
**ABSTRACTS OF
DAYS OF VETERINARY MEDICINE 2011**



EVALUATION OF DAIRY COWS ENERGY STATUS BY BIOCHEMICAL ANALYSIS OF ORGANIC COMPONENTS OF MILK

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Introduction

The energy status of cows was estimated on the basis of milk urea, protein and fat concentrations and its relationships.

Material and methods

Concentrations of urea, fat and protein were determined in 62 early lactation Holstein-Friesian dairy cows milk samples from 4 farms (11 samples from farm A, 16 from farm B, 15 from farm C, 20 from farm D). Graphic presentations are given for the relationship between urea and protein, as well as protein and fat concentrations for the individual milk samples. Such an analysis provides a more detailed picture of the cow energy status. If milk urea concentration is lower than 4 mmol/L and protein concentration higher than 32 g/L, feeding is proper. If protein concentration is higher than 32 g/L, urea concentration higher than 4 mmol/L moderate deficit of ratio energy is present. If urea concentration is higher than 4 mmol/L, protein concentration lower than 32 g/L ratio is insufficient in energy. If urea concentration is lower than 4 mmol/L, protein concentration lower than 32 g/L deficit of both ratio energy and protein is present. Additionally, if milk protein is higher than 32 g/L, milk fat lower than 4.5 g/L energy supply is proper. If milk fat concentration increases and protein concentration decreases there is energy deficit in ratio.

Results

The fat concentration in all milk samples was 23 ± 13 g/L (Farm A- 38.9 ± 7.7 g/L; Farm B- 16.6 ± 7.5 g/L; Farm C- 33.8 ± 9.3 g/L; Farm D- 10.7 ± 2.6 g/L), the protein concentration was 29 ± 3 g/L (Farm A- 29.9 ± 3.3 g/L; Farm B- 26.9 ± 3.1 g/L; Farm C- 28.4 ± 4.5 g/L; Farm D- 30.5 ± 1.7 g/L), the urea concentration was 4.6 ± 1.3 mmol/L (Farm A- 3.2 ± 7.7 mmol/L; Farm B- 16.6 ± 7.5 mmol/L; Farm C- 33.8 ± 9.3 mmol/L; Farm D- 10.7 ± 2.6 mmol/L). Urea concentration in milk from morning milking on farm D (5.05 ± 1.59 mmol/L) was significantly higher than in evening

milking (4.43 ± 1.06 mmol/L) indicating on energy deficit during night. Graphs are present. Most cows on farm A had a deficiency of energy and protein (8 from 11 milk samples had protein concentration under 35 g/L and urea concentration under 4 mmol/L), cows on farms B and C had a deficiency of energy with a relative surplus of proteins (13 of 16 milk samples on farm B and 12 milk samples from 15 on farm C had protein concentration under 35 g/L and urea concentration higher than 4 mmol/L). Cows from farm D had a deficiency of energy, with an equal number of cows with a deficiency and with a relative surplus of proteins.

Conclusions

The determination of urea, fat and protein concentrations in milk is a reliable indicator of the energy status of cows. It is rational and financially acceptable. The obtained results provide grounds for making recommendations for correcting the feed rations and improving the health of the cows.

VECTOR-BORNE DISEASES OF DOMESTIC ANIMALS IN HUNGARY

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The increasing frequency of vector-borne diseases (VBDs) of domestic animals is most probably due to a joint action of several different factors. Climate change has influenced the geographical distribution, density and seasonal activity of many blood-sucking arthropods. Changes in habitats, social and leisure activities of humans have also affected the epidemiology of vector-borne diseases. For the last two decades the knowledge about VBDs of livestock and companion animals has been extended in Hungary using different methods for diagnosis and PCR to identify these agents in vectors collected from animals and/or environment.

Studies on anaplasmosis of ruminants have resulted in internationally new data. Examination of sheep and cattle in the northern part of Hungary with a competitive ELISA and PCR revealed first time the endemicity of *Anaplasma ovis* and *A. marginale* in the country. These data extend the northern latitude in the geographical occurrence of ovine anaplasmosis in Europe. During an outbreak of 25 bovine anaplasmosis acute disease was observed in five animals, two of which died. *Anaplasma*-carrier state was diagnosed in 92% of cattle. Concurrent infections were detected with *Mycoplasma wenyonii* and 'Candidatus *M. haemobos*'. *Anaplasma marginale* was detected in one and six pools of *Ixodes ricinus* and *Dermacentor reticulatus*

males, respectively. In the same *I. ricinus* pool *A. ovis* was also identified. In an attempt to identify the main vector(s) and possible transmission routes of *Anaplasma* spp. DNA was extracted from 316 hard ticks, tabanids and hornflies. The results provided the first molecular evidence for the potential mechanical vector role of *Tabanus bovinus* in the transmission of *A. marginale*. Anopluran lice of ruminants and pigs were evaluated for the presence of anaplasma, rickettsia and haemotropic mycoplasma DNA. The study first molecularly confirmed of bovine and ovine *Anaplasma* spp. in *Linognathus vituli*, *L. stenopsis* and *Haematopinus suis*.

Our knowledge has also been extended concerning VBDs of horses and companion animals. *Babesia caballi* antibodies were detected with a competitive ELISA in blood samples of 29 horses came only from one region of the country that is endemic focus for this piroplasma species. *Theileria equi* infection of horses was identified in many parts of the country. It was the first serological evidence of horses being naturally infected with *Babesia canis* of dogs. On the basis of morphological identification of microfilariae 18 of 195 horses were infected with mosquito-borne *Setaria equina*, and the infection was confirmed in 10 animals by PCR and sequencing. Serological and/or molecular studies on dogs, cats, hard ticks and fleas gave up-to-date information about the prevalence of babesiosis, Lyme borreliosis and granulocytic anaplasmosis, leishmaniosis of dogs and dirofilariosis and bartonellosis of dogs and cats, some of which have zoonotic potential.

EU-FUNDED SUPPORT FOR THE CONTROL AND ERADICATION OF CLASSICAL SWINE FEVER IN MACEDONIA

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Strengthening measures to control and eradicate Classical Swine Fever (CSF) in Macedonia is a key component of the EC-funded IPA programme 2008¹. The Food and Veterinary Agency is committing significant resources, both in terms of staff and finance, to achieving the goal of national disease freedom. The Agency is supported in this work by the IPA CSF Project Team. However, the collaboration and cooperation of the wider veterinary community is a prerequisite for success: Veterinary practitioners perform disease

prophylaxis and surveillance activities on domestic pig farms and in backyard holdings; veterinary professionals in the National Reference Laboratory conduct the diagnostic and monitoring tests needed to implement control and eradication programmes and hunting veterinary colleagues assist in the collection of samples from shot wild boar. Colleagues in neighbouring countries roles also cooperate with control and public awareness activities. The 'Days of Veterinary Medicine' conference, organised by the Faculty of Veterinary Medicine at the University "St. Cyril and Methodius", offers a unique and valuable opportunity for the VFA and Project Team to engage with veterinarians practising in all relevant fields, both at national and regional level.

¹IPA Programme 2008 – Capacity building of the veterinary service for implementation of EU Acquis

ANIMAL INFECTIOUS DISEASES AND METHODS OF THEIR PREVENTION

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Republic of Tajikistan implements a policy to ensure its food security and public health, and with that in mind takes preventive measure against animal infectious diseases like foot-and-mouth, anthrax, brucellosis, TB and cattle plague by state supply of necessary vaccines and monitoring epizootic situation of the country.

It is appropriate to mention close cooperation between the interested state and private institutions, international non-government organizations in this field, especially the State Veterinary Service and the Tajik Agency on Standardization. In addition such international organizations like International Epizootic Bureau, UN FAO, closely cooperate and make contribution in ensuring public health.

The activity of FAO together with other related institutions to support Tajikistan structures dealing with animal health is based and closely connected with several projects like, improving cattle production and rehabilitation of pastures, improving living conditions in the rural areas, combating brucellosis, monitoring of Avian Influenza, preparedness to react to pandemic, monitoring spread of the diseases within the borders of Central Asian states and projects on management of trans-border rivers' basins.

The aim of these projects is in supporting the State Veterinary Service to promote private veterinary service, improve food security issue (especially among vulnerable households), increase income and improve living conditions of the residents of the country. It is also aimed to set the program of preventive measures as

well as a better cooperation between five neighboring states to set barriers on the area of distribution of diseases.

In accordance with the Program of FAO the Association of Veterinarians of Tajikistan (AVT) was created with its branches being set in all regions to provide support and set training programs for all members of the association. Now the AVT and its service units are able to operate independently.

The Veterinary Institute of Academy of Agricultural Science of the Republic of Tajikistan (AASRT) is an important institution with its research laboratories of virusology, brucellosis, honey bee and poultry diseases, horse diseases and TB. To the activity the institute has close ties with SVST, scientific-research institutes of the country and foreign veterinary centers, like Russian Scientific Research Institute of Virusology, Microbiology and Veterinary, Pokrov City, Russian Experimental Veterinary Institute, Moscow, Russian Scientific Research Institute on Cattle Health. In accordance with the project TAD /5/003 training of 6 experts on brucellosis detection on cattle was conducted at the Veterinary Faculty of the University of Kirill and Methodius, Macedonia, in 2007-2009. This training gave the experts an opportunity to gain new skills and practices on detection of other infectious diseases. The Veterinary Institute conducts the epizootic situations of the farms and economies under the bilateral agreements. The results of the activity of the experts of the institute would be even more beneficial by using modern technologies in their activity and being able to update their knowledge and skills.

In general current and future of the veterinary service of the country depends on close cooperation with veterinary structures, in particular Veterinary Institute of AASRT with other developed veterinary centers. In short, development of the veterinary service is the requirement of current period demanding training and preparation of highly qualified specialists and updating the knowledge of both the experts and the public.

CEREBELLAR HYPOPLASIA AND HYDROCEPHALUS IN LAMBS

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Introduction

Malformations of the nervous system are common and their variety is greater than in the other tissues. Cerebellar hypoplasia seen in many species is one of the most common congenital nervous system defects in domestic animals. Hydrocephalus is characterized by an abnormal accumulation of fluid in cranial cavity. The aim of the present study was to determine the prevalence and the pathological morphology of

cerebellar hypoplasia and hydrocephalus in lambs during 2 lambing seasons in this region.

Materials and Methods

For this reason, cerebellar hypoplasia and hydrocephalus were investigated in a total of 11700 births whose 472 abortion/stillbirth cases from 26 sheep flocks during 2 lambing seasons and brain samples were examined histopathologically.

Results

These brain anomalies were only diagnosed in 6 flocks mainly performing extensive breeding. The prevalences of cerebellar hypoplasia and hydrocephalus in abortion/stillbirth cases were 2.33% and 1.69% respectively and were relative high for congenital defects. Three histological (microscopic, intermediate and severe) types of cerebellar hypoplasia were identified, the most frequently observed being the intermediate type (56.25% of hypoplasia cases). Three microscopic and 2 intermediate types were encountered also in alive lambs as well as one case of hydrocephalus.

Conclusion: These results suggest that the two brain anomalies were probably linked to inherited disorders coupled to some toxic and infectious causes.

Key words: Cerebellar hypoplasia, hydrocephalus, lamb, pathology

THE IMPACT OF COPPER AND ZINC DEFICIENCY ON MILK PRODUCTION PERFORMANCES IN DAIRY COWS FROM THE NORTH-EAST OF ROMANIA

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The incidence of copper and zinc deficiencies in dairy cows are little known in Romania. It is, though, known that some soils, e.g. those in the North-Eastern Romania, are trace elements unbalanced and thus, the forages harvested from such areas are deficient in some minerals. Feeding dairy cows with such feedstuffs can induce some mineral deficiencies. Once animals are deficient, their products, especially milk, are also deficient. Supplementation of the cattle feeds with trace elements can improve the mineral status of

animals and thus their products are more minerally balanced.

A 20-week experiment was undertaken on a farm in North-East of Romania. Forty subjects groups of a local Holstein-Friesian mix bred lactating cows (copper, zinc, copper-zinc and control groups) were formed. The goal of the experiment was the evaluation of the zinc and copper status during the daily feeding of the improved diets (8.5 ppm copper and 42 ppm zinc). Correlations of the Cu and Zn plasma values with some animal performance criteria of health and production were done. During the 140 days of the experiment, the two added minerals caused a statistically significant increase ($P < 0.05$) of their plasma values after the peak of the cows' lactations. It was also observed that subjects that have received copper and zinc supplements had the lowest number of somatic cell counts in milk. The Pearson correlation test showed a positive correlation ($P = 0.007$, $r = +0.451$) between the plasma Zn and the milk production. The improvement of the nutritional status improved the milk production performances of the cattle as well as their health performances.

Key words: dairy cows, copper, zinc, health, milk production

BLOOD GLUCOSE LEVELS IN DOGS WITH VARIOUS FORMS OF ACUTE PANCREATITIS

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Introduction:

The early diagnosis of pancreatic necroses is essential for adequate and efficient treatment. Contrast-enhanced computed tomography, accepted as a reference method for detection of necrotic pancreatic foci in human medicine, is very expensive for veterinary medicine. The purpose of this study was to establish the diagnostic and prognostic value of blood glucose concentrations in various forms of canine acute pancreatitis.

Material and Methods:

Seventeen dogs with spontaneous pancreatitis (group A), and 12 dogs with experimentally induced pancreatitis (groups B and C) were used. Group A was further divided into three subgroups depending on the pancreatitis severity: subgroup A₁: 7 dogs with acute pancreatitis (AP); subgroup A₂: 6 dogs with acute necrotising pancreatitis (ANP) and subgroup A₃: 4

dogs with acute necrotising haemorrhagic pancreatitis (ANHP). Group B included 6 dogs with experimental pancreatitis induced by ligation of *ductus pancreaticus*. Group C comprised 6 dogs with experimental pancreatitis induced by introduction of oleic acid.

Results:

The blood glucose concentrations in dogs with spontaneous acute pancreatitis were significantly higher than the usual values determined in dogs. Glycaemia was superior to 8.0 mmol/L in 11 dogs from this group (64.7%). It was observed that the blood glucose concentrations were dramatically elevated in dogs with acute necrotising pancreatitis: 5 (83.33%) and 3 (75%) dogs from the subgroups A₂ and A₃ respectively exhibited a glycaemia above 8.0 mmol/L. In spontaneous cases, 72.2% of dogs with blood glucose levels over 8 mmol/L had pancreatic necroses, whereas those with blood glucose < 8 mmol/L, exhibited necroses in only 11%.

When acute pancreatitis was surgically induced by ligation of the pancreatic ducts (group B), the mean blood glucose concentrations were markedly depressed 24 and 48 hours post surgery compared to initial values ($p < 0.01$) then slightly increased at 72 and 96 hours but still remained significantly below the basal values ($p < 0.01$). By contrast, in the group C in which the acute pancreatitis was chemically induced, glycaemia tended to increase since the 48 hours and reached maximal value (6.4 mmol/L) at the 72nd hours but differences with the basal values were not significant.

As a conclusion, although the glycaemia presents a low diagnostic value and has to be determined simultaneously than other biochemical and haematological parameters such as amylase and lipase activities, concentrations of acute phase proteins and blood leukocyte counts for example, high circulating glucose concentrations in spontaneous acute pancreatitis may be considered as a bad prognostic sign and corollary the probability that a subject with glycaemia included into the usual ranges develops necrotizing pancreatitis appears as very weak.

COMPARATIVE IMAGING ANATOMIC STUDY OF DOMESTIC RABBIT LIVER (ORYCTOLAGUS CUNICULUS)

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Intorduction

Ultrasonography and computed tomography are widely applicable, conventional, non-invasive methods for visualization the normal characteristics of the small animals' abdominal organs. The aim of the present study is to present some imaging anatomic features of the rabbit liver and to compare them with the native morphology of this organ.

Materials and methods

The liver of 7 sexually mature, healthy male New Zealand White rabbits, at the age of 12 months, weighing 2,8 kg - 3,2 kg was studied.

The ultrasonographic study was made following anesthesia. The animals were positioned in dorsal recumbence. The sonographic approach was transabdominal, peicutaneous hypochondriac.

The computed tomographic study was performed via axial computed tomography. The animals were sedated and positioned in dorsal recumbence. The abdominal cavity was scanned in the transversal planes from the eighth thoracic vertebra (Th8) to the third lumbar vertebra (L3) and the cuts' thickness was 8 mm.

Frozen transversal cuts of the cranial abdominal part were taken from five animals. The liver was extirpated and studied out of the trunk. The imaging anatomic features of the organ were compared with these of its normal morphology.

Results

The ultrasonographic study showed that the liver was a hypoechoic structure compared to the adjacent soft tissues. It was located close to the hyperechoic diaphragm and its limits were regular and smooth. The parenchymal echogeneity was heterogeneous and there were hyperechoic linear findings with lateral position. The portal vein's branches were with more echoic walls and lumen, compared to the same structures of hepatic veins.

The computed tomographic study found that the rabbit liver was massive, heterogeneous, normodense, soft tissue finding with well outlined limits. There wasn't visible limit between Lobus hepatis sinister lateralis et medialis and Lobus hepatis dexter. The liver parenchyma was normodense, compared to the hypodense hepatic veins.

Conclusions

The results of the ultrasonographic and computed tomographic study were analogous with the morphological features of the liver in the transversal abdominal frozen cuts. The obtained data from these investigations could be use as a basis for interpretation of some rabbit and human liver diseases.

