing of the data, no statistically significant difference was observed if the time

required to reduce the number of bacteria was observed, but if the action itself was observed, the only statistically significant difference was

observed between the groups of rats that were given Compound 2 in the concentration of 0,626 mg/g and control group that received vaseline ($p=0,009$, $p<0,05$). All other groups did not differ statistically (Table 3).

Table 3 Effect of Compounds 1 and 2 on *Pseudomonas aeruginosa*

Tested compounds*	p value (time)	p value (compounds)
1A-1B	$4,68 \cdot 10^{-7}$	0,12
2A-2B	$7,9 \cdot 10^{-8}$	0,352
1A-2A	$2,23 \cdot 10^{-7}$	0,09
1B-2B	$4 \cdot 10^{-6}$	0,192
1A-Gentamicin	$1,1 \cdot 10^{-5}$	0,452
1B-Gentamicin	$1,98 \cdot 10^{-4}$	0,327
1A-Vaseline	0,001	0,06
1B-Vaseline	$5,4 \cdot 10^{-5}$	0,571
2A-Gentamicin	$4 \cdot 10^{-6}$	0,416
2B-Gentamicin	0,001	0,922
2A-Vaseline	$1,19 \cdot 10^{-4}$	0,009**
2B-Vaseline	$2,33 \cdot 10^{-4}$	0,067

*A=0,626mg/g; B=1,0 mg/g;
1=Compound 1;2=Compound
** statistically significant

DISCUSSION AND CONCLUSION

P. aeruginosa is a bacterium that cancels the effect of antibiotics by different mechanisms so that the resistance develops very easily. In 2017, the World Health Organization recognized this problem, and put *Pseudomonas aeruginosa* on the list of priorities for research and development of new antibiotics that will overcome the increasingly common problem of resistance (WHO, 2017).

The skin is an organ that represents a barrier against various types of microorganisms. As long as it is not damaged in some part, *Pseudomonas*

aeruginosa cannot cause an infection (Coates et al., 2018). However, if a suitable environment is developed, *Pseudomonas aeruginosa* will very quickly form colonies and develop an infection (Tosh et al., 2011). on which basis the design of this experiment was developed.

In this study, all rats were infected with 10^6 CFU/ml (except two rats), and from the obtained results it can be observed that already on the 2nd day after the infection, Compound 1 in the concentration of 0,626 mg/g also worked in two rats, while the number of bacteria in the swabs was 360, i.e. 300. Even better result is observed in the group treated with Compound 1 in the concentration of 1 mg/g, where after the 2nd day in one rat the swab was sterile, and in other the number of bacteria was 4176. If we compare this with the effect of Compound 2 in both concentrations, we will see that there were no changes in the number of bacteria after the 2nd day. Also, after the 2nd day, there were no changes in either the comparative or the control group. Only on the 3rd day after infection, the changes are observed in all tested groups. In the group in which Compound 1 was used in the concentration of 0,626 mg/g, the wounds of two rats were sterile, while in the group where Compound 1 was used in the concentration of 1 mg/g, the number of rats with a sterile wound remained the same, but a significant decrease in the number of bacteria was observed in other rats from the group. Also, in the comparative group, the number of bacteria is significantly lower compared to the previous swabs, so that the number is below 200. After the 3rd day, in the groups in which Compound 2 was applied in both concentrations, a slight decrease in the number of bacteria was observed, but still not a single rat had a sterile wound. After the 7th day of infection, three rats had the sterile wounds, which were treated with Compound 2 in the concentration of 0,626 mg/g and one tested Compound 2 in the concentration of 1mg/g. After taking the last swab in the group to which Compound 1 was applied, four rats had a sterile wound, two from the group to which Compound 1 was applied in concentration of 0,626 mg/g and two from the group to which Compound 1 was applied in the concentration of

1mg/g.

Bearing in mind the time period that should have passed to obtain sterile wounds in at least one animal, we can conclude that Compound 1 has a better antimicrobial effect than Compound 2.

However, by statistical processing of the data (Table 3), the only statistically significant difference was observed between the groups of rats that were administered Compound 2 compared to the control group ($p < 0,05$), but we should not ignore the results between the groups to which Compound 1 was applied in the concentrations of 0,626 mg/g and 1 mg/g ($p = 0,09$), groups that were administered Compound 1 in the concentration of 0,626 mg/g and vaseline ($p = 0,06$), as well as between the groups that were administered Compound 2 in the concentration of 1 mg/g and vaseline ($p = 0,067$).

From this, it can be concluded that the results of our test indicate that the compounds have a potential antimicrobial effect. Taking into account the length of the test time and the decrease in the number of bacteria during that time, the conclusion

is that, most likely, a more realistic picture of the antimicrobial effect of the compounds would be obtained if the treatment time of the infected wounds were extended, while considering an increase in the concentration of the tested compounds, as well as testing a larger number of differently substituted xanthene derivatives. In accordance with the above, further research is necessary.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

AS: 1,2,3,4,5,6,7,8,9,10; EV: 1,8 10; MČ: 1,2,6,10.

Concept (1); Design (2); Supervision (3); Resources (4); Materials (5); Data Collection and/or Processing (6); Analysis and/or Interpretation (7); Literature Search (8); Writing Manuscript (9); Critical Review (10)

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EFEKTI NOVOSINTETIZIRANIH KSANTEN-3-JEDAN NA *PSEUDOMONAS AERUGINOSA* – EKSPERIMENTALNA STUDIJA NA STAHORIMA

SAŽETAK

Potreba za novim lijekovima koji imaju antimikrobno dejstvo je svakim danom sve veća. Ksanteni su ciklični organski spojevi koji mogu biti prirodnog, polusintetskog ili sintetskog porijekla i koji su u mnogobrojnim studijama pokazali dobro antimikrobno, antioksidantno, antiproliferativno, antidijabetično, neuroprotektivno i drugo biološko djelovanje te koji su od interesa za daljnje istraživanje. U ovim istraživanju je ispitivano antimikrobno dejstvo dva novosintetizirana ksantenska spoja: 2,6,7-trihidroksi-9-(2- hidroksi-5-bromofenil)-3H-ksanten-3-jedan (SPOJ 1) i 2,6,7-trihidroksi-9-(-(3-bromofenil)-3H-ksanten -3-jedan (SPOJ 2) kod infekcije rane izazvane bakterijskim sojem *Pseudomonas aeruginosa* (ATCC 10145) na pacovima (n=36). Životinje su podijeljene u šest grupa. Prva grupa je tretirana sa spojem 1 u koncentraciji od 0,626 mg/g, druga grupa sa spojem 1 u koncentraciji od 1 mg/g, treća sa spojem 2 u koncentraciji od 0,626 mg/g i četvrta sa spojem 2 u koncentraciji od 1 mg/g. Peta i šesta grupa su komparativna (gentamicin) i kontrolna (vazelin). Spojevi su pripremljeni u obliku dermalnog preparata u koncentraciji od 0,626 mg/g i 1 mg/g koji su aplicirani na ranu svaki dan u količini od 1 mg.

1, 2, 3. i 7. dana nakon infekcije su uzimani brisevi rana kako bi se odredilo da li je došlo do smanjenja broja bakterija. Rezultati su pokazali da su u konačnici oba spoja dovela do povlačenja infekcije, ali je statistički signifikantna razlika uočena samo između grupa koje su tretirane sa spojem 2 u koncentraciji od 0,626 mg/g u usporedbi sa kontrolnom grupom.

Ključne riječi: Antimikrobni efekt, ksanteni, *Pseudomonas aeruginosa*

RESEARCH ARTICLE

QUALITY PROPERTIES AND FATTY ACIDS COMPOSITION OF BREAST MEAT FROM JAPANESE QUAILS WITH DIFFERENT VARIETIES GROWN UNDER WARM CLIMATE

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ABSTRACT

As climate change is expected to worsen in the future, raising livestock animals used in meat production with maximum efficiency in warm climate conditions may become a necessity to meet food needs. To this end, the experiment was carried out to determine the quality properties of breast meat from different colored Japanese quails (*Coturnix coturnix Japonica*) grown under the warm climate conditions. A total of 100 quails were used, with 25 each of black, white, golden, and gray. After the quails were grown under the warm climate conditions from the 15th-42nd Day, they were slaughtered, and the chemical-physicochemical properties, along with the fatty acid composition of the breast muscles, were determined. The values of b* (yellowness) were significantly lower in the black quails ($P<0.01$). The lowest fat content of meat was determined in black and gray quails, and the highest fat content was determined in white quails ($P<0.001$). The proportion of total saturated fatty acids was the highest in the gray quails, and total monounsaturated fatty acids were found to be the highest in the golden and the lowest in the gray quails ($P<0.05$). The highest proportion of n-3 polyunsaturated fatty acids was detected in gray quails and the lowest in golden and white quails ($P<0.01$). The white and golden quails had the highest n-6: n-3 proportions, while the gray quails had the lowest ($P<0.01$). This study concluded that quail varieties had distinct traits that arise due to varying environmental circumstances. Quail varieties growing in warm climates may have different meat quality parameters.

Keywords: Fatty acids, meat quality characteristics, quail varieties, warm climate

INTRODUCTION

Climate change is a global menace to the food and nutritional safety of the world. Every year, the seasons change because of global warming, and the average temperature keeps going up. The average global temperature has increased at an approximate rate of 0.15-0.20 °C per decade since 1975 and is expected to increase by 1.4-5.8 °C over the next few years (Malhi et al., 2021). Raising species, breeds, and even varieties that are resistant to warm weather will have great importance in the near future. Publications that have come out recently show that rising temperatures can affect many populations of species across large areas of land (Lehikoinen et al., 2021). Various studies report that it is more difficult for bird communities to adapt to high exposure to warming caused by climate change than for mammals (Riddell et al., 2021).

The Japanese quail is a small avian species in the *Coturnix* genus, and its consumption as food has increased. Its main advantages are that the time between generations of quails is short, they yield more products per unit area, their raising is cost-effective, and they are resistant to many diseases (Lukanov, 2019; Tarhyel et al., 2012). However, exposing poultry to warm climate conditions has a negative impact on their performance and causes economic losses (Kumar et al., 2021).

Quails are homeothermic animals that conserve a stable internal body temperature without considering environmental effects. When the environmental temperature exceeds the thermoneutral distance, respiratory activity increases, since panting for air is a necessity for birds in order to increase the heat loss by evaporation. This allows quails to maintain body temperature. This situation may suffer from extreme physiological disorders, such as decreased efficiency of feeding or low specific gravity (Nawab et al., 2018; Batol et al., 2021). There have been many ideas for how to make birds more tolerant of heat, such as creating new hybrids.

Most of the color changes in the plumage within and between species have a powerful genetic component. The plumage color phenotype is considered the principal aspect of the complex

genetic composition of Japanese quails (Sayed and El Shoukary, 2021). Also, it can be considered an economically important trait that is attributed to multiple genes, gene-gene interactions, and environmental factors. Furthermore, latent relationships might exist between plumage color and body weight (Davoodi, 2021). Also, Sezer and Tarhan (2012) tried to find a link between the color of Japanese quails' feathers and how they grew. They concluded that morphological characteristics, especially feather color, played a big role in both the identification process and the choice of quail varieties.

Quail meat is one of the best alternative animal protein sources due to its low fat and cholesterol content (Nasar et al., 2016). Although quail meat has less fat content compared to other poultry meats, it has a high amount of unsaturated fatty acids. It is also rich in terms of conjugated linoleic acid (anti-inflammatory, antithrombotic, and anti-atherosclerotic), which has many beneficial effects on human physiology (Genchev et al., 2008; Nasr et al., 2017). Besides the composition of the meat, color, texture, and taste are among the other quality traits affecting the acceptability of the product by consumers before and after the sale. The appearance of meat depends on the skin and meat color, and it mostly affects the choice of consumers. In terms of storage and processing, meat quality is affected by factors such as pH value, water holding capacity, and cooking loss. It has been determined that there are varieties of Japanese quail based on plumage color (Mir et al., 2017). In terms of yield characteristics, different Japanese quail varieties have varying growth rates, feed conversion efficiency, and egg-laying capacity. Some varieties may be more resilient to climate change-induced stress and have better productivity under suboptimal environmental conditions. However, this may also depend on other factors such as genetic makeup, nutrition, and management practices. Changes in climate can also affect the yield quality of Japanese quail. Heat stress can cause oxidative stress and lipid peroxidation, leading to decreased meat quality (Gonzalez-Rivas et al., 2020).

The studies to date have focused on demonstrating that different varieties of Japanese quail have comparable meat quality (Inci et al., 2015; Nasr et al., 2016; Sabow, 2020). Climate change is expected to worsen in the near future (Malhi et al., 2021). Raising the livestock animals used in meat production with maximum efficiency in warm climate conditions may be a necessity shortly to meet the food need. In this context, the quality parameters of quail strains under warm climate conditions have been a matter of curiosity. So, the goal of this study was to compare the meat quality of black, white, golden, and gray Japanese quails that were raised in warm climates.

MATERIAL AND METHODS

Ethical statement

This study was approved by the Elazığ Veterinary Control Institute Poultry Unit. Approval was received from Elazığ Veterinary Control Institute Local Ethics Committee (EVKEM: 2020/01).

Experimental design

In the study, a total of 100 Japanese quails (*Coturnix coturnix Japonica*), with 25 from each

plumage color (black, white, golden, and gray), were included in the test period by being matched with their initial live weights (Aslan et al., 2022). The study was planned with 5 replications, with 5 quails in each repetition. Throughout the test period (15th-42nd Days), the quails were housed in special broiler quail cages. A control group was not formed in this study because it was desired to examine the differences between the variances in only warm climatic conditions. An environmentally-controlled room, including the cages, was arranged, and the temperature and humidity values in the room were monitored with a digital gauge. The warm climate condition was set to be 34°C for 8 h a day (from 9:00 a.m. to 05:00 p.m.) and 22 °C for the remaining 16 h throughout the experiment (Aslan et al., 2022). Water cans were placed in front of the heaters, and the ambient relative humidity (RH) was maintained at 55-60%. The light/dark period was set up as a 23/1 h feed, and water was given to the quail ad libitum, as shown in Table 1 (NRC, 1994). At the end of the experiment, all the quails were weighed and taken to be slaughtered. For slaughter, a total of 10 quails (5 females and 5 males), reflecting the average of each group (1 female and 1 male from

Table 1 Composition and nutritional properties of the feed used in quail nutrition

Feed Materials	%	Chemical Composition	%
Corn, Yellow	51.50	KM, %	89.9
Soybean meal, 48%	28.00	HP, %	24.0
Corn Gluten, 43% HP	12.00	HY, %	2.48
Wheat Bran, Coarse	5.00	HK, %	5.52
Vegetable Oil	0.51	HS, %	3.32
Dicalcium Phosphate	0.70	ME, kcal/kg	2900
DL-Methionine	0.01		
Limestone	1.35		
L-Lysine Hydrochloride	0.27		
L-Threonine	0.16		
Salt	0.25		
Vitamin-Mineral Mix*	0.25		

* Vitamin-mineral premix (For each 1 kg): Vitamin A, 12.000 IU; Vitamin D3, 3.300 IU; vitamin E, 20 mg; Vitamin K3, 4 mg; Vitamin B1, 3.0 mg; Vitamin B2, 7.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.015 mg; Niacin, 25.0 mg; D-calcium pantothenate, 10.0 mg; folic acid, 1.0 mg; D-biotin, 0.05 mg; choline chloride, 175 mg; manganese, 100 mg; iron, 60 mg; zinc 60 mg; copper, 5 mg; cobalt, 0.5 mg; iodine, 2 mg; selenium, 0.15 mg; phytase, 300.

each replication), were selected. The slaughter, carcass, and breast weights of the quails were measured using a balance with g precision. Half of the breasts were separated, and *M. pectoralis major* was kept at -20 °C for fatty acid and basic nutrient analyses. The rest of the breast was kept at +4 °C for 24 h, and then the color, ultimate pH (pHu), water holding capacity, and cooking loss analyses were performed.

Chemical and physicochemical analysis

The crude protein content of the samples was determined using the Dumatherm protein analyzer and the Dumas method (Gerhardt, Germany). Crude fat, dry matter, and crude ash analyses were performed based on the Association of Official Agricultural Chemists (AOAC) guidelines (AOAC, 2000). In determining the pHu value of the *M. pectoralis major* muscle 24 hours after the slaughter, the probe of the standardized pH meter was stuck to three different points of the breast meat, and the pHu value was recorded by taking the average of every three measurements. Water holding capacity (WHC) was determined as reported previously (Hamm, 1961). Cooking loss (CL) analysis was performed at the 24th hour after the slaughter based on the method reported by Honikel (1998). The color of the meat was analyzed with a colorimeter device (Chen et al., 2021). Instrument calibration was performed on the white plate and black hole provided by the producer. The average of the measurements performed in three different parts of the breast meat was recorded.

Determination of fatty acid profile by gas chromatography

Briefly, a 0.5-1 g tissue sample was homogenized in a 5 ml hexane-isopropanol mixture at a rate of 3:2 (v/v) for 30 sec. The homogenization vessel was washed with 2 ml of lysis solvent and taken to the centrifuge tubes. Then, the upper supernatant parts were taken from the tissue samples and centrifuged at 4500 rpm for 10 min, and they were put into covered test tubes (Hara and Radin, 1978). The collected lipid extract from the hexane/isopropanol phase was taken to the 30 ml test tubes.

Subsequently, 5 ml of 2% methanolic sulfuric acid was added to them, and they were vortexed. This mixture was left for methylation for 15 h in a drying oven at 50 °C. Then, they were cooled to ambient temperature and mixed thoroughly by adding 5% (5 ml) sodium chloride. The produced fatty acid methyl esters were extracted with 5 ml of hexane, and the hexane phase was taken by pipette, treated with 5 ml of 2% KHCO₃, and kept for 4 h for the phases to separate. Then, the mixture, including the methyl esters, was dissolved with 1 ml hexane, the solvent of which was evaporated at 45 °C and under a nitrogen stream, and they were taken into 2 ml covered autosampler vials and analyzed in gas chromatography. After the fatty acids in the lipid extract were converted into methyl esters and analyzed with Macherey-Nagel capillary columns in Shimadzu GC-17 Ver. 3 gas chromatography (GC-17 series, Shimadzu, Japan). During the analysis, the column temperature was kept at 120-220 °C, the injection temperature was kept at 240 °C and the detector temperature was kept at 280 °C. The column temperature was set from 120 °C to 220 °C. The temperature increase was determined as 5 °C/ min until 200 °C and 4 °C/ min from 200 °C to 220 °C. It was kept at 220 °C for 8 min, and the total period was determined to be 35 min. Nitrogen gas was used as the carrier gas. During the analysis, the mixtures of the standard fatty acid methyl esters were injected, and the retention time of each fatty acid was determined before the analysis of the fatty acid methyl esters of the samples. After this process, the required scheduling was performed, and the fatty acid methyl ester mixtures of the samples were analyzed. The results were determined as a percentage for each fatty acid among the total fatty acids. The calculations were made using the GC Solution 2.3 software.

Statistical analyses

After the normality and homogeneity tests were performed, all data were subjected to a one-way ANOVA using analysis of variance. Significant differences have also been subjected to Tukey HSD testing. All analyses were performed using SPSS 22 for Windows. The results were considered significant when P values were less than 0.05.

RESULTS

Performance

Slaughter weights, carcass weights, and breast weights of black, white, golden, and gray-colored Japanese quails raised in warm climate conditions are given in Figure 1. The different varieties of Japanese quails were found to be similar in terms of the characteristics examined ($P>0.05$).

Chemical and physicochemical characteristics of breast meat

The composition of the breast meat of different colored quail varieties is given in Table 2. In terms of protein and ash content, there was no significant difference between the groups in the chemical analysis of breast meat ($P>0.05$). The effect of varieties on intramuscular fat content was significant ($P<0.001$), and the meat of white quails had a higher fat content than the black ones. The dry matter content of white quail meat was lower than that of golden quail meat ($P<0.05$).

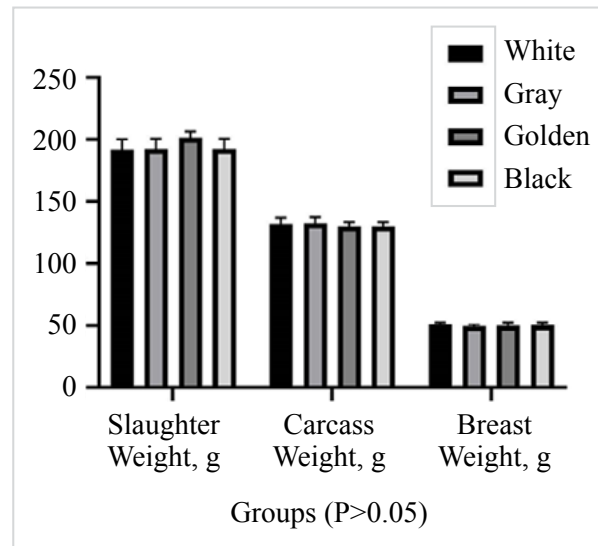


Figure 1 Slaughter, carcass, and breast weights of Japanese quails with different varieties grown under warm climate conditions (Mean±SE)

Table 2 Basic breast meat compositions and meat quality characteristics of Japanese quails with different varieties grown under warm climate conditions

Variables	Quails with Different Varieties (Mean±Std Error)				P
	White	Gray	Golden	Black	
Protein, %	19.96±0.86	21.97±1.51	22.60±0.85	20.64±0.88	0.293
Fat, %	3.20±0.49 ^a	1.87±0.19 ^{ab}	2.21±0.76 ^{ab}	1.13±0.51 ^b	0.000
Dry matter, %	29.09±0.34 ^b	31.73±1.29 ^{ab}	33.61±2.46 ^a	30.87±1.24 ^{ab}	0.023
Ash, %	1.66±0.12	1.60±0.15	2.10±0.15	1.77±0.13	0.069
Ultimate pH (pH _u)	6.22±0.02	6.19±0.03	6.29±0.03	6.16±0.05	0.169
WHC* (%)	21.05±1.38	21.87±0.82	22.16±0.72	21.53±1.04	0.886
Cooking loss (%)	18.57±0.78	21.52±1.45	18.35±0.38	20.43±1.13	0.102
Lightness (L*)	44.17±0.57	42.61±1.41	42.92±1.62	42.85±0.86	0.792
Redness (a*)	8.66±0.89	10.46±1.88	8.85±1.23	9.08±0.97	0.768
Yellowness (b*)	12.88±0.51 ^a	12.91±0.66 ^a	13.19±0.64 ^a	10.29±0.45 ^b	0.003

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

* WHC: Water Holding Capacity

When Table 2 was viewed, there was no significant difference among the groups in terms of pHu, water holding capacity, and cooking loss ($P>0.05$). Among the color parameters, the L^* (lightness) and a^* (redness) values of the groups were found to be similar ($P>0.05$), and the difference between the groups in the b^* (yellowness) value was found to be significant ($P<0.01$). While the b^* value was similar in white, golden, and gray varieties, this value was significantly lower in black quails ($P<0.01$).

Breast meat fatty acid compositions

When the results of fatty acids are examined (Table 3), cis-10 pentadecenoic acid (C15:1) ($P<0.01$), heptadecenoic acid (C17:0) ($P<0.001$), cis-10 heptadecenoic acid (C17:1) ($P<0.01$),

stearic acid (C18:0) ($P<0.05$), eicosatrienoic acid (C20:3) ($P<0.05$), eicosapentaenoic acid (C20:5 n-3) ($P<0.001$), docosahexaenoic acid (C22:6 n-3) ($P<0.05$), nervonic acid (C24:1) ($P<0.05$) values were found to be higher in gray quails. The total monounsaturated fatty acid (Σ MUFA) proportion was the highest in golden and significantly lowest in gray quails ($P<0.05$). Total polyunsaturated fatty acids (Σ PUFA) were similar among the groups. The highest proportion of n-3 polyunsaturated fatty acids was detected in gray quails and the lowest in white quails and golden quails ($P<0.01$). The proportion of n-6 unsaturated fatty acids was similar among the groups ($P>0.05$). The n-6:n-3 proportion was calculated as the highest in the golden group and the lowest in the gray group ($P<0.01$).

Table 3 Fatty acids profiles of breast meat in Japanese quails with different varieties grown under warm climate conditions (g/100 g fatty acids)

Variables	Quails with Different Varieties (Mean $\pm$ Std. Error)				P
	White	Gray	Golden	Black	
Myristic acid (C14:0)	0.66 $\pm$ 0.03	0.55 $\pm$ 0.03	0.51 $\pm$ 0.05	0.57 $\pm$ 0.02	0.150
cis-10 Pentadecenoic acid (C15:1)	0.84 $\pm$ 0.10 ^b	1.50 $\pm$ 0.18 ^a	0.87 $\pm$ 0.14 ^b	1.21 $\pm$ 0.13 ^{ab}	0.008
Palmitic acid (C16:0)	20.45 $\pm$ 0.24	20.23 $\pm$ 0.43	19.20 $\pm$ 0.73	20.40 $\pm$ 0.53	0.303
Palmitoleic acid (C16:1 n-7)	6.40 $\pm$ 0.65	4.82 $\pm$ 0.34	5.30 $\pm$ 0.51	6.05 $\pm$ 0.30	0.106
Heptadecenoic acid (C17:0)	0.49 $\pm$ 0.08 ^{ab}	0.87 $\pm$ 0.17 ^a	0.25 $\pm$ 0.06 ^b	0.12 $\pm$ 0.01 ^b	0.000
cis-10 Heptadecenoic acid (C17:1)	0.40 $\pm$ 0.05 ^{ab}	0.59 $\pm$ 0.07 ^a	0.30 $\pm$ 0.06 ^b	0.47 $\pm$ 0.03 ^{ab}	0.006
Stearic acid (C18:0)	7.34 $\pm$ 0.65 ^{ab}	9.37 $\pm$ 0.56 ^a	6.90 $\pm$ 0.53 ^b	8.10 $\pm$ 0.37 ^{ab}	0.016
Oleic acid (C18:1 n-9)	27.15 $\pm$ 3.25	26.06 $\pm$ 1.11	32.90 $\pm$ 2.67	26.33 $\pm$ 1.39	0.139
Linoleic acid (C18:2 n-6)	24.76 $\pm$ 1.79	22.49 $\pm$ 0.39	22.22 $\pm$ 1.21	22.55 $\pm$ 0.40	0.370
α -Linolenic acid (C18:3 n-3)	1.09 $\pm$ 0.11	0.90 $\pm$ 0.05	1.00 $\pm$ 0.08	0.94 $\pm$ 0.03	0.356
Eicosatrienoic acid (C20:3)	0.41 $\pm$ 0.06 ^b	0.67 $\pm$ 0.06 ^a	0.44 $\pm$ 0.07 ^b	0.62 $\pm$ 0.06 ^{ab}	0.024
Arachidonic acid (C20:4 n-6)	4.84 $\pm$ 0.77	7.02 $\pm$ 0.70	4.54 $\pm$ 0.73	6.56 $\pm$ 0.78	0.057
Eicosapentaenoic acid (C20:5 n-3)	0.21 $\pm$ 0.03 ^b	0.42 $\pm$ 0.03 ^a	0.10 $\pm$ 0.02 ^c	0.13 $\pm$ 0.01 ^{bc}	0.000
Docosahexaenoic acid (C22:6 n-3)	1.14 $\pm$ 0.18 ^{ab}	1.81 $\pm$ 0.17 ^a	1.06 $\pm$ 0.18 ^b	1.62 $\pm$ 0.20 ^{ab}	0.016
Lignoceric acid(C24)	0.33 $\pm$ 0.05	0.44 $\pm$ 0.05	0.28 $\pm$ 0.04	0.36 $\pm$ 0.05	0.170
Nervonic acid (C24:1)	0.37 $\pm$ 0.07 ^b	0.60 $\pm$ 0.09 ^a	0.32 $\pm$ 0.05 ^b	0.55 $\pm$ 0.04 ^{ab}	0.033
Others ¹	3.10 $\pm$ 0.21 ^{ab}	1.66 $\pm$ 0.14 ^b	3.80 $\pm$ 0.46 ^a	3.42 $\pm$ 0.35 ^a	0.000
Σ SFA ²	27.28 $\pm$ 1.64 ^b	31.48 $\pm$ 0.51 ^a	27.20 $\pm$ 0.98 ^b	29.61 $\pm$ 0.56 ^{ab}	0.017
Σ MUFA ³	39.80 $\pm$ 1.73 ^{ab}	34.66 $\pm$ 1.23 ^b	43.00 $\pm$ 2.46 ^a	37.45 $\pm$ 1.31 ^{ab}	0.014
Σ PUFA ⁴	32.90 $\pm$ 2.19	33.85 $\pm$ 0.90	29.79 $\pm$ 1.57	32.93 $\pm$ 1.08	0.276
Σ PUFA n-3	2.87 $\pm$ 0.24 ^b	3.91 $\pm$ 0.24 ^a	2.68 $\pm$ 0.23 ^b	3.54 $\pm$ 0.28 ^{ab}	0.004

Variables	Quails with Different Varieties (Mean±Std. Error)				P
	White	Gray	Golden	Black	
∑PUFA n-6	29.96±1.99	29.94±0.71	27.06±1.41	29.33±0.85	0.390
n-6:n-3 proportion	10.84±0.74 ^a	7.88±0.43 ^b	10.44±0.67 ^a	8.63±0.58 ^{ab}	0.004

¹Others = (C12:0, C14:1, C15:0, C18:1 n-7, C18:3 n-6, C20:0, C20:1, C20:2, C22:2, C22:4 n-6, C22:5 n-6, C22:5 n-3)

²∑SFA (saturated fatty acids) = sum of C14:0 + C16:0 + C17:0 + C18:0 + C24:0

³∑MUFA (monounsaturated fatty acids) = sum of C15:1 + C16:1 n-7 + C17:1 + C18:1 n-9 + C18:1 n-7 + C24:1

⁴∑PUFA (polyunsaturated fatty acids) = sum of C18:2 n-6 + C18:3 n-3 + C20:3 n-6 + C20:3 n-3 + C20:4 n-6 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05

DISCUSSION AND CONCLUSION

Performance

The carcass yield in Japanese quail is managed by the slaughter age, breed, line, sex, raising conditions, and feeding programs (Alkan et al., 2013). Furthermore, heat stress has been shown in many studies to reduce feed intake and weight gain in quails (Orhan et al., 2020; Orhan et al., 2021). Quails exposed to heat stress try to maintain body temperature by reducing metabolism (Alagawany et al., 2017). Quail carcass weight was found to be similar in different varieties in current research. In other studies, without heat stress, Nasr et al. (2017) reported carcass weight to be high in the white, golden, gray, and brown varieties, respectively. Inci et al. (2015) have reported carcass weight to be high in wild-type dark brown, white, and golden quail, respectively. Sabow (2020) reported that the carcass weight of black and brown quails was similar, and the white quails had the lowest carcass weight. The superiority of different varieties based on the examined trait may be an indicator that these varieties have different adaptations for different environmental conditions, as postulated by Jeke et al. (2018).

Chemical and physicochemical characteristics of breast meat

The protein and ash values between groups were found to be similar. Likewise, Nasr et al. (2017) and Sabow (2020) reported that the protein content of the quails with different plumage colors was similar. In this study, the fat content was the lowest in black quails and the highest in white

quails, and the dry matter content was the highest in golden quails (Table 2). It is known that the meat composition is significantly affected by the adaptation process of genotypes under heat stress (Al-Sagan et al., 2020). It has been reported that the ash value decreased due to mineral loss (Xing et al., 2019), and therefore, the dry matter level may be affected. In this study, the breast-meat fat content of the quails was found to be lower compared to the studies by Sabow (2020). High temperatures reduce fat metabolism in poultry during the growing stage (Kumar et al., 2021). Also, it was considered that this difference was associated with different feeding programs or slaughter ages.

Genetic factors are effective on meat quality, and the variation between the breeds or varieties of poultry used in global breeding is important. Meat pH is obtained as the most important factor that affects poultry meat's physical and chemical properties, including water-holding capacity, color, and storage life (Nasr et al., 2017; Sabow, 2020).

The average ultimate pH for quails was reported as 5.30-6.58 by various researchers (Genchev et al., 2008; Narinc et al. 2013; Zerehdaran et al., 2012). In the study, the pH of the pectoral major muscle in Japanese quail varieties had similar values to the varieties examined in other studies (Genchev et al., 2008; Nasr et al., 2017). Also, Sabow (2020) reported that, similarly, there was no difference between the quails with white, black, and brown plumage colors in the pH_u. The meat of Japanese quail possessed the highest ultimate pH levels when

compared with other poultry meats (Karakaya et al., 2005). The difference in ultimate pH might be based on a difference in intramuscular glycogen content, amount of anaerobic glycolysis after slaughtering, and breed factors (genetic difference between breeds) (Nasr et al., 2017; Ribarski and Genchev, 2013). As Walasik et al. (2006) reported, the quail muscles were characterized by a slower progression of rigor due to higher mitochondrial levels and, consequently, higher pH levels than other poultry muscles.

Studies have shown that low pH levels in meat reduce the WHC and tenderness quality (pale, soft, and exudative) of the meat and enhance the cooking loss percentage (Barbut, 1997). No significant difference was found in water holding capacity or cooking loss among the experimental groups of the present study (Table 2). It was considered that this similarity was caused by the pHu similarity among the groups. Besides, the detected levels of WHC and cooking loss in the four different plumage colors are nearly similar to the results of other studies (Genchev et al., 2008; Nasr et al., 2017; Ribarski and Genchev, 2013; Zerehdaran et al. 2012).

Meat color is an organoleptic parameter that directly expresses the freshness of meat. Meat color is affected by many factors, such as myoglobin amount, intramuscular fat content, genotype, sex, age, the diet of animals, the pre-slaughter applications, and boiling temperature (Fletcher, 2002). There are different findings on the effect of plumage color on L^* , a^* , and b^* values. Some researchers have reported an interval between L^* : 48.00–61.54, a^* : 13.1–19.20, and b^* : >19.81 (Boni et al., 2010; Ponnampalam et al., 2017), and some researchers determined some lower values: L^* : 35.89–52.34, a^* : 5.1–14.4, and b^* : 3.32–13.4. The values found in this study are similar to those found by other researchers who found low values (Narinc et al., 2013; Nasr et al., 2017; Ribarski and Genchev, 2013). Also, L^* and a^* values were similar between the groups, and the b^* value, demonstrating yellowness, was the lowest in black quails. The reason for this state may

be the concentration of pigments, the antioxidant potential of meat, and the level of intramuscular fat in the meat (Ponnampalam et al. 2017). Indeed, in this study, the lowest fat content was determined for this variety.

Breast meat fatty acid compositions: The lipids of the muscle involve phospholipids, triacylglycerol, and cholesterol. While triacylglycerol is made up mainly of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), a part of phospholipid has a great proportion of polyunsaturated fatty acids (PUFA) (Abdulla et al., 2018). The major FA that was detected in breast meat was oleic acid in all varieties, as expected. In addition, palmitic acid was the main fatty acid in total SFA in all varieties, followed by stearic acid. Similarly, Sabow (2020) and Kurekci (2021) observed quail meat and other poultry species such as broiler chicken (Ölmez et al., 2021) or duck meat (Huo et al., 2021). In this study, while there was no difference in palmitic acid between quail varieties, the highest amount of stearic acid was found in gray quails. Sabow (2020), stated the amounts of stearic acid in white, black, and brown quails grown under normal conditions as 9.57, 9.55, and 9.66, respectively. Also, the amount of stearic acid in quail varieties reared in warm climate conditions was lower than the amount indicated by Sabow (2020), and only 9.37 percent of gray quails had the same amount. As known, compared with palmitic acid, stearic acid lowers levels of total cholesterol, LDL cholesterol, as well as HDL cholesterol (van Rooijen et al., 2021). Furthermore, stearic acid in saturated fatty acids has anti-thrombogenic and anti-atherosclerotic properties and is biocompatible with the human body (Attia et al., 2017). According to current research, MUFA and n-6 are important, but the n-3 proportion was highest in golden quails and lowest in gray quails. On the contrary side, the highest proportion of n-3 polyunsaturated fatty acids, EPA, and DPA levels were detected in gray quails. In another study, the fatty acid composition of quail varieties under normal conditions was found to be similar (Sabow, 2020). It seems that warm climate conditions may affect the fatty acid composition of

golden and gray quail varieties. The fact that n-3 Σ PUFA proportions were high in gray and black quails may be associated with the fact that these varieties had more powerful antioxidant systems, since the relationship of the melanin pigment with the antioxidant defense system has been previously determined in another study (Galvan et al., 2010). Erişir (2018) determined a higher level of kidney and liver malondialdehyde in the golden genotype, among the golden and gray quails, which were kept under heat stress. The low malondialdehyde level in the gray genotype was associated with the fact that this genotype had a better adaptation to heat stress, which supported this thesis. Similarly, Vitousek (2013) demonstrated that dark-colored swallows were more resistant to oxidative damage.

The results of the current study indicate that most meat quality parameters obtained from the four quail strains were comparable. Some researchers suggest that white quails possess the best carcass traits and meat quality (Inci et al., 2015, Sabow, 2020). Some of them recommend that brown quail could be preferred for meat production purposes (Nasr et al., 2017). These recommendations are valid for normal conditions. However, it is important to know the traits of quail strains under adverse conditions before making any other recommendations. The current study discovered

that the meat quality parameters of different quail varieties growing in warm climate conditions were changeable. On the other hand, the highest n-3 PUFA ratio was obtained from dark-colored quails, especially gray-colored quails. Overall, the adaptation ability, yield characteristics, and yield quality changes of different Japanese quail varieties in the face of climate change depend on various factors, including genetic makeup, environmental conditions, and management practices. Further research is needed to better understand the effects of climate change on Japanese quail and to develop effective adaptation strategies to mitigate the negative impacts on their productivity and welfare.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept –AG, GG, ÜGS, SA.; Design – AG, GG, ÜGS, SA; Supervision – ÜGS; Resources –ÜGS, SA; Materials –AG, GG, ÖY, MB; Data Collection and/or Processing – AG, GG, ÖY, MB; Analysis and/or Interpretation – AG, GG, ÜGS; Literature Search – AG, GG; Writing Manuscript – AG, GG, ÜGS; Critical Review – ÜGS.

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SVOJSTVA KVALITETE I SASTAV MASNIH KISELINA MESA PRSA RAZLIČITIH VRSTA JAPANSKIH PREPELICA UZGOJENIH U UVJETIMA TOPLE KLIME

SAŽETAK

S obzirom na očekivano pogoršanje klime u budućnosti, maksimalno učinkovit uzgoj životinja za proizvodnju mesa u uslovima tople klime će postati neophodan kako bi se zadovoljile potrebe za mesom. Iz ovog razloga smo izveli eksperiment kako bismo odredili svojstva kvalitete mesa prsa različitih obojenih japanskih prepelica (*Coturnix coturnix Japonica*) uzgojenih u uslovima tople klime. Uključeno je ukupno 100 prepelica, po 25 od svake vrste, crnih, bijelih, zlatnih i sivih. Nakon što su prepelice uzgojene u uvjetima tople klime od 15. do 42. dana i zaklane, određena su hemijsko-fizikohemijska svojstva, zajedno sa sastavom masnih kiselina u prsnim mišićima. Vrijednosti *b (žutilo) su bile znatno niže kod crnih prepelica ($P < 0.01$). Najniži sadržaj masti u mesu je pronađen kod crnih i sivih prepelica, a najviši sadržaj masti kod bijelih ($P < 0.01$). Omjer ukupnih zasićenih masnih kiselina je najviši kod sivih prepelica, a najviše ukupnih mononezasićenih masnih kiselina je pronađeno kod zlatnih, a najmanje kod sivih prepelica ($P < 0.05$). Najviši omjer n-3 polinezasićenih masnih kiselina je pronađen kod sivih prepelica, a najniži kod zlatnih i bijelih prepelica ($P < 0.01$). U ovom istraživanju je zaključeno da različite vrste prepelica imaju različita svojstva zahvaljujući različitim uvjetima koji vladaju u okolišu. Vrste prepelica koje se uzgajaju u uslovima tople klime mogu imati različite parametre kvaliteta mesa.

Ključne riječi: Masne kiseline, svojstva kvaliteta mesa, topla klima, vrste prepelica

RESEARCH ARTICLE

HEMATOLOGICAL PROFILE IN HOLSTEIN DAIRY COWS ACCORDING TO THE DIFFERENT PHYSIOLOGICAL STAGE

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ABSTRACT

This study aims to investigate the variations of hematological and ionic parameters of Holstein dairy cows according to the physiological stage, as an indicator of their health status. The survey was conducted between January and March, 2020 and carried out on 4 lots of 15 dairy cows each. The analysis of blood parameters included: red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), lymphocytes (LYM), monocytes (MONO), neutrophils (NEU), eosinophils (EOS), basophils (BASO) and ionic parameters such as potassium (K), chlorine (Cl), calcium (Ca), phosphorus (P) and sodium (Na).

The RBC level was lowest in the 2nd third of gestation ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) and in postpartum period ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$), in comparison to the 1st third ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) and 3rd third (dry period) of gestation ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$). The same observation was noted for HCT ($p < 0.0001$). MCV was highest in dry cows ($p < 0.01$). MCH and MCHC were highest in cows that were in the postpartum period ($p < 0.001$). For WBC, the count was higher in the 1st third of gestation compared to the other stages, ($11.3 \pm 2.2 \times 10^3/\mu\text{L}$) versus ($6.4 \pm 4.1 \times 10^3/\mu\text{L}$), ($5.2 \pm 1.3 \times 10^3/\mu\text{L}$), and ($8.0 \pm 4.9 \times 10^3/\mu\text{L}$) for the 2nd third, 3rd third of gestation, and postpartum, respectively ($p < 0.001$).

In postpartum cows, the ionic parameters had the lowest values of potassium (K), calcium (Ca) and phosphorus (P) compared to the other stages ($p < 0.001$).

This investigation can be used as a basis for information on the hematological and ionic profile of dairy cattle in order to determine the health status before the onset of clinical signs.

Keywords: Algeria, dairy cows, hematological parameters, ions, physiological stage

INTRODUCTION

Through the various phases of the production cycle, dairy cows are confronted with changes in diet, management and environment, as well as changes in physiological parameters. These changes are reflected by hematological analyses (Herdt, 2000). Therefore, the evaluation of the hematological profile at the herd level can help in the diagnosis and prognosis of diseases, and provide an overview of the feeding and management strategies adopted in dairy farms, allowing the rapid assessment of animal responses to the different phases of the lactation cycle (Bezerra et al., 2017; Herdt, 2000; Roland et al., 2014). It is also useful for the classification of certain anemias (Brockus, 2011).

The hematological parameters can be influenced by several factors including age, breed, sex, physiological phases of the animal, season and farming system (Brucka-Jastrzebska et al., 2007; Farooq et al., 2012; 2017; Shehab-El-Deen et al., 2010).

Throughout the different physiological phases, dairy cows are confronted with multiple subclinical disorders (like subclinical hypocalcemia, subclinical ketosis) that are often associated with severe changes in several blood parameters (Duffield, 2000; Goff, 2008; Mezzetti et al., 2019). Variations in the hematological parameters of dairy cows during different physiological phases have been investigated in several studies (Brscic et al., 2015; Cozzi et al., 2011; Moretti et al., 2017).

In Algeria, the cattle herd represents about 6% of the total livestock population (sheep, goats, cattle, camels, and horses) with a total of 1.9 million cattle of which 52% are dairy cows (MADR, 2018).

This study aims to investigate the variations of the hematological and ionic parameters of Holstein dairy cows, according to the physiological stage (first and second thirds of gestation, dry period and post-partum) as an indicator for physiological status assessment. The results obtained for the first time in Algeria could be used as reference data.

MATERIAL AND METHODS

Study area

The study was conducted between January and March, 2020 on a dairy cattle farm covering an area of 1500 hectares in the wilaya of Mila, northeast Algeria (36°20'47'' N 6°20'19'' E). This region has an altitude of 486 m, known for its warm Mediterranean climate with dry summers. The average annual precipitation in this region is 501.7 mm.

Animals

The study was conducted on Holstein cows aged between 2 and 5 years. The milk production varies from 30 to 40 liters/cow/day. The dry period is at the end of the 7th month of gestation. Cows are artificially inseminated.

For the purpose of the study, the cows were divided into lots in a homogeneous way with regard to health status, age groups and season of study in order to keep only one factor of variation, which is the physiological stage. Clinically sick cows were excluded from our study.

The breeding system of the farm is intensive. The animals receive their feed (hay, barley, concentrate, silage and straw) *in situ*. The watering is *ad libitum*. Milking is done in the milking room twice a day (morning and evening).

Blood samples

Four lots of 15 cows each were selected in the: 1st third period of gestation (1T), 2nd third period of gestation (2T), 3rd third period of gestation or dry period (3T) and postpartum (PP). Each animal was sampled only once.

Blood samples were taken from the coccygeal vein. Animals should be calm to avoid stress-related changes of the hemogram (Jones and Allison, 2007). The samples were transported under isothermal conditions respecting the cold chain, and the time frame for analysis (within 24 hours) was respected to ensure accurate and reliable results (Adewoyin, 2014; Warren et al., 2013).

Blood samples were collected in the morning and divided into two tubes: one has EDTA for blood count formula and another is heparinized for ion analysis. Blood smears for white blood cells were made at the farm and dried, then placed in the boxes for later staining.

Laboratory analysis

The following hematological analyses were performed: red blood cell count (RBC, $\times 10^6/\mu\text{L}$), hemoglobin concentration (HGB, g/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin content (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), platelets (PLT, $\times 10^3/\mu\text{L}$), and white blood cell count (WBC, $\times 10^3/\mu\text{L}$).

All these parameters were measured using a hematology automat (MINDRAY BC3000 Plus, Ref SKU-BC-3000Plus, China) and (BIOTA, Ref VABIO 360, Turkey).

Relative WBC values (neutrophils (NEU), eosinophils (EOS), basophils (BAS), lymphocytes (LYM), and monocytes (MON)) were determined on the basis of microscopic observation of blood smears stained by May-Grunwald/ Giemsa staining technique (RAL 555 kit, RAL diagnostic, Martillac, France).

For ions, the dosage of sodium (Na), potassium (K), chlorine (Cl), calcium (Ca) and phosphorus (P) was determined by:

- The EasyLyte electrolyte analyzer (EasyLyte plus ANALYZER, USA) which is an automated system that was used to make measurements of Na, K and Cl ions.
- Thecolorimetry for Ca and P ions.

For each physiological stage, average values of each blood parameter were calculated.

Data analysis

The statistical program used was R i386 3.0.2 for Windows Front-end GUI. The ANOVA test supplemented by the Chi-square test and multiple range tests (multiple ranges) were used for statistical analysis. The threshold value of different tests was $p < 0.05$.

RESULTS

The data concerning changes in hematological and ionic parameters according to the physiological stage in dairy cows are presented in Tables 1, 2 and 3.

The results showed that the changes were not significant for HGB and PLT (Table 1). However, the changes were significant for RBC, HCT, MCV, MCH and MCHC.

RBC were lower in 2T ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) and PP ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$) compared to 1T ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) and 3T ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$) (Table 1).

Table 1 Red blood cell parameters and erythrocyte indices according to cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P value
RBC ($\times 10^6/\mu\text{L}$)	6.6 ^a (6 – 7.2)	6.0 ^b (5.2 – 6.8)	6.7 ^a (6.2 – 7.2)	5.6 ^c (4.8 – 6.4)	0.0001
HGB (g/dL)	10.6 ^a (10 – 11.2)	10.2 ^a (9.3 – 11.1)	10.6 ^a (9.9 – 11.3)	10.2 ^a (10.1 – 10.3)	-
HCT (%)	30.6 ^b (28.6 – 32.6)	28.1 ^b (23.4 – 32.8)	33.6 ^a (31.4 – 35.8)	26.8 ^c (22.4 – 31.2)	0.0001
MCV (μm^3)	46.4 ^b (43.7–49.1)	47.0 ^b (43.4 – 50.6)	49.9 ^a (47.5 – 52.3)	47.7 ^b (45.5 – 49.9)	0.01
MCH (pg)	16.0 ^c (14.7 – 17.3)	17.2 ^b (16.1 – 18.3)	15.8 ^c (14.6 – 17)	18.2 ^a (17.5 – 18.9)	0.0001
MCHC (g/dL)	34.5 ^a (33.7 – 35.3)	36.8 ^a (32.6 – 41)	31.7 ^b (30.3 – 33.1)	37.1 ^a (31.5 – 42.7)	0.001

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P value
PLT ($\times 10^3/\mu\text{L}$)	332.7 ^a (250 – 415.4)	319.6 ^a (191.4 – 447.8)	288.4 ^a (207.5 – 369.3)	347.9 ^a (225.1 – 470.7)	-

1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Values that have different letters in the same line are significantly different ($p < 0.05$)

HCT was lower in PP ($26.8 \pm 4.4\%$), compared to 1T, 2T and 3T, ($30.6 \pm 2.0\%$) ($28.1 \pm 4.7\%$), ($33.6 \pm 2.2\%$), respectively ($p < 0.0001$) (Table 1).

Dry cows had the highest MCV values ($49.9 \pm 2.4 \mu\text{m}^3$) ($p < 0.01$). MCH ($18.2 \pm 0.7 \text{ pg}$) and MCHC ($37.1 \pm 5.6 \text{ g/dL}$) were highest in PP ($p < 0.001$) (Table 1).

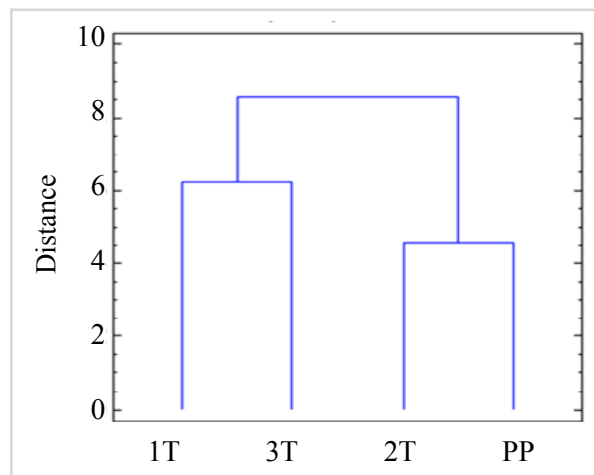


Figure 1 Red blood cell dendrogram and erythrocyte indices according to cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

The dendrogram presented in Figure 1 shows that the changes of the hematological parameters of red

blood cells, according to the physiological stage, are distributed into 2 groups of which the first includes 1T and 3T and the second includes 2T and PP (Figure 1).

Hematological analysis of white blood cells presented in Table 2 showed no significant variation for EOS and BASO. The highest values of WBC, LYM, MONO and NEU were observed in 1T ($p < 0.001$).

The neutrophil/lymphocyte ratio (NEU/LYM) was equal to 1.04, 1.15, 0.91, 0.96 at 1T, 2T, 3T and PP respectively.

The dendrogram shows that the changes of WBC hematological parameters according to the physiological stage were divided into three groups (Figure 2). The first group is represented by 1T, which is significantly different from the other two groups. The second is composed by 2T and 3T, which are very close to the third group, which is PP (Figure 2).

Regarding the ionic elements presented in Table 3, there was no significant difference for Na. For K, Ca, and P, cows showed the lowest values in PP ($3.8 \pm 0.3 \text{ mmol/L}$), ($86.3 \pm 7.5 \text{ mmol/L}$), and ($48.2 \pm 10.7 \text{ mmol/L}$), respectively ($P < 0.001$). Cl was highest in 2T and 3T ($P < 0.01$) (Table 3).

Table 2 Values of the WBC according to the cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P values
WBC ($\times 10^3/\mu\text{L}$)	11.3 ^a (9.1 – 13.5)	6.4 ^{bc} (2.3 – 10.5)	5.2 ^c (3.9 – 6.5)	8.0 ^b (3.1 – 12.9)	0.001
LYM ($\times 10^3/\mu\text{L}$)	5.10 ^a (4.08 – 6.12)	2.69 ^{bc} (0.96 – 4.42)	2.33 ^c (1.59 – 3.07)	3.80 ^b (1.67 – 5.93)	0.0001
MON ($\times 10^3/\mu\text{L}$)	0.51 ^a (0.14 – 0.88)	0.20 ^c (0.04 – 0.36)	0.32 ^{bc} (0.02 – 0.62)	0.45 ^b (0.20 – 0.70)	0.05
NEU ($\times 10^3/\mu\text{L}$)	5.28 ^a (3.79 – 6.77)	3.10 ^{bc} (0.87 – 5.33)	2.11 ^c (1.44 – 2.78)	3.63 ^b (0.81 – 6.45)	0.001

EOS	0.42 ^a (0.02 –	0.34 ^{bc} (0.00 –	0.40 ^b (0.14 –	0.11 ^c (0.00 –	-
(x10³/μL)	0.82)	0.78)	0.66)	0.25)	
BAS	0.0	0.0	0.0	0.0	-
(x10³/μL)					

1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Values that have different letters in the same line are significantly different ($p < 0.05$)

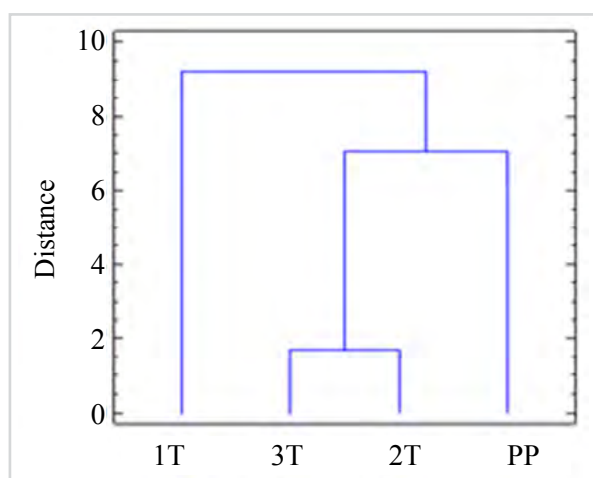


Figure 2 Dendrogram of the variation of WBC according to the cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

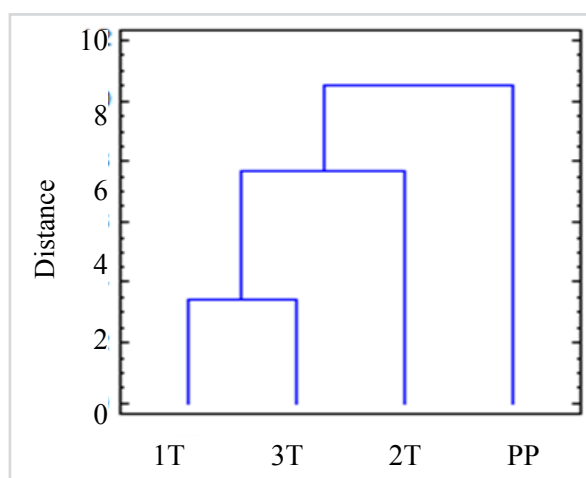


Figure 3 Dendrogram of the ions according to the cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Table 3 Variations in ion concentrations according to the cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P values
Na (mmol/L)	139.9 ^a (138.1 – 141.7)	140.6 ^a (138.9 – 142.3)	139.0 ^a (137.1 – 140.9)	139.8 ^a (138 – 141.6)	-
K (mmol/L)	4.2 ^a (4.0 – 4.4)	4.2 ^a (3.8 – 4.6)	4.3 ^a (4.1 – 4.5)	3.8 ^b (3.5 – 4.1)	0.001
Cl (mmol/L)	98.7 ^c (97.2 – 100.2)	100.5 ^{ab} (98.4 – 102.6)	102.1 ^a (100.5 – 103.7)	100.3 ^{bc} (96.5 – 104.1)	0.01
Ca (mg/L)	97.9 ^a (95.6 – 100.2)	99.0 ^a (94.7 – 103.3)	96.5 ^a (93.4 – 99.6)	86.3 ^b (78.8 – 93.8)	0.001
P (mg/L)	62.4 ^b (54.2 – 70.6)	70.2 ^a (62.9 – 77.5)	68.7 ^b (60.6 – 76.8)	48.2 ^c (37.5 – 58.9)	0.001

It appears that the mineral content varies in three groups (Figure 3). The first group is represented by 1T and 2T, whose values are quite close. The second group, composed of dry cows, differs significantly from the previous group and from the group that follows, composed of cows from the PP (Figure 3).

DISCUSSION AND CONCLUSION

Performing a hematological profile of clinically normal cattle must take into account many factors such as age, physiological status of the animal, season and climate (Brucka-Jastrzebska et al., 2007; Dzavo et al., 2020; Farooq et al., 2017).

The results of the investigated blood parameters reported in the present survey were within the range of reference values for healthy dairy cattle (Bellier and Cordonnier, 2010; Krimer, 2011).

In this study, RBC, HGB and HCT contents were high in the beginning of gestation (1T). This may be associated with increased erythrocyte release from the spleen due to the stress of gestation (Kumar and Pachauri, 2000).

The lowest RBC and HCT values were reported in cows in PP. This finding could be due to anemia caused by calving (Brockus, 2011) and increased production of pro-inflammatory cytokines that could cause a reduction in erythropoiesis (Chikazawa and Dunning, 2016).

The findings of this investigation are consistent with others (Herman et al., 2018; Paiano et al., 2020).

For MCV, dry cows have the highest values, while lactating cows (PP) have the lowest values. This is consistent with the results of Sattar and Mirza (2009). This observation suggests a change in plasma volume induced by the cessation of milk production (van den Brink et al., 2023).

MCH and MCHC contents in parturient cows were the highest. The same observations were found by Sattar and Mirza (2009).

The total number of platelets is influenced by the quantity of consumption, production, sequestration,

and loss (Russell, 2010). The change in platelet count during the four stages investigated in this study was not significant. The same finding was made by Mirzadeh et al. (2010).

The WBC count is higher in early pregnancy cows (1T) compared to the other groups. Our results are consistent with Abdelatif and Alameen (2012) and Ouahrani and Bordjah (2016).

The increase in the number of WBC in PP compared to 3T observed in this survey is due to the increase in antepartum cortisol (Preisler et al., 2000). In addition, cows must satisfy the needs of their fetus at the end of gestation, which increases the energy demand reaching the peak around one month before parturition, to synthesize colostrum and milk (Esposito et al., 2014).

In the present study, NEU and LYM levels were higher in early lactation (PP) than in the third trimester of gestation (3T), and EOS, BAS and MON were not affected by the periparturient period. This is consistent with the results of Ate et al. (2009) and Sattar and Mirza (2009).

Regarding ions, K, Ca and P, recorded the lowest levels in postpartum cows (PP). This is explained by the occurrence of hypocalcemia coupled with hypophosphatemia during calving due to the high demand by the mammary gland, a few days before calving. In the absence of any health problems, this hypocalcemia is physiological (Goff, 2014; Goff, 2008; Horst et al., 2005). This finding is in accordance with Cerutti et al. (2018), Mukača and Katica (2018) and Hadzimusic and Krnic (2012).

Ca and P concentration is regulated by parathormone, which stimulates Ca reabsorption from the urine and bone and stimulates P absorption from the intestine (Cavestany et al., 2005; Goff et al., 2014; Peterson et al., 2005). The sudden high mobilization of circulating Ca and P by the mammary gland at the onset of lactation, however, leads to a decrease in their blood concentration (Goff and Liesegang, 2014).

However, the Na level was not significantly different between the different stages investigated. This observation is consistent with that of Mukača

and Katica (2018).

An increase in plasma electrolyte concentration in the postpartum period has been reported in cattle affected by renal dysfunction or related to stress conditions caused by adrenocorticotropin hormone release and inflammatory conditions occurring around calving (Harshfield et al., 2009). In our study, all cows were healthy and were raised under optimal conditions of which the lowest ion values were observed in PP.

Significantly strong variation was noted for the majority of hematological parameters according to the physiological stage (RBC, HCT, MCV, MCH, WBC, and number of LYM, MON and NEU) and ions (K, Cl, Ca and P). The most marked variations were noted around parturition (peripartum period) by a decrease in red line and ion values, while white line cells show an increase.

Consequently, this study may be useful in providing baseline information on the hematological profile

of dairy cattle for the assessment of physiological status. This survey requires additional investigation concerning the differences in hematological parameters according to age and sex.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design – MM, ON; Supervision – TA, ALN, MA, ON; Analysis and/or Interpretation – KTNA, ON; Literature Search – MM, ON; Writing Manuscript – MM, KTNA, TA, ALN, MA, ON; Critical Review – KTNA, ON.

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HEMATOLOŠKI PROFIL HOLSTEIN MLIJEČNIH KRAVA PREMA RAZLIČITIM FIZIOLOŠKIM STADIJIMA

SAŽETAK

Cilj ovog istraživanja jeste ispitati varijacije hematoloških i jonskih parametara kod holstein mliječnih krava u različitim fiziološkim stadijima kao indikatora zdravstvenog stanja. Istraživanje je provedeno između januara i marta, 2020. godine na 4 grupe od po 15 mliječnih krava. Analiza krvnih parametara je uključivala: eritrocite (RBC), hematokrit (HCT), hemoglobin (HGB), srednji korpuskularni volumen (MCV), srednji korpuskularni sadržaj hemoglobina (MCH), srednju korpuskularnu koncentraciju hemoglobina (MCHC), trombocite (PLT), leukocite (WBC), limfocite (LYM), monocite (MONO), neutrofile (NEU), eozinofile (EOS), bazofile (BASO) i jonske parametre kao što su kalijum (K), hlor (Cl), kalcijum (Ca), fosfor (P) i natrijum (Na).

RBC su bili najniži u 2. trećini gestacije ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) i u postpartalnom periodu ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$) u odnosu na 1. trećinu ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) i 3. trećinu (suhi period) gestacije ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$). Isto je uočeno za HCT ($p < 0.0001$). MCV je bio najviši kod suhih krava ($p < 0.01$). MCH i MCHC su bili najviši kod krava u postpartalnom periodu ($p < 0.001$). WBC su bili najviši u 1. trećini gestacije u usporedbi s ostalim stadijima ($11.3 \pm 2.2 \times 10^3/\mu\text{L}$) prema ($6.4 \pm 4.1 \times 10^3/\mu\text{L}$), ($5.2 \pm 1.3 \times 10^3/\mu\text{L}$), i ($8.0 \pm 4.9 \times 10^3/\mu\text{L}$) u 2. trećini, 3. trećini gestacije i postpartalno ($p < 0.001$).

Kod postpartalnih krava od jonskih parametara su najniži bili kalijum (K), kalcijum (Ca) i fosfor (P) u usporedbi sa drugim stadijima ($p < 0.001$).

Ovo istraživanje se može koristiti kao osnovna informacija o hematološkom i jonskom profilu mliječnih krava kako bi se odredilo njihovo zdravstveno stanje prije pojave kliničkih znakova.

Ključne riječi: Alžir, fiziološki stadij, hematološki parametri, joni, mliječne krave

RESEARCH ARTICLE

FORENSIC MEANING OF CORE TEMPERATURE - AN INDICATOR FOR ASSESSING THE SEVERITY OF HEATSTROKE IN AN ANIMAL MODEL

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ABSTRACT

Sudden deaths during efforts that are multifactorial and associated with exposure of the body to high temperatures beyond the power of thermoregulatory mechanisms are increasingly common. Autopsies are often performed, but the evidence is insufficient and non-specific. The research is aimed to determine the core temperature values of rats exposed to different water temperatures (37°C, 41°C, 44°C), before the start of the experiment (T_b), after immersion in water (T_u), after 20 minutes of exposure and at death (T_s) in rats for hyperthermia and heat stroke.

Forty rats were divided into five groups depending on the temperature and length of exposure to water: control group- CG37, G41-hyperthermia- group which exposure time was 20 minutes at 41°C, G41-heat stroke- group exposed until death at 41°C, G44- hyperthermia- group which exposure time was 20 minutes at 44°C, G44- heat stroke- group exposed until death at 44°C. A RET-4 probe was used to measure the core temperature of rats.

Significant changes in the body temperature of rats were observed during the lethal outcome, $p < 0.0005$. After exposure to water temperature for a period of 20 minutes, depending on the group, it was observed that the body temperatures of rats differed significantly between G37 and G41, CG37 and G44, $p < 0.0005$ and G41 and G44, $p < 0.0005$. A significant difference was also observed in the *post mortem* temperature of groups G41 and G44, $p = 0.01$, a significant difference between body temperatures in groups CG37, G41-hyperthermia, G41- heat stroke, G44-hyperthermia and G44-heat stroke ($p < 0.0005$), and the significance of the differences in the CG37 group was $p = 0.044$.

Exposure of albino rats to different water temperatures also led to the changes in the internal temperature; normothermia was established through thermoregulation in the control group, and in the other groups, hyperthermia and heat stress occurred.

Keywords: Heat stroke, rats, temperature, variation

INTRODUCTION

A large number of deaths both in Europe and in the United States of America have been attributed to exposure to high temperatures (Haines et al., 2006; Luterbacher et al., 2004). Central Europe experienced its hottest summer in 2003 since 1500, with average temperatures around 3.5°C above the upper air temperature limits. The number of deaths was between 22,000 and 45,000 in a period of two weeks, according to data published by the Red Cross Organization (IFRC, 2004). Common causes of death were dehydration and heat stroke in hospitalized patients in France (Ferron et al., 2006). A 7% increase in mortality recorded during the same period in Switzerland was associated with heat stroke (Grize et al., 2003). The core temperature of internal organs is regulated at approximately 36.6°C (Obermeyer et al., 2016), but due to exposure to high temperatures, it can vary significantly, from the lowest recorded temperature of 13.7°C experienced by human survivors (Gilbert et al., 2000) to a maximum of 41.5°C without any complications for the bodies (Racinais et al., 2019). Core body temperature in humans is the main regulated variable in thermoregulation (Kramer et al., 2022). Body core temperature is the result of local thermal balance depending on the place of measurement (Taylor et al., 2014). It has been proven that the influence of high air temperature triggered certain processes in the organism and that compensatory mechanisms failed. At autopsy, in cases of sudden death after exertion, atherosclerosis of the coronary vessels was found in more than 60%, left ventricular hypertrophy of unknown etiology in 7.8%, and valvular heart disease in 7.1% (IFRC, 2004; Ferron et al., 2006; Grize et al., 2003). Sudden death during exertion can depend on the predisposing heart disease. In comparison with the control group, a statistically significant association was observed between the subjects' heat exhaustion and changes in the heart tissue. Establishing absolute evidence in support of the diagnosis of death caused by heat stroke is a major problem in forensic pathology. Primarily, it is not routine to always measure the temperature of the deceased. In addition, macroscopic findings

are not site-specific, especially if the survival time was short. The diagnosis of hyperthermia is based on investigative actions, circumstances, and reasonable exclusion of other causes of death.

The research aimed to determine the core temperature values of rats exposed to different water temperatures before the start of the experiment (Tb), after water immersion (Tu), after 20 minutes of exposure (Tu) and at death (Ts) rats for hyperthermia and heat stroke.

MATERIAL AND METHODS

The study was processed as a prospective experimental, randomized study based on the albino Wistar rat model of hyperthermia. The research was conducted at the Faculty of Medicine, University of Sarajevo, by the Principles for the Care of Laboratory Animals (De Labra, 2021). After the approval of the Ethics Committee of the Faculty of Medicine of the University of Sarajevo (registration number 02-3-4-1253/20, Bosnia and Herzegovina), the total number of adult Wistar rats with body weight from 200 to 300 g were included in the experiment. Target temperature points in the water bath were determined, and one rat at a time was exposed to a total of 40 rats included in the experiment. Rats were divided into five groups depending on the temperature and length of exposure to water: control group exposed to a water temperature of 37°C (CG), groups exposed to a water temperature of 41°C and 44°C for 20 minutes (G41-hyperthermia; G44-hyperthermia), groups exposed to a water temperature of 41°C and 44°C with exposure time until death (G41-heat stroke; G44-heat stroke). Heat stroke is defined as an increase in core temperature above 40.5 °C (Benzinger, 1969; Racinais et al., 2019).

The rats were anesthetized with ketamine in a dose of 1.2 mL/kg bw + /- 10% (USP Rotexmedica-Germany) (Režić-Mužinić et al., 2018). The experimental protocol was made according to the previously described methodology of induction of hyperthermia and heat shock (Dervišević et al., 2022). A probe (RET-4 probe for mice and rats) was used to measure the core temperature

of rats, and the core temperature was read on a thermometer (Physitemp Instruments Clifton, Physitemp Thermalert Model TH-8, USA).

RESULTS

The basal temperature was the lowest in group G44 at $38.02 \pm 0.55^\circ\text{C}$, and the highest in G37 at

$38.25 \pm 0.12^\circ\text{C}$. The immersion temperature ranged from 37.84°C in G41 to $38.67 \pm 0.90^\circ\text{C}$ in G44. In group CG37, the lowest temperature of 37.75°C was observed after 20 minutes of exposure, and the highest in G44, $43.09 \pm 0.79^\circ\text{C}$. The death temperature was lower in G41 $38.34 \pm 3.59^\circ\text{C}$ compared to $41.51 \pm 2.73^\circ\text{C}$ in G44 (Table 1).

Table 1 Mean values of measured body temperature of rats of experimental groups at time points

T ($^\circ\text{C}$)	Group	$\bar{x}$	$\pm\text{SD}$	95% IP	
				DG	GG
T-b ($^\circ\text{C}$)	CG37	38.25	0.12	38.13	38.37
	G41	37.80	0.48	37.53	38.07
	G44	38.02	0.55	37.72	38.32
T-u ($^\circ\text{C}$)	CG37	38.17	0.23	37.95	38.39
	G41	37.84	0.50	37.56	38.12
	G44	38.67	0.90	38.19	39.15
T-20 ($^\circ\text{C}$)	CG37	37.75	0.38	37.40	38.11
	G41	40.04	0.43	39.79	40.28
	G44	43.09	0.70	42.71	43.46
T-s ($^\circ\text{C}$)	G41	38.34	3.59	36.35	40.33
	G44	41.51	2.73	40.05	42.97

$\pm \bar{x}$ - mean value; $\pm\text{SD}$ -standard deviation; IP-Confidence Interval; DG-Lower Bound; GG- upper limit; T-b- basal temperature of rats; T-u immersion temperature of rats; T-temperature in the 20th minute; T-s- temperature of death; p-probability CG37-control group of rats exposed to water temperature of 37°C ; G41-group exposed to water temperature of 41°C ; G44-group of rats exposed to a water temperature of 44°C

By analyzing the mean values of body temperature measured at four-time time points, showed that the basal body temperature of rats of group CG37 was not significantly different from the basal temperature of rats in G41 and G44, $p=0.125$, so multiple comparisons between groups were not performed. The temperature after water immersion depending on the group was significantly different between groups G41 and G44, $p=0.005$. After

exposure to water temperature for a period of 20 minutes, depending on the group, it was observed that the body temperatures of rats differed significantly between G37 and G41, CG37 and G44, $p<0.0005$ and G41 and G44, $p<0.0005$. A significant difference was also observed in *post mortem* temperature of groups G41 and G44, $p=0.01$ (Table 2).

Table 2 The difference in body temperature values of rats of experimental groups at three-time time points

T (°C)	Group	Group	p	95% IP	
				DG	GG
T-u (°C)	G44	G41	0.005	-1.42	-0.22
T-20 (°C)	CG37	G41	<0.0005	-2.91	-1.65
		G44	<0.0005	-5.95	-4.71
T-s (°C)	G44	G41	<0.0005	2.56	3.54
	G41	G44	0.01	-5.86	-0.46

T (°C) - Temperature in degrees Celsius; p-probability; IP-Confidence Interval; DG-Lower Bound; GG- upper limit; T-u immersion temperature of rats; T-temperature in the 20th minute; T-s- temperature of death; p-probability; CG37 (-control group of rats exposed to water temperature of 37°C; G41-group exposed to water temperature of 41°C; G44-group of rats exposed to a water temperature of 44°C)

Repeated measurements of body temperature at three time points (T-b, T-u, T-20) by group showed a significant difference between body temperatures in all three groups. In the control group (CG37), a significant difference was observed in three measurements ($p=0.044$), and

in groups G41 and G44, a significant difference in the body temperature of the rats was observed in three measurements ($p<0.0005$) with a drop in the temperature of CG37 group in 20 minutes and an increase in temperature in groups G41 and G44 (Table 3).

Table 3 Multicomparison of body temperature of rats of groups at three-time time points**a)**

T (°C)	Group	Group	p	95% IP	
				DG	GG
T-u (°C)	G41-AM	G41-PM	0.04	-2.07	-0.03
	G41-PM	G44-AM	0.03	-2.03	-0.6
T-20 (°C)	CG37	G41-AM	<0.0005	-3.00	-1.25
		G41-PM	<0.0005	-3.2	-1.57
		G44-AM	<0.0005	-6.08	-4.39
		G44-PM	<0.0005	-6.27	-4.58
	G41-AM	G44-AM	<0.0005	-3.95	-2.26
		G44-PM	<0.0005	-4.14	-2.45
	G41-PM	G44-AM	<0.0005	-3.64	-2.00
		G44-PM	<0.0005	-3.82	-2.19

b)

T (°C)	Group	Group	p	95% IP	
				DG	GG
	CG37	G41-PM	<0.0005	-9.19	-4.29
		G44-AM	<0.0005	6.99	-409
		G44-PM	<0.0005	-12.01	-7.11
	G41-AM	G41-PM	<0.0005	-8.56	-3.66
T-s		G44-AM	0.001	-6.36	-1.46
		G44-PM	<0.0005	-11.38	-6.48
	G41-PM	G44-PM	0.013	-5.19	-4.58
	G44-AM	G44-PM	<0.0005	-7.39	-2.65
	G44-PM	G44-AM	<0.0005	2.65	7.39

T (°C) - Temperature in degrees Celsius; p-probability; IP-Confidence Interval; DG-Lower Bound; GG- upper limit; T-b- basal temperature; T-u- rat immersion temperature; T-s- temperature of death; CG37-control group of rats exposed to a water temperature of 37°C; G41-AM (G41-hyperthermia)- *ante mortem* group exposed to water temperature 41°C (length of exposure 20 minutes); G41-PM (G41-heat stroke) – *post mortem* group exposed to water temperature 41°C (length of the exposure until death); G44-AM (G44-hyperthermia)-*ante mortem* group of rats exposed to a water temperature of 44°C (length of exposure 20 minutes); G44-PM (G41-heat stroke) –*post mortem* group of rats exposed to a water temperature of 44°C (length of the exposure until death)

DISCUSSION AND CONCLUSION

Large temperature fluctuations and climate changes affect the global temperature, with the period between 2011 and 2020 being the warmest in the last 140 years (Asheville, 2021; Global Climate Report-Annual, 2020; Périard et al., 2021; WMO, 2020). Since 2015, the warmest years are 2016, 2019, and 2020. The mean global temperature was 1.2°C above the level in 2020, WMO 2020 and between 2030 and 2052, it is predicted to rise by another 1.5 °C (Masson-Delmotte et al., 2018). These temperature changes in the environment directly affect the internal temperature of the organism because the basic temperatures increase and the loss of water in the body also increases the reserve consumption of the compensatory mechanisms of the organism (Masson-Delmotte et al., 2018).

Heat stroke leads to oxidative cellular stress and impaired thermo-compensatory mechanisms, and can range from heat exhaustion to death. An organism engages compensatory mechanisms of heat- release through sweating, tachycardia, and ultimately the activation of pro-inflammatory and

inflammatory cytokines. Heat stroke is defined as a state of elevated body temperature of 40.05°C and above this value, which is a consequence of overemphasized compensatory mechanisms and exhausted body reserves with interindividual variations (Nzvere et al., 2020; Suzuki and Hori, 2014).

Heat stroke, a condition occurring in the range of 10 to 50%, leads to death through cardiac dysfunction with global hypokinesia (Rajan et al., 2017). Given that the number of deaths caused by heat stroke is increasing, it is necessary to understand the behavioral response of the organism to increased environmental temperature, which is directly related to global warmings (Barney and Kuhrt, 2016). In the world, the number of deaths that occur in closed spaces with an increase in air temperature inside the room, for example during bathing, in saunas, Turkish baths, and among athletes due to strenuous training, especially in the summer months, is increasing. Two significant causes of sudden death in athletes are death due to arrhythmia and heat stroke. Death caused by arrhythmia is more in the center of medical attention, while heat stroke is less considered

(Yankelson et al., 2014). Autopsy rates remain low and vary between countries, and protocols for performing autopsies in cases of suspected sudden death also differ (Illing et al., 2019).

Determining heat stroke as the cause of death is very difficult in forensic medicine due to the non-specificity of biochemical analyses. In our rat model of thermal climate, a thermoregulatory response was included by exposing rats to different water temperatures (37, 41, and 44°C). An esophageal probe was used to measure core temperature. The proven physiological body temperature of rats is 37.5-37.7°C (Bathini et al., 2020), and the research aim was to determine the core values of rats exposed to different water temperatures (37, 41, and 44°C) before the start of the experiment (T_b), after immersion (T_u), after 20 minutes of exposure (T_u) and at the moment of death (T_s) in rats with hyperthermia and heat stroke. Basal temperature was the lowest in group G44 and the highest in the control group, which is in correlation with inter-individual variations. The death temperature was lower in G41 compared to G44, which considers the physiological state and the absence of pathological processes in the rats before the experiment started. Given that the analysis of basal temperatures in the grower was not significant, this points to the conclusion about the organism's physiological state and normothermia in rats before the start of the experiment. A significant difference appears during water immersion, after 20 minutes, and at the moment of death, which points to the control mechanisms of the thermoregulation center. Heat stroke was detected in rats exposed to the highest temperature of 44 degrees.

Our results are in accordance with the literature data that indicate that the minimum time required for the onset of heat stroke with the compensatory mechanisms of the organism involved is 20 minutes from the moment of the target temperature (Suzuki and Hori, 2014). Basal temperature is the result of the organism's physiological thermoregulation itself, while increased ambient temperature and the length of exposure of the body affect the increase in internal temperature and engage the compensatory mechanisms of the organism with

the exchange of heat between the body and the environment. The temperature of rats at the time of death was $44.02 \pm 0.38^\circ\text{C}$ in group G44-heat stroke, and $41.20 \pm 0.76^\circ\text{C}$ in G41. The rats exposed to water temperatures of 41°C and 44°C had longer survival in group G41 of 175.50 minutes, compared to rats in group G44 of 4.14 minutes ($p=0.001$). Therefore, the period of exposure to high temperature caused more rats of group G44 not reaching the temperature threshold for heat stroke. In a study by Quinn et al. (2014), a predetermined temperature is used as the temperature standard of heat stroke, which was also done in our research, and is considered an inter-individual variation (Danzl, 2002; Hori, 2014; Sweeney, 2002; Yoder, 2004). In a study of healthy individuals, the internal temperature reached values of 40°C after 10 minutes of exposure. In addition, deterioration of brain and heart function was observed after immersion in water at a temperature of 44°C, but no brain and heart damage was observed after immersion in water heated to 40°C. The degree of damage depends on the temperature of the environment and the length of exposure of the body to that environment (Hori, 2014). Heat stress induces systemic inflammation through increased cytokine and white blood cell concentrations, which contributes to hyperthermia and reduced metabolic activities, as metabolic reservoirs are depleted to deploy homeostatic mechanisms. Excessive inflammation in heat stress induces the release of damage-associated molecular ligands from apoptotic cells in the final stage of pyroptosis (Geng et al., 2015).

The modern epoch of climate change also caused research on the topic of thermoregulation of the organs with adaptation to high temperatures. Thermoregulation of heat is described by the central integrator model of peripheral and central heat inputs that activate thermoeffector reactions when the core temperature moves above physiological. However, further research should consider more complex models that include several integrators and other afferent signals taking into account the stages of the heat flow mechanism and the inter-individual variations of the response that

cannot be excluded.

Exposure of albino rats to different water temperatures also led to a change in the internal core temperature. In the control group, normothermia was established through thermoregulation, and in the other groups, hyperthermia and heat stress occurred.

Taking into account the hypotheses of our research, we confirmed the working hypothesis. In addition, there are certain limitations to the study. It is necessary to work on the development and expansion of existing protocols for the diagnosis of heat stroke, given that the climate changes are becoming more frequent, compensatory mechanisms are unpredictable, and that heat stroke is an increasingly common cause of death

at autopsy. It would be necessary to validate the results on human material and create registers of causes of death.

CONFLICT OF INTEREST

None declared.

CONTRIBUTION

Concept – ED, MD, AB; Desing – LD, AS, EM; Supervision – ED, MD, AB; Materials – ED, ZA, AB; Data Collection and/or Processing – AS, LD; Analysis and/or Interpretation – ED, LD, ZA; Literature Search – AB, ZA, ED, MD; Writting Manuscript – ED; Critic Review – EM, ZA, AS.

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FORENZIČKO ZNAČENJE UNUTRAŠNJE TEMPERATURE – INDIKATOR PROCJENE TEŽINE TOPLOTNOG UDARA NA ŽIVOTINJSKOM MODELU

SAŽETAK

Iznenadne smrti tokom napora, koje su multifaktorijske i povezane s izlaganjem tijela visokim temperaturama, a prevazilaze snagu termoregulatornih mehanizama, su sve učestalije. Obdukcije se često izvode, ali su dokazi nedostatni i nespecifični. Cilj istraživanja je određivanje vrijednosti unutrašnje temperature pacova izloženih različitim temperaturama vode (37°C, 41°C, 44°C), prije početka eksperimenta (T_b), nakon potapanja u vodu (T_u), nakon 20 minuta izlaganja i u trenutku ugibanja (T_s) kod hipertermije i toplotnog udara.

Četrdeset stahora je podijeljeno u pet grupa prema temperaturi i dužini izlaganja vodi: kontrolna grupa-CG37, G41-hipertermija - grupa čije je izlaganje iznosilo 20 minuta na 41°C, G41 - toplotni udar – grupa izložena do ugibanja na 41°C, G44 - hipertermija- grupa čija je dužina izlaganja iznosila 20 minuta na 44°C, G44 - toplotni udar - grupa izložena do ugibanja na 44°C. Za mjerenje osnovne temperature pacova je korištena RET-4 proba.

Kod smrtnih ishoda su zabilježene signifikantne promjene u tjelesnoj temperaturi stahora, $p < 0.0005$. Nakon izlaganja temperaturama vode u trajanju od 20 minuta, u ovisnosti od grupe, uočeno je da su se tjelesne temperature stahora signifikantno razlikovale između G37 i G41, CG37 i G44, $p < 0.0005$ i G41 i G44, $p < 0.0005$. Također je uočena signifikantnost razlika u *post mortem* temperaturama između grupa G41 i G44, $p = 0.01$, kao i signifikantnost razlika u tjelesnim temperaturama između grupa CG37, G41 - hipertermija, G41 - toplotni udar, G44 - hipertermija i G44 - toplotni udar ($p < 0.0005$), dok je signifikantnost razlika u CG37 grupi iznosila $p = 0.044$.

Izlaganje albino stahora različitim temperaturama vode je, također, dovelo do promjene unutarnje temperature; normotermija je u kontrolnoj grupi uspostavljena termoregulacijom, dok je u ostalim grupama došlo do pojave hipertermije i toplotnog stresa.

Ključne riječi: Stahori, toplotni udar, temperatura, varijacija

RESEARCH ARTICLE

EFFICACY OF AUTOGENOUS FORMALIN KILLED WHOLE CELLS VACCINE TO AGAINST EDWARDSIELLOSIS IN STRIPED CATFISH (*Pangasionodon hypophthalmus*)

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ABSTRACT

This research aims to make a vaccine from *Edwardsiella tarda* isolated from local isolate. It was carried out at Aquaculture Laboratory of Aquaculture Department, Sriwijaya University. Bacterial isolate was a stock from Fish Quarantine and Inspection Agency, South Sumatera, Indonesia. Prior to vaccination, fish had already adapted to pellets and aquaria conditions. After 14 days post-vaccination (dpv) catfish was infected by *E. tarda* 10⁷ CFU.ml⁻¹ administrated by injection. The result revealed that vaccination can protect fish from edwardsiellosis better than non-vaccinated fish with Relative Percent Survival (RPS) value was 63.64 % and antibody titer increased from 7 dpv and greater at 14 dpv. In fact, the RPS value was less than 70% but there were no hematological disorders after vaccination. It means the whole cells vaccine can be used as a vaccine candidate to protect striped catfish from edwardsiellosis. Furthermore, some experiments need to be developed to increase RPS value in the future.

Keywords: Autogenous vaccine, *Edwardsiella tarda*, *Pangasionodon hypophthalmus*, whole cell vaccine

INTRODUCTION

South Sumatera is the largest province that produce striped catfish (*Pangasionodon hypophthalmus*) in Indonesia. In 2019, South Sumatera striped catfish production almost 50% from total production of Indonesia (<https://statistik.kkp.go.id>). However, infectious diseases such as edwardsiellosis are a major problem in striped catfish culture because its infection can cause more than 50% mortality depends on virulency, environment and fish health status. Edwardsiellosis caused by bacteria *Edwardsiella tarda* and *E. ictaluri*. Moreover, both

bacteria were reported to attack striped catfish in Indonesia which caused extensive mortalities in striped catfish cultured on cage, in Cirata Lake (Mawardi et al. 2018; Susanti et al. 2016). Thus, fish health management including prevention and treatment needs to be developed to avoid disease outbreaks in striped catfish.

Fish vaccination is already well known as prevention action in aquaculture to minimize risk of failure due to disease outbreaks (Ma et al. 2019). Some commercial vaccines have been released to fish farmers with various types of vaccine and their efficacy for some fish species. Vaccine efficacy is strongly influenced by several variables including: (i) antigen dose, exposure and absorption, (ii) improving vaccination strategies, (iii) adjuvant, (iv) water temperature, (v) fish size and life stage and (vi) type of vaccine, virulence and administration (Evensen 2020; Ma et al. 2019; Miccoli et al. 2021). The simplest type of vaccine or traditional vaccine is a killed whole cells vaccine. The advantages of this type are no risk of inducing diseases as they do not contain live component, provide sources of potential antigen and cheaper to produce than any type of vaccines (Adams, 2019) (Ma et al. 2019). Autogenous vaccine is custom made that are produced based on local pathogen or isolated from farm which they are to be used in small to medium scale (Bwalya et al. 2020).

This is preliminary research to produce autogenous vaccine that expected able to protect local striped catfish from *E. tarda* infection. As hypothesis, the vaccinated fish has higher immunity than non-vaccinated fish. Furthermore, this research can be used as basic information to enhance vaccine efficacy in order to protect striped catfish better by boosting cellular immune response from edwardsiellosis.

MATERIAL AND METHODS

Time and Place

This research was carried out in June – August 2021 at Aquaculture Laboratory, Department of Aquaculture and Genetics and Biotechnology

Laboratory, Department of Biology, Sriwijaya University.

Research Materials

Juvenile striped catfish were bought from local fish farmers with 10-11 cm in length for vaccination and 17-18 cm for collecting virulence type of bacteria. Fish were fed with commercial pellet (HI Pro-Vite 781-2, CP Prima). *E. tarda* isolate were collection from Fish Quarantine and Inspection Agency, South Sumatera. Medium culture was Brain Heart Infusion Agar (BHIA) and Brain Heart Infusion Broth (BHIB) (Oxoid, CM1136B, CM1135, ThermoFisher Scientific, UK). Neutral Buffer Formalin 10% (v/v) was made from 37% formaldehyde (Sigma Aldrich, Merck, Germany). Fish IgM Elisa kits (E0025FI, Bioassay Technology Laboratory, Sanghai Korain Biotech Co, Ltd). The equipment was nanospectrophotometer (NanoDrop™ Lite Spectrophotometer, ThermoFisher, UK), thermal centrifuge (microfuge 20R- Beckman Coulter Life Science), Laminar air flow (Robust Laminar air flow), microhematocrit (Marinefeld Micro Hematocrit) and aquarium 50 x 50 x 50 cm³.

Research Design

The research design was experimental study with two treatments which was vaccinated and non-vaccinated fish. As control of rearing, there was negative control which was no infection and no vaccination. Each treatment has 3 replications and 2 repetitions (Figure 1).

Work Procedure

The research divided into 4 parts including bacterial and growth conditions, vaccine preparation, fish vaccination and bacterial challenge. All procedures are described below.

Bacterial and growth conditions

The *Edwardsiella tarda* were used for antigen preparation and infection respectively. One colony from the stock isolate cultured on BHIA were transferred into 20 ml BHIB. It was grown in BHIB with hand shaking regularly for 24 h at room

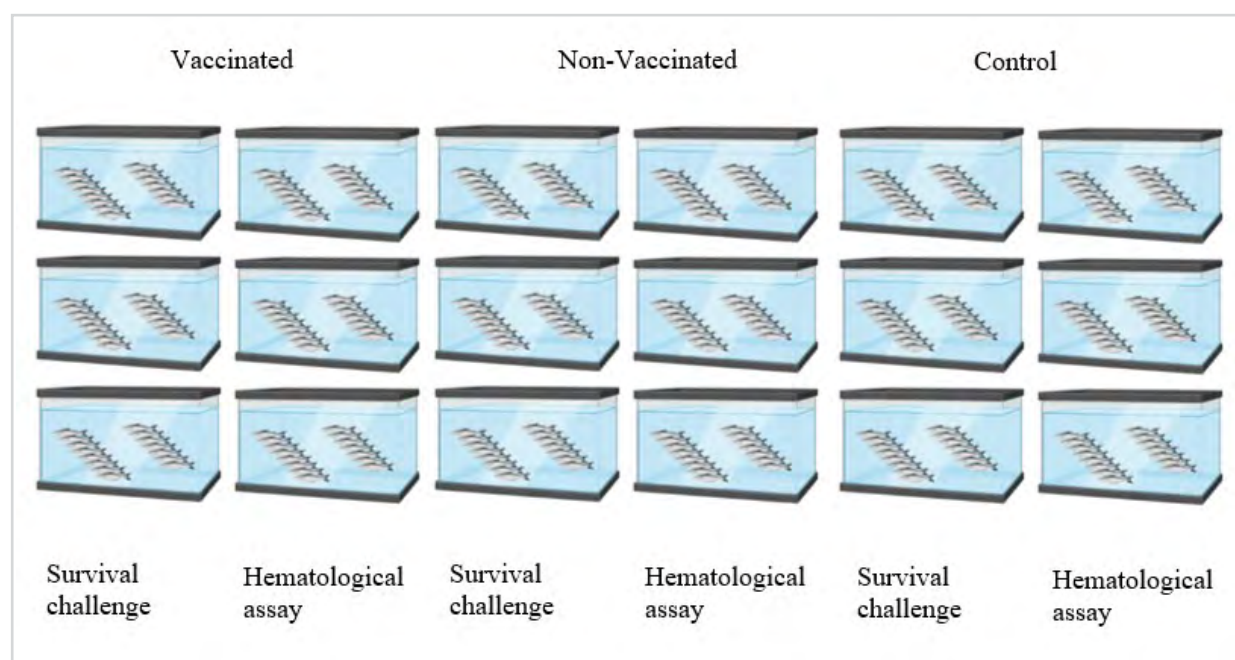


Figure 1 Research design of experiment created by BioRender.com (n=15 fish/aquarium)

temperature (25-26°C). 10 ml of pre-cultured cells were inoculated into 90 ml BHIB with the same condition. Prior vaccine production, the isolate had been tested to striped catfish for virulence factor. Re-isolation of bacteria from infected fish has been done with postulate Koch approach on BHIA. The total bacteria were counted with Total Plate Count (TPC) method.

Vaccine preparation

Whole cells vaccine with formalin-inactivated or killed methods was made as previously described by Cao et al. (2015) and Dwinanti and Fitriani (2016) with modifications as follows. Inoculant were diluted into final concentration 10^{11} CFU.mL⁻¹. Bacterial cells were inactivated by addition of 10% (v/v) neutral buffer formalin and incubated at room temperature for 24 h. The suspension was washed twice with phosphate buffer saline. Supernatant was separated by centrifugation at 10.000 rpm for 5 minutes 4°C. Sterility of vaccine was confirmed by viability test which was no growth of bacteria on BHIA plate. Absence of toxicity was confirmed by injecting the vaccine candidate into 10 striped catfish and if no death the vaccine ready to used.

Fish vaccination

The healthy fish were reared in aquarium with water volume was 15 L and fish density was 1 individu.L⁻¹. The vaccine was injected intramuscular 0.1 mL.Individu⁻¹ while negative and positive were injected by phosphate buffer saline with same volume. Hematological tests (hematocrit, total leukocytes, titer antibody) were measured at day 7 and 14 post-vaccination (dpv).

Bacterial challenge

Striped catfish fingerling reared for 14 days post-vaccination (dpv) was injected with *E. tarda* (10^4 CFU.mL⁻¹) 0.1 ml per individual. The percentage of mortality, relative percent survival (RPS) and prevalence of infection on fingerlings were calculated 7 days after the challenge test.

Parameters

Hematological (hematocrit, total leukocytes and antibody titer)

Antibody titer was measured by using fish serum and ELISA kits by Bioassay Technology Laboratory. The curve standard was made before

measuring the sample. All protocols followed the manual procedure from the kit. Total leukocytes and hematocrit were measured according to Blaxhall and Daisley (1973).

Efficacy of vaccine (Relative Percent Survival, Prevalence Rate, Survival Rate)

Relative percent survival, prevalence rate and survival rate were calculated in the end of experiment. Relative percent survival (RPS), prevalence rate and survival rate were calculated using the following formula:

$$\text{RPS (\%)} = \frac{1 - \% \text{mortality of vaccinated fish}}{\% \text{mortality of non vaccinated fish}} \times 100$$

$$\text{Prevalence rate (\%)} = \frac{\text{total of infected fish}}{\text{total of fish}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{number of survived fish}}{\text{total initial of fish}} \times 100$$

Water quality

The water quality during research was well maintained such as pH, water temperature, ammonia and dissolved oxygen.

Data Analysis

This study used a completely randomized design method. Data obtained was collected using Microsoft Office Excel 2010 and analyzed using one way ANOVA method.

RESULTS

The efficacy of the vaccine can be represented by the relative percent survivor value (RPS) which is calculated from mortality in control group and vaccinated group after infection. Based on experimental, survival rate and prevalence rate are presented in Table 1 while Relative Percent Survivor (RPS) value of vaccine is presented in Table 2.

Vaccinated fish have better protection than unvaccinated fish. Based on the results of statistical tests, it was seen that the vaccinated fish produced significantly different survival rates and prevalence rate than the unvaccinated fish. During the study, fish that were not infected with the bacteria also showed high survival rates. This explains that the catfish rearing technique has been implemented properly.

Table 1 Percentage fish both survival and prevalence rate infected with *E. tarda* after 7 days post infection (dpi)

Treatments	Survival rate (%) $\text{LSD}_{0.05}$	Prevalence rate (%) $\text{LSD}_{0.05}$
Control -	93.33 ^c ± 6.67	-
Control +	51.11 ^a ± 3.85	48.89 ^b ± 5.44
Vaccinated	82.22 ^b ± 3.85	22.22 ^a ± 3.44

Note: different manuscripts in the same column indicate significantly different (at least $p < 0.05$)

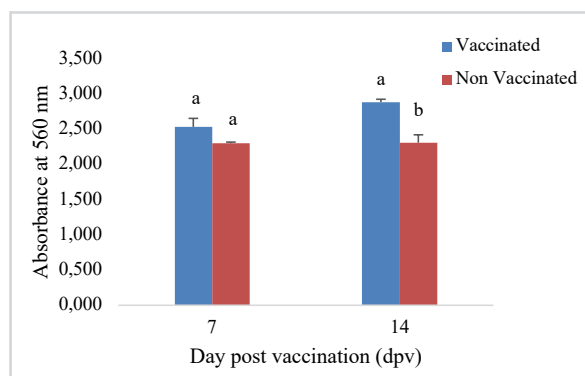
Infected fish by *E. tarda* clearly show clinical sign like whitish necrotic abscesses which is showed in Figure 2.

The antibody level after vaccination was increase after 14 days post vaccination (Figure 4). The experiment revealed that vaccinated fish produce antibody since 7 dpv and almost doubled increase in 14 dpv. The first 7 dpv, showed that antibody

titer not significantly different ($p < 0.05$) which means during the period the antibody not produced optimum yet. However, in 14 dpv titer antibody significantly different which vaccinated fish had higher 1.2-fold antibody titer than non-vaccinated. By using regression on standard curve (Figure 3), the amount of antibody for 7 dpv and 14 dpv were 237.7 mg.ml⁻¹ and 589 mg.ml⁻¹ respectively.

Table 2 Relative Percent Survivor (RPS) value of vaccine

Treatment	Mortality rate (%)	RPS (%)
Control -	6.77	63.64
Control +	48.89	
Vaccinated	17.78	

**Figure 2** Striped catfish infected by *E. tarda* (→: whiteish necrotic abscesses)**Figure 4** Antibody titer of striped catfish after vaccination

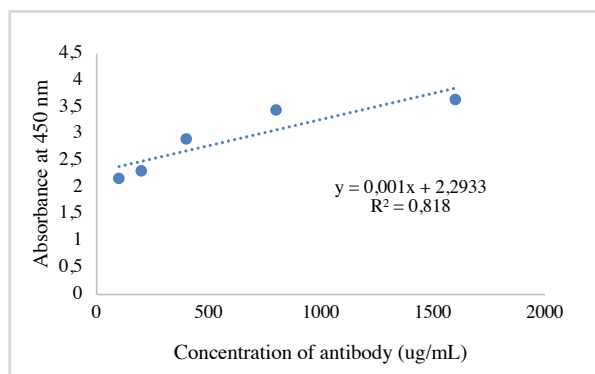
DISCUSSION AND CONCLUSION

Fish vaccination is revealed for some commercial fishes as a tool to prevent diseases outbreaks and avoid mass mortality due to pathogen infection (Gudding 2014). Vaccine is made from pathogen (antigen) or its any derivatives that able to stimulate fish immunity to produce antibody in order to protect fish from specific infection (pathogen is same with material vaccine). Various types of vaccine has been researched, developed,

commercialized, successfully applicated and reviewed (Collins et al. 2019, Dwinanti et al. 2014; Ma et al. 2019, Nor et al. 2020).

Edwardsiellosis or *Edwardsiella septicaemia* in fish has clinical sign such as necrotic abscesses in the muscle that can be whiteish or show petechia and mortality pattern may be acute or chronic depending on environment, virulency factor and fish immunity. *Edwardsiella tarda* that were used in this experiment was virulence type and clinical sign were observed after striped catfish injected (Figure 2). Transmission of *E. tarda* in catfish might be through the water from an infected source (carrier animal feces, water or mud) to susceptible fish since this bacterium is common in the aquatic environment (Evans et al. 2011).

Nowadays, fish farmers emphasizing on diagnosis and prevention of edwardsiellosis infections and promote health and production efficiency by environmental manipulation, proper nutrition and immunological protection such as vaccination (Hoque et al. 2020).

**Figure 3** Curve standard of antibody titer

Vaccination in fish can trigger the fish immune response to produce antibody to protect fish from specific pathogen.

In general, the specific system of fish defense requires the presence of an antigen (vaccine). The antigen will initiate reactions and culminate in the increase of circulation of specific antibodies, besides promoting immune memory. Moreover, antigens that enter the body will be recognized and processed by the innate system by antigen presenting cells (APC-macrophages, dendritic cells and B lymphocytes), to process microorganisms in molecular units, and at first trigger immune response of proliferation, and in a second moment, the response of memory (Biller-Takahashi and Urbinati 2014). In line with antibody titer, the relative percent survival (RPS) revealed that the autogenous formalin killed whole cell vaccine able to protect striped catfish from edwardsiellosis (Table 2). RPS value in this experiment reached about 63.64% which indicates that the vaccine can be used for striped catfish farmer or developed to increase the protection value in the next future research. The similar result about whole cells vaccine also gives the RPS value of around 50-75% in different fish but the same type of vaccine (Sughra et al. 2021; Taukhid et al. 2018).

During experiment, water quality was maintained into certain condition which were temperature 28-30 °C, pH 6-7, dissolved oxygen 4-5 mg.L⁻¹ and ammonia 0.07-0.17 mg.L⁻¹. The behavior of striped catfish was also monitored as control to environment disorder. Based on Nasional Standard of Indonesia, the conditions of fish rearing are 27-30 °C, 6.5-8.5 and >5 mg/l for temperature, pH and

dissolved oxygen respectively (Standar Nasional Indonesia 2000).

To sum up, the formalin killed cells vaccine from autogenous bacteria had a good performance with efficacy of vaccine was 63.64%. It means that the vaccine can protect striped catfish from edwardsiellosis caused by *Edwardsiella tarda* and might cause mortality around 36.36 % when the outbreak occurs. The efficacy value needs to be increased in order give more protection to striped catfish. Thus, some researches are needed to be developed such as application of adjuvant into vaccine or modification of material for vaccine.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – SHD, T; Design – SHD, T, NAU; Resources – SP, SHD, MAR; Materials – SHD, T, NAU; Data collection and/or processing – NAU, MAR, RCM; Literature research - NAU, SP, SHD; Writing Manuscript – SHD, T, NAU; Critical Review - SHD, T, RCM

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EFIKASNOST AUTOGENOG CJELOSTANIČNOG CJEPIVA TRETIRANOG FORMALINOM PROTIV EDWARDSIELLOZE KOD PRUGASTOG SOMA (*Pangasionodon hypophthalmus*)

Cilj istraživanja je napraviti cjepivo korištenjem *Edwardsiella tarda* iz lokalnog izolata. Istraživanje je izvedeno u Laboratoriji za akvakulturu Katedre za akvakulturu Univerziteta Sriwijaya. Bakterijski izolot potječe iz zaliha Agencije za karanten ribe i inspeksijski nadzor iz Južne Sumatere u Indoneziji. Prije cijepjenja, ribe su već bile prilagođene na pelete i akvarijske uvjete. 14 dana nakon cijepjenja (dpv), somovi su inficirani sa *E. tarda* 107 CFU/mL-1 po injekciji. Rezultati su pokazali da je riba cijepljena protiv edwardsieloze bolje zaštićena od necijepjene ribe, pri čemu je vrijednost relativnog procenta preživljenja (RPS) iznosila 63,64%, a titar antitijela povećan sa 7 dpv na više od 14 dpv. Ustvari, RPS vrijednost je iznosila manje od 70%, ali nisu zabilježeni hematološki poremećaji nakon cijepjenja. Možemo zaključiti da su cjelostanična cjepiva potencijalni kandidati za zaštitu prugastog soma od edwardsieloze. Nadalje, u budućnosti je potrebno provesti određene eksperimente u svrhu povećanja RPS vrijednosti.

Ključne riječi: Autogeno cjepivo, cjelostanično cjepivo, *Edwardsiella tarda*, *Pangasionodon hypophthalmus*

RESEARCH ARTICLE

GROSS BETA COUNTING METHOD IN RADIATION PROTECTION SYSTEM OF ANIMAL PRODUCTION CYCLE

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ABSTRACT

The research investigated the potential application of the gross beta counting method in radiation protection of animal production under conditions of environmental contamination. Mushroom samples, animal and agricultural products as well as samples from proficiency tests were analyzed using both the gross beta counting method and the gamma spectrometric analysis as a control.

The findings revealed that the quantitative measurements of total beta emitters in the tested samples, obtained with the gross beta counting method, can be effectively employed as a triage approach in scenarios involving radioactive contamination. It is important to notice that successful implementation of this method relies on prior knowledge of the average levels of total potassium or its isotope ⁴⁰K in the samples being analyzed.

Control measurements and assessments based on the results obtained from the gamma spectrometric method demonstrated an acceptable uncertainty for the triage of contaminated samples as well as for the implementation of protective measures within the animal production system under conditions of environmental contamination.

Keywords: Beta activity, food production chain, radioactive contamination

INTRODUCTION

Gross beta activity measurements are quantitative analyses used to determine the overall content of beta-emitting radionuclides in a given sample. The method does not provide qualitative results as it detects the impulses or energies released by beta particles (electrons), which are indistinguishable regardless of the specific origin of the beta-emitting radionuclides. Therefore, the results are expressed as the cumulative activities of all beta-emitting radionuclides present in the tested sample (Duong Van et al., 2020).

This method offers advantages such as straightforward implementation, particularly in emergency situations, and suitability for field con-

ditions. These benefits make it a preferred method for rapid assessments of contamination levels in the field (Bari et al., 2011).

However, the measurement uncertainty of this method comes from limitations in analyzing solid samples, including the lack of standardized preparation and measurement procedures to ensure result reproducibility. These limitations become more significant in cases of low-level contamination, where specific laboratory methods such as gamma spectrometry, alpha spectrometry, and radiochemical separation of radionuclides are recommended. Consequently, the measurement of gross beta activity is not currently recommended as an independent approach for monitoring radioactivity in food.

Qualitative results obtained from this method can be significant depending on the context in which they are used. The measurement of gross beta activity in water samples is the official standard method for assessing the radiological safety of drinking water. In accidental situations, this method can provide rapid and efficient triage information regarding the extent and significance of environmental contamination, as well as its impact on animal and agricultural production. Since the majority of biologically important artificial radionuclides (e.g., ^{89}Sr , ^{90}Sr , ^{134}Cs , ^{137}Cs , and ^{131}I) are beta emitters, measuring total beta activity plays a crucial role in rapid field assessments of radioactivity and contamination levels. This method has been extensively used in response to past accidents such as Chernobyl (Hanfi et al., 2020). Beta counters like LARA 5, LARA 10, and LARA 86 were employed in the former Yugoslavia for triage field assessments of food and environmental samples following the Chernobyl accident (Gradašćević, 2017).

In situations with high contamination, the results obtained through this method can serve as a basis for implementing protective measures in agricultural and animal production. For applying certain protective measures, understanding the contamination status is essential. Accordingly, field measurements of gross beta activity provide

a rapid and accessible way of obtaining that information (Bari et al., 2011). For that purpose, it should be necessary to provide prompt education and training for veterinarians and other biotechnical experts. The experiences following the Fukushima accident have also emphasized the importance of establishing a system for protection against radioactive contamination, where rapid triage methods such as total beta activity measurements hold a special place (Naletoski et al., 2021).

It is evident that there is a need to enhance existing knowledge and experiences in utilizing this method during emergency situations. While the development of other radioanalytical methods and reduced demand for rapid field measurements of radioactivity have somewhat marginalized this method, it remains a logical choice in response to the existing challenges posed by the increasing risk of nuclear accidents and general political unpredictability surrounding the use of nuclear weapons.

When considering potential scenarios of global radioactive contamination, it becomes evident that existing laboratories for radioactivity measurements would be overwhelmed with samples, leading to delays in obtaining results, which has been recognized by other authors (Bari et al., 2011). This delay could result in errors and poor decision-making in the food production process. To avoid this issue, field measurements of total beta activity performed by trained veterinary and other experts could be introduced. It should prevent time gaps and enable timely measures in the food production process. Considering the urgency of information required to implement adequate protection measures, the aforementioned slight unreliability of total beta activity measurement results under conditions of high environmental contamination becomes practically negligible.

Furthermore, in the context of food production protection, the absolute reliability of results is not crucially important compared to the timeliness, considering the wide range of available protective measures. By combining the use of total beta activity measurements with subsequent analyses

using more sophisticated laboratory methods, comprehensive information can be obtained to evaluate the effectiveness of implemented measures and the safety of agricultural and animal products.

The aim of the work is to examine the possibility of using the gross beta counting method in conditions of contamination of animal production. To achieve this, the results obtained under normal circumstances need to be extrapolated and evaluated for use in scenarios involving environmental contamination. Plant samples and animal products with normal and higher activity concentrations of one or more artificial radionuclides would be used as models.

MATERIAL AND METHODS

Sample preparation

Samples of agricultural (pumpkin, potato, grass, corn, spinach, beans, onion) and animal (meat, milk) products as well as wild mushrooms were used for the evaluation of methods used in the research. The levels of artificial radiocesium were in the following ranges: below the detection limit (0.5 Bq kg^{-1}), medium (up to 200 Bq kg^{-1}), and higher (above 200 Bq kg^{-1}). The ranges have been chosen in order to represent the scenarios of low and higher contaminations. Mushrooms were used as the model of feeding stuff according to their significant participation in wild animals' diets.

Water samples were evaporated to dryness, crushed in a mortar, and weighed for planchets. Agricultural and animal samples were mineralised at 400°C , homogenised by crushing in a mortar and approximately 500 mg of the ash was dispersed with acetone on the planchets. The sample preparation procedure for wild mushrooms involved several steps, including drying, homogenization, and packaging. Initially, the samples were crushed using a blender and subsequently subjected to drying at a temperature of 80°C while periodically stirring. Once dried, the samples were further pulverized in the mill until a fine powder consistency was achieved. Completely dried and homogenized samples were

then sieved using a $150 \mu\text{m}$ pore diameter sieve. Following sieving, the samples were appropriately packed based on the specific measurement method. Plastic containers weighing 100 g were utilized for gamma spectrometric measurements, whereas stainless steel planchets were used for measuring total beta activity. Each sample was prepared on a planchet with a mass of approximately 500 mg by slurring with a few drops of acetone to ensure the formation of a uniform layer suitable for counting on the alpha/beta counter.

Measurements

The first group included samples whose radioactivity was coming primarily from the presence of potassium, where isotope ^{40}K produced an average of 31 Bq g^{-1} of total potassium content in the sample (Samat et al., 1997). Those samples were used as a model for regular conditions without significant contamination and whose radioactivity was coming due to the naturally occurring ^{40}K .

The second group consisted of the samples of mushrooms whose radioactivity levels were a combination of potassium (^{40}K) and additional radioactivity related to the presence of ^{137}Cs to different extents (up to 198.6 Bq kg^{-1}). This group was used as the model for low to medium contamination levels.

The third group included higher contaminated mushroom samples as well as samples from different proficiency tests with significant activities of ^{137}Cs (428 to 1823 Bq kg^{-1}), together with background levels of naturally occurring ^{40}K .

The total beta activity was measured using the Ortec MPC 9604 multi-detector alpha/beta counter, which enables the measurement of very low activities. The counter is equipped with Vista 2000 software and allows simultaneous measurement of up to four samples. As stated by the manufacturer, the counter has four pancake-style gas flow proportional sample detectors and a large area gas flow proportional cosmic guard detector. The instrument was calibrated in accordance with BAS EN ISO 9697:2020 standard method, using a solution of potassium chloride (KCl), and a

reference material with known activity of ^{137}Cs in a calcium sulfate (CaSO_4) matrix. Since beta particles emitted by ^{40}K and ^{137}Cs have different energies (1300 keV and 514 keV, respectively), their attenuation through the sample on planchets of varying masses differs. Hence, a calibration procedure was conducted beforehand, involving planchets with masses varying from 100 mg to 1000 mg of the matrix, in order to establish efficiency-mass curves of ^{137}Cs and ^{40}K used in the calculation of activity concentrations in this study (Alharbi, 2018).

Considering the typical composition and characteristics of mushrooms, the presence of the other beta-emitting radionuclides (apart from ^{40}K and ^{137}Cs) in significant concentrations was not expected. Therefore, the accredited gamma spectrometry method was used as a comparative analysis for the quantitative determination of ^{137}Cs and ^{40}K activity concentrations and their contribution to the total beta activity with relatively low uncertainty.

The gamma spectrometric analysis was conducted using a BSI (Baltic Scientific Instruments) HPGe gamma spectrometer equipped with a P-type detector. The detector has a relative efficiency of 50% at the energy of 1332 keV emitted by ^{60}Co , and it provides a resolution (FWHM) of 1.9 keV at 1332 keV. The levels of ^{40}K and ^{137}Cs were determined at 1460.8 keV and 661.6 keV, respectively. Quality control procedures were included in the operating activities of the laboratory accredited by BAS EN ISO/IEC 17025:2018.

RESULTS

The results are displayed through tables and graphs arranged by the groups of analyzed samples.

1. Uncontaminated samples

Uncontaminated samples from several villages around Tuzla were analyzed and the results are presented in Table 1. ^{40}K activities were calculated on the fresh masses (f.m.) of the samples.

Table 1 Gross beta activities in animal and agricultural products and water samples with ^{40}K levels measured by gamma spectrometry method (Bq kg^{-1})

	A_{β} (Bq kg^{-1})	^{40}K (Bq kg^{-1})	Ratio ($^{40}\text{K}/A_{\beta}$)	^{40}K % in gross β
Meat 1	83.54±0.25	89.20±6.01	1.068	100
Meat 2	85.98±0.25	81.70±6.67	0.935	99.6
Milk 1	44.74±0.14	43.70±3.57	0.977	99.4
Milk 2	46.53±0.15	45.00±3.67	0.967	99.1
Pumpkin	51.93±0.14	52.90±4.31	1.019	100
Potato	127.27±0.30	128.00±10.50	1.006	100
Grass	181.84±0.58	179.00±12.00	0.987	94.5
Corn	91.75±0.28	96.80±7.91	1.055	100
Spinach	142.08±0.50	130.00±7.16	0.915	98.5
Beans	362.61±0.99	418.00±34.20	1.153	100
Onion	60.82±0.16	61.10±4.79	1.005	100
Water	0.0313±0.0004	0.0441±0.0153	1.409	100

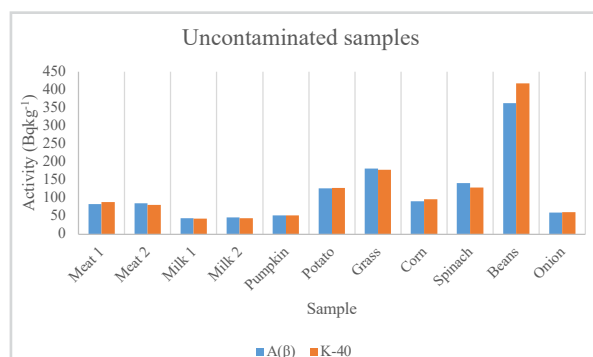


Figure 1 Gross beta activities and ^{40}K levels in animal and agricultural samples (Bq kg^{-1})

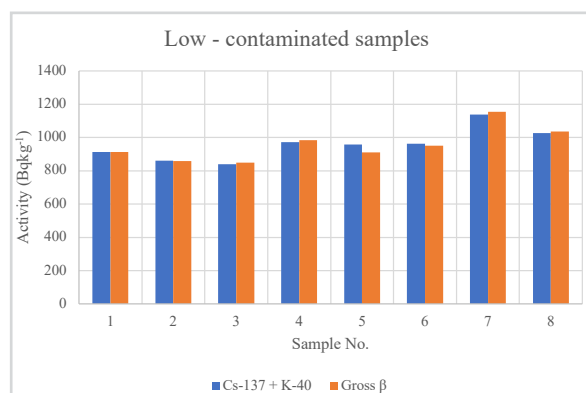


Figure 2 Gross beta activities and the sum of the ^{137}Cs and ^{40}K activity concentrations obtained by gamma spectrometry method in mushrooms (Bq kg^{-1})

2. Low-contaminated samples

Mushrooms were chosen considering their affinity for ^{137}Cs sorption from the soil and, consequently, their higher levels in observed samples. Taking

into account that the described method is intended for the triage of samples in field conditions, the measurement uncertainty was not shown in the estimated results in Tables 2 and 3.

Table 2 Activity concentrations of ^{40}K and ^{137}Cs measured by gamma spectrometer and gross beta activities in mushrooms (Bq kg^{-1} d.m.)

Sample	^{40}K	^{137}Cs	Gross beta	Gross $\beta - ^{40}\text{K}$ (^{137}Cs)
1	754.8 ± 15.6	156.9 ± 2.5	911.8	157.0
2	766.4 ± 16.4	95.4 ± 1.6	858.6	92.2
3	776.7 ± 16.6	63.5 ± 1.2	849.5	72.8
4	781.4 ± 16.7	190.0 ± 3.0	983.7	202.3
5	884.4 ± 18.0	73.0 ± 1.3	909.8	25.4
6	901.2 ± 17.3	62.2 ± 1.0	950.4	49.2
7	940.1 ± 19.9	198.6 ± 3.1	1154.7	214.5
8	1009.7 ± 19.1	16.1 ± 0.4	1036.8	27.1

3. Higher contaminated samples

Higher contaminated samples were used as a model for higher contamination scenarios.

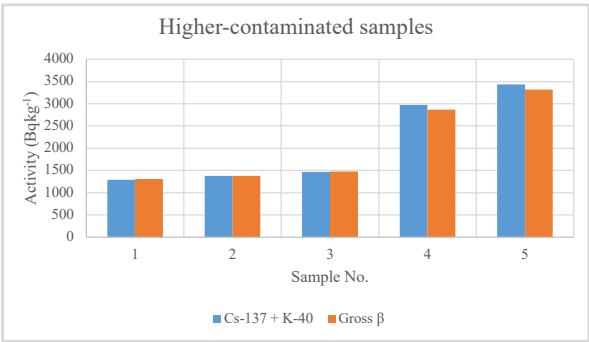


Figure 3 Gross beta activities and the sum of ¹³⁷Cs and ⁴⁰K activity concentrations obtained by gamma spectrometry analysis in samples from the proficiency tests

Table 3 Activity concentrations of ⁴⁰K and ¹³⁷Cs measured by gamma spectrometer and gross beta activities in contaminated samples (Bq kg⁻¹ d.m.)

Sample	⁴⁰ K	¹³⁷ Cs	Gross beta	Gross β – ⁴⁰ K (¹³⁷ Cs)
1	859.0 ± 17.0	428.5 ± 6.4	1305.2	446.2
2	847.6 ± 17.8	529.1 ± 7.9	1374.9	527.3
3.	539.0 ± 32.0	931.9 ± 78.0	1477.8	938.8
4.	1508.3 ± 59.2	1462.8 ± 126.4	2870.6	1362.3
5.	1614.4 ± 63.7	1823.2 ± 149.1	3322.3	1707.9

DISCUSSION AND CONCLUSION

The evaluation of the applicability of the gross beta activity measurement method relies on comparing the obtained results with those derived from the accredited gamma spectrometry method. Generally, for the successful use of the method, it is necessary to know the levels of ⁴⁰K which are the basic parameters of the assessment. The levels of ⁴⁰K should be provided by measurement or calculated from the total potassium content in field conditions.

Considering the fact that potassium is homeostatically controlled in biological systems, its average contents in agricultural and animal samples do not extremely vary (except the mushrooms) and it is well described in the literature. Therefore, literature values can be used for the assessment of the activity concentration of ⁴⁰K in those samples. That could be done by simply multiplying the average potassium content from literature, e.g. 1.5 g in cow milk (Nogalska et al., 2020) by 31, which is an average activity of ⁴⁰K in 1 g of natural potassium (Samat et al., 1997).

The results presented in the first two columns of Table 1 show values of gross beta activity measured by alpha/beta counter and values of ⁴⁰K obtained by accredited gamma spectrometry method. It was obvious that obtained results were very similar for both methods, indicating that in the case without contamination, gross beta values correspond to the naturally occurring ⁴⁰K in solid samples. An average level of ⁴⁰K contributed to the gross beta activity of more than 94.5%. Statistical analysis proved the null hypothesis about the similarity of the results (t-test: two tails P- values equal - 0.923; Pearson coefficient r²=0.98, significance p < 0.05).

In the presence of other beta-emitting radionuclides, gross beta activities would show higher levels than those recorded in the above samples, whilst the percentage of the ⁴⁰K should be lower depending on the activities (mass) of the additional beta emitters. For the samples of water, the same approach could not be applied considering the fact of low ⁴⁰K levels in drinking water. The national legislation requires the limit value of gross beta activities that should not be exceeded in drinking water (1 Bql⁻¹) and should

be used as the limit value for consideration of the water samples (“Regulation on the monitoring of radioactivity in the environment”).

Results in column 5 (right) of Table 2 represent the levels of ^{137}Cs obtained from the gross beta activity after subtracting the ^{40}K levels. Comparing those values with values of the ^{137}Cs obtained by gamma spectrometry measurements (column 3), it is obvious that they are similar if the uncertainties of the ^{40}K results would be considered. The exemption in sample 5 was probably due to differences in used efficiencies for ^{40}K and ^{137}Cs and their specific ratio in the observed sample. It could appear in cases in which the yields of ^{40}K might vary in a wide range like in mushrooms (Falandysz and Borovička, 2013), and should be anticipated. For other types of agricultural and animal samples, the potassium content is more stable with no possibility for significant under- or over-estimation of its concentrations (Nasreddine et al., 2008). Additionally, the yields of the rest of the radionuclides potentially present in the contaminated samples (^{90}Sr , ^{131}I , ^{134}Cs , etc.) during the radioactive contamination should be available from the general assessment of contamination.

The values in Table 2 showed compliance among the used methods. Results for ^{137}Cs measured by gamma spectrometry method were in well accordance with those obtained after subtracting ^{40}K activity concentrations from the gross beta activities. Statistical analysis of the gross beta activities and the sum of the values for ^{137}Cs and ^{40}K obtained by the gamma spectrometry method (Figure 2) indicated equal values (t-test: P value 0.972). The results approved the possibility of using the described method for the estimation of the ^{137}Cs levels in contaminated animal and agricultural samples.

Higher contaminated samples were used as a model for higher contamination scenarios. Generally, all samples with gross beta activities above average activity concentration of ^{40}K might be considered as contaminated except for samples significantly rich in potassium (e.g. dried products: mushrooms, bananas, potatoes, etc.).

Results presented in Table 3 showed values above 1000 Bq kg⁻¹ of gross beta activities (1305.2 – 3322.3). The higher values were recorded due to the high levels of ^{40}K (847.6 – 1614.4 Bq kg⁻¹) as well as the presence of significant activities of ^{137}Cs shown in column 3 (428.5 – 1823.2 Bq kg⁻¹). Calculated levels of ^{137}Cs that were obtained by subtracting the ^{40}K from the gross beta activities (column 5-right) corresponded to those obtained by the direct gamma spectrometry measurements. Small differences in activities between calculated and measured levels of ^{137}Cs were in the ranges of measuring uncertainties of gamma spectrometry results for ^{137}Cs and ^{40}K .

The results presented in Figure 3 were statistically equal (t-test: P value 0.95) with a statistically significant Pearson correlation coefficient ($r^2=0.99$, $p < 0.05$).

In the case of presence of other beta emitters, more accurate estimates could be possible if efficiency calibration would be performed on existing radionuclides and the data about the quantitative-qualitative composition of contamination would be available.

During environmental radioactive contamination, the food production chain is especially endangered; therefore, appropriate protective measures must be applied. The selection of the appropriate protective measures depends on the contamination levels and it is specific for each production line. The best solution in such circumstances is to have appropriate methods for fast assessment of the contamination levels in field conditions. Therefore, the study was carried out in order to estimate the use of the gross beta counting method for the approximative evaluation of food chain contamination by artificial radionuclides in field conditions.

The results for the first group showed that gross beta counting method provided values that statistically corresponded to the naturally occurring ^{40}K in uncontaminated samples of animal and agricultural products. The possible use of the gross beta counting method in such conditions would be based on the ^{40}K levels, which means

that every significant excess of the gross beta activities above the average ^{40}K values should be considered as possible contamination. The levels of ^{40}K in specific samples could be calculated from the average potassium content (grams) provided by literature and multiplied by 31. The instrument should be calibrated by ^{40}K standard.

The samples contaminated by the low levels of ^{137}Cs (second group) exhibited excellent accordance among the gross beta activities and the sum of the levels of ^{137}Cs and ^{40}K obtained by the gamma spectrometry method. Almost the same statistical significance was obtained among the levels of gross beta activities and high levels recorded in proficiency testing samples which were spiked with the mixture of ^{137}Cs and ^{40}K .

Therefore, the use of the method in contamination circumstances should be based on the subtracting of the ^{40}K levels from the gross beta activities and the rest would be considered as a mixture of artificial beta emitters (mainly Cs, Sr, I). For more accurate assessments in field conditions, it would be necessary to provide the two important

requirements: prepared efficiency calibration for the artificial radionuclides of interest (Cs, Sr, I, etc.) as well as general information about the quantitative-qualitative composition of the contamination.

The findings proved the assumption that the gross beta counting method could be used for fast triage in conditions of low and high artificial radioactive contamination. Obtained results could be used as milestones for decisions about protective measures in food production processes.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – NG; Design – NK, NM, NG; Supervision – NG, NM; Resources – NG, LT; Materials – LT, NK; Data Collection and/or Processing – NM, NK, LT; Analysis and/or Interpretation – NG, NM, NK; Literature Search – LT, NG, NK; Writing Manuscript – NG, NK; Critical Review – NG, NM.

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METODA MJERENJA UKUPNOG BETA ZRAČENJA U SISTEMU ZAŠTITE OD ZRAČENJA U ANIMALNOM CIKLUSU PROIZVODNJE

SAŽETAK

U studiji je ispitana mogućnost primjene metode mjerenja ukupnog beta zračenja u radijacionoj zaštiti animalne proizvodnje u uslovima kontaminacije okoliša.

Rezultati su pokazali da se kvantitativna mjerenja ukupnih beta emitera u testiranim uzorcima koji su dobijeni metodom određivanja ukupne beta aktivnosti mogu uspješno koristiti u trijaži u slučajevima koji uključuju radioaktivnu kontaminaciju. Važno je primijetiti da se uspješna primjena ove metode zasniva na prethodnom poznavanju prosječnih koncentracija ukupnog kalija ili njegovog izotopa ^{40}K u analiziranim uzorcima.

Kontrolna mjerenja i procjene zasnovane na rezultatima istraživanja korištenjem gama spektrometrijske metode su pokazala prihvatljivu nepouzdanost u trijaži kontaminiranih uzoraka. Korištenjem ove metode u terenskim uslovima omogućila bi se primjena ostalih mjera zaštite sistema animalne proizvodnje tokom radioaktivne kontaminacije okoliša.

Ključne riječi: Beta zračenje, lanac proizvodnje hrane, radioaktivna kontaminacija

RESEARCH ARTICLE

PARTIAL REPLACEMENT OF SOYBEAN MEAL BY BROAD BEANS OR FIELD PEAS IN JAPANESE QUAILS' DIET

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ABSTRACT

This study was conducted to determine feed formulas specific to the Japanese quail *C. japonica* by partially substituting imported soybean meal with locally produced and cheaper protein-crops. Each formula contained either Entire Broad Beans (EBB), Heat-treated Decorticated Broad Beans (HDBB) or Entire Field Peas (EFP), each one at several replacement levels (10, 20 and 30%) of the whole formula. The results showed that feed containing HDBB led to better growth performances as compared to all the other formulas. Substitution with EFP recorded close effects to the control formula, but its high fiber and average protein contents represent a limitation to its incorporation at higher levels. EBB had adverse impacts, especially on growth performances. HDBB incorporation led to a significant hypoglycemia (batches 20% and 30%) ($p=0.0039$ and $p=0.0049$, respectively), and a considerable reduction of uremia (all batches) ($p=0.0047$, $p=0.0009$ and $p=0.0227$, in that order) with hypercalcemia (batches 20 and 30%) ($p=0.0285$ and $p=0.0208$, respectively), hyperphosphatemia (all batches) ($p=0.0064$, $p=0.0163$ and $p=0.0027$, respectively) and normal cholesterolemia, triglyceridemia, proteinemia, serum creatinine, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GTP) amounts. EBB induced a very significant hypoglycemia (at 30% replacement) ($P=0.0015$), and hypouremia (batch 10%) ($p=0.0019$) with hypercalcemia (batch 30%) ($p=0.0035$), hyperphosphatemia (batch 20%) ($p=0.0048$) and normal cholesterolemia, triglyceridemia, proteinemia, serum creatinine, GOT and GPT. On the other hand, EFP had no effect ($p>0.05$) on the biochemical parameters of *C. japonica* except a non-significant decrease of uremia in batch 10% ($p=0.49$). Accordingly, it can be suggested that Field Peas and Treated Broad Beans could be possible substitutes to soybean meal in quails' feed production.

Keywords: Biochemical profile, *Coturnix japonica*, feed formulation, growth performances

INTRODUCTION

Since their domestication in Japan as singing birds (Hrncar et al., 2014), Japanese quails *Coturnix japonica* have gained an economic importance as agricultural species (Djitie-Kouatcho et al., 2015). They have many distinctive characteristics such as low body weight (80 to 300 g), short generation interval (3 to 4 generations per year), disease resistance, high egg production, rapid sexual maturity and adaptability to several breeding conditions (Nikpiran et al., 2014; Okuliarova et al., 2014).

Because of their high energy and protein contents, corn-soy based diets were considered for a long time as staple feed for birds despite the production type. In poultry, like any other livestock production, feed accounts for almost 60-80% of the total production cost (Olugbenga et al., 2015; Shamna et al., 2013). Thus, nutritionists had attempted to establish feed formulas balanced in energy and proteins at cheapest prices using locally available ingredients. In this context, local protein crops (like broad beans and field peas) can be inexpensive and more available alternatives to soybean meal, which remains the most costly constituent.

Broad bean (*Vicia faba var. minor*) is a leguminous plant that contains relatively high amounts of energy and proteins (25-33% of Dry Matter DM) rich in lysine, but fairly poor in sulfur amino acids and tryptophan. It has also moderate fibercontent (crude fiber: 7-11% DM) (Heuzé et al., 2013).

Field pea (*Pisum sativum*) is an annual herbaceous plant containing two major families of soluble proteins (globulins, soluble in saline buffers and albumins, soluble in water) and 10-15% insoluble proteins. Globulins are the main storage proteins (55-65%) in this crop (Adebiyi and Aluko, 2011; Potter, 1995).

On the other hand, broad beans enclose many anti-nutritional factors (protease inhibitors, lectins, phenolics and especially tannins/proanthocyanidins) which interfere with digestibility and nutrients' uptake (Kaysi and

Melcion, 1992; Kumar et al., 2015) and require appropriate treatments to guarantee a better nutritional use. As well, field peas have fairly low amounts of anti-nutritional factors (protease inhibitors, tannins, alkaloids, lectins, phytic acid, saponins and oligosaccharides) (Hickling, 2003; Konieczka et al., 2014).

In this study, the effects of partial substitution of soybean meal (by entire or heat-treated decorticated broad beans or field peas) on growth parameters and biochemical profiles of Japanese quails were assessed.

MATERIAL AND METHODS

Animals and diets

The trials took place in 2016 at the Institut Technique des Élevages of Constantine (Northeastern Algeria). Fertile quail eggs were incubated, and chicks were started on the same feed under the same conditions prior to being placed on test diets. At 07 days old, about 450 chicks were weighed and allotted to 10 treatment groups (with 03 replicates of 15 chicks for each treatment) in a way to get approximately the same mean body weight and body weight range between all replicates.

Field peas and broad beans (from a local market) were washed, cleared of all impurities then air-dried. Entire broad beans and field peas were then ground, and while treated, broad beans were placed in hot water (80°C) for 30 minutes to facilitate removal of their coats. Next, their almonds were allowed to air dry for one day, after what they were placed in an autoclave at 120°C for about 20 minutes, air dried and finally ground as described by Brufau et al. (1998).

Feed formulations were prepared using Windows User-Friendly Feed Formulation tool (WUFFDA ver. 1.02, 2004) to meet all nutritional requirements of quails (at growth and finishing periods) established by the National Research Council (NRC, 1994).

As presented below in Tables 1 and 2, diets were prepared with no substitution of soybean meal (Control diet CTRL) or with partial replacement

levels of it (at 10%, 20% and 30% of the whole formula) using either Entire Broad Beans (EBB), Heat-treated Decorticated Broad Beans (HDBB) or Entire Field Peas (EFP).

Birds of each replicate were housed in a separate diets during growth period (from 7th to 21st day)

galvanized-wire cage with feed and water *ad-libitum* and under the same breeding conditions (temperature, relative humidity, lighting program and hygiene).

Table 1 Ingredients and nutrient composition of

	Feed formulas	CTRL	EBB			HDBB			EFP		
			10%	20%	30%	10%	20%	30%	10%	20%	30%
Ingredients (%)	Corn	51.25	47.00	45.50	40.50	47.00	45.50	40.50	48.50	43.05	37.95
	Entire beans		10.00	20.00	30.00						
	Treated beans					10.00	20.00	30.00			
	Field Peas								10.00	20.00	30.00
	Wheat bran	8.25	7.00	2.25	2.00	7.00	2.25	2.00	3.35	2.50	1.00
	SBM 44% CP	38.75	34.25	30.5	25.75	34.25	30.50	25.75	36.40	32.70	29.30
	Limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
	DCP	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
	MV premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Total	100	100	100	100	100	100	100	100	100	100
Nutrient Composition (%)	Dry matter (%)	88.08	88.04	88.00	87.95	88.04	88.00	87.95	88.26	88.42	88.59
	ME (kj/g)	11.63	11.63	11.63	11.63	11.63	11.63	11.60	11.70	11.67	11.63
	Proteins (%)	23.05	23.05	23.06	23.05	23.05	23.06	23.05	23.00	22.99	22.99
	Fat (%)	2.58	2.52	2.49	2.49	2.52	2.49	2.49	2.45	2.32	2.19
	Crude fiber (%)	3.55	3.89	3.94	4.37	1.75	1.77	1.96	3.46	3.70	3.89

CTRL: Control diet. CP: Crude Proteins. DCP: DiCalcium Phosphate. EBB: Entire Broad Beans. HDBB: Heat-treated Decorticated Broad Beans. EFP: Entire Field Peas. ME: Metabolizable Energy. MV: Mineral-Vitamin. SBM: Soybean Meal.

Table 2 Ingredients and nutrient composition of diets during finishing period (from 22nd to 42nd day)

	Feed formulas	CTRL	EBB			HDBB			EFP		
			10%	20%	30%	10%	20%	30%	10%	20%	30%
Ingredients (%)	Corn	58.05	55.00	51.90	48.70	55.00	51.90	48.70	53.65	50.05	46.20
	Entire bean		10.00	20.00	30.00						
	Treated bean					10.00	20.00	30.00			
	Field peas								10.00	20.00	30.00
	Wheat bran	10.00	7.25	4.55	2.00	7.25	4.55	2.00	7.20	3.90	0.92
	SBM 44% CP	29.95	25.75	21.55	17.30	25.75	21.55	17.30	27.15	24.05	20.88
	Limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
	DCP	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
	MV premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	Total	100	100	100	100	100	100	100	100	100	100
Nutrient Composition (%)	Dry matter	88.02	88.98	87.94	87.89	88.98	87.94	87.89	88.19	88.36	88.53
	ME (kj/g)	11.75	11.75	11.75	11.75	11.75	11.75	11.75	11.71	11.71	11.71
	Proteins	20.01	20.01	20.01	20.01	20.01	20.01	20.01	20.00	20.00	20.00
	Fat	2.81	2.73	2.64	2.56	2.73	2.64	2.56	2.67	2.54	2.41
	Crude fiber	3.55	3.83	4.12	4.43	1.72	1.85	1.99	3.63	3.67	3.73

CTRL: Control diet. CP: Crude Proteins. DCP: DiCalcium Phosphate. EBB: Entire Broad Beans. HDBB: Heat-treated Decorticated Broad Beans. EFP: Entire Field Peas. ME: Metabolizable Energy. MV: Mineral-Vitamin. SBM: Soybean Meal.

Performances measurement and biochemical analyses

During the experiment, the following data were collected:

- Individual body weights (BW): measured daily before feed distribution early in the morning, and used to calculate average weekly weight of all males, all females separately and of the whole lot along the rising stages.
- Average Daily Body Weight Gain (BWG) (of each batch, all males and all females separately): calculated for the growing and finishing periods and for the entire rearing period.
- Feed Intake (FI) of each batch: recorded daily and used to calculate Feed Conversion Ratio (FCR) during growing, finishing and entire rearing periods.
- Mortality rates (M%): dead birds were counted daily and taken into consideration when calculating FCR and BWG.

By the experiment end, 10 males and 10 females of each group were sacrificed and blood samples taken in heparinized tubes. Plasmas were then harvested by centrifugation at 3000 rpm for 10 minutes to measure glycemia, cholesterolemia, triglyceridemia, proteinemia, uremia, creatininemia, calcemia, phosphatemia, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) amounts, using a Kenza Max-Biolabo Diagnostics® semi-automated spectrophotometer with commercial kits (Spinreact®).

Statistical analysis

Growth parameters were expressed as mean±SE and biochemical ones as median±IQR.

Graph Pad InStat prism 6.04 (*GraphPad Software, Inc., San Diego, CA, USA, 2014*) statistical software was used to compare the growth performances and biochemical profiles of birds of the different batches.

At first, the distribution of all obtained data

was analyzed using the D'Agostino-Pearson test. Disparities between the different batches (including the CTRL one) were analyzed by applying Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test for parametric data, and Kruskal-Wallis test followed by Dunn's multiple comparison test for non-parametric one. As well, the results recorded in males and females of each batch were compared using either Student's *t* test or Mann-Witney's *U* test.

Statistical significance was set at $p < 0.05$.

RESULTS

Growth performances

As shown in Table 3, a significant ($p < 0.0001$) body weight (BW) increase was recorded in birds of all HDBB batches during the growing and finishing periods; while a significant ($p < 0.0001$) BW decrease was observed in all EBB batches throughout the growing phase and only in EBB 10% and 20% batches along the finishing phase, as compared to the CTRL. Quails' BWs of EFP batches were higher than EBB ones. A substantial difference in BW was observed between males and females of all HDBB batches ($p < 0.0001$, $p = 0.0008$ and $p = 0.0012$, respectively). Furthermore, many significant disparities were also observed in BW of quails of different batches as compared to each other.

HDBB batches had displayed a significant increase in feed intake (FI) through the growing ($p < 0.0001$) and the finishing periods ($p = 0.0007$, $p = 0.0013$ and $p = 0.0009$, respectively), leading to a sensitive augmentation ($p = 0.0005$, $p = 0.0017$ and $p = 0.0009$, respectively) in their body weight gain (BWG) as compared to CTRL quails. On the other hand, a significant ($p < 0.0001$) drop in quails' BWG was noted in all EBB batches along the growing period. Quails of all EBB batches and EFP showed no difference ($p > 0.05$) in FI as compared to the CTRL.

Furthermore, a significant increase ($p = 0.0056$) was discerned in feed conversion ratio (FCR) of HDBB 20% batch along the growth phase,

and it became significant for all HDBB batches in the finishing phase ($p=0.0035$, $p=0.0115$ and $p=0.0082$, respectively). FCR increased also significantly in EBB 20% batch ($p=0.0021$) in growing period and in EFP 10% and 20% batches in the finishing one ($P=0.0008$ and $p<0.0001$, in that order), as compared to the CTRL.

The highest mortality rate during the growing period was recorded in all EBB batches ($p=0.0335$, $p=0.0005$ and $p=0.0027$, respectively), while HDBB fed batches showed relatively low rates compared to the CTRL in the growth and finishing phases ($p>0.05$).

Table 3 Effect of soybean meal substitution by EBB, HDBB or EFP on growth parameters of *C. japonica* (Mean $\pm$ SE)

Batch	Growing period					Finishing period				
	BW (g)	FI (g/d)	BWG (g/d)	FCR	M (%)	BW (g)	FI (g/d)	BWG (g/d)	FCR	M (%)
CTRL	61.98 ± 6.36	6.28 ± 0.78	3.46 ± 0.05	1.78 ± 0.02	0.00 ± 0.00	142.51 ± 7.25	18.74 ± 1.47	4.01 ± 0.04	4.60 ± 0.05	2.27 ± 1.43
EBB	10% $\pm 3.93^{*ab}$	5.05 $\pm 0.93^{*ab}$	1.97 $\pm 0.11^{*aC}$	2.58 $\pm 0.15^{*B}$	10.68 $\pm 1.66^{*}$	122.79 $\pm 9.58^{*ab}$	15.82 $\pm 1.94^{*a}$	4.11 $\pm 0.08^a$	4.01 $\pm 0.09^{*ab}$	3.03 $\pm 2.13^a$
	20% $\pm 4.49^{*ab}$	5.44 $\pm 1.03^a$	1.87 $\pm 0.11^{*aC}$	2.99 $\pm 0.19^{*AC}$	20.38 $\pm 1.35^{*AB}$	123.37 $\pm 10.59^{*ab}$	16.52 $\pm 2.08^a$	4.27 ± 0.11	3.80 $\pm 0.14^{*ab}$	0.00 $\pm 0.00^a$
	30% $\pm 3.93^{*ab}$	5.51 $\pm 1.04^a$	2.02 $\pm 0.02^{*aC}$	2.41 $\pm 0.03^{*c}$	16.27 $\pm 2.85^{*B}$	130.78 $\pm 9.92^{*ac}$	17.89 $\pm 2.38^{*ab}$	4.48 $\pm 0.10^C$	3.99 $\pm 0.09^{*abC}$	6.66 ± 2.35
HDBB	10% $\pm 4.11^{*A}$	11.57 $\pm 1.19^{*A}$	5.55 $\pm 0.22^{*A}$	2.16 $\pm 0.09^a$	5.55 $\pm 1.95^a$	180.42 $\pm 8.54^{*A}$	26.78 $\pm 2.89^{*A}$	4.58 $\pm 0.10^B$	6.01 $\pm 0.13^{*A}$	0.00 $\pm 0.00^a$
	20% $\pm 4.85^{*A}$	11.93 $\pm 1.07^{*A}$	5.07 $\pm 0.07^{*A}$	2.35 $\pm 0.03^{*a}$	0.00 $\pm 0.00^{ab}$	177.14 $\pm 8.59^{*A}$	26.45 $\pm 2.71^{*A}$	4.59 $\pm 0.18^B$	5.77 $\pm 0.23^{*A}$	3.03 $\pm 2.13^a$
	30% $\pm 4.26^{*A}$	11.24 $\pm 1.25^{*A}$	5.21 $\pm 0.16^{*A}$	2.16 $\pm 0.08^a$	5.80 ± 2.05	177.41 $\pm 10.33^{*A}$	26.61 $\pm 3.11^{*A}$	4.76 $\pm 0.11^B$	5.83 $\pm 0.14^{*A}$	0.00 $\pm 0.00^a$
EFP	10% $\pm 6.89^{*ab}$	6.29 $\pm 0.51^a$	3.16 $\pm 0.05^{*aBc}$	1.98 $\pm 0.03^{*abc}$	2.77 $\pm 1.95^{*ab}$	143.38 $\pm 9.76^{*ab}$	14.50 $\pm 1.56^{*ab}$	4.29 ± 0.10	3.43 $\pm 0.08^{*aBcd}$	5.55 ± 3.92
	20% $\pm 4.53^{*ab}$	6.79 $\pm 0.45^{*ab}$	2.94 $\pm 0.13^{*ac}$	2.32 $\pm 0.11^{*c}$	7.40 $\pm 5.23^b$	152.16 $\pm 7.79^{*abc}$	14.55 $\pm 1.37^{*ab}$	4.97 $\pm 0.15^{*AC}$	2.92 $\pm 0.03^{*aBcD}$	0.00 $\pm 0.00^a$
	30% $\pm 4.82^{*ab}$	6.97 $\pm 0.51^{*ab}$	3.83 $\pm 0.14^{*abc}$	2.03 $\pm 0.07^{*c}$	8.33 $\pm 2.94^b$	136.90 $\pm 7.07^a$	16.26 $\pm 1.84^a$	3.78 $\pm 0.13^{*bc}$	4.31 $\pm 0.15^{*ad}$	19.04 $\pm 3.36^{*A}$
ANOVA (p-value)	0.0001	0.0001	0.0001	0.0001	0.0004	0.0001	0.0001	0.0005	0.0001	0.0091

BW: Body Weight. BWG: Daily Body Weight Gain. CTRL: Control. EBB: Entire Broad Beans. EFP: Entire Field Peas. FI: Daily Feed Intake. FCR: Feed Conversion Ratio. HDBB: Heat-treated Decorticated Broad Beans. M: Mortality rate. *: significant difference compared to CTRL. Letters within the same column indicate significant differences at $p<0.05$ probability level by Tukey test: means labelled by an upper-case letter are significantly different from those labeled by the same lower-case letter. There are no significant differences between means that share the same upper-case or the same lower-case letter.

Biochemical parameters

The biochemical traits of the different batches are summarized in Table 4. A significant decrease in glycemia was observed in HDBB 20% and 30% groups ($p=0.0039$ and $p=0.0049$, respectively)

and in EBB 30% group, as compared to the CTRL ($p=0.0015$). However, we did not record any serum cholesterol variations in our batches except a significant difference ($p=0.0079$) between males and females in the control group, in favor

of females. Furthermore, no disparities ($p>0.05$) in protein levels were observed in the different batches as compared to the CTRL; nevertheless, differences were noticed in favor of EBB and EFP batches compared to HDBB ones. In addition, blood urea decreased in EBB batch 10% significantly ($p=0.0019$), and in EFP 10% batch, but in a non-significant way ($p=0.49$). This decrease was very significant in HDBB 10%, 20% and 30% batches ($p=0.0047$, $p=0.0009$ and $p=0.0227$, in that order), as compared to the CTRL. On the other hand,

creatinine levels were unaffected by dietary changes ($p>0.05$), in contrast with calcemia that increased in a significant manner in HDBB 20% and 30% ($p=0.0285$ and $p=0.0208$, respectively) and EBB 30% batches ($p=0.0035$) in comparison to CTRL. Phosphatemia of all HDBB groups ($p=0.0064$, $p=0.0163$ and $p=0.0027$, respectively) and EBB 20% ($p=0.0048$) was higher than the CTRL. Finally, the partial replacement of soybean meal by EBB, HDBB and EFP had no impact on plasma GOT and GPT amounts.

Table 4 Effect of soybean meal substitution by EBB, HDBB and EFP on the biochemical profile of the Japanese quail (Median $\pm$ IQR)

Batch	Glucose (g/l)	Cholesterol (g/l)	Triglycerides (g/l)	Total Proteins (g/l)	Urea (g/l)	Creatinine (g/l)	GOT (U/l)	GPT (U/l)	Calcium (mg/l)	Phosphate (mg/l)
CTRL	3.63 ± 0.43	2.35 ± 1.52	1.20 ± 0.87	27.05 ± 9.49	0.10 ± 0.05	5.45 ± 0.77	280.00 ± 177.80	25.50 ± 6.75	75.00 ± 21	64.70 ± 10.75
EBB	10% 2.83 ± 0.41	2.05 ± 0.88	0.78 ± 0.41	34.45 $\pm 6.55^a$	0.03 $\pm 0.03^{*a}$	4.75 ± 1.14	252.00 ± 78.80	34.50 ± 15.75	81.00 $\pm 11.25^a$	83.50 ± 21.00
	20% 3.51 $\pm 1.10^a$	2.22 ± 0.75	1.44 ± 1.24	36.25 $\pm 5.42^{ab}$	0.07 ± 0.02	4.45 ± 0.65	247.00 ± 85.20	44 ± 35.25	88.5 ± 13.50	93.75 $\pm 32.50^*$
	30% 1.66 $\pm 0.55^{*A}$	2.08 ± 0.42	0.74 ± 0.78	28.20 ± 5.38	0.10 $\pm 0.03^A$	5.70 ± 3.40	218.00 ± 36.50	39.50 $\pm 17.75^a$	100.00 $\pm 6.30^*$	80.00 ± 20.39
HDBB	10% 3.05 ± 0.23	2.17 ± 0.36	1.21 ± 0.80	19.45 $\pm 6.37^A$	0.05 $\pm 0.02^{*a}$	4.85 ± 2.30	157.50 ± 87.00	15.50 ± 17.50	117.00 $\pm 10.50^A$	115.00 $\pm 51.70^{*A}$
	20% 2.81 $\pm 0.51^*$	2.56 ± 0.89	1.90 ± 1.50	22.95 $\pm 10.53^B$	0.04 $\pm 0.03^{aB}$	6.20 ± 1.47	165.00 ± 24.00	37.00 ± 20.50	125.00 $\pm 55.30^{*A}$	109.00 $\pm 49.50^{*B}$
	30% 2.83 $\pm 0.28^*$	2.20 ± 1.04	1.21 ± 0.84	21.75 ± 17.60	0.05 $\pm 0.02^*$	5.40 ± 0.87	162.00 ± 99.50	34.00 ± 11.75	142.00 $\pm 81.50^{*A}$	119.30 $\pm 15.90^{*C}$
EFP	10% 3.94 $\pm 0.51^a$	1.88 ± 0.43	1.36 ± 0.36	27.70 ± 6.65	0.06 ± 0.03	4.25 ± 0.72	245.50 ± 90.00	18.50 ± 12.00	88.0 ± 11.50	64.40 $\pm 21.00^{ac}$
	20% 3.18 ± 0.48	2.41 ± 0.74	1.25 ± 0.39	26.85 ± 5.70	0.08 $\pm 0.02^b$	5.83 ± 0.28	268.50 ± 135.00	15.50 $\pm 4.75^A$	85.00 $\pm 13.50^a$	67.80 $\pm 5.43^{ac}$
	30% 3.19 ± 0.32	2.23 ± 0.39	0.98 ± 0.74	39.65 $\pm 6.90^{ab}$	0.07 ± 0.03	3.95 ± 3.30	240.00 ± 58.70	46.00 ± 47.00	85.50 $\pm 20.75^a$	53.00 $\pm 29.30^{abc}$
Kruskal-Wallis (p-value)	0.0001	0.58	0.15	0.0001	0.0001	0.009	0.029	0.0006	0.0001	0.0001

CTRL: Control. EBB: Entire Broad Beans. EFP: Entire Field Peas. GOT: glutamic-oxaloacetic transaminase. GPT: glutamic-pyruvic transaminase. HDBB: Heat-treated Decorticated Broad Beans.

*: significant difference compared to CTRL. Letters within the same column indicate significant differences at $p<0.05$ probability level by Dunn test: medians labeled by an upper-case letter are significantly different from those labeled by the same lower-case letter. There are no significant differences between medians that share the same upper case or the same lower-case letter.

DISCUSSION AND CONCLUSION

The disparities recorded in quails' weights of our several batches, could be related to variations in nutrients' contents, others than energy and proteins, of the various used formulas. Furthermore, the higher content of EFP and EBB (to a less extent) in antinutritional factors, as described by Hickling (2003), negatively affected weights of birds fed on them, as compared with those fed on HDBB.

Differences in BW between males and females may be related to sexual dimorphism. This same observation was made by Aytac and Karabayir (2012) and Ojedapo and Amao (2014).

FI noted in our CTRL was similar to the one reported by Guluma and al. (2014) in their CTRL and may be linked to similarities in used quail strains and rearing conditions (Ipek et al., 2007). The increase in feed consumption is consistent with the work of Magoda and Goss (2011), in laying hens fed on dehulled broad beans, and it was related to the absence of antinutritional factors after shelling and to an unidentified deficiency that incited birds to consume more in order to get enough nutrients. However, it is believed that shelling and even autoclaving do not eliminate all antinutritional factors since the thermostable α -galactosides found in the bean almonds will persist. Higher organisms, like birds, do not possess α -galactosidase and the presence of these sugars results in digestive discomfort (flatulence and diarrhea). This can diminish FI and glucose absorption, and leads to hypoglycemia and accelerated intestinal transit (Cunha and Freire, 1993; Kaysi and Melcion, 1992). Therefore, and in order to compensate for the limiting element (glucose), feed consumption increased. In addition, the elimination of tannins with a major part of other antinutritional factors by treating broad beans has positive effects on the improvement of palatability. According to Gemede and Ratta (2014), tannins are known to be responsible for decreasing feed consumption, dietary efficiency and protein digestibility. They are also the cause of decreased palatability and reduced growth rate. Moreover, Kanyinji and Moonga (2014) reported

that the increase in consumption index might have been due to spillage, while its reduction could be related to increased fiber content.

The effect of antinutritional factors would be the cause of the FCR increase observed during growing period in EBB and EFP batches. These factors are known to cause growth retardation, reduced feed efficiency and protein digestibility in animals (Gemede and Ratta, 2014). To this, we can also associate the effect of fibers contained in EBB and EFP. The decreased FCR in EBB batches in the finishing period can be explained by the fact that quails probably became more resistant to its antinutritional factors.

The high mortality recorded in EBB batches may be attributed to the adverse effects of antinutritional factors as observed in the work of Koivunen et al. (2014), and the fact that HDBB- fed batches showed relatively less mortalities supports the idea that heat treatment of broad beans eliminates a big amount of them.

The nutrient profile of broad bean shows a high content of thiamine (5.5mg/kg), compared to soybean meal and field peas (1.7 and 1.8mg/kg, respectively). This vitamin is known to play a fundamental role in the metabolism of carbohydrates and the production of ATP within cells. Thus, it can be suggested that its high content in broad bean was probably the cause of the hypoglycemia observed in quails that fed on it. However, it is worth noting that the animal organism does not store thiamine, and the excess is excreted in urine or feces (Mc Dowell, 2000). Furthermore, diurnal variations in blood glucose levels had been well documented in terms of rapid ingestion cycle effect and its interference with internal rhythms (Scholtz et al., 2009). Another hypothesis that may explain the hypoglycemia observed in broad bean batches is related to the presence of α -galactosides, as previously explained. In the literature, there are also similar observations that are still normal for the Japanese quail glycemia standards noted by Sholtz et al. (2009) and Tufan et al. (2015).

The difference in blood glucose between males and females may be due to increased steroid hormones

secretion with birds aging in preparation for laying period (Ali et al., 2012). Elnagar and Abd-Elhady (2009) reported that reproductive and pre-sexual development led to 13-15% reduction in glycemia during sexual maturity and peak production, respectively.

Fibers contained in whole seeds contribute in cholesterol absorption decrease in the intestine (Potter, 1995). Additionally, in birds, most of the circulating cholesterol is transported by HDL and LDL. The effect of legume (including *Vicia faba*) proteins on serum cholesterol is related to their amino acid composition that reduces the number of LDL particles available for cholesterol transport in the plasma (Kingman et al., 1993). In preparation and during the laying period, hepatic synthesis of triglycerides, phospholipids and cholesterol is increased in quails (Walzem et al., 1999). These lipids are incorporated in lipoproteins of blood and ovary oocytes. This makes females, especially the laying ones, to have extraordinarily high blood cholesterol concentrations, unlike males (König et al., 2007). This is in concordance with our CTRL records.

Proteinemia of our CTRL is consistent with those described in previously published works (Ali et al., 2012; Nazifi and Asasi, 2001; Tufan et al., 2015). Nevertheless, it is important to note that a passive and transient hyperproteinemia can occur following dehydration (Hochleithner, 1994).

It is known that hypoproteinemia can be caused by the antinutritional factors present in the broad beans, especially the tannins (concentrated mainly in the teguments), in addition to trypsin and chymotrypsin inhibitors, lectins and saponins that reduce the digestibility and use of nitrogen (Cunha and Freire, 1993). This may explicate the decrease in blood urea remarked in EBB and EFP batches.

Plasma creatinine levels of our study did not vary between the batches, and they are very similar to those of Scholtz et al. (2009).

Serum calcium values of CTRL quails are consistent with Coenen et al. (1994) records. The hypercalcemia observed in HDBB and EBB

batches is, interestingly, close to those recorded in CTRL batches of previous studies (Nazifi and Asasi, 2001; Vali, 2010). Unfortunately, we could not find any explanation to this increase, except that in female birds, the formation of woven medullary bone is stimulated by the actions of oestrogens and androgens concomitantly with the maturation of the ovarian follicles. Resorption of this medullary bone provides 40% of the calcium required for shell formation, which explains the increase in blood calcium at the onset of spawning (Dacke et al., 1993; Thorp et al., 1993). In fact, we have observed an early sexual maturity in broad bean batches.

Increase in phosphoremia of HDBB and EBB groups can be physiological, as concluded by Hassan (2010) who attributed it to estrogens. Indeed, at the beginning of the laying period, this increase is due to higher calcium and phosphorus requirements, that lead to in-kidney vitamin D activation and bone resorption increase to meet the quails' needs (Proszkowiec-Weglarz and Angel, 2013).

Finally, the plasma amounts of GOT and GPT of our birds are still analogous to those obtained in CTRL quails of other studies (Babazadeh et al., 2011; Mahmoud et al., 2012).

To sum up, our feeding trials demonstrated that entire broad beans should not be incorporated in feed formulas for Japanese quails, especially during the growing period because of their richness in antinutrient factors; however, treated broad beans and field peas could be potential alternatives to solve the soybean-meal importation problem in Algeria and elsewhere. Nevertheless, the extensive studies are needed to determine up to which amounts these crops can be used.

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CONFLICT OF INTEREST

None.

CONTRIBUTIONS

AB: Data Collection and Processing; Analysis and Interpretation of the Data; Literature Review; Writing. AA: Conception; Design; Supervision; Analysis and Interpretation of the Data; Critical Review.

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DJELOMIČNA ZAMJENA OBROKA OD SOJE SA BOBOM ILI GRAŠKOM U PREHRANI JAPANSKIH PREPELICA

SAŽETAK

Cilj istraživanja jeste odrediti prehrambene formule specifične za Japansku prepelicu *C. japonica* djelomičnom zamjenom uvoznog sojinog obroka sa lokalno proizvedenim i jeftinijim proteinskim žitaricama. Svaka formula je sadržavala ili cjeloviti bob (EBB), toplotom oguljeni bob (HDBB) ili cjeloviti grašak (EFP) sa različitim udjelima (10, 20 i 30%) u cjelokupnoj formuli. Rezultati su pokazali da je prehrana koja sadrži HDBB imala bolje performanse rasta u odnosu na sve druge formule. Zamjena sa EFP-om je pokazala učinke slične kontrolnoj formuli, ali je visok sadržaj vlakana i proteina u njoj predstavljao ograničenje za dodatno povećanje udjela u ukupnoj formuli. EBB je pokazao negativne učinke, posebno na performanse rasta. Dodavanje HDBB-a je izazvalo signifikantnu hipoglikemiju (serije 20% i 30%) ($p=0.0039$ i $p=0.0049$) i znatno smanjenje uremije (sve serije) ($p=0.0047$, $p=0.0009$ i $p=0.0227$, tim redoslijedom) sa hiperkalcemijom (serije 20 i 30%) ($p=0.0285$ i $p=0.0208$), hiperfosfatemijom (sve serije) ($p=0.0064$, $p=0.0163$ i $p=0.0027$) i normalnom holesterolemijom, trigliceridemijom, proteinemijom, serumskim kreatininom, glutamat oksalacetatnom transaminazom (GOT) i glutamat piruvatnom transaminazom (GTP). EBB je izazvao veoma signifikantnu hipoglikemiju (pri zamjeni od 30%) ($P=0.0015$) i hipouremiju (serija 10%) ($p=0.0019$) sa hiperkalcemijom (serija 30%) ($p=0.0035$), hiperfosfatemijom (serija 20%) ($p=0.0048$) i normalnom holesterolemijom, trigliceridemijom, proteinemijom, serumskim kreatininom, GOT i GPT. S druge strane, EFP nije imao učinka ($p>0.05$) na biohemijske parametre *C. japonice*, osim nesignifikantnog smanjenja uremije u seriji 10% ($p=0.49$). Prema tome, može se zaključiti da grašak i tretirani bob mogu biti korišteni kao zamjene za obroke od soje u proizvodnji prepelica.

Ključne riječi: Biohemijski profil, *Coturnix japonica*, formula, performanse rasta

RESEARCH CASE REPORT

A CASE OF SIMULTANEOUSLY DETECTED RECTOVAGINAL FISTULA AND ATRESIA ANI IN A KITTEN

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ABSTRACT

A 45-day-old female kitten was admitted to the Obstetrics and Gynaecology Clinics of Faculty of Veterinary Medicine at Istanbul University-Cerrahpaşa with complaints of vomiting, abdominal swelling and difficulty in defecation after switching to dry food. The mucous membranes were pale pink, the palpable lymph nodes were of normal size, the furs were dull, the abdomen was swollen, intestinal fullness, and the normal posture on all fours were noticed. On clinical examination, it was seen that the anus was completely closed, and the faeces was coming from the vagina. In this case, Type II atresia and recto-vaginal fistula were observed simultaneously. General anesthesia was administered to the kitten whose vital signs and hemogram values were within the normal reference range, and an anal opening surgery was performed. In this case report, the surgical intervention of the rarely seen congenital defects and the post-surgery process were discussed. It is concluded that the atresia ani and recto-vaginal fistula cases, which are successfully treated by a surgical intervention, may simultaneously exist in 45-day-old kittens, and both clinical and surgical approaches at an early age would have great importance in such congenital cases.

Keywords: Cat, congenital defect, surgical therapy

INTRODUCTION

Atresia ani is a rare congenital defect in puppies and kittens associated with a rectovaginal or rectovestibular fistula between the distal urogenital tract and the rectum (Matthiesen and Maretta, 1993). Anorectal abnormalities associated with urogenital malformations result from abnormal embryonic development of the cloacal region (Rothenberger and Goldberg, 1983; Santulli et al., 1965). The gastrointestinal, urinary, and genital tracts initially coexist in one cloacal opening. This embryonic cloaca consists of the rectum, which is separated by the urorectal fold and urogenital sinus. The urogenital sinus is divided into the bladder and urethra. The cranial part of the

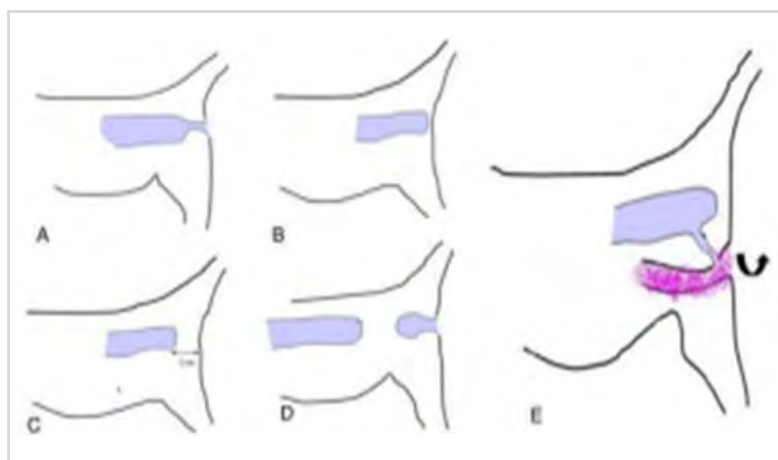


Figure 1 (A): Schematic representation of type I atresia ani (B): Schematic representation of type II atresia ani (C): Schematic representation of type III atresia ani (D): Schematic representation of type IV atresia ani (E): Schematic representation of type II atresia ani and rectovaginal fistula (Papazoglou and Ellison, 2012).

anal canal is formed by the terminal end of the hindgut, while the anus is formed by the ingrowth of the ectoderm and perineum. In females, the Müllerian ducts form the cranial two-thirds of the uterus and vagina. The caudal part of the vagina develops from sinovaginal buds originating from the epithelium of the dorsal wall of the urogenital sinus. Division of the cloaca is completed early in pregnancy in the mammalian embryo (Amand, 1974; Arey, 1974). Four types of atresia ani have been reported: congenital anal stenosis (Type I); imperforate anus alone (Type II), or combined with more cranial termination of the rectum as a blind pouch (Type III) and discontinuity of the proximal rectum with normal anal and terminal rectal development (Type IV) (Aronson, 2003; Papazoglou and Ellison, 2012) (Figure 1). Typically, a rectovaginal fistula is accompanied with type II atresia (Maretta and Matthiesen, 1989).

Fistula, ventral terminal of which is usually ending as a blunt sac, connects the rectum to the dorsal wall of the vagina (Suess et al., 1992). The fistula allows the evacuation of faeces so that the animal survives the postnatal period (Kibar Kurt and Turan, 2021). In this case, surgical treatment of type II atresia ani and rectovaginal fistula was discussed.

CASE PRESENTATION

A 45-day old female Scottish kitten was forwarded from a private clinic to the Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa with complaints of difficulty in defecation, swelling in the abdomen and vomiting one day after weaning and switching to dry food. As a result of the clinical examination, the mucous membranes were pale pink, the palpable lymph nodes were

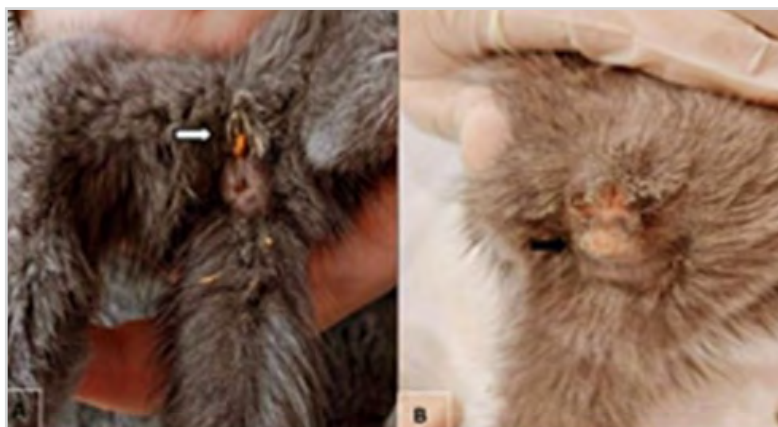


Figure 2 A Evacuation of faeces from rectovaginal fistula (white arrow) B Closed anus (black arrow)



Figure 3 Positive contrast radiography and fistulography in laterolateral position
Absence of retrograde contrast agent flowing out from the anus due to the absence of anal opening

in normal size, the furs were dull, the abdomen was swollen, and the normal posture on all fours was observed. Intestinal fullness was noticed on abdominal palpation. There was no anal opening detected in clinical examination, it was observed that the faeces was evacuated through the vagina (Figure 2).

Lateral plain positive contrast radiography and fistulography were performed after catheterization of the bladder and colon through the vaginal canal (Figure 3). As a result of the examination, type II atresia and rectovaginal fistula were diagnosed.

Blood tests were performed to avoid the anesthesia risk before the surgical intervention. Hemogram and some blood biochemistry parameters were within the normal reference ranges. Since the vital signs were found to be normal, anal opening surgery was decided to perform. After intravenous

catheterization, infusion was started with Ringer's lactate at a rate of 7 ml/kg/hour (Medifleks, Koçak Farma, Turkey) (Figure 4). Amoxicillin+clavulanic acid at a dose of 8.75 mg/kg (Synulox suspension for injection, Haupt Pharma, Italy) was administered subcutaneously.

For induction of the anesthesia, medetomidine (0.08 ml/kg) (Domitor 1 mg/ml, Zoetis, USA) was administered intramuscularly. General anesthesia was maintained with isoflurane at a dose of 2.5% (Isoflurane USP, Piramal Critical Care, USA) and oxygen at a dose of 0.5 - 1% by the inhalation mask. The kitten was positioned in sternal recumbency. Asepsis and antisepsis were provided at perianal and perivaginal area. The closed anus region was incised approximately 1 cm in length (Figure 5).



Figure 4 Serum infusion in the pre-operative term



Figure 5 Incision of closed anus and visualization of anal opening after incision

An anal opening was seen under the skin cut with the incision, and the overlying skin was sutured to the edges of the anal opening. A vertical mattress suture was used to inclination of the wound lips towards the anal opening. Then, the fistula hole opened from the rectum to the vagina was revised and the operation area was closed with a sultan (x) suture. Absorbable and 4/0 surgical suture material (Monocryl, Medeks, Turkey) was used for all sutures (Figure 6).

After surgery, the kitten was fed with wet food (Hill's A/D) for 7 days. Sutures were cleaned daily with 0.9% NaCl isotonic serum (Polifleks, Polifarma, Turkey). Epithelializing skin cream (Bepanthen Plus Cream, Bayer, Germany) was applied to the wound line for 5 days. Duphalac



Figure 6 Post-operative image of anus

syrup (2 ml/kg/oral) (Abbott 670 mg/ml, Illinois, USA) was given to ease faeces output with the transition to dry food. Normal urine and faeces output were observed regularly. Cystitis, vaginitis or fecal incontinence were not observed. Sutures were removed on Day 14. To complete the tissue healing, the treatment was continued for 7 days using epithelializing skin creams (Denovo, Farmalion, Turkey), (Bepanthen Plus Cream, Bayer, Germany).

DISCUSSION AND CONCLUSION

The diagnosis of atresia ani can be made based on anamnesis, age, and physical examination. Additionally, radiographic examination and ultrasonography are considered to be the ideal methods for the confirmation of the rectal abnormality (Salari Sedigh et al., 2010). Although there was no radiographic examination in this case, the case was classified as atresia ani type II and recto-vaginal fistula anomaly, since the rectal opening was not observed, and the vaginal fistula was seen during palpation, while the anal fistula was not evident.

In cases where the recto-vaginal fistula and atresia ani coexist together, faeces are evacuated from the vagina throughout the fistula by maternal stimulation. Small amounts of watery faeces may pass through the fistula and exit from the vulva and consequently, it may cause the secondary ulceration of the labia, while harder faeces cannot pass through the fistula and remain in the colon,

thus causing abdominal distension (Rahal et al., 2007; Vianna and Tobias, 2005). This condition is usually noticed after weaning (4-6 weeks after birth) (Salari Sedigh et al., 2010). However, the kitten which had both atresia ani and recto-vaginal fistula was approximately 7 weeks old (45 days old) in this case. The reason why this frequently encountered case occurred after a period of 4-6 weeks might be the coexistence of two cases together. In cases of atresia ani, severe tenesmus, vomiting, anorexia and developmental delay are clinically observed (Salari Sedigh et al., 2010). The clinical findings seen in this case are consistent with the previous case reports (Rahal et al., 2007; Salari Sedigh et al., 2010; Jardel et al., 2013).

The only available curative treatment is surgical correction. If the defects are left uncorrected, type II atresia ani and recto-vaginal fistula always lead to secondary complications such as megacolon, urinary tract infection, vaginitis, and chronic kidney injury; therefore, the surgical treatment of these congenital defects is compulsory (Jardel et al., 2013). The anoplasty is referred to the opening of the rectum and the reconstruction. There have been cases where the anomaly is corrected with different surgical techniques. Complications have been reported in cases due to widely dissected soft tissue where the complete excision of the fistula was performed (Bornet, 1990; Suess et al., 1992). In line with Jardel et al. (2013) who suggested the importance of surgical methods for the treatment of the congenital defects, anoplasty was performed for the treatment of type II atresia ani and rectovaginal fistula in this case. After anoplasty intervention, no faecal discharge from the vagina was observed. Moreover, no postoperative complications were seen in the present case, although the postoperative complications such as faecal incontinence, constipation and megacolon, or anal stenosis due to incorrect suture techniques which are performed during surgery, have been reported (Salari Sedigh

et al., 2010). It is suggested that the surgery technique is likely the predisposing factor for the possible complications.

Other congenital anomalies with anorectal malformations may be present in one-third of patients (Aronson, 2003). Partial tail agenesis associated with recto-vaginal fistula has been observed in dogs (Aronson, 2003; Vianna and Tobias, 2005). In cats, atresia ani and recto-vaginal fistula have been reported in association with sacro-caudal dysgenesis and hydrocephalus (Suess et al., 1992). There are cases suggesting the defect is inherited; therefore, neutering is recommended to prevent these congenital anomalies (Suess et al., 1992). Neutering was not applied to the 45-day-old cat that constituted the present case, but in accordance with Suess et al. (1992), it was recommended that the cat might be neutered instead of prepubertal gonadectomy during its adulthood.

In this case report, it is concluded that the atresia ani and recto-vaginal fistula cases which are successfully treated by surgical intervention, may simultaneously exist in 45-day-old kittens, and both clinical and surgical approaches are of importance in such congenital cases.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – İK, ZGU.; Design – İK; Supervision – İK, ZGU, BG; Resources - MY; Materials – RBE; Data Collection and/or Processing – İK,BG; Analysis and/or Interpretation – İK,ZGU,MY; Literature Search – AB,BG; Writing Manuscript – BG,ZGU; Critical Review – RBE,ZGU.

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SLUČAJ ISTOVREMENOG DIJAGNOSTICIRANJA REKTOVAGINALNE FISTULE I ATREZIJE ANUSA KOD MAČETA

SAŽETAK

45-dnevno žensko mače je primljeno na Kliniku za opstetriciju i ginekologiju Veterinarskog fakulteta Cerrahpaşa Univerziteta u Istanbulu sa povraćanjem, abdominalnim otokom i otežanom defekacijom nakon prelaska na suhu hranu. Mukozne membrane su bile blijedoružičaste, palpabilni limfni čvorovi uredne veličine, krzno bez sjaja, abdomen otečen uz vidljivu intestinalnu napetost i normalan položaj na sve četiri noge. Kliničkim pregledom je uočen potpuno zatvoren anus, dok je feces izlazio kroz vaginu. U ovom slučaju su tip II atrezije i rektovaginalna fistula otkriveni istovremeno. Mače je uvedeno u opću anesteziju, dok su vitalni parametri i hemogram bili u granicama referentnih vrijednosti, pri čemu je izvedena operacija otvaranja. Ovaj prikaz slučaja pokazuje operativnu intervenciju rijetko viđenih kongenitalnih defekata i postoperativni proces. Zaključeno je da slučajevi atrezije anusa i rektovaginalne fistule koji se operativno uspješno tretiraju, mogu istovremeno postojati kod 45-dnevnih mačića, kao i da klinički i operativni pristupi u ranoj dobi mogu imati izuzetan značaj u takvim kongenitalnim slučajevima.

Ključne riječi: Kongenitalni defekt, mačka, operativna terapija

PROFESSIONAL PAPER

ANALYZING THE DISTRIBUTION OF SLAUGHTERED CATTLE BREED IN EDİRNE SLAUGHTERHOUSE

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ABSTRACT

It was aimed to determine the cattle breed distribution, which were slaughtered in the Edirne slaughterhouse between 2017-2021. Slaughterhouse data were used as the material for this study that accounted for a number of 49,473 slaughtered cattle. Cattle were first grouped into pure breeds and cross-breeds, and then, sub-genotypes of cross-breeds were determined.

Twenty-one pure breeds and nineteen cross-breeds were determined corresponding to the identified genotypes. Furthermore, 148 sub-genotypes in crosses according to sire breed were observed, which accounted for 169 genotypes in total. It was determined that most of the slaughtered cattle were Holstein-Black (53.8%), followed by Simmental crosses (13.9%), pure Simmental (11.2%), Brown Swiss crosses (4.6%), pure Brown Swiss (4.2%) and Holstein-Black crosses (2.8%). Among the crossbreeds, the highest rate was found for the Simmental crosses (54.8%). Brown Swiss (18.0%), Holstein-Black (10.9%), Aberdeen-Angus (6.0%) and Belgium Blue (2.4%) crosses followed the Simmental crosses.

Considering the results, beside of the cattle breeds mostly grown in the region, other breeds that were used for the meat production would contribute to identify the farmers' breed preferences. Determination of the wide variety of cattle breeds or genotypes implies that farmers may have fattened various breeds without a proper breeding programme. Improper implementation of a breeding programme may cause herds to loss its uniformity and efficiency. We suggest policy makers to determine a proper breeding and/or crossbreeding programme by considering expectation and available resources of the farmers.

Keywords: Breed, carcass, cattle, crossbreeds, frequency

INTRODUCTION

Animal husbandry practices can differ due to the farmers' preferences, which are mainly directed by the geographic and economic conditions of the provinces (Ozturk et al., 2022). Marmara Region is in the north-west of Turkey, and it is one of the significant regions for the Turkish animal husbandry. Edirne is a province in the Marmara region, and cattle husbandry is among the main economic activities for the rural people of the region. Besides being important for the regional economic circulation, cattle husbandry provides nutritional demands for the nation (Lorcu and Bolat, 2012).

According to the FAO (2023), Turkey has a significant number of cattle population. Among 193 countries in the world, Turkey ranks 22nd; and

among 40 countries in Europe, Turkey ranks 3rd in terms of the number of cattle.

Between 2017-2022, a total number of cattle population in Turkey corresponding to breed classification and their share among the total cattle population was presented in Table 1. According to this, improved breeds and crossbreed were preferred for the animal husbandry throughout Turkey (TSI, 2023a). In Table 2, number of cattle population in Edirne corresponding to breed classification, their share and Edirne's cattle population share within Turkey was presented. Farmers in Edirne preferred to use improved breeds more commonly, and the share of local breeds among the total cattle population in Edirne was below 1%. Furthermore, cattle population in Edirne contributed at around 1% to the total national cattle population (TSI, 2023b).

Table 1 Number of cattle population in Turkey

Years	Improved Breeds	%	Cross Breeds	%	Local Breeds	%	Total
2017	7 804 588	49.0	6 536 073	41.0	1 602 925	10.1	15 943 586
2018	8 419 204	49.4	7 030 297	41.3	1 593 005	9.3	17 042 506
2019	8 559 855	48.4	7 554 625	42.7	1 573 659	8.9	17 688 139
2020	8 838 498	49.2	7 594 127	42.3	1 532 857	8.5	17 965 482
2021	8 824 784	49.4	7 641 100	42.8	1 384 659	7.8	17 850 543
2022	8 295 825	49.2	7 324 866	43.5	1 231 265	7.3	16 851 956

Table 2 Number of cattle population in Edirne province and its share within Turkey

Years	Improved Breeds	%	Cross Breeds	%	Local Breeds	%	Total	%*
2017	116,720	75.7	36,348	23.6	1,042	0.7	154,110	0.97
2018	117,527	75.2	37,705	24.1	971	0.6	156,203	0.92
2019	119,993	74.6	39,941	24.8	888	0.6	160,822	0.91
2020	117,262	72.9	42,719	26.6	894	0.6	160,875	0.90
2021	118,018	73.1	42,597	26.4	855	0.5	161,470	0.90
2022	111,200	71.4	43,829	28.1	747	0.5	155,776	0.92

*Edirne's share among Turkish cattle population

Number of slaughtered cattle and meat production in Turkey between 2017-2021 were shown in Table 3 (TSI, 2023c), as well as the number of slaughtered cattle and meat production in Edirne

and its share among the Turkish slaughtered cattle population and meat production were shown in Table 4 (HBS, 2023).

Table 3 Number of slaughtered cattle and cattle meat production in Turkey

Years	Slaughtered cattle (number)	Cattle meat production (tonnes)
2017	4,334,034	1,093,841
2018	4,844,711	1,281,234
2019	4,856,517	1,330,169
2020	4,812,902	1,341,446
2021	5,134,441	1,460,719
2022	*	*

* Data for 2022 is not available.

Table 4 Number of slaughtered cattle and meat production in Edirne province and their share in Turkey

Years	Slaughtered cattle		Cattle meat production	
	Number	%	Tonnes	%
2017	24,652	0.57	6,190	0.57
2018	18,725	0.39	5,955	0.46
2019	29,452	0.61	8,002	0.60
2020	23,360	0.49	6,336	0.47
2021	23,819	0.46	6,472	0.44
2022	22,149	*	5,968	*

* 2022 data for total is not yet available, it could not be calculated.

As it is known, in addition to total amount of meat production, individual meat production per animal is essential. Beside the environmental factors, genotype is a determining factor for individual meat production (Ariturk and Yalcin, 1966). In general, local breeds are less productive as compared to the improved breeds (Arpacik, 1997; Evrim and Gunes, 2000).

Even though the number of cattle corresponds to approximately 1% of Turkey's cattle population in Edirne province, Edirne's share in the slaughtered cattle and cattle meat production is half of this. These values indicate that cattle raised in Edirne province have also been slaughtered in another cities.

In Turkey, each year a number of cattle and their milk production is presented per improved breeds, cross-breeds, and local breeds by the Turkish Statistical Institute and it is publicly available.

However, there is no information regarding the slaughtered cattle, whether they are improved, cross-breed and local breed. Also, a detailed breed information is not available, which corresponds to the numbers for animals, milk and meat production.

Due to the short duration of animal breeding, especially in meat production, many commercial cross-breeds can be formed together with purely-raised breed. Considering the economic conditions, cross-breeds can be more beneficial for meat or milk production (Evrin and Gunes, 2000). However, random crossbreeding is regulated by laws (Resmi Gazete, 2001).

In this study, it was aimed to identify the breed distribution of slaughtered cattle in the Edirne slaughterhouse. Considering the results, beside the mostly used pure breeds in the region, other breeds that were preferred for the meat production would contribute to identify the farmers' breed

preferences. Furthermore, by taking the outcomes of this study into consideration, future studies on the determinants of the farmers' breed preferences, and observed and expected production yields can be compared.

MATERIAL AND METHODS

Data consisting of information on slaughtered cattle breeds in the Edirne slaughterhouse between 2017 and 2021 were used as the study material. During this period, a total of 49,473 cattle were slaughtered. Number of slaughtered cattle between 2017-2021 was given in Table 5.

Table 5 Number of slaughtered cattle in Edirne slaughterhouse between 2017-2021

Years	Number of slaughtered cattle
2017	9,417
2018	9,659
2019	12,106
2020	8,914
2021	9,377
Total	49,473

Number of slaughtered cattle in the Edirne slaughterhouse constituted approximately half of the total slaughtered cattle in Edirne. Therefore, determination of the slaughtered cattle breeds is sufficient for sampling and represents the Edirne province.

In the study, the breeds of the slaughtered cattle were determined from the records according to their ear tags. In addition to 21 pure breeds and 19 crossbreeds in general, sub-genotype groups were formed from the cow and bull records of the crosses. The creation of cross-breed groups was based on bull breeds and 148 sub-genotype groups emerged from 19 cross-breed groups. Thus, a total of 169 genotype groups were determined together with pure breed groups. The ratios of these groups were calculated according to the total number of slaughtered animals in the slaughterhouse, as well as pure breed and mixed breed groups. Due to the numerical differences of the distributions, no statistical analysis was performed between the groups.

RESULTS

In the study, 40 genotype groups (21 pure breeds and 19 cross-breeds) were identified. In Table 6, number, and frequency of the slaughter cattle breed in total, in pure breed and in cross-breed were shown. The distribution of the identified genotypes was arranged from the highest to lowest cattle breed.

According to our results, most of the slaughtered cattle breeds were Holstein-Black (53.9%), followed by Simmental crosses (13.9%), pure Simmental (11.2%), Brown Swiss crosses (4.6%), pure Brown Swiss (4.2%) and Holstein-Black crosses (2.8%), and farmers dominantly preferred to use breeds which specialized in both dairy and meat production. Charolais and Limousine were two most slaughtered meat-type breeds by 0.9% and 0.8%, respectively, following the Aberdeen Angus (2.6%). Number of slaughtered cattle from Aubrac, Normande, Piedmontese cross and Salers breed were reported only one each between 2017-2021. Gray Breed was the only identified local cattle breed that was slaughtered in the mentioned period. However, the number of slaughtered Gray Breed was only 18.

Holstein-Black (72.1%), Simmental (15.0%), Brown Swiss (5.6%), Aberdeen-Angus (2.6%), Charolais (1.2%) and Limousine (1.1%) had the highest percentages among the pure breeds that were slaughtered. Furthermore, among the slaughtered cross-breeds, the highest rate was

found for Simmental crosses (54.8%). This was followed by Brown Swiss (18.0%), Holstein-Black (10.9%), Aberdeen-Angus (6.0%), Belgium Blue (2.4%), as well as Charolais, Hereford, Holstein-Red and Montbeliarde crosses at lower rates (around 1%).

Table 6 Number and frequency of slaughtered cattle breed (pure and cross-breed)

Breeds		Years						Frequency (%)		
								total	in pure breed	in crossbreed
Pure	Cross-breed	2017	2018	2019	2020	2021	Total	49473	36901	12572
Holstein-Black		6282	5513	5576	4707	4527	26605	53.78	72.10	-
Simmental C		755	1276	1881	1447	1534	6893	13.93	-	54.83
Simmental		738	1006	1404	1082	1291	5521	11.16	14.96	-
Brown Swiss C		273	399	506	468	611	2257	4.56	-	17.95
Brown Swiss		445	402	435	349	437	2068	4.18	5.60	-
Holstein-Black C		184	165	253	346	428	1376	2.78	-	10.94
Aberdeen-Angus		134	38	626	135	30	963	1.95	2.61	-
Aberdeen-Angus C		126	309	196	67	51	749	1.51	-	5.96
Charolais		39	51	271	23	45	429	0.87	1.16	-
Limousine		38	45	252	22	47	404	0.82	1.09	-
Belgium Blue C		29	58	117	40	55	299	0.60	-	2.38
Hereford		91	5	139	7	24	266	0.54	0.72	-
Brangus		8	150	92	-	-	250	0.51	0.68	-
Charolais C		10	20	66	51	88	235	0.48	-	1.87
Hereford C		110	92	25	-	-	227	0.46	-	1.81
Holstein-Red C		22	25	22	36	32	137	0.28	-	1.09
Montbeliarde C		7	17	45	34	28	131	0.26	-	1.04
Red-Angus		78	10	25	2	5	120	0.24	0.33	-
Montbeliarde		4	8	61	14	7	94	0.19	0.25	-
Limousine C		4	11	25	17	26	83	0.17	-	0.66
Holstein-Red		15	6	10	13	29	73	0.15	0.20	-
Swedish Red C		17	14	17	9	13	70	0.14	-	0.56
Norwegian Red C		-	13	21	9	7	50	0.10	-	0.40
Normande C		-	1	10	10	15	36	0.07	-	0.29
European Red		4	6	7	5	12	34	0.07	0.09	-
Belgium Blue		-	1	9	3	8	21	0.04	0.06	-
Gray Breed		3	6	5	3	1	18	0.04	0.05	-
Swedish Red		1	2	4	5	6	18	0.04	0.05	-
European Red C		-	-	-	2	7	9	0.02	-	0.07
Red-Angus C		-	1	3	2	-	6	0.01	-	0.05

Breeds		Years						Frequency (%)		
								total	in pure breed	in crossbreed
Pure	Cross-breed	2017	2018	2019	2020	2021	Total	49473	36901	12572
Shorthorn C	-	1	1	1	3	6	0.01	-	0.05	
Blonde d'Aquitaine	-	5	-	-	-	5	0.01	0.01	-	
Norwegian Red	-	-	-	1	4	5	0.01	0.01	-	
Parthenaise C	-	1	-	3	1	5	0.01	-	0.04	
Angler	-	-	-	1	3	4	0.01	0.01	-	
Angler C	-	-	1	-	1	2	0.00	-	0.02	
Aubrac	-	-	1	-	-	1	0.00	0.00	-	
Normande	-	1	-	-	-	1	0.00	0.00	-	
Piedmontese C	-	-	-	-	1	1	0.00	-	0.01	
Salers	-	1	-	-	-	1	0.00	0.00	-	
Total		9417	9659	12106	8914	9377	49473	100.00	100.00	100.00

C: Crossbreed

Even though a regular trend was not observed for the slaughtered cattle breed, numbers of slaughtered cattle were similar between 2017-2021, except for 2019. Between 2017-2019, the number of slaughtered cattle was observed to increase, but between 2019-2020, the number of slaughtered cattle was decreased by 26.3%, most probably due to the COVID-19 pandemic.

Beyond classifying slaughtered cattle as pure and cross-breeds, it is also important from which

breeds the crosses are obtained. For example, the Simmental breed, which has the highest number of crossbred cattle (6893 animals), has crosses from 19 different genotypes. Among these cross-breeds, there are also backcross cross-breeds formed by combining even with hybrids of their own breed. In Table 7, genotypes of crossbred slaughtered cattle in the Edirne slaughterhouse, and their numbers and percentages were given. The breeds were listed starting from the most used crossbred breed.

Table 7 Number of slaughtered cross-breed cattle in Edirne province and their percentages (%) according to the sub-genotype

Genotype of crossbreed's gender		Years						Frequency (%)	
Male ("n" of total group)	Female	2017	2018	2019	2020	2021	Total	in total (12572)	in group "n" of male
Simmental	Holstein-Black	493	955	1248	765	790	4251	33.81	61.67
(6893)	*Simmental C	262	318	398	476	505	1959	15.58	28.42
(54.83%)	Brown Swiss	-	2	106	77	95	280	2.23	4.06
	Brown Swiss C	-	-	46	44	74	164	1.30	2.38
	Holstein-Black C	-	-	51	55	34	140	1.11	2.03
	Holstein-Red C	-	-	7	11	10	28	0.22	0.41
	European Red	-	-	9	2	1	12	0.10	0.17
	Montbeliarde C	-	-	5	3	1	9	0.07	0.13

Genotype of crossbreed's gender		Years						Frequency (%)	
Male ("n" of total group)	Female	2017	2018	2019	2020	2021	Total	in total (12572)	in group "n" of male
	Charolais C	-	-	3	1	4	8	0.06	0.12
	Gray Bred	-	-	2	4	2	8	0.06	0.12
	Holstein-Red	-	1	1	1	5	8	0.06	0.12
	Charolais	-	-	-	4	2	6	0.05	0.09
	Swedish Red	-	-	3	1	1	5	0.04	0.07
	Belgium Blue C	-	-	1	-	3	4	0.03	0.06
	Limousine C	-	-	-	1	3	4	0.03	0.06
	Aberdeen-Angus C	-	-	1	1	1	3	0.02	0.04
	Swedish Red C	-	-	-	-	2	2	0.02	0.03
	Limousine	-	-	-	-	1	1	0.01	0.01
	Red-Angus C	-	-	-	1	-	1	0.01	0.01
Brown Swiss (2257) (17.95%)	*Brown Swiss C	257	133	166	209	291	1056	8.40	46.79
	Holstein-Black	13	227	292	199	225	956	7.60	42.36
	Simmental C	-	10	16	20	45	91	0.72	4.03
	Holstein-Black C	-	10	12	17	9	48	0.38	2.13
	Gray Bred	3	3	10	3	11	30	0.24	1.33
	Simmental	-	3	4	10	11	28	0.22	1.24
	Charolais	-	1	1	4	6	12	0.10	0.53
	Holstein-Red C	-	5	2	4	-	11	0.09	0.49
	Charolais C	-	3	-	-	6	9	0.07	0.40
	Swedish Red C	-	2	1	-	3	6	0.05	0.27
	European Red	-	1	1	1	1	4	0.03	0.18
	Aberdeen-Angus C	-	-	1	1	1	3	0.02	0.13
	Belgium Blue C	-	-	-	-	1	1	0.01	0.04
	Limousine C	-	1	-	-	-	1	0.01	0.04
	Montbeliarde C	-	-	-	-	1	1	0.01	0.04
Holstein-Black (1376) (10.94%)	*Holstein-Black C	184	114	186	262	300	1046	8.32	76.02
	Simmental C	-	15	11	32	55	113	0.90	8.21
	Brown Swiss	-	10	21	13	21	65	0.52	4.72
	Brown Swiss C	-	9	10	20	17	56	0.45	4.07
	Simmental	-	6	13	8	15	42	0.33	3.05
	Holstein-Red C	-	4	6	2	6	18	0.14	1.31
	Aberdeen-Angus C	-	-	-	2	5	7	0.06	0.51
	European-Red	-	-	1	1	4	6	0.05	0.44
	Gray Bred	-	2	2	1	-	5	0.04	0.36
	Montbeliarde C	-	-	-	2	3	5	0.04	0.36
	Belgium Blue C	-	1	-	1	1	3	0.02	0.22
	Charolais	-	1	-	2	-	3	0.02	0.22
	Holstein-Red	-	2	1	-	-	3	0.02	0.22
	Charolais C	-	-	2	-	-	2	0.02	0.15
	Limousine	-	1	-	-	-	1	0.01	0.07
	Limousine C	-	-	-	-	1	1	0.01	0.07

Genotype of crossbreed's gender		Years						Frequency (%)	
Male ("n" of total group)	Female	2017	2018	2019	2020	2021	Total	in total (12572)	in group "n" of male
Aberdeen-Angus (749) (5.96%)	Aberdeenshire	109	250	84	-	-	443	3.52	59.15
	Holstein-Black	9	44	92	41	28	214	1.70	28.57
	*Aberdeen-Angus C	3	1	5	7	7	23	0.18	3.07
	Brown Swiss C	-	4	-	8	9	21	0.17	2.80
	Brown Swiss	4	1	6	4	4	19	0.15	2.54
	Simmental	1	2	1	2	2	8	0.06	1.07
	Belgium Blue C	-	1	3	-	-	4	0.03	0.53
	Holstein-Red C	-	4	-	-	-	4	0.03	0.53
	Limousine	-	-	2	1	1	4	0.03	0.53
	Holstein-Black C	-	-	3	-	-	3	0.02	0.40
	Simmental C	-	1	-	2	-	3	0.02	0.40
	Charolais C	-	-	-	1	-	1	0.01	0.13
	Limousine C	-	1	-	-	-	1	0.01	0.13
	Red-Angus C	-	-	-	1	-	1	0.01	0.13
Belgium Blue (299) (2.38%)	Holstein-Black	29	48	99	32	41	249	1.98	83.28
	Brown Swiss	-	6	12	5	3	26	0.21	8.70
	*Belgium Blue C	-	-	4	1	2	7	0.06	2.34
	Holstein-Black C	-	3	-	-	4	7	0.06	2.34
	Simmental	-	1	2	-	2	5	0.04	1.67
	Brown Swiss C	-	-	-	2	1	3	0.02	1.00
	Aberdeen-Angus C	-	-	-	-	1	1	0.01	0.33
	Simmental C	-	-	-	-	1	1	0.01	0.33
Charolais (235) (1.87%)	Holstein-Black	10	12	36	19	20	97	0.77	41.28
	*Charolais C	-	-	6	9	27	42	0.33	17.87
	Brown Swiss C	-	-	8	12	18	38	0.30	16.17
	Brown Swiss	-	7	8	7	13	35	0.28	14.89
	Simmental C	-	-	6	-	3	9	0.07	3.83
	Holstein-Black C	-	-	-	3	5	8	0.06	3.40
	Simmental	-	1	1	1	2	5	0.04	2.13
	European Red	-	-	1	-	-	1	0.01	0.43
Hereford (227) (1.81%)	*Hereford C	106	92	25	-	-	223	1.77	98.24
	Aberdeen Angus	4	-	-	-	-	4	0.03	1.76
Holstein-Red (137) (1.09%)	Holstein-Black	-	18	13	25	12	68	0.54	49.64
	*Holstein-Red C	22	5	6	7	8	48	0.38	35.04
	Simmental	-	2	2	2	1	7	0.06	5.11
	Simmental C	-	-	-	-	7	7	0.06	5.11
	Brown Swiss	-	-	1	-	1	2	0.02	1.46
	Belgium Blue C	-	-	-	-	1	1	0.01	0.73
	European Red	-	-	-	1	-	1	0.01	0.73
	Montbeliarde C	-	-	-	-	1	1	0.01	0.73
	Norwegian Red C	-	-	-	-	1	1	0.01	0.73

Genotype of crossbreed's gender		Years						Frequency (%)	
Male ("n" of total group)	Female	2017	2018	2019	2020	2021	Total	in total (12572)	in group "n" of male
	Red-Angus C	-	-	-	1	-	1	0.01	0.73
Montbeliarde	Holstein-Black	7	14	36	23	17	97	0.77	74.05
(131)	*Montbeliarde C	-	2	3	4	3	12	0.10	9.16
(1.04%)	Simmental C	-	-	2	1	4	7	0.06	5.34
	Simmental	-	1	-	4	1	6	0.05	4.58
	Brown Swiss	-	-	1	2	2	5	0.04	3.82
	European Red	-	-	1	-	1	2	0.02	1.53
	Holstein-Red	-	-	2	-	-	2	0.02	1.53
Limousine	Holstein-Black	-	5	11	8	2	26	0.21	31.33
(83)	Simmental	4	4	1	-	6	15	0.12	18.07
(0.66%)	Brown Swiss	-	1	4	3	3	11	0.09	13.25
	Brown Swiss C	-	-	2	3	5	10	0.08	12.05
	Simmental C	-	-	3	2	4	9	0.07	10.84
	*Limousine C	-	-	1	-	3	4	0.03	4.82
	European Red	-	-	2	1	-	3	0.02	3.61
	Holstein-Black C	-	-	-	-	3	3	0.02	3.61
	Aberdeen-Angus C	-	-	1	-	-	1	0.01	1.20
	Charolais	-	1	-	-	-	1	0.01	1.20
Swedish Red	European Red	17	7	3	3	-	30	0.24	42.86
(70)	Holstein-Black	-	1	10	5	3	19	0.15	27.14
(0.56%)	*Swedish Red C	-	5	1	-	6	12	0.10	17.14
	Brown Swiss C	-	-	-	1	1	2	0.02	2.86
	Holstein-Red C	-	-	-	-	2	2	0.02	2.86
	Simmental C	-	-	2	-	-	2	0.02	2.86
	Brown Swiss	-	1	-	-	-	1	0.01	1.43
	Holstein-Red	-	-	1	-	-	1	0.01	1.43
	Simmental	-	-	-	-	1	1	0.01	1.43
Norwegian Red	Holstein-Black	-	8	12	4	1	25	0.20	50.00
(50)	European Red	-	1	4	2	1	8	0.06	16.00
(0.40%)	Swedish Red C	-	3	2	1	-	6	0.05	12.00
	Simmental	-	-	1	2	-	3	0.02	6.00
	Brown Swiss	-	-	2	-	-	2	0.02	4.00
	Holstein-Red C	-	-	-	-	2	2	0.02	4.00
	*Norwegian Red C	-	-	-	-	2	2	0.02	4.00
	Brown Swiss C	-	-	-	-	1	1	0.01	2.00
	Holstein-Red	-	1	-	-	-	1	0.01	2.00
Normande	Holstein-Black	-	1	8	7	9	25	0.20	69.44
(36)	Brown Swiss	-	-	2	-	2	4	0.03	11.11
(0.29%)	Simmental C	-	-	-	1	3	4	0.03	11.11
	European Red	-	-	-	1	-	1	0.01	2.78
	*Normande C	-	-	-	-	1	1	0.01	2.78
	Simmental	-	-	-	1	-	1	0.01	2.78

Genotype of crossbreed's gender		Years						Frequency (%)	
Male ("n" of total group)	Female	2017	2018	2019	2020	2021	Total	in total (12572)	in group "n" of male
European-Red (9) (0.07%)	Holstein-Black	-	-	-	1	3	4	0.03	44.44
	*European Red C	-	-	-	-	2	2	0.02	22.22
	Swedish Red C	-	-	-	1	1	2	0.02	22.22
	Limousine C	-	-	-	-	1	1	0.01	11.11
Shorthorn (6) (0.05%)	Holstein-Black	-	-	1	1	1	3	0.02	50.00
	Simmental	-	1	-	-	1	2	0.02	33.33
	Brown Swiss	-	-	-	-	1	1	0.01	16.67
Red-Angus (6) (0.05%)	Brown Swiss	-	-	3	-	-	3	0.02	50.00
	Brown Swiss C	-	-	-	2	-	2	0.02	33.33
	Holstein-Black	-	1	-	-	-	1	0.01	16.67
Parthenaise (5 - 0.04%)	Holstein-Black	-	1	-	2	1	4	0.03	80.00
	Brown Swiss	-	-	-	1	-	1	0.01	20.00
Angler (2 - 0.02%)	Holstein-Black	-	-	-	-	1	1	0.01	50.00
	Limousine	-	-	1	-	-	1	0.01	50.00
Piedmontosa (1-0.01%)	Brown Swiss	-	-	-	-	1	1	0.01	100.00
Total		1537	2403	3189	2542	2901	12572		

C: Crossbreed, * Backcross group of male genotype

When Table 7 was examined, it was observed that most cross-breeds were obtained by using the Simmental (6893) bulls. Simmental crosses were a breed in which females from other breeds were mainly used (19 breeds), beside of its numerical majority. Among the cross-breeds of the Simmental breed, Holstein-Black (33.81%) and their crosses took the first place, while followed by the Simmental own cross-breeds (*i.e.*, backcrossed to the Simmental breed) and the Brown Swiss and their crosses (2.41%). Crosses from the other 14 genotypes were not in significant numbers among the total Simmental crosses.

Interestingly, backcrosses of Brown Swiss (in total 2257 crosses) and Holstein-Black (in total 1376 crosses) had the highest rate (46.79% and 76.02%, respectively). It has been determined that the Holstein-Black breed, which is grown in the region and in the Edirne province, was not really preferred in the production of crossed-use animals.

It has been observed that the crosses of Aberdeen-Angus cattle, which is a well-known and popular

beef cattle breed, consist of crosses with the Abendenshire breed at a large rate by 59.15%. In addition, it was determined that Holstein-Black crosses, one of the main breeder breeds, took the second place by 28.57%. Females of the Holstein-Black breed were combined with the bulls from meat breeds such as Belgium Blue, Charolais and Montbeliarde, and hybrids were bred. Here, some meat breeds such as Limousine, Swedish Red, Norwegian Red and Normande were also used, albeit in small numbers, in crosses with females, mainly Holstein-Black. In addition, it was determined that crosses of some meat breeds (Aberdeen-Angus, Belgium Blue, Charolais, Hereford, Montbeliarde, Limousine, etc.) were combined with the same breed to form backcrosses and be used in meat production.

DISCUSSION AND CONCLUSION

In this study, the distribution of slaughtered cattle in the Edirne slaughterhouse according to their breed was investigated. Edirne slaughterhouse is the largest slaughterhouse among the five

slaughterhouses in the province. Holstein-Black was identified as the most prevalent of slaughtered cattle breeds, followed by the Simmental and Brown Swiss breeds and crosses of these breeds. Identified cattle breeds predominantly slaughtered emphasize that farmers prefer to use breeds of both milk and meat types. However, when farmers do not need breeding animals or when the meat prices are higher comparing to milk prices, meat-type sires are preferred in order to increase farm profit in the short term. This fact may be the reason of various genotypes that were identified in this study.

It was expected that the Holstein-Black breed is used as female material for breeding because it is the most abundant breed in the region. However, Simmental breed was the most used breed as a sire line in the five-year period studied. Simmental is specialized in beef-dairy and has improved dairy traits in the last years. The data show that the Simmental breed is preferred not only for the cattle raised in Edirne, but also for the cross-breeds brought for slaughter by import. In this study, genotypes were classified according to the sire line in the analysis of breed distribution. If the same data had been classified according to the breed of the cow line, it could be more clearly seen which cow breed crossed with which sire breed.

In this study, beside of the well-known meat and dairy specialised breeds, we have identified twelve genotypes (*i.e.* European Red crosses, Red-Angus crosses, Shorthorn crosses, Blonde d'Aquitaine, Norwegian Red, Parthenaise crosses, Angler, Angler crosses, Aubrac, Normande, Piedmontese crosses, and Salers) which were reportedly slaughtered in numbers below ten. In fact, during the study period, only one animal from some genotypes (*i.e.* Aubrac, Normande, Piedmontese cross and Salers) was reported to be slaughtered. Smallholders in developing countries have often failed to follow recommendations from veterinarians and breeding associations, when motivated by other extrinsic factors such as physical appearance of the cow (large mature size dairy cows), temperament, advice from the neighbour, and/or marketing opportunities (Bebe

et al., 2003; Desta et al., 2010; Traore et al., 2017). In this study, observation of some genotypes only once during the five-year period could also be explained by the geographical condition of Edirne, which is a border city, therefore the import could be preferred by some farmers. It should be noted that upon importing live animals, the following processes should be carefully carried out. For instance, for some time, the replacement of the earrings of imported animals with local earrings made it difficult to follow up the breed, causing an irrational existence of various breeds in the national database. For this reason, abandoned was to change the earrings afterwards, which is a correct practice.

When the currently existing cattle breeds in Edirne are examined, it is seen that almost all the breeds brought to be slaughtered by import had been used by farmers for breeding or fattening. On the other hand, twelve breeds which were not observed in our data, *i.e.* Ayrshire cross, Gray Breed cross, Domestic Black, Chianina crosses, etc. (total 192 cattle) were found. However, they have no numerical significance (HBS, 2023).

Another interesting issue is the presence of backcrosses in some breeds, including breeds that are in minority. This may be due to the female material remaining in the farm after a cross is not sent to slaughter but is used for breeding and obtaining crossbreeds of the same breed. In this case, it is necessary to question why the intermediate breed is used. In fact, the official registration of crossbreeding with different breeds is important for the protection of breed characteristics, and this issue is under control by law. Regardless of the chosen breeding programme, farmers should plan for several generations, not just a few years ahead and should be aware of the negative circumstances of productivity and profitability if the breeding programmes are not fully contemplated before implementation (Garcia et al. 2019).

When the imported cattle breeds are examined, it will be observed they are also obtained through different crossbreeding. Generally, variability between the breeds is higher than between

individuals in a breed. All breeds have strengths in terms of some economically important traits, but there is no breed showing excellence for all traits (Weber, 2008). A crossbreeding programme is helpful to capitalize on the traits that each of the parent brings to crosses. It is essential to consult veterinarians when performing crossbreeding to maximize hybrid vigor (Garcia et al. 2019).

The existence of a large number of animals of different breeds shows that the breeders are trying to produce different solutions in order to increase their income from meat. It should be kept in mind that the effect of genotype on yields is smaller than the effect of environmental conditions. Improving environmental conditions or providing optimum conditions will ensure high yields from breeds with high genetic potential (Ariturk and Yalcin, 1966; Evrim and Gunes, 2000).

When the distribution of slaughtered cattle in the Edirne slaughterhouse according to breeds was examined, it was observed that Holstein-Black, Simmental and Brown Swiss breeds and their crosses were used as majority. In addition, it was observed that some of the imported cattle breeds for slaughter were continued to be bred. It is known that these crosses are made to benefit from the slaughter characteristics by using hybrid vigor. Here, it should be separately evaluated which animals are slaughtered and what proportions are available for slaughter (either by fattening or directly), and the level of productivity of the hybrid females groups. To increase the income of the farms, it should be determined what benefit will be gained by using

many breeds as the female material. It is important to compare the performances of cattle imported for slaughter and those slaughtered after being imported and fattened.

As a result, in a five-year period, the high number of breeds or genotypes shows that the farmers may have fattened various breeds without a proper breeding programme to improve their profitability. However, improper implementation of a breeding programme may cause herds to lose their uniformity and efficiency and the extinction of local breeds. Before applying a breeding programme, farmers should consider their available sources (i.e. labour, feed, pasture, financial) and optimum herd size. We suggest policy makers to determine and motivate farmers to apply for a proper breeding and/or crossbreeding programme by considering the expectations and available resources of the farmers to prevent uncontrolled breeding.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – H.G.; Design – H.G.; Supervision – H.G.; Resources –S.A.; Materials – S.A.; Data Collection and/or Processing –S.A.; H.G.; Analysis and/or Interpretation – H.G., N.O.; Literature Search – S.A., N.O.; Writing Manuscript – S.A.; Critical Review – H.G., N.O.

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SAŽETAK

Cilj istraživanja je odrediti distribuciju stočnih vrsta u klaonici u Edirneu u periodu od 2017. do 2021. godine. Kao materijal za istraživanje su korišteni podaci iz klaonice koji su obuhvatili ukupno 49.473 zaklanih grla. Grla su prvenstveno podijeljena na čistokrvna i križana, nakon čega su određeni podgenotipovi križanih grla.

Uključeno je 21 čistokrvno i 19 križanih grla prema identificiranim genotipovima. Nadalje, određeno je 148 podgenotipova križanaca prema pasmini bikova i ukupno 169 genotipova. Ustanovljeno je da je većina zaklanih grla pripadala pasmini Holstein-Black (53.8%), potom križancima pasmine Simmental (13.9%), punokrvnoj Simmental pasmini (11.2%), križancima smeđeg švicarskog goveda (4.6%), čistokrvnom smeđem švicarskom govedu (4.2%) i križancima Holstein-Black pasmine (2.8%). Među križanim pasminama, najbrojniji su bili križanci pasmine Simmental (54.8%). Slijedili su ih križanci smeđeg švicarskog goveda (18.0%) te pasmina Holstein-Black (10.9%), Aberdeen-Angus (6.0%) i belgijskog plavog goveda (2.4%).

Iz rezultata je vidljivo da, osim stoke koja se uglavnom uzgaja u regiji i ostale pasmine koje se koriste za proizvodnju mesa doprinose identifikaciji pasmina koje uzgajivači najviše vole. Određivanje velikog broja uzgajanih vrsta ili genotipova znači da su uzgajivači mogli udebljati različite vrste bez odgovarajućeg uzgojnog programa. Nepravilna provedba uzgojnog programa može uzrokovati gubitak uniformnosti i efikasnosti stada. Naš prijedlog kreatorima politika jeste odabir odgovarajućeg uzgojnog programa.

Ključne riječi: Goveda, križanci, pasmine, trupovi, učestalost



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Review Articles may be commissioned or proposed. Authors wishing to submit a review article are advised to contact the Editor. In general, reviews are written in support of original research. They may cover any relevant aspect of veterinary science or comparative medicine. The first author of the review article should have at least three published papers from that field. Reviews should follow the layout for Research Article, but with the main text sub-divided as appropriate to the subject matter. Review articles should be no longer than 50 pages, should have an abstract of 300 words at most, should contain a limit of 120 references.

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„This is in agreement with results obtained later (Bregoli et al., 2006).“

References by the same author(s) in the same year: (Michalek et al., 2004a, 2004b)

Multiple references cited in the same sentence: (Knott, 1987; Güneş et al., 2002; Jones et al., 2004)

Journal articles:

Journal names in the reference list should be written in accordance with the Journal Title Abbreviations list available at:

https://images.webofknowledge.com/images/help/WOS/A_abrvjt.html

Harvey N, Kingsley M, Terek F. 2019. Anatomical aspect of the red fox lung. Veterinaria, 64(3), 20-5. Include the doi number if one exists

When more than a six authors:

Catanese G, Grau A, Valencia JM, Garcia-March JR, Vázquez-Luis M, Alvarez E, et al. 2018. Haplosporidium pinnae sp. nov., a haplosporidian parasite associated with mass mortalities of the fan mussel, Pinna nobilis in the Western Mediterranean Sea. J Invertebr Pathol, 157, 9-24.

Books:

Brown C, Laland K, Krause J. 2011. Fish Cognition and Behavior. 2nd ed. Oxford, UK: Wiley-Blackwell.

Chapters in books:

King AS. 1993. Apparatus urogenitalis. In Baumel JJ, King AS, Breazile JE, et al (Eds), Handbook of Avian Anatomy: Nomina Anatomica Avium

(2nd ed., pp. 329-90). Cambridge, USA: Nuttall Ornithological Club.

Conference proceedings:

Bregoli M, Dediassi K, Pasolli C. 2006. Antibiotic resistant *E. Coli* in free-ranging alpine wild ruminants, in: VII Conference of the European Section of the Wildlife Diseases Association. p. 78.

Web Adresses:

Papazoglou LG, Basdani E. 2015. Exploratory laparotomy in the dog and cat. <https://www.cliniciansbrief.com/article/exploratory-laparotomy-dog-cat> (accessed 4.18.17).

Theses:

Donker SA. 2010. Arctic ground squirrels in the Southwest Yukon Territory: evidence for habitat specific demography and source-sink dynamics. MSc, University of British Columbia, Vancouver, Canada.

Watts AJR. 2012. Nutritional status and trophic dynamics of the Norway lobster *Nephrops norvegicus* (L.). PhD, University of Glasgow, Glasgow, UK.

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