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*Corresponding Author: Tegegn Dilbato
E-mail: dilbatot@gmail.com

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Parasitary infestation in three tiger cubs

Case Report
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Burçak Özkan*

Kadıköy, Istanbul, Turkey

ABSTRACT

Being an apex predator, the tiger is known as an indicator of healthy ecosystems. This animal's crucial role causes a significant decrease in the population, in case death occurs the ecosystem devastation also occurs. This highlights the importance of this species' protection. Apart from hunting, traps, road causalities, starvation and unidentified reasons, diseases, mostly parasites are one of the most significant reason of these animals' death. There are different researches explaining the most important and frequent parasites detected in tigers. *Toxocara* spp. is one of them. This case report explains the treatment period of three tiger cubs suffering from *Toxocara* spp. One female cub and a male cub revealed gastrointestinal signs. Anemia accompanied these signs in male cub. The third cub was asymptomatic. Apart from supportive treatment, pyrantel pamoate was administrated to all cubs. The treatment was judged as successful. The therapy procedure and preventive measurements were evaluated.

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Introduction

Most of the feline species are between endangered ones because of their decreasing habitats due to anthropogenic effects. As a result, loss of several individuals may worsen the species' extinction (Natalia et al., 2017). It is documented that only 3500 wild tigers survive in very small isolated populations which are prone to extinction (Walston et al., 2010). The dramatic diminution in tiger population highlights that there is an urgent necessity to recognize, prevent and treat big cats' diseases.

A better knowledge about parasitary infestations will help to protect these rare and precious animals (Natalia et al., 2017). Captive carnivores suffer a wide array of parasitic infestations; which reflects the diversity of seven different carnivore families. Yet, there is only few parasitic infestations known to cause diseases in carnivores and these may be prevented and cured with appropriate husbandry, precautions and therapy (Williams and Thorne, 1996). In contrary, felids live in large areas in their natural habitat; which makes them to face lesser parasitic agents and consequently having low resistance against parasitary infections. In addition to this, captive lifestyle facilitates the susceptibility to infectious diseases (Raja et al., 2014).

Krone et al. (2008), explicate that reports about parasitary infestation spectrum on wild cats are seldom and generally based on very small groups. Their research exhibited that 8 endoparasite species infested wild cats; those species being listed as *Toxocara mystax*, *Toxascaris leonina*, *Petrowospirura petrowi, Capillaria aerophila*, *Capillaria plica*, *Capillaria feliscati*, *Taenia taeniaeformis*, *Mesocestoides litteratus* (Krone et al., 2008).

In general, data concerning parasites in tigers are obtained from various researches realized in zoos.

*Corresponding Author: Burçak Özkan
E-mail: burcakozkan1974@yahoo.com

Literature explains 25 helminth species in tigers. Protozoan parasites causing infection in tigers are listed in various reports, the pathogens being identified such as *Trypanosoma evansi*, *Babesia* species, *Isospora felis*. Tigers are frequently infected with *Trypanosoma evansi* and symptoms include anorexia, pyrexia, panting, occasional convulsions and sudden death (Moudgil et al., 2015).

Reports explicate that the most commonly encountered trematode infection in free-living and captive wild felids is paragonimiasis. Royal Bengal Tigers were reported to be infected with *Paragonimus westermani* (Moudgil et al., 2015). Sano et al. (1994) recovered Paragonimus eggs from four tigers and they explicated these eggs differed from the eggs of *Paragonimus westermani*; which raised a debate about whether a new species of Paragonimus was identified (Sano et al., 1994).

Reports explain that domestic and wild species interaction plays an important role in spreading of infectious disease (Furtado and Filoni, 2008). On the other hand, the parasitary infestation rate (93 %) found for wild cats was higher than the same percentage existed for domestic cats. The difference was related to the anthelmintic prophylaxis provided by the owners of domestic cats. In addition, feeding exclusively on wild prey augments the probability of being infested by parasites. Thus the higher parasite burden and diversity in wild cats are possibly related to wider potential intermediate host diversity in wild cats' diet than in domestic cats (Krone et al., 2008).

Natalia et al.'s research shows that parasite (helminth) diversity in close-related species may differ according to habitat use and climatic properties. In addition to climate characteristics, snow may cover and protect parasite eggs form the cold. Another important factor is that spatial and social organization of the felid species; those who have a low frequency of contact with the others influence the parasitic infestation. In conclusion, species-specific differences in parasites (helminths) were possibly related to the species' evolution in different habitats (Natalia et al., 2017).

An interesting finding explicit sex-specific differences between parasite diversity exist in wild cats. Reasons why male-biased parasitism occurs are hypothesized as either by ecologic or physiologic factors. These causes may be endocrine system, immune system, behavioral, territorial, movement, social or diet related. The association of testosterone and the immune system may cause a higher susceptibility to parasitary infestations in sexually matured males in wildlife (Krone et al., 2008).

The burden of parasites depends on the length of time since the animals were taken from wildlife, how they are adjusted to captivity, quality of life in captivity, the proximity to other species with which they could interchange parasitary agents. Sources of infectious agents for captive carnivores include individuals of same or different species, humans, food, water, fomites and iatrogenic introduction (Williams and Thorne, 1996).

Endoparasitism results in emaciation, weakness, inappetence and produces predisposition to other secondary illnesses. The more parasitary infestations become a significant reason of losses, the more it's high time to organize detailed researches about the subject (Nimisha et al., 2017).

The most important part of fighting against wildlife diseases is to detect the early stages of a new disease; which is hard to realize since wild animals are very difficult to be found when died. This is what makes difficult to get the appropriate biological material for epidemiologic studies. In general, most of the studies concerning wildlife include either case reports or cross-sectional serologic surveys which form when reunited, a fragmentary data. Comparison between different data is problematic because of the different lab methods used and the pathogen selection which is generally arbitrary, opportunistic or determinate by the foundation. Thus, correlation between the infectious agent and the development of the disease necessities further information. This wide information consists of clinical examinations, necropsies and histopathologic evaluations (Furtado and Filoni, 2008).

Case

Clinical examination and findings

Three six weeks old tiger cubs of a circus, two of which were females and the other one was a male, were brought to the internal medicine clinic as soon as some complaints had started. The cubs were staying with their mother in captivity.

Upon arrival at the clinic, complete physical exam of each cub were performed. The examination started with rectal body temperature measurement. All animals' temperatures, heart rates and respiration rates were compared to domestic cats' reference values (Kahn and Line, 2005; Reilly et al., 2014). All of these values remained between normal intervals and didn't show any anomaly during the treatment.

External examination of eyes, ears, pelage, feet and claws followed by examination of oral cavity,

pharynx, gingiva pursued this first step. Lymph nodes and thoracic auscultation were also realized. No pathologic finding was found.

The first female cub was reported to have a slight diarrhoea. No anomaly except a slight abdominal sensitivity was observed. The second female cub was asymptomatic except smooth stool formation. The male cub was reported to have vomited twice. A slight diarrhoea also had been observed. Exam revealed a slight abdominal distension accompanied with mild abdominal sensitivity. Body weights were recorded for each cub. The first female weighed 3.8 kg., the second one weighed 4.3 kg. and the male's weight was 5.4 kg.

It is well known that V. cephalica saphena, V. jugularis and V. femoralis is used in order to collect blood in cats, dogs and non human primates. But in tigers, these areas are inconvenient since the blood collector must stand in front of the tiger; thus V. caudalis is safer and convenient when blood must be taken in a tiger (Shrivastav et al., 2011). Blood may also be taken from dorsal coccygeal vein (Sajjad et al., 2012). Yet, in this case, jugular venous blood samples for haemogram could be obtained as similar to one literature (Reilly et al., 2014). Blood samples were collected on the first day, at the end of first treatment period and on the last day of the second treatment period. The blood samples collected on the day the cubs had been brought to the hospital showed that only a mild anemia were present for the male cub. The abnormal values ameliorated as the therapy went on. Other cubs' haemogram values remained within normal intervals from the beginning to the end of the therapy.

Gaita samples for parasitologic examination were also collected. To maintain normal body temperature, the cubs were maintained in a warm, dry and clean environment as required during treatment in hospital.

Diagnosis and Treatment

Toxocara spp. was detected in first female and male cubs' stool samples. The gaita exam of the second female was negative. Dehydration was checked out for each cub by pulling up on the skin on the back of the neck for checking out whether the skin does retract immediately or stays suspended.

None of the cubs exhibited dehydration. On the other hand, the first female cub and the male cub were supported with fluidotherapy since the first had a slight diarrhoea and the second had vomited twice apart from having had a slight diarrhoea. IV catheter was placed in medial saphenous vein for fluid administration so that fluid losses that may occur with ongoing diarrhoea and vomiting should be prevented. Lactaded Ringer's solution were given (Hoskins, 2001; Reilly et al., 2014).

The male cub had been deprived of both food and water for 24 hours so that mucosal integrity restoration and more rapid return of gastrointestinal function realize (Hoskins, 2001). The vomitus discontinued on the second day and didn't reoccured.

The fluidotherapy was discontinued on the 3th. day as the cubs' diarrhoea turned into normal stool and the vomitus of the male cub had already ceased (on the second day) and normal feeding could be started by increasing gradually the amount of food and water (Hoskins, 2001).

Pyrantel pamoate (Kontil^{TM}; Hüsnü Arsan) was administrated 5 mg/kg. PO. for 5 consecutive days as suggested in literature (3-5 mg/kg. PO for 3-5 consecutive days) in all cubs. For the male cub, the administration of the drug started when vomitus ceased. Moreover as recommended in literature, follow-up treatments with the aim of remove larval stages was realized. The treatment was repeated 7 days later as recommended for domestic cats (Kahn and Line , 2005).

In order to support immune system which might be weakened due to parasitary infestation, to help prevent secondary infections and to combat anemia and weakness, vitamin B complex (0.5ml/day IM) (Dodex^{TM}; Vetaş) support was supplied during the first treatment period as agreed with Katona and Katona-Apte, (2008).

At the end of both first and second treatment periods, gaita samples were re-collected and re-examined. No parasites were found. The therapy was judges as successful.

Table1. Hemogram values of the male cub represented with abnormalities and amelioration during the treatment

Parameters	First day	The end of first treatment period	Last day of the treatment period
RBC (X 10^6 /µL)	3.76	5.86	7.38
PCV (%)	21	28	32

Discussion

Gastrointestinal parasites recorded in tigers in a research are listed such as *Paragonimus westermani* (41.6 %), *Diphyllobothrium latum* (55 %), *Strongyle* sp. (6.6 %), *Taenia* sp. (10 %), *Trichuris* sp. (3.33 %), *Toxocara* sp. (3.33 %) and *Nematode* larvae (3.33 %) respectively (Arjun et al., 2017). Ascarididae nematodes, *T. canis*, *T. cati* and *T. leonina* are very important parasites of canids and felids. The large roundworms of dogs and cats, especially in kittens and puppies are common. Between *T. canis*, *T. cati* and *T. leonina*, the most important ones are *T. cati* and *T. canis* be cause they cause larvae migration in humans and *T. canis* may be lethal. Definitive hosts for *T. canis* are canidae and rarely feline species, definitive hosts for *T. cati* are felidae including tigers. In felids, co-occurence of Both *T. cati* and *T. leonina* are detected. This co-occurrence seen both in domestic and wild felids is variable depending on climate, environmental conditions, age of the host and the season (Kahn and Line, 2005; Okulewicz et al., 2012). Roundworms may be acquired via four ways known as transplacental migration, mil-borne transmission, infective egg ingestion, paratenic or intermediate host ingestion (Leib and Monroe, 1997; Oculewicz et al., 2012).

In a survey conducted in a national park, three zoo parks and a circus, 31.25 % of the tigers were found to be parasitary infested. The tigers were kept in captivity only in one zoo, in the park and in the circus. The incidence of parasitary infestations were respectively 0 %, 33.33 % and 50 %. The high incidence observed in captivity was related to pasture contamination and close association of animals in captivity (Kashid et al., 2002). In captivity, the health status of the animals depends on feeding, keeping conditions, animal management, and environmental conditions including humidity and temperature. The staff may also transmit the infections through their shoes, clothes, hands, foods or working tools. Another transmission route is the animals themselves when being transmitted from one area to another; mixing different species brings additional infection risk (Atanaskova et al., 2011). In this case, all three cubs were living in a circus and in captivity with different animal species and with a huge amount of people. Thus, there were a multifactorial situation which may have influenced the contamination. Captive lifestyle makes it easy to get parasitary infestation in wild felids (Raja et al., 2014). First of all, the circus is mobile and keeps visiting various geographic areas with various animal and human populations and various food and water sources. The species-species interactions might as well have played an important role in the cubs' infestation. The staff and all the tools around also must be taken into consideration.

Roundworm infection should be suspected in all puppies and kittens (Leib and Monroe, 1997). In cats and dogs, infection is diagnosed by detection of eggs in feces (Kahn and Line, 2005).

In this case, three cubs' scatologic analyses revealed *Toxocara* spp. The disease was managed to be detected in early stage. This result was confirmed by the literature revealing *Toxocara* spp. as one of the most often detected parasitary agents in both free and captive wild felids (Krone et al., 2008; Marathe et al., 2002; Nimisha et al., 2017; Okulewicz et al., 2012). The negative result of fecal examination doesn't proof the absence of a parasitary infestation. The most important part of fighting against wildlife diseases is to detect the early stages. Parasites are the most frequent reason why gastrointestinal diseases occur. Parasitary reason must be taken into consideration in puppies and kittens. This is why it is very important for the staff to observe even a little change in animals' routine. In this case, three cubs were brought for treatment as soon as the signs had been noticed (Furtado and Filoni, 2008; Hoskins, 2001, Leib and Monroe, 1997).

Scatology is a non-invasive, easy and cheap way to gain information about parasitary infestations of free-ranging wildlife's individuals. Wild animals' faeces being the most significant sign of their presence, the faeces of carnivores contain anal glands secretion which represents specific properties in every species. The gaita produced by each individual varies with age, ingested food, this individual's absorption capacity and health situation. But, due to the difficulty to study parasite dynamics of wildlife, little information exists about the subject (Gorman and Trowbridge, 1989; Marathe et al., 2002; Chame, 2003; Arjun et al., 2017). In this case gaita analysis provided a fast, non invasive and reliable diagnosis method.

Wild felids may hide clinical signs of illness until the disease becomes severe. This is why it is vital that the staff be astute to subtle behavioural and physiologic changes suggesting any pathology. Any change in appetite, urination, defecation, hair coat or mucous membranes the dryness of which shows dehydration, breathing pattern or general behaviour must be carefully observed.

Many infected animals suffering this parasitic infestation remain asymptomatic. The first indications in young animals are lack of growth and loss of

Table 2. Normal values of the RBC and HCT represented with abnormalities in the male cub (In tigers)

Parameters	Duncan and Prasse, (1986)	Shrivastav and Singh, (2012)	Macree and Ramsay, (2013)	Sajjad et al., (2012) (Lahore Zoo)	Sajjad et al., (2012) (Lahore Wildlife Park)
RBC (X 10^6/µL)	5-10	4.66 - 9.15	--	9.60 - 11.0	7.64-12
PCV (%)	--	36—45	31.8 - 49.2	59.6 - 65.46	46.46 - 66.23

condition. Vomiting, diarrhoea, abdominal pain, pot-bellied appearance and failure to thrive, showing a dull coat are most common signs. In the early stages of the disease larvae migration may cause eosinophilic pneumonia which may be associated with cough. Cough, fever, mucoprulent nasal discharge and respiratory distress can accompany extensive larvae migration. Mucous containing diarrhoea may be observed. Worms are either vomited or seen in the feces. Cortical kidney granulomas containing larvae also are noted. Intestinal obstruction may realize occasionally. Heavy infestations may lead to stillbirth or pneumonia in newborns. These severe infestations result in verminous pneumonia, ascites, fatty liver and mucoid enteritis. *Toxascaris leonina* differs from the others; severe clinical signs may be detected in prepatent infections; thus diagnosis must be based on anamnesis, signalement and physical examination. Eosinophilia may also be present (Leib and Monroe, 1997; Kahn and Line, 2005).

In this case one female cub was asymptomatic while the other two were representing the signs that could have been detected in early stages. All were in concordance with the literature.

There are many well-known anthelmintic used against roundworms. In cats, drugs licensed for the disease's treatment are listed as dichlorvos, diethylcarbamazine, fenbendazole, flubendazole, mebendazole, piperazine, pyrantel, selamectin and the combination of praziquantel/pyrantel. Despite not being approved in cats, pyrantel pamoate use (20 mg/kg.) is safe and efficacious. Heartworm prevention provided by milbemycin or selamectin is also effective to control ascariasis in cats. Kittens can be treated several times at 2- to 3- week intervals up to 3 to 4 months of age. In cats piperazine should be used as 55 -62 mg/kg. PO. The patient should be re-treated 10 days after first cure. Another cure choice is to use piperazine hydrate, 80-100 mg/kg. PO. Praziquantel may also be used as 5 mg/kg. PO. or 5.68 mg/kg. SC, IM. A pyrantel dosage of 20 mg./kg. PO is another choice. The treatment must be repeated 7-10 days later. Another dosing schedule may be organized as 5 -10 mg./kg. PO and re-treatment 2 weeks later is necessary (Kahn and Line, 2005). The prognosis for dogs and cats are excellent. Yet, mixed infections with other parasites (especially with hookworms) or viral infections may worsen the situation. On the other hand, dealing with human infection is a serious challenge since environmental contamination is generally occured by the time puppies or kittens are first presented to vet (Leib and Monroe, 1997).

In tigers the most often identified species are Ascarididae and Strongyloidae (*Toxocara*, *Toxascaris* and *Ancylostoma* spp.). A total parasitary elimination in tigers is barely possible. Yet, the parasites may be controlled with the help of periodic oral anthelmintic administration.

Anthelmintic use is found to be more effective when full recommended dose is administrated more than one day. A three consecutive days' treatment schedule instead of adopting a single day treatment is suggested. Follow-up treatments with the aim of remove larval stages is also recommended. Post treatment fecal exams are necessary in order to evaluate the efficacy of initial cure. All animals' feces must have two follow-up exams at weekly intervals 1-2 weeks post therapy. Routine monthly heartworm prevention treatment is suggested year-round. A fecal exam for all animals must be provided every six months. In this case the staff were warned about follow-up treatments, routine prophylactic antparasitic administrations, lifestyle conditions and hygiene necessities.

Table 3. Normal values of the haemogram parameters represented with abnormalities in the male cub (In domestic cat).

Parameters	Kahn and Line (2005)	Sodikoff, (2001)
RBC (X 10^6/µL)	5-10	--
PCV (%)	30-45 24-34[a]	26-45[b] 30-45[c] 20-25[d] 15-19[e] 10-14[f]

a: 5-to 6-wk-old kittens. b: < 6 Months. c: Adults, d:Mild anemia, e: Moderate anemia, f: Severe anemia

The effective and safe anthelmintics for tigers are listed such as; febantel (6 mg/kg. PO, once a day for 3 days; re-treatment in 2 weeks); fenbendazole (5-10 mg/kg. PO; 3 consecutive days); ivermectin (0.2 mg/kg. SC/PO) (an injectable cattle dose may be used orally at this dose for 1-3 days. Limited use is suggested in tigers via parenteral route); praziquantel (5.5-6.6 mg/kg.; either PO or parenteral form for cestodes); pyrantel pamoate (3-5 mg/kg. PO for 3-5 consecutive days); sulfadimethoxine (50 mg/kg. PO or parenteral for coccidositat). Pyrantel pamoate administration was useful in this case.

It is also important to take into consideration that most endoparasites detected in tigers are relatively common and ubiquitous in ex situ conditions (Bush, et al., 1987). Not all larvae or eggs found in fecal examination may be parasitic to the tigers. Be cause tigers serve as a transport host depending on what they had been fed with; which highlights the importance of the diet. Yet in this case, Toxocara known to be pathologic and ongoing with gastrointestinal symptoms was successfully treated.