Key words: liver, ultrasonography, computed tomograohy, anatomy, rabbit

COMPARISON OF DIFFERENT ANAESTHETIC PROTOCOLS IN CARP (CYPRINUS CARPIO) FOR MEASUREMENT OF GROWTH PERFORMANCES

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Introduction

Measurement of growth performances in fish is carried out in stressful and unnatural environment. To avoid any damage to the fish and, in some cases to the handler, anaesthesia is useful tool to the fish practitioner and enables various tasks to be performed. Fish often struggle in most forms of restrain and handling, so anaesthesia can greatly facilitate examination, transport and diagnostic sampling as well as reducing stress to the fish. Waterborne anaesthesia is the most widely used method of fish anaesthesia and is analogous to gaseous anaesthesia in mammals. The drug solution is ventilated by the fish and drug molecules diffuse rapidly into the blood spaces in the secondary lamellae, which drain into the efferent arterial blood from where it is a very short route to the central nervous system.

Materials and Methods

The experiment was performed in the Experimental Aquaculture Base, Trakia University – Stara Zagora, during May-June 2009. The fish were divided into four groups and stocked at a density of 3 in each of the glass tanks (50X35X30 cm.) containing 40 L of dechlorinated bore water. Adequate level of oxygen in each tank was maintained through aeration. The average weight of fish (Ave. wt. 40.3±0.12 g) at the beginning of the experiment was recorded. During experiment water quality parameters (pH, O₂, T, NO₃) were recorded. Water temperature range (t°) from 25.7° – 26.1° C, nitrate concentration (NO₃) from 10 – 12 ppt, free and total chlorine (Cl₂) 0.00 ml, oxygen concentration (O₂) between 9.2 – 10.9 ppm and (pH) 6.8 – 7.3. Prior to anaesthesia, food was withhold for 24 hours to minimize risk of vomiting. For first group, clove oil and its active ingredient- eugenol dissolved in 95% ethanol is used. Anaesthetic agents used for other three groups are as follows: second group- lidocaine 1%, III group- isoflourane, IV group- halothane. The depth of anaesthesia was monitored by observing the behavior of the fish in water. The desired concentration of anaesthetics was established and induction time, maintenance and recovery time were recorded.

Results

In two groups out of four (II and IV) time of induction was longer approximately with 4 minutes and time of recovery was shorter with 2 min and 30 seconds in comparison with other two groups (I and III) where the time for both was 3 minutes.

Conclusion

In our study, it could be concluded that each of the used anaesthetic protocols ensures enough anaesthetic time of approximately 4-5 min for measurement of growth performances. On the basis of anaesthetic effectiveness, fish welfare, environment safety and economical effect, we recommend the anaesthetic protocol using clove oil.

DETECTION OF *PAENIBACILLUS* LARVAE – THE CAUSE OF AMERICAN FOULBROOD DISEASE BY MULTIPLEX POLYMERASE CHAIN REACTION (MULTIPLEX PCR)

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Introduction

Paenibacillus larvae is the causative agent of American foulbrood, the most deleterious bacterial honey bee disease worldwide. The disease gives rise to death to affected bee colonies, causing considerable economic losses. Timely detection of *Paenibacillus larvae* is of great importance to prevent dissemination of infection. Therefore, in recent years interest of scientists has been focused on recovering new PCR techniques for establishment of the etiological agent. The present study was performed to develop a fast and reliable multiplex polymerase chain reaction for diagnosing of American foulbrood.

Materials and methods

A reference strain *Paenibacillus larvae* LMG 9820, 40 clinical isolates from bee combs suspicious for American foulbrood and four strains of closely related bacterial species were included in the experiment as well as putrid masses. Three pairs of primers specific for 16S RNA and one pair of primers specific for 35

kDa metalloprotease genes of *Paenibacillus larvae* were tested. DNA was isolated by prepGem extraction kit for gram-positive and gram-negative bacteria and by heat treatment of suspensions following by a centrifugation step. Amplified products were electrophoresed in 1.0 % agarose containing ethidium bromide and visualized on gel documentary system. The sensitivity of the method was also determined.

Results

The used PCR primers selectively amplify 973-bp; 965-bp and 665-bp amplicon of 16S RNA and 273-bp amplicon of 35 kDa metalloprotease genes. We used these primers in different combinations for multiplex PCR protocol for detection of *Paenibacillus larvae*. The suitable annealing temperature was determined by gradient PCR. The highest specificity was detected for multiplex PCR with primers giving amplicons with length of 973-bp and 273-bp, simultaneously for 16S RNA and 35 kDa metalloprotease. A positive reaction was also observed when DNA was extracted directly from putrid masses. No specific PCR products were obtained when control PCR analyses were performed with closely related bacterial species. The sensitivity of the method was less than 15 ng/μL DNA.

Conclusion

The developed multiplex PCR protocol could be successfully used for rapid detection of *Paenibacillus larvae* in both isolated colonies and putrid masses

COMBINED VETERINARY COMPETENCIES IN FOOD SAFETY, ANIMAL HEALTH AND WELFARE

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Veterinary public health (VPH) is an essential part of public health and includes various types of cooperation between the disciplines that link the health triad, people-animals-environment, and all of its interactions. VPH is defined by World Health Organisation (WHO) as “the sum of all contributions to the physical, mental and social well-being of humans through an understanding and application of veterinary science” (WHO website). By its very nature, the VPH has been closely linked with various aspects of the work of other international umbrella organisations, Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) in relation to zoonoses, food safety, and the public health aspects of trade in animals and animal products.

Veterinarians, and allied professionals, play crucial – indeed central – role in maintaining and improving

veterinary public health globally. Nearly 75% of the new diseases that have affected humans over the past 10 years have been caused by pathogens originating from an animal or from products of animal origin. Many of these diseases have the potential to spread through various means over long distances and to become global problems. In dealing with the new developments and challenges in today's world, veterinarians are therefore required to be competent for the jobs they are expected to perform.

There are many definitions of "competence" and many views about how it can be developed and assessed. In general terms, however, competence is a concept that integrates knowledge, skills and attitudes, the application of which enables the professional to perform effectively, and to respond to contingencies, change and the unexpected. The Royal College Of Veterinary Surgeons, UK's veterinary registration body and regulator of educational, ethical and clinical standards, takes a broad definition of competence as being "the ability to perform the roles and tasks required by one's job to the expected standard" (Eraut & Boulay, 2000). The essential competences have been broken down into three main areas, and these are reflected in both "day one" and "year one" requirements.

These are:

- 1) General professional competences and attributes
- 2) Underpinning knowledge and understanding
- 3) Practically- based veterinary competences.

With the progression of one's veterinary career, the veterinary competence level improves and in many cases becomes more specialised. The required competence level will broadly depend on roles and tasks that are required to be performed by a veterinarian. World's administrative terminology generally describes animal health, public health and/or welfare of animals. However, by looking at a sick animal and its interaction with other animals, humans and environment it is not always easy for a veterinarian to find an appropriate "administrative box" for the case being dealt with. An approach will simply depend on whether one lives in the developing or developed world.

In my talk I will explain how veterinary competence in public health, animal health and welfare has been defined and agreed in the UK. I will base my talk on roles, responsibilities and current challenges of the Food Standards Agency, the UK CA for food and feed, and DEFRA, the UK CA for animal health and welfare. I will also briefly describe the way how the veterinary profession in the UK cooperates, collaborates and improves its professional standing and reputation both nationally and internationally.

Further reading/sources of information:

<http://www.who.int/en/>

• <http://www.who.int/zoonoses/vph/en/>

• http://whqlibdoc.who.int/trs/WHO_TRS_907.pdf

<http://www.fao.org/>

<http://www.oie.int/en/>

<http://www.rcvs.org.uk/home/>

• <http://www.rcvs.org.uk/education/professional-development-phase-pdp/day-and-year-one-competences/>

• <http://www.informatics.sussex.ac.uk/users/bend/doh/>

<http://www.food.gov.uk/>

<http://www.defra.gov.uk/>

VALIDATION OF TWO RECEPTOR TESTS: TWINSSENSOR AND TETRASENSOR FOR SCREENING ANTIMICROBIAL SUBSTANCES IN MILK AND HONEY

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Antimicrobial drugs have been used in dairy industry for more than five decades. They are principally administered to prevent or treat udder infections. Residues of antibiotics in honey are found mostly due to use of tetracyclines in apiculture for the treatment of bacterial brood diseases (American foulbrood and European foulbrood). The use of antibiotics in apiculture is not authorized in the European Union (EU). The presence of antimicrobial drug residues in food is a public health issue.

A new competitive receptor test Twinsensor^{BT} and an improved Tetrasensor test (Unisensor S.A., Belgium) were validated. Both tests were designed for screening antimicrobial substances in the food of animal origin: Twinsensor^{BT} test is used for β -lactams and tetracyclines in milk and Tetrasensor for tetracyclines in honey. The performance criteria described by the Commission Decision 2002/657/EC and Guide for analytical validation of screening methods (AFSSA, Fougères) were used for the validation study.

The Twinsensor^{BT} test was found to be easy to use, with very short incubation period (6 minutes), robust and sensitive to all certified β -lactams and tetracyclines at or lower concentrations than EU maximum residue limits (MRLs) except for Nafcillin.

Tetrasensor has a longer incubation period (half an hour) and detection capabilities (CC β) below 15 μgkg^{-1} were obtained, depending on the type of tetracycline and the honey sample. A provisional MRL for oxytetracycline in honey is 25 μgkg^{-1} .

Keywords: *Twinsensor, Tetrasensor, Receptor test, Milk, Honey, Antibiotics, Veterinary drug residues, Food safety*

STAPHYLOCOCCUS AUREUS MASTITIS - DO WE REALLY HAVE TO LIVE WITH IT?

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Staphylococcus aureus (*S. aureus*) is still one of the most prevalent mastitis pathogens in dairy herds all over the world. Effective and economic *S. aureus* control programs rely on prevention rather than treatment. Since the introduction of the standard mastitis prevention program, much progress has been achieved in decreasing the prevalence of intramammary infections (IMI). However, at the farm level, staphylococcal mastitis remains the disease causing the highest financial losses.

Among *S. aureus* strains isolated from the bovine mammary gland resistance to penicillin increased rapidly from approximately 20% in 1965 to 45% in the mid 70s and decreased again in the 1990s to approximately 30%. Although the therapeutic value of penicillin is limited in many countries, there are still sufficient antimicrobials available for treatment of *S. aureus* IMI. Currently there are no founded indications that methicillin-resistant *S. aureus* (MRSA) strains are involved in bovine mastitis. To control *S. aureus* mastitis at the farm level complex measurements are needed, which involves strategies for treatment of existing infections and also prevention of new mastitis cases should be implemented.

Key words: *Staphylococcus aureus*; mastitis; cows

FINDINGS AND THE SENSITIVITY OF COAGULASE-NEGATIVE STAPHYLOCOCCI IN THE HERD WITH AN INCREASED NUMBER OF SOMATIC CELLS

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Mastitis is one of the most common and expensive diseases in dairy production. One of the control measures against mastitis is constantly monitoring the number of somatic cells in bulk milk, since the number of somatic cells in milk from farms is in correlation with subclinical mastitis in the herd. In recent years,

it is increasingly proving that coagulase-negative staphylococci are most dominant cause of subclinical and clinical mastitis.

From individual udder quarters where mastitis test (previously performed on 112 cows) showed an increase of somatic cell, samples of milk were taken from individual udder quarters. Milk samples were immediately streaked after sampling on blood agar with esculin and ferric citrate and Endo agar. Incubation was performed for 24 hours at 37°C, but in cases where there was no growth on the plates the incubation was prolonged for up to 48 hours. Identification of microorganisms grown colonies was based on colony appearance, hemolysis, morphological findings, catalase test and the test of free and bound coagulase.

For all isolates antibiogram disk diffusion method was performed according to the Kirby Bauer method. For the susceptibility testing of gram-positive microorganisms next drives were used: penicillin 6µg, amoxicillin/clavulanic acid (20+10 mg), cloxacillin 25µg, amoxicillin 30µg, cephalexin 30µg, ceftiofur 30µg, lincomycin 15µg, gentamicin 30µg and tetracycline 30µg. Susceptibility of microorganisms was evaluated on the basis of inhibition zone diameters according to the manufacturer and was marked as sensitive (S) moderately sensitive (I) or resistant (R).

The results of the mastitis test, done on the farm where the number of somatic cells in milk increased, showing that of 112 examined cows mastitis test positive reactions found in milk from 52 (11.60%) quarters of 42 cows. Bacteriological examination of 52 milk samples taken from individual udder quarters resulted in identification of gram positive microorganisms from 37 (71.15%) milk samples, while the gram negative organisms were isolated from only two (3.84%) milk samples.

A total of 39 pathogens were isolated: 24 (61.53%) coagulase-negative staphylococci, 8 (20.51%) coagulase-positive staphylococci, 5 (12.82%) *St. agalactiae*, 1 (2.56%) *Klebsiella spp.* and 1 (2.56%) *E. Coli*.

The highest resistance of coagulase-negative staphylococci was found to penicillin G. Complete sensitivity of coagulase-positive staphylococci was found to amoxicillin / clavulanic acid, and good sensitivity is determined to ceftiofur, gentamicin and tetracycline.

DIFFERENT LEVELS OF *LISTERIA* *MONOCYTOGENES* IN READY-TO- EAT FOODS AND THE RELATED RISK FOR HUMAN ILLNESS

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Increase in the number of listeriosis cases, since 2000, is predominantly a food borne disease. The foods which could be associated with transmission of listeriosis were mostly ready-to-eat foods that support growth of *Listeria monocytogenes*. Application of microbiological criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for humans. Microbiological criteria will assist in control link the levels of *Listeria monocytogenes* e.g. absence in 25 g or ≤ 100 cfu/g at the point of consumption. Recent risk assessment concluded that most listeriosis cases are due to foods markedly above the latter limit.

The observation that some ready-to-eat foods categories were more frequently contaminated with *Listeria monocytogenes* than others does not imply that these food categories are more likely to cause listeriosis in particular it would be necessary to estimate whether these foods support growth of *Listeria monocytogenes* when sampling accrued in relation to shelf life and whether they undergo any listericidal treatment before consumption. Consumption data of ready-to-eat foods that support growth of *Listeria monocytogenes* are needed for better assessment of risks.

Key words: Ready-to-eat foods, *Listeria monocytogenes*, criteria

COMPARISON OF THE SURVIVAL AND TRANSFER ABILITY OF *SALMONELLA ENTERITIDIS* AND *SALMONELLA TYPHIMURIUM* FROM DOMESTIC FOOD CONTACT SURFACES

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Food contact surfaces in the domestic kitchens can easily be contaminated with pathogens, which can present a potential source for cross-contamination during food preparation.

In our study, we inoculated *S. enteritidis* and *S. typhimurium* strains on 3 food contact surfaces (stainless steel, plastic and wooden cutting board), in the presence and absence of organic matter (BPW). We analysed the concentrations of the strains on the surfaces during a period of 6 hours (from the inoculation up to every hour) at room temperature

by swab sampling technique and the transfer of the pathogen to a food model (chicken ham) at the 6-th hour.

Regarding all three contact surfaces, the concentration of the *Salmonella* strains was decreasing during the period of 6 hours, which was most evident in the first two hours, particularly for the wooden surface. In the presence of organic matter (BPW), the concentrations were increasing significantly after 3 hours. The recovery of the bacteria from the wooden surface was less than those from steel and plastic surface, because of the rapid absorption of the inoculum. There was a small difference between the *S. enteritidis* and *S. typhimurium* strains analysed in this study.

The results demonstrate the survival of the pathogens on various types of food contact surfaces and their ability easily to be transferred to food and cause a foodborne disease.

Key words: food contact surfaces, pathogens, *S. enteritidis*, *S. Typhimurium*

ANIMAL WELFARE – WHAT IT IS AND HOW TO MEASURE IT

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Animal welfare is a difficult and contentious subject in modern society and so it is important to explore what is meant by the term. Many consider that welfare relates principally to an animal's feelings (see Duncan and Fraser 1997) such that only sentient animals can experience good or poor welfare. Defining animal welfare in this way is difficult, as feelings cannot be measured directly. Approaches to welfare assessment that measures animal emotions or animal preferences and priorities are the best methods currently available that give us some possible insight into animal feelings. An alternative idea, closest to the position held by many members of the public, is that the key element for good welfare is whether or not the animal is leading a natural life. However, genetic and experiential differences between modern and ancestral species, together with empirical evidence, suggests that farm animals can have good welfare in what we might think of as 'unnatural' environments, or poor welfare in 'natural' environments. A third approach to the assessment of animal welfare asks whether farm animals are coping with their environment (Broom, 1986), something that can be assessed by examining physical health, physiological and behavioural responses. Phillips (2009) argues that we should assess whether animals are thriving in their environment rather than merely coping, but the principle remains the same.

Despite differing cultural attitudes and conceptual

difficulties, some key points are generally agreed upon when it comes to measuring animal welfare. First, animal welfare is a concept that relates to individual animals not to groups or populations, so herd or flock-level data have to be interpreted with great care. Second, animal welfare can range from very good to very poor; it is not an all-or-nothing characteristic. Third, animal welfare can best be assessed by taking animal-based measures rather than simply recording the dimensions of housing systems or the presence or absence of facilities or resources. Fourth, a wide range of measures should be taken to cover both physical and mental well-being (Botreau et al. 2007). This is highlighted by many practical schemes that have been devised for on-farm use, from the Five Freedoms approach (Webster 1995) to the newer Welfare Quality® protocols (<http://www.welfarequality.net>). Fifth, expert opinion can provide important practical insights for developing auditing methods that work (Phythian et al., 2011). However, cross-validation of measures taken from different approaches e.g. by linking physical welfare indicators with the choices or preferences expressed by farm animals (Nicol et al., 2009), or by assessing how expert opinion correlates with independent measures is still needed to provide increased confidence in animal welfare assessment methodology.

SHEEP WELFARE: CONCEPTS, ISSUES AND RESEARCH

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Sheep are the most diverse farmed species by purpose (milk, meat, fibre), breed, and husbandry. However, with the exception of dairy sheep in some systems, sheep are always farmed in systems that are extensive (outdoors and able to obtain at least some of their diet from the environment) for all or part of the year. Welfare assessment protocols for sheep, therefore, need to be effective across this diversity of production systems. The welfare of sheep is generally assumed to be good, particularly in comparison to other species farmed under more confined conditions. This appears to be because of their greater freedom to express most of their natural behavioural repertoire. They are, however, subject to a lower frequency of inspection, leaving them vulnerable to untreated disease and injury, variable provision of food, water, and variable thermal and physical comfort. In addition, sheep are subject to painful husbandry procedures (e.g. castration, tail-docking) and can experience fear and distress through predation and during handling and management. Unlike more intensively managed species, where the human-animal relationship is expressed in daily

contact, in extensively managed species farmers may manage the environment and expect the sheep to cope without daily interactions. The welfare state of the animal in this situation depends on how effectively it can cope with resulting environmental challenges. Breed variation in, for example, responses to predation or expression of maternal care and the formation of mother-offspring attachment, can affect welfare, thus good welfare depends on appropriate matching of animals to the environment. In addition, the low level of human interaction in extensive systems can create welfare problems when animals need to be inspected, or in the provision of individualised care. Gathering and animal movement are usually brought about by inducing fear and a flight response, often by using dogs, and most handling is aversive. Although handler behaviour can reduce the stress caused by these interactions, many farmers under-estimate the impact that gathering or handling can have on the sheep. Although the sheep have opportunities to express positive welfare, and may be in very good welfare in some husbandry systems, they are also vulnerable to extremely poor welfare, which can be exacerbated by infrequent inspection and lack of treatment. Provision of good welfare relies on effective environmental management and risk assessment, good stockperson skills to understand, recognise and identify welfare problems, and a prompt response to provide treatment or mitigate welfare challenges.

PAIN IN CATTLE: A REVIEW OF RESEARCH FROM THE LAST DECADE WITH PARTICULAR REFERENCE TO LAMENESS IN DAIRY CATTLE

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The 18th century philosopher Jeremy Bentham said of animals "...the question is not, Can they reason? nor, Can they talk? but, Can they suffer?". This widely used quote from Bentham describes the view that it is not necessary to judge animals' abilities by our own standards, i.e. whether they have speech or sophisticated decision making capacities, but that we should be most concerned about how they feel and whether they themselves are alright. The International Association for the Study of Pain (IASP) definition outlines that; [pain is] "*an unpleasant sensory and emotional experience with actual or potential tissue damage*" (1). It is important to note that this definition recognizes that pain has an emotional as well as physical component; this implies that some level of

consciousness is required to fully experience pain in the way that humans do. Interestingly, despite the amount of value put on whether animals can have experiences akin to humans, it is only relatively recently that medical science has recognized that all adult humans experience pain to a similar degree regardless of race, gender and wealth. Even now the debate continues as to the levels of pain experienced by neonates. This uncertainty about whether neonates can experience pain illustrates the problem that we have to overcome when trying to understand whether non-human animals feel pain. It means that a) despite the obvious merit of exercising the precautionary principle it is still not standard practice in all neonatal care units to provide analgesia when dealing with poorly babies, and b) when examining the reason for this uncertainty about human neonates ability to suffer pain much of the problem seems to be that because young children cannot communicate through language there is room for doubt as to their actual pain experiences.

This paper will consider some aspects of the ethical debate and scientific evidence that contribute towards our now widely held belief that animals do suffer pain. It will look at the effects of pain in cattle and review an effective integrated approach to the management of pain associated with lameness in dairy cattle. Further to this it will consider how the perceptions and attitudes of humans towards pain in animals influence their actions and the likelihood of them taking action to relieve suffering.

STRATEGIES TO IMPROVE ANIMAL WELFARE AT FARM LEVEL

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Animal welfare is an important aspect of sustainable animal production. A high level of animal welfare may primarily be achieved through appropriate housing conditions and management including the use of appropriate breeds, feeding and herd management practices. Welfare improvement strategies in commercial farms generally require three steps: 1) welfare assessment, 2) implementation of housing and management changes and 3), re-evaluation of the welfare state. Feasible on-farm welfare assessment protocols such as the Welfare Quality® protocols or the Bristol Welfare Assurance Programme protocols have recently been developed. They are based on a complimentary list of valid animal-based welfare indicators and have been tested with regard to reliability and feasibility. Such mostly animal-related information on the welfare state may be accompanied by information on herd

management and housing conditions e.g. using check-lists and questionnaires. Successful implementation of measures with the aim to improve animal welfare requires the joint evaluation of welfare outcomes and potential risk factors potentially leading to the welfare problems. Several studies have shown that compliance in terms of actual implementation of changes is crucial for welfare improvement, and there may be several ways to achieve this compliance. Interventions have to motivate the farmer or stockperson to make changes for the benefit of their animals. Herd health and welfare plans, which build on the steps described above, are one promising approach. Finally, re-assessment of the welfare state is important in order to assess whether a welfare improvement has actually been achieved and if interventions may have to be adapted or new welfare problems have developed since the last assessment.

*This presentation is a part of **AWARE project**
(FP7-KBBE-2010.1.3-04) roadshow

FARM ANIMAL WELFARE TEACHING IN THE EU

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The Federation of Veterinarians in Europe recently summarised the state of farm animal welfare teaching in European Veterinary Schools. Their analysis revealed a very large variation in both the hours devoted to this area of teaching, and in the specific subjects covered. The challenges and opportunities facing European veterinary schools in developing a more integrated animal welfare curriculum will be presented.

Farm animal welfare teaching may also take place in institutions other than veterinary faculties, for example in agricultural or zoological faculties. Overall, there is very little collated information about the nature and scope of farm animal welfare teaching across a range of different faculties in Europe. A mutual aim of the AWARE project and the EU VII Framework programme AWIN (<http://www.animal-welfare-indicators.net/>) is to investigate and record the extent of farm animal welfare teaching activities across EU and other European countries. This mapping exercise will be followed by suggestions for improving and integrating the teaching of farm animal welfare. In this talk I will describe some of the methods used to undertake the mapping task, and present any preliminary information available from initial questionnaires distributed as part of the AWARE programme.

Preliminary evidence suggests that many institutions are expanding their teaching of animal welfare and in some countries it is now possible to complete a full undergraduate degree in this topic. One example is

the Animal Behaviour and Welfare BSc degree taught at the University of Bristol in the UK (http://www.bristol.ac.uk/prospectus/undergraduate/2011/sections/XABW/dept_intro).

The curriculum and methods of teaching used in this programme will be described as one example of how the subject can be approached at an undergraduate level. Examples of post-graduate programmes now available for veterinarians and others within the EU will also be presented.

*This presentation is a part of **AWARE project**
(FP7-KBBE-2010.1.3-04) roadshow

GENERAL ATTITUDES FOR FARM ANIMAL WELFARE STANDARDS IN R. MACEDONIA

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Understanding current position of the main actors in the supply chain considering farm animal welfare is the groundwork for implementation and improvement of animal welfare in Macedonia. Therefore, the main goal of this paper is to present their attitudes and opinions regarding acceptance of farm animal welfare in the society and development of appropriate legislation. Representatives from different categories in the supply chain (farmers, producers and consumers) participated at the organized “National workshop for current and upgraded standards of animal welfare in the supply chain” where answering the questions from the developed questioner expressed their views. This paper presents the processed data and analysis obtained from the answers of main actors in the supply chain.

All categories of the production chain (farmers, processors and manufacturers) presented very low rating to society attitude toward farm animal welfare. Particularly important is the low level of media attention to this issue, which is the basis for the absence of initiatives in the area of welfare, implementation of legal measures and the education of consumers. At the moment all representatives, are agreeable that particular attention should be given to implementation of welfare standards in almost all categories of farm animals in Macedonia (especially poultry and pigs). A positive approach is given to labelling the products manufactured by animal welfare standards. There is a conflict between farmers and producers/consumers regarding the requirements for improving welfare standards – the requirements are too high for farmers,

contrary to the opinion of producers and consumers.

Regarding Macedonian farm animal welfare legislation, general impression is the lack of participation in the development process by the key actors. The main advantages of the legislation are primarily related to the existence of a law that regulates the protection and animal welfare, thus the initiative for its implementation in everyday farm practice is welcomed. One of the major disadvantages of the legislation is the reduced implementation of the law and regulations for protection and welfare of animals. As a main obstacle in the process of implementation are the high investments and costs for achieving the required standards. Therefore, farmers are requesting prolongation of the deadlines for implementation of certain requirements of the legislation. The absence of ethical aspects of animal protection at the outlay of the economic aspects is evident in the Macedonian legislation for farm animal welfare.

INTRODUCTION TO THE “AWARE” - PROJECT

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The Protocol on Protection and Welfare of animals annexed to the EC Treaty by the Amsterdam Treaty obliges the European Institutions to pay full regard to the welfare requirements of animals when formulating and implementing Community legislation. EU citizens have expressed a growing appreciation for high farm animal welfare standards as animal welfare can have both a direct and indirect impact on food safety and quality. However, there are marked differences between states and between geographical regions in the general perception and understanding of farm animal welfare as well as in the feeling of being able to influence animal welfare through the willingness to pay, the informed consumer choice and through political and societal (e.g., NGOs) means. These differences, which often amount to clear gaps or divisions between Western and Eastern countries, and also between the countries in the north and the south of the European Union, also exist in implementation of animal welfare legislation. Similar differences in attitudes towards, and awareness of, animal welfare issues may be expected in associated countries, alongside significant differences in the actual welfare of farm animals. The division between the member states is partly caused by factors such as variation in living standards and production systems, the time of EU membership and differences in ‘cultural’ sensitivity and concepts about food quality. Nevertheless, the differences also stem from

informational factors like the level of knowledge about animal welfare, including its scientific basis, about animal housing conditions, and about identification and labelling systems in the farm-to-fork chain.

Overall project objective

The project has one main goal: to develop sustainable and actively expanding Europe-wide networks of farm animal welfare scientists, of farm animal welfare university lecturers and students, and of stakeholder platforms active in farm animal welfare knowledge transfer and implementation. In order to achieve this, AWARE will develop open networks of resources and information platforms in farm animal welfare science that will enhance information transfer and further improve networking ability and provide networking possibilities for farm animal welfare scientists, lecturers, students and professionals across the enlarged EU and candidate countries.

CYSTIC OVARIAN FOLLICLES

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Reproduction is one of the key targets in dairy production. Reproductive disorders including cystic ovarian follicles (COF) present a great problem in dairy cow's management. COF in cows and heifers traditionally have been defined as an anovulatory fluid-filled hollow structures having diameter ≥ 2.5 cm that persist for 10 or more days in the absence or in presence of a corpus luteum which persist in one or both ovaries and accompanied by abnormal oestrus behavior. COF are considered to be one of the most frequent and important causes of ovarian disorders and reproductive failure in modern high yielding dairy cows. COF etiology is very complex. There is no single cause of COF, an interaction between hereditary predisposition, milk yield, age, season, nutrition management, stress and negative energy balance is important in COF development. Negative energy balance are thought to be one of the most important factors contributing to the formation of COF during early puerperium. COF could be single and multiple, follicular and luteal. Clinical signs of COF depend on the extent of luteinisation of the cyst, in most cases cows with COF are anoestrus. Rectal palpation, ultrasonography and determination of progesterone concentrations in plasma or milk are common diagnostic tools for COF. By careful genetic selection, improvements have been by eliminating bulls that have sired daughters subsequently suffering from COF. Prevention of COF can be approached by identifying and eliminating the contributory causes of disorder and prophylactic use of GnRH 12-14 days after calving. By using the most recent treatment

methods it is possible to cure numerous affected cows. The choice of the treatment and success will depend on some extent upon the cyst type. It can be used GnRH, hCG alone or in combination with progestagens, progestagens and PGF_{2 α} for treatment of luteal cyst. Maybe the most efficiency treatment is the use of GnRH and PGF_{2 α} 9-11 days post treatment with GnRH no matter of cysts type. Clear understanding of COF etiology, pathogenesis and prevention contribute to better approach to reproductive management of dairy cows avoiding its incidence as much as possible.

Key words: *Cystic ovarian follicles, dairy cows, reproduction*

APPLICATION OF SEX-SORTED SEMEN IN BOVINE REPRODUCTION

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Long time the science was searching for the reliable method for controlling the sex of mammalian offspring. Recently, application of certain modern cellular methodologies has led to development of a flow cytometric system capable of differentiating and separating living X- and Y-chromosome-bearing sperm in amounts suitable for AI and therefore, commercialization of this sexing technology. Nevertheless this method is not 100% reliable, there is still significant reliability in the sex offspring. This is on the level of 90% female offspring in cows inseminated with X bearing sperm.

Why someone should use sexed sperm? Certainly, due to numerous advantages that are offered with this method. The implementation of sexed semen for the dairy industry creates many positive opportunities for rapid genetic improvement both within specific herds, as well as the entire industry. By taking advantage of sexed semen technology, heifers will be born as often as 95% of the time, instead of 49% of the time when using semen that is not sorted for sex. Therefore, using sexed semen can produce offspring of a selected sex in a reduced amount of time when compared to using unsexed semen. Advantages of using sexed semen include an increased return on investment of offspring when marketing for specific genetic traits or purposes. For a single producer breeding with sexed semen has definite strengths and advantages. Using sexed semen has been proven to produce genetically superior daughters, rapidly increase desired traits within a

herd, and subsequently creating more opportunities for the sale of dairy genetics (embryo sales and bull marketing). Additionally, sexed semen can be utilized to breed the top cows of the herd which will result in increased genetic base of the herd. Also, by using sexed semen in the top cows of the herd will help the producer to potentially receive higher return per dollar invested in this technology.

Unfortunately, as well as other biotechnological methods, using of sorted semen has some disadvantages. A major limitation remains the short viable lifespan of spermatozoa after sorting, with fertility results in all species suggesting a reduced viability in the female genital tract compared with unsorted spermatozoa, making inseminations close to the time of ovulation necessary. The other is the price of the semen. Sex sorted semen is couple of times more expensive than unsorted. Because of the higher cost and reduced conception rate, sexed semen seems more appropriate for virgin heifers, which have naturally higher conception rate than adult cows.

Instead of conclusion, we can only say that the use of sexed sementechology would have the double advantage of reducing the number of low value male dairy calves and increasing the number of more valuable beef cross calves for the beef industry.

MODIFIED OVSYNCH SYNCHRONISATION PROGRAMME COMBINED WITH VITAMINS AND MINERALS IN COWS: BIOCHEMICAL, HORMONAL AND REPRODUCTIVE TRAITS

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Introduction

The aim of present study was to investigate the effects of modified Ovsynch synchronisation programme combined with vitamins and minerals upon biochemical, hormonal and reproductive parameters for improving the reproductive performance in underfed dairy cows postpartum (pp).

Materials and Methods

Thirty-six healthy cows, aged 3-10 years-old, were used with a minimum 43 days pp. After balancing the body condition scores, BCSs (2.56 ± 0.03 , mean; 1-5 scale), animals were then divided into four trial groups, as control (Group I, n=7), vitamin ADE (II, n=10), mineral mix (III, n=9) and combination group (IV, n=10). Fourteen days prior to the synchronisation, the injections of *placebo*, vitamin (Ademin®), mineral (Minerasol®), and combined doubling injections were made accordingly. For synchronisation, the 1st GnRH, Lecirolin acetate (75 mg) was given on the 1st day (Day_{GnRH} zero). On Day_{GnRH} 8th, a PGF_{2α}, Dinoprost tromethamine- THAM salt (25 mg) was injected. The first intrauterine artificial inseminations (AIs) were performed using frozen semen between the 20th-26th h after the 2nd GnRH injection on Day_{GnRH} 10th. On Day_{AI} 12th of the 1st AI, 25 mg injection of the 3rd GnRH was given. The 2nd (n=35) and 3rd (n=15) AIs were also performed, as appropriate. Totally, three blood samples were collected on Day_{vit-min} -11th, as baseline-BL, Days_{vit-min} 22nd and 46th (vit.-min. injection as Day_{vit-min} zero). Plasma vitamins, minerals and metabolic parameters were analysed by spectrophotometry and of P₄ levels by EIA. The initial ovarian cyclicity (by pedometry), Graaf follicle/corpus luteum (CL) were monitored following each AIs. The ultimate pregnancy rate was determined at Day_{AI} 108th. For feeding, a diet (of grass hay, silage, concentrate and whole barley-trefoil forage) was given daily, but in varying contents, amounts and durations.

Results

A significant ($p < 0.05$) increase of vitamin A was observed on Day_{vit-min} 46th in Group II. Mineral levels remained virtually unchanged, except for Zn. VLDL and TG levels increased significantly on Day_{vit-min} 46th in Groups III and IV. Creatinine levels decreased significantly on Day_{vit-min} 46th, except for Group IV. Final P₄ levels were numerically higher in Group I and II than to those in others. The cyclicity varied from 50% (Group IV) to 71.4% (Group I). The cows in Group IV had 70% CL on Day_{AI} 63rd (2nd AI), as compared to those (42.9-60%) in other groups. Nevertheless, 30% of cows in Group II sustained embryogenesis.

Conclusion

Overall, the results of vitamins and/or minerals plus modified Ovsynch synchronisation programme suggest that; i) only the plasma levels of vitamin A affected favourably, ii) desirable effects of supplements upon the CL and pregnancy rate were evident in vitamin

group only, iii) the levels of glucose (tendency), some protein and lipids were changed by negative energy balance (NEB), and iv) the low initial cyclicity and poor BCS seemed to diminish the overall success rate of oestrus synchronisation in underfed dairy cows pp.

Key words: cow, synchronisation, vitamin, mineral, metabolism, reproduction, NEB

ARTIFICIAL INSEMINATION OF DAIRY GOATS

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Artificial insemination (AI) is method of improving the genetic base of the herd, diminishing number of bucks on the farm, decrease transmission of diseases and improvement of herd management. If a producer's objective is only to impregnate the goats, less costly option is to invest in a quality buck. When oestrus and ovulation are induced, does can be inseminated at any time of the year (if frozen-thawed semen is provided). Daylight or photoperiod is the most important factor related to reproduction. Oestrus appears when days get short; goats cycle every 18-21 days (seasonal polyestrous) until the days become longer or pregnancy occurs. The cycling start in August and end in January. The length of the breeding season is influenced by photoperiod, breed type and nutrition levels.

Generally speaking, AI should take place 12-15 hours after onset of oestrus or standing heat (cervical mucus changes from clear to slightly cloudy and stringy) if detected by teaser buck (buck with apron) and re-inseminated again 12h later if still in heat. Goats ovulate at late estrous or shortly after the end of standing heat. AI in goats is performed (trans)cervically (very gently!; better deposit the semen at the cervical entrance then provoke lesions in order to reach deeper). The semen is deposited 1-2cm deep into the cervical canal in a 60% of goats or even into the uterus (10-30% goats). The goat's hindquarters are elevated. The lubricated vaginal speculum with light source is introduced (perineal region well washed, rinsed and dried). After cervix is visualized, the insemination pipette is introduced into the cervix.

The straws are thawed in water bath (37-38°C, 15-30s). The desired number of motile sperm is 200 million sperm in frozen semen (150 fresh). The most standardised procedures for the IA are progestagen based methods.

When "long treatment" is performed, sponges are withdrawn 17-21 days after insertion with injection of 400-500 i.u. of PMSG (depending on milk production and seasonal period, doelings half-doses) at sponge removal during breeding season or 24-48h before in transition period or out of breeding season. Optimal time for fixed-time AI is at 43±2h after sponge removal. Better results are achieved if "short treatment" is used. The sponges are inserted for 11 days and PMSG and a PGF_{2α} (50µg; 0.2mL) 48h before withdrawal. The heat is detected 28-30h after removal of sponges and AI performed 43±2h after sponge withdrawal.

The general fertility rates ranges from 50-70% with higher rates when short treatment is applied. The fertility rates after IA are low (30%) if oestrus appears after 30h after sponge removal. It is important to inseminate only the animals that are confirmed to be in oestrus!

The stress should be avoided before, during and after AI!

INFERTILITY IN THE BITCH - CURRENT APPROACHES

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Infertility in the bitch refers to the inability to conceive and produce viable offspring. The majority of bitches that owners bring to a veterinarian for reproductive problem are actually healthy because the most common cause of infertility is inappropriate breeding management. Anamnestic data of bitches examined for infertility most commonly reveal failure to conceive after several breeding attempts, or failure to cycle.

Examination of a bitch thought to be infertile should occur after an evaluation of the dog. Before any medical evaluation, it is important to obtain detailed historical information. Important components of history for the male include semen evaluations, insemination techniques, semen handling, data from other breedings, general health status, brucella and date(s) of assessment. Important components of history for the bitch include reproductive and breeding management history, general health history including the environment, other animals, housing arrangements, routine prophylactic therapy, current medications and travel history. After these preliminary steps, fertility problems are most commonly considered in one of these categories: abnormal estrous cycles, normal estrous cycles, failure to breed, or failure to carry a litter to term.

Because conception depends not only on the health of animals but also on appropriate management of the environment and breeding, many factors contributing

to reproductive success can be manipulated. However, some factors contributing to fertility, such as uterine pathology, may not be treatable. Therefore, it is reasonable to have lower reproductive expectations for a bitch more than 8 years old due to predictable changes that occur as a consequence of repetitive exposure to sex hormones. Low conception rates or apparent inability to carry a litter to term may also represent an abnormality of the reproductive organs in the bitch or the male.

The majority of diagnostic tests required are available to the veterinary practitioners (complete blood count, chemistry profile, ultrasonography, vaginal cytology, vaginal endoscopy, radiography). Specific treatment for the most common causes of infertility is centered on appropriate breeding management. Potential missed breeding opportunities or conception failure, primary or secondary anestrus and synchronization of ovulation for embryo transfer programs present indications for estrus induction. Methods for estrus induction in bitches include the use of synthetic dopamine agonists, estrogens, exogenous gonadotropins, GnRH agonists and opiate antagonists. These methods vary in efficacy of inducing estrus as well as in the conception rates for the induced estrus. The use of some of them for clinical practice is questionable.

In conclusion, historic evaluation of the bitch and the dog, in conjunction with previous management techniques, assists identification of problem areas. Physical examination and diagnostics add the final details which permit therapeutic and management changes to be developed and instituted.

FLOCK-REPROD: HORMONE-FREE NON-SEASONAL OR SEASONAL GOAT REPRODUCTION FOR A SUSTAINABLE EUROPEAN GOAT-MILK MARKET

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FLOCK-REPROD is a European project co-financed by the Seventh Framework Programme (GA n°243520, Capacities, Research for SME Associations). It began in December 2009 and brings together 15 partners including research institutes and SMEs: CAPGENES (France, coordinator of the project), ACRIMUR, KPRA and CABRAMA (Spain), ANCRAS (Portugal), ARAL (Italy), CAPRIROM (Romania), OLYMPOS (Greece) and OPG Moravec (Croatia). In the European context of increased restrictions in the use of exogenous hormones (currently used by the majority of dairy goat breeders using artificial insemination (AI)) in breeding systems (96/22/EC), this project aims at providing the dairy goat industry with an innovative and sustainable technology to ensure the hormone-free production of goats' milk and related products, in full conformity with EC regulation. The work plan has been structured in 6 work packages (WP). The first three ones are dedicated to "Research and Technological Development": experiments based on the use of the male effect and light treatments are carried out to obtain a high-level of oestrus synchronisation and thus to optimize AI conditions, in and out of the breeding season. Seasonal variations of sexual activity and ovulatory response to the male are studied in breeds for which those physiological data are lacking (WP1). In other breeds, photoperiodic treatments without melatonin (WP2) and progestagen-free (prostaglandin-based) / hormone-free AI protocols (WP3) are already being tested. Moreover, protocols will be delivered according to breeders' technical and geographical situations. The different breeds concerned are: Alpine goats in France; Saanen goats in Croatia; Murciano-Granadina goats in Spain; Sarda goats in Sardinia; Serrana goats in Portugal; Capra-Prisca, Damascus and Skopelos goats in Greece; White of Banat and Carpathian goats in Romania. Once standardized "male effect + AI" protocols developed, they will be tested and validated in large-scale field conditions by SMEs (WP4) before dissemination and technological transfer (WP5). Finally, technical and practical guides will be produced (for technicians and breeders), as well as a

training DVD and a trademark. The last work package (WP6) is dedicated to the management of the project. The first results show that the male effect is a very promising non-pharmacological alternative to the use of hormones for inducing and synchronising oestrus in some breeds of goats. Depending on the breed and/or the time of the year, a photoperiodic treatment of the females and/or the males might be necessary to optimize the response to the male effect - <http://www.flock-reprod.eu>.

QUALITATIVE MOTILITY PARAMETERS OF BOAR SPERMATOZOA

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The widespread application of AI and the implementation of its full potential depends largely on the use of good quality semen. The production procedure requires compliance with high technological standards, in order to ensure the customers in its quality. The semen quality is highly dependable on many factors, and the breed of the sire is one of them. The objective of this study was to compare the qualitative motility parameters obtained by computer assisted semen analyser (CASA) in different porcine breeds. Total of 90 ejaculates, obtained from 15 boars of 3 different breeds (Landrace, Yorkshire and Durock) were investigated in order to establish the basic standards of quality motility parameters of boars spermatozoa, in commercially prepared semen for artificial insemination (AI). The assessed parameters were: velocity of average path (VAP), velocity of straight line path (VCL), velocity of curvilinear path (VCL), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF), straightness (STR) and linearity (LIN). Also, two additional morphological parameters were assessed: elongation and area of the head, and the tests were performed by computer assisted semen analysis (CASA). Obtained results are in accordance with available literature data, which, due to high market prices of mentioned devices are not very seldom. However, the obtained results are giving a firm base in establishment of quality control in production of boar semen for AI, which would result with satisfactory pregnancy rates, and sufficient litter size.

Key words: spermatozoa, motility parameters, boar, CASA, semen, artificial insemination.

INDI_SHEEP TRADI_CHEESE: SUSTAINABLE PRODUCTION OF TRADITIONAL CHEESES FROM LOCAL SHEEP MILK, IN THE BALKANS: 1. IMPROVED REPRODUCTIVE MANAGEMENT OF INDIGENOUS-SHEEP-BREEDS POPULATIONS, 2. ENSURING OF TRADITIONAL CHEESES HYGIENE AND QUALITY

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INDI_SHEEP TRADI_CHEESE is a research project under the SEE-ERA.NET PLUS Call for Joint European Research Projects, focused on the topics AgroFood and ICT of the Seventh Framework Programme (FP7). The project is within the subtopic

a.) Preservation of indigenous species and traditional food products (in SEE/WBC).

The objective of this project is to support the local producers in making safe and certified traditional cheeses from indigenous-sheep-breeds milk through

A. Ensuring the supply of milk from indigenous sheep, which will be achieved on two ways:

Approach 1. Rapid improvement of productivity of the indigenous-sheep-breed population by application of A.I. and MOET programs, in order to encourage breeders to use exclusively sheep of the local breed. Initially, suitable protocols for the induction of superovulation in local sheep should be established and freezability of ram semen and embryos should be investigated in the view of formation of a germ cell bank.

Approach 2. *Determination of the breed's reproductive profile.* Application of natural/ alternative methods for the control of estrus and increase of fecundity (ram-effect/light manipulation/flushing), suitable for extensive and semi-extensive, environmentally viable sheep breeding, in the view of the increasing demand for organic products in the EU market.

B. Ensuring the hygiene and underscoring the qualitative superiority of traditional cheeses:

1. *Evaluation of raw milk quality*: microbiological examination and chemical analysis of milk

2. *Evaluation of cheese quality*: a) microbiological examination of cheese: effect of milk pasteurization on microbial evolution, composition of NSLAB during ripening, evolution of pathogens b) biochemical analysis of cheese: chemical and biochemical characteristics during ripening and c) organoleptic evaluation of cheese. Four traditional cheeses will be studied: Ladotyri Mytilinis, Sir iz Mišine, Ovčo belo sirenje, Sjenički sir.

Fundamentals for *Improvement of reproductive management* of indigenous sheep breeds: Lesvos and Pramenka (Ovcepolian, Dalmatian / Lika and Sjenicki strains) will be realized during:

a. Investigation of the superovulatory response to different FSH preparations, in terms of follicular development, hormone concentrations, ovulation rate and embryo yield; and embryo cryopreservation using antioxidants in slow-freezing and in vitrification solutions,

b. Cryopreservation of ram semen by freezing diluents with different antioxidants; using modified diluting method (two-stage addition of cryoprotectant glycerol); and testing soy

bean extract based freezing extender. Quality of the semen will be evaluated during transcervical and laparoscopic intrauterine artificial insemination (IUI) following either estrus synchronization or superovulation treatment.

We expect that the implementation of this project a) could be the first step for the future establishment of a "germ cell bank" for important indigenous sheep breeds in the region and b) will contribute to the confirmation of the quality and hygienic status of local traditional cheeses production.

INTRATUMORAL ADMINISTRATION OF GROSS FUNGI EXTRACT OF T10-1 STRAINS OF CLAVICEPS PURPUREA FOR ANTINEOPLASTIC EFFECT - CASE STUDY

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Claviceps purpurea is a fitoparazite fungus from Clavicipitaceae family, genus *Claviceps*, whose

parasitize different grains, especially rye, hence was called "ergot." In general ergots containing alkaloids. Some alkaloids are partial agonists, while others are antagonists, affecting both serotonin and catecholamines. Representatives are clavins alkaloids: agroclavie, festuclavie, elimoclavine. Ergot alkaloids and their derivatives have either agonist or antagonist activity at different receptors: adrenergic, serotonin and dopamine. Ergot alkaloids and their derivatives have the ability to inhibit the growth of certain hormone-dependent tumors by inhibiting the secretion of prolactin from the anterior pituitary gland. Gross fungal extract was used for its vasoconstrictor effect pursued the reduction of external tumors (rectal or breast) with difficult surgical approach in dogs. After 16 repeated once in a week, it was observed a decrease in the volume of tumor tissue, reduce peritumoral edema and clear delineation of tumor from healthy tissue. Side effects of gross fungal extract in tumor administered was seen especially in the liver and kidney by significant increases of most important parameters of liver and kidney. Side effects of fungic gross extract administered in tumor could be attenuated following a general supportive treatment by powerful antibiotics with broad spectrum and administering a more liver protective.

Key words - *Claviceps purpurea*, fungus, antitumor

BLOOD PLASMA PROTEIN PROFILES IN EXPERIMENTALLY INDUCED INFECTION WITH STAPHYLOCOCCUS AUREUS IN DOGS

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Introduction

Staphylococcus aureus is a medically important bacterial pathogen that is responsible for a broad and divergent range of human and dog infections. Toxin-mediated diseases caused by *S. aureus* include range from cutaneous infections to infections of wounds, osteomyelitis, endocarditis, bacteremia with metastatic complications, toxic shock syndrome. The bacterial components and secreted products that affect the pathogenesis of *S. aureus* infections are numerous and include surface-associated adhesins, exoenzymes,

exotoxins, and capsular polysaccharide. This infection was chosen because *Staphylococcus aureus* presented in a lot of animals – dogs, horses, cats, pigeons. It is established, that this is mostly 100% pathogenic cause for piodermitis and this is the most described skin disease in this species. The aim of the present study was define changes in blood proteins in dog with *S. aureus* infection-total protein (TP), albumin (as an acute phase protein), globulin and A/G ratio.

Materials and Methods

The study was performed on 9 mongrel dogs at the age of 2 years and body weight 12-15 kg. The dogs were housed in metal cages. They were exposed to a 12h light-dark cycle at room temperature (20-22°C). They were fed a commercially available diet of dog pellet twice daily and had free access to water. The infection was reproduced by inoculation of 5 ml 24h broth culture of *S. aureus* strain with density of $3,1 \times 10^9$ c.f.u./ml. Blood samples were collected into heparinized tubes before inoculation (hour 0) then at hours 24, 48, 72 and on days 7, 14, 21. TP and albumin were determined with a commercial kit Human-GmbH, Germany. Globulins were determined by subtraction the albumin concentration from the total protein concentration. The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The results were processed with software Statistica v.6.1 (StatSoft Inc., 2002).

Results

At the start of experiment, the amount of TP in plasma of dogs was $71,8 \pm 1,19$ g/l, albumin – $37,2 \pm 5,2$ g/L, globulin - $31,6 \pm 5,07$ g/l, A/G ratio was $1,12 \pm 0,25$. *Staphylococcal* infection caused a rapid and marked fall in serum albumin levels-the concentration of albumin statistically decreased at 72h, 7 and 14 days after infection vs. baseline. At the same time, the globulin level showed tendency to be higher at 72h, 7 and 14 days after inoculation. Albumin/globulin (A/G) ratio started to decline ($0,87 \pm 0,14$) and on days 7 and 14 was statistically lowest than baseline. The value of TP was within normal range (about 69-71 g/L) throughout the experiment and on the 7th day was noted lower levels, which was not statistically significant.

Conclusion

The result obtained in this study indicate that in dogs exposed to *S. aureus* infection, the concentration of globulin increase at 72h and the same time observed decrease in albumin level support relating of an albumin as a negative acute phase protein. A tendency for slightly reduction of A/G ratio noted of 7 day after infection.

EFFECT FROM THE ADDITION OF VEMOZIM F (PHYTASE) ON DIETS WITH DECREASED CONTENT OF PHOSPHORUS ON THE MICROSTRUCTURE OF TIBIA IN CHICKENS BROILERS

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Introduction

The subject of the performed study was to investigate the effect of the addition of different doses from new bulgarian enzyme preparation – *VemoZim F* (activity 5000 FTU/g) to diets with 30% decreased content of available phosphorus on the microstructure of tibia in chickens broilers.

Material and methods:

The experiment was performed with 180 one day old male chicks broilers (Ross 308), up to age 38 days, bred in batteries, distributed in 5 groups, with 36 numbers in each one, equalized on body weight. The chicks from the control group obtained balanced diet, according to the requirements of the used hybrid, and these from the experimental groups, with 30% deficit of phosphorus diets (0,35, 0,32 and 0,30% in the starter, grower and finisher respectively). The combined diets IInd group of chicks were without addition of *VemoZim F*, compared to IIIrd, IVth and Vth experimental groups, in whose diets, was added 150 g/t *VemoZim F* correspondly (recommended doze, providing 750 FTU/kg mix), 1500g/t from the investigated additive (10 ways higher doze of fitase) and in the chicks from Vth group 100 ways more phytase from the recommended doze, via the addition of 1500 g/t concentrate of phytase in *VemoZim*.

Results:

The results of made hisological analysis of tibial bones' epiphysis showed, that in chickens, obtained 30% reduced content of available phosphorus, without addition of *VemoZimF*, diversions in the ossification processes were observed, characteristic for phosphorus

deficiency, compared to the control. Remarkable differences in the chickens' tibial microstructure from the control group and these, obtained 150 g/t *VemoZimF* (recommended doze, providing 750 FTU/kg feed) weren't found. The increased level of phytase 10 and 100 ways (respectively 7 500 and 75 000 FTU/kg feed) in the chickens' diet from experimental group led to different degree structure alterations, with identical kind and localization in the epiphyseal cartilage of the tibial bones.

Conclusion:

The results from our histological and morphometric studies proved, that the addition of recommended doze *VemoZim F* (fitase) in the diets of chickens broilers compensates 30% phosphorus deficiency, that is demonstrated with alterations in the lengths of the cartilaginous, proliferative and hypertrophic zones, as the first two are elongated, and the last is widened considerably. These changes are manifested with presence of normally passing processes of mineralization. The repeated increase of *VemoZim F* - 10 and 100 ways, led to alterations in the histostructure of the epiphyseal cartilage, observed in IIIrd experimental group (obtained recommended doze *VemoZim F* – 750 FTU/kg feed), but in intensified degree, without negative influence on the bone structure.

Key words: broilers, phytase, microstructure, tibial bones.

DISTURBNCE OF HAEMOSTASIS LIKE POSIBLE COMPLICATION DURING SAMPLING BLOOD AT SWINE

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Blood sampling at swine were usually innocuous act and complication were rarely occurred. Usually complication were injury of *N. phrenikusa*, during deep punctation when we may injure heart and during punctation of *A. carotis*. Disturbance of haemostasis were not argue like problem during blood sampling at swine.

Few month ago we sampling blood of 950 swine at quarantine. During sampling temperature in object were 25- 28°C. Sampling were performed using

vakutejner system with needle 1,2*38 mm.(18g1,5”). During sampling we ascertain unusually number of complication – at numerous number of swine we had not homeostasis and blood were seeping up to 15 minutes after sampling. At floor we had a numerous large blood spots. Three swine were died owing to extinct. During necropsy we confirmed extinction and occurred haematoma in neck region. Injured of blood vessels during sampling of blood induced extravasations and heap of blood and develop of oedema which compressed glottis and induced extinction.

Swine were obtained dihidrostreptomycin before transport. From 5 board and 15 saw from various group we use a blood to biochemical and cytomorphological examination and examination of coagulation factors PT and P-aPTT . Those result don't shown any dysfunction of coagulation. At same time we found decreased of haematocrit value at 18 animals (90%) and haemoglobin at 12 (60%) animals. Increased numbers of leukocyte were found at 8 animals, deficiency of K at 9 and sufficiency of Ca at 4 swine. Sufficiency of P were found at 12 animals and balance of Ca and P in blood sera were unusually.

At base of our results we don't may to conclude which factor were predominate to disturbance of homeostasis and died of 3 swine and presumed that all found factors (high temperature, given dihidrostreptomycin, disbalance between Ca and P and etc.) influence together to accrued of that problem.

THE CASE OF OSTEOSARCOMA (OSA) IN FEMALE SAMOYED

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Introduction

In this case, is described the occurrence of osteosarcoma in the back left leg in female dog Samoyed's breed, aged about 2 years. Although this tumor mostly occur in dogs from 7-9 years of age, however rarely it may occur in young dogs 1-2 years old. As malignant disease, osteosarkoma is often with primary tumor

characteristics. Males have with negligible affinity for it. The appearance of this tumor is more common on the epiphyses of the long bones of the front limbs, unlike the long bones of the hind limbs.

Materials and methods

For radiological diagnostic of the osteosarkoma, the bitch was put in the trocho position (laterolateral recumbency), with medio-lateral (ML) view of the rear leg. As a method of testing was used initial radiography. Imaging was performed on 30x40 x-ray films, with an average exposure of 65-70 kV with 6-8 mAs.

Results

The bitch was brought for radiological diagnostics showing the following clinical signs: lameness on the hind left leg during active movement, accompanied with obvious discomfort as a result of pain caused by movement. The presence of pain was confirmed by manual passive movements of the extremity. For temporarily pain relief and possible prevention of tumor aggression analgesics and corticosteroid medicaments were administered before and after radiological examination.

Discussion and conclusion

Within two months, bone destructions progressed to the extent of occurrence of various distinct mottled or mosaics appearing of the cortical and medullary bone destruction of the affected proximal epiphysis of the tibia. During the next month the medicament treatment was continued but did not result with stagnation and control of the process. Finally, amputation of the leg was performed and the material for pathohistological confirmation was taken. Short after the amputation of the leg, the bitch died as result of the aggressiveness and metastasis of the tumor.

THORACOMPHALOPAGUS ET HAERNIA UMBILICALIS - CONJOINED TWINS IN SIMMENTAL CATTLE

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Complete conjoined twins (*duplicitas completa*) are conjoined twins whose spinas are totally separated. Approximately half are stillborn, and a smaller

fraction of pairs born alive have abnormalities incompatible with life. The case under report was a *thoracomphalopagus* conjoined twins which had been delivered by caesarean operation on a Simmental cow. Conjoined twins whose vertebral columns were totally separated and two thoracic cavities were merged into one "thorax". In the shared thoracic cavity two hearts in its own pericardium were found. There were a lungs well developed. Twins were died before delivery. The diaphragms were separated thoracic and abdominal cavities. Each of twins had own heart, lungs, liver, spleen, stomach, guts, kidneys. There was umbilical haernia in both of twins. The pedigree of the affected animal showed neither inbreeding nor any other affected animal in relationship. A wide range of different chemicals and environmental factors are suspected or are known to be teratogenic agents in humans and in animals, for example: drugs, radiation, some infections or some environmental chemicals such as polychlorinated biphenyls, dioxin, organic mercury, etc. This cow wasn't treated with any drugs or medication during or before pregnancy. Most of exploitation fields of natural gas in Croatia are very closed to this farm. The process of getting natural gas from underground has the potential of being environmentally destructive because it contains some dangerous substances (mercury) and small amounts of radioactive elements, so it could cause harmful effects on population living near. Redundant use of different pesticide in agriculture and forestry, also could be harmful for animals and humans. Cause of malformation in this case is unknown.

COCCIDIOSIS IN CAGED EGG LAYERS AND POSSIBILITIES FOR CONTROL

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Introduction

Coccidiosis in caged egg layers is not a rare event and usually is unpredictable. It has been increasingly reported shortly after the hens start to lay, particularly around 23 to 24 week of age [1]. Coccidiosis may increase mortality and reduce feed efficiency, and recovered birds may demonstrate lack of uniformity throughout the life of the flock [2]. Ingestion of viable sporulated oocysts is the only natural method of transmission. The appearance of diseases can vary

from a subclinical effect on performance to severe mortality. Birds can be infected through contaminated feed and water. Prepatent period is 4 - 6 days while sporulation occurs at 21 - 32°C [3].

Materials and Methods

Commercial white layers reared in cages at 24 weeks of age were subject to investigations. Post mortem examination and parasitological examination were performed on all carcasses submitted to the laboratory. Intestinal lesions were noted and scored using the technique of Johnson and Reid [4]. Counting of the number of infective oocysts in 1 gram of feces was done by modified McMaster method [5]. Identification of oocysts was done by morphological characteristics, micrometer measurements and location of the lesion.

Results

History and clinical signs: outbreak occurred in a flock of layers reared in cages aged 24 weeks. Birds were presented with bloody diarrhea, mottled feathers, anemia of the comb and wattles, somnolence, decreased feed intake and egg production and increasing mortality reaching up to 15%. Clinical signs became evident 1 week after adding of new compounds in to the feed meal. Post mortem examination revealed distended ceca filled with blood and caseous cores. Blood vessels of the mesentery were hyperemic and other parts of the intestines were characterized with pseudo-membranous and dysphenteric inflammation. Parasitological examination of cecal scrapings revealed mass presence of infective oocysts of *Eimeria tenella* with average length 22 µm and width 19 µm. There were 2500 infective oocysts in 1 gram of feces counted with McMaster method. Bacteriological examination of the intestines showed presence of *Clostridium perfringens*.

Conclusion

Two-day treatment course at 7 mg/kg body weight daily with Toltrazuril (Baycox® 2.5% - Bayer) proved to be effective in the treatment of severe clinical form of coccidiosis. After the treatment number of infective oocysts declined drastically to 300 in 1 gram of feces. The situation worsens when coccidiosis outbreaks are complicated by a mixed, concurrent necrotic enteritis infection caused by the Gram-positive bacterium, *Clostridium perfringens*. Control programs for coccidiosis in the egg industry are variable, but when no anticoccidial program is used in caged reared pullets and layers the emphasis should be put on biosecurity measures especially on control of the feed and water and movement of the people.

Key words: coccidiosis, caged egg layers, toltrazuril, biosecurity

FIRST RESULTS ON THE SITUATION WITH CLASSICAL SWINE FEVER (CSF) IN WILD BOARS IN R. of MACEDONIA

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Introduction

The aim of this paper is presentation of the results of the monitoring for the presence of the virus of classical swine fever (CSF) in the population of wild boars in the territory of Republic of Macedonia, as well as presentation of the implemented methods for molecular diagnosis of the CSF virus in the laboratory of FVMS.

Epidemiological links between cases of CSF in wild boars and domestic pigs are often reported. In areas where infections occur in wild boars, CSF should be taken into consideration as a possible source of infection among domestic pigs. The clinical and pathological findings in wild boars and domestic pigs are comparable and can be assumed that both groups are equally susceptible to the virus.

Materials and Methods

As a working material, tissue samples from spleen, kidneys, lymph nodes and tonsils, taken immediately after shooting of the wild boars, were used. Materials from 77 wild boars were received, coming from almost all regions of the Republic of Macedonia. Method used to test the received samples was RT-qPCR. Extraction of the nucleic acid (RNA) was made according to the protocol of the manufacturer of the commercial kit, QIAGEN RNeasy Midi kit.

Results

From the total of 77 wild boars, as a diagnostic material, we received: 77 spleen samples, 15 mandible lymph nodes and 6 tonsils. All 98 samples (from 77 animals) were negative on RT-qPCR.

Conclusions

The results gained from the testing of this 77 wild boars, represent first results related to the situation with the CSF in wild boar population in Republic of

Macedonia. Although the samples arrived from almost all regions in Macedonia, their number was too small to reflect the true situation in these regions, and they are not sufficient to make sound conclusions about the real situation with the CSF in wild boars in R. Macedonia. Therefore, forced efforts should be made to upgrade on these initial results, by taking more extensive study for gaining more valuable information about circulation of the CSF virus in wild boar population in R. Macedonia. This could be achieved especially by sending an appropriate material from animals which shows clinical signs of disease or dead animals.

INFLUENCE OF THE NUMBER OF INTRODUCED LARVAE IN THE BREEDING COLONIES ON THE YIELDS OF ROYAL JELLY

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The objective of the study was to determine the effects of the number of introduced larvae in the honey bee breeding colonies both queen right or queen less, on the acceptance percentage of the larvae and quantity of royal jelly produced per queen cell. The study was conducted on a commercial bee hive yard with 100 bee hives located in the village of Tabanovce, Kumanovo region in the Republic of Macedonia. Bee hives into Dadant- Blat hive types were involved in the investigation, grouped into two groups according to the type of breeding colonies (queen right or queen less). Each group was divided into three sub-groups of breeding colonies, according to the number of introduced larvae for royal jelly production (20,40,60). The highest percentage of acceptance of introduced larvae $79,699 \pm 0,128\%$ is determined in the honey bee breeding colonies with 60 larvae. Lower percentage was determined in the breeding colonies with 40 introduced larvae ($77,677 \pm 0,158$), and lowest in the breeding colonies with 20 larvae ($75,742 \pm 0,226$). The estimated marginal mean for quantity of royal jelly produced per queen cell for the main effect - 20 larvae introduced is $0,300 \pm 0,003$ g, in breeding colonies with 40 introduced larvae is $0,310 \pm 0,002$ g, and in 60 introduced larvae is $0,311 \pm 0,002$ g per queen cell. Following the results, it can be concluded that queen right breeding colonies with 60 introduced larvae are recommended for commercial production of royal jelly.

Key words: royal jelly, breeding colonies, larvae, beekeeping

MODIFIED TOGGLE-PIN TECHNIQUE IN SALTER-HARPIS TYPE I FEMORAL HEAD FRACTURE REPAIR: CLINICAL CASE

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Incidence of femoral fracture in small animal practices is about 20-25 % of all fractures and represents 45 % of all long-bone fractures, more than double rate than all other bones. Traumatic injuries of the femur includes fractures of the proximal and distal femur and diaphyseal fractures. Proximal femur fractures includes: capital fractures, capital growth plate fractures, femoral neck fracture and fracture of greater trochanter. Capital femoral phiseal fractures usually occurs through the capital phiseal growth plate between femoral epiphysis and the neck. According to Salter and Haris, physeal fracture are classified into five groups (type I-V) according to the growth plate separation and the adjacent metaphysis and epiphysis. Open reduction and internal fixation are indicated for almost all femoral fractures. The aim of this report is to present surgical technique of modified toggle pin fixation of femoral head Salter-Harris fracture type I, usually used in hip luxation. Eight-month old, female, Golden retriever brought to the clinic shortly after a traffic accident. The clinical examination have shown high degree of lameness of the right hind leg, pain during palpation and the limb held semi-flexed adducted under the body. Standard ventro-dorsal and lateral radiograph revealed capital physeal fracture. The patient was premedicated by i/m application of 0,2 mg/kg Acepromazine maleate. Surgical anesthesia (TIVA) was achieved by bolus infusion of 10 mg/kg Ketalar 10 % and 1 mg/kg Xylazine and maintained with intermittent boluses of the same combination in total of 1/2 of the induction dose. In order to provide sufficient intraoperative analgesia, 4 mg/kg Carprofen i/v was applied during the premedication. A cranio-lateral approach was used to expose the hip joint. The acetabulum was cleared of debris, fibrin, blood clots

and the femoral head. After removal of the femoral head, a tunnel was drilled through the acetabular fossa using a hand drill. The toggle comprises of a looped Kirchner wire 1,0 mm and a 3 USP double braided polyester suture (Dermafil, Krusse). The toggle was inserted into the acetabular tunnel with a forceps and pushed into the pelvic canal. Another tunnel was drilled through the femoral neck exiting ventrally to the great trochanter. Two filaments of the polyester suture were driven through the tunnel and the other were placed around the great trochanter and knotted together. The leg was immobilized with Ehmer's sling for 3 weeks. Light exercise was recommended in the following period. No intra or postoperative complications associated with this surgical technique or material used, were noticed. Postoperative pain was reduced by s/c injection of 2 mg/kg carprofen (Norocap, Norbrook) for the following 5 days after surgery. Skin sutures were removed at day 10 after the surgery, the patient was fully recovered without evidence of pain and lameness. Minimal postoperative care and early return to function of the fractured leg are the main goals of this toggle-pin technique.

Key words: toggle-pin, dog

A SURVEY OF GASTRO-INTESTINAL PARASITES IN ANIMALS IN SKOPJE ZOO, R. MACEDONIA

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Background

Zoo-animals are often exposed and susceptible to various parasitic infections, despite living in captivity. Presence of different parasites in zoological gardens is due to introduction of new animals not being previously treated against parasites and not conducting proper decontamination of the contaminated enclosures with faces which abounds with parasitic eggs. Certain risk is laying in the transfer of the parasites forms in the footwear of zoo keepers and equipment. Wild animals in captivity can be susceptible to different diseases, among which gastro-intestinal parasitic infections can frequently represent a major health problem.

Materials and Methods

Fresh fecal samples were collected from 29 different species of animals, kept in separate enclosure, as

well as from once kept in mixed species enclosures. All fecal samples were analyzed for the presence of parasitic eggs or other parasitic forms using the ZnSO₄ flotation and sedimentation technique described by Foreyt (2001). The follow up of the gastro-intestinal parasites in the zoological garden in Skopje was conducted in 2008 and 2009 respectively.

Results

Helminth infection in 2008 was present in 34,4%, while in 2009 helminth eggs were found in 31% of the examined animals. Coccidian oocysts were found in zebu cattle in 2008 (3.4%), while in 2009 it was present in peafowl and goat (6,8%). In 2008 in zebu cattle a multiple infection with high number of *Nemethodirus* spp. eggs and infective oocysts of *Eimeria* sp. were found. Horses and zebras had a presence of *Strongylus* sp. infective eggs. *Baylisascaris transfuga* eggs were diagnosed in bear. In peafowl, *Capilaria* sp. eggs and *Eimeria* sp. oocyst were registered.

In two animal specieses (tiger and lion) in 2008 (6.8%) eggs of *Toxocara* sp. were found. In 2009, *Toxocara* sp. eggs were found in 3 different animal species (tiger, lion and panthera) (10.3%). In the same year in peafowl *Capilaria* sp. eggs were registered. In 2008 in 3 animals (pony horse, yak and deer) (10.7%) *Trichostrongylus* sp. eggs were present. In 2009 *Trichuris* sp. eggs were found in deer, while in berber monkey *Oesophagostomum* sp. eggs were clearly distinguishable.

In carnivores, percentage of invasions with gastro-intestinal parasites was 13.7% in 2008 and 2009, while in herbivores in 2008 was represented with 10.7%, and in 2009 with 10,3%, There was no infestation with parasites in primates in 2008, while in 2009, 3,4% were infected with gastro-intestinal parasites.

Conclusion

Gastro-intestinal parasites in animals kept in zoological garden in Skopje were present in high percentage and they indicate a significant potential risk of developing related diseases. They can be controlled by suitable anthelmintic therapy and adequate management procedure, but the risk of their recurrence is always present, due to densely populated enclosures and difficult facilitating of proper decontamination of the living arias inside the zoological garden.

Key words: gastro-intestinal parasites, zoo-animals

IMPLEMENTATION OF MOLECULAR ASSAY FOR DETECTION OF BLUETONGUE VIRUS

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Introduction

Bluetongue (BT) is a reportable disease that affects ruminants and has great socioeconomic impact. Its epidemiology has changed dramatically in the last ten years, so the disease which has been considered as exotic in Europe until 1998, has expanded its range of occurrence in areas of Europe that has never been affected, managing to survive in many of them, causing the greatest epidemic ever recorded. These recent outbreaks of BT in Europe have highlighted a need for rapid, sensitive and specific diagnostic methods for detection of bluetongue virus (BTV). Molecular methods has proved to be very useful for this purpose, enabling rapid detection of viral nucleic acid and Real Time Polymerase Chain Reaction (RT qPCR) has become one of the most widely used methods for molecular detection of BTV. So far, few assays that are able to detect all serotypes of BTV are published and two of them were implemented.

Materials and methods

Primers and probes used for BTV detection are described in publications of Touissaint et al. (2007) and Hoffman et al. (2008). QIAGEN Viral RNA kit was used for RNA extraction. Reverse Transcription and Polymerase Chain Reaction were performed in one step, using QIAGEN One Step RT PCR kit. Test protocols were adopted following instructions from the kit manufacturer. PCR tests were optimized using standard BTV RNA with concentration of 2×10^5 copies/ μ l (provided by Dr. Bernd Hoffmann, Friedrich Loeffler Institute). Two separate series of 10-fold dilutions of standard RNA ranging from 10^{-1} to 10^{-6} were tested by two test protocols and standard curves were generated. Optimization of the protocols

was assessed using obtained values for efficacy of the reaction (E), coefficient of determination (R^2) and slope of the standard curves. For purposes of external evaluation of implemented protocols, 16 blind samples from experimentally infected sheep and cattle were tested (provided by Dr. Bernd Hoffmann, Friedrich Loeffler Institute). Finally, laboratory participated in the following annual BTV Proficiency testing, organized by Community Reference Laboratory (CRL) for BTV, Institute of Animal Health, Pirbright.

Results

Two implemented protocols gave satisfactory results for efficacy of the reaction (100.3% and 101.8%), coefficient of determination (0.998 and 0.995) and slope of the standard curves (-3.314 and 3.280) which were within recommended limits for well optimized PCR test. Calculated limit of detection was approximately 4 RNA copies/ μ l for both test protocols. All 16 blind samples were correctly identified, including the sample with lowest titer (only 3 RNA copies/ μ l). Participation on BTV Proficiency testing was successful, with correct interpretation of all 10 samples.

Conclusion

Early and rapid detection of BTV incursions is of great importance for BT control. Implementation of molecular assays has enabled prompt BT diagnostics of all suspicious cases, thus improving the efficacy of control program and reducing the economic losses related to disease occurrence.

EPIDEMIOLOGY OF CLOSTRIDIAL INFECTION IN SMALL RUMINANTS AND CATTLE IN R.MACEDONIA IN PERIOD OF 1992 – 2010

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The clostridial bacteria are group of microorganisms which cause a wide range of diseases, classified according to the affected organ/tissue in three groups as muscle and soft tissue infections, enteric diseases and neurotoxic diseases. The bacteria are widespread in the environment and cannot be avoided or eradicated so the vaccination is the most effective method of control. The most important clostridial diseases that cause death in small ruminants and cattle are: blackleg, pulpy

kidney (enterotoxaemia), black disease, malignant oedema, tetanus and botulism.

This paper presents the retrospective epidemiological data of two most frequent clostridial diseases in R. Macedonia - enterotoxaemia(s) and blackleg. The data have been collected during the past 18 years, as a result of the bacteriological investigations on routinely received samples from small ruminants and cattle in the bacteriology lab of the Veterinary institute/Faculty of Veterinary Medicine in Skopje.

CYTOLOGICAL DIAGNOSTIC OF CANINE TRANSMISSIBLE VENEREAL TUMOR - CASE REPORT

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Canine Transmissible Venereal Tumor (CTVT) also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor, is a benign reticuloendothelial (histiocytic) tumor of the dog that mainly affects the external genitalia. The tumor occurs naturally on the genitals of both male and female dogs. In male dogs it is located on the penis or prepuce, and in females is present on the vagina or labia. CTVT has cauliflower-like shape, and it could be pendular, nodular, papular, or multilobular. Its surface is often with erosions, ulcers and inflamed. Also, this tumor could be solitary or multiple, but always is located on the external genitalia. It is transmitted from animal to animal during copulation. Cytoplasmic inclusions found in the tumoral cells caused this tumor to be attributed to a viral agent by some authors, although the tumor could not consistently be transmitted by cell free extracts. Presently, the consensus view is that CTVT arise from allogenic cellular transplants and that the abnormal cells of the neoplasm are the vectors of transmission. The exfoliation and transplantation of neoplastic cells during physical contact provide the main mode of transmission onto genital mucosa. All tumors examined

so far have a long interspersed nuclear element (LINE-1) insertion near *c-myc* oncogen. LINES are long DNA sequences that represent reverse-transcribed RNA molecules (also called retrotransposons), and they can be used as a diagnostic marker to confirm that a tumor is CTVT. This tumor was found in male 2 years old mongrel dog. According the anamnestic data, there was no visible change of its general clinical status, except the presence of blood in the urine. Ultrasound evaluation of the abdomen did not indicated any changes in the urinary tract, but spontaneous bleeding with blood drops on the prepuce was evident. With clinical examination a multilobular mass on radix penis mucosa was found, which was in p-phase i.e. proliferative phase, which actually caused pseudohemorrhagia. The material for cytological diagnostic was taken with the imprint method and 4 cytological films were prepared and stained. With microscopy a homogenous population of large round cells, with centrally located nucleus, and with moderate anisocytosis and anisokariosis was found. These cells had a moderate amount of light blue cytoplasm, with several small punctuated vacuoles. Their nuclei were round, with dense chromatin, and single prominent nucleolus. Also, a moderate mitotic activity was found. Behind in some neutrophils, lymphocytes, plasma-cells, normal plate epithelial cells, and many erythrocytes were found. The conclusion of the cytological diagnosis was CTVT. The tumor cause only local disturbances, and the differential diagnosis of the other "round cells tumors", histiocytomas, plasmacytomas, lymphoma, some melanomas and especially lymphosarcomas, which could be located on the external genitalia, had a big significance. Although some authors are mentioning spontaneous regression, however, because this is invasive tumor, a complete surgical excision was made.

Key words: canine transmissible venereal tumor, dogs, cytology

ASSESSMENT OF DEGREE OF OXIDATIVE STRESS AND ANTIOXIDANT CONCENTRATIONS IN DOGS WITH MAMMARY CARCINOMAS

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Reactive oxygen species (ROS) and oxidative stress play an important role in carcinogenesis, tumor initiation and tumor progression. It is suggested that

the concept of 'persistent oxidative stress in cancer' may open up a new research area, explaining part of the characteristic tumor biology of cancer such as activated transcription factors and proto-oncogenes, genomic instability, chemotherapy-resistance, invasion and metastasis.

The purpose of this study was to evaluate serum malondialdehyde (MDA), serum catalase (CAT), erythrocyte superoxide dismutase (SOD), erythrocyte reduced glutathione (GSH), serum vitamin C and vitamin E concentrations, ferric reducing ability of plasma (FRAP) and oxidative stress index (OSI) in bitches with spontaneous mammary adenocarcinomas, with comparison with healthy controls.

Studies were performed in 21 bitches aged 6–14 years, weighing 4–29 kg, with histologically confirmed mammary gland carcinoma and 14 clinically healthy dogs.

Serum malondialdehyde (MDA) concentrations and oxidative stress index (OSI) were significantly increased in dogs with mammary adenocarcinomas ($p \leq 0,001$) compared to control dogs. Superoxide dismutase concentrations also were found to be statistically significantly higher in this dogs ($p \leq 0,01$). Ferric reducing ability of plasma (FRAP) were significantly lower ($p < 0,01$) in dogs with mammary tumours compared to the control group.

In conclusion, dogs with mammary adenocarcinoma appear to be under persistent oxidative stress.

Key words: oxidative stress, dogs, mammary tumors, malondialdehyde, ferric reducing ability of plasma, oxidative stress index

CHEMICAL COMPOSITION AND MICROBIAL POPULATIONS OF SLAVONIAN SAUSAGE

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Slavonian sausage is traditional pork meat product produced in households of Eastern Croatia. The aim of this study was to investigate its changes in chemical properties and dominant microbial flora during the ripening and drying. Sausages were sampled at 0, 7, 14 and 45 days of production. Quality parameters monitored were moisture (ISO 1442:1997), total protein content (HRN ISO 937:1999), hydroxyproline

(HRN ISO 3496:1999), fat (HRN ISO 1443:1999), ash (ISO 936:1998) and pH (pHmeter MP220). Coagulase negative staphylococci and lactic acid bacteria counts were determined using manitol salt agar and de Man Rogosa Sharpe agar, respectively, incubated for 48 hours at 30 °C. Selected bacterial strains were identified by means of biochemical tests API Staph (n=14) and API 50 CHL (n=14). In final products average values for moisture were 23.2 %, total proteins 31.80 %, meat proteins 28.97 %, hydroxyproline 0.35 %, fat 40.01 %, ash 5.22 % and pH 5.34. Population of coagulase negative staphylococci remain at stable counts of 3 log CFU/g during the ripening, while lactic acid bacteria increased for 5 log CFU/g reaching 8 log CFU/g in final product. Dominant strains in selected populations were *Staphylococcus xylosus* (n=13; 92.8 %) and *Lactobacillus fermentum* (n=10; 71.4 %).

ANTIMICROBIAL RESISTANCE OF *E. coli* STRAINS ISOLATED FROM RAW MEAT IN SLOVENIA IN 2010

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Introduction

Discovery of antimicrobials in human and veterinary medicine was one of the most significant achievements of the 20th century. Before the antibiotic treatment, many of bacterial infectious diseases were lethal. Unfortunately, bacteria have rapidly developed different mechanisms of resistance to antimicrobial drugs. Antimicrobial resistance of common pathogens, including some commensal bacteria, like *Escherichia coli* (*E. coli*), is now rising concern all over the world. The *E. coli* strains are widely spread. They are also part of the normal intestinal flora in humans and animals. But they are an important zoonotic agent, causing different infections. For this reason *E. coli* strains are also used as an indicator microorganisms for the presence/detection of antimicrobial resistance.

In this paper we wish to present the results of antimicrobial susceptibility for *E. coli* strains, isolated from raw meat in Slovenia for 2010.

Materials and Methods

105 *E. coli* strains were tested for antimicrobial resistance at the National Veterinary Institute of

Veterinary Faculty in Ljubljana. All strains were isolated from raw meat samples of healthy food producing domestic animals. 26 of them were isolated from beef, 32 from turkey meat and 47 from pork. All of them were sampled within the official monitoring programmes of zoonoses. They were tested with disk-diffusion method with 17 different antibiotics from 8 antimicrobial groups.

According to the CLSI standard the following antimicrobials were tested: ampicillin, amoxicillin – clavulanic acid, cephalotin, cefotaxime, ceftazidime, cefpodoxime, gentamicin, kanamycin, streptomycin, tetracycline, nalidixic acid, enrofloxacin, ciprofloxacin, chloramphenicol, sulfonamides, trimethoprim and trimethoprim- sulfamethoxazole combination.

Results

Among *E. coli* isolates many sensitivity patterns were found. There was resistance to all antimicrobial groups. More than 55% of isolates were resistant to at least one of the tested antibiotics. The highest percentage of resistance were obtained in group of tetracyclines, penicillins and fluoroquinolones. *E. coli* isolates from turkey meat seemed to be more resistant than those from beef and pork.

The lowest resistance was to 3rd generation cephalosporins, amoxicillin with clavulanic acid and gentamicin (less than 2%). We also found one strain from sample of pork belonging to the group of extended-spectrum β -lactamase-producing *E. coli* (ESBL).

Conclusion

E. coli are commensal bacteria common to all animals and humans, but can also serve as an indicator bacterium, that easily become resistant to antimicrobials. Furthermore, changes in the resistance in *E. coli* can be an early warning for resistance of others potentially pathogenic bacteria. *E. coli* is also considered to constitute reservoir of resistance genes, which may be transferred to pathogenic bacteria causing disease in animals or humans. Our results indicate the importance of a rational use of antimicrobials both in animals and humans.

MICROBIOLOGICAL QUALITY OF POTABLE WATER USED IN FOOD PROCESSING PREMISES IN REPUBLIC OF MACEDONIA

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Water is the most used compound in the food processing industry, and as such it presents a potential source of contamination with pathogen bacteria to the final product. This study was made to determine the microbiological quality of the water that is used in the food processing premises.

We've examined 227 samples of water from local water supply network, 10 samples of ground water and 15 samples of ice. The samples were tested according to Book of rules on water safety (Official Gazette of RM No.46/2008), on the following parameters: total number of bacteria at 37°C and 22°C, intestinal enterococci, coliform bacteria, *E.coli*, *Pseudomonas aeruginosa* and sulfide-reductive anaerobic bacteria.

After the analysis 89,87% of the tested samples gave satisfactory results. Of the samples that were not satisfactory on the criteria 7 were ground water, 1 was ice, and 16 were from the local water supply.

Most of the water used in the food processing premises is in compliance with the water safety regulations. The only concern is the ground water that gave a high percentage (70%) of unsatisfactory results.

Key words: microbiological quality of water, enterococci, *E. coli*, *Pseudomonas aeruginosa*

MICROBIOLOGICAL QUALITY AND OCCURRENCE OF ANTIBIOTIC RESIDUES IN EWE MILK

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The first aim of this study was to evaluate the main factors that influence the microbiological quality of the milk: somatic cell count and total bacteria count. The second aim was to estimate the occurrence of antibiotic residues in raw milk.

Ewe milk samples (in total 302) from five ovine farms from the Central region of R. Macedonia were collected from collection point tanks. Somatic cell count, bacterial count and antibiotic residues were analyzed by routine accredited methods. Milk somatic cell count and bacterial levels were determined, respectively, with Fossomatic 5000 and BactoScan 8000S (Foss Electric, Hillerod, Denmark).

The analysis showed an average bacterial count of 1.980.000 cfu/ml and 485.000 scc/ml. The screening of the 302 raw milk samples by the Delvotest SP-NT

test (DSM Food Specialties, Delft, The Netherlands) showed 4.3% "positive" and 2.64% "doubtful" results.

Key words: ewe milk, antibiotic residues, bacterial count, somatic cells

MILK FATTY ACID COMPOSITION FROM TWO DAIRY BREEDS IN MACEDONIA

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Present study evaluates the milk fatty acid (FA) composition and cholesterol content of two main Macedonian dairy breeds Simmental and Holstein Frisian (n = 25 for each breed). The cows were housed together and received the same diet. The results show a significant variation ($P < 0.05$) in the FA content of the two breeds. The milk fat of Simmental breeds was found to contain significantly lower ($P < 0.05$) amount of saturated fatty acid content than Holstein Frisian breeds (66.96 and 69.09 g/100 g).

Determined mean monounsaturated fatty acid (MUFA) contents (27.62 vs. 25.20 g/100g) and total trans fatty acids (3.48 vs. 2.48) were significantly elevated ($P < 0.05$) in the milk fat of Holstein Frisian breeds. Amount of fat and conjugated linoleic acid content was higher ($P = 0.04$) in Holstein Frisian cows as compared to Simmental cows (7.00 vs. 7.78 g /100g and 0.80 vs. 0.71g / 100g), while cholesterol content was not different among both breeds ranging from 8.89 vs. 10.24 mg /dl.

Present studies show that in future genetic selection programs along with altered breed nutrition may be able to result in optimum levels of various fatty acids in milk.

Keywords: Simmental, Conjugated linoleic acid, Milk fat, Holstein Frisian

DETERMINATION OF CLENBUTEROL IN MEAT SAMPLES WITH ELISA METHOD

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Clenbuterol is used in human and veterinary medicine for its broncholytic and tocolytic action. In 5 to 10-fold the therapeutic doses, clenbuterol acts as a repartitioning agent to improve the performance of food-producing animals. Such a growth-promoting dose of clenbuterol influences animal growth and carcass composition by increased muscle mass and decreased fat mass, presumably through a direct effect of BAA on skeletal muscle and adipose tissue, indirect effect on many other tissues, or a combination of both. Because of these effects, clenbuterol has been illegally used in food producing animals. It is essential from the public health point of view, that the Macedonian regulatory agencies implement a monitoring surveillance program that only uses preliminary methods for the determination of clenbuterol.

Thus, the aim of this work was to monitor the presence of clenbuterol residues in bovine meat muscle collected from the veterinarian inspectors. The limit of detection (LOD) and limit of quantification was obtained by adding 3 and 10 times standard deviation of 20 blank samples analyzed to the mean blank value, respectively. The method recovery was determined at four different levels by spiking meat samples with clenbuterol standard to yield clenbuterol concentrations equivalent to 0.5, 1, 5 and 10 µg/kg (six replicates per concentration level) and calculated from seven-point calibration curves.

From 55 bovine meat samples analyzed by the ELISA test, 1 (1,8%) presented clenbuterol residues was 1.19 µg/kg, 7 (12,7%) from 0.5 to 1.0 µg/kg, 40 (72,73%) from 0.1 to 0.5 µg/kg and only 7 (12,7%) were below the limit of detection (LOD) of 0.1 µg/kg. The one sample with the highest clenbuterol residues was subjected to GC-MS-MS for confirmation. The results showed that clenbuterol was not used as a bovine growth promoter.

Keywords: Clenbuterol, ELISA method, Meat, validation

IMPLEMENTATION OF EU LEGISLATIVE IN MACEDONIA RELATED TO FEED SAFETY

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As a reaction on food and feeding stuff safety crises at the beginning of new millennium European Union adopted sets of new rules establishing the new safety system of the food chain from farm to fork to increase the level of protection of both human and animal health and to prevent these crises. The key Regulation in this area No.178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety has established the principle of risk analysis which consists of three interrelated components (risk assessment, risk management and risk communication) and they all provide the basis for food and feedingstuff law as appropriate to the measure under consideration.

Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed came into force on the 1 September 2010. The approximation of Macedonian legislative and fulfilling of the requirements and implementation of the selected resolutions of this particular regulation, particularly the new elements which had not been regulated earlier is already done. These include the catalogue of EU feed materials, the method of notifying feed materials not mentioned in the catalogue, the arrangement and adoption of criteria for distinguishing feed materials and additives, permitted tolerance regarding labeled feed products and estimating the chances of meeting the requirements by feed manufacturers and official control laboratories.

In compliance with article 24 act 2 of regulation 767/2009 published by regulation of the Council (EC) No 242/2010 of 19 March 2010, the EC feed catalogue of feed materials includes only basic feed materials listed in directives 96/25/EC and 82/471/EU, amounting to a total of 171 materials. Much more feed materials have been available on the market and their producers are interested in including these materials in the catalogue.

In order to prepare future modifications of the catalogue, the obligation has been introduced for the feed material producers to inform immediately representatives of the feed sector, declare and register materials (notification) not mentioned in the catalogue, under article 24 act 6 of regulation 767/2009. The notification and registration

of such materials on line is done by the European Feed Manufacturers Federation, FEFAC. The list has been available since the 1 September 2010 at www.fefac.org or directly at www.feed-materialsregister.eu.

Creating a catalogue of feed materials indirectly resulted in developing the criteria for distinguishing feed materials. The criteria were used to publish the regulation of the Council (EC), No 892/2010 of 8 October 2010, on the status of certain feed additives.

According to food law it is not allowed to put on the market unsafe feed or feeding animals used for food production with such feed. The feed is unsafe if it has a negative effect on human and animal health, and makes the food derived from animals used for food production, unsafe for human consumption. Therefore, when feeding animals used for food production, feed bussiness operators must take measures and adopt procedures (HACCP) in order to avoid the risk of biological, chemical or physical hazards i.e. to reduce contamination of feed, animals and products of animal origin to a minimum. In order to further ensure the safety of feed, feed operators must apply to the competent authority for registration or approval of any facility for production, processing and distribution of feed.

Key words: food safety, feed safety, EU food law

SCREENING OF RAW MILK SAMPLES FROM REPUBLIC OF MACEDONIA FOR RESIDUES OF SULFONAMIDES

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Background

As antimicrobials sulfonamides are one of the most frequently used at food producing animals for prevention and treatment of infection diseases caused by bacteria or as a feed additives to improve feed efficiency and growth. The presence of residues of these drugs might have various adverse effects for humans: allergic reactions in some hypersensitive individuals, possible disruption of the intestinal microflora and problems related with drug persistency at the microorganisms. The maximum residue limits (MRL's) for tetracycline according to Commission Regulation 37/2010/EU are set at 100 µg/kg. The

most widely used screening methods for detection of residues of sulfonamides in food are microbiological and immunochemical.

Materials and methods

In this paper a screening for presence of total sulfonamides in 1233 raw milk samples (915 of cow's, 245 of sheep's and 83 of goat's) was performed, applying indirect competitive ELISA test. The samples originated from 251 farms and 78 dairy houses. Sulfonamides extraction and the procedure of the assay was performed according to the producer's instruction (Europroxima, Netherlands). The method validation was previously performed and published [2], and the method was "fit-for-purpose".

Results

The screening analysis of total sulfonamides obtained a values over LOD in 182 samples (14.76 %). Fifteen of them were suspicious for a violative presence of sulfonamides over the determined CC β value. Concerning the different types of milk, the prevalence of total sulfonamides was 14,64 %, 13,92 % and 18,07 % for cow's, sheep's and goat's milk, respectively. 13 samples of cow's milk contained sulfonamides over the CC β value, while one sample of sheep's and one of goat's milk were suspicious for MRL violation.

Discussion

The presented results of screening indicates that some possibility for inappropriate administration of sulfonamides at food producing animals is present. There is no significance between the presence of total sulfonamides in cow's milk samples originated from farms and dairy houses, 13.12 % and 13.42 % ($p=0.05$), as well as between the sheep's milk samples 14.29 % and 12.80 % ($p=0.05$). The prevalence of suspicious samples varies from 1.42 % for cow's milk samples, 1.20 % for goat milk samples to 0.42 % for sheep's milk samples. These figures are comparable to the results from screening (0,81 %) published by Abjean et al. [3]. To make the final judgment for the compliance of the milk samples in which the amount of total sulfonamides is over the CC β value, it is obligatory to perform confirmatory analysis of the suspicious samples according to the requirements laid down in the Decision 2002/657/EC.

Key words: Sulfonamides, screening, milk, ELISA, detection capability

MILK PRODUCTIONS CONDITIONS IN REPUBLIC OF MACEDONIA – IFCN REVIEW

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What is international farm comparison networking for dairy products (IFCN)? – It is international network for comparison of dairy farms, with vision to develop international research network for economical analysis of dairy production world wide. IFCN mission is to create better understanding of dairy production world wide. Participation in this networking provides information about global development of milk production and analysis of competitive positions of each country in region, identifying critical point which needs corrections.

Who has benefits of IFCN? – Users of information are farmers like direct milk producers, milk processors, and other stakeholders in milk supply chain, agrarian policy makers and research organisations. For the first time, since 2008 till now, Republic of Macedonia is represented with field dates in annual reports of IFCN. Where Macedonia is regards basic dairy productions parameters?

We can notice that almost all neighbouring countries has lower prices compared with world price of milk. It means that competitively of Macedonian milk is in a lower level than other regional countries. From the other side Macedonia is on the last place in the region and wider regard milk production and productivity. The reasons for this are in insufficient development of this branch, but in the low productivity either. In the milk consumption per capita we are in the last place, too. Low milk consumption is signaling country with low income per capita. For more serious conclusions we should analysing full consumer's basket, but one of the possible conclusion is low consumer culture of the inhabitants.

The most interesting is percentage of self supplying of domestic milk market which is very near to the other countries in the region (95%), but to the double lower milk production and consumption. Dairy productions sector shows many characteristics of low developed economy and in depth analysis is obligatory for detecting weak points and suggesting improvement measures.

Key words: milk production, dairy market, IFCN, conditions

DETERMINATION OF TOTAL SUGAR CONTENT AND PERCENTAGE OF ALCOHOL IN RED AND WHITE WINES ORIGINATING FROM REPUBLIC OF MACEDONIA

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With the advent of sophisticated techniques and growing consumer demand, gives a contribution to ensuring the quality of wine. In his paper we will show results obtained by analysis of two important parameters in wine, the percentage of alcohol and the content of total sugars. The results for the second parameter were obtained by a newly introduced potentiometric method at the Food Institute.

Within 7 months, analysis were carried out on 12 red wines, Vranec, and 8 white wines, Smederevka, from three geographical areas of Macedonia: Skopje, Ovcepole and Strumica-Valandovo. The amount of total sugars was determined potentiometrically, with Metrohm titrator 848 Titrino plus-Universal manufacturer Metrohm Ltd. (Switzerland) and was used Combine Pt electrode. The percentage of alcohol was determined with pycnometer, for which were used previously obtained distillates from the wines. Preparation of samples and performance of the methods was performed according to methods proposed by the International Organization of Vine and Wine (OIV) contained in its Compendium of internal methods of wine and must analysis (2009).

The content of total sugars in white wines ranged between 1,1 g / l and 3,1 g / l, while in the red wines between 1,41 g / l and 2,94 g / l. In three red wines were observed greater values of 3,59 g / l, 4,73 g / l and 6,86 g / l, and in the same wines is recorded a greater percent of alcohol, which was still within the limits of the declared value. The mean value for the percentage of alcohol in the white wines was 11.01% Vol., while in the red wines was 12,29% Vol.

Based on the obtained results all wines meet the prescribed values for quality wines. From the results we can conclude that white wines have a lower sugar content and consequently a lower percentage of alcohol as opposed to red wines. We were ensure that with potentiometric determination of the total sugar content can get faster and more accurate results than classical titrimetric determination.

Key words: wine, quality control, potentiometric determination, total sugar

LEVEL OF ACTIVITY OF ¹³⁷Cs IN MUSHROOMS FROM DIFFERENT REGIONS OF R. OF MACEDONIA

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Wild mushrooms have become popular delicates in many countries including Macedonia. Some of these varieties of mushrooms are capable of accumulating high levels of certain heavy metals and radionuclei. There is a shortage of investigations in Macedonia referring to radioactive contamination of mushrooms. For that reason the aim of this investigation was to determine the degree of radioactive contamination of certain varieties of mushrooms.

During the investigation, 139 samples of mushrooms were taken from different locations in Macedonia. Based on the acquired results, an evaluation of the condition of specific eco systems was performed from the aspect of radioactive contamination. The comparison with the Regulations for maximum allowed quantity of radio nuclei in food, showed that all samples fulfill the given criteria.

Key words: radioactivity; radio nuclei; mushroom analysis; gamma spectrometry

INTERNATIONAL STANDARDS AND METHODOLOGY FOR EVALUATION OF VETERINARY SERVICES – THE OIE PVS PATHWAY

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The World Organisation for Animal Health OIE promotes animal health and public health in international trade of animals and animal products by issuing harmonised sanitary standards for international trade and disease control, by improving resources and legal framework of Veterinary Services and by helping Members comply with the OIE standards, guidelines and recommendations, consistent with the SPS Agreement

of the World Trade Organization (WTO). The OIE PVS Pathway is a global programme for sustainable improvement of national Veterinary Services' in compliance with OIE standards. The activities of the Veterinary Services are an "international public good" and are consequently eligible for appropriate national, regional or international funding support.

A specific methodology has been developed "OIE Tool for the Evaluation of Performance of Veterinary Services" OIE-PVS Tool ("diagnosis tool"), as the basis for evaluating performance against the international standards published in the Terrestrial Animal Health Code.

The OIE PVS Gap Analysis Tool ("prescription tool") is a quantitative evaluation of a country's needs and priorities based on the outcome of the independent external evaluation of the country Veterinary Services using the OIE PVS Evaluation Tool. The key objective of an OIE PVS Gap Analysis mission is to define a five-year programme for the sustainable strengthening of a country's Veterinary Services' compliance with OIE quality standards, suitably adapted to national constraints and priorities.

Veterinary legislation is an essential prerequisite that enables Veterinary Services to carry out their key functions efficiently, including epidemio-surveillance; early detection and reporting of diseases, including zoonoses; rapid response to and prevention and control of sanitary emergencies; animal production food safety; animal welfare and the certification of animals and animal products for export.

The increasing of the global trade, climate change and the emergence and re-emergence of diseases that can rapidly spread across international borders, the Veterinary Services must be supported by effective and modern legislation. Sustainably improving a country's Veterinary Services' compliance with OIE standards is a precondition for improving animal health and public health, at both the national and the international level. With the development of the OIE Tool for the Evaluation of Performance of Veterinary Services (OIE PVS Tool), the OIE enables any country that so wishes to determine its level of advancement and compliance in terms of 40 different critical competencies grouped in four fundamental components. The use of the PVS Tool thus provides a way of measuring in absolute terms the progress that countries have made in sustainably improving their compliance with the OIE quality standards set out in the OIE Terrestrial Animal Health Code (the Code).

Good governance of animal health systems based on a close public/private partnership is the responsibility of all governments. If one country fails, it may endanger its neighbouring countries, the region, the continent and potentially the entire planet.

Key words: OIE Pathway, OIE PVS Tool, OIE PVS GAP Analysis

PREVALENCE, SEROTYPING AND ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA SPP. ISOLATED FROM POULTRY HOUSES IN REPUBLIC OF MACEDONIA IN 2009

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Abstract

Prevalence of *Salmonella* spp. in poultry flocks for rearing different ages categories was examined in total of 44 houses with 246 samples. Samples were taken according to requirements of national program for control of salmonella in poultry in 2009. During testing 162 samples of faeces were taken from egg hatchery houses, 72 samples of dust from houses for laying hens and broilers and 32 samples booth swabs from deep liter from broiler houses.

Samples were tested in laboratory according to ISO 6579: Annex D 2007 for detection and typisation of *Salmonella* spp. in poultry faecal samples. Obtained isolates were tested toward antimicrobial susceptibility with antibiotics most often used in veterinary medicine. During research *Salmonella* spp. was isolated from 79 (32,11%) of samples, from which 35 (44,30%) isolates from dust samples, 36 (45,57%) from faecal samples and 8 from booth swabs from deep liter. After performed serotyping using salmonella antisera, highest prevalence had *S. enteritidis* with 35 isolates, followed by *S. hadar* with 9 isolates, *S. virchow* with 6, *S. blegdam Ser. II*, *S. infantis*, *S. newport* and *S. glostrup* with 3 isolates each, *S. cleveland*, *S. dublin* and *S. lamberthurst* with 2 each, and *S. blockley*, *S. gueuletapee*, *S. istambul*, *S. typhimurium* with 1 isolates, however 7 isolates count been typed.

Achieved isolates were further on tested for antimicrobial susceptibility toward 9 antimicrobials using the method of agar-gel diffusion inhibition test. Gained results showed differences in resistance between isolates from the same bacterial strain with presence of strains with multi-drug resistance.

Key words: *Salmonella* spp., poultry, serotyping, antimicrobial susceptibility

POTENTIAL MICROBIOLOGICAL RISKS IN DRIED HERBS AND SPICES

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From the randomly collected 200 samples of spices and herbs mostly found in retail, the main output of this microbiological study considers the total amount of *enterobacteriaceae*, *staphylococcus aureus*, yeast and molds, as well as the total viable count.

The basic asset of this examination will implicate the number of hygiene problem occurring in spice and herbs which can be exposed to wide range of microbial contaminants. This food hygiene pathogens present a significant portion of the total count of microbial causes in the determined samples of substances that were examined.

From the overall number of herbs and spices that were tested, the final results indicate the following percentage of bacteria and yeasts and molds, from the total, 30% of the samples were unsatisfactory for *enterobacteriaceae*, in 1 % of the samples was found *Clostridium perfringens*, in 3% of the total number of samples was found *Bacillus cereus*, 10 % were unsatisfactory for the total viable count, and in many of them were found potentially hazardous molds (genera *Aspergillus*, *Fusarium* and *Penicillium*).

These unsatisfactory results showed a poor microbiological quality. Spices and dry herbs are used as ingredients in a variety of products in a different way prepared, this fact suggests the need to provide a control system to improve the quality of herbs and spices, especially because of the high temperature tolerance of the toxins produced by bacteria and molds.

Key words: herbs, spices, *Bacillus cereus*, *enterobacteriaceae*, molds

RELATION BETWEEN BACTERIA AND SOMATIC CELL COUNT IN COWS MILK

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This study was conducted to screen mammary gland health status. Milk samples were collected from each quarter of 66 Holstein and Brown Swiss cows at days in milk 20 to 275. Somatic cell counts (SCC) were determined and microbiological culture was performed. 7 quarters were blind, and bacteria *Bacillus* spp., *S.agalactia*, *Proteus* spp., and *S.intermedius* grew in 2, 2, 2, and 1 quarters, which were excluded from statistical analyses due to their small population size. LogSCC and bacterial profile were subjected to one-way ANOVA and chi-square test (n=250). 73.2% of the quarters were healthy, and *Staph.aureus* (24.0%) and coagulase negative staphylococci (CNS) (2.8%) were predominant bacteria infecting quarters. There was no difference in logSCC across the quarters (P<0.48), and mean logSCC was 4.40. LogSCC for the quarters infected with CNS (5.56±0.45) and *Staph. aureus* (4.86±0.15) was greater than healthy quarters (4.20±0.09) (P<0.0001). Distribution of bacteria infecting quarters was similar among quarters. The frequency of quarters infected by CNS was 4.9, 1.6, 3.1, and 1.6% and of quarters infected with *Staph. aureus* was 21.3, 28.6, 26.6, and 19.4%, and of non-infected quarter was 73.8, 69.8, 70.3, and 79.0% in rear-right, front-right, rear-left, and front-left quarters (P<0.74). These results ascertain importance of routine test for SCC and culture to improve mammary gland health status and productivity.

Key words : mastitis, SCC, culture, screening.

BODY WEIGHT AND SOME BODY MEASUREMENTS OF MASTIFF DOGS IN TURKEY

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This study was aimed at determining the body weight and some body measurements of Mastiff Dogs for Turkish natural dogs. Mastiff dogs are trained as hobby, guard and shepherd dogs in different regions of Turkey, including especially Aksaray, Sereflikochisar and Nevsehir provinces. Kangal and Akbas dogs are well known breeds in Turkey. Mastiff dog's color and body structure similar to Kangal but have wider head and thicker muzzle than Kangal. Capar (tiger pattern- black mixed yellow) and Ala (yellow mixed white) colors seen in these dogs and black mask color in Kangal dogs may not be present in this dog. In this study, body weight, head and body measurements were taken from twelve months of age and older, of total 277 dogs (77 female and 200 male), in different regions of Turkey and especially Aksaray, Sereflikochisar and Nevsehir regions. Values obtained in this research from Mastiff dogs from head and body traits were found to be higher than Kangal breed except tail length.

Key words: body measurement, dog, head measurement, mastiff, Turkey

DIAGNOSTIC TECHNIQUES AND CONTROL STRATEGIES FOR THE RELEVANT HEREDITARY DISEASES OF HOLSTEIN CATTLE

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The availability of the bovine genome sequence and the use of genetic markers have broadened our understanding of a number of hereditary diseases in cattle, leading to the development of techniques for their early detection. By extracting DNA from nucleated cell samples, followed by *in vitro* amplification of specified sequence and digestion with restriction enzymes, it is now possible to detect the presence of mutant alleles for a specific phenotype. Such techniques are already being used for the study of genetic diseases of dairy cattle such as BLAD (Bovine leukocyte adhesion deficiency), CVM (Complex vertebral malformation), DUMPS (Deficiency of uridine-monophosphate synthase) and BC (Bovine citrullinemia), among others, where the disorder is caused by recessively inherited autosomal genes. The characteristic feature of these genes is that they are expressed in the phenotype only if their both mutant alleles are present in homozygous form. Although the frequency of these alleles in the population is usually low, it can be easily increased if heterozygous (carrier) sire is used for artificial insemination thus

resulting in significant economic losses. On the other hand, testing and certifying the absence of such mutations in sires increases their value. Since there is a worldwide tendency towards the implementation of monitoring programs for several genetic diseases in cattle, it is important to update the researchers and veterinary doctors involved in cattle breeding, as well as the farmers, on the use and importance of genetic screening in animal health.

Keywords: cattle hereditary disease, recessive alleles, molecular diagnosis.

USES OF LOW DOSE GnRH AND eCG TREATMENT OF STATIC OVARIES IN THE BEGINNING OF THE TRANSITION FEEDING PERIOD IN HIGH YIELDING DAIRY COWS

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Failure in resumption of the cyclic ovarian activities in postpartum period is one of the important causes of infertility in dairy cows. A static ovary (small ovaries with non dominant follicles and absence of corpus luteum) and transition feeding period (winter-summer) are some of the reasons of this condition. The aim of this study was to evaluate efficiency of the ovarian response to application of low doses of GnRH and eCG, in cows more than 60 days after calving, in the begging of the transition feeding period. During routine ultrasound examination on one commercial dairy farm, 15 cows out of 120 (12.5%) were diagnosed bearing static ovaries. Cows were divided into 3 groups (Group 1. n= 5, Group 2. n= 5, and control group n=5), and subjected to the treatment with 100 µg GnRH, 750 IU eCG, and no treatment (control), respectively. Consecutively, the animals were monitored by real-time B-mode ultrasound machine with 7.5 MHz transrectal probe, every 3 day for next 15 days. Feeding – transition period, was done by substituting corn silage and alfa-alfa with oats hay, haylage and fresh alfalfa lasting 20 days. Treatment was effective in 40% of the cows (2/5) for Group.1, 20% (1/5) Group.2, and 40% (2/5) for the control group. Differences between the groups were statistically non significant. In Group 1, the growing rate of the follicles was 1.3 ± 0.1 mm. and the diameter of the dominant follicle was 14 ± 0.6

mm. In two cows corpus lutea in size of 19.1 and 20.3 mm were diagnosed on day 6. In the Group 2 the growing rate and the diameter of the dominant follicle were 1.1 ± 0.8 mm and 13 ± 1.4 mm respectively. Preovulatory size follicle (17,3mm) was achieved in only one cow, whereas rest of the cows did not resume ovarian activity. In the Control group the growing rate and the diameter of the dominant follicle were 1.0 ± 0.8 mm and 15 ± 1.4 mm respectively. 2/5 cows were diagnosed with CL with diameter 21.6 mm and 20.8 mm on day 9. Our results indicated that uses of lower doses of GnRH and eCG did not stimulate sufficiently resumption of cyclic activity. The reason for this might be in extended NEB period thus further investigation must be done, but this research point as to conclude that it is absolutely essential to optimize feed intake to avoid excessive NEB and maintain satisfactory fertility.

BIOCHEMICAL AND HISTOLOGICAL CHANGES IN NEONATAL BROILER LIVER AFTER DELAYED FEEDING

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Introduction

The importance of early feeding of newly hatched chicks on their performance and postembryonic development was adequately demonstrated by several studies. However, in commercial poultry operations chicks hatch over two days and are transferred from the hatchery when majority of them have hatched. Thus, in practice, newly hatched chicks spend substantial time without access to feed or water (48-72 h), causing poor viability and slow growth. Delayed access to nutrients can result in depressed immune response, increased early mortality and reduced overall performance. In neonate broilers, minimizing the post-hatch holding time and providing early access to feed is desirable to initiate not only growth, but also the development of intestines, liver, pancreas and immune system.

Material and methods

The aim of this paper was to examine the effect

of delayed feed access upon liver function and morphology in neonatal broiler chicks. For that reason, we used a total of 150 unsexed day-old, Cobb 500 broiler chicks, randomly divided into three groups. One group considered as control received feed and water ad libitum; second group-experimental group 1, was restrained from feed access starting from day 1 until day 5 after hatch and received water ad libitum, and third group-experimental group 2, received water ad libitum and was restrained from feed starting from day 3 until day 7 after hatch. Biochemical, histological and pathomorphological examination were done on day 3, 5 and 7. Serum activity of alanine aminotransferase (ALT) and aspartat aminotransferase (AST) were measured spectrofotometrically. on day 1, 3, 5 and 7 post hatch.

Results

Results obtained showed significant ($p < 0.01$) increase of serum activities of both enzymes, due to changes in hepatocyte's cell membrane. Patomorphological changes such as icteric liver and prominent gallbladder were observed in experimental group 1 in 80% of the chicks, while in group 2, on the second day of starvation in 40% of the chicks we found icteric liver. Histological liver analyses showed increased hepatocyte volumen in experimental group 1, whereas in experimental group 2, only enlarged vacuoles were observed. Furthermore, early starvation significantly ($P < 0.01$) reduced weight of liver, compared to those with early access to feed, and short term starvation.

Conclusions

The embryo to neonate transition is a critical period of development that has significant impact on broiler production. Neonates are constantly exposed to different kinds of stressors, and delayed feeding as one of the unavoidable stress factors in the first 24-48 hours after hatch, changes chick's homeostasis, followed by changes in different organ morphology and in many biochemical and hematological parameters.

Key words: starvation, broiler chicks, liver, aminotransferase

COMPARATIVE VARIABILITY OF MICROSATELLITE DNA IN SARHPLANINIAN SHEPHERD

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DNA Microsatellites are codominant genetic markers and they are widely used in the characterisation of biodiversity.

Genetic variability of sarplaninian shepherds populations was determined on the basis of analyses of ten DNA microsatellites loci (FH2361, DGN10, FH3287, FH3924, FH3608, FH3023, FH3489, FH3721, FH4027 and FH2141).

In this study we have included 37 representative individuals from sarplaninian shepherd (19 lowland sarplaninians and 18 mountain sarplaninians).

Informativnes of DNA microsatellites loci was determinate with parametar (Polymorphism Informativ Content-PIC), and according to the determinate number of alleles for each locus. All ten DNA microsatellite loci were highly polymorphic.

In the genome of sarplaninian shepherd loci FH2141 showed highest PIC value (0.975), and the loci with lowest PIC value was FH3924 locus (0.812).

Number of detected alleles for each locus in the genom of sarplaninian shepherd varies from 16 (FH2361) to 49 (FH2141).

Intrapopulation genetic variability was determinate according to the number alleles for each locus separately in each population, mean number of alleles for all eight loci and with number of the characteristic alleles.

The most common alleles in populations of sarplaninian shepherds were detected in FH2141 loci, 49 (34 heterozygos and 3 homozygos)

Key words: dog, wolf, DNA microsatellites, genetic variability

Urea is a byproduct of the protein catabolism. In order for the body to obtain normal energy balance, glucose is required. If there is a condition of hypoglycemia, such as in the first stages of lactation, the body resorts to secondary energy resources, among which are the amino acids. Elevated level of urea nitrogen is an indicator of intensified glyconeogenetic process in which amino acids are deaminized and ammonia is released. This is especially evident after the second week post-partum when the milk production increases and the energy requirements surpass the energy production capacity of the body. The first objective of this research was to determine the average serum levels of glucose and urea nitrogen in cows which are in first and middle stage of lactation, and their correlation. The second objective was to examine the possibility to determine the hypoglycemic status of the animal by monitoring urea nitrogen. This method could be utilized as monitoring system on dairy farms, which could help the farmers detect nutritional deficiencies and take corrective measures on time, in order to avoid loss in their production. For this purpose we used 30 healthy cows of the Holstein-Freisian breed, with average body mass of 450-500 kg, and average milk yield of 25 kg per day, kept in standard microclimatic, zoo-hygienic and zoo-technical conditions. The animals were grouped by the stage of lactation into two groups. Group A (n=15), which were at 0-4 weeks in lactation, and group B (n=15), which were between 16-20 weeks in lactation. Biochemical parameters in the serum were analyzed spectrophotometrically. The results showed that glucose level in group A were 2.0 mmol/l, and BUN were 8.8 mmol/l, which can be related to hypoglycemia or inadequate nutrition due to increased amino acid deamination. Results in group B showed expected levels of glucose and BUN for that stage of lactation. The practical application of this research requires variables that are easily measured on-farm and can be gathered easily. Additional research with more cows is needed to examine these relationships and validate the method as a reliable tool.

Key words: urea, BUN, glycemia, dairy cows

SERUM LEVELS OF GLUCOSE AND UREA NITROGEN IN EARLY AND MIDDLE STAGES OF LACTATION IN DAIRY COWS

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IMMUNOPOSITIVE GH CELLS IN ADULT MALE RATS AFTER EXPOSURE TO HIGH AMBIENT TEMPERATURE

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The effects of exposure to high ambient temperature on morphofunctional characteristics of pituitary somatotrophs (GH cells) in adult male Wistar rats was examined. The experimental group of animals was exposed to $35\pm 1^\circ\text{C}$ for 4 days, whereas the control group was kept at room temperature ($20\pm 2^\circ\text{C}$), under a 12:12 h light-dark regime. GH cells were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. The body weight of animals in the experimental group was significantly decreased by 19.8% compared to the controls. Immunocytochemically identified GH cells in control rat pituitaries were usually situated along the sinusoids. They were intensely stained, oval in shape, with a centrally located spherical nucleus. In rats exposed to high ambient temperature, neither the shape nor the localization of GH cells was significantly changed. GH cells were mostly organized in groups with darker cytoplasmic granules throughout the whole cytoplasm. The cellular and nuclear volume of GH cells, as well as the volume density were significantly ($p<0.05$) decreased by 18.4%, 9.5% and 23.7% respectively, in comparison with the controls. These findings suggest that four days exposure of adult male rats to high ambient temperature has an inhibitory effect on the morphofunctional characteristics of GH cells.

Key words: GH cells, high temperature, rat

ANATOMICAL CORRELATION BETWEEN DOG TRANSVERSAL S10 PLASTINATED THIN SECTIONS AND COMPUTER TOMOGRAPHY (CT) IMAGES

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Introduction

Interpretation of split images produced by computer tomography (CT) requires precise knowledge about

topographical anatomy of the body cavities. In past, three-dimensional models based on CT scan data have been used extensively to study the anatomy of animals. However, it has been shown that it is less desirable to use CT data as the basis for models due to the poor resolution of soft tissues.

Recently, the thin plastinated specimens were affirmed as models for study the topographic anatomy. Thus the most of combined anatomic and diagnostic explorations using CT scans use plastinated tick 1-2 cm S10 specimens that yielded good correlation of the tissue with CT image. But thick plastinated slices cannot offer precise correlation of anatomical structures on identical level with CT images due to the differences in level slice.

Therefore we develop protocol to produce thin 3-5 mm S10 tissue slices that can be combine with CT images to better understanding the transversal anatomy of dog.

Materials and Methods

One dog, 3 year aged was used in the study. The dog was euthanized according approved protocol from animal welfare and ethical committee and fixed in a desired position for computer tomography scanning and sheet plastination.

The dog was scanned on SHIMADZU SCT 6800TXL scanner and immediately deeply frozen at -80°C to preserve the correct position needed for obtaining the slices during the plastination.

The 3-5 mm transversal slices from whole dog body were cut with band saw. Each slice was labeled with a serial number and placed between two plastic grids (2x2 mm). The dehydration was carried out with freeze acetone substitution method in a pure acetone (ratio 10:1) at -25°C for 3 weeks. For impregnation a mixture of silicon's S10/S3 in ratio 100:0.5 for two weeks at -20°C was performed.

Finally, the slices were embedded in a mixture of S10/S3 with additional S6 hardener using a stretch folia method and pictured from a both sides.

Results

Accurate alignment of the structures in the serial macroscopic sections was obtained by the plastination technique. The S10 thin plastinated slices were perfectly preserved without noticeable shrinkage. The quality of the reconstructed images was distinct and perfect, specifically the spatial positions and complicated adjacent relationships of various structures can be shown in direct viewing. Clear distinction between individual structures was easily noted and remarkably useful when viewing and comparing to one another. The position of organs, nerves, vessels as well skeletal muscles with surrounding adipose tissue and skin allows easy correlation with anatomical structures on CT images at identical level.

The computer tomography (CT) images could be used to obtain cross-sectional images with better differentiation of soft tissues, on which resolution of CT images was limited. Discrete anatomical structures

were not delineated due to the ambiguous relationship between signal intensity and tissue composition. It was noticed that the plastinated transverse sections yielded much information especially in skeletal muscles, vessels and nerve arrangement that were difficult to differentiate on the CT images.

Conclusion

The results showed that a combined use of semitransparent slices thin S10 slices from the imaged specimens allows an accurate evaluation in CT images of many anatomic structures and should be used as a valuable tool for interpretation the diagnoses in clinical reveals.

The possibilities of three-dimensional plan-view of whole dog anatomy can help for better understanding the specific anatomical details of the 2 - 3 mm thick CT scans.

Key words: S10 sheet plastination, CT-scans, topographic anatomy, dog

PLASTINATION OF THE CHICKEN EMBRYOS WITH C10 TECHNIQUE IN DIFFERENT EMBRYONIC STAGE OF DEVELOPMENT

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Background

Plastination is a method that provides an ideal presentation of anatomical and histological structures of animal tissue. Plastination of chick embryos has not been made so far.

Thus the aim of our study was the preservation of chick embryos in their natural condition at the age of 9, 11, 12, 13, 14, 17, 19, 20 days of development.

Materials and Methods

Eight chicken embryos were plastinated with standard technique of plastination S-10. The embryos were fixed by injecting 5% formalin at a stage when they were still in the egg shell. Dehydration was performed by the method of freeze substitution on -25°C for 27 days. Impregnation with a mixture of silicon S10/S3 in a ratio of 100:1 was performed during 25 days at - 25 ° C. Embryos were fixed with gas curing agent S6 at room temperature for 3 days.

Results

Plastinated embryos were dry, non-toxic and flexible.

Their shrinkage was not observed. In all embryos their natural color was preserved. Their external shape and size varied, depending of the days of embryonic development. Starting with 9th day, the embryo was big as grain with expressed eyes. On 11th, 12th, 13th, 14th of embryonic development, blood vessels were initiated in the yolk sac. On 13th and 14th day coverage of embryos with feathers was evident. At this stage they were bent in the shape of a sickle. On 14th day the eyes were clearly differentiated, as well as the nails and the wings. In the period of 17th day yolk sac was the size of a walnut, coverage with white feather was even, the eyes were fully developed, whether the beak was not fully developed and the nasal openings were noticeable. On 19th day they acquired yellow feathers, beak was more elongated, and yolk sac was the size of half a walnut, with initiated blood vessels. Fingers were extended and collected. There was no yolk sac on the 20th and the feathers dark yellow color. There was a complete development of the legs and ledge on top of the beak.

Conclusion

Plastinated embryos are durable educational materials that can help for studying the anatomical structures. At the same time they present durable models that allow monitoring of embryonic development of the birds species.

Key words: chick embryos, S10 plastination, education

ANIMAL WELFARE DURING STUNNING AND SLAUGHTER OF PIGS AND LAMBS IN APPROVED SLAUGHTERHOUSES IN MACEDONIA

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Animal welfare during stunning and slaughtering of animals was assessed in 4 approved slaughterhouses in Republic of Macedonia. Duration of electrical stunning and stick to bled interval were followed in two pig slaughterhouses and two lambs slaughterhouses.

Stunning and slaughter of 140 pigs in total was observed in both slaughterhouses with 70 pigs from each. Electrical current of 1,2 A and 1,4 A was used, with average times of application duration of 6,3s and 14,5s. Longer than recommended current application

times were detected in 18% and 100% of the animals respectively. Stun to bleed interval was short enough in 92% and 90% of animals, to guarantee killing of unconsciousness animals.

Animal welfare during lamb slaughtering was assessed for 200 lambs in two slaughterhouses. Electrical current of 1,1 A in first and 0,5 A in second slaughterhouse was recorded, with average application duration longer than needed in 22% and 83% of animals respectively.

Interval from stun to bleed in 98% of the slaughtered animals was in compliance with animal welfare rules in both slaughterhouses.

These data put emphasis on necessity for every slaughterhouse-operator to implement procedures according the legislation for animal welfare in order to assure humane and efficient slaughter.

Key words: *animal welfare, slaughter, stunning haken.*