FOLIA VETERINARIA, 51, 1, 2007

CONTENTS

FOLIA VETERINARIA

PUBLISHED BY
THE UNIVERSITY OF VETERINARY MEDICINE
KOŠICE, THE SLOVAK REPUBLIC

Folia veterinaria
Vol. 51, 2007

VYDALA UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA
KOŠICE 2007

E D I T O R I A L

In the year 2007 the scientific journal FOLIA VETERINARIA entered the second fifty-years of its existence. Short evaluation and history of the first fifty years of this journal were presented in the first issue of its last (50th) volume on pages 3—4. This short review described its gradual transformation from a year-book, issued in Slovak with only abstracts in foreign languages, up to its full-English version starting from 1982 (Vol. 26), which increased interest in this journal also outside Slovakia. In addition to the regular four issues per year, there were also special issues — supplements (in irregular intervals) which contained information about some important (particularly at international level) scientific events organised at our alma mater.

The editorial office plans to follow the same trend (set during the first fifty years of its existence) in the future. However, the journal will also reflect some important changes related to reorganisation of our University. The University extended its study programmes by bachelor study of pharmacy and bachelor study of cynology which will inevitably influence the contents of FOLIA VETERINARIA and increase interest in this journal in concerned specialists involved in research and education activities.

In relation to this it appears very important to make every effort so FOLIA VETERINARIA, as the only Slovak veterinary journal issued in English, can become one of CURRENT CONTENTS journals. We hope that the new management of the University will succeed in this direction within its present term of office which would benefit the entire veterinary community in the Slovak Republic and particularly our alma mater.

Reaching the above mentioned goals will require much effort on the side of UVM in Košice regarding the activities of the new editorial board, university teaching staff, contributors and reviewers of papers sent for publication. I believe that we will all contribute effectively to this programme and our joint effort will be crowned with success.

Prof. MVDr. Emil Pilipčinec, PhD
Editor in chief of FOLIA VETERINARIA
The Rector of the University of Veterinary Medicine in Košice

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 5–8, 2007

HISTOLOGICAL ANALYSIS OF SIZE, STRUCTURE AND APPEARANCE OF GIANT FIBRES IN SKELETAL MUSCLES OF PIGS

Makovický, P.[1], Makovický, P. Jr.[2], Kulíšek, V.[2], Debrecéni, O.[2], Haščík, P.[2]

[1]Teaching Hospital, Institution of Pathology, Špitálska 6, 949 01 Nitra
[2]Slovak Agricultural University, Tr. Andreja Hlinku 2, 949 76 Nitra
The Slovak Republic

pmakovicky@email.cz

SUMMARY

Giant fibres are object of histopathological investigations and they are also in correlation with metabolism defect of cross striated skeletal muscles. The present work describes occurrence, expression, appearance and also morphological structure of giant fibres of pigs. Results shows that giant fibres were detected for first time in 48-old days pigs. They are not always oval because they have often angular shape. As pigs age has increased, so too number of giant fibres in differently muscle was detected. Paper gives information about their most manifestations in *m. rectus femoris* (MRF) and their lows occurrence in *m. longissimus dorsi* (MLD). Results of morphometric analysis demonstrated, that thickness of giant fibres was a higher value as average thickens of normal muscular fibres from 48 until 192-days old pigs. At 1055-days old pigs was the thickness of giant fibres identical with average thickness of their intermediate (α-Red), or white (α-White) muscular fibres. Growth of giant fibres can be limited. Giant fibres occurrences not stop growth process of normal muscular fibres in muscles.

Key words: centronuclear myopathy; giant fibres; muscular fibres; skeletal muscle; pigs

INTRODUCTION

A cause of long-term trends breeding of pigs is that their muscle now created approximately 40 to 55 % from weight of their body. The cross striated skeletal muscle, which has contraction ability is in the process from quantitatively point of view in pigs body dominant place. Basic processors of skeletal muscles are muscular fibres, which are parallel arranged. Each fibre contains several elongated, oval nuclei, in the fiber periphery (10). They length can be to 40 cm, they have cylindrical shape and their thickness have approximately 10 to 150 µm (7). However enzymatic activity and typical metabolic profile of skeletal muscle of pigs can affect their higher growth of weight condition. It is evident that also microscopic structure of muscle is one from lot of indicator of meat quality. In dependence on age, state of health organism, nutrition, but also on breeding style may be observed in muscles some systematic microscopically changes of muscular and connective tissues. During postnatal growth of muscular fibres it is possible from microscopically point of view observe varied situations and meet varied influences on giant fibres (5, 9).

Great giant fibres in microscopically image were observed first time by Wohlfart (20). Subsequently they were observed frequently in skeletal muscles of pigs and their occurrence was give in connection with stress skeleton muscular syndrome of pigs (13, 14, 19). A relationship between PSE meat and occurrences of giant muscular fibres was observed too (4). Its was documented, that giant muscular fibres detection can by supposed as some special metabolism defect of skeletal muscles and that they are indicators of decreased quality of pork (6, 12, 15, 16).

However the giant fibres are present by variable age category of pigs. In our work are described from light microscopic view their occurrence, expression, appearance and also their morphological structure.

MATERIAL AND METHODS

The giant fibres were detected in 48 (1 months and 2 weeks), 84 (2 months and 3 weeks), 192 (6 months and 1 weeks) and 1055-old days (3 years) Landrace breed pigs. Each group represent ten pigs, only in 1055-old day pigs it was three pigs. The average weight of group were at 48-old days pigs 17.20 kg, at 84-old days pigs 31.00 kg, at 192-old days pigs 96.30 kg and at 1055-old days pigs 319.73 kg. All pigs were male sex, only last group were created by female sex. The animals were killed with standard methods. For euthanasia of 48-old days pigs special T-G1 solution was used, which is especially designated for domestic and farm animals. Killing of animals was brought about the intracardial application of 0.50 ml T-G1 solutions. The muscle samples were obtained from three muscles: *m. triceps brachii* (MTB), *m. longissimus dorsi* (MLD) and *m. rectus femoris* (MRF). The muscle samples were taken by 30 minutes *post mortem* according to the methodology of Kulíšek *et al.* (8). All samples were marked and fixed in nitrogen medium. Than were the samples located in a container with nitrogen medium and sent to a histological laboratory. The samples were stored in a freezing box in a temperature below −30 °C.

All samples were cute on freezing microtome (cryocut—MTB) at the temperature below −18 to −21 °C with thickness of 10 µm. Afterwards were the slices stained by haematoxylin-eosin. To detect the process of dehydrogenisation for the visualisation of individual muscular fibres according to the methodological processes recommended by Stein and Padykula (17).

The microscopically preparation were evaluated subjectively and also objectively. At subjective evaluation were writed special protocols with the microscopically data of muscle structure in all sections of three muscles. Precisely was measured the value of average thickness of the individual muscle fibres. Average thickness of all individual types of muscular fibres and average thickness of giant fibres were measured. The differences was compared and also commented. The subjectively, also objectively evaluations were realised on light microscope Olympus Provis with special analyser.

RESULTS

The subjective evaluation of giant fibres in the pig skeletal muscles

In muscles of 48-old days pigs in some microscopically preparations singleness hypertrophic giant fibres were detected. In MTB giant fibres were missed and in MLD their number was sporadic. Great number of giant fibres was in MRF, where in primary muscular fascicles it was to find two or four giant fibres. Giant fibres were generally located in periphery of primary muscular fascicles. They are most shaped, but any have angular shape with numerous vacuoles. Nucleuses of giant fibres are oval shaped and they are multiply. Some or several nucleuses are directed into the centre of fibres, exceptionally they may by found in the centre of fibres. Cytoplasm of giant fibres have deep colour and with visible tightly grooves.

In 84-old days pigs age category the giant fibres are detected in all muscles investigated, but mainly in the MRF and MLD. Their occurrence is lower in the MTB. Thickness variability of muscular fibres is distinctive especially in MRF. Giant fibres have hypertrophy characters, they are always oval shaped with deep homogenous sarcoplasm with numerous vacuoles. Central movements of nucleus from sarcolemma to the centre of muscular fibres are distinct. Nucleus have grouping into the centre of sarcoplasm and they are in immediate contact. Central nucleuses have chain structure. Some spontaneous marks of grooves are visible on periphery of giant fibres, common in immediate contact with nucleolus movements.

In all samples of muscles from 192-old days pigs the occurrence of giant fibres is evident. Thickness disproportionateness of normal and giant muscular fibres is major. Giant fibres are oval shaped, with always deep vacuolar sarcoplasm. They are unlike to normal muscular fibres surrounded with bigger layer of endomysium, which contain numerous capillaries and reticular, or reticular fibres. The giant fibres are in most cases located on periphery of some muscular fascicles in immediate contact with *perimysium internum*. In the contact of primary fascicles of muscular fibres it is often visible also two giant fibres from opposite fascicles. It is not exception, that two giant fibres are visible in immediate contact in one muscular fascicle. Several central nucleuses are often present, but it is visible also individual central nucleus in active movement. It is visible some central movements of muscular nucleus from periphery of giant fibres. They have vertically or spiral moving. In the centre, or near the sarcolemma of giant fibres is visible some grooves.

The size of muscular fibres of 1055-old days pigs is a continuum and their proportionality is not noticeably different. The giant fibres are in muscles represented only sporadic. Most of muscular fibres have an angular shaped and only sometimes in individual fascicles it is visible some oval muscular fibres. These fibres unlike another ages category of pigs are not different with other normal muscular fibres. The thickness of these muscular fibres is utterly in correlation with thickness of intermediate (α-Red), or white (α-White) muscular fibres. On these characteristics it is not possible categorised these fibres between giant fibres.

Cytoplasm of muscular fibres is fragmented, myofibrils are right detected and the fibres are not vacuolated. Cytoplasms of some oval fibres are homogenous, darker and they are often evidently vacuolated.

On difference another category it is in these fibres observable evidently numerous number of nucleuses and central nucleus, which are detected in all three types of altered muscular fibres. At point of primary muscular fascicles have touched to *perimysium internum*, respectively interstitial tissue is befallen practically all muscular fibres with this phenomenon. These fibres with visage can corresponde whit giant fibres. The nucleuses are

travelling individually, or collectively in such quantity that the report comes up to centronuclear myopathy. In the middle of muscular fibres the nucleuses create some specially grouping like chain structure. Like this arranged nucleus has ranged almost continuously on entire perimeter of muscular fibres. Clear marks of scissions are not detected, but on periphery of giant fibres it is observed involuntarily linear scissions, which is localised near the central nucleus.

The objectively evaluated of giant fibres in skeletal muscle of pigs

The subjectively evaluations were appended with objectively morphological measuring. It was detected a value thickness of all (normal and giant) and only giant muscular fibres. The value about thickness was divided according to individual ages category of pigs and according to concrete skeletal muscles (MTB, MLD, MRF). Real results were established in especially tables, which values inform about size and structural changes of muscular fibres of pigs by variable ages category. It was confirmed, that exist some differences between average thickness of normal and giant muscular fibres (Table 1).

From all samples of 48-old days pigs followed, that total average thickness of muscular fibres was 30.17 μm. For all that the average thickness of all muscular fibres in MTB was presented 29.43 μm, in MLD 29.56 μm and in MRF 31.53 μm. In MTB was detected any giant fibres. In MLD was thickness value of giant fibres 30.27 μm and in MRF presented 33.42 μm.

For morphological measurement were subjugated also samples of skeletal muscles from 84-old days pigs. Results shows that total average thickness of all muscular fibres make 36.22 μm. The average thickness of all muscular fibres in MTB was making 35.93 μm, in MLD 34.80 μm and in MRF 37.93 μm. The average thickness of giant fibres in MTB was making 102.96 μm, in MLD 107.53 μm and in MRF 133.41 μm.

From all samples of 192-old days pigs followed, that total average thickness of muscular fibres was 78.47 μm. The average thickness of all muscular fibres in MTB was 71.35 μm, in MLD 77.40 μm and in MRF 86.66 μm. The average thickness of giant fibres in MTB was 124.42 μm, in MLD 126.44 μm and in MRF 141.20 μm.

As is documented the morphological analysis of 1055-old days pigs, total average thickness of all muscular fibres was 151.70 μm. The average thickness of all muscular fibres in MTB was 153.97 μm, in MLD 150.07 μm and in MRF 151.07 μm. The average thickness of giant fibres in MTB was 220.69 μm, in MLD 152.30 μm and in MRF 172,41 μm.

Table 1. Thickness of muscular fibres by variable ages category of pigs (μm)

Age of pigs in days and number of group	Muscles	ϕ thickness of all muscular fibres	ϕ thickness of giant fibres	Thickness limits of giant fibres
48-days old n = 10	MTB	29.43	Nondetectable	nondetectable
	MLD	29.56	30.27	20.42 till 54.68
	MRF	31.53	33.42	20.59 till 50.48
84-days old n = 10	MTB	35.93	102.96	76.22 till 159.46
	MLD	34.80	107.53	74.50 till 175.80
	MRF	37.93	133.41	94.56 till 192.43
192-days old n = 10	MTB	71.35	124.42	89.62 till 167.71
	MLD	77.40	126.44	108.62 till 158.53
	MRF	86.66	141.20	109.45 till 186.35
1055-days old n = 3	MTB	153.97	220.69	177.72 till 276.51
	MLD	150.97	152.30	99.21 till 222.46
	MRF	151.07	172.41	148.25 till 197.55

n – number of group, MTB – *m. triceps brachii*
MLD – *m. longissimus dorsi*, MRF – *m. rectus femoris*

DISCUSSION

Long-term effort for rationalisation of nutrition trends of broad populations has consisted also in surety of qualitative parameters of food chain. Microscopically analysis of skeletal muscles is one instrument for detection of meat quality. The meat quality term is corresponding with indicator resume of variable requirement form. Some knowledges on this level make possibility for valuate changes in standard microscopically structure of skeletal muscle. It is clearly, that changes of muscle fibres and their effect on function of skeletal muscles come to the fore. Giant fibres as object of intensive research become important from her effect on skeletal muscles function and on the meat quality.

Our results and results others authors are comparable. Sosnicki (16) has detected, that giant fibres are oval shaped and his thickness has exceed average thickness of normal muscular fibres. Similarly has commented also Dutson *et al.* (3) and Rehfeldt and Ender (12). Our results documented, that the giant fibres are not always oval because they are often of angular shape. At 1055-days old pigs was the thickness of giant fibres identical with average thickness of their intermediate (α-Red), or white (α-White) muscular fibres.

This fact has demonstrated, that normal muscular fibres show continual growth, but the growth of giant fibres can be limited. Giant fibres occurrence not stops growth process of normal muscular fibres in muscles. These facts have documented some microscopically imaging of 1055-old days pigs with a number of giant fibres scissions. Also work Dubowitz (2) and Carpenter and Karpathi (1) maybe state, that scission of muscular fibres is in correlation with above diameter value.

Fiedler *et al.* (6) has noticed that occurrences of giant fibres are in correlation with stress skeleton muscular syndrome of pigs. Similarly facts has stated before Sosnicki (16). Weiler *et al.* (19) has compared muscular sample of domestic and wild pigs, but giant fibres were detected only in skeletal muscles of domestic pigs. It is possible that occurrences of giant fibres may be determined by nutrition, also by way breeding of pigs and by another variable conditions. In our experiment, where we have go out from the study of skeletal muscles and interstitial tissue by variable ages category were breed all pigs by standard and similar conditions. Our results shows that giant fibres were detected for first time in 48-old days pigs after born, but in lower category we have detected any giant fibres.

Biologically, physically, chemically, mechanically and other characters are the point of much works, which deals with problems about giant fibres. Among others works are analysed effects of giant fibres on organisation of skeletal muscle from his functionality and also muscular fibres functionality. In addition to it is possible meet with some specially works, which are oriented on genesis of giant fibres. Dutson *et al.* (3) have stated that giant fibres are not capable to normal contractions. Weiler *et al.* (19) have stated that giant fibres occurrences are one from primary indicator of skeletal muscles degeneration. Stephan and Dzapo (18) has declared that giant fibres occurrences are responsible of muscles disordered metabolism. Pilegaard *et al.* (11) have predicted, those giant fibres geneses are results of lactate intracellular accumulations in muscle fibres.

REFERENCES

1. Carpenter, S., Karpati, G., 1984: *Pathology of Skeletal Muscle*. Churchill and Livingstone, New York.

2. Dubowitz, V., 1965: Enzyme histochemistry of skeletal muscle. Part I. Developing animal muscle. *J. Neurol. Neurosurg. Psychiat.*, 28, 516–519.

3. Dutson, T. R., Merkel, R. A., Pearson, A. M., Gann, G. L., 1978: Structural characteristics of porcine skeletal muscle giant myofibers as observed by light and electron microscopy. *J. Anim. Sci.*, 46, 1212–1220.

4. Fazarinc, G., Candek-Potokar, M., Ursic, M., Vrecl, M., Pogacnik, A., 2002: Giant muscle fibres in pigs with different Ryr1 genotype. *Anat. Histol. Embryol.*, 31, 367–371.

5. Fiedler, I., Ender, K., Wicke, M., Maak, S., von-Lengerken, G., Meyer, W., 1999: Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. *Meat Sci.*, 53, 9–15.

6. Fiedler, I., Kuhn, G., Hartung, M., Kuchenmeister, U., Nurnberg, K., Rehfeldt, C. *et al.*, 2001: Effects of the malignant hyperthermia syndrome (MHS) on meat quality, muscle fibre characteristics end metabolic traits of the Longissimus muscle in Pietran pigs. *Arch. Tierz. (Archiv. Anim. Breed.)*, 44, 203–217.

7. Goldring, K., Patridge, T., Watt, D., 2002: Muscle stem cells. *J. Pathol.*, 197, 457–467.

8. Kulíšek, V., Dvořák, J., Makovický, P., Vykoukalová, Z., Civáňová, K., 2003: Methodological process samples intake from pigs embryos for geneticall and histological analysis. *Infovet,* 10, 39–41.

9. Linke, H., 1972: Histologische Untersuchungen bei wafrigem, blassem Schweinefleisch. *Fleischwirtschaft*, 52, 493–512.

10. Picard, B., Jurie, C., Cassar-Malek, I., Hocquette, J. F., Lefaucher, L., Berri, C. *et al.*, 2003: Myofibre typing and ontogenesis in farm animal species. *Product. Animal*, 16, 117–123.

11. Pilegaard, H., Mohr, T., Kjaer, M., Juel, C., 1998: Lactate/H+ transport in skeletal muscle from spinal-cord-injured patients. *Scand. J. Med. Sci. Sports*, 8, 98–101.

12. Rehfeldt, C., Ender, K., 1993: Skeletal-muscle cullularity and histochemistry in response to porcine somatotropin in finishing pigs. *Meat Sci.*, 34, 107–118.

13. Rehfeldt, C., Ender, K., 1995: Somatotropin action on skeletal-muscle and backfat cellularity in pigs of different breed and halothane sensitivity. *Arch. Tierz. (Archiv. Anim. Breed.)*, 38, 405–415.

14. Scheper, J., 1979: Influence of enviromental and genetic factors on meat quality. *Acta Agr. Scand.*, 21, 20–28.

15. Sosnicki, A., 1985: Histological characteristics of stress-induced myopathy of the *musculus longissimus dorsi* in swine. *Patol. Pol.*, 36, 302–320.

16. Sosnicki, A., 1987: Histopathological observation of stress myopathy in *m. longissimus* in the pig and relationships with meat quality, fattening and slaughter traits. *J. Anim. Sci.*, 65, 584–596.

17. Stein, J. M., Padykula, H. A., 1962: Histochemical classification of individual skeletal muscle fibers of the rat. *Am. J. Anat.*, 110, 103–123.

18. Stephan, E., Dzapo, V., 1997: Histometric investigation of the breast muscle of laying and meat genotype in the course of gaining weight. *Arch. Geflüge*, 61, 62–65.

19. Weiler, U., Appell, H. J., Kremser, M., Hofacker, S., Claus, R., 1995: Consequences of selection on muscle composition. A comparative study on gracilis muscle in wild and domestic pigs. *Anat. Histol. Embryol.*, 24, 77–80.

20. Wohlfart, G., 1937: Über das Vorkommen verschiedener Arten von Muskelfasern in der Skeletmuskulatur des Menschen und einiger Säugetiere. *Acta Psychiat.*, 13, 100–119.

Received September 13, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 9–13, 2007

AN EVALUATION OF SERUM GLUCOSE, BHB, UREA AND CORTISOL CONCENTRATIONS IN PREGNANT EWES

Ramin, A. G., Asri-Rezaie, S., Macali, S. A.

Large Animal, Vet. Sci., Urmia Univ., Urmia
Iran

ABSTRACT

A study was conducted to determine the distribution of subclinical pregnancy toxaemia by evaluation of the serum glucose, beta-hydroxybutyrate (BHB), urea and cortisol concentrations in pregnant ewes. 809 blood samples were collected from the jugular veins of 497 pregnant, 242 lambed and 70 aborted ewes. They were apparently healthy without any signs of disease. The concentrations of BHB, glucose and urea were assessed by a spectrophotometer and cortisol by ELISA. Mean serum glucose ($mg.dl^{-1}$), BHB ($mmol.l^{-1}$), urea ($mg.dl^{-1}$) and cortisol ($ng.ml^{-1}$) concentrations in pregnant ewes were 35.1, 0.45, 33.1 and 22.4, in lambed ewes were 35.7, 0.44, 30.0, 13.5 and in aborted ewes were 41.4, 0.4, 28.6, 24, respectively. Mean urea and BHB concentrations in pregnant ewes were greater and glucose was lower than in lambed and aborted ewes. Cortisol concentrations in pregnant ewes were higher than in the lambed but lower than in aborted ewes. Mean comparison of parameters showed a significant difference ($P < 0.01$) except for BHB in that the concentrations were similar in all groups. A negative correlation coefficient in urea concentration was observed between pregnant and lambed ewes ($r = -0.14$, $P < 0.05$). There were also correlations between BHB and glucose concentrations in pregnant ewes ($r = -0.16$, $P < 0.01$), between BHB and cortisol concentrations in lambed ewes ($r = 0.25$, $P < 0.01$) and between BHB and urea concentrations in aborted ewes ($r = 0.28$, $P < 0.05$). With regard to the references, the concentration of BHB > 0.7 $mmol.l^{-1}$ is considered as subclinical pregnancy toxaemia, the distribution and percent of ewes in pregnant, lambed and aborted groups were 61 (12.3%), 17 (7%) and 6 (8.57%), respectively. Similarly, for glucose < 20 $mg.dl^{-1}$ were 76 (15.3%), 38 (15.7%), 10 (14.3%) and for cortisol > 52 $ng.ml^{-1}$ were 28 (8.54%), 1 (0.6), 5 (10.2%), respectively. Significant differences were found for the BHB and cortisol groups. The highest subclinical pregnancy toxaemia occurs among pregnant and aborted ewes. Mean comparison of parameters in the group with BHB > 0.7 $mmol.l^{-1}$ showed a significant difference ($P < 0.01$) for urea and cortisol concentrations and in the group with glucose < 20 $mg.dl^{-1}$ and cortisol > 52 $ng.ml^{-1}$ showed differences just for BHB concentration. Thus, it is concluded that hypoglycaemia, uremia and high cortisol concentration in late pregnancy could be considered as subclinical pregnancy toxaemia up to 12% that should be seriously considered in order for prevention or treatment.

Key words: BHB; cortisol; glucose; pregnant, lambed, aborted ewes; subclinical pregnancy toxaemia; urea

INTRODUCTION

Blood glucose, BHB, urea and cortisol concentrations are mainly recommended to diagnose pregnancy toxaemia in ewes and does. These substances are the sources of energy, final product of fat, protein metabolisms and stress phenomenon, respectively, which are predisposing factors of pregnancy toxaemia. Determination of these parameters and their correlations will facilitate the therapeutic and prevention methods for pregnancy toxaemia in pregnant ewes.

Reported values for blood glucose, ketone bodies, cortisol and urea concentrations in non-pregnant ewes were 35–45 $mg.dl^{-1}$ (15), 0.7 $mmol.l^{-1}$ (17), 10 $ng.ml^{-1}$ (7) and 6.92 $mmol.l^{-1}$ (23), respectively. The values were varied according to preg-

nancy, parturition and lactation (3), stress (7) and pregnancy toxaemia (22). Blood urea in pregnant ewes is reported to be more than in lactating and non-pregnant ones (22), whereas Firat and Ozpinar (2) have reported no difference between them. Ramin *et al.* (16) have reported that urea in non-pregnant ewes to be more than in pregnant ewes. Hamadeh *et al.* (16) have concluded that blood glucose in pregnant ewes is less than that in non-pregnant and lactating ewes, while Firat and Ozpinar (3) have recorded no difference in glucose or BHB before and after pregnancy and the lactation period. The values for ketone bodies were also different (16), but for cortisol values were not (8).

West (2) has shown a negative correlation between glucose and BHB in pregnancy toxaemia, but it was not confirmed in a study conducted by Bickhardt *et al.* (1). In pregnancy toxaemia hypoglycaemia was recorded by hyperketonaemia (16). Firat and Ozpinar (2) have established a positive relationship between blood glucose, urea and dietary protein intake. Finally, a positive relationship has been found between ketone bodies and urea in pregnancy toxaemia (16). Presence of the correlations between one or more parameters in pregnancy toxaemia not only show their diagnostic importance, but also directs essential attention towards priority in disorders occurring in pregnancy toxaemia.

Schlumbohm and Harmeyer (19) believe that hyperketonaemia will not cause hypoglycaemia unless it is coupled with hypocalcaemia. However, Wastney *et al.* (21) believe that a defect in glucose haemostasis control is the main cause of hyperketonaemia. Ford *et al.* (4) have stated that in 80% of pregnancy toxaemia blood cortisol concentration is high. Henze *et al.* (7) have referred to the increase in BHB and cortisol amount but have not mentioned urea, whereas West (22) has reported a significant increase in urea concentration following pregnancy toxaemia.

With the consideration of the reported results and values for relevant parameters either in subclinical or clinical pregnancy toxaemia and in order to present an appropriate diagnosis, treatment, prevention and prognosis for pregnancy toxaemia in ewes, this study was conducted with following objectives:

1) Determination and comparison of the concentrations of blood glucose, BHB, cortisol, and urea in pregnant, lambed and aborted ewes.