Parasites alter their hosts' survival and reproduction either directly by pathological influences (blood loss, tissue damage, spontaneous abortion, congenital malformations, death) and indirectly by diminishing the host's immune system and affecting the physical situation. In addition, some parasites are zoonotic (Thawait et al., 2014). In this case no symptoms had occurred except some mild gastrointestinal signs in two cubs and mild anemia in one. This was probably because the disease had been detected in an early stage.

Since pathogens alter normal physiology, bloodwork interpretation is a useful tool for health evaluation (Shrivastav and Singh, 2012). Although data about haematological and biochemical parameters of wild animals is meagre, some explications about blood values of captive tigers was published (Sajjad et al., 2012). Domestic cat hemogram reference values also can be used in order to evaluate tiger clinical pathology (McRee and Ramsay, 2013).

Though CBC alone is never accepted to confirm totally the presence or the absence of any infection or infestation, it is a useful tool in order to identify whether the nature of the disease is bacterial, viral, fungal or parasitic (Khan et al., 2015). The changes noted in various haemogram findings may be useful in diagnosing the disease and in evaluating the prognosis (Sajjad et al., 2012). In this case only a mild anemia was noted in one female cub. The mild anemia was thought to be related to the infestation and could be normalized with appropriate therapy. WBC measurements which remained always within normal intervals didn't show any secondary infection.

The therapeutic management of parasitic infections in captive wild felids mainly consists of drug application targeting the specific parasite (Sur et al. 2001). But environmentally resistant larvated eggs form the main problem since they are the main infection reservoirs. The ways how to reduce perinatal transmission or minimize egg output are listed (Kahn and Line, 2005). Especially in unnatural environments such as zoos, the transmission of *Toxocara* spp. and *Toxascaris* occur inter alia by rodents. Moreover, the infections may be persistent. Although the initial treatment leads to an elimination, the parasite is re-detected after two months. A report explicates *T. leonina* persistence in tigers both in autumn-winter and spring-summer periods while another one reveals a re-infection occurred in lions after 30 days of treatment in a zoo. *Toxocara* spp. and *T. leonina* elimination from zoos is very difficult (Oculewicz et al., 2012). In this case the appropriate anthelmintic deworming provided an effective cure. The follow-up scatologic exams were negative. Yet, as suggested by the literature and as agreed with it, it was recommended to the staff to regularly realize parasitary prophylaxis and gaita exams for all species living in the circus. Or as a better choice, to take the wild species to wildlife, at least to a zoo park where they belong.

Infections be cause they may enter adjacent territories and either eat anything found or come in contact with domestic species from which they take infectious agents (Shirbhate, 2007).

As an apex predator, the tiger is accepted as an indicator of healthy ecosystems. Thus a significant decrease in tiger population will devastate ecosystem. This is why their protection is very important. Be cause of being an apex predator the parasitary diversity and load in tiger is likely to be higher compared to the other higher vertebrates (Arjun et al., 2017).

Zoo gardens exhibit wild animals for various reasons such as aesthetic, educational and conservation purposes. Zoos, sanctuaries and wildlife parks, the main of which are protecting endangered species also serve as a seat of education, research and recreation. But human-made environments are not suitable for wild animals to carry out their instinctive behaviours and they hence result in physiologic and homeostatic alterations (Sajjad et al., 2012). Especially helminth infestations play a major role in parasitary

diseases and they may even result in death. In their natural wild habitat the animals may gain a natural resistance against parasites or live in balance with their parasites while in captivity, the altered environmental conditions damage the animals' ecology and may increase their sensitivity against parasitary infestations. The wildlife which is well adapted to parasites lacks in adapting to adverse effects of parasitism (Goossensa et al., 2005; Borghare et al., 2009; Thawait et al., 2014). Wild animals living under natural conditions have evolved to develop resistance to parasitic diseases in a way that parasites co-evolved with their host and wouldn't cause much overt diseases (Gairola, 1986). Apart from playing a major role in population regulation, parasites have other powerful effects such as parasite mediated host competition, sex and sexual selection, social behaviour including xenophobia and sexual fidelity, foraging strategies and predator prey interactions. When it comes to tiger populations, parasites have a twofold influence. First of all, animals which are deprived of predatory pressure have greater parasite loads. Between all species, tigers show maximum load. Such a huge amount of parasites impacts deeply the health status, behaviours and reproductive success of the individual. Moreover, parasites influence the cub mortality, the most important factor of population growth. Secondly, parasites with an intermediate host as a prey species and with a definitive host as a predator, influence predator-prey dynamics (Marathe et al., 2002).

Detailed epidemiological studies targeting parasites of the felids, regular faecal examinations, improvement of parasite identification techniques and the use of novel methods such as molecular techniques supplemented with post mortem findings when necessary, effective drug use with wide safety margins targeting a specific parasitic species, better management practices such as routine cleaning and disinfection, correct disposal of waste and clean food presentation should be adopted wisely as each one plays an important role in reducing parasitary infections in captive wild animals (Mougil et al., 2015) According to some authors, parasitic diseases are the main reason why wild animals in captivity die (Rao and Acharjyo, 1984). This is why establishing precautions and appropriate treatment protocols against these infestations are of crucial importance (Atanaskova et al., 2011). If parasitic infestations are to be prevented, effective precautions such as appropriate antiparasitic treatment, increased hygiene, good animal and staff management, regular parasitary controls of food and water, quality food, appropriate vitamins and vitamins supplement need to be organized. It is also important to take into consideration that every parasitary treatment may cause stress and increase the possibility of infection (Atanaskova et al., 2011; Borghare et al., 2009).

The best approach that could be adopted would be an interdisciplinary one interconnecting population, geographic, etiologic, pathologic studies. Such a perspective obligates reuniting biologic, clinical, geographic data while monitoring closely the diseases and using the appropriate diagnostic techniques. Adequate worker, laboratory equipment and constant funding are also very important (Furtado and Filoni, 2008).

As a result, wild species must live in their natural habitat, not in captivity for any reason. Appropriate researchers, veterinarians, biology and veterinary education, staff and budget must be provided so that their extinction could be prevented and their health problems could be both prevented and managed.

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Comparative determination of digestibility and energy contents of heliz and parzuk with traditional forages by in vivo and in vitro methods*

Mahmut Ekinci[1], Suphi Deniz[2], Selçuk Altaçlı[2]

1.Project Monitoring and Evaluation Center, Governorship Gürpınar, Van, Turkey
2.Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Yuzuncu Yil University, Van, Turkey.

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ABSTRACT

The aim of this study was to determine digestibility and energy contents of plants heliz (Prangos pabularia) and parzuk (Hippomarathrum microcarpum) with in vivo and in vitro methods and also to compare them to traditional. Seven male Red Karaman hoggets were used in the trial. Digestibilities of four different forages were determined with classical and two-stage digestibility methods. Moreover, energy contents of forages were also estimated using both digestibility values. In situ degradation characteristics of these forages were also evaluated. In the in vivo trial for digestibility, the degrees of digestibility of four different forage plants were determined by ''missing block trial pattern''. In vivo organic matter digestibility (OMD) of parzuk and heliz (75.52% and 73.46%) were higher than those of dried meadow grass (68.94%) and dried alfalfa hays (65.81%), (P<0.05). These difference were reflected in energy contents and DE (digestible energy) (3.33 and 3.24Mcal/kg dry matter (DM)), ME (2.73 and 2.65 Mcal/kg) and NEL (net energy lactation) (1.73 and 1.68 Mcal/kg DM) values of parzuk and heliz were higher than those of meadow grass (3.04, 2.49 and 1.57 Mcal/kg DM) and dried alfalfa (2.90, 2.38 and 1.49 Mcal/kg DM) hays, (P<0.05). In the two-stage digestibility method, OMD values were 71.88, 68.85, 66.99 and 58.52% for parzuk, heliz, meadow grass hay and dried alfalfa hay, respectively (P<0.05) The highest OMD was observed in parzuk with two-stage digestibility method, while OMD of heliz and dried meadow grass were similar to that of parzuk, OMD of dried alfalfa was similar to that of dried meadow grass but less than those of parzuk and heliz (P<0.05). In in situ experiment, degradability of dried alfalfa, parzuk and heliz were rapid after 4 hours incubation but degradability of meadow grass hay were slow. Dry matter digestibility of meadow grass hay, dried alfalfa hay, parzuk and heliz was 72.45, 76.36, 88.36 and 84.21%, respectively at end of 48 hours incubation period (P<0.05). In conclusion, parzuk and heliz grown at highlands, in case of cultivation, these plants can be alternative forages to other high quality forages such as alfalfa hay.

Keywords: dried meadow hay, dried alfalfa, parzuk, heliz, in vivo/in vitro

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Introduction

Prangos pabularia and Hippomarathrum microcarpum, which grow widely in the high plateaus of the East Anatolia Region and are used by the local people to feed their sheep, have a considerable potential in meeting the high-quality forage requirements of livestock in the region. It is important to determine the nutrient contents of these plants, present their consumption by livestock and their availability, and to increase their production by cultivation. Belonging to

*Corresponding Author: Selçuk Altaçlı
E-mail: selcukaltacli@yyu.edu.tr

the family Apiaceae, these plants grow naturally in many parts of the world, including the Balkans, Sicily, the Caucasus and Iran. In Turkey, it grows in high regions at an altitude of 1,000 to 2,800 m, such as Kastamonu, Giresun, Kars, Erzurum, Ağrı, Konya, Kahramanmaraş and Hakkari. It is a perennial plant with a height of 50 to 150 cm (Davis, 1972). In a study conducted in Iran by Eilami and Noroozian (1995), it is reported that the replacement of dried alfalfa, which constitutes 60% of the ration for Karakul lambs, with this plant at a ratio of 12%, 24%, 48% and 60% does not affect the fattening performance and carcass quality. In a study conducted by Coşkun et al. (2005) to find the chemical composition and nutritional value of Prangos ferulacea, the metabolic energy value of the plant is reported to be 12.2 Mj/kg DM. In a study conducted by Önal et al. (2004) to research the effect of Ferula communis on the reproductive functions of hoggets, it was reported that the estrogen content of this plant has a positive impact on the reproductive yield of livestock. Alfalfa is called the queen of the forage plants because it contains many basic and active nutrients. In Turkey, alfalfa is mainly used as a dried fodder in the nutrition of livestock (Çerçi et al., 2011).

The purpose of this study is to determine, through in vivo and in vitro methods, the digestibility and energy contents of P. pabularia and H. microcarpum, which grow widely on the high plateaus of the East Anatolia Region and are used by the local people to feed their sheep, and to compare them with the traditional forage (meadow grass and dried alfalfa) commonly used in the region.

Materials and methods

The dried meadow grass and dried alfalfa used in the trial was procured from Van. H. microcarpum and P. pabularia were obtained from Gevaş, Van and Hakkari. In the in vivo trial for digestibility, the degrees of digestibility of four different forage plants were determined by "missing block trial pattern" (Düzgüneş et al., 1983). Seven male Red Karaman hoggets at one year of age were used in the trial. The DE, ME and NEL contents of the forage were calculated on the basis of the crude nutrient digestibility of the forage (Van ES, 1978; MAFF, 1975; Öğretmen and Kılıç, 1991).

To this end, seven cages were used in the experiment that were produced specifically for digestion experiments in a special workshop, containing portable mangers and water bowls on the front side, suitable for placement of sheep and goats. For the collection of fertilizer in the experiment, we used a polyester tent fabric measuring 25x40 cm with bonding belts on four sides, one-side zippered fertilizer pouches that were specially sewed, and belts resembling horse harness that were fixed to the animals. All of the animal manure was collected.

For the purpose of the study, a 10-day pre-exercise period was applied to allow the animals to adapt to the cage environment and trial feeds, and to determine feed consumption. Throughout the following 10-day main exercise period, the animals were fed different roughages in amounts that they could fully consume.

Each experiment period was arranged as 10 days of exercise and seven days of sample collection. The amount of feed given to the animals in two meals was arranged to be around 90 percent of their ad libitum consumption. Attention was paid to meeting the nutritional needs for the survival of the animals (NRC, 1985).

During the sample collection periods, the fertilizer was emptied from the fertilizer bags and weighed separately at the same time each day, and 10 percent of the material was deep frozen for later analysis. At the end of each period, the specimens of each animal were combined and mixed homogeneously, and after the amount required for the crude protein analysis was separated, the remaining fertilizer was dried using the method reported by Bratzler and Swift (1959).

In the in vitro trial, the version modified by Marten and Barnes (1980) of the two-stage digestion method reported by Tilley and Terry (1963) was employed. DE, ME and NEL contents of the forage were calculated on the basis of the organic matter digestibility of the forage (NRC, 1989; Ishler et al., 2000).

In the first step, in two repetitions from each feed sample, 0.25 g feed samples were weighed into 50 mL centrifuge tubes, and 25 mL of a buffer solution filled with CO_2 gas, along with 5 mL of rumen fluid in a CO_2 gas medium were added. The tubes were then closed with special caps containing injector needles to allow gas to be discharged from the tubes, and the tubes were left in a water bath at 38°C for 48 hours of incubation. During the incubation period, the caps on the tubes were opened and 1 mL of 5% $HgCl_2$ solution was added to each tube, and centrifuged for 15 minutes at 2500–3000 rpm/min, after which, the clear liquid layer over the sedimentation was removed.

In the second step, 25 mL of a 0.2% pepsin + HCL solution was added to each tube, and the tubes were

left for incubation for 24 hours in a water bath at 38° C. At the end of the incubation period, the tubes were filtered through Gooch crucibles, of which the tare weight had been determined beforehand. They were then dried at 105°C, weighed and then burned for eight hours in an ash oven at 550°C. Calculations were made after the burnt samples were cooled and weighed again.

Using the nylon bag technique, the characteristics regarding the rumen degradability of the forage plants were explored. For this purpose, the rumen degradation of dried meadow grass, dried alfalfa, H. *microcarpum* and P. *pabularia* in 4, 8, 16, 24 and 48 hours were determined (Deniz and Tuncer, 1995). In the trial, two White Karaman rams at three years of age, which had rumen fistula, were used. The animals were fed with sainfoin throughout the trial.

For this purpose, each feed sample was applied in two repetitions for each animal. The samples of the used feed materials were ground to a 2 mm size and placed in pouches after weighing. The nylon pouches were washed in a mini washing machine for 15 minutes after each usage, and then dried in a drying cabinet at 80°C for 24 hours. The tare weight of the pouches were determined after cooling in a desiccator.

Approximately 3 g of feed sample was placed in the pouches, of which the tare weights had been determined, and the pouches were tied tightly with rubber band. Then, the pouches were attached to thin plastic hoses measuring 25 cm in length and perforated at even intervals using 20 cm nylon thread. The pouches were left in the rumen in a way that one end of the plastic hose remained in the fistula cover.
At the end of the incubation periods, the pouches collected from the rumen were rinsed with tap water to remove coarse dirt, and then washed in a mini washing machine for 15 minutes and left to dry at 80° C for 24 hours. The pouches taken from the drying cabinet were cooled in a desiccator, and their weights were recorded (Deniz and Tuncer, 1995).

Variance analysis was employed to statistically assess the data obtained from the study. Duncan's multiple comparison test was used to determine the differences between the groups (Steel and Torrie, 1980). The calculations were made using the SPSS 17.0 package program.

Results and Discussion

The ratio of OM was found to be lower than other forage because the DM contents of the forage used in this study are similar, but the ratio of ash in dried alfalfa is high (Table 1). The crude protein (CP) content of H. microcarpum and P. pabularia are similar to the CP contents of dried meadow grass, whereas their neutral detergent fiber (NDF) and acid detergent fiber (ADF) content are lower than those in dried meadow grass and dried alfalfa. As the harvest period of forage directly affects its nutrient content, it would not be appropriate to make a general judgment based on a single forage sample. For this reason, the assessments made regarding the nutrient content of these forage plants are limited to these plants only.

The DM values found in the studies on H. microcarpum and P. pabularia are close to 90%, similar to the findings in this study. The DM values reported by Hakan et al. (2009) for H. microcarpum and P. pabularia are 90.58% and 89.13%, respectively, whereas the DM value reported by Coşkun et al. (2005) for P. pabularia is 91.56%. The CP values of H. microcarpum and P. pabularia found as 9.09% and 8.84% in this study are reported to be 8.98% and 9.41% by Hakan et al. (2009). The CP value of P. pabularia was reported to be 9.98% by Coşkun et al. (2005) and 11.44% by Eilami and Noroozian (1995).

The NDF values of H. microcarpum and P. pabularia obtained in this study are 41.93% and 42.88%, respectively. The ADF values are 26.84% and 26.97%, respectively. The NDF values of H. microcarpum and P. pabularia reported by Hakan et al. (2009) are 45.38% and 30.86%, respectively. The ADF values are 27.91% and 20.01%, respectively. The NDF and ADF values reported by Coşkun et al. (3) for P. pabularia are 34.78% and 28.45%.

Table.1. Nutrient content of feeds, %

Forage plants	DM	Ash	OM	CP	EE	CF	NDF	ADF
Dried meadow grass	92.19	8.60	83.60	8.66	2.07	41.28	61.67.	33.04
Dried alfalfa	91.22	10.20	81.02	15.61	1.73	34.29	46.66	28.77
H. microcarpum	91.36	8.06	83.30	9.09	2.64	37.03	41.93	26.84
P. pabularia	91.28	7.40	83.88	8.84	1.72	40.21	42.88	26.97

DM: Dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fiber, NDF: neutral detergent fiber, ADF: acid detergent fiber

Table 2. Nutrient digestibility (%) and energy contents (Mcal/kg DM) of forage plants determined by in vivo method (feeding trial).

Forage plants	Dried meadow grass	Dried alfalfa	H. microcarpum	P. pabularia
DM	66.50±1.08^{b}	62.53±1.43^{c}	72.81±1.05^{a}	67.73±0.71^{b}
OM	68.94±1.01^{b}	65.81±1.39^{b}	75.52±1.06^{a}	73.46±0.63^{a}
CP	55.68±2.15^{b}	71.02±1.07^{a}	61.79±2.75^{b}	55.32±1.95^{b}
EE	48.29±2.76^{b}	35.83±3.70^{c}	72.18±1.05^{a}	45.16±1.01^{b}
CF	76.31±1.23^{a}	65.41±1.84^{b}	78.41±1.36^{a}	78.43±1.13^{a}
NDF	69.60±1.16^{a}	56.09±2.40^{b}	65.87±1.67^{a}	65.99±1.03^{a}
ADF	67.57±1.10^{a}	52.13±2.12^{b}	65.36±1.62^{a}	65.62±1.63^{a}
DE	3.04±0.10^{b}	2.90±0.14^{b}	3.33±0.11^{a}	3.24±0.06^{a}
ME	2.49±0.08^{b}	2.38±0.12^{b}	2.73±0.09^{a}	2.65±0.05^{a}
NE_L	1.57±0.02^{b}	1.49±0.03^{c}	1.73±0.03^{a}	1.68±0.02^{a}

H. microcarpum and P. pabularia were found to have better digestibility than dried meadow grass and dried alfalfa, in terms of the digestibility of OM (Table 2). The OM digestibility of dried meadow grass, dried alfalfa, H. microcarpum and P. pabularia were found to be 68.94%, 65.81%, 75.52% and 73.46%, respectively ($p<0.05$). Therefore, the energy content of the forage plants is also different. DE, ME and NEL values for H. microcarpum and P. pabularia were found to be higher than those for dried meadow grass and dried alfalfa. DE, ME, and NEL values of dried meadow grass, dried alfalfa, H. microcarpum and P. pabularia were calculated to be 3.04, 2.90, 3.33 and 3.24 Mcal/kg DM, 2.49, 2.38, 2.73 and 2.65 Mcal/kg DM, and 1.57, 1.49, 1.73 and 1.68 Mcal/kg DM, respectively.

In the in vivo trial for digestibility, the digestibility of crude fiber (CF), NDF and ADF, the structural carbohydrates of forage plants, were found to be similar for dried meadow grass, H. microcarpum and P. pabularia, whereas these values are lower for dried alfalfa. In terms of the digestibility of ether extract (EE), H. microcarpum has the highest and dried alfalfa has the lowest digestibility.

The highest OMD value of the forages obtained through the in vitro method belongs to H. microcarpum (Table 3). OMD values of P. pabularia and dried meadow grass are similar to that of H. microcarpum. OMD value of dried alfalfa was found to be similar to that of dried meadow grass but lower than that of H. microcarpum and P. pabularia. OMD values of dried meadow grass, dried alfalfa, H. microcarpum and P. pabularia were found to be 65.99%, 58.52%, 71.88% and 68.85%, respectively ($P<0.05$). The OM digestibility value found by Coşkun et al. (2005) for P. pabularia using the gas production technique is 80.60%. In vitro organic matter digestibility determined by Coşkun et al. (2005) for P. pabularia is higher than the value determined for P. pabularia in this study. However, since the values obtained from both studies were found using different in vitro methods, it may be more meaningful to account for this difference by the variation of the methods, rather than the variation of the feeds.

Table 3. Digestibility (%) and energy contents (Mcal/kg DM) of the forage plants determined by in vitro method (two-stage method)

Forage plant	Dried meadow grass	Dried alfalfa	H. microcarpum	P. pabularia
OMD	65.99±0.98^{ab}	58.52±0.84^{b}	71.88±4.02^{a}	68.85±3.15^{a}
DE	2.91±0.43^{ab}	2.58±0.35^{b}	3.17±0.18^{a}	3.04±0.14^{a}
ME	2.39±0.07^{ab}	2.12±0.06^{b}	2.60±0.25^{a}	2.49±0.20^{a}
NE_L	1.50±0.05^{ab}	1.31±0.04^{b}	1.64±0.17^{a}	1.57±0.13^{a}

a, b: Values with different letters in the same row found to be different ($P<0.05$). OMD: organic matter digestibility, DE: digestible energy, ME: metabolic energy, NE_L: net energy lactation.

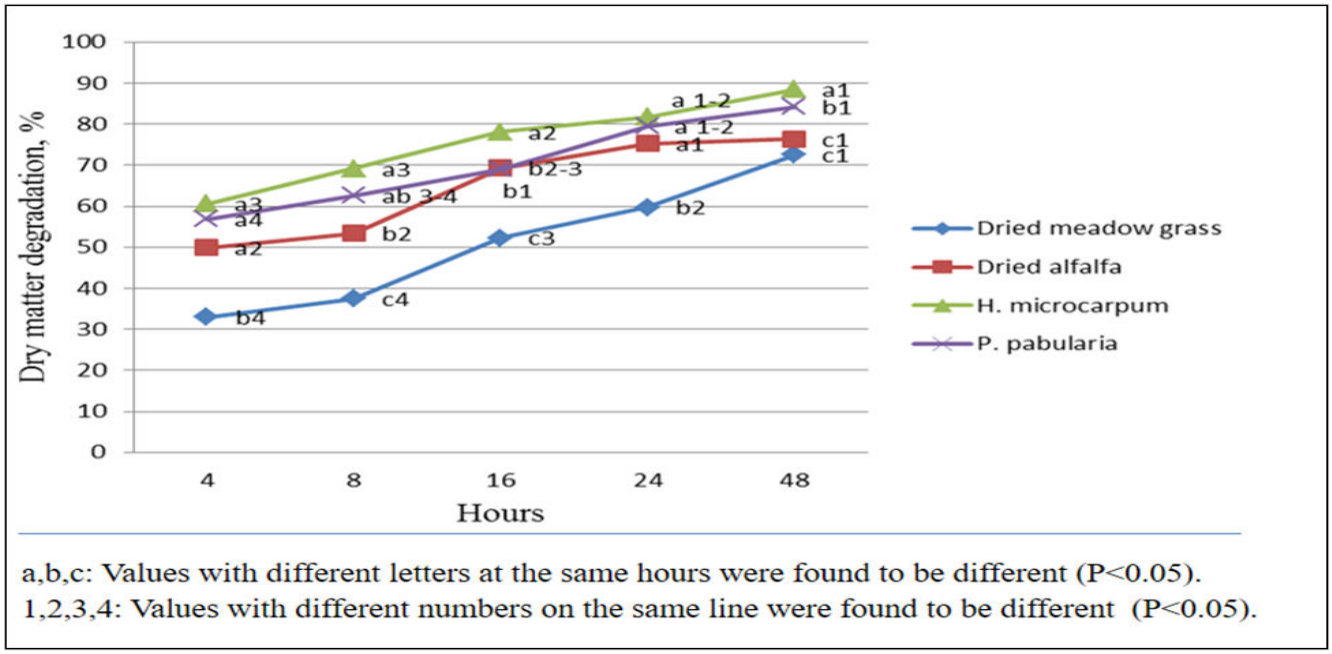

Figure 1. Degradability values for forage plants determined using the in situ (nylon bag) technique, %

The same situation in the OMD value of forages also applies to the energy values obtained from OMD values. With regard to the energy values of these forages, H. microcarpum and P. pabularia have the highest DE, ME and NEL values. The energy values of dried meadow grass are similar to those of these forage plants. However, the values of dried alfalfa are lower than those of H. microcarpum and P. pabularia, but are similar to those of dried meadow grass. ME values obtained from H. microcarpum and P. pabularia (2.60 and 2.49 Mcal/kg DM, respectively) were found to be lower than the value determined by Coşkun et al. (2005) for P. pabularia (2.906 Mcal/kg DM).

The values for in situ dry matter degradability of the forage plants are given in Figure 1. It was observed that dried alfalfa, H. microcarpum and P. pabularia were highly degraded after an incubation of four hours, whereas meadow grass showed a slower degradation trend. As for the levels of degradation at the same hour, it was observed that H. microcarpum and P. pabularia had a higher level of degradation at almost every hour, except for their similarity to the degradation of dried alfalfa at the 4th hour. The DM degradation values at the 8th hour, which were determined to be 37.49% and 53.37% for dried meadow grass and dried alfalfa, were found to be 69.14% and 62.50% for H. microcarpum and P. pabularia, respectively. The DM degradation values at the end of an incubation of 24 hours, which were determined to be 59.73% and 75.21 for dried meadow grass and dried alfalfa, were found to be 81.74% and 79.48% for H. microcarpum and P. pabularia, respectively. At the 48th hour, which was the last incubation in the trial, the dry matter degradation values for dried meadow grass, dried alfalfa, H. microcarpum and P. pabularia were found be 72.45%, 76.36%, 88.36% and 84.21%, respectively (P<0.05). When we examine the rumen degradation of H. microcarpum and P. pabularia between the 4th and 48th hours, we can see that it follows a very regular trend.

Taking into account the in vivo and in vitro OMD values determined for H. microcarpum and P. pabularia and the energy values calculated, based on those values, these plants were found to have a higher energy value than that of dried alfalfa and dried meadow grass, as they have a high a OMD value. The results show that the energy content of these plants are similar to that of barley. Indeed, Coşkun et al. (2005) report that P. pabularia has a relatively high level of energy for a forage, and this value is comparable to the energy level of barley.

Conclusion

It is concluded that if cultivated, the high organic matter digestibility and energy content of H. microcarpum and P. pabularia, which grow in the high plateaus of the East Anatolia Region, could be an alternative to the other high-quality forage plants.

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Rhinoscopy in three dogs

Case Report
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Duygu sezer[1]*, Kemal Altunatmaz[1]

1. Istanbul University Cerrahpasa, Veterinary Faculty, Surgery Department, Avcilar, Istanbul, Turkey.

ABSTRACT

Rhinoscopy is a significant procedure which explores patients upper airway problems. It is easy to implement, provides important information, saves patient from invasive procedures like rhinotomy and obtains substantial hints during diagnostic process. It was aimed to define to advantages of rhinoscopy in diagnosis and treatment of epistaxis and usage of rhinoscopy routinely in clinical examination in Istanbul University Faculty of Veterinary Medicine, Department of Surgery.

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Introduction

Rhinoscopy's meaning is the diagnostic examination of nasal cavity with endoscopy. Rhinoscopy is a significant procedure which explores patient's upper airway disorders (Adaszek et al., 2014). It is easy to implement, provides important information, saves patient from invasive rhinotomy and obtains substantial hints with the patient when a diagnosis is reached (Noone, 2001). Furthermore, rhinoscopy provides direct inspection of almost all nasal mucosa surface, and more importantly it provides to have tissue samples to perform cytological, histopathological and microbiological examination. Rhinoscopy simplifies the diagnosis of nasal neoplasia, lympho-plasmocytic rhinitis and differentiation of orofungal and bacterial rhinitis. The best examination of upper airway can be done with using several techniques together; rhinoscopy, bacteriological, cytological, histopathological and diagnostic technique (Clerx et al., 1996; Meler et al., 2008). The of endoscopic examination is more comprehensible in small animals nasal cavity and upper airway disorders, especially in neoplasma, foreign body, anatomic abnormality and some infections (Willard and Radlinsky, 1999; Sapikowski, 2006). Endoscopy of nasal cavity reveals mucosal congestion and existence of mucus in many chronic rhinit cases. Other diagnosis are foreign bodies, nasal polips, granulamatous rhinitis, oronasal fistulas and nasopharyngeal stenosis (Adaszek et al., 2014). Foreign bodies like fishbone and green ear of grain can be removed mechanically by using and endoscopic forceps. Granulomas are prone to bleed but usually stops bleeding in 3-60 minutes (Knotek et al., 2001). Anterior examination of nasal cavity can be done by using a rigid or elastic endoscope. The indications of rhinoscopy includes; persistent unilateral or bilateral nasal discharge, recurrent nose bleedings, progressive nasal discharge, deformation of nasal region, nasal sounds, tenderness of nasal region,

*Corresponding Author: Duygu Sezer
E-mail: m.duygu.sezer@gmail.com

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wheezy respiration, foreign body and suspicion of neoplastic mass (Elie and Sabo., 2006; Venker-Van Haagen., 2005). It also has been reported that detection of bilateral changes and application of systemic antifungal agents in mucotic rhinosinusitis (Sharman., 2010).

This region splits as anterior and posterior rhinoscopy. In anterior rhinoscopy rigid endoscopes varies between 0, 30 degrees, with 50 centimeter length and 2-5 mm of diameter is frequently used. Arthroscopic equipment can be used in this implementation; 2,7 mm arthroscopes are suitable for many patients and it provides well illumination and good quality of vision (Tappin, 2011). Flexible endoscopes are used less frequently and 6-10 mm ones should be preferred (Marcin et al., 2010). Anterior rhinoscopy; should be moved gently and carefully throughout nasal septum. All three air sections can be examined in this way. Posterior rhinoscopy allow to inspect soft palate, nasopharynx, concha and auditory tube opening. Retrograde (posterior) rhinoscopy is applied in retroflexion; endoscope should be pushed in parallel to oral opening and soft palate through the caudal of pharynx. After entering to soft palate, it should be turned over 90 degrees of angle. However, all manipulations in this region in highly irritant (Tappin, 2011; Knotek., 2000).

The quality of patients rhinoscopy assisted with the history of the patient, clinical examination findings and additional data, blood analysis (CBC -complete blood count- and serum biochemistry), radiological and nasal examination (Marcin et al., 2010; McKiernan, 2001).

Nasal cavity and corona regions mucosal condition (color, moisture, brittleness), blood vessels, foreign body and hypertrophic changes should be considered during rhinoscopy (Marcin et al., 2010; Harcourt-Brown, 2006; Tams, 1990; Venker-Van Haagen, 2005).

It was aimed in this study to show the advantages of rhinoscopy in routine examination of upper airways.

Case

In the present study, a total of 3 dogs of different breeds, age and genders which underwent rhinoscopy were evaluated. Food and water intake of the patients were restricted 8 hours and 1 hour before the induction of anesthesia, respectively and was taken under general anesthesia with propofol. (8 mg/kg, Propofol , %1 Fresenius, Germany). The patients weren't intubated because the implementation took short time. Rhinoscopy was performed in lateral or sternal recumbency. (Butorphanol (0.02 mg/kg, Butomidor, Richter Pharma AG, Austria) was used for analgesia.

In this study, a Weidler branded rigid endoscope with 0-degree angle, 11 cm lenght, 2.7 mm diameter was used.

The first case is a 2.5-year-old German Shepherd Dog and was taken to the clinic for epistaxis. As a result of rhinoscopy, bleeding and focal abscesses were seen. Patient came back positive for *Ehrlischia* and *Drofilaria* according to blood parasite analysis. (Figure 1)

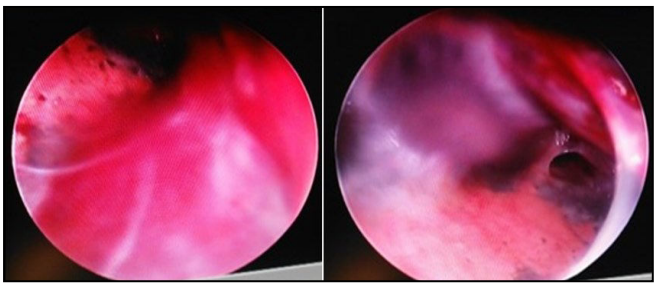

Figure 1. Focus of bleeding (a) and abscess (b) in first case's concha.

The second case is a 2 year old Belgian Shepherd Dog which was brought in to the clinic for unilateral nose bleeding. Foreign body (false barley/ Hordeum murinum) was seen in rhinoscopy and removed by a forceps (Figure 2).

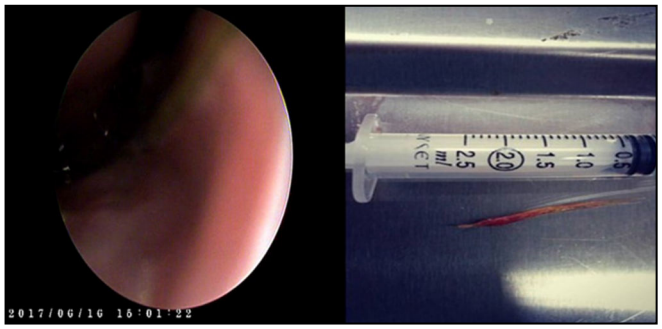

Figure 2. Foreign body's rhinoscopy imaging (a) and false barley (Hordeum murinum) removal via alignment forceps (b) in second case.

The third case is a 1,5-year-old female Pitbull Terrier and admitted to the clinic with unilateral epistaxis and reverse sneezing. A nasal foreign body (wild barley) was detected in rhinoscopy and removed by a foreign body forceps (Figure 3).

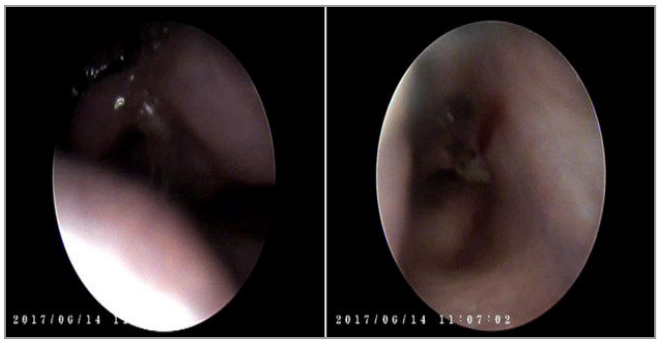

Figure 3. False barley (Hordeum murinum) in cocha (a) and focus of bleeding (b) in third case.

Results and Discussion

In a study carried out by Knotek et al. (2000 and 2001), foreign bodies can be removed mechanically via forceps and prone to bleeding. In two of in this study two cases a foreign body (false barley) was detected and removed with forceps. Although it has been stated in previous studies (Knotek et al., 2000; Knotek et al., 2001), that the bleeding would stop in half an hour without any implementations. It was applied local adrenaline and tampons in the cases to control the bleeding.

According to the study of Pietra et al. (2010), blood parasites take an important place in epistaxis. Although this study (Pietra et al., 2010) points out that endoscopy is not definitive in blood parasites, the focus of bleeding and abscess that have encountered during rhinoscopy made to think about blood parasites and thoughts were confirmed according to analysis.

Rhinoscopic examination provides some beneficial data in a large scale in patients who were brought to the clinic with unilateral or bilateral epistaxis. Furthermore, it's invasive act is scarcely any when its performed carefully. For this reason, conclude that routine rhinoscopic examination of similar patients is minimally invasive during diagnosis and treatment. As a result; it has been found that rhinoscopy is quite useful in the diagnosis and treatment of most problems encountered in the nasal region.

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The effect of processing factors on detection of genetically modified soy in flour by ELISA assay

Ozge OZGEN ARUN[1], Funda YILMAZ EKER[1], Karlo MURATOGLU[1] *

1.Istanbul University, Veterinary Faculty, Department of Food Hygiene and Technology, Avcilar, Istanbul/Turkey

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ABSTRACT

Genetic modification (GM) techniques have been an important research area of food and feed industry since the 19th century. There is a strong consumer concern over genetically modified organisms (GMOs) because of their potential risks on health and environment. For this purpose, various countries including Turkey have released labelling regulations for products derived from GMOs. These legal enforcements brought the necessity for reliable detection methods. The aim of our study was to evaluate the effect of processing factors on the detection possibility of GMOs by using a commercial Enzyme Linked Immunosorbent (ELISA) assay. For this, flour mixtures containing 0.5%, 1%, 5%, 10%, 100% were prepared by mixing the appropriate amount of RUR-GM and non GM standard soy flour and main processing techniques most used in the food industry (baking, autoclaving and freezing) were applied. According to our results, the detection of GMOs was possible at all concentrations of autoclaved and frozen samples. In dry heated samples, GMOs could not be detected containing below 5% GMOs. ELISA method cannot be recommended as a reference method for evaluation of the compliance with the regulations, but it can serve as a practical alternative to be used as an online monitoring tool in production lines for raw and mildly processed foods.

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Introduction

Genetic modification (GM) techniques have been an important research area of food and feed industry since the 19th century. The most significant product of this field is Genetically Modified Organisms (GMOs). “GMOs are organisms that genetic materials (DNA) have been altered in a way that does not happen in normal ways” as defined by World Health Organization (WHO). In this context, it is intended to get edible vaccines or medicines, functional foods, enhanced shelf life and nutritional composition, also growing adaptable and strong plants such as herbicide tolerant or insect resistant in various environmental conditions via using GM technology. Improving the quality of certain crops is believed to be the most significant advantage of GMOs (Arun Ozgen et al., 2015).

Since they were first approved, GMOs have received a worldwide demand and GM crops have been planted on a very large scale. The plantation area of GM crops have increased 110-fold from 1996 to 2016 and finally reached 2.1 billion hectares (ISAAA, 2016). Soybean, maize, cotton and canola are the most cultivated GM crops in 26 countries which grows GM crops. Most of these crops have been approved to be used in food/feed in several countries and thus enters the food chain (Anonymous, 2018).

Despite this huge market share, there is a strong

*Corresponding Author: Karlo MURATOGLU
E-mail: karlomrt@istanbul.edu.tr

consumer concern over GMOs because of their potential risks on health and environment. For this purpose, various countries including Turkey have released labelling regulations for products derived from genetically modified organisms (GMOs). The main policy of such regulations are to leave the decision making to the consumer (Anonymous, 2003; Anonymous, 2009; Anonymous, 2010). These regulations lay emphasise on that; GM food or feed must not have any adverse effect on the environment or human/animal health. Also, labelling must not misguide the consumer (Varzakas et al., 2007).

These legal enforcements brought the necessity for reliable detection methods. For this purpose, numerous analytical methods, supporting the regulations have been carried out to monitor and verify the presence of GMOs in food or feed samples (Anklam et al., 2002). These methods mainly based on detection of novel DNA or protein present in the product. DNA-based methods widely used both qualitatively and quantitatively for the detection of GMOs product in transgenic raw or unprocessed soy products (Meyer et al., 1996; Lipp et al., 2000; Lipp et al., 2001; Taverniers et al., 2001). Qualitative testing is used to identify GM or non GM material or to distinguish certified or noncertified material. Quantitative testing, on the other hand is used for confirming the official thresholds (Quist and Chapela, 2001; Windels et al., 2001 and 2003; Chowdhury et al., 2003a and 2003b ; Hernandez et al., 2004; Collonnier et al., 2005; Nielsen et al., 2005; Ortiz-Garcia et al., 2005; Saji et al., 2005; Taverniers et al., 2005; Aono et al., 2006; Messean et al., 2007). DNA based methods are proved to be the most reliable methods for detection of GMOs in food and feed. However, PCR methods need an expensive laboratory infrastructure and experienced staff. In such cases, protein based methods may be used as an alternative cheap and easy testing especially for quality control laboratories performing routine process monitoring. ELISA is the most widely used protein based method for detection of the proteins expressed by GMOs especially in raw food (Vollenhofer et al., 1999; Ahmed, 2002; Arun Ozgen and Garrett, 2009; Suchitra and Ali, 2013). The method can be used for qualitative and quantitative purposes.

Food processing causes serious degradation of proteins in food. In many studies, it was reported that heat processing such as cooking, baking, drying, sterilizing or freezing causes a severe denaturation on food proteins (Asensio et al., 2008).

Taking these into consideration the aim of our study was to evaluate the effect of processing on the detection possibility of GMOs by using a commercial ELISA assay.

Materials and methods

Raw Material Preparation : Flour mixtures containing 0.5%, 1%, 5%, 10%, 100% were prepared by mixing the appropriate amount of Roundup Ready® (RUR) GM and non GM standard soy flour (SDI diagnostics, USA) and main processing techniques most used in the food industry were applied to the mixtures. Utmost care was taken to avoid contamination between samples and different steps.

Dry Heat Treatment (Baking): For baking 0.5 g of each flour mixture containing RUR GM soy were mixed with 1000 µl milli Q water and cooked at 100°C for 20 min in a sterilizer (Murray, 2007).

Wet Heat Treatment (Autoclaving): For autoclaving process; 0.5 g of each flour samples were mixed with 3750 µl milli Q water and autoclaved at 121°C, 15 Ibs pressure for 20 min (Hirayama, HV-50L, Japan) (Murray, 2007).

Freezing: For freezing; 1 g of each flour mixture were mixed with 1000 µl milli Q water and stored at –18°C for three days (Murray, 2007).

All of these preparations were performed in duplicate and analysed with ELISA method.

GMO detection with ELISA method: For detection and quantification of GMOs in the treated and raw samples were performed with Romer Labs Agraquant Toasted Meal Plate Kit (No: 7099999). The ELISA kit is designed to detect the CP4 EPSPS protein in RUR soybeans in toasted meals. According to the manufacture© instructions 100 mg of each standard (0%, 0.3%, 1.25%, and 2.5% RUR soy flour) and samples were mixed with 16 and 13 ml extraction buffer, respectively and vortexed for 1 min. The wells of the plate were filled with 100 µl of these extracts in duplicate and processed incompliance with the manufacturer's instructions. The absorbance of the developed colour was read at 450 nm using a plate reader (ELISA Plate Reader ELX 800, Biotek-Inst, ABD).

Results and Discussion

The main objective of this study was to evaluate the detection and quantification capability of a commercial ELISA based GMO detection assay on heat treated samples. For this; five different concentrations of RUR soya (0.5, 1, 5, 10 and 100%) flour samples were heated in two different conditions (baking, autoclaving) and also they were frozen to simulate the common processes in the industry. Detection and

quantification of GMOs in these samples were performed with Romer Labs Agraquant Toasted Meal Plate ELISA assay. The results of this study are summarised in Table 1 and 2.

Table1. Detection results of ELISA assay

Samples	100 °C Dry Heat Treatment	121 °C Wet Heat Treatment	-18 °C Freezing
0.5% GMO	Not detected	Detected	Detected
1% GMO	Not detected	Detected	Detected
5% GMO	Detected	Detected	Detected
10% GMO	Detected	Detected	Detected
100% GMO	Detected	Detected	Detected

GMO = Genetically modified organism

According to these results, the detection of GMOs was possible at all concentrations of autoclaved and frozen samples. In dry heated samples, GMOs could not be detected in samples containing GMOs below 5%.

The quantification results of frozen samples were significantly closer to the true values while the results of autoclaved samples were still close although slightly deviated compare to frozen samples. However, there was a significant bias between true values and results of dry heated samples as in detection.

Table 2. Quantification results of ELISA assay

Samples	100 °C Dry Heat Treatment	121 °C Wet Heat Treatment	-18 °C Freezing
0.5% GMO	<0.00%	0.44%	0.49%
1% GMO	<0.00%	0.74%	1.17%
5% GMO	0.26%	>2.50%	>2.50%
10% GMO	0.70%	>2.50%	>2.50%
100% GMO	>2.50%	>2.50%	>2.50%

GMO = Genetically modified organism

Our previous studies performed with PCR also showed that baking (dry heating) process has a significant effect on detection of GM DNA in food samples (Arun Ozgen et al., 2016). Similarly, several other researchers indicated the degradation/ denaturation effect of heat on DNA and proteins (Asensio et al., 2008, Arun Ozgen and Garrett, 2009). Although autoclaving is performed at 121°C detection and quantification was more successful at these samples. Several other studies also proved that the degradation effect of dry heat is stronger than wet heat (Corbisier et al., 2005; Vijayakumar et al., 2009; Bergerová et al., 2010; Ballari and Martin, 2013). This was incompliance with our study on novel protein.

Conclusion

Despite the detection and quantification is effected from dry heating and detection limit of the method is significantly higher compared to PCR based methods, it can still produce reliable results on wet heated (autoclaved) and frozen samples. ELISA method for sure cannot be recommended as a reference method for evaluation of the compliance with the regulations. However, it should be taken into consideration that, ELISA does not require a sophisticated laboratory infrastructure and expertise and thus it can serve as a practical alternative to be used as an online monitoring tool in production lines for raw and mildly processed foods.

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Non-waste hydrobionts management

Nina DANKEVYCH[1]*

1. Odessa State Agrarian University, Odessa, Ukraine

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ABSTRACT

The aim of the study was to investigate the possible use of mussels and algae wastes processed into high quality and safe food additives for the poultry. Black sea mussels (*Mytilus galloprovincialis* Lamarck) and algae (*phyllophora*) were used as a waste product. The samples were taken from the households where mussels were initially processed at an agar plant. Waste samples and derived additives were tested for bacterial load, quality and chemical composition. In addition, protein-mineral and mineral additive were produced from waste products. Their possible uses in poultry meat production were investigated. It was found that contamination with mesophilic bacteria and facultative anaerobic organisms fluctuates within current requirements. Sometimes even substantially exceeds them depending on waste storage conditions. The contamination with *E. coli*, *Salmonella*, and other pathogenic microorganisms is also observed within existing requirements or exceeding them. Chemical analysis reveal that intact mussels consist of average 80% wet matter and 20% dry matter, 9.4% protein, 1.2 % fat, 4.8 % nitrogen free extractive substances, 0.18% ash, 24.5 g/kg calcium, 1.0 g/kg phosphorus, 0.18 g/kg potassium. On the other hand, mussel valves consist of % 12 wet matters and % 88 dry matters, 37.2g/kg calcium, 0.2 g/kg phosphorus, 0.1 g/kg potassium. Use of additives in poultry production positively affects development and slaughter-out percentage and does not reduce the quality and biological value of their meat. As a conclusion it can be said that non-waste product from processed mussels represent a kind of raw material which can be used as food additive in animal industry after being technologically processed.

Keywords: sea hydrobionts, body weight gain, mineral additive, protein-mineral additive.

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INTRODUCTION

Ukraine is a maritime stat; it benefits from the bio resources of the black sea like other coastal countries. These bio resources contribute to the increase of food stuff, reduction of production costs and strengthening of the national economy (Kupynets, 1986; Amystyslavskyi, 1984]. One of the bio resources derived from the black sea is hydrobionts. The hydrobionts in the Black sea and its lagoons are one of the supplemental sources of food and feed protein. When used for food industry a lot of wastes are left causing environmental pollution. It is known that, wastes contain a certain amount of protein and mineral substances. Thus, researches conducted in this field showed, not only marine fish but also marine hydrobionts such as bivalve molluscs-mussels and algae are of great value.

Numerous studies by Odesa State Agrarian University researchers led by professor Kovbasenko found that mussels and algae (especially *Phyllophora nervosa*) are of great practical interest (among numerous bio resources of the sea) to agrarian production namely animal husbandry. If properly processed, they can be use as a protein and mineral sources for farm animals and poultry (Kovbasenko, 1993; Tarnazgenko, 2005; Kovbasenko, 2005).

The aim of the study was to investigate the possible use of mussels and phyllophora wastes processed into high quality and safe food additives for the poultry.

*Corresponding Author: Nina Dankevych
E-mail: dankevych82@gmail.com

Material and methods

Waste materials : The waste materials derived from the Black sea mussels were taken from a household where mussels were initially processed at an agar plant. Mussel's meat, valves and agar production waste (Algae after being processed into agar) were used as a material.

Waste samples and derived additives were tested for bacterial contamination (toxicity), quality and chemical properties.

Bacterial examination: Bacterial content of raw material for feed production is practically the main indicator of the possibility of use in fodder production. Bacterial contamination was studied using a microbiological method involving *Colpoda steinii* infusorium which was approved by the State Veterinary Medicine Department [Kovbasenko, and Melnyk, 2005; Kovbasenko, et al. 1982; Leonorm, 2000). The mesophilic aerobic and an optional anaerobic bacterial content of mussels should not exceed (CFU in 1 g) 5×10^4. The number of coliform (*E. coli*), golden staphylococci (*S. aureus*) and other pathogenic microorganisms does not exceed standards.

Qualitative assessment: For qualitative evaluation of the waste product, current state standards (which includes the rules of pre-slaughter veterinary and sanitary inspection of animals, meat and meat products) taken into account. In addition, pH of mussel's meat, reaction of mussels with sulfate, reaction of determination of amino-ammoniacal nitrogen and reaction on ammonia from reactive Nessler were performed.

Chemical properties: For the determined chemical composition of waste products moisture, dry substance, protein, fat, REM, ashes, calcium, phosphorus and potassium level were analyzed. In addition, magnesium, sodium, iron, manganese, zinc, copper, aluminum, molybdenum, nickel, iodine, silicon, barium, titan and cobalt were measured in the mussel's meat samples.

Production of protein-mineral and mineral feed additive: Protein-mineral additive was produced from only non-standard mussels and agar production wastes while mineral additive was produced from valves only (Kovbasenko and Dronova, 2008; Kovbasenko and Karaivan, 2009). To produce, mussels with no more than 40-45% of empty valves are mixed with agar production wastes in the ratio 5:1, crushed and hydrolyzed by hydrochloric acid. To produce mineral additive only valves are used being mixed with 10% of sea water, crushed and partially hydrolyzed by the acid. The paste gained after hydrolysis is mixed with 40% of decontaminated water and subjected to a short heat treatment. (15s, at 100℃).

Birds, feed and experimental groups: Totally 10 days old 240 ducklings were used in the study. The ducklings were divided into 2 groups as a protein mineral additive (PMA) and mineral additive (MA). Each consists of 3 subgroups (1 control, and 2 experimental groups). All bird had identical conditions and received the main staple ration, which was balanced by the basic specific substances in accordance with the applicable norms. Experimental groups and birds ration were presented Table 1. In accordance with the study methodology control and test groups of ducks were provided with staple ration for 8 days (aged from 12 to 20 days). From the 21st day till the end of the period (60 days in total) their ration was enriched by paste-like additives in the amount of 10% to the staple ration. We used the additives in 2 ways: 10% replacement of the staple ration with additives and adding extra 10% of additives to enrich the staple ration. At the end of the study birds were slaughtered and the meat quality was investigated.

Table 1: Research scheme

Feed additives	Study groups and ration	
	Comparative (12 – 20 days)	Basic (21 – 60 days)
Protein-mineral additive	PMA- control	SR - control
	PMA-I	90%SR+10% PMA
	PMA-II	100%SR+10% PMA
Mineral additive	MA-control	SR- control
	MA-I	90%SR+10% MA
	MA-II	100%SR+10% MA

SR= staple ration; **MA**= mineral additive; **PMA-I** = 90%SR+**10%** PMA ; **PMA-II**= 100%SR+**10%** PMA

Biological value of duck meat: Biological value of meat was defined on VASKhNIL methods (Lenin all-union Academy of Agricultural Sciences) using WH strain paramecium as the test object. Relative biological value (RBV) was defined on the scale for nominal product totaling to 100 per cent. RBV was calculated by the ratio of paramecia grown on experienced product multiplied by 100 or by the ratio of control product ABV (Absolute biological value) multiplied by 100.

The effects of feed additives from aquatic organisms on the biological value of broiler chicken meat in a complex way were studied: based on the amino acid composition of the protein, with the deduction of the protein quality index and the determination of biological value using the *Tetrachimena piriformis* infuzoria as a test object.

Results and discussion

According to the studies initial processing of mussels accumulates up to 70-80% of wastes: valves and small mussels polluting the environment. Without being disposed and properly treated they are left at fishing and initial processing positions. Most valves are intact of 4-6 cm with specific odor without sand or other impurities. The proportion of valve mass to mussel mass made up from 45 to 50%. Being processed, mussels result in smaller ones of not more than 3 cm. According to organoleptic characteristics small fresh mussels must have clean surface without sand or sludge, some of them having threads and intact valves. The valves are tightly closed releasing cloudy liquid when opened with an effort. Mussel body is moist and shiny firmly attached to the valves. The valves are closed tightly. Waste mussels have a typical subtle specific odor.

The researches on microbiological indicators of wastes according to the requirements of «Compulsory minimum list of researches on raw materials, vegetable and animal products, compound feedstuff, vitamin supplements etc.» found that contamination with mesophilic bacteria and facultative anaerobic organisms fluctuates within current requirements from 5×10^3 and sometimes even substantially exceeds them depending on waste storage conditions. The insemination with bacteria of *E. coli*, *Salmonella*, and other pathogenic microorganisms is also observed within existing requirements or exceeding them. It was found that bacterial contamination of wastes depends on the fisheries, marine environment and freshness of wastes. As the toxicity tests of initially processed mussels showed, fresh and doubtfully fresh mussels satisfy the veterinary and sanitary requirements.

For a full assessment of initially processed mussels we determined their main chemical substances responsible for their fodder and biological values. Chemical composition of intact mussels and valves are presented in the Table 1. It was found that processed mussel wastes contain proteins and mineral substances which can be used as food additives but their content is not stable and depends on many factors (season, natural conditions, etc.). On average overall, wastes from processed mussels consist of raw material which can be used as food additive in animal feeds after being technologically processed.

Table 2: Chemical composition of intact mussels and valves

Chemical composition	Moisture content			
	Intact mussels		Valves	
	Variation rate	Average rate	Variation rate	Average rate
Wet matter (%)	74.2 – 86.4	80.0	9.8- 12.6	12.0
Dry matter (%)	25.8 – 13.6	20.0	90.2 – 87.4	88.0
Protein (%)	8.7 – 10.6	9.4	-	-
Fat (%)	0.7 – 1.6	1.2	-	-
NFES (%)	4.2 – 5.4	4.8	-	-
Ash (%)	0.16 – 0.2	0.18	0.78 – 0.92	0.90
Calcium (g/kg)	22.1 – 26.3	24.5	32.4 – 38.6	37.2
Phosphorus (g/kg)	0.9 – 1.2	1.0	0.18 – 0.23	0.2
Potassium (g/kg)	0.18 – 0.19	0.18	0.1 – 0.03	0.1

NFES –nitrogen-free extractive substances

Table 3. Impact of protein-mineral hydrobiont additive (PMA) on broiler ducks productivity (n = 40).

Production parameters	Control	PMA-I	PMA-II
Initial live body weight (g)	510 ± 1.6	508 ± 2.4	504 ± 1.2
Final live body weight (g)	2290 ± 2.34	2300 ± 3.26	2450 ± 2.3
Live body weight gain (g)	1780 ±3.62	1792 ± 4.12	1946 ± 4.21
Survival rate (%)	92.6	95.4	98.2

SR= staple ration; **MA**= mineral additive; **PMA-I** = 90% SR+**10%** PMA ; **PMA-II**= 100% SR+**10%** PMA

Both food additives represent grayish suspension with specific smell typical of mussels. Produced on the proposed technology food additives basically contain a complex of mineral substances essential for animals but at the same time protein-mineral additive is also a source of protein in the amount from 8.2 to 9.1%. As regards health, food additives produced according to the proposed technologies satisfy veterinary and sanitary requirements of current standards.

The effects of protein-mineral additives (PMA) on production performance of duckling are presented in Table 3. It was found that PMA addition increased live body weight gain approximately 6.8-9.32 % in PMA-I and PMA-II groups respectively. In addition, survival rate (conversation rate) was increased 2.8% and 5.6% in PMA-I and PMA-II groups respectively. Therefore, it can be say that, using protein-mineral hydrobiont additives has a positive effect on broiler ducks growth regardless the way used.

The effects of -mineral additives (MA) on production performance of ducks are presented in Table 3. The data indicated that body weight gain and relative growth rate do not differ from those in the control group in MA-I group. Therefore, it can be say that, replacement of the ration with 10% mineral additive does not have a significant impact on growth and development of ducks. But adding extra 10% of mineral to the ration increases the body weight gain by 1.73 % compared to the control group. The survival rate is also increased by 3.9% in this group.

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Biological value of duck meat is presented in Table 5. Meat quality parameters showed that, using food additives from hydrobionts doesn't affect qualitative and quantitative composition of ducks meat and using hydrobiont food supplements does not reduce biological value of meat.

It can be clearly seen from the given results that the use of hydrobiont food additives when feeding broilers following our method does not reduce the quality or biological value of their meat. In the overall, it must be noted that in modern conditions when catching and initially processing mussels up to 80% of wastes accumulate without utilization. It leads to environmental pollution at fishing and initial processing areas. It was revealed that these wastes can be used for food additives production for animals and birds. Our food additives produced from wastes are being successfully used in poultry production.

Table 4: Impact of mineral hydrobiont additive on ducks productivity (n = 40)

Production parameters	Control	MA-I	MA-I
Initial body weight, g	502±1,21	504±3,26	505±1,36
Final body weight	2292±2,12	2286±3,24	2406±2,24
Body weight gain, g	1790±3,69	1782±1,12	1821±2,32
Daily body weight gain, g	44,7±0,6	44,5±0,3	45,5
Relative growth rate, g	127,0	126,4	129,2
Survival rate (%)	93,4	93,6	97,3

SR= staple ration; **MA**= mineral additive; **MA-I** = 90%SR+**10%** MA ; **MA-II**= 100%SR+**10%** PMA

Table 5: Biological value of meat for both control and test groups (n = 5)

Indicators	Content in groups in %		
	Control group	Test group 1	Test group 2
ABV	45.8 ± 0.2	45.6 ± 0.2	45.8 ± 0.3
RBV	69.9 ± 0.3	69.9 ± 0.3	70.0 ± 0.4
CBV	100.0	101.2	101.6

ABV = absolute biological value; **RBV**= relative biological value; **CBV** = comparative biological value

Conclusions

Due to their chemical composition and biological value marine mussel's wastes can be used as raw material to produce food additives for animals.

A simple waste management technology was designed to initially process mussels into fodder additives: protein-mineral additive and mineral one.

Use of additives in poultry production positively affects development and slaughter-out percentage and does not reduce the quality and biological value of their meat.

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