2) Determination of a relationships between intragroup and intergroups.

3) Distribution and percentage of probable subclinical pregnancy toxaemia and

4) Determination of the priority of parameter abnormalities that could happen in subclinical pregnancy toxaemia.

MATERIAL AND METHODS

Flocks and ewes

A total of 809 blood samples was taken from 497 pregnant ewes (in the last month of pregnancy), 242 lambed ewes (one week after lambing) and 70 aborted ewes that were selected from among 145 flocks located at village 45 in the Urmia suburbs in 2005. On average five pregnant and three lambed ewes were selected from each herd. If there were aborted ewes in the flocks, blood samples were also collected from them. Blood samples were taking mainly early in the morning when they were in the pen or around the village. They were fed lucerne and grass hay.

Sample collection procedure

Samples were taken by a five ml disposable syringe from the jugular vein of pregnant, lambed and aborted ewes. Blood was collected in 10 ml test tubes and were carried immediately to the laboratory centre. The ewes were apparently healthy with no special clinical signs, aged over one year old and supplementary information as to the location of the village, breeder name, date of sampling and nutritional program was recorded. Samples were centrifuged at 3 000 *g* for fifteen minutes and serum was separated to assess the glucose, urea, BHB and cortisol concentrations. Blood from aborted ewes was tested for brucellosis and were all negative.

Laboratory tests

Blood glucose ($mg.dl^{-1}$), urea ($mmol.l^{-1}$) and BHB ($mmol.l^{-1}$) concentrations were measured by spectrophotometer (RA-1000, UK) using commercial glucose, urea (Pars Azemon Iran) and BHB (Runbut, UK) kits. Cortisol ($ng.ml^{-1}$) was measured by ELISA using a commercial cortisol kit (Human, Germany).

Statistical method

SPSS software program (Version 13) and case summaries were used to determine mean, standard deviation and standard error. ANOVA was used for comparison of the mean blood parameters among pregnant, lambed and aborted ewes. Student *t*-test was carried out for measuring the difference between blood parameters. Pearson correlation test was applied to establish relationship between parameters under study within and between groups. Assuming BHB < 0.7 $mmol.l^{-1}$ (17, 10), glucose < 20 $mg.dl^{-1}$ (16) and cortisol < 52 $ng.ml^{-1}$ (7), the distribution and percentage of ewes in each group was determined and analysed by the Chi-square test to find out the differences among those distributions. Mean and standard deviation of other parameters were measured and compared with AVOVA among those distributions.

RESULTS

Mean ± SE and the range for blood glucose, urea, BHB and cortisol concentrations are shown in Table 1. Mean urea and BHB concentrations in pregnant ewes were greater and glucose was lower than in lambed and aborted ewes. Cortisol concentration in pregnant ewes was higher than in lambed ewes but lower than in aborted ewes.

Blood parameters showed a significant difference ($P < 0.01$) in glucose ($F = 6.9$), urea ($F = 7.3$) and cortisol ($F = 6.7$) concentrations but the difference in BHB among groups was insignificant. The *t*-test results revealed a difference in glucose concentration between pregnant and lambed ewes ($P < 0.01$) and in urea concentration between

Table 1. Mean, standard error and range of blood glucose, urea (mg.dl^{-1}), BHB (mmol.l^{-1}) and cortisol concentrations in pregnant, lambed and aborted ewes

Parameters	Pregnant ewes		Lambed ewes		Aborted ewes	
	$\bar{x}$ ± SE (No.)	Range	$\bar{x}$ ± SE (No.)	Range	$\bar{x}$ ± SE (No.)	Range
Glucose	35.1 ± 0.6 (497)	8.80	35.7 ± 0.9 (242)	10.86	41.4 ± 1.7 (70)	13.73
Urea	33.1 ± 0.6 (497)	9.87	30.3 ± 0.7 (242)	10.72	28.6 ± 1.3 (70)	11.54
BHB	0.45 ± 0.1 (476)	0.1 – 1.3	0.44 ± 0.1 (122)	0.1 – 1.0	0.4 ± 0.1 (59)	0.03 – 0.99
Cortisol	22.4 ± 1.7 (328)	2.200	13.5 ± 1.1 (164)	1.67	34 ± 4.9 (49)	11.54

No. – Number of ewes

Table 3. The results of ANOVA in ewes with BHB over 0.7 mmol.l^{-1}, glucose less than 20 mg.dl^{-1} and cortisol over 52 ng.ml^{-1} concentrations

Parameters	Glucose		Urea		Cortisol	
	F	SS	F	SS	F	SS
BHB > 0.7	611	1.7	1652	5.6**	5854.7	5.6**
	BHB		Urea		Cortisol	
Glucose < 20	0.29	3.3*5	225	0.8	574.8	0.69
	Glucose		Urea		BHB	
Cortisol > 52	249	0.9	327	1.2	0.56	4.8*

** – P < 0.01, – P < 0.05

Table 2. Mean±SE and frequency (No) of blood parameters in pregnant, lambed and aborted ewes with BHB > 0.7, glucose < 20 mg.dl^{-1} and cortisol < 52 ng.ml^{-1} concentrations

Parameters	BHB > 0.7 mmol.l^{-1}			Glucose < 20 mg.dl^{-1}			Cortisol < 52 ng.ml^{-1}		
Ewes	Glucose	Urea	Cortisol	BHB	Urea	Cortisol	Glucose	BHB	Urea
Pregnant	31.8±1.8 (61)	34.2±1.7 (61)	22.2±3.6 (41)	0.5±0.03 (70)	32±1.38 (76)	18.3±3.3 (46)	37.5±2 (28)	0.5±0.1 (28)	35.4±2.8 (28)
Lambed	37.7±3.0 (17)	28.6±2.3 (17)	16.3±4.7 (13)	0.37±0.1 (18)	30±1.75 (17)	17.3±3.1 (13)	52 (1)	1.09 (1)	17 (1)
Aborted	39.5±3.6 (6)	45.2±4.3 (6)	75.0±3.5 (6)	0.37±0.1 (8)	28±3.27 (10)	29±10.1 (5)	42.2±8 (5)	0.3±0.1 (5)	34.2±4.3 (5)

pregnant with and lambed and aborted ewes (P < 0.01). Cortisol concentration in lambed ewes was significantly (P < 0.01) lower than in aborted and pregnant ewes. BHB concentration demonstrated a significant difference between aborted and pregnant ewes (P < 0.01).

Negative correlation was found in urea concentration between pregnant and lambed ewes (r = –0.14, P < 0.05). There were also correlations between BHB and glucose concentrations in pregnant ewes (r = –0.16, P < 0.01), between BHB and cortisol concentrations in lambed ewes (r = 0.25, P < 0.01) and between BHB and urea concentrations in aborted ewes (r = 0.28, P < 0.05).

If the concentrations of BHB > 0.7 mmol.l^{-1}, glucose < 20 mg.dl^{-1} and cortisol > 52 ng.ml^{-1} are considered as subclinical pregnancy toxaemia, therefore, the distribution and percentage of ewes in pregnant, lambed and aborted groups is demonstrated in Table 2. Chi-square tests show significant differences for the BHB and cortisol groups only. The highest subclinical pregnancy toxaemia occurs among pregnant and aborted ewes. Mean comparison of parameters in group with BHB > 0.7 mmol.l^{-1} showed a significant difference (P < 0.01) for urea and cortisol concentrations and in group of glucose < 20 mg.dl^{-1} and cortisol > 52 ng.ml^{-1} showed differences just for the BHB concentration (Table 3).

DISCUSSION AND CONCLUSION

The glucose results being high in aborted and low in pregnant ewes confirm that continuation of pregnancy accumulates hypoglycaemia. The values reported for glucose (20) was in agreement with this study for lambed and aborted ewes, but not in pregnant ones. The value below 20 mg.dl^{-1} considered as subclinical pregnancy toxaemia (17), means that the ewes in this study with less than 20 mg.dl^{-1} must be suspected as having subclinical pregnancy toxaemia.

Blood glucose is known as a metabolic profile test, thus, it has distinguishable value in pregnancy toxaemia, retarded growth, weight loss, production and reproduction defects (16). It is varied in pregnancy, lactation and non-lactation (20, 3, 6). This variation is related to nutrition, production and reproduction (16). The differences in glucose concentration among pregnant, lambed and aborted ewes reveal the consumption of glucose by the foetus and milk yield, so glucose administration before and after parturition results reduction in hypoglycaemia and pregnancy toxaemia (12).

The mean BHB in pregnant ewes is somewhat more than in lambed and aborted ewes. The BHB concentration should not exceed 0.7 mmol.l^{-1} (17) which is consistent in this study. The increase to 0.86 and 1.6 mmol.l^{-1} will lead

to subclinical and clinical pregnancy toxaemia, respectively (4, 11). Hyperketonaemia will not appear clinically unless it is accompanied by hypoglycaemia and hypocalcaemia (13, 19). In other words, there is a negative correlation between blood BHB and glucose (22).

The signs of hypoglycaemia and hyperketonaemia are the cause of abortion, brain defects, pregnancy toxaemia and immune repression (11). Some believe there is neither correlation (1) nor differences among pregnant, lambed and lactating ewes (3, 7). The highest BHB distribution over 0.7 $mmol.l^{-1}$ observed in pregnant ewes could be considered as subclinical pregnancy toxaemia. Other factors affecting hyperketonaemia include long term starvation, poor nourishment and reproduction disorders (18) in which nutrition was known as the important factor (22).

The highest urea concentration was observed in pregnant ewes. A value was of up to 30 $mg.dl^{-1}$ (23, 16) was recorded, which was consistent with our results. Urea production rises to 67 % during pregnancy and falls to 36 % following parturition and lactation (20) as demonstrated in this study. Dehydration (9) and starvation (5) result in non-clinical uremia, while diarrhoea (9), renal disorders (15) and pregnancy toxaemia cause clinical uremia (16, 22). The reason for a high urea concentration in pregnant ewes could be related to either high protein metabolism during pregnancy or nutritional management. In this study, in some pregnant and lambed ewes it is three and two fold respectively, which are together with hypoglycaemia and hyperketonaemia, would be considered as subclinical pregnancy toxaemia.

Blood cortisol is recorded up to 10 $ng.ml^{-1}$ (4, 8) which was inconsistent with the results found in this study. It increases within a hundred days of pregnancy, then reduces towards the end of pregnancy and early lactation (3). Cortisol products following pregnancy, abortion, parturition (3), poor nutrition (22) and veterinary handling as it was observed in this study. Henze *et al.* (1998) have reported cortisol up to 52 $ng.ml^{-1}$ as subclinical pregnancy toxaemia, thus some ewes in pregnant and aborted groups are suspected of disease. The differences in blood cortisol among groups underline the rule of stress in pregnancy and abortion. In individual surveys the cortisol level in pregnant and aborted ewes rises up to 200 and 160 $ng.ml^{-1}$, respectively as observed by Ford *et al.* (4) in 80 % of ewes with pregnancy toxaemia. Cortisol terminates with hypoglycaemia, hyperketonaemia and uremia in pregnant ewes.

Results of correlations among the parameters indicate a physiological relationship among hypoglycaemia, uremia and cortisol in hyperketonaemia. The mechanism could be the substitution of fats and proteins in energy production, results in an increase in urea and BHB and depletion of glucose in blood, liver and muscles. These mechanisms are confirmed in bovine ketosis, pregnancy toxaemia, fat cow syndrome and liver lipidosis (14), while no correlation has been reported between glucose and BHB in non-pregnant ewes (16) though a relationship between glucose and urea has been reported by Firat and Ozpinar (2).

The ultimate objective of this study is to evaluate the rate of subclinical pregnancy toxaemia based on the assessment of glucose, cortisol and BHB concentrations as reported in the literature. Urea changes seem to be not specific following disease. The highest distribution of subclinical pregnancy toxaemia based on glucose and BHB levels are related to pregnant ewes and that based on cortisol level is found in aborted ewes.

The chi-square result shows the high susceptibility of pregnant ewes to subclinical pregnancy toxaemia and also reveals that the relevant parameters are BHB and cortisol and then it might be glucose and urea as demonstrated in Table 3. Thus, it can be concluded that subclinical pregnancy toxaemia rates in Urmia pregnant and aborted ewes are 12.3 % and 10.2 % The glucose concentration in susceptible pregnant ewes declines to subnormal and causes abortion at the rate of 10.2 %. Therefore, adjusting carbohydrates toward the end of pregnancy simultaneously monitoring BHB and cortisol tests would reduce the occurrence of subclinical pregnancy toxaemia.

REFERENCES

1. Bickhardt, K., Grocholl, G., Koning, G., 1989: Glucose metabolism in sheep in different reproductive stage and with ketosis using the intravenous glucose tolerance test (IVGTT). *Zentralbl. Veterinärmed*. A, 36, 514–529.

2. Firat, A., Ozpinar, A., 1996: The study of changes in some blood parameters (glucose, urea, bilirubin, AST) during and after pregnancy in association with nutritional conditions and litter size in ewes. *Turk Veterinerlik ve Hayvancilik Dergisi,* 20, 387–393.

3. Firat, A., Ozpinar, A., 2002: Metabolic profile of pre-pregnancy, pregnancy and early lactation in multiple lambing Sakiz ewes. 1. Changes in plasma glucose, 3-hydroxybutyrate and cortisol levels. *Ann. Nutr. Metab.*, 46, 57–61.

4. Ford, E. J., Evans, J., Robinos, N. I., 1990: Cortisol in pregnancy toxaemia of sheep. *Br. Vet. J.*, 146. 539–542.

5. Goal, T., Mezes, M., Miskucka, O., Ribiczey, S. P., 1993: Effect of fasting on blood lipid per-oxidation parameters of sheep. *Res. Vet. Sci.*, 55, 104–107.

6. Hamadeh, M. E., Bostedt, H., Failing, K., 1996: Concentration of metabolic parameters in the blood of heavily pregnant and nonpregnant ewes. *Berliner and Münchener Trierärztliche Wochenschrift*, 109, 81–86.

7. Henze, P., Bickhardt, K., Fuhrmann, H., Sallmann, H. P., 1998: Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. *Zentralbl. Veterinärmed. A*, 45, 255–266.

8. Houdeau, E., Raynal, P., Marnet, P. G., 2002: Plasma levels of cortisol and oxytocin, and uterine activity after cervical artificial insemination in the ewe. *Reprod. Nutr. Dev*., 42, 381–392.

9. Igbokwe, I. O., 1993: Haemoconcentration in Yankasa sheep exposed to prolonged water deprivation. *Small Ruminant Res.*, 12, 99–105.

10. Lacetera, N., Bernabucci, U., Ronchi, B., Nardone, A., 2001: Effects of subclinical pregnancy toxaemia on immune response in sheep. *Am. J. Vet. Res.*, 62, September (9), 1446–1449.

11. Lacetera, N., Franci, O., Scalia, D., Bernabucci, U., Ronchi, B., Nardone, A., 2002: Effects of nonesterified fatty acids and BHB on functions of mononuclear cells obtained from ewes. *Am. J. Vet. Res.*, 63, 414–418.

12. Lemosquet, S., Rideau, N., Rulquin, H., Farerdin, P., Simon, J., Verito, R., 1997: Effect of a duodenal glucose infusion on the relationship between plasma concentration of glucose and insulin in dairy cow. *J. Dairy Sci.*, 2854–2865.

13. Marteniuk, J. V., Herdt, T. H., 1988: Pregnancy toxaemia and ketosis of ewes and does, *Vet. Clin. North Am. Food Anim. Pract.*, 4, 307–315.

14. Palmer, M. V., Smith, S. C., 2002: Hepatic lipidosis in pregnant captive American bison (bison-bison), 14, 542–550.

15. Radostits, O. M., Gay, C. C., Blood, D. C., Hinchcliff, K. W., 2000: *Veterinary Medicine* (9th edn.). Harcourt Publishers Ltd., London, pp.1417–1420.

16. Ramin, A. G., Asri-rezaie, S., Majdani, R., 2005: Correlations among serum glucose, beta-hydroxybutyrate and urea concentrations in non-pregnant ewes. *Small Ruminant Res.*, 57, 265–269.

17. Robinson, J. J., 1980: Energy requirements of ewes during late pregnancy and early lactation. *Vet. Rec.*, 106, 282–284.

18. Rook, J. S., 2000: Pregnancy toxaemia of ewes, does, and beef cows. *Vet. Clin. North Am. Food Anim. Prac.*, 16, 293–317.

19. Schlumbohm, C., Harmeyer, J., 2003: Hypocalcaemia reduces endogenous glucose production in hyperketonaemic sheep. *J. Dairy Sci.*, 86, 1953–1962.

20. Shetaewi, M., Daghash, H. A., 1994: Effects of pregnancy and lactation on some biochemical components in the blood of Egyptian coarse-woolewes. *Istitute Vet. Med. J.*, 30, 64–73.

21. Wastney, M. E., Arcus, A. C., Bickerstaffe, R., Wolff, J. F., 1982: Glucose tolerance in ewes and susceptibility to pregnancy toxaemia. *Aust. J. Biol. Sci.*, 35, 381–392.

22. West, H. J., 1996: Maternal under nutrition during late pregnancy in sheep. Its relationship to maternal condition, gestation length, hepatic physiology and glucose metabolism. *Br. J. Nutr.*, 75, 593–605.

23. Zadnik, T., Pengov, A., Mijovic, A., Lipuzic, E., Pogacnik, M., 1993: Somatic cell count and ewe milk composition. *Prvi Slovenski Vet. Kongres*, pp. 18–20.

Received November 14, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 14–18, 2007

LACTATE DEHYDROGENASE ISOENZYME PATTERN IN SERUM OF CATTLE AT SLAUGHTER

Heinová, D., Vaško, L.

University of Veterinary Medicine Komenskeho 73, 041 81 Košice
The Slovak Republic

heinova@uvm.sk

ABSTRACT

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) (LDH) isoenzymes pattern in serum of cattle at slaughter was investigated. An extraordinarily intensive diffuse zone was found at the cathodic site of LDH_4 by gradient polyacrylamide gel electrophoresis. 71% of total LDH activity was concentrated in this zone while the catalytic intensity of LDH_1 to LDH_3 decreased proportionaly (4.4-, 3.2- and 1.8-times, respectively). This profile of serum lactate dehydrogenase isoenzymes was greatly different from that of the normal. Similar patterns were observed in some human serum/plasma where diffused LDH zones were identified as macroglobular complexes of lactate dehydrogenase isoenzymes with immunoglobulins. Using polyacrylamide gel isoelectric focusing technique in a pH range of 3.0 to 9.0 revealed that the diffused LDH zone had not been homogeneous. It consisted of two to four extra fractions which were localized in a relatively high and narrow pH area of 8.1 to 9.0. Their localization on the isoelectrophoretogram was different from that of the true LDH isoenzymes molecules with pI values of 4.2 to 6.7. As the extra LDH fractions occurred in sera of animals at slaughter we presume a relationship to the stress exercise.

Key words: cattle; extra-LDH; isoelectrofocusing; lactate dehydrogenase isoenzyme; macroenzyme; slaughter

INTRODUCTION

Four to five isoenzymes of lactate dehydrogenase (LDH) are usually present in normal sera of animal and human beings. Their quantitative distribution in the serum is different and relatively characteristic for a particular biological species. When the LDH isoenzymes are liberated from tissue to serum, as on cell injury, the LDH isoenzyme pattern of the serum changes in favour of the LDH profile of an affected organ (16). The change of the LDH isoenzyme pattern often serves as a valuable adjunct indicator in diagnosis of heart, skeletal muscle and liver disorders both in veterinary and human medicine which is well documented in the literature.

Damage to the skeletal muscle cells is known to be accompanied with an increased LDH_5 activity in serum of an affected individual. Such a pattern can be observed, for example, in pigs at slaughter (12, 20, 21, 32, 33) and an idea of its relationship to the physical stress is generally accepted. Little is kown, however, about lactate dehydrogenase isoenzymes in the serum of cattle at slaughter.

The aim of our work was to examine LDH isoenzyme pattern in serum of cattle at slaughter and to identify an increased LDH_5 activity, if any, in those sera.

MATERIAL AND METHODS

Animals and serum sample. Blood serum of bulls at slaughter (n = 20) (Slovak spotted cattle), each weighing about 500 kg and appearing clinically healthy, were investigated. Serum of rested and clinically healthy cattle served as a control (n = 8). Blood samples of the animals at slaughter were collected on exsanguination, those of the control group were collected from *vena jugularis*. No haemolyzed sera were used for examinations. Blood samplings and examinations of the cattle slaughtered sera with the aim to detect the LDH_5 activity were made in three separated series during two years.

Table 1. Total proteins, LDH activities and relative distribution of the LDH isoenzymes in normal and slaughtered cattle sera (native PAGE 8% to 25%, pH 8.8)

Serum	Total proteins ($g.l^{-1}$)	Total LDH ($U.l^{-1}$)	Relative distribution of LDH activity (%) (x ± SD)
normal (n = 8)	80 (± 4.0)	403 (± 15)	LDH1 53 (± 2.0)
			LDH2 35 (± 1.0)
			LDH3 11 (± 1.0)
			LDH4 1 (± 0.2)
slaughtered (n = 20)	87 (± 2.0)	570 (± 14)	LDH1 12 (± 1.0)
			LDH2 11 (± 0.8)
			LDH3 6 (± 0.1)
			LDH4 1 (± 0.1)
			extra-LDH
			zone 71 (± 2.0)

Determination of total serum lactate dehydrogenase activity as well as separations of its isoenzymes were performed in the same day as the blood collection. Total catalytic activity of LDH ($U.l^{-1}$) was determined using a colorimetric method according to Ševela and Továrek (29) at 37 °C. Total serum protein was estimated according to Bradford (3).

Electrophoretic separations. Serum proteins and lactate dehydrogenase isoenzymes were separated using an electrophoretic system PhastSystem (Pharmacia LKB, Sweeden). Separation techniques were used as follows:

1. Gradient polyacrylamide gel electrophoresis with a continuous 8% to 25% gradient gel zone and 2% crosslinking (PAGE 8%–25%). Separation conditions: 400 V, 10.0 mA, 45 min separation time. Separations were carried out with cooling at 4 °C with a buffer system of 0.88 $mol.l^{-1}$ L-alanine/0.25 $mol.l^{-1}$ Tris pH 8.8.

2. Isoelectric focusing with a pH range of 3.0 to 9.0 (IEF 3–9) in homogeneous polyacrylamide (PAA) (5%) containing Pharmalyte® carrier ampholytes. Separation conditions: 2000 V, 2.5 mA, 15 °C, 15 min separation time.

Detection and quantification of the electrophoretograms. Lactate dehydrogenase isoenzymes were detected with nitroblue tetrazolium (NBT) and sodium lactate (Sigma, USA) as substrate using a method of Michálek and Marcaník (18). Proteins were stained with Coommassie Brilliant Blue R (Pharmacia LKB, Sweeden). PhastImage system (Pharmacia LKB, Sweeden) served for densitometric scanning (613 nm) and quantification of patterns. Relative distribution of the isoenzymes was expressed in % of total lactate dehydrogenase activity.

Table 2. pI values of lactate dehydrogenase activities in slaughtered cattle serum (IEF 3–9)

Peak No.	ckp*	pI value	Identity of substance: LDH fraction	Identity of substance: pI value
1	Trypsinogen	9.00	extra-LDH4	8.98
2	lentil lectin-basic band	8.65	extra-LDH3	8.83
3	lentil lectin-middle band	8.45	extra-LDH2	8.52
4	lentil lectin-acidic band	8.15	extra-LDH1	8.09
5	myoglobin-basic band	7.35	LDH4	6.74
6	human carbonic anhydrase B	6.55	LDH3	5.58
7	bovine carbonic anhydrase B	5.85	LDH2	4.67
8	β-lactoglobulin A	5.20	LDH1	4.20
9	soyabean trypsin inhibitor	4.55		
10	amyloglucosidase	3.50		

Legend: isoelectric points (pI)-values of lactate dehydrogenase activity fractions were estimated using calibration kit proteins (cpk) for pI determinantion by gel isoelectric focusing (pI 3.0 to 9.0) (Pharmacia LKB, Sweeden)

RESULTS

Fig. 1 shows an electrophoretic pattern of proteins and LDH isoenzymes in normal bovine serum after their separation in a continuous gradient of polyacrylamide (PAGE 8%–25%). Three to four fractions of the enzyme (i.e. LDH_1, LDH_2 and LDH_3) were detected on the LDH enzymogram in the area between β- and γ-globulins. Their quantitative distribution in the serum is listed in Table 1. Fig. 2 shows a changed lactate dehydrogenase isoenzyme pattern in blood sera of animals at slaughter.

An extraordinarily intensive diffuse extra-LDH zone can be seen on the electrophoretogram after separation of LDHs in the gradient of polyacrylamide at the cathodic site of LDH_4. Its presence caused a dramatic change in quantitative distribution of LDH_1 to LDH_3 isoenzymes in sera of affected animals (Table 1). Predominant portion of the LDH activity in slaughtered serum was concentrated in this extra-LDH zone (71%) while the other fractions, especially LDH_1 to LDH_3, decreased proportionally (4.4-, 3.2- and 1.8-times, respectively).

Homogeneity of the diffused LDH zone was checked using gel isoelectric focusing method (IEF 3–9). After separation in a gradient of pH 3.0 to 9.0 three to four new extra bands appeared on the LDH enzymogram (Fig. 3). They were focused in the area with a relatively high and narrow pH values of 8.1 to 9.0 (Table 2). On the contrary, pI values of true cattle LDH_1 to LDH_4 molecules were situated in a more acidic pH range of 4.2 to 6.7.

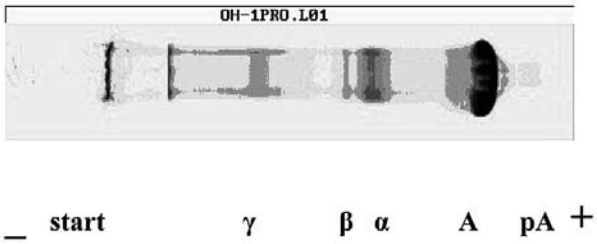

Fig. 1. An electrophoretic pattern of normal bovine serum proteins (a) and lactate dehydrogenase isoenzymes (b) (PAGE 8–25 %, pH 8.8)

Legend: pA – prealbumin; A – albumin
α-, β-, γ-globulins, respectively

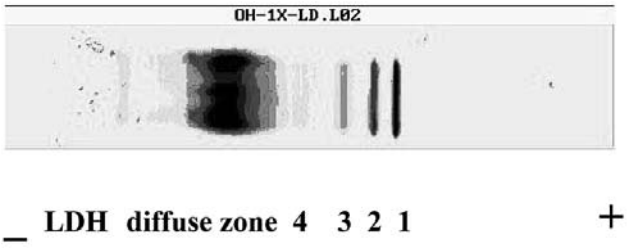

Fig. 2. Lactate dehydrogenase isoenzyme pattern in serum of cattle at slaughter (PAGE 8–25 %, pH 8.8)

Table 3. Lactate dehydrogenase activities relative distribution (%) in normal cattle serum and in serum of cattle at slaughter (IEF 3–9)

Serum	Identity of substance	LDH activity (%) ($\bar{x} \pm SD$)
Normal (n = 8)	LDH_1	65 (± 2.0)
	LDH_2	23 (± 1.0)
	LDH_3	12 (± 2.0)
	LDH_4	1 (± 0.2)
slaughtered (n = 20)	LDH_1	7 (± 2.0)
	LDH_2	5 (± .0)
	LDH_3	4 (± 0.7)
	LDH_4	1 (± 0.3)
	extra-LDH_1	2 (± 0.5)
	extra-LDH_2	9 (± 2.0)
	extra-LDH_3	26 (± 2.0)
	extra-LDH_4	45 (± 6.0)

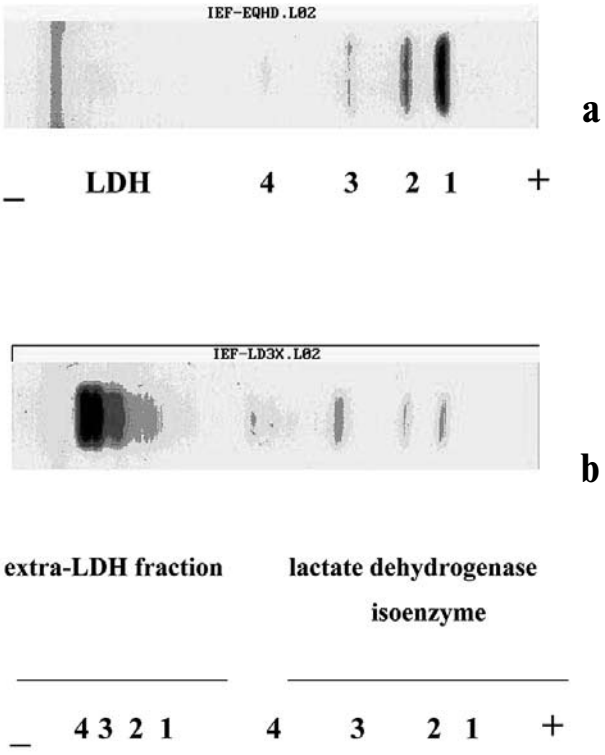

Fig. 3. Lactate dehydrogenase isoenzyme pattern in: a – normal and b – slaughtered bovine serum after separation in a pH gradient of 3.0 to 9.0 (IEF 3–9)

As the new fractions with the LDH enzymatic activity differed by their electrophoretic properties from the true LDH isoenzyme molecules we denoted them extra-LDH proteins. To designate these substances we applied a principle used for an identification of normal LDH isoenzymes, i.e. their relative position in respect to the anode. Thus, the fraction nearest to the anode was designated the extra-LDH_1, the fraction nearest to the cathode the extra-LDH_4. Table 3 lists a relative distribution of lactate dehydrogenase activities in both of normal and slaughtered cattle sera after their separation in the gradient of pH 3.0 to 9.0. Predominant portion of slaughtered LDH activity migrated in the most basic extra-LDH_4 fraction (45 %) having been followed by the extra-LDH_3 (26 %).

The extra-LDH_2 and the extra-LDH_1 as well as true LDHs occurred in low amounts with a fairly similar proportion (all bellow 10 %). Both of the extra-LDH_4 and extra-LDH_3 appeared in the all of the investigated cattle slaughtered sera, while the extra-LDH_2 as well as LDH_1 were present there less frequently.

DISCUSSION

Our results show that in serum of Slovak spotted cattle at slaughter an unusual change of lactate dehydrogenase isoenzymes pattern was detected after their separation in

a continuous gradient of polyacrylamide 8 % to 25 % gel electrophoresis (Figs. 1 and 2). This change was characterized by a presence of an extraordinarily intensive diffuse zone in the area of the LDH_4 cathodic site which corresponded to the area of γ-globulins. Its presence caused a remarkable change in quantitative distribution of the other lactate dehydrogenase forms (Table 1). Similar patterns were observed in some human serum/plasma where diffused LDH zones were identified as macroglobular complexes of lactate dehydrogenase isoenzymes with immunoglobulins IgG (LDH-IgG macroenzyme) (2, 8, 13, 24), IgA (LDH-IgA macroenzyme) (4, 14, 22, 27, 30) and IgM (LDH-IgM macroenzyme) (14, 15).

Enzyme-linked immunoglobulin complexes known as macroenzymes have been detected in most of the serum enzymes routinely used in biochemical tests: macrogamma-glutamyltransferase, macroalanine aminotransferase, macroalkaline phosphatase (1), macrocreatine kinase (17, 23, 25, 31), macrolactate dehydrogenase (24), macroaspartate aminotransferase (5, 6, 7, 10, 19, 28), macroamylase and macrolipase (11, 34).

In general, macroenzymes are serum enzymes of a higher molecular mass and a longer plasma half-time than the corresponding enzymes normally found in serum under physiologic or pathologic conditions (9, 10, 26). Macroenzymes can persist in serum/plasma for month or even years (10).

There is a lack of information concerning macroenzymes in animals. As the serum lactate dehydrogenase isoenzyme pattern with the abnormal extra-LDH zone found in cattle at slaughter was similar to the ones observed in human individuals we connected it with a presence of lactate dehydrogenase-immunoglobulin(s) linked complexes.

After electrophoretic separation of this extraordinarily intensive and diffused LDH zone using polyacrylamide gel isoelectric focusing in a pH-gradient of 3.0 to 9.0 (IEF 3–9), three to four new extra bands appeared on the isoelectrophoretogram (Fig. 3). They were focused in an area with a relatively high and narrow pH values of 8.1 to 9.0 (Table 2). The pI values of these fractions were situated in an apparently different range of pH than those of true normal LDH molecules and quantitative distribution of true lactate dehydrogenase were changed by a presence of the extra LDH bands (Table 3). These observations proved that a diffused LDH-zone found after native 8–25 % PAGE (Fig. 2) had not been one protein. As they occured in serum of animals at slaughter we presume a relationship with stress exercise.

The formation of complexes between the enzyme protein and plasma immunoglobulins seems to be a rather common phenomenon. However, neither the mechanism involved nor the pathological significance are clear since they have been observed in patients suffering from different diseases as well as in clinically healthy humans.

Nevertheless, it is important to recognize these macroglobular forms because they cause high levels of enzyme activities thus leading to the possibility of diagnostic errors. Awarness of macroenzymes may also save the patient from performance of unnecessary tests and sometime invasive investigations.

REFERENCES

1. Artur, Y., Sanderink, G. J., Maire, I., 1987: Macroenzymes in human plasma. 2. Macrogamma-glutamyltransferase, macroalanine aminopeptidase, macroalkaline phosphatase, macroaminotransferases and other macroenzymes. *Ann. Biol. Clin.* (Paris), 45, 277–284.

2. Biewenga, J., Feltkamp, T. E. W., 1975: Lactate dehydrogenase (LDH)-IgG3 immunoglobulin complexes in human serum. *Clin. Chim. Acta*, 64, 101–116.

3. Bradford, M. M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing. The principle of protein-dye binding. *Anal. Biochem.*, 72, 248–254.

4. Buda, E. M., Rosen, S. W., 1987: Myeloproliferative syndrome evolving in a patient with macromolecular lactate dehydrogenase. *Arch. Intern. Med.*, 147, 390–392.

5. Collins, J., Ritter, D., Bacon, B. R., Landt, M., Creer, M. H., 2002: Macro-aspartate aminotransferase in a female with antibodies to hepatitis C virus. *Liver*, 22, 501–506.

6. Fortunato, G., Iorio, R., Esposito, P., Lofrano, M. M., Vegnente, A., Vajro, P., 1998: Macroenzyme investigation and monitoring in children with persistent increase of aspartate aminotransferase of unexplained origin. *J. Pediatr.*, 133, 286–289.

7. Foust, R. T., O'Brien, J. F., Schiff, E. R., 1990: Isolated aspartate aminotranferase elevation due to macroenzyme formation with liver biopsy correlation. *Am. J. Gastroenterol.*, 85, 88–90.

8. Fujita, K., Sakurabayashi, I., Kusanagi, M, Kawai, T., 1987: A lactate dehydrogenase-immunoglobulin G1 complex, not blocked by antiidiotype antibody, in a patient with IgG1-lambda type M-proteinemia. *Clin. Chem.*, 33, 1478–1483.

9. Galasso, P. J., Litin, S. C., O'Brien, J. F., 1993: The macroenzymes: a clinical review. *Mayo. Clin. Proc.*, 68, 349–354.

10. Goenner, S., Corriat-Bourtron, A., Pelletier, G., Legrand, A., Buffet, C., 1998: Macroaspartate aminotransferase. Study of 5 cases and review of the literature. *Gastroenterol. Clin. Biol.*, 22, 549–553.

11. Goto, H., Wakui, H., Komatsuda, A., Imai, H., Miura, A. B., Fujita, K., 2002: Simultaneous macroamylasemia and macrolipasemia in a patient with systemic lupus erythematosus in remission. *Intern. Med.* (Tokyo, Japan), 39, 1115–1118.

12. Heinová, D., Blahovec, J., Kováč, G., 1994: New aspects of lactate dehydrogenase isoenzyme pattern in the serum of pigs at slaughter. *Vet. Med.-Czech*, 39, 287–296.

13. Liu, Z., He, C., Huang, X., Wei, M., 1998: Lactate dehydrogenase-immunoglobulin G complex of the postburn patient. *Clin. Chem.*, 44, 2368–2369.

14. Liu, Z., Wang, W., Wei, M., 1999: LD-IgG, IgA, IgM complex in a postburn patient. *Clin. Chim. Acta*, 285, 195–198.

15. Liu, Z. J., Zhang, Y., 2000: Macro lactate dehydrogenase in a patient with myocarditis. *Clin. Chem. Lab. Med.*, 38, 307–308.

16. Maekawa, M., 1988: Review. Lactate dehydrogenase isoenzymes. *J. Chromatogr.*, 429, 373–398.

17. Maire, I., Artur, Y., Sanderink, G. J., 1987: Macroenzymes in human plasma 1: Macroamylase, macrokreatine kinase, macrolactate dehydrogenase. *Ann. Biol. Clin.* (Paris), 45, 269–276.

18. Michálek, A., Marcaník, J., 1975: Activities values of lactic dehydrogenase in sera of domestic animals (In Slovak). *Vet. Med.*, 20, 199–205.

19. Monfort-Gouraud, M., Hamza, A., Nacer, K., Barjonnet, G., Tranie, V., Devanlay, M., Sauvageon, G., 1999: Hypertransaminasemia in a adolescent. *Arch. Pediatr.,* 6, 1191–1192.

20. Mori, Ch., Maeda, H., Yuasa, A., 1990: Relationship between liver degeneration and the production of dark firm dry meat in slaughtered pigs. *Jpn. J. Vet. Sci.*, 38, 613–620.

21. Moss, B. W., McMurray, C. H., 1979: The effect of the duration and type of stress on some serum enzyme levels in pigs. *Res. Vet. Sci.*, 26, 1–6.

22. Nagamine, M., 1972: Lactate dehydrogenase isoenzymes linked to immunoglobulin A in two cases. *Clin. Chim. Acta*, 36, 139–144.

23. Perez-Calle, J. L., Marcos, I. M., Carneros, J. A., Barrio, J., Trascasa, C., Munoz, E., Mancheno, E., Gonzalez-Lara, V., 2001: Macromolecular creatine kinase in patients diagnosed with ulcerative colitis. *Gastroenterol. Hepatol.*, 24, 16–19.

24. Perry, C., Peretz, H., Ben-Tal, O., Eldor, A., 1997: Highly elevated lactate dehydrogenase level in a healthy individual: a case of macro-LDH. *Am. J. Hematol.*, 55, 39–40.

25. Perry, C., Peretz, H., Graf, E., Ben-Tal, O., Eldor, A., 1997: Macroenzymes: an interesting laboratory finding, without clinical relevance. *Harefuah*, 133, 359–362.

26. Remaley, A. T., Wilding, P., 1989: Macroenzymes: biochemical characterization, clinical significance, and laboratory detection. *Clin. Chem.*, 35, 2261–2270.

27. Shibasaki, T., Kaguchi, Y., Ohno, I., Ishimoto, F., Sakai, O., 1993: Lactic dehydrogenase anomaly in a patient with chronic renal failure. *Intern. Med.* (Tokyo, Japan), 32, 316–318.

28. Stasia, M. J., Surla, A., Renversez, J. C., Pene, F., Morel-Femelez, A., Morel, F., 1994: Aspartate aminotransferase macroenzyme complex in serum identified and characterized. *Clin. Chem.*, 40, 1340–1343.

29. Ševela, M., Továrek, J., 1959: A method for determinantion of lactic dehydrogenase in body liquids (In Czech). *Čas. Lék. Českoslov.*, 98, 844–848.

30. Tozuka, M., Hidaka, H., Okumura, N., Ichikawa, T., Furihata, K., Katsuyama, T., 1996: A case of immunoglobulin A-lambda conjugated with lactate dehydrogenase-5 isoenzyme, causing an extremely high enzyme activity in serum. *Clin. Chem.*, 42, 1288–1290.

31. Venta, R., Geijo, S. A., Sanchez, A. C., Bao, C. G., Bartolome, L. A., Casares, G., Lopez-Otin, C., Alvarez, F. V., 1989: IgA-CK-BB complex with CK-MB electrophoretic mobility can lead to erroneous diagnosis of acute myocardial infarction. *Clin. Chem.*, 35, 2003–2008.

32. Weeding, C. M., Hunter, E. J., Guise, H. J., Penny, R. H. C., 1993: Effects of abattoir and slaughter handling systems on stress indicators in pig blood. *Vet. Rec.*, 133, 10–13.

33. Young, J. F., Rosenvold, K., Stagsted, J., Nielsen, J. H., Andersen, H. J., 2005: Significance of vitamine E supplementation, dietary content of polyunsaturated fatty acids, and preslaugter stress on oxidative status in pig as reflected in cell integrity and antioxidative enzyme activities in porcine muscle. *J. Agricul. Food Chem.*, 53, 745–749.

34. Zaman, Z., Van-Orshoven, A., Marien, G., Fevery, J., Blanckaert, N., 1994: Simultaneous macroamylasemia and macrolipasemia. *Clin. Chem*., 40, 939–942.

Received January 24, 2007

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 19–25, 2007

FATTY ACIDS AND MINERAL ELEMENTS IN BULK MILK OF HOLSTEIN AND CZECH SPOTTED CATTLE ACCORDING TO FEEDING SEASON

Janů, L., Hanuš, O., Macek, A., Zajíčková, I., Genčurová, V., Kopecký, J.

Research Institute for Cattle Breeding, Ltd., Rapotín, Výzkumníků 267, 788 13 Vikýřovice The Czech Republic

libor.janu@vuchs.cz

ABSTRACT

Knowledge of milk fat composition from the point of view of fatty acids (FAs) and of cow's milk from the point of view of mineral substances (MSs) is important for the consumers' nutrition and health. Some of the breeding factors affect the conditions inside the FAs and MSs of milk. In most MSs there are no differences ($P > 0.05$) between winter (W) and summer (S) seasons the same as between the Holstein (H) and the Czech spotted (C) breeds. An exceptions are the Zn concentrations, where there was a difference between H and C ($P < 0.05$), 5.16 mg.kg^{-1} in H and 5.52 mg.kg^{-1} in C. The Ca, Mg and Na values were within the limits quoted in literature. Higher values were only for P (79.8 mg.100 g^{-1}) and for K (164 mg.100 g^{-1}). The Cu, Zn, Ni and Fe contents were under the level of the highest permissible quantity. The results confirm a hypothetically prognosticated possible growth of Mg content in milk towards the original values. This may point to a healthier way of dairy cow nutrition and milk production. There were no significant differences ($P > 0.05$) in FAs. The only difference was in the monounsaturated FAs in H, where there were higher in S (26.28 %) than in W (24.19 %; $P < 0.05$).

Key words: bulk milk sample; cattle breed; cow; fat; fatty acids; macroelements; microelements; raw milk; season

INTRODUCTION

Fatty acids of milk fat

The accent on healthy nutrition and its publicizing are the topical questions in the last years. With the constantly developing analytic methods at the same time possibilities of explaining a detailed composition of food raw materials and products as well as possibilities of explaining the connections of their incidence and health consequences in their consumption are growing. Equally the effort to preserve the value of foodstuffs during their production, preparation and distribution also increases. Further to that, the control of the above mentioned processes is made stricter, too. The lay public also seek more and more information about usefulness, harmfulness or quality of the individual foodstuffs and their components, whichever reason they may have for it. Not only for this reason does the interest of specialists also concentrate, apart from other things, just on quality of foodstuffs and food sources as minutely as molecular level.

A lot of works are engaged not only in securing and controlling their quality, but also in possibilities how to improve them. Relations of the particular food components to possibility of influencing the population's health and/or trying to avoid or decrease the most various illnesses are studied. Moreover, the research and development in the animal production area, in concrete terms, in the dairy industry, just the same the practice, have made a decisive progress. Thus e.g. genetic improvement, nutrition enriching and so the increase of the mean milk yield capacity of milk cows in the Czech Republic (CzR) from 4680 kg to 6285 kg during the lactation, thus by 34 % during 6 years, are the reasons why to keep aiming at these indicators and relations (3).

One of the most variable milk components is the milk fat, not only in the sense of proportional representation, but also in the sense of chemical composition. As known, the basis of the milk fat are the esters of fatty acids with glycerol. As the triacylglycerols consist of 95 % of fatty acids (FAs) and 5 %

of glycerol and as the fatty acids are also bonded in further components of milk fat, the FAs can be considered as the proper building stones of the milk lipids. Therefore, the nutrition and technological properties of the milk fat depend on their representation (1). It follows from the said that the FAs are found in the milk fat predominantly in bonded form. The free FAs occur in fat only in trace concentrations, nevertheless, they participate in the typical taste of the milk fat and of milk in general.

After their structure FAs can be divided into saturated FAs (SAFA), monounsaturated FAs (MUFA) and polyunsaturated FAs (PUFA). Another criterion to distinguish them is their quantitative representation in fat. The acids occurring in the milk fat in a greater quantity than 1 % are termed as majority acids. The acids of lesser occurrence than 1 % are called minority acids. Milk is also a source of essential FAs, that is such ones which the body itself is not able to create and it must receive them in food. Linoleic and linolenic acids belong to the named.

The most important of the saturated fatty acid group are the palmitic and stearic acids. The palmitic acid increases the LDL level-cholesterol, and is therefore responsible for the origin of cardiovascular diseases. That is also the reason of the generally widespread fear of milk fat consumption. Cholesterol, however, is of course also a biologically very important component of animal fat and cell membranes. For this reason fatty meat contains a comparable quantity of cholesterol just as lean meat.

The egg yolk contains the most of it. Cholesterol in milk (0.010–0.015 %) is concentrated in the fat phase, i.e. in the membranes of fatty globules and in the proper globule fat. By skimming it is therefore concentrated into butter (about 20 times more than in milk) and naturally decreases its quantity in milk with a lowered fat content (3). On the contrary, PUFAs, especially linoleic acid, are thought to have antiatherogenic effects by decreasing the cholesterol level. The conjugated linoleic acid (CLA) is at present in centre of attention of scientists because of its potential anticarcinogenic effect, the same as its effects on the immune system and lipide metabolism: (CLA) is a group of isomers of linoleic acid (C18:2 n-6; 13).

Milk fat is also unique among animal fats due to a high fatty acid content with a short and medium chain. These acids are digested much faster and more effectively than those with a long chain. FAs, especially those with a short and medium chain, have antimicrobial effects (12). Some studies have shown that the depression and postpartum spleen are connected with the changes of FA levels in the blood (21).

It is obvious that besides indisputably positive effects the milk fat consumption also implies certain risks. Lowering these risks, however, depends to a large extent first of all on an individual's lifestyle because not only the food composition but mainly ample exercise (which, unfortunately, is reduced to a necessary evil and then even distorted to an activity dangerous to health) are the important factors contributing to the favourable forms and levels of cholesterol in the organism.

Hermansen and Lund (1990) (4) found very small impacts of the dairy cow diet, which was supplemented by higher doses of Ca saponified palm oil (1 kg of soap per day), on shortening of time for enzymatic coagulation of milk and there was noted an increase of C16:0 proportion only in fatty acid pattern of milk fat in Danish Black and White cow breed as well. It is nourishment, season and breed that most influence the changes of milk fat content and composition. Researchers have proved that e.g. by means of appropriate nutritional supplements and properly adapted fodder rations the percentage of unsaturated FAs (USFA) can be increased and thus the saturated FA level (SAFA) in the cow's milk lowered (15). There is no need to mention again the possible favourable consequence of this move on further prevention of the occurrence of cardiovascular diseases.

Mineral substances in milk

Milk is also a source of a number of mineral substances (MSs). The human body is formed for a great part by organic substance. First of all it is water, but there are important quantities of calcium, magnesium, potassium, natrium, phosphorus, chlorides, sulphur, ferrum, and other minerals. Each of these elements is important for a correct function of cells and. that is why their well-balanced receipt is very important (19). The bones e.g. contain 99 % of the total Ca in the body, over 50 % of Mg and approximately 80 % of phosphates. Even if the mineral fraction is represented in milk in a lesser amount than the main organic components (fat, proteins, lactose), milk may be considered as a suitable donor of mineral nutrition.

It is interesting that milk can contain, in proportion to blood, as much as a twentifold quantity of Ca and a fourfold. quantity of P (10). Moreover, Ca, as generally known, plays an important role in forming bone substance, and has a positive influence on the state of health of teeth (3). It is represented in milk for the most part in organic form, i.e. as Ca phosphocaseinate, which contributes to its better usability than inorganic forms. With organically bonded Ca also the occurrence of citric acid is closely connected, for it interferes in its balance and facilitates Ca absorption through the intestinal mucous membrane in the presence of the D vitamin which prevents it from oxidation (10).

In addition to Ca and, already mentioned P a number of other minerals in quantity of units up to hundreds of mg.100 g^{-1} are represented in milk. They are K, Na, chlorides and Mg. Among the trace elements they are molybdenum, manganese, cuprum, ferrum and zincum. These are represented in lesser quantities, i.e. in units up to tenths of mg.100 g^{-1}. Milk and milk products are therefore considered as suitable sources of MSs, whose usability for the organism is improved by numbers of amply present organic substances such as proteins and lactose (5).

The aim of this work was to evaluate the stock and season influence on the quantity of MSs and FAs in raw cow's milk.

MATERIALS AND METHODS

Set of bulk milk samples

The representation of FAs in the milk fat and of MS in the raw cow`s milk was monitored in two main milked breeds in the CzR, namely in the Holstein (H) and Czech spotted (C) breeds for a period of three years. Bulk samples were taken

from eight different farms in the CzR and sorted out after breed in C and H and further after season, in which they were taken, in winter (W) and summer (S) seasons. Further, there were stated their combinations, H-winter (HW), H-summer (HS), C-winter (CW) and C-summer (CS). In this way sixteen bulk milk samples arose. After being taken the samples were immediately analysed for the content of FAs and MSs. The different but typical versions of nourishment and feed systems of dairy cows were applied in the herds: in lowlands – alfalfa silage with maize silage; in highlands – clover-grass silage, grass silage with maize silage and grass pasture as well. The concentrates were fed according to milk yield level and demands of relevant nutrition standards. The dairy herds were kept in altitudes from 215 up to 605 m.

Investigated milk indicators

The following milk indicators were investigated in widespreaded spectrum: DMY = daily milk yield (kg of milk per day); FAT = fat ($g.100\,g^{-1}$); LAC = lactose (monohydrate; $g.100\,g^{-1}$); SNF = solids non fat ($g.100\,g^{-1}$); SCC = somatic cell count ($103\,ml^{-1}$); U = urea ($mg.100\,ml^{-1}$); AC = acetone ($mg.l^{-1}$); CA = citric acid concentration ($mmol.l^{-1}$); pH acidity; EC = electrical conductivity ($mS.cm^{-1}$); AL = alcohol stability (in ml of 96 % ethanol up to creation of first protein flakes in 5 ml of milk); SH = titratable acidity (in ml 0.25 $mol.l^{-1}$ NaOH solution, used to titration of 100 ml of milk); TEC = time for enzymatic coagulation (in seconds); RCQ = subjective estimation of the rennet curds quality (in classes, 1 good, 4 poor); RCF = rennet curds firmness (in mm, opposite relationship to firmness); WV = whey volume after coagulation (in ml); CP = crude protein (Kjeldahl total N × 6.38; $g.100\,g^{-1}$); CAS = casein (casein N × 6.38; $g.100\,g^{-1}$); TP = true protein (protein N × 6.38; $g.100\,g^{-1}$); WP = whey protein (difference TP-CAS; $g.100\,g^{-1}$); NPN = non protein nitrogen matters (CP-TP nitrogen × 6.38; $g.100\,g^{-1}$); URN = urea nitrogen/non protein nitrogen ratio (%); F/CP = fat/crude protein ratio; the casein numbers as possible indexes of cheesemaking yield of milk on the basis of crude and true protein were expressed in % (CN-CP, CN-TP). The following concentrations were investigated about elements: Ca, Mg, Na, K, P as macroelements ($mg.100g^{-1}$); Mn, Fe, Cu, Zn, Ni as microelements ($mg.kg^{-1}$).

The macro- and microelements were stated by the method of atomic absorption spectrometry. The samples were mineralised by boiling on a heating plate with nitric acid and hydrogene peroxide. For determining macroelements the technique of flame AAS (Ca, Mg) and flame AES-emission spectrometry (K, Na) was used. For determining other elements a method according to their assumed concentration in the sample was adopted. Ferrum and zinc were measured by the flame AAS, manganese, cuprum and nickel by AAS with electrothermic atomisation. All measurements were made on the atomic absorption spectrometer SOLAAR S4 with the graphitic measuring cell GFS97.

Phosphorus (total P) was determined in the mineralizate spectrophotometrically as phosphomolybdenum blue. For determination a reduction by ascorbic acid in the sulphuric acid medium in the presence of antimonite ions as used. The blue colouring intensity was measured on the spectrophotometer with a wavelength of 750 nm.

The occurrence of fatty acids in milk fat

In addition to the individual FAs the representation of fatty acid groups, i.e. saturated (SAFA), monounsaturated. (MUFA) and polyunsaturated (PUFA) FAs was monitored. The fat was isolated by extraction in petroleum ether and re-esterified and methyl esters of the FAs were analysed by means of gas chromatography (GC). From the total number of 37 FAs detected on the chromatogram, 27 FAs were identified.

Other chemical, biochemical and physical analyses and statistical evaluation

The analyses of another named milk indicators were made according to the methodes described in another previous work (9). From the obtained results the arithmetic mean ($\bar{x}$) and the standard deviation (sd) were calculated for each of the indicators. The results were then divided according to the season-winter (W), summer (S) and according to the breed-H, C. Their values are given in Table 1. Mean values within the frame-work of the evaluated groups were tried out by a significance test between two means. The individual FAs determined within the framework of this experiment are shown in Table 2. The values are given in % and they were added up as arithmetic means of the results of the identified FAs within the bounds of the evaluated groups. The differences between groups were tested as well.

RESULTS AND DISCUSSION

Milk quality indicators and mineral substances

Within the framework of the breed from among the usual milk indicators (Tabs. 1, 3 and 4) log SCC ($P < 0.05$), WV ($P < 0.05$), RCQ ($P < 0.01$), CP ($P < 0.01$), CAS ($P < 0.01$) and TP ($P < 0.01$) statistically differed significantly. When compared within the bounds of the season the parameters LAC ($P < 0.05$), SNF ($P < 0.05$), pH ($P < 0.05$) and EC ($P < 0.001$) showed themselves to be significantly different. The other differences between the means were statistically insignificant ($P > 0.05$). The testing results are resumed in Tabs. 3 and 4. For Ca, P, Mg, K and Na the values 117–140, 40–79, 100–165, 118–138 and 44–80 $mg.100\,g^{-1}$ (2, 11, 12) are given in the literature. The established values were higher for P ($79.8\,mg.100\,g^{-1}$) and for K ($164.7\,mg.100\,g^{-1}$). No significant differences appear between the sample groups from W and S seasons in the samples of the different breeds in most results of analyses of MSs determination. A significant difference ($P < 0.05$) was only found in the zinc concentrations between the breeds ($5.16\,mg.kg^{-1}$ in H and $5.52\,mg.kg^{-1}$ in C). For P a lower value in S samples than in W ones was found, but their difference is insignificant (Tab.1).

Continually, a decrease of Mg content in milk by as many as 38 % was reported in comparison of time during 40 years of an intensive development of agriculture in the last century. Later, according to a prognostic hypothesis (6, 7, 8) a reverse growth of Mg content in milk towards the original values (about $16\,mg.100\,g^{-1}$) was predicted in

the CzR due to the consequence of the so-called "overall ecologization" of the CzR's agriculture. It was about an economically forced dramatic reduction of the use of all agrochemicals, especially industrial fertilizers in the past fifteen-year period from 1989. That means a lower supply of K into the soils, where potassium fertilization causes a reduction of Na, Mg and Ca contents in the fodders.

In view of this opinion the Mg content in milk was even recommended as a thrift indicator of the natural milk production (6, 7, 8). It is very interesting that the here presented research results may suggest a confirmation of the validity of the given hypothesis. That would correspond to a healthier way of animal nutrition and milk production. But more detailed confirmations are still necessary. The Mg values were relatively high under a low variability (16.2 ± 0.6 mg.100 g^{-1}; variation coefficient 3.7 %) (Tab. 1). These days, however, the use of mineral fodder additives with a higher Mg level in the animal nutrition also increases (Tab. 1).

The stated Cu, Zn, Ni and Fe contents are under the level of the highest permissible quantity which the Regulation of the Ministry of health No. 298/1997 Coll. states. For Cu this value is 0.4 mg.kg^{-1}, Ni 0.1 mg.kg^{-1}, Zn 10 mg.kg^{-1} and for Fe 50 mg.kg^{-1} (for foodstuffs generally). There were not any noticeable differences between the individual groups in the stated microelements, either. The CA concentration values are near the physiologic interval, which is 8–10 mmol.1^{-1}.

Milk fat composition

The results of the FAs representation and their groups in the milk fat are given in Table 2. The results are sorted together after breed (H, C) and season (W, S). There were evaluated the occurrence differences of FAs and their groups in the milk fat between H and C, then between the season in which the sample was taken, and. finally the influence of the breed and with the season influence simultaneously. As can be read from Table 2, palmitic acid (C16 : 0), oleic acid (C18 : 1) and stearic acid (C18 : 0) are most represented from among all FAs in both breeds, namely in quantities of 31.40 %, 21.84 % and 12.73 %, which is comparable with the data presented e.g. by Pešek *et al.* (2005; 15) in their work.

The palmitic acid (C16 : 0) and stearic acid (C18 : 0) are consequently most represented also within the frame of the SAFA group. The oleic acid (C18 : 1) is in percentages most represented in the group of the MUFAs. The linoleic acid (C18 : 2n-6) is the most occurring acid (2.69 %) of the PUFAs group. Greater differences in the occurrence of individual FAs between both breeds were registered in the SAFAs group, namely in the case of stearic acid (C18 : 0) where the content in H was higher than in C (13.86 % *vs.* 11.60 %, $P > 0.05$). In the contrary a higher percentage in C appeared in the case of palmitic acid (C16 : 0; 32.13 % in C and 30.67 % in H, $P > 0.05$), lauric acid (C12 : 0; 3.68 in C and 3.34 % in H, $P > 0.05$) and myristic acid. (C14 : 0; 11.23 % in C and 10.77 % in H, $P > 0.05$) (Tab. 2).

Higher percentages of palmitic, lauric and myristic acids in C was also presented by Pešek *et al.* (2005, 2006; 15, 16). For interest's sake it can be mentioned. that Palmquist *et al.* (14) compare the same figure between H and the Jersey breed, where the percentage of unsaturated FAs C6 : 0 up to C14 : 0 was higher in the Jersey breed (17).

No significant differences were found between the breeds in evaluation of the other individual FAs. A difference in representation of FAs in W and S season in both evaluated breeds was only found. out in oleic acid, whose percentage in W season was 21.37 % and in S season 22.31 % ($P > 0.05$). Quantity differences of oleic acid together with stearic acid also appeared in evaluation within the bounds of H, where different percentages of oleic acid in S season (22.86 %) as compared with W season (21.08 %) were proved again.

On the contrary, stearic acid shows a higher quantity in W season (14.41 %) than in S season (13.32 %). On the other hand, as more different showed themselves the contents of palmitic acid (31.72 % in W, 32.54 % in S) and myristic acid (11.58 % in W and 10.89 % in S) in C. All these influences, however, are insignificant ($P > 0.05$). The season influence did not show itself in the representation of linoleic acid significantly. Nevertheless, according to the last works the content of this acid in cow's milk varies in the course of the year and it is assumed that it is positively influenced by a longer S season and a shorter W season (20).

The influence of the breed did not show itself very much in the representation evaluation of the FA groups (SAFA, MUFA, PUFA) where there were minimal differences. But on the contrary, in some available data (15) a conclusively lower representation of SAFA is mentioned in C breed than in H. Higher percentages were registered in the case of the season influence, namely both within the frame of both the breeds and within the bounds of C. The highest differences were in H, where the season influence was shown, in SAFA and MUFA categories in particular. Here the percentage of saturated FAs was higher in W season (67.94 % in W season *versus* 66.00 % in S season, $P > 0.05$) and on the contrary, the percentage of MUFAs was higher in S season (26.28 %) than in W season (24.19 %; $P < 0.05$).

CONCLUSION

No significant differences occur in most analysis results of MS establishment between the sample groups from W and S seasons, even in the samples of the evaluated breeds. An exception are only the measured zinc concentrations, where a significant difference ($P < 0.05$) was proved between the breeds, in concrete terms 5.16 mg. kg^{-1} in H and 5.52 mg.kg^{-1} in C. Within the framework of the compared groups the other resulting means of macro- and microelement concentrations did not differ significantly. The Ca, Mg and Na values ranged within

Table 1. Arithmetical means and standard deviations for individual indicators – in groups according to season (W, S) and breed (H, C) and together

Indicator		Season W	Season S	Breed H	Breed C	Together	Indicator		Season W	Season S	Breed H	Breed C	Together
DMY	$\bar{x}$	27.1	26.0	27.7	25.3	26.5	TP	$\bar{x}$	3.14	3.08	3.03	3.20	3.11
	sd	3.7	4.3	4.9	2.6	4.1		sd	0.06	0.16	0.10	0.09	0.13
FAT	$\bar{x}$	3.93	3.93	3.86	4.00	3.93	WP	$\bar{x}$	0.49	0.50	0.49	0.50	0.49
	sd	0.12	0.42	0.23	0.35	0.31		sd	0.01	0.03	0.02	0.03	0.03
LAC	$\bar{x}$	5.02	4.95	5.00	4.97	4.98	NPN	$\bar{x}$	0.18	0.20	0.18	0.20	0.19
	sd	0.04	0.06	0.06	0.06	0.06		sd	0.03	0.03	0.02	0.03	0.03
SNF	$\bar{x}$	8.90	8.72	8.73	8.89	8.81	URN	$\bar{x}$	44.8	42.2	39.7	47.3	43.5
	sd	0.05	0.19	0.18	0.10	0.17		sd	7.8	10.0	7.4	9.0	9.1
SCC	$\bar{x}$	207.3	262.4	265.2	204.5	234.9	F/CP	$\bar{x}$	1.19	1.21	1.21	1.18	1.20
	sd	135.9	157.8	127.8	163.4	149.8		sd	0.03	0.15	0.11	0.11	0.11
log SCC	$\bar{x}$	1.78	1.94	1.96	1.77	1.86	CN-CP	$\bar{x}$	79.7	78.9	79.3	79.3	79.3
	sd	0.17	0.15	0.17	0.14	0.18		sd	0.7	0.8	0.5	1.1	0.9
U	$\bar{x}$	25.9	27.1	22.3	30.6	26.5	CN-TP	$\bar{x}$	84.3	83.9	83.9	84.2	84.1
	sd	6.2	10.9	6.6	9.0	8.9		sd	0.5	0.7	0.6	0.7	0.7
AC	$\bar{x}$	3.18	2.83	3.29	2.73	3.01	Ca ($mg.100\,g^{-1}$)	$\bar{x}$	122.0	122.3	120.5	123.8	122.2
	sd	1.07	1.49	1.53	0.97	1.31		sd	8.8	11.7	9.5	10.8	10.3
log AC	$\bar{x}$	0.29	0.24	0.28	0.26	0.27	Mg ($mg.100\,g^{-1}$)	$\bar{x}$	16.2	16.3	16.1	16.4	16.2
	sd	0.21	0.26	0.26	0.21	0.24		sd	0.7	0.4	0.6	0.5	0.6
pH	$\bar{x}$	6.77	6.70	6.75	6.73	6.74	Na ($mg.100\,g^{-1}$)	$\bar{x}$	49.6	48.8	48.3	50.1	49.2
	sd	0.05	0.04	0.06	0.05	0.05		sd	3.6	1.9	2.6	2.9	2.9
EC	$\bar{x}$	4.07	4.55	4.40	4.22	4.31	K ($mg.100\,g^{-1}$)	$\bar{x}$	164.1	165.2	166.1	163.2	164.7
	sd	0.13	0.14	0.30	0.21	0.27		sd	4.1	3.9	5.2	1.4	4.0
AL	$\bar{x}$	1.44	1.15	1.30	1.29	1.30	P ($mg.100\,g^{-1}$)	$\bar{x}$	83.9	75.7	80.9	78.8	79.8
	sd	0.40	0.09	0.30	0.35	0.33		sd	3.3	16.7	2.8	17.7	12.7
SH	$\bar{x}$	7.46	7.45	7.33	7.58	7.45	Mn ($mg.kg^{-1}$)	$\bar{x}$	0.11	0.09	0.10	0.10	0.10
	sd	0.26	0.28	0.30	0.15	0.27		sd	0.03	0.01	0.02	0.02	0.02
TEC	$\bar{x}$	112.6	110.1	115.5	107.2	111.3	Fe ($mg.kg^{-1}$)	$\bar{x}$	0.85	1.04	1.01	0.87	0.94
	sd	13.2	10.7	12.0	10.8	12.1		sd	0.19	0.32	0.29	0.25	0.28
RCQ	$\bar{x}$	2.57	2.55	2.74	2.37	2.56	Cu ($mg.kg^{-1}$)	$\bar{x}$	0.12	0.13	0.12	0.12	0.12
	sd	0.18	0.38	0.20	0.26	0.30		sd	0.01	0.02	0.02	0.01	0.02
RCF	$\bar{x}$	16.6	17.3	17.3	16.7	17.0	Zn ($mg.kg^{-1}$)	$\bar{x}$	5.32	5.35	5.16	5.52	5.34
	sd	0.8	0.9	1.0	0.8	0.9		sd	0.39	0.29	0.30	0.29	0.35
WV	$\bar{x}$	35.1	35.9	35.9	35.1	35.5	Ni ($mg.kg^{-1}$)	$\bar{x}$	0.032	0.034	0.037	0.028	0.033
	sd	0.6	0.8	0.8	0.5	0.8		sd	0.011	0.015	0.017	0.006	0.013
CP	$\bar{x}$	3.32	3.28	3.21	3.39	3.30	CA ($mmol.l^{-1}$)	$\bar{x}$	9.81	10.40	10.1	10.1	10.1
	sd	0.07	0.19	0.11	0.10	0.14		sd	1.90	1.09	1.2	1.9	1.6
CAS	$\bar{x}$	2.65	2.59	2.54	2.69	2.62							
	sd	0.06	0.14	0.10	0.07	0.11							

* – $P \leq 0.05$

Table 2. The concentrations (% of total observed FAs) of individual fatty acids and their groups (SAFA, MUFA, PUFA and USFA) in milk fat of Czech pied (C) and Holstein (H) cattle breed in summer (S) and winter (W)

FA	H	C	W	S	HW	HS	CW	CS	x
C4:0	1.43	1.41	1.41	1.43	1.38	1.47	1.43	1.39	1.42
C6:0	1.40	1.40	1.42	1.38	1.40	1.41	1.44	1.36	1.40
C8:0	1.10	1.16	1.17	1.10	1.13	1.08	1.21	1.12	1.13
C10:0	2.73	2.99	3.00	2.72	2.83	2.63	3.17	2.82	2.86
C11:0	0.24	0.24	0.24	0.24	0.23	0.24	0.25	0.24	0.24
C12:0	3.34	3.68	3.66	3.35	3.48	3.21	3.85	3.50	3.51
C13:0	0.09	0.10	0.10	0.10	0.09	0.09	0.11	0.10	0.10
C14:0	10.77	11.23	11.34	10.66	11.09	10.44	11.58	10.89	11.00
C15:0	1.14	1.20	1.17	1.17	1.15	1.14	1.20	1.20	1.17
C16:0	30.67	32.13	31.14	31.66	30.55	30.78	31.72	32.54	31.40
C18:0	13.86	11.60	12.95	12.51	14.41	13.32	11.50	11.71	12.73
C20:0	0.21	0.17	0.19	0.19	0.21	0.21	0.17	0.17	0.19
SAFA	66.97	67.32	67.78	66.51	67.94	66.00	67.62	67.02	67.15
C14:1	0.92	0.89	0.89	0.92	0.89	0.95	0.88	0.90	0.90
C15:1	0.23	0.28	0.26	0.25	0.23	0.22	0.29	0.28	0.26
C16:1n9	1.83	1.76	1.69	1.90	1.72	1.95	1.66	1.85	1.80
C18:1	21.97	21.72	21.37	22.31	21.08	22.86	21.67	21.77	21.84
C20:1	0.29	0.26	0.26	0.29	0.28	0.31	0.25	0.27	0.28
MUFA	25.24	24.91	24.47	25.67	**24.19***	**26.28***	24.75	25.06	25.07
C16:2n4	0.64	0.64	0.63	0.66	0.62	0.67	0.64	0.64	0.64
C16:3n4	0.34	0.32	0.31	0.35	0.33	0.35	0.30	0.35	0.33
C18:2n6	2.73	2.65	2.65	2.73	2.79	2.68	2.50	2.79	2.69
C18:3n6	0.18	0.16	0.17	0.17	0.19	0.18	0.16	0.16	0.17
C18:3n6	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.07
C18:3n4	0.12	0.12	0.12	0.12	0.11	0.12	0.12	0.12	0.12
C18:3n3	0.58	0.61	0.61	0.58	0.60	0.57	0.63	0.59	0.59
C20:2	0.09	0.09	0.10	0.08	0.10	0.08	0.10	0.08	0.09
C20:3n6	0.10	0.11	0.10	0.10	0.10	0.09	0.11	0.11	0.10
C20:4n6	0.16	0.18	0.17	0.17	0.16	0.16	0.18	0.18	0.17
PUFA	5.00	4.94	4.93	5.01	5.06	4.94	4.80	5.08	4.97
USFA	30.24	29.85	29.40	30.67	29.25	31.22	29.55	30.14	30.04

FA – fatty acid, HW – Holstein cattle-winter, HS – Holstein cattle-summer, CW – Czech pied cattle-winter, CS – Czech pied cattle-summer
x – FAs concentrations in milk of both breeds during all year (%); * – $P \leq 0.05$

the limits usually presented in literature. The established values were higher only for P (79.8 mg.100 g^{-1}) and for K (164.7 mg.100 g^{-1}). The stated Cu, Zn, Ni and Fe contents are under the level of the highest permissible quantity.

The results prove a hypothetically prognosticated possible growth of Mg content in milk towards the original values, which point to a healthier way of cow nutrition and milk production. No significant differences were proved in the individual FA types. A conclusive difference was only found in MUFAs in H, where a higher percentage in S season 26.28 %) than in W season 24.19 %; $P < 0.05$).

ACKNOWLEDGEMENT

This paper was supported by project 1B44036 of MZe-ČR, NAZV and also carried out in the framework of the activities of National Referential Laboratory for Raw Milk in Rapotín.

REFERENCES

1. Forman, L., 1998: Milk fat in technology and nutrition. (In Czech). *Mlékařské listy*, 14,189–196.

2. Hanuš, O., Genčurová, V., Ponížil, A., Hlásný, K., Gabriel, B., Míčová, Z., 1993: The effects of year season, urea, acetone

Table 3. Statistical *t*-test of chosen milk indicators between C and H breed

Indicator	Statistical significance	Indicator	Statistical significance
DMY	ns	RCF	ns
FAT	ns	WV	*
LAC	ns	CP	**
SNF	ns	CAS	**
log SCC	*	TP	**
U	ns	WP	ns
AC	ns	NPN	ns
pH	ns	URN	ns
EC	ns	F/CP	ns
AL	ns	CN - CP	ns
SH	ns	CN - TP	ns
TEC	ns	Zn ($mg.kg^{-1}$)	*
RCQ	**		

Statistical significance of differences: ns – non-significant $P > 0.05$; * – $P \leq 0.05$; ** – $P \leq 0.01$; *** – $P \leq 0.001$

Table 4. Statistical *t*-test of chosen milk indicators between W and S season

Indicator	Statistical significance	Indicator	Statistical significance
DMY	ns	RCQ	ns
FAT	ns	RCF	ns
LAC	*	WV	ns
SNF	*	CP	ns
log SCC	ns	CAS	ns
U	ns	TP	ns
AC	ns	WP	ns
pH	*	NPN	ns
EC	***	URN	ns
AL	ns	F/CP	ns
SH	ns	CN - CP	ns
TEC	ns	CN - TP	ns

Statistical significance of differences: ns – non-significant $P > 0.05$; * – $P \leq 0.05$; ** – $P \leq 0.01$; *** – $P \leq 0.001$

and nitrate additions and native content of microelements on cow`s milk fermentation (In Czech). *Živočišná Výroba*, 38, 753–762.

3. Hanuš, O., Hlásný, J., Genčurová, V., Bjelka, M., 2004: Milk, versatile important foodstuff. Physiology of rise, taking care of production and value for human (In Czech). *Appendix, Osteological bulletin*, 9, 3–15.

4. Hermansen, E. J., Lund, P., 1990: Fatty acid composition and milk quality related to feeding Ca-saponified palm acid oil to different breeds of dairy cows. *J. Dairy Res.*, 57, 23–31.

5. Hlásný, J., 1996: To importance of calcium and magnesium in cow milk (In Czech). *Výzkum v chovu skotu*, 38, 1–11.

6. Hlásný, J., 1999a: The important ecological changes and their relationships with Czech milk advertising (In Czech), *Výzkum v chovu skotu*, 41, 46–48.

7. Hlásný, J., 1999b: Was the magnesium content in feeding plants changed? (In Czech). *Agrární noviny*, 43, 8.

8. Hlásný, J., 1999c: Cows milk shows on more ecological production (In Czech). *Agrární noviny-Zěmědělec*, 44, 2–3.

9. Janů, L., Hanuš, O., Frelich, J., Macek, A., Zajíčková, I., Genčurová, V., Jedelská, R., 2006: Influences of lactation physiology on Holstein high milk yielding cows on milk components and properties and milk health state indicators in the Czech Republic. A*cta Vet. Brno* (In opponent procedure).

10. Klíčník, V., 1978: *Animal Product Technology I, Dairying* (In Czech), Scriptum VSZ Brno, SPN Praha, 270pp.

11. Kováč, G., Nagy, O., Seidel, H., Jesenská, M., Hiščáková, M., Zachar, P., Hisira, V., 2003: The status of macro- and micro-elements in the blood serum, milk, rumen fluid, faeces and urine in a farm with increasing milk production. *Folia Veterinaria*, 47, 124–129.

12. Obermaier, O., 1995: The nutritional value of milk fatt (In Czech). *Mliekarstvo*, 1, 28.

13. Oprzadek, J., Oprzadek, A., 2003: Modifications of fatty acids composition in ruminants. *Medycyna-Weterynaryjna*, 59, 492–495.

14. Palmquist, D. L., Bealieu, A., Barbano, D. M., 1993: Feed and animal factors influencing milk fat composition. *J. Dairy Sci.*, 76, 1753–1771.

15. Pešek, M., Špička, J., Samková, E., 2005: Comparison of fatty acids composition of milk fat of Czech Pied cattle and Holstein cattle. *Czech J. Anim. Sci.*, 40/3, 122–128.

16. Pešek, M., Samková E., Špička J., 2006: Fatty acids and composition of their important groups in milk fat of Czech Pied cattle. *Czech J. Anim. Sci.*, 41/5, 181–188.

17. Peters, E. J., Medrano, J. F., Reed B. A., 1995: Fatty acid composition of milk fat from three breeds of dairy cattle. *Canadian J. Anim. Sci.*, 267–269.

18. Sikiric, M., Brajenovic, N., Pavlovic, I., Havranek, J. L., Plavljanic, N., 2003: Determination of metals in cow's milk by flame atomic absorption spectrophotometry. *Czech J. Anim. Sci.*, 48, 481–486.

19. Sumegi, B., Szakaly, S., 2001: Role of intake of inorganic elements in maintenance of normal human cell function-relationships of metabolism of calcium, iron and sodium. *Calcium-dairy-foods-health*, 109–121.

20. Thorsdottir, I., Hill, J., Ramel, A., 2004: Seasonal variation in cis-9, trans-11 conjugated linoleic acid in milk fat from nordic countries. *J. Dairy Sci.*, 87, 2800–2802.

21. Vriese de, S. R., Christophe, A. B., Maes, M., de Vriese, S. R., 2003: Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life-Sci.*, 73, 3181–3187.

Received November 17, 2006

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 51, 1: 26–29, 2007

HERITABILITY OF HUNTING PERFORMANCE TRAITS OF DACHSHOUND IN SLOVAKIA

Kasarda, R., Chudejová, I., Chudej, P., Kadlečík, O.

Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra
The Slovak Republic

radovan.kasarda@uniag.sk

ABSTRACT

The aim the paper was to estimate heritability of hunting performance traits in population of dachshound (also known as Teckel or German Basset) in Slovakia. Study was carried out on results of hunting performance examinations, executed by Slovak Hunting Association in cooperation with Dachshound Breeders Club of Slovakia in 2002–2004 years. Data included 898 judgment protocols of 646 individuals (some animals were classified in more tests). Ability traits nose, loudness and courage were evaluated. Permanent and random effects were tested using GLM procedure of SAS V 9.1. Linear model included fixed effects of breed group, sex, mother, father and permanent environmental effects of year, season, place, type of examination. Significant effect was observed for breed (R^2 = 1.6 %) in loudness, mother and father in courage (R^2 = 65.5 %, resp. 58.6 %), year (R^2 = 7 %) for nose, season of exam (R^2 = 53.8 %), place (R^2 = 87 %) and type of exam (R^2 = 70 %). Mixed model was used for estimation of heritability using AS REML including all significant fixed effects and random effect of individual. Estimated heritability of traits in mark was low (h^2 = 0.04 for loudness to h^2 = 0.172 for nose), higher heritability was observed in point evaluation where nose was 0.31, loudness 0.182 and courage 0.191. Phenotypic correlations between evaluated traits were positive.

Key words: correlations; heritability; dachshound; hunting performance

ADDRESS FOR CORRESPONDENCE
Kasarda, R. Ing., PhD, Slovak University of Agriculture
Dept. of Genetics and Breeding Biology, Tr. A. Hlinku 2
949 76 Nitra, the Slovak Republic
E-mail: radovan.kasarda@uniag.sk

INTRODUCTION

Genetic determination of hunting performance traits is on high importance in practical breeding of hunting dogs. That way are early examinations of instincts in most of dog breeds indicators of future performance of young dog and his competence for given type of performance. Some abilities of dog are inherited, many of them are in general the result of good and intensive practice. To basic hunting performance traits of dachshound belong nose, courage and loudness.

Aim was to estimate heritability of nose, loudness and courage of dachshound on the base of results from hunting trials in Slovakia. Our research followed informations available. Olfactive abilities of dogs are inherited, present from early age and are fundamentally impossible to affect them (2). Association of olfactive abilities with life skills are long life saved in memory of dog and motivating its performance also after long time (4). Nose of dogs is 100 times more sensitive as human (9) and has 40 times more cells in brain centre of odor detection. Loudness on track is inherited and is impossible to practice if this instinct is missing (4).

Training of dog with low or missing instincts of this ability is according to Báča (1) ineffective. Result is low reliable and time consuming performance.

Courage is defined as ability of dog, to confront situations evaluated as danger, irrespective of self-preservation instinct. According to Vochozka (6) courage is important and inseparable part of hunting performance. Courage dog is hard, fearless, resistant to effort and pain.

MATERIAL AND METHODS

Study was carried out on results of hunting performance examinations executed by Slovak Hunting Association in cooperation with Dachshound Breeders Club of Slovakia in 2002–2004 years. Data included 898 judgment protocols of 646 individuals (some animals were classified in more tests). In database were collected informations from ability exams, kennel exams, driving exams, trailing trials and forest exams of small breeds. There were 443 animals attended only one type of examination, 160 twice, three exams attended 38 dogs, 4 dogs attended four exams and only one dog all types of exams.

In year 2002 were examined 104 males and 114 females, in year 2003 169 males and 157 females, in year 2004 172 males and 182 female animals. According to breed group, in year 2002 were examined 191 hardhair, 10 longhair and 17 shorthair dogs. In year 2003 were examined 289 hardhair, 19 longhair and 18 shorthair dogs. In year 2004 were examined 307 hardhair, 36 longhair and 9 shorthair dogs.

Several factors were tested to estimate their effect on variability of results: sex, breed group, place of exams, year of exams, month of exams (season), evaluator, type of exam, father and mother of animal as basic preposition for setting up suitable mixed model for genetic determination of ability traits. Data pretreatment, basic processes and analyses were provided using SAS 9.1 packages BASE, CORR and GLM. Results of GLM procedure for permanent and environmental effects were tested under F-test and coefficient of determination (R^2) were estimated to evaluate their impact and significance on variability of ability traits. Significant effects were then introduced to model equation for genetic evaluation.

For estimation of genetic parameters, mixed model was established to take under consideration effect of individual and its pedigree information (BLUP animal model), method of random estimated maximum likelihood (REML) was used and equations were solved using AS REML (14) software. Mixed model equation design:

$$yijk = \mu + ai + bj + ck + ... + eijk$$

yijk – estimated trait [nose, loudness, courage]
μ – mean
ai – random effect [individual]
bj, ck... – fix effects [sex, breed group, year, month,...]
eijk – random error

RESULTS AND DISSCUSION

Only less scientific literature exists in studied area. As expected, sex has no effect on ability traits, statistical differences between sex were very low (0–0.003), not significant. Differences in behavior, temperament and workability of three groups of dachshound (longhair, shorthair and hardhair) were mentioned by Foogle (3) and Varhallen-Verhoef (5). Effect of breed group on variability of ability traits was very low and only loudness (both mark and points) significant ($R^2 = 0.015$, resp. 0.016) for hardhair dachshounds over longhair, resp. shorthair animals. Breed group as one of important effect in their statistic model for estimation of genetic parameters took under consideration also Krepsová *et al.* (11).

According to exam place, in average 4.5 dogs were examined (minimum 1 and maximum 34 at each exam). Lowest effect of place was observed in courage ($R^2 = 0.279$, resp. 0.314), higher in loudness ($R^2 = 0.768$, resp. 0.827) and highest on nose ($R^2 = 0.87$, resp. 0.828).

Year of exam showed low effect on variability of ability exams, only in nose significant ($R^2 = 0.07$, resp. 0.026).

More important was season of exams represented by month. In the database exams were held during whole year except February and March. Most of dogs were present on exams in September (n = 195), June (149), May (139) and October (137). Lowest effect was observed in mark for courage $R^2 = 0.09$ and mark for nose $R^2 = 0.054$, higher in mark for loudness $R^2 = 0.265$, highest in points for nose $R^2 = 0.598$ and points for loudness $R^2 = 0.70$. Karjalainen *et al.* (10) and Boeningk *et al.* (7) considered season of examination as important effect influencing result of exam too.

Type of exam has significant impact on variability of results. In our database 77 animals attended ability exams, 378 animals attended kennel exams, 122 forest exams, 281 driving exams and 40 animals trailing trials. Lowest effect was observed in mark for courage $R^2 = 0.09$ and highest for points of loudness $R^2 = 0.70$.

Animals in database were progeny of 146 fathers with minimum 1 progeny per father and maximum 105 progeny per father attending different exams. Impact of father on variability was from $R^2 = 0.164$ to $R^2 = 0.586$, significant only for courage, both mark and points ($R^2 = 0.22$, resp. 0.586).

Animals in database were progeny of 261 mothers with minimum 1 and maximum 30 progeny per mother attending different exams. Analogically as by father mother impact from $R^2 = 0.275$ to 0.655 significant only in courage, both mark and points ($R^2 = 0.354$, resp. 0.655).

Low positive phenotypic correlations of ability traits were observed between mark for nose and courage, both in mark and points; points for nose and mark for loudness resp. points for loudness and courage, both in points and mark.

Medium positive phenotypic correlations were observed between mark for nose and points for nose resp. mark and points for loudness, points for nose and courage both mark and points and between mark for loudness and courage, both for mark and points.

High phenotypic correlations were observed between mark for loudness and points for loudness and between mark for courage and points for courage. Detailed view is in Table 1. Brenoe *et al.* (8) estimated genetic correlations to be higher than phenotypic correlations between ability traits of three breeds of hunting dogs and to be near to 1.0 and proposed those correlated traits as input parameters for selection.

Table 1. Phenotypic correlations (rP) of ability traits (over diagonal) and coefficients of heritabity (h^2) (diagonal)

	Nose mark	Nose points	Loudness mark	Loudness points	Courage mark	Courage points
Nose - mark	**0.172**	0.3749^{+++}	0.4465^{+++}	0.3742^{+++}	0.2523^{+++}	0.2548^{+++}
Nose - points		**0.310**	0.2260^{++}	0.6440^{+++}	0.4263^{+++}	0.6343^{+++}
Loudness - mark			**0.042**	0.7554^{+++}	0.2654^{+++}	0.2617^{+++}
Loudness - points				**0.182**	0.4320^{+++}	0.6316^{+++}
Courage - mark					**0.098**	0.9213^{+++}
Courage - points						**0.191**

According to estimates of coefficients of heritability, ability traits were low heritable. Lower coefficients were estimated for results evaluated by mark (from 0.042 for loudness to 0.172 for nose).

Variability of evaluation in points was higher than in mark and this resulted to higher coefficients of heritability from 0.182 for loudness to 0.310 for nose. Schmutz *et al.* (13) achieved comparable results for heritability of nose (0.25 to 0.39). Karjalainen *et al.* (10) estimated higher heritability of loudness from 0.15 to 0.17 in comparison to our study. Brenoe *et al.* (8) published heritability of ability traits from 0.06 to 0.28.

Our results were higher in comparison to Liinamo *et al.* (12), who also estimated low heritability of ability traits (from 0.02 to 0.15). Authors mentioned relatively high standard error of estimates and recommend increase of objectiveness of evaluation system on examination. Krepsová *et al.* (11) estimated genetic parameters of ability traits tending to zero, in population of Weimar pointer in the Czech Republic. The main reason of low reliability was subjective evaluation on exams. Relatively high standard errors were observed also in our research. There is possibility to have more accurate estimates after enlarging database with more detailed evaluation and appended pedigree information.

Development of evaluation concepts for exams was studied by many authors in past. We can agree with Krepsová *et al.* (11) and Liinamo *et al.* (12) that mark given by trials is insufficient measure for estimation of real hunting abilities and is low effective as the selection criterion. This is in agreement with our results, where by points evaluation, which copies evaluation in mark were achieved higher coefficients of heritability.

Breeders association increase ambition of exacting results as qualification criterion for stud animals as criterion of increased hunting performance. Increased objectiveness of results could be possible *via* suitable selection index which could correlate performance of given dog with its genetic premises. Liinamo *et al.* (12) and also Krepsová *et al.* (11) assumed need to improve present system of evaluation of dogs using objective BLUP animal model based method, where genetic parameters of estimated hunting traits will be the measure, and prerequisite for selection index and selection of mates.

CONCLUSIONS

Permanent and random effects were tested using GLM procedure of SAS V 9.1. Linear model included fixed effects of breed group, sex, mother, father and permanent environmental effects of year, season, place, type of examination. Significant effect was observed for breed group ($R^2 = 1.6\,\%$) in loudness, mother and father in courage ($R^2 = 65.5\,\%$, resp. $58.6\,\%$), year ($R^2 = 7\,\%$) for nose, season ($R^2 = 53.8\,\%$), place ($R^2 = 87\,\%$) and type ($R^2 = 70\,\%$).

Mixed model was used for estimation of heritability using AS REML including all significant fix effects and random effect of individual. Estimated heritability of traits in mark was low ($h^2 = 0.04$ for loudness to $h^2 = 0.172$ for nose), higher heritability was observed in evaluation with points where nose was 0.31, loudness 0.182 and courage 0.191. Between evaluated traits were observed positive phenotypic correlations.

Mark given by exams is insufficient measure for estimation of real hunting abilities and as the selection criterion are low effective. Evaluation in points, which copies evaluation in mark, leaded to achievement of higher coefficients of heritability.

Improvement of present system of evaluation of dogs is needed, where genetic parameters of estimated hunting traits will be the measure, and prerequisite for selection index and selection of mates. Important preposition for increased exaction of results are complexity of evaluation and increased objectiveness of examination. Further research will be oriented on using multitrait models which will allow estimation of other genetic parameters.

ACKNOWLEDGEMENT

Authors would like thank to Scientific Agency of the Slovak Republic for financial support under project No. 1/3449/06 and Slovak Hunting Association for cooperation by data collection.

REFERENCES

1. Báča, J., 1967: *Training of Hunting Dogs* (In Czech). Praha: Státní zemědělské nakladatelství, 362 pp. ISBN 07-052-67.

2. Červený, J., Hell, P., Slamečka, J. *et al.*, 2004: *Hunting Encyclopedia* (In Slovak). Praha: Ottovo nakladatelství, 591 pp. ISBN 80-7181-902-6.

3. Fogle, B., 1997: *Dachshound* (In Slovak). Bratislava: Art Area, 77 pp. ISBN 80-88879-04-3.

4. Švec, J., Hrabák, P., Hrabáková, O., 1988: *Small Hunting dogs* (In Czech). Praha: Státní zemědělské nakladatelství, 296 pp. ISBN 07-087-88.

5. Varhallen-Verhoef, E., 2001: *Encyclopedia of Dogs* (In Czech) (4th edn.). Dobřejovice: REBO productions, 270 pp., ISBN 80-7234-172-3.

6. Vochozka, V., 2000: *Dachshounds in Hunting Praxis* (In Czech). České Budejovice: Dona, 196 pp. ISBN 80-86136-77-9.

7. Boenigk, K., Hamann, H., Distl, O., 2006: Genetic influences on the outcome of the progeny tests for behaviour traits in Hovawart dogs. *Dtsch. tierärtzl. Wschr.*, 113, 182–188.

8. Brenoe, U. T., Larsgard, A. G., Johannessen, K. R., Uldal, S. H., 2002: Estimates of genetic parameters for hunting performance traits in three breeds of gun hunting dogs in Norway. *Appl. Anim. Behav. Sci.*, 77, 209–215.

9. Dalton, C., 2004: *The working dog – breeding, training, and welfare*. www.lifestyle block.co.nz/articles/working_dog/16_breeding.htm

10. Karjalainen, L., Ojala, M., Vilva, V., 1996: Environmental effects and genetic parameters for measurements of hunting performance in the Finnish Spitz. *J. Anim. Breed. Genet.*, 113, 525–534.

11. Krepsová, Z., Přibyl, J., Soukupová, Z., 2002: Breeding value estimation of hunting traits of weimar pointer (In Czech). *Proceedings of XX Genetic Days, Brno, the Czech Republic.* pp. 209–211.

12. Liinamo, A. E., Karjalainen, E., Ojala, M., Vilva, V., 1997: Estimates of genetic parameters and environmental effects of measures of hunting performance in Finnish hounds. *J. Anim. Sci.*, 75, 622–629.

13. Schmutz, S. M., Schmutz, J. K., 1998: Heritability estimates of behaviors associated with hunting in dogs. *J. Hered.*, 89, 233–237.

14. Gilmour, A. R. *et al.*, 1999: AS REML Reference Manual. *NSW Agriculture Biometric Bulletin*, No. 3. NSW Agriculture, Orange, NSW, Australia, 210 pp.

Received February 2, 2007

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 51, 1: 30–33, 2007

A TRIAL OF A PROPOFOL AND KETAMINE COMBINATION ON DOMESTIC SHORT HAIRED CATS

Ajadi, R. A., Agbesinu, A. J. B., Adetunji, A., Akinrinmade, J. F.

Department of Veterinary Surgery and Reproduction
University of Ibadan, Ibadan
Nigeria

ade_vsr@hotmail.com

ABSTRACT

Trials were undertaken on domestic short haired cats to compare the anesthetic indices and haemodynamic changes of the combination of a 15 mg.kg^{-1} intramuscular injection of ketamine (Ketalem®) and 4 mg.kg^{-1} intravenous single bolus injection of 1% propofol (Propovan®) to that of an i.m. injection of 15 mg. kg^{-1} of 5% ketamine alone. In the course of this trial, the onset and duration of analgesia were respectively 2.3 ± 0.3 minutes and 54.2 ± 5.7 minutes for the ketamine and propofol combination. However, there was no analgesia in cats that received only a ketamine injection. The duration of recumbency was significantly ($P < 0.05$) longer in cats that received ketamine and propofol combination (64.2 ± 6.5 minutes) than cats that received ketamine alone (46.5 ± 7.8 minutes). However, there is no significant differences ($P > 0.05$) in the heart rates (HR), respiratory rate (RR) and rectal temperature (RT) of the two groups of cats. It was therefore concluded that combination of ketamine and propofol may be additive at the hypnotic and anaesthetic end points in domestic short haired cats. The combination is therefore recommended for surgical procedures of short duration in cats.

Key words: anesthesia; domestic short haired cats; Ketalem®; ketamine hydrochloride; propofol; Proporan®; trial

INTRODUCTION

The technique of administering two or more drugs to facilitate the induction and maintenance of general anaesthesia has gained considerable popularity (2). The goal of this combination is to achieve more specific "target" responses and minimize the side effects of individual drugs.

To date, benzodiazepines and propofol have emerged as one of the most popular combinations for the induction and maintenance of general anaesthesia (10,11) though these drugs behave in a synergistic manner to induce hypnosis, it has been shown recently that midazolam and propofol do not interact synergistically with respect to other end points, including prevention of movement to noxious stimuli (10,13,15)

Interactions between propofol and opioids have also been reported and are more marked during surgery than during the induction of anaesthesia (9). Propofol interferes with opioid metabolism to increase the circulating concentration of opioid (14)

Propofol and ketamine had been paired for anaesthesia induction and total intravenous anaesthesia in man (3, 5, 7). The combination was found to be additive at both hypnotic and anaesthetic end-points (5). However, the interactions between ketamine and propofol have not been evaluated in the domestic short haired cats. The aim of this study therefore, was to assess the anaesthetic indices and haemodynamics of propofol and ketamine combination in domestic short haired cats.

MATERIALS AND METHODS

Ten adult domestic short-haired cats comprising of six intact toms and four non-pregnant, non-lactating queens were used. The cats had a mean body weight of 1.5 ± 0.2 kg. They were housed individually in a concrete floor kennel throughout the duration of the experiment. All the cats were judged to be in good health before the commencement of the study by physical examination and the results of complete blood counts.

During the study, the cats were fed on rice and fish while water was provided *ad libitum*. However, food alone was withheld for twelve hours prior to the commencement of each trial.

Two series of trials were carried out at a one week interval. The cats were assigned randomly into each trial. The first trial comprised of cats that received only a single intramuscular (i.m.) injection of 5 % ketamine hydrochloride (Ketalem®) at 15 mg.kg^{-1} body weight. The second trial involved cats that received a single i.m. injection of 5 % ketamine hydrochloride at 15 mg.kg^{-1} body weights followed by an intravenous injection of a single dose of 1 % propofol (Propovan®) at the rate of 4 mg.kg^{-1} body weight. Venous access was gained *via* the cephalic vein with a 23 gauge needle for the injection of propofol. The access was kept patent by the injection of 0.9 % normal saline. After the induction of anaesthesia, an endotracheal tube was inserted to ensure patent airways. The cats were then placed in a laterally recumbent position.

In this study, the onset of the analgesia, duration of analgesia, duration of recumbency and recovery time were determined for each cat. Analgesia was assessed by the cats' reaction to artery forceps applied to the interdigital space (limb withdrawal reflex).

The onset of analgesia was defined as the time interval between drug administration and the disappearance of limb withdrawal reflex. Duration of analgesia is the time interval between the disappearance and reappearance of limb withdrawal reflex. The duration of recumbency was defined as the time interval between the assumption of sternal recumbency and when the cat can maintain sternal recumbency. Recovery time is the time between cat's assumption of sternal recumbency and when the cat can maintain standing.

During the course of each trial, the cats' heart rates (HR), respiratory rates (RR), and rectal temperatures (RT) were taken immediately after ketamine administration, immediately after propofol administration and at 10, 20, 30, 40, 50 and 60 minutes after propofol administration. Heart rate was determined in beats/min with the aid of precordial stethoscope. Respiratory rate was counted in breaths/min by visual movement of the chest wall. Rectal temperature was measured in degrees centigrade (°C) using a digital clinical thermometer.

In addition, previously reported unusual reactions linked with the administration of propofol and ketamine (Briggs *et al.*, 1982) were also looked for and recorded. Limb withdrawal during propofol injection was considered to be a sign of pain perception. Apnoea was defined as a sign of complete cessation of spontaneous breathing efforts.

Data were expressed as mean ± SEM. Anesthetic indices were compared using the Student *t*-test. A value of $p \leq 0.05$ was considered significant in all cases. Physiological variables were compared using analysis of variance (ANOVA) and then tested with Least Square Difference (LSD) method of agreement.

RESULTS

Administration of either 15 mg.kg^{-1} intramuscular (i.m.) injection of ketamine or a combination of 15 mg.kg^{-1} (i.m.) injection of ketamine and 4.0 mg.kg^{-1} intravenous single bolus injection does not produce any adverse reaction in the domestic short-haired cats except salivation (Table 1).

The anaesthetic indices of a cat receiving a ketamine injection alone or the combination of ketamine and propofol is shown in Table 2. There was no analgesia in cats receiving ketamine alone while the onset and duration of analgesia were respectively 2.3 ± 0.3 minutes and 54.2 ± 5.7 minutes in cats that received the combination of ketamine and propofol.

The changes in heart rates (HR), respiratory rates (RR) and rectal temperatures (RT) following intramuscular injection of ketamine alone or the combination of ketamine and propofol is shown in Fig. 1. There is no significant difference ($p > 0.05$) in the HR, RR and RT between cats that received only ketamine and those that received the combination of ketamine and propofol.

Table 1. Comparison of adverse effects following intramuscular injection of either ketamine alone or a ketamine and propofol combination

Adverse effect	Ketamine only	Ketamine + propofol
Apnoea	-(10)	-(10)
Pain on injection	-(10)	-(10)
Perivascular reaction	-(10)	-(10)
Muscle rigidity	-(10)	-(10)
Cyanosis	-(10)	-(10)
Salivation	+(10)	+(10)
Vomition	-(10)	-(10)
Retching	-(10)	-(10)
Arrythmias	-(10)	+(1)
Excitement	-(10)	-(10)
Urination	+(1)	-(10)
Defaecation	-(10)	-(10)

Table 2. Anaesthetic indices following intravascular administration of ketamine alone or a combination of ketamine (i.m.) and propofol (i.v.)

Anaesthetic indices (min)	Ketamine only	Ketamine + propofol Combination
Onset of analgesia	–	2.3 ± 0.3
Duration of analgesia	–	54.2 ± 5.7
Duration of recumbency	46.5 ± 7.8	64.2 ± 6.5*
Recovery time	23.2 ± 4.6	48.3 ± 5.8*

* – $p < 0.05$

DISCUSSION

Drug interaction may occur as the result of synergy, addition, potentiation or inhibition. The nature of the

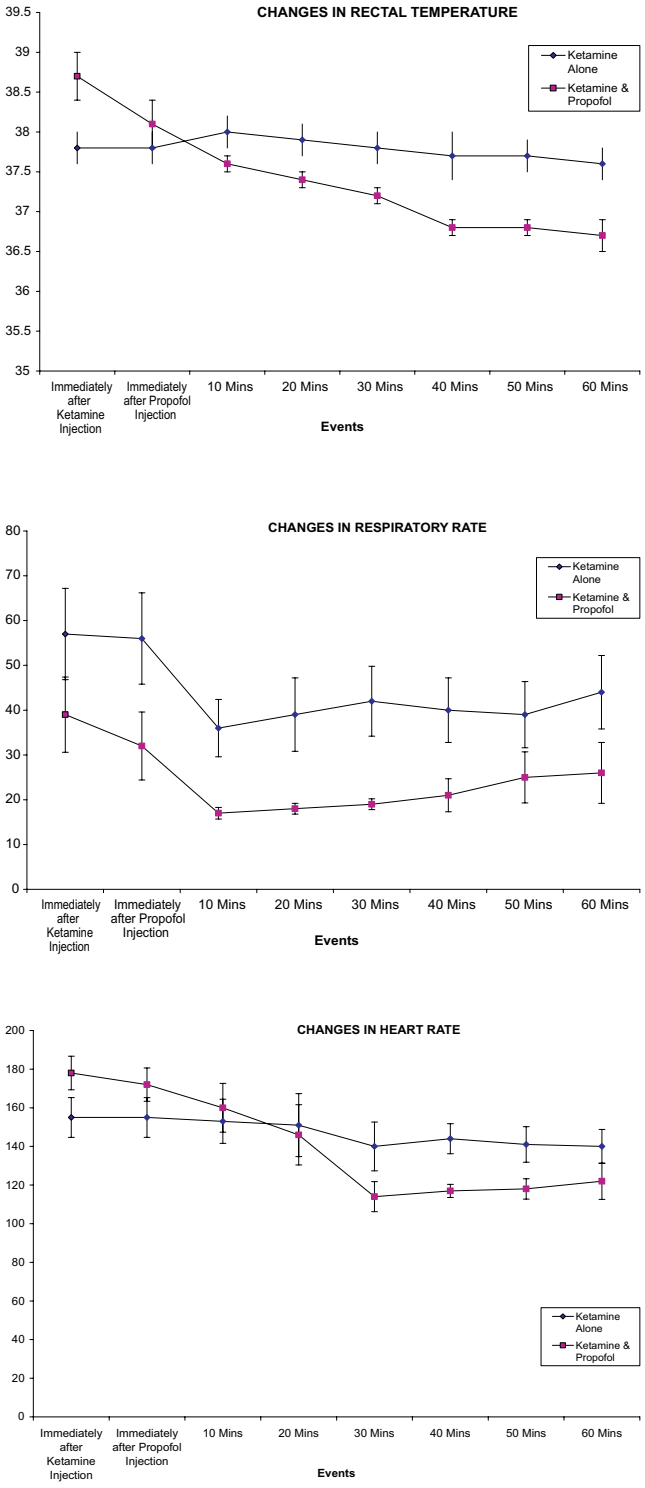

Fig. 1. Mean heart rates, respiratory rates and rectal temperatures of ten cats sedated with either 15 mg.kg^{-1} of 5% ketamine alone or a combination of 15 mg.kg^{-1} of 5% ketamine and 4.0 mg.kg^{-1} single bolus intravenous injection of 1 % propofol

interaction may reflect the underlying mechanism so that two drugs acting on the same receptor are likely to have an additive rather than synergistic effect (14). In this trial, the administration of a single bolus injection of 1 % propofol (4 mg.kg-^{1}) to cats sedated with 15 mg.kg^{-1} body weight of 5 % ketamine was characterized by rapid induction, moderate duration of analgesia and minimal net haemodynamic effect.

Adverse effects associated with propofol injection include apnoea, nausea and pain on injection (1). It is note worthy that none of the cats receiving propofol after ketamine sedation show pain response to injection. Ketamine has been reported to have somatic analgesia (4). The only adverse effect observed in the cats is excessive salivation. However, this can be controlled by the concurrent administration of an anti-cholinergic agent (16).

In this trial, there are no significant differences ($p > 0.05$) in the HR, RR and RT between cats that received ketamine alone and those that received the combination of ketamine and propofol. This suggests that the mixture has a minimal net haemodynamic effect. Ketamine stimulates the cardiovascular system in healthy dogs causing increased heart rates, cardiac output and pulmonary blood pressure (6). However, concurrent use of sedatives or hypnotics blocks this effect (4, 12).

The addition of a single bolus injection of 4 mg.kg^{-1} body weight of propofol to cats sedated with 15 mg.kg^{-1} body weight of 5 % ketamine injection produced analgesia with a mean duration of 54.2 ± 5.8 minutes. This is in agreement with the earlier study that propofol and ketamine are additive at both hypnotic and anaesthetic end-points (7). Since cats receiving ketamine alone responded to noxious stimuli, and propofol injection alone has been reported to virtually lack analgesia, the result of this trial suggested that ketamine and propofol interacts synergistically with respect to the analgesia end-point.

In conclusion, this trial suggested that propofol and ketamine combination appeared to be more beneficial for a day case surgery of short duration in a feline patient.

REFERENCES

1. Adetunji, A., Ajadi, R. A., Adewoye, C. O., Oyemakinde, B. O., 2002: Total intravenous anesthesia with respect to propofol: intermittent bolus *versus* continuous infusion techniques in xylazine premedicated dogs. *Israel Journal of Veterinary Medicine*, 57, 139–144.

2. Beverly, A. Orser, Miller, D. R., 2001: Propofol-benzodiazepine interactions: insights from a bench to a bedside approach. *Canadian Journal of Anaesthesia*, 48, 431–432.

3. Guit, J. B., Koning, H. M., Coster, M. L., Mackie, D. P., 1991: Ketamine as analgesia for total intravenous anaesthesia with propofol. *Anaesthesia*, 46, 24–27.

4. Hall, L. W., Clark, K. W., 1991: *Veterinary Anaesthesia*. (9th edn.). Balliere Tindall, London.

5. Hernedez, C., Parramon, F., Garcia-Velasco, P. *et al.*, 1999: A comparative study of three techniques for total intravenous anesthesia: midazolam-ketamine, propofol-ketamine and propofol-fentanyl. *Rev. Esp. Anestesiod. Reanim.*, 46, 154–158.

6. Herwets, D. D., 1977: Effects of intravenous agents on left ventricular function in dogs. *Am. J. Vet. Res.*, 232, 1444–1448.

7. Hui, T. W., Short, T. G., Hong, W. *et al.*, 1995: Additive interactions between propofol and ketamine. *Anesthesiology*, 22, 641–648.

8. Briggs, L. P., Clarke, R. S., Watkins, S. B., 1982: Adverse reaction to the administration of propofol (Diprivan). *Anaesthesia*, 37, 1099–1101.

9. Mora, C. T., Henson, M., Bailey, J. *et al.*, 1992: Propofol plasma concentration affects alfetanil requirements for cardiac surgery. *Anesthesiology*, 77, A408.

10. Oxorn, D. C., Ferris, L. E., Harrington, E., Orser, B. A., 1997: The effects of midazolam on propofol-induced anesthesia; Propofol dose requirements, mood profiles, and peri-operative dream. *Anesthesia and Analgesia*, 85, 553–559.

11. Short, T. G., Chui, P. T., 1991: Propofol and midazolam act synergistically in combination. *Br. J. Anaesth.*, 67, 539–545.

12. Trim, C. M., 1987: Sedation and anaesthesia. In **Holsworth, J.** (ed.): *Diseases of the Cat*. W.B. Saunders Co., Philadelphia.

13. Vinik, H. R., Bradley, E. L., Kissin, I., 1994: Triple anaesthetic combination: Propofol-midazolam-alfetanil. *Anaesthesia and Analgesia*, 78, 354–358.

14. Vuyk, J., 1998: Supplementation and drug interactions. *Anaesthesia*, 43, 35–41.

15. Wilder-Smith, O. H. G., Ravussin, P. A., Decosterd, L. A., Despland, P. *et al.*, 2002: Midazolam premedication reduces propofol dose requirements for multiples anesthetic end-points. *Canadian Journal of Anesthesia*, 48, 439–445.

16. Wright, M., 1982: Pharmacologic effects of ketamine and its use in veterinary medicine. *J. Am. Vet. Med. Assoc.*, 180, 1462–1471.

Received October 23, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 34–37, 2007

COMPARISON OF TWO METHODS TO COUNTERACT THE EFFECTS OF DEOXYNIVALENOL

Faixová, Z.[1], Faix, Š.[2], Bořutová, R.[2], Leng, Ľ.[2]

[1]University of Veterinary Medicine, Komenského 73, 041 81 Košice
[2]Institute of Animal Physiology Slovak Academy of Sciences, 040 01 Košice
The Slovak Republic

faixova@uvm.sk

ABSTRACT

The aim of the study was to evaluate the effects of adsorbent modified glucomannan and dietary selenium to counteract toxicity of deoxynivalenol in growing broiler chicks. Eighty four, 1-d old male broiler chicks were fed 1 of 6 diets containing deoxynivalenol (DON) for 42 days. The diets included: (1) positive control A (0.2 ppm deoxynivalenol), (2) deoxynivalenol-contaminated (3 ppm deoxynivalenol), (3) deoxynivalenol-contaminated (3 ppm deoxynivalenol) plus glucomannan (2 g.kg^{-1} diet), (4) positive control B (0.2 ppm deoxynivalenol, 0.4 mg selenium.kg^{-1} diet), (5) deoxynivalenol-contaminated (3 ppm deoxynivalenol, 0.4 mg selenium.kg^{-1} diet) and (6) deoxynivalenol-contaminated (3 ppm deoxynivalenol) plus selenium-enriched yeast (1.4 selenium.kg^{-1} diet). All birds were sacrified and blood samples for biochemical analyses were collected. Dietary supplementation of DON significantly altered plasma total protein, calcium, magnesium, triglycerides and free glycerol levels and alanine aminotransferase activity. Supplementation of selenium-enriched yeast to DON-contaminated diet reversed plasma levels of calcium, magnesium and alanine aminotransferase activity of chicks induced by dietary deoxynivalenol. Inclusion of glucomannan in the diet restored plasma levels of magnesium, triglycerides, free glycerol, total protein and alanine aminotransferase activity induced by dietary deoxynivalenol. Inclusion of adsorbent glucomannan to DON-contaminated diet, however, did not prevent toxic effect on calcium metabolism. The inclusion of selenium to DON-contaminated diet did not completely alleviate toxic effect on protein and lipid metabolism.

Key words: adsorbent; deoxynivalenol; glucomannan; plasma indices; selenium-enriched yeast

INTRODUCTION

During the past few decades there has been a steady increase in global production of poultry meat and eggs. As mycotoxins are one of the major factors suppressing poultry productivity, control of their impact is critical.

According to the FAO, approximately 25 % of the world's grain supply is contaminated with mycotoxins.

Mycotoxins of importance in poultry are mainly produced of fungi of the genera *Aspergillus, Fusarium* and *Penicillium* either pre-harvest, during harvest, or in storage or during feed processing whenever conditions are favourable.

Although more than 100 *Fusarium* mycotoxins are known, those fusariotoxins of most concern based on toxicity and occurrence are trichothecenes, zearalenone, fumonisins and moniliformin.

Deoxynivalenol (DON, vomitoxin) is the most prevalent trichothecene in crops used for food and feed production. DON affects cellular immune response, the gastrointestinal tract, proteosynthesis and is also known to interfere with metabolism of membrane phospholipids and to increase liver lipid peroxides (10).

In order to avoid mycotoxicosis, several strategies have been investigated.

One of the methods for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind mycotoxins effectively in the gastrointestinal tract. Many compounds have been tested for adsorptive effects on mycotoxins, but only few have been proven successful (16, 4).

Since some mycotoxins are known to produce membrane damage through increased lipid peroxidation, the protective

properties of antioxidant substances have been extensively used.

The objective of the study was to compare and evaluate the efficacy of modified glucomannan and selenium-enriched product to alleviate toxicity of deoxynivalenol in growing broiler chicks.

MATERIAL AND METHODS

Animals, diets and treatments. Eighty four, 1-d old male broiler chicks of a commercial strain Ross 308, Párovské háje, the Slovak Republic were distributed randomly into groups of 14 chicks in each one. The birds were maintained on the floor for the course of study. Chicks were initially maintained at 31°C, the temperature was gradually lowered by 2 °C/wk to reach 21 °C by the end of wk 5, and this temperature was maintained for the duration of the experiment. Continuous lighting and water *ad libitum* were provided throughout the experiment.

Chicks were fed the diets from the day of hatch to 42-d of age. The six diets included (1) positive control A (0.2 ppm DON), (2) DON-contaminated (3 ppm DON), (3) deoxynivalenol-contaminated (3 ppm DON) plus modified glucomannan Mycosorb® (Alltech Inc., USA) (2 g.kg^{-1} diet), (4) positive control B (0.2 ppm DON, 0.4 mg selenium.kg^{-1} diet), (5) deoxynivalenol-contaminated (3 ppm DON, 0.4 mg selenium. kg^{-1} diet) and (6) deoxynivalenol-contaminated (3 ppm DON) plus selenium-enriched yeast (Sel-plex®) (Alltech Inc., USA) (1.4 mg selenium.kg^{-1} diet).

To provide stable dietary contents of mycotoxins throughout the experiment, the chicks were fed only one type of diet HYD-02.

The final diets were obtained by mixing the basal diet supplied by Agrokonzult, s.r.o., the Slovak Republic (the part of HYD-02 diet before addition of 40 % portion of maize) with positive control or contaminated maize. Maize used for the diets of positive control contained DON background level 0.5 ppm, while zearalenone and 15-acetyldeoxynivalenol were below detection limits. Diets for group 2 and 3 contained DON contaminated maize at concentration 7.5 ppm. Zearalenone and 15-acetyldeoxynivalenol levels in the contaminated maize were 0.3 and 0.6 ppm, respectively. Concentration of other mycotoxins were below detection limits in both positive control and contaminated maize.

The mycotoxin contents in the basal diet (the part of HYD-02 diet before addition of 40 % portion of positive control or contaminated maize) were found to be 0.05 and 0.0026 ppm for zearalenone and total aflatoxins, respectively.

The selenium content of HYD-02 diet for each group of birds was 0.4 mg.kg^{-1}. The diet for group 6 was supplemented with Se dose 1.4 mg.kg^{-1} in the form of selenized yeast.

All experimental procedures with animals were in accordance with European Guidelines for Care and Use of Animals for Research Purpose and they were approved by a local ethic committee.

Sample analysis. Mycotoxins in maize were detected using GC-MS method (13). The mycotoxin contents in the basal diet (the part of HYD-02 diet before addition of 40 % portion of control or contaminated maize) were analyzed using NOACK kits for ELISA with spectrophotometric evaluation.

The concentration of selenium in diets were measured using the fluorometric method of Rodriguez *et al.* (14).

All birds were sacrified and blood samples were collected. Plasma was separated by centrifugation at 1 600 *g* for 10 min and stored at –20 °C until analysis.

The concentration of chloride (CL 90 T, BIO-LACHEMA-TEST, CzR), calcium (Ca 10 KX, BIO-LACHEMA-TEST, CzR), magnesium (MG 208, BIO-LACHEMA-TEST, CzR), total protein (TP 450, BIO-LACHEMA-TEST, CzR), triglycerides and free glycerol (TR 210, RANDOX, UK) were assayed in the plasma according to procedures outlined in respective commercial kits using a spectrophotometric sets. The enzymes, alkaline phosphatase (ALP 2x60, BIO-LACHEMA-TEST, CzR) and alanine aminotransferase (ALT 360, BIO-LACHEMA-TEST, CzR) were assayed in the plasma according to procedures outlined in respective commercial kits using a spectrophotometer.

Statistical analysis. The results are expressed as mean ± S.E.M. Statistical significance was evaluated by one-way ANOVA test.

RESULTS AND DISCUSSION

The toxicity of DON was expressed through decreased plasma triglycerides and free glycerol of broiler chicks (Table 1 and 2). These findings are in agreement with the previous reports of Kubena *et al.* (8). These authors decribed decreased plasma triglycerides and cholesterol in White Leghorn chicks fed a 9 and 18 mg.kg^{-1} DON contaminated diet for 35 days. Later Huff *et al.* (5) reported a significant decrease in serum triglycerides in chicks fed a diet containing contaminated wheat (16 mg.kg^{-1} DON in feed) for 3 weeks. Inclusion of modified glucomannan in the DON-contaminated diet provided a significant protective effect against changes in lipid metabolism of broiler chicks. Our results showed that selenium in the diet was ineffective in the adverse effects of DON on lipid metabolism.

Dietary supplementation of DON significantly decreased plasma total protein level of chicks (Table 1 and 2). One of the toxicities of DON is thought to be derived from the inhibition of protein synthesis (15). These data were later confirmed by Mikami *et al.* (11).

Our data showed that adsorbent in the DON-containing diet was able to provide a significant protective effect against changes in proteosynthesis by the liver. However, we failed to demonstrate a protective effect of organic selenium against changes in protein metabolism in the liver induced by DON. Burguera *et al.* (3), however, reported that selenium supplementation was effective in reducing the adverse effects of aflatoxin in turkey poults expressed by higher values of total protein in blood.

Dietary inclusion of 3 ppm DON resulted in increase plasma alanine aminotransferase activity, indicating liver damage and leakage of the enzymes into the blood (Table 1 and 2). Supplementation of organic selenium and inclusion of glucomannan to the DON-contaminated diets reversed

Table 1. Effect of dietary inclusion of deoxynivalenol and glucomannan on plasma chemistry in growing broiler chickens

Item	Control diet A (0.2 ppm DON)	Contaminated diet (3 ppm DON)	Contaminated diet (3 ppm DON) plus glucomannan (2 g.kg^{-1} feed)
Chloride (mmol.l^{-1})	104.30 ± 4.88	114.40 ± 4.30	103.40 ± 3.34
Calcium (mmol.l^{-1})	2.171 ± 0.07^{b}	3.36 ± 0.34^{b}	2.73 ± 0.19
Magnesium (mmol.l^{-1})	0.86 ± 0.05^{b}	0.34 ± 0.01^{a}b	0.92 ± 0.079^{a}
Alkaline phosphatase (μkat.l^{-1})	8.06 ± 0.81	10.72 ± 0.12^{a}	6.36 ± 0.09^{a}
Alanine amino-transferase (μkat.l^{-1})	0.25 ± 0.02^{b}	0.47 ± 0.004^{ab}	0.18 ± 0.09^{a}
Total protein (g.l^{-1})	39.11 ± 1.52^{b}	27.59 ± 1.92^{ab}	38.70 ± 1.57^{a}
Triglycerides (mmol.l^{-1})	1.02 ± 0.06^{b}	0.34 ± 0.02^{ab}	0.69 ± 0.05^{a}
Free glycerol (mmol.l^{-1})	0.91 ± 0.06^{b}	0.23 ± 0.02^{ab}	0.58 ± 0.05^{a}

Values are mean ± S.E.M., n = 14. Significant differences within a row are indicated by using the same superscript letter, P < 0.01

Table 2. Effect of dietary inclusion of deoxynivalenol and selenium-enriched yeast on plasma indices in growing broiler chickens

Item	Control diet B (0.2 ppm DON, 0.4 mg Se.kg^{-1} diet)	Contaminated diet (3 ppm, DON, 0.4 mg Se.kg^{-1} diet)	Contaminated diet (3 ppm DON) plus 1.4 mg.kg^{-1} Se diet
Chloride (mmol.l^{-1})	81.90 ± 5.37^{ab}	112.41 ± 2.37^{a}	107.95 ± 1.28^{b}
Calcium (mmol.l^{-1})	1.73 ± 0.13^{a}	3.26 ± 0.24^{ab}	1.69 ± 0.09^{b}
Magnesium (mmol.l^{-1})	1.01 ± 0.07^{a}	0.35 ± 0.02^{ab}	0.89 ± 0.05^{b}
Alkaline phosphatase (μkat.l^{-1})	8.13 ± 0.91	6.72 ± 1.26	8.54 ± 0.62
Alanine ami-notransferase (μkat.l^{-1})	0.24 ± 0.00^{a}	0.46 ± 0.00^{ab}	0.25 ± 0.02^{b}
Total protein (g.l^{-1})	35.54 ± 1.53^{ab}	18.59 ± 1.59^{b}	25.06 ± 1.28^{a}
Triglycerides (mmol.l^{-1})	0.59 ± 0.04^{a}	0.35 ± 0.01^{a}	0.50 ± 0.02
Free glycerides (mmol.l-^{1})	0.48 ± 0.04^{a}	0.24 ± 0.01^{a}	0.39 ± 0.09

Values are mean ± S.E.M., n = 14. Significant differences within a row are indicated by using the same superscript letter, P < 0.01

plasma alanine aminotransferase activity. Similar results were observed by Atroshi *et al.* (1) in rats.

In the present study, the administration of 3 ppm DON to diets altered plasma calcium and magnesium (Table 1 and 2). Inclusion of organic selenium in DON-enriched diet of growing broiler chicks provided a significant protective effect against toxic changes in calcium and magnesium metabolism. Inclusion of glucomannan to DON-contaminated diet, however, did not prevent toxic effect on calcium metabolism.

The role of dietary antioxidants such as vitamin C, E and selenium in preventing mycotoxin toxicity has attracted increasing attention at the present time. Peng and Yang (12) observed that sodium selenite is able to reduce *in vitro* toxic effect of DON on cultured cardiomyocytes. Studies of Jakhar and Sadana (6) showed that supplementation of selenium (5 ppm sodium selenite) had some protective effect against the toxic effect of 1 ppm aflatoxin B1 in Japanese quail. However, McLeod *et al.* (9) reported that rats fed a selenium deficient diet were more resistent to aflatoxin B1 that those fed a selenium-sufficient diet. According to the authors, the protection conferred by selenium deficiency against aflatoxin B1 is associated with the hepatic expression of an aldo-keto-reductase and a glutathione S-transferase subunit that efficiently metabolizes the mycotoxin.

Atroshi *et al.* (2) reported a decrease of the GSH activity after two-week-treatment with ochratoxin in mice. Treatment of mice with the combined antioxidants could enhance hepatic oxidant/detoxification system, as indicated by increase in hepatic reduced glutathione level. Authors suggest that use of the combined antioxidants may be of interest in conditions when certain toxin-mediated forms of cell death/apoptosis contribute significantly to toxicity.

Adsorbent of mycotoxins, a glucomannan derived from yeast cell wall was used in the experiment to reduce toxic effect of DON in broiler chicks. Dosage of a glucomannan in DON-contaminated diet was chosen on the basis of several trials (13, 4).

The high adsorptive capacity of modified glucomannans for mycotoxins has been reported by many researchers. Karaman *et al.* (7) reported that Mycosorb™ was effective in diminishing of adverse effect of aflatoxin (2 mg.kg^{-1} diet) on growing chicks and the higher concentration of yeast glucomannan (1 g.kg^{-1} feed) was more effective than lower concentration (0.5 g.kg^{-1}). Modified glucomannan supplementation was found to be effective in reducing the adverse effects of *Fusarium* mycotoxin in broilers (16).

Diaz *et al.* (4), however, reported that the only feed additive capable of counteracting the adverse effects on performance caused by the dietary administration of 2 ppm T-2 toxin in broiler chickens was the additive based on the enzymatic inactivation of the 12, 13-epoxide ring of the trichothecenes (Mycofix) while Mycosorb, Mycoad and Zeolex were not effective.

CONCLUSION

Our data show that modified glucomannan and selenium-enriched yeast are beneficial in reversing adverse effects of deoxynivalenol in broiler chicks since they are able to improve the most plasma biochemical parameters.

ACKNOWLEDGEMENT

This work was supported by the grant VEGA projects No 1/2443/05 and No 2/7046/27 and APVV 51 004804.

REFERENCES

1. Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Saloniem, H., Sankari, S. *et al.*, 1999: Fuminisin B1-induced DNA damage in rat liver and spleen: effects of pretreatment with coenzyme Q10, L-carnithine, alpha-tocopherol and selenium. *Pharmacol. Res*., 40, 59–67.

2. Atroshi, F., Biese, I., Saloniemi, H., Ali-Vehmas, T., Saari, S., Rizszo, A. *et al.*, 2000: Significance of apoptosis and its relationship to antioxidants after ochratoxin administration in mice. *J. Pharm. Pharmaceut. Sci.*, 3, 281–291.

3. Burguera, J. A., Edds, G. T., Osuna, O., 1983: Influence of selenium on aflatoxin B1 or crotalaria toxicity in turkey poults. *Am. J. Vet. Res.*, 44, 1714–1717.

4. Diaz, G. J., Cortés, A., Roldán, L., 2005: Evaluation of the efficacy of four feed additives against the adverse effects of T-2 toxin in growing broiler chickens. *J. Appl. Poult. Res.*, 14, 226–231.

5. Huff, W. E., Kubena, L. F., Harvey, R. B., Hagler, W. H., Swanson, S. P., Phillips, T. D. *et al.*, 1986: Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poult. Sci.*, 65, 1291–1298.

6. Jakhar, K. K., Sadana, J. R., 2004: Sequential pathology of experimental aflatoxicosis in quail and the effect of selenium supplementation in modifying the disease process. *Mycopathologia*, 157, 99–109.

7. Karaman, M., Basmacioglu, H., Ortatatli, M., Oguz, H., 2005: Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopathology. *Br. Poult. Sci.*, 46, 394–400.

8. Kubena, L. F., Swanson, S. P., Harvey, R. B., Fletcher, O. J., Rowe, L. D., Phillips, T. D., 1985: Effect of feeding deoxynivalenol (vomitoxin)-contaminated wheat to growing chicks. *Poult. Sci.*, 64, 1649–1655.

9. Mc Leod, R., Ellis, E. M., Arthur, J. R., Neal, G. E., Judah, D. J., Manson, M. M. *et al.*, 1997: Protection conferred by selenium deficiency against aflatoxin B1 in the rat associated with the hepatic expression of an aldo-keto-reductase and glutathione S-transferase subunit that metabolize the mycotoxin. *Cancer Res.*, 57, 4257–4266.

10. Mezes, M., Barta, M., Nagy, G., 1999: Comparative investigation of the effect of T-2 mycotoxin on lipid peroxidation and antioxidant status in different poultry species. *Res. Vet. Sci.*, 66, 19–23.

11. Mikami, O., Yamamoto, S., Yamanaka, N., Nakajima, Y., 2004: Porcine hepatocytes apoptosis and reduction of albumin secretion induced by deoxynivalenol. *Toxicology*, 15, 241–249.

12. Peng, S. Q., Yang, J. S., 2003: Effect of deoxynivalenol on action potentials of cultured cardiomyocytes and the protective effect of selenium. *Zhonghua Yu Fang Yi Xue Za Zhi*, 37, 423–425.

13. Raymond, S. L., Smith, T. K., Swamy, H. V. L. N., 2003: Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficay of a polymeric glucomannan mycotoxin adsorbent. *J. Anim. Sci*., 81, 2123–2130.

14. Rodriguez, E. M., Sanz, M. T., Romero, C. D., 1994: Critical study of fluorometric determination of selenium in urine. *Talanta*, 12, 2025–2031.

15. Rotter, B. A., Prelusky, D. B., Pestka, J. J., 1996: Toxicity of deoxynivalenol (vomitoxin). *J. Toxicol. Environ. Health*, 48, 1–34.

16. Swamy, H. V., Smith, T. K., Cotter, P. F., Boermans, H. J., Sefton, A. E., 2002: Effects of feeding blends of grain naturally contaminated with *Fusarium* mycotoxins on production and metabolism in broilers. *Poult. Sci.*, 81, 966–975.

Received March 1, 2007

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 38–42, 2007

COMPARISON OF THE ANTIBODY RESPONSE OF NIGERIAN INDIGENOUS AND EXOTIC CHICKENS TO INFECTIOUS BURSAL DISEASE VIRUS INFECTION

***Oladele, O. A., Emikpe, B. O., Ohore, O. G. Oluwayelu, D. O., Agboola, O. O.**

University of Ibadan
***Department of Veterinary Medicine, Ibadan**
Nigeria

lade_ed@yahoo.co.uk

SUMMARY

The infectious bursal disease (IBD) antibody response of Nigerian indigenous and exotic chickens to experimental infection was investigated. Twenty indigenous chickens and forty Harco cockerels at four weeks of age were inoculated with 80 µl of IBD virus inoculum, equivalent to $2 \times 10^{3.5}$ chicken LD_{50}, *via* conjunctival instillation. The IBD virus antibody response was determined by the agar gel precipitation test (AGPT) and the Enzyme-linked imunnosorbent assay (ELISA) for a period of eight weeks post infection (pi). Precipitin lines were observed from six weeks to eight weeks pi in the indigenous chickens while none was observed in the exotic chickens for the period of observation. On the other hand, ELISA detected the IBD virus antibody from five weeks to eight weeks pi in indigenous chickens, while it was detected between one week and eight weeks pi in the exotic chickens. Average ELISA unit (EU) values ranged from 0.15 to 50.3 peaking at six weeks pi in the indigenous chicken while it ranged from 3.7 to 13.0 peaking at three weeks pi in the exotic chickens. From this study, it could be concluded that antibody response to IBD virus infection in the indigenous chicken is slower but the level higher, than in the exotic chickens.

Key words: antibody response; bursa Fabricii; infectious bursal disease; Nigerian indigenous chicken.

INTRODUCTION

Infectious bursal disease is an acute, highly contagious viral disease of chickens characterized by enlargement of the bursa Fabricii, whitish diarrhoea and moderate to high mortalities. It results in the destruction of lymphocytes, specifically in the bursa Fabricii but also in the thymus, spleen and caecal tonsils (12).

There are several strains of the IBD virus, which vary in virulence ranging from 50 % mortality to subclinical infections (6). In Nigeria, mortality in affected flocks ranges between 25 and 45 % (8, 15,16, 20). IBD is of significant economic importance in terms of losses due to mortalities and immunosupression precipitated by damage to the bursa Fabricii in subclinical infections (3). Both neutralizing antibodies detectable in a virus neutralization test – VNT (23) and precipitating antibodies detectable by counter-immuno-electroosmophoresis – CIEOP and agar gel precipitation test – AGPT (9, 23) are produced in response to natural and experimental infections in chickens.

The Nigerian indigenous chicken make up about 80 % of the 150 million total chicken population in Nigeria (22). They are more adapted to tropical conditions of high temprature and humidity, as well as poor nutrition than the exotics, which perform sub-optimally in the tropics (13). According to Okoye *et al.* (17), the hardiness or survival trait of the indigenous chicken has been assumed to infer a better disease resistance ability than the exotic breeds. There is however little or no evidence to support this assumption. Although there have been reports of IBD outbreaks in indigenous chicken flocks (1, 2, 18, 21), these are fewer than in exotic chickens.

Running Title: IBDV antibody response in Nigerian indigenous and exotic chickens.

*Corresponding author: lade_ed@yahoo.co.uk

This study was therefore carried out to determine and compare IBD virus antibody response in the Nigerian indigenous and the exotic breeds of chickens as a measure of survival traits.

MATERIALS AND METHODS

Experimental chickens

Ninety indigenous chicken eggs obtained from different sources in Ibadan, Nigeria were set for hatching in a commercial hatchery. Thirty-two, one-day old indigenous chicks were hatched and 28 of them were reared up until four weeks of age. The chicks were placed on vitamins-antibiotics for the first five days post-hatching. Fifty-five, one-day-old Harco cockerels obtained from a commercial hatchery were also reared up until four weeks of age in a separate cage. These chicks were also placed on vitamins-antibiotics for the first five days post-hatching.

Infectious bursal disease virus inoculum

The IBD virus (Serotype 1) inoculum used was a 50 % bursal homogenate in PBS. It was prepared from bursa Fabricii from a confirmed case of IBD outbreak in five-week old pullets with 40 % mortality.

Experimental infections

Twenty indigenous and forty exotic (Harco breed) chicks at four weeks of age were inoculated with 80 µl each of virus inoculum equivalent to 2.103.5 chicken LD_{50}, *via* conjunctival instillation. The remaining eight indigenous and fifteen exotic chicks served as uninoculated controls. Prior to experimental grouping and infections, ten chicks each randomly selected from both the indigenous and exotic flocks were bled and the sera harvested were subjected to agar gel precipitation test (AGPT) for the detection of the IBD virus antibody.

Serology

Eleven chicks were randomly bled from each infected group one week post-infection (pi) and then at weekly interval for eight weeks pi Two mls of blood was collected by venupuncture into plain universal bottles. Blood samples were kept at room temperature to clot before they were kept in the refrigerator at 4 °C overnight for serum to separate. Serum was harvested into eppendorf tubes and subjected to both qualitative and quantitative AGPT as well as ELISA for the detection of IBD virus antibody within 48 hours.

Agar Gel Precipitation Tests (AGPT)

Qualitative AGPT

Sera harvested from all blood samples were subjected to qualitative AGPT as described by Oladele (19) using IBD virus serotype 1 antigen and purified agar. Results were read after twenty-four hours. A positive result was shown by the presence of a precipitin line within the space between the central antigen well and any of the peripheral wells.

Quantitative AGPT

Two-fold serial dilutions of serum samples that were positive by qualitative AGPT were diluted from 1 : 2 to 1 : 64. Equal volumes of the diluted sera were used to fill peripheral wells while the central wells were filled with a known IBD virus antigen as in qualitative AGPT (7).

Enzyme-linked immunosorbent assay (ELISA)

An ELISA kit produced by Affinitech, Ltd., Bentonville, AR. USA, for the detection of an antibody to infectious bursal disease virus was used. Serum samples obtained from both indigenous and exotic chicks pi were subjected to ELISA as described by the manufacturer.

Control positive and control negative (provided in kit) as well as test sera were tested at 1 : 400 dilution and in duplicates. Optical density values were read on a Multiscan® ELISA reader (Titertek) using dual wavelengths (405 nm and 630 nm).

Assay validation

Each test run included positive and negative controls. The results were considered valid when the difference between the means of the positive and negative controls (Pcx-Ncx) was 0.7 O.D. (optical density) or greater as recommended by the manufacturer. Also the average absorbance value of the negative control wells had to be less than 0.4 O.D.

Calculation and interpretation of results

The reading for each sample was calculated as follows:

$$\frac{\text{Average O.D. test sample} - \text{Average O.D. negative}}{\text{Average O.D. positive} - \text{Average O.D. negative}} = \text{Sample to positive (Sp) ratio}$$

Sp. ratio × 100 = ELISA unit (EU)

Serum samples with EU values of five or less were considered negative for antibodies to IBD virus; five to fifteen EUs were considered weakly positive while 15 to 75 EUs were considered moderately positive as directed by the manufacturer of the ELISA kit (Affinitech, Ltd., Bentonville, AR. USA).

RESULTS

Clinical signs consisting of anorexia, ruffled feathers, droopy wings, diarrhoea were observed in both groups of chickens from day two pi. Mortality rates were 10 % and 22.5 % in the indigenous and exotic chickens respectively with gross and histopathological lesions typical of IBD. No intercurrent infection was observed.

Agar gel precipitation tests (AGPT)

Precipitin lines were observed from six weeks to eight weeks pi in the indigenous chickens but not between one week and five weeks pi. The results of qualitative and quantitative AGPT from six to eight weeks pi in indigenous chickens are presented in Tables 1 and 2 respectively. At six and seven weeks pi, 90.9 % of serum samples were positive for IBD virus antibody while it was 100 % at

Table 1. Qualitative Agar gel precipitation test (AGPT) on sera from IBD virus infected indigenous chickens 6 to 8 weeks post- infection

Sample No.	6 weeks pi	7 weeks pi	8 weeks pi
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	-	-	+
% Positive	**90.9**	**90.9**	**100**

Table 2. Quantitative Agar gel precipitation test (AGPT): Titres (log2) of sera from IBD virus-infected indigenous chickens 6 to 8 weeks post-infection

Sample No.	6 weeks pi	7 weeks pi	8 weeks pi
1	4	5	5
2	5	2	4
3	5	4	1
4	5	5	2
5	4	1	5
6	5	3	5
7	3	5	5
8	3	0	2
9	5	5	5
10	5	4	3
11	0	5	5
Average titre	**4**	**3.55**	**3.82**

Table 3. ELISA Units (EU) of serum samples from IBD virus infected indigenous and exotic chickens

Weeks Post-infection	Breed of Chicken	Sample No. 1	2	3	4	5	6	7	8	9	10	11	Average EU values
1	**Ind.**	0	0	0	0.9	0.6	0.6	0.06	2.7	0.8	0	2.6	**0.75**
	Ex.	2.2	3.3	4.9	4.8	3.1	0.8	7.0[b]	4.0	2.7	3.9	-	**3.7**
2	**Ind.**	1.3	0.6	0.5	0	0	0	0	0	0	0.2	0	**0.24**
	Ex.	11.2[b]	11.4[b]	2.5	0	4.4	2.2	5.5[b]	-	-	-	-	**5.3[b]**
3	**Ind.**	0.4	0.3	0	0	0	0.3	0	0	0.2	0.4	0	**0.15**
	Ex.	7.1[b]	8.8[b]	24.9	17.9[a]	4.8	11.0[b]	9.0[b]	9.4[b]	35.1[a]	5.5[b]	9.2[b]	**13.0[b]**
4	**Ind.**	0.6	0.7	0.1	0	0	0.7	0.2	0.2	1.0	0	0.1	**0.33**
	Ex.	3.6	4.6	16.2[a]	31.8[a]	10.2[b]	10.1[b]	28.[a]	12.1[b]	0	3.0	13.8[b]	**12.1[b]**
5	**Ind.**	0.6	9.6[b]	0.5	6.0[b]	7.6[b]	0	1.3	7.1b	0.8	0.3	-	**3.38**
	Ex.	5.4[b]	14.0[b]	2.7	19.3[a]	4.4	3.7	4.6	0.9	4.6	5.0[b]	6.6[b]	**7.1[b]**
6	**Ind.**	52.4[a]	62.7[a]	61.6[a]	59.6[a]	50.6[a]	64.2[a]	37.4[a]	35.4[a]	58.9[a]	59.2[a]	11.5[b]	**50.3[a]**
	Ex.	2.3	7.5[b]	5.6[b]	7.2[b]	2.1	4.9	1.8	0	-	-	-	**3.9**
7	**Ind.**	58.7[a]	46.5[a]	55.1[a]	64.1[a]	20.3[a]	37.6[a]	60.8[a]	3.8	52.4[a]	43.1[a]	59.3[a]	**45.6[a]**
	Ex.	16.0[a]	2.5	6.8[b]	6.0[b]	4.5	7.6[b]	-	-	-	-	-	**7.2[b]**
8	**Ind.**	64.0[a]	41.3[a]	20.2[a]	28.6[a]	60.8[a]	55.5[a]	59.2[a]	16.2[a]	64.1[a]	24.1[a]	61.8[a]	**45.1[a]**
	Ex.	4.2	2.3	9.9[b]	4.6	7.1[b]	4.5	11.3[b]	4.0	2.5	3.5	13.9[b]	**6.2[b]**

- – No sample, [a] – moderately positive, [b] – weakly positive.

eight weeks pi (Table 1). Average antibody titers at six, seven and eight weeks pi were 4 $\log_2$, 3.5 $\log_2$ and 3.82 $\log_2$ respectively by quantitative AGPT (Table 2).

None of the sera from exotic chickens was positive by AGPT from one to eight weeks pi.

Enzyme-linked immunosorbent assay (ELISA)

ELISA units (EU) of serum samples obtained pi from the IBD virus infected indigenous and exotic chickens are presented in Table 3.

ELISA detected IBD virus antibody from five to eight weeks pi in the indigenous chickens while it was detected from one week pi up to eight weeks pi in the exotic chickens. Amongst the indigenous chickens, 40 % (4 out of 10) were positive for IBD virus antibody at five weeks pi, 100 % at six and eight weeks pi (all eleven samples each week) and 90.9 % at seven weeks pi (10 out of 11). In the exotic chickens, serum samples were 10 % positive at one week pi (1 out of 10), increased to a maximum of 91 % at three weeks pi (10 out of

11) and decreased to 36.4% at eight weeks pi. Average EU values ranged from 0.15 to 50.3 with the peak at six weeks pi in the indigenous chicken while it ranged from 3.7 to 13.0 peaking at three weeks pi in the exotic chickens (Fig. 1).

DISCUSSION

The results of this study showed that the IBD virus precipitating antibody was detectable in the serum of indigenous chickens from six weeks to eight weeks pi by AGPT while it was not detected for the eight weeks of observation in the exotic chickens. On the other hand, ELISA detected antibody in the serum of indigenous chickens from five weeks to eight weeks pi and from one week to eight weeks pi in the exotic breed (Fig. 1). Thus, some serum samples that tested negative for the IBD virus antibody with AGPT tested positive with ELISA. This further stresses the relative sensitivity of ELISA over AGPT (14).

It could be observed that the six to eight weeks pi period, during which AGPT was able to detect IBD antibody in the serum of the indigenous chicken, coincided with the period of high antibody titre detectable by ELISA, thus, confirming the report of Yondem (24) that AGPT detects medium to high antibody levels while ELISA in addition, detects low levels not detectable by AGPT.

The average EU values obtained per week pi in both indigenous and exotic breeds, shows a slower antibody response by the indigenous chickens when compared with the exotic breed (Fig. 1). Sa'idu *et al.* (21) in a retrospective study of diseases of Nigerian indigenous chickens have observed that the age at greatest risk of IBD was eight weeks. This is contrary to the known susceptible age of three to six weeks in exotic breeds.

According to Ibe (11) the Nigerian indigenous chicken is characterized by such traits as small body size, slow growth, late maturity and poor production ability. Thus, the slower or late antibody response in the indigenous chickens compared with the exotic chickens, could be the result of the slower development of the bursa Fabricii due to a slower growth rate. Also, average EU values in the indigenous chickens with a peak of 50.3 at six weeks pi, were generally higher than the values obtained in the exotic chickens, which peaked (13.0) at three weeks pi (Fig. 1). This indicates a higher level of antibody response in the indigenous chickens, which could be responsible for the infrequent encounter of clinical IBD in this breed of chickens (21).

Aire (5) has shown that the bursa Fabricii of the Nigerian indigenous cockerels attained a greater relative organ to body weight than White Leghorn cockerels. Glick (10) earlier reported that birds with bigger bursa Fabricii have a greater resistance to disease. This could explain the higher level of antibody response in the indigenous chickens used in this study. In a similar study by Aire and Ojo (4), it has been observed that the Nigerian indigenous cockerels were more resistant to *Salmonella gallinarum* infection than the White Leghorn as judged by values in haemoglobin, haematocrit, erythrocytes and leukocyte counts.

In a similar study by Okoye *et al.* (17), in which serum sampling was performed within four weeks pi, with only two samplings for the detection of the IBD virus antibody, no significant difference was observed between mean titres of the IBD antibody of the two breeds of chickens and AGPT was the only serological method used. Detailed antibody monitoring was not possible in that study as in the present study.

This study has shown that the Nigerian indigenous chickens are as susceptible as the exotic breed to IBD virus infection as evident in antibody response in both breeds. However, IBD antibody response in the indigenous chickens was slower, and the level was higher than in the exotic chickens.

ACKNOWLEDGEMENTS

We thank Leigh Nogy and Raul Felman of AFFINITECH, LTD. Bentoville, AR., U.S.A., for donating the ELISA kit used in this study.

REFERENCES

1. Abdu, P. A., 1988: Infectious bursal disease in a flock of broilers and local Nigerian chickens. *Bull. Anim. Prod. Afr.,* 36, 269–271.

2. Abdu, P. A., George, J. B., 1986: Outbreak of infectious bursal disease among local chickens in Zaria, Nigeria. *Trop. Vet.*, 4,143–144.

3. Adene, D. F., Oyejide, A., Owoade, A. A., 1984: Studies on the possible roles of naturally infected local chickens and vaccine virus in the epidemiology of infectious bursal disease. *Rev. Elev. Med. Vet. Pays. Trop.*, 38, 122–126.

4. Aire, T. A., Ojo, M. O, 1974: Response of White Leghorn and Nigerian cockrels to experimental *Salmonella* infection. *Trop. Anim*. Hlth Prod., 6, 111–116.

5. Aire, T. A., 1973: Growth of the bursa of Fabricius and thymus gland in the Nigerian and White Leghorn cockrels. *Res. Vet. Sci.*, 15, 383–385.

6. ByGrave, A. C., Faragher, J. T., 1970: Mortality associated with Gumboro disease. *Vet. Rec.*, 86, 758–759.

7. Cullen, G. A., Wyeth, P. J., 1975: Quantitation of antibodies to infectious bursal disease. *Vet. Rec.*, 97, 315.

8. Durojaiye, O. A., Ajibade, H. A., Olafimihan, G. O., 1984: An outbreak of infectious bursal disease in 20 wk-old birds. *Trop. Vet.*, 2, 175–176.

9. Durojaiye, O. A., Adene, D. F., Owoade, A. A., 1985: Counter immunoelectroosmophoresis in the diagnosis of infectious bursal disease of poultry. *Trop. Anim. Hlth. Prod.*, 17, 225–229.

10. Glick, B., 1955: Growth and function of the bursa of Fabricius in chickens. *Poult. Sci.*, 34, 1196–1201.

11. Ibe, S. N., 1990: In *Rural Poultry in Africa (Proc. of an International Workshop on Rural Poultry Development in Africa)*. (Ed. **Sonaiya, E. B.**). African Network on Rural Poultry Development. Obafemi Awolowo University, Ile-Ife, Nigeria.

12. Lukert, P. D., Saif, Y. M., 1991: Infectious bursal disease In *Diseases of Poultry (9th edition.)*. Ames, IA, USA, Iowa State University Press, 648–663.

13. Marks, H. C. Moore, C. H., Gyles, N. R., Wilson, H. R., Tindell, L. D., Johnson, W. A., Dressen, I. J., Blow, W. L., Rrueger, W. F., Siegel, P. B., 1969: Genotype and environment interaction in egg production stocks of chickens: minor effects and interaction of parent-flock, location, parent stock and growing location. *Poult. Sci.*, 48, 1553.

14. Marquardt, W., Johnson, R. B., Odenwald, W. F., Schlothober, B. A., 1980: An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Av. Dis.*, 24, 375–385.

15. Nawathe, D. R., Onunkwo, O., Smith, I. M., 1978: Serological evidence of infections with the virus of infectious bursal disease in wild and domestic birds in Nigeria. *Vet. Rec.*, 102, 444.

16. Okoye, J. O. A., Uzoukwu, M., 1981: An outbreak of IBD among chickens between 16 and 20 weeks old. *Av. Dis.*, 25, 1034–1038.

17. Okoye, J. O. A., Orajaka, L. J. E., Nwosuh, C. J., 1992: Antibody response of local and exotic Nigerian chickens to infectious bursal disease virus In *Proc. 29th Annual Conference of the Nigerian Veterinary Medical Association*, 56–59.

18. Okoye, J. O. A., 1987: The pathology of infectious bursal disease in indigenous Nigerian chickens. *Rev. Elev. Med. Vet. Pays Trop.*, 40, 13–16.

19. Oladele, O. A., 2001: Comparison of seroprevalence rate of infectious bursal disease antibody in village chickens and ducks in Southwest Nigeria. *Afr. J. Clin. Exp. Microb.*, 2, 49–51.

20. Onunkwo, O., 1975: An outbreak of infectious bursal disease (IBD) of chickens in Nigeria. *Vet. Rec.*, 97, 443.

21. Sa'idu, L., Abdu, P. A., Abdullahi, U. S., Umoh, J. U., 1994: Diseases of Nigerian indigenous chickens. *Bull. Anim. Prod. Afr.*, 42, 19–23.

22. Sonaiya, E. B., 1990: The systems approach to rural poultry development. In *Rural Poultry in Africa (Proc. of an International Workshop on Rural Poultry Development in Africa)*. (Ed. **Sonaiya, E. B.**). African Network on Rural Poultry Development. Obafemi Awolowo University, Ile-Ife, Nigeria.

23. Weisman, J., Hitchner, S. B., 1978: Virus neutralization *versus* agar gel precipitation tests for detecting serological response to infectious bursal disease virus. *Av. Dis.*, 22, 598–603.

24. Yondem, B., 1994: Comparison of enzyme linked immunosorbent assay and agar gel precipitation test for virus (D-78). *HayVan-Asilarduriugu-Dergisi*, 18, 32, 1–16.

Received October 23, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 43–46, 2007

HAEMATOLOGY OF THE NIGERIAN TURKEY

***Olayemi, F. O.[1], Ojo, O. E.[2]**

[1]Department of Veterinary Physiology and Pharmacology, University of Ibadan Ibadan
[2]Department of Veterinary Microbiology and Parasitology, University of Agriculture Abeokuta, Ogun State
Nigeria

funsho_olayemi@yahoo.com

ABSTRACT

Haematological data were determined in apparently healthy Nigerian turkeys. Significant sex differences were not found ($P > 0.05$) in RBC, WBC, PCV, Hb, MCH, MCV and MCHC values. The differential WBC counts also did not differ significantly ($P > 0.05$) between the male and female, but the percentage value of the monocyte was higher ($P < 0.05$) in the female than in the male. The haematological values obtained from the Nigerian turkey were compared with the values reported in the same tropical environment in the White England turkey, Nigerian local duck, local chicken and the ostrich. This is with a view to establishing breed and species differences.

Key words: haematology; Nigerian turkey; sex; species

INTRODUCTION

There are reports on the haematology of White England turkeys in the tropical environment (6, 17, 11). However to the best of our knowledge there has not been any report on the Nigerian turkey. There are about 200,000 of these local turkeys in Nigeria – Arowolo (1) and they are reared under the semi-intensive management system. They are black in colour and weigh between seven and ten kilograms.

The Nigerian local turkeys are now being raised intensively by turkey farmers and it is hoped that this effort will assist in alleviating the acute protein deficiency experienced by the vast majority of low-income earners. Also the intensive production of this local breed of turkeys will help in conserving the much-needed foreign exchange that is currently being expended on the procurement of White England turkeys.

The development of the Nigerian local breed of turkey would depend heavily on research information on the physiology of the bird, unfortunately such information are currently not available. That is why in this paper we present the influence of sex on the haematology of the Nigerian local turkey.

We have also attempted a comparison of the haematology of the Nigerian local turkey with the values that have been previously reported for some avian species in the same tropical environment.

MATERIALS AND METHODS

Nineteen apparently healthy Nigerian turkeys (ten males and nine females) were used for the present study. They were reared in a deep liter pen and fed commercially prepared growers mash (14.5 % protein, 4.8 % fat, 7.2 % fibre and 0.8 % calcium) produced by Bendel feeds and flour mill Ltd, Benin, Edo State. The feed and water were given *ad libitum*. The turkeys were treated against nematodes using Piperazine hydrochloride (Wormazine®) (Alfasan International BV3440AB Woerden, Holland) at 1 g.l^{-1} of water, three weeks before the commencement of the study.

Blood was obtained from the jugular vein of each of the

*Corresponding author: Dr F. O. Olayemi
Department of Veterinary Physiology and Pharmacology
University of Ibadan, Ibadan, Nigeria
E-mail address: funsho_olayemi@yahoo.com

Nigerian local turkeys into a bottle containing ethylene diamine tetraacetic acid (EDTA) (2 mg.ml^{-1} of blood). Red blood cells (RBC) were counted with haemocytometers. Packed cell volume (PCV) was determined using the microhaematocrit method. Haemoglobin (Hb) concentration was measured by the cyanmethaemoglobin method. From the values obtained the haematimetric indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)) were calculated (5). Total WBC count was determined with haemocytometer using the WBC diluting fluid (2). Blood smears, made in duplicate, were fixed in alcohol and stained with Giemsa stain for differential WBC counts.

The results were statistically evaluated using Student's *t*-test.

RESULTS

Table 1 shows the erythrocyte values of the male and female Nigerian turkeys. The values of MCV and MCH were significantly higher ($P < 0.05$) in the male. However, the RBC, WBC, PCV, Hb and MCHC values were similar ($P > 0.05$) in both sexes.

Table 2 reveals that the effect of sex on the leukocyte values of the Nigerian turkeys. The total WBC, lymphocyte, heterophils, eosinophil and monocyte counts were similar ($P > 0.05$) in the male and female, but the percentage value of monocyte was higher ($P < 0.05$) in the female than in the male.

In Table 3 a comparison erythrocyte parameter in Nigerian local turkey with values reported in the same environment in the white England turkeys – Oyewale and Ajibade (17), Nigerian local duck – Oyewale *et al.* (20), local chicken and the ostrich – Olowookorun and Makinde (14). The Nigerian local turkey had significantly higher RBC counts than the white England turkey ($P < 0.01$), Nigerian local duck ($P < 0.001$), local chicken ($P < 0.01$) and ostrich ($P < 0.001$). The PCV was also significantly higher ($P < 0.001$) in the Nigerian local turkey than the white England turkey, Nigerian local duck and local chicken but the PCV of the ostrich was similar to that of the Nigerian local turkey.

Table 1. Erythrocyte values (mean ± SD) of male and female Nigerian turkey

PARAMETERS	MALE (n = 10)	FEMALE (n = 9)
RBC (.10^{6}.µl^{-1})	2.72 ± 0.56	3.06 ± 0.40
PCV (%)	48.20v6.43	44.66 ± 6.70
Hb (g.dl^{-1})	12.25 ± 1.07	11.58 ± 1.72
MCH (pg)	47.35 ± 12.93	38.40 ± 7.70
MCHC (g.dl^{-1})	25.71 ± 2.83	26.39 ± 4.97
MCV (fl)	87.06 ± 58.02	148.28 ± 31.96

All parameters are not significantly different ($P > 0.05$)

The Hb concentration in the Nigerian local turkey was similar with that of the White England turkey but it was significantly lower ($P < 0.001$) in the Nigerian local turkey than the Nigerian local duck, ostrich and the local chicken. The MCV value of the Nigerian local turkeys was similar to that reported for the white England turkey and it was lower ($P < 0.01$) than that of the Nigerian local duck. The MCV value of the Nigerian local turkey was however higher than that of the local chicken ($P < 0.01$) and ostrich ($P < 0.001$).

The MCH value in the Nigerian local turkeys was similar to that of the White England turkey but it was lower ($P < 0.01$) than that of the Nigerian local duck. The MCHC value in the Nigerian local turkey was also lower than that of the White England turkey ($P < 0.01$) and the Nigerian local duck ($P < 0.001$).

Table 4 shows a comparison of the WBC values in the Nigerian local turkey with those of the Nigerian local duck (13), local chicken and ostrich – (14). The total WBC of Nigerian local turkey was significantly lower ($P < 0.001$) than the total WBC of Nigerian local duck, local chicken and ostrich. The lymphocyte count was lower ($P < 001$) in the Nigerian local turkey than the Nigerian duck. The percentage of lymphocytes in the Nigerian local turkey was also significantly lower than that of the Nigerian duck ($p < 0.01$), domestic chicken ($P < 0.05$), but higher ($P < 0.001$) than that of ostrich. The heterophil count of the Nigerian local turkey was lower ($P < 0.001$) than that of the Nigerian duck. However the percentage value of heterophil was higher in the Nigerian local turkey than the value of the Nigerian duck ($P < 0.01$) and local chicken ($P < 0.02$), but it was lower than the value of the ostrich ($P < 0.001$). The eosinophil count of the Nigerian local turkey was similar to that of the Nigerian duck. Also, the percentage of eosinophil in the Nigerian local turkey was similar to that of the Nigerian duck and local chicken but it was higher ($P < 0.02$) than that of the ostrich. The monocyte count of the Nigerian

Table 2. Leukocyte values (mean ± SD) of male and female Nigerian turkey

PARAMETER	MALE (n = 10)	FEMALE (n = 9)
TOTAL WBC (.10^{9}.l^{-1})	3.28 ± 1.88	2.73 ± 1.46
LYMPHOCYTE (.10^{9}.l^{-1})	1.48 ± 0.79 (46.30 ± 13.58)^{a}	1.19 ± 0.73 (44.33 ± 9.58)^{a}
HETEROPHIL (.10^{9}.l^{-1})	1.79 ± 1.25 (53.30 ± 13.78)^{a}	1.52 ± 0.81 (56.00 ± 10.63)^{a}
EOSINOPHIL (.10^{9}.l^{-1})	0.01 ± 0.01 (0.30 ± 0.48)^{a}	0.01 ± 0.01 (0.22 ± 0.44)^{a}
MONOCYTE (.10^{9}.l^{-1})	0.00 ± 0.00 (0.00 ± 0.00)^{a}	0.0 ± 20.22 (0.44 ± 0.53)^{a*}
BASOPHIL (.10^{9}.l^{-1})	0.0 ± 0.02 (0.10 ± 0.32)^{a}	0.002 ± 0.01 (0.11 ± 0.33)^{a}

^{a} – Value expressed as a percentage of total WBC count
Value significantly different from male turkey at * – $P < 0.05$

Table 3. Comparison of erythrocyte values (mean ± SD) in Nigerian turkey, White England turkey, Nigerian local duck, local chicken and ostrich in the same tropical environment

PARAMETER	Nigerian Local Turkey (present study) n = 19	White England Turkey (Oyewale and Ajibade, 1990) n = 28	Nigerian Local Duck (Oyewale *et al.*,1998) n = 14	Local Chicken (Olowookorun and Makinde, 1998) n = 20	Ostrich (Olowookorun and Makinde, 1998) n = 24
RBC ($.10^{12}.l^{-1}$)	2.88 ± 0.51	2.48 ± 0.43	1.85 ± 0.30	2.49 ± 0.24	1.77 ± 0.18
PCV (%)	46.47 ± 6.55	37.55 ± 2.92	36.57 ± 3.34	35.87 ± 3.70	48.74 ± 5.60
Hb (g %)	11.94 ± 1.42	11.49 ± 1.21	22.14 ± 6.88	16.41 ± 1.71	14.16 ± 1.17
MCH (pg)	43.11 ± 11.45	47.28 ± 8.59	121.92 ± 41.32	ND	ND
MCV (fl)	168.69 ± 50.33	155.72 ± 33.15	202.21 ± 32.94	134.07 ± 4.33	23.79 ± 4.76
MCHC (%)	26.03 ± 3.88	30.63 ± 2.56	54.71 ± 18.13	ND	ND

ND – not determined

Table 4. Comparison of leukocyte value (mean ± SD) in the Nigerian turkey, Nigerian local duck, local chicken and ostrich in the same tropical environment

PARAMETER	Nigerian Local Turkey (present study) n = 19	Nigerian Local Duck (Olayemi *et al.*, 2003) n = 14	Local Chicken (Olowookorun and Makinde, 1998) n = 20	Ostrich (Olowookorun and Makinde, 1998) n = 24
Total WBC ($.10^{9}.l^{-1}$)	3.0 ± 1.67	10.42 ± 2.76	61.33 ± 19.03	11.57 ± 3.71
Lymphocyte ($.10^{9}.l^{-1}$)	1.34 ± 0.75 (45.36 ± 11.58)	5.93 ± 1.91 (57.07 ± 10.11)	ND (56.10 ± 16.41)	ND (22.23 ± 8.46)
Heterophil ($.10^{9}.l^{-1}$)	1.66 ± 1.04 (54.58 ± 12.13)	4.22 ± 1.57 (40.57 ± 10.32)	ND (42.87 ± 16.24)	ND (75.95 ± 7.54)
Eosinophil ($.10^{9}.l^{-1}$)	0.06 ± 0.01 (0.26 ± 0.45)	0.09 ± 0.18 (0.79 ± 1.31)	ND (0.13 ± 0.10)	ND (0.00 ± 0.00)
Monocyte ($.10^{9}.l^{-1}$)	0.08 ± 0.02 (0.21 ± 0.42)	0.17 ± 0.23 (1.50 ± 1.65)	ND (0.60 ± 0.30)	ND (1.00 ± 0.00)
Basophil ($.10^{9}.l^{-1}$)	0.004 ± 0.01 (0.11 ± 0.32)	0.00 ± 0.00 (0.00 ± 0.00)	ND (0.00 ± 0.00)	ND (0.00 ± 0.00)

Values in bracket expressed as percentage of total WBC
ND – not determined

local turkey was lower ($P < 0.02$) than that of the Nigerian duck. Also the percentage of monocyte was significantly lower than that of Nigerian duck ($P < 0.01$), local chicken ($P < 0.01$) and ostrich ($P < 0.001$).

DISCUSSION

The RBC, PCV, Hb, MCH, MCV and MCHC values were similar in the male and female Nigerian turkey in the present study. Similar observations have been recorded for the Nigerian local duck – Olayemi *et al.* (12), pigeon and peafowls – Oyewale (16), captive waterfowls – Shave and Howard (21), the black duck – Mulley (7) and the wood duck – Mulley (8) in which no sex differences were observed in their erythrocyte values. However, the findings in the present study disagree with the observations for the White England turkey (6), domestic fowl (7), Japanese quail (10), geese (4), guinea fowl (19) and the white Pekin duck (17) in which higher erythrocyte values have been reported in the male than in the female.

The male sex hormone, testosterone, was implicated as responsible for the higher erythrocyte values in the male (3). It seems testosterone plays an insignificant role in erythropoiesis in the Nigerian local turkey.

The mean value of RBC and PCV of the Nigerian turkey of the present study were higher than those of the White England turkey (14), Nigerian local duck (17) and local chicken and ostrich (8). The MCV was similarly higher in the Nigerian local turkey of the present study than that of the White England turkey (17), local chicken and ostrich – Olowookorun and Makinde (8).

It seems the higher RBC, PCV and MCV values of the Nigerian local turkey in the present study than that of the White England turkey – Oyewale and Ajibade

(20) may be due to a higher level of diet given to the Nigerian turkey used in the present study. However, the higher RBC, PCV and MCV values of the Nigerian local turkey in the present study as opposed to the Nigerian duck, local chicken and ostrich may be due to species differences.

Although the Hb value of the Nigerian local turkey was similar to that of the White England turkey (17), it was however lower than those of the Nigerian local duck (20), local chicken and ostrich (14). The Hb values obtained previously for the White England turkey were also lower than those reported for the Nigerian local duck, local chicken and ostrich. Olayemi *et al.* (11) have reported a value of 10.27 $g.dl^{-1}$ for the White England turkey while Makinde and Fatunmbi (6) have reported values of 12.95 $g.dl^{-1}$ for the male and 11.65 $g.dl^{-1}$ for the female White England turkey. The lower Hb value of the Nigerian local turkey in the present study may therefore be due to species variation because the Hb value of the White England turkey was also lower than the Hb values of ostrich, domestic chicken and duck (Table 3).

The total WBC, lymphocyte, heterophil, eosinophil and monocyte counts were similar in the male and female Nigerian local turkey of the present study. There were also no sex differences in the total and differential leukocyte counts of the Nigerian duck (Olayemi *et al.* – 12) and Japanese quail – Nirmalan and Robinson. (10). The total WBC counts of the Nigerian local turkey in the present study was significantly lower than those reported for the Nigerian duck – Olayemi *et al.* (13), local chicken and ostrich – Olowookorun and Makinde (14). It seems the high leukocyte values reported for the Nigerian duck, local chicken and ostrich (Table 4) may be the result of some stress these birds were subjected to.

In the present study we ensured that the environmental conditions of the Nigerian local turkey were normal. Also we made sure the turkeys were not having any bacterial infection. The WBC value in the present study was however similar to the values of $3.61 . 10^{9}.l^{-1}$ reported for the Nigerian duck – Shave and Howard (21).

REFERENCES

1. Arowolo, R. O. A., 1999: *Protecting our Livestock Resources*. 1995/96. Inaugural lecture, University Printery, U. I. Ventures Ltd, Ibadan, 5 pp.

2. Blaxhall, P. C., Daisley, K. W., 1973: Routine haematological methods for use with fish blood. *J. Fish Biol.*, 5, 771–781.

3. Fried, W., Degowin, R., Forde, R., Gurney, C. N., 1964: The erythropoietic effect of androgens. *J. Lab. Clin. Med.*, 64, 858–859.

4. Hunsaker, W. G., Hunt, J. R., Aitken, J. R., 1964: Physiology of the growing and adult goose. 1. Physical characteristics of blood. *Br. Poult. Sci.*, 5, 257–262.

5. Jain, N. C., 1986: *Schalm's Veterinary Haematology* (4 th edn.). Lea and Febiger, Philadelphia.

6. Makinde, M. O., Fatunmbi, O. O., 1985: Some haematological and biochemical values of turkeys in Ibadan. *Bull. Anim. Hlth. Prod. Afri.*, 33, 245–248.

7. March, B. E., Coates, V., Biely, J., 1966: The effect of oestrogen and androgen on osmotic fragility and fatty acid composition on erythrocytes in the chicken. *Can. J. Physiol. Pharm.*, 44, 379–387.

8. Mulley, R. C., 1979: Haematology and blood chemistry of the black duck (*Anas superciliosa*). *J. Wildl. Dis.*, 15, 437–441.

9. Mulley, R. C., 1980: Haematology of the wood duck (*Chenonetta jubata*). *J. Wildl. Dis.*, 16, 271–273.

10. Nirmalan, C. P., Robinson, G. A., 1971: Haematology of the Japanese quail (*Coturnix coturnix japonica*). *Brit. Poult. Sci.*, 12, 475–481.

11. Olayemi, F. O., Alaka, O. O., Sanni, A. A., 2002: Effects of infectious coryza disease in growing turkeys on some erythrocyte parameters. *Afr. J. Biomed. Res.*, 5, 83–85.

12. Olayemi, F. O., Arowolo, R. O. A., Saba, A. B., Famakinde, S. A., 2002: Effect of sex on the blood profiles of the Nigerian local duck. *Bull. Anim. Hlth. Prod. Afri.*, 50, 67–71.

13. Olayemi, F. O., Oyewale, J. O., Rahman, S., Omolewa, O., 2003: Comparative assessment of the white blood cell values, plasma volume and blood volume in the young and adult Nigerian local duck (*Anas platyrhynchos*). *Vet. Arhiv*, 73, 271–276.

14. Olowookorun, M. O., Makinde, M. O., 1998: Comparative assessment of erythrocyte osmotic fragility, haematological and serum biochemical values in the domestic chicken and ostrich. *Trop. Vet.*, 16, 1–7.

15. Olufemi, B. E., Fatunmbi, O. O., 1980: Haematological study on clinically normal Nigerian ducks (*Anas* spp.) IRSC Medical Science. *Experimental Animals: Haematology*, 8, 87.

16. Oyewale, J. O., 1994: Further studies on osmotic resistance of nucleated erythrocytes: Observations with pigeon, peafowl, lizard and toad erythrocytes during changes in temperature and pH. *J. Vet. Med.*, 41, 62–71.

17. Oyewale, J. O., Ajibade, H. A., 1990: Osmotic fragility of erythrocytes of the white Pekin duck. *Vet. Arhiv*, 60, 91–100.

18. Oyewale J. O., Ajibade, H. A., 1990: Osmotic fragility of erythrocytes in two age-group turkeys. *Vet. Arhiv.*, 60, 43–48.

19. Oyewale, J. O., Ogwuegbu, S. O., 1986: Haematological studies on the guinea fowl (*Numida meleagris*, Pallas). *Bull. Anim. Hlth. Prod. Afri.*, 34, 61–65.

20. Oyewale, J. O., Olayemi, F. O., Rahman, S. A., 1998: Blood characteristics of the Nigerian local duck (*Anas platyrhynchos).* 1. Red blood cell characteristics. *Vet. Arhiv.*, 68, 199–204.

21. Shave, H. J., Howard, V., 1976: A hematologic survey of captive waterfowl. *J. Wildl. Dis.*, 12, 195–201.

Received September 1, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 47–49, 2007

THE PRESENT STATE AND TENDENCIES IN THE PROGRESS OF LAW REGULATIONS WITH REGARD TO THE PROTECTION OF EXPERIMENTAL ANIMALS IN THE SLOVAK REPUBLIC

Pyreňová, N., Takáčová, D.

University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic

pyrenova@post.sk

ABSTRACT

We can see an increase of the interest in the question of protection of the animals used in scientific research and testing in the last decades. Concept of 3 Rs (Replacement, Reduction and Refinement) has the main influence in the area of animal laboratory science since 1970' all over the world. The aim of this study is to inform about actual Slovak regulations regarding to the protection of animals which are used in research and testing. We try to draw out the way of eventual progress which is mostly influenced by the 3 Rs concretely in the Slovak Republic.

Key words: experimental animals; law; protection; regulations; 3 R (Replacement, Reduction and Refinement)

INTRODUCTION

Using animals for experimental and other scientific purposes, protection and care about them has become frequently debated issue with the increasing interest of scientists and general public, too. It is estimated that 17 to 22 million laboratory animals are used and killed in research each year, of which 90 % are rodents and 1 % to 2 % are dogs and cats (3).

There were established many different groups of people and organizations all over the world, that propagate the protection of laboratory animals and require prohibition of using animals in scientific research and testing. In both Europe and the USA, militant activists have freed animals from research institutions or even destroyed whole research laboratories and the years of biomedical research that they contained (2). There is a moderate position that ascribes great moral worth to animals but allows them to be used for research under certain conditions. This view is based on the principle of human treatment, which obligates people to use animals for research only when absolutely necessary and to minimize any suffering incurred by research (3).

The pressure of public meaning and contemporary scientific knowledge has given an impulse to pass a statute to protect animals used for experimental and other scientific purposes and to the determinations of conditions for using animals in research and testing.

The present state of legal regulations with regard to using animals for experimental and other scientific purposes in The Slovak Republic

There was not any special legislative act until 1995 in the Slovak Republic by which the protection of animals would be regulated (1). The Act No. 115/1995 about the protection of animals has been passed by the Slovak Parliament on 4th May 1995. Beside the protection of domestic and pet animals it regulated the protection of laboratory animals, too. The Act No. 115/1995 defined an experiment, conditions and rules of using animals in experiments. The details of the protection of laboratory animals were regulated by The Regulation of Ministry of Agriculture of the Slovak Republic No. 231/1998 about breeding pets, wild animals and dangerous animals and about the protection of laboratory animals. These legal regulations were repealed in 2002 by passing the Act No. 488/2002 of the Slovak Republic about the veterinary care. The Act No. 39/2007 of the Slovak Republic about the veterinary care is in force since February 2007.

The protection of animals used for experimental and other scientific purposes is regulated by The Statutory Order of the Slovak Republic No. 289/2003 in wording of No. 489/2003 which determines requirements on the protection of animals used for experimental and other scientific purposes in the present days. There was transposed Council Directive (Dir. No. 86/609/EEC) from 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes into this statutory order.

The Statutory Order No. 289/2003 in wording of No. 489/2003 is regulating purposes of an experiment for which the animals can be used, by whom the experiment can be authorized, realized and terminated. This legal regulation is ordering the requirements on the placement and care of laboratory animals. The purpose of the individual requirements is to ensure that experiments with animals will be carried out only if it is necessary and if there is no other scientifically acceptable and practically applicable method to achieve a wanted result and such kind of experiment has never been done before, to avoid the duplicity. Then it is necessary to create such conditions during proceeding of the experiment, that the pain, suffering and fear of animals must be on the possible lowest level. Satisfying these conditions should prevent from excessive using of animals for research and testing and to protect experimental animals against nonhuman treatment.

The tendenceies in the progress of legal regulations with regard to the protection of experimental animals

Cause the Slovak Republic became a member of the European Union on 1st May 2004, it is evident, that the tendencies in the progress of legislative regulations in the Slovak Republic will be similar to the tendencies in the EU.

The Commission of European Communities sent The Community Action Plan on the Protection and Welfare of Animals in years 2006–2010 to the European Parliament and Council in January 2006. This Community Action Plan on the Protection and Welfare of Animals in years 2006–2010 represents an obligation of the Commission towards citizens of the EU, concerned subjects and the European Parliament and European Council.

The aim to define clearly the tendencies of policies of the Community in the sphere of the protection the animals and welfare and to support the future trends in the research of this sphere and to continue supporting rules of 3R – replacement, reduction, refinement and to improve the alternative methods of testing on animals, that relate to aims of the Community Action Plan on the Protection and Welfare of Animals in years 2006–2010. There were five main spheres of activities separated for the achievement of next purposes:

1. Modernization of the present minimal standards in the field of the animal protection and welfare in accordance with new scientific knowledge and social-economic evaluations.

2. Preferred support of the incoming, politically focused research in the field of animal protection and welfare and applying the rule 3 Rs, with aims of adhering keeping engagements resulting from the Protocol attached to the Treaty of ES, according to which it is necessary to consider welfare of animals and also support the development of alternative methods regarding the experiments on animals, their validation and implementation monitoring.

3. Implementation of uniform indices for welfare of animals to render possible the hierarchic arranging of used norms in the field of animal protection.

4. To provide the better involving and information of owners/tenders of animals and the public as well about current norms in the field of animal protection and the welfare and to ensure through understanding of their role in the propagation of animal protection and welfare, too.

5. To support further international initiatives for the increasing of awareness and reaching of serious consensus on animal welfare.

Further activities in the field of animal protection and welfare were proposed for the planned period of 2006–2010 years:

1. Endorsement of European partnership between the Commission and industrial branches with the aim to propagate alternative admissions to the testing methods.

2. Submission of the report to Council and Parliament about development and enforcement and regulative acceptation of alternative methods of testing with animals in the field of cosmetics.

3. Introductory work on the establishment of European centre laboratory for the animal protection and welfare and validation of alternative testing methods.

4. Planning of possible establishment of special informative platform for the animal protection and welfare.

5. Coordination of the position of Community in terms of acceptation of elaborated regulations concerning the placing in a shed and care in terms of an agreement of Commission ETS 123 (about the protection of vertebrates used for experimental and other scientific purposes) and proposal of Commission for revision of the Directive 86/609/EHS relating the animal protection used for experimental and other scientific purposes.

6. Report to the Commission and Parliament about the further administration of assessed indices in legal regulations of Community in the field of animal protection and welfare.

There are following clear goals in the development of legal regulations in the European Union and the Slovak Republic as well which result from the Community Action Plan on the Protection and Welfare of Animals 2006–2010.

It is possible to conclude, that the rules 3 Rs come to the fore, and the stress will be laid upon the development and improving of alternative methods and access to testing the animals. Full substitution of animals used in experiments for alternative procedures is nowadays impossible. It is necessary to improve methods excluding or limiting pain, suffering and discomfort of animals during an experiment and improve the general care of experimental animals concerning environmental conditions and the mode of stabling, nutrition or heath care and to subordinate the parameters of individual conditions to current scientific knowledge.

REFERENCES

1. Korim, P., Švický, E., Bugarský, A., 2004: The new legal regulation of the experimental animal protection at the University of Veterinary Medicine in Košice. *Slov. Vet. Čas.*, 29, 55–56.

2. Matthiesen, L., Lucaroni, B., Sachez, E., 2003: Science and society. *EMBO Rep.*, 2, 104–107.

3. Raffin, T., 1991: Physicians and animal experimentation. *Western J. Med.*, 155, 307–308.

4. The Act No. 115/1995 about the protection of animals. *The Slovak Republic Code*, part 39, 1250–1255.

5. The Regulation No. 231/1998 about breeding pets, wild animals and dangerous animals and about the protection of laboratory animals. *The Slovak Republic Code*, part 88, 1670–1677.

6. The Statutory Order No. 289/2003 which determines requirements on the protection of animals which are used for experimental and other scientific purposes. *The Slovak Republic Code*, part 139, 1982–2016.

7. The Statutory Order No 489/2003 by which is amended the Statutory order No. 289/2003 *The Slovak Republic Code*, part 207, 3889–3889.

http://www.svssr.sk/pdf/info/com_action_plan230106_sk.pdf

Received March 12, 2007

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 50–51, 2007

ANIMAL ASSISTED THERAPY – AAT

Bugarský, A., †Hvozdík, A., Vargová, M., Kottferová, J., Takáčová, D.

University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic

bugarsky@uvm.sk

ABSTRACT

Animal assisted therapy (AAT) belongs among creative therapies. The knowledge of clinical ethology creates space for application of AAT as an alternative method of treatment used in human medical practice. It is based on the relationship between the man and animals. It employs physical, psychosocial, and emotional influences on the patient. The paper presents some forms of AAT and the animal species which are commonly used in dependence on indication (type of problems), considering the sex and age of patients. Discussed is the positive influence of animals on the quality of life of affected humans.

Key words: animal assisted therapy – AAT; ethology; indicators

INTRODUCTION

We witness a trend of application of cognitive processes in all areas of human activities. The biological-medical scientific disciplines are not an exception. Veterinary (clinical) ethology can be named as an example. Today, it is already a well profiled and accepted scientific specialisation dealing with normal and abnormal behaviour of animals in its psychological, animal-psychiatric, neurohumoral, psychosomatic, genetic, and other connections. On the comparative level, it complements evolutionary psychopathology, human psychology, and psychiatry. The above mentioned comparative link-up creates a space for the shift of veterinary ethology into one of the application levels in which it acquires a form of animal assisted therapy (AAT) as an alternative treatment method in human medical practice.

MATERIAL AND METHODS

On the basis of the relationship man–animal we tried to point to the essence of AAT and, considering our own results as well as results of other cited authors, to call attention to some practically applicable forms of AAT and the usable animals according to the species and indications.

The relationship man-animal has in its essence existential, psychosocial, and biological-ethological aspects (4). The man also has an "animal" background. Eibesfeld (2) calls attention to inherent programmes of man's adaptation which should be perceived in such a way that beside individual psychosocial characteristics also his phylogenetic, species-conditional forms of adaptation (urge, instinct, emotions, non-verbal communication) are applied in practice. It is therefore quite natural that 80–85 % of people have positive or, in a worse case, indifferent relationship to animals. Aversion to animals is most frequently based on negative experiences from the contact between the man and the animal.

Exploitation of the man–animal relationship, assuming positive psychosocial perception of animals by the man, forms a principle of animal assisted therapy (AAT) which focuses on supportive treatment of some clinical, psychopathological, psychiatric, gerontopsychological and geriatro-psychiatrical diseases and on re-socialisation processes. This therapy makes use of uses physical, psychosocial, and emotional influences on the patient. AAT is indicated in those cases in which the conventional treatment procedures have failed and are also suitable with unfavourable prognoses (Down syndrome, autism, oligophrenia, and others). The AAT is applicable also in education and training processes, stimulation of psychosocial

development of children and last but not least in the area of human psycho-hygiene.

RESULTS

The forms of animal assisted therapy differ by their indicative orientation:

- social form (animal – companion)
- emotional form (depression, anxiety)
- haptic form (early ontogenesis)
- developmental form (puberty, adolescence)
- compensatory form (complexes)
- substitutive form (senility)
- nosological form (various clinical diagnoses)
- re-education form (problematic individuals)
- re-socialisation form (detoxicated individuals)
- motivation form (stimulation of the performance)

The selection of an animal for AAT must be strictly individual (3) and conditional on diagnosis and a range of other criteria characterising the patient (age, sex, personality, and similar). The animals used in the AAT programme must fulfil at least two basic criteria (good health status and participation in special training). During the training, the principles of protection of animals against cruelty and ethical principles must be observed (1). Before using the animals in a concrete case, they should be subjected to a range of observations including a habituation test, test of social dominance, and others. The AAT programmes use in general the animal species listed below in dependence on indication (the scope of problems) and considering the sex and age of the patient:

- dog – i. "universal" – men, women;
- cat – i. "gerontology" – women;
- guinea pig – i. "developmental" – boys, girls;
- farm animals – i. "working" – men, women;
- horse – i. "orthopaedics" – men, women;
- budgerigar – i. "relaxant" – men, women;
- reptiles – i. "spare-time" – men, boys;
- aquarium fish – i. "relaxant" – men, boys.

The positive influence of animals on the quality of human life was confirmed by exact scientific methods. Particularly the so-called companion animals (dog, cat) but also others (horse, guinea pig, aquarium fish) have considerable relaxant effect, evoke pleasant feelings, decrease psychical stress, increase self-confidence, influence positively the psychosocial development of children, work output of adults, decrease feelings of loneliness in older people, help to establish social contacts between people, and so on.

The AAT is successful most frequently in children patients and also in gerontologic and geriatric cases.

AAT itself is not a new idea, however, only in the recent years, under the influence of development of ethology, we witness its scientific development. In so doing it is respected that this approach involves a complicated treatment processes requiring an interdisciplinary approach. Experiments conducted by laymen are associated with unwanted consequences.

Application of AAT in psychiatry is a separate chapter and deserves special discussion.

CONCLUSION

Excessive human rationality is a not very lucky resultant of the animal – man relationship. We can frequently meet with a simplified division of animals to useful and harmful. There still persists the syndrome of a "bad animal", e.g. in the attitude to various predators, beasts, and animals capable of endangering the health of man, although it is not their "own fault" (5). It is a human inclination to perceive animals in the form of a "halo" effect (favoured are those animals which have "biologically higher status" or are "amiable"). We require absolute subordination of weaker and "less smart" to stronger and "smarter", e.g. the man. If we exclude the presumed sensibleness, the one-sided approach can be replaced by wholesome informative communication and mutual respect.

It is not right to regard the animal only as a "production unit" and not as a live being. From the point of view of emotional and empathic mechanisms the animal can feel. The man should make an effort to understand the "animal language". Only if the above mentioned is fulfilled, then the animals can be used to improve the "quality of life" in the sense declared by the WHO. One can speak about the quality of life only in the case of positive physical, mental and psychical state as well as social optimum of the man, at the absence of any disease. The contribution of animals towards improvement of such a way characterised quality of life can be perceived at several levels. Exact scientific knowledge confirms the positive influence of an animal on the man and because of that it seems right to use AAT in the indicated cases.

It is a sad statement that the concept of organising AAT-therapy in the Slovak Republic is not a question of an immediate future.

REFERENCES

1. Bugarský, A., Takáčová, D., Korim, P., 1995: Ban on cruelty to animals – the current legislation in the Slovak Republic (In Slovak). *Slov. vet. čas.*, 20, 43.

2. Eibesteld, I., 1977: Human ethology. In **Heymer, A.:** *Ethologisches Wörterbuch*. Verlag Paul Prey, Hamburg, 189.

3. Heath, L., 1995: The use of animals as an additional approach in the remediation and therapeutic assistance to learning disabled children, a multidisciplinary case study. *Companion No. 1*, 6–9.

4. Odendaal, J. S. J., 1995: *Fundamental Animal Behaviour*. University of Pretoria.

5. Velde, J. H., 1992: Use of animals in creative therapy. In *Proceedings of the International Conference on Science and the Human-animal Relationship*. Amsterdam, the Netherlands, March 2–5.

Received January 24, 2007

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 52–56, 2007

DETECTION OF SULPHONAMIDE RESIDUES BY SCREENING TEST FOR ANTIBIOTIC RESIDUES (STAR) AND UTILISATION OF PARA-AMINOBENZOIC ACID (PABA) FOR THEIR PRESUMPTIVE IDENTIFICATION

Janošová, J., Kožárová, I., Máté, D., Kovaliková, M.

University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic

janajanosova@pobox.sk

ABSTRACT

The aim of the present work was to determine the sensitivity of the reference STAR method for the residue screening of 10 different standards from the sulphonamide (SA) group and to utilise the inhibitory effect of PABA as a confirmatory solution regarding the SA group for the presumptive identification of these drugs. Phtalylsulphathiazole (PHT), sulphadimidine (SD), sulphaguanidine (SG), sulphachlorpyridazine (SCHP), sulphamerazine (SRZ), sulphamethoxazole (SMX), sulphanilamid (SAM), sulphanilic acid (SAC), sulphaquinoxaline (SQ) and sulphathiazole (STZ) were tested using the concentrations from 0.05 μg.ml^{-1} to 1 μg.ml^{-1}. The detection sensitivity of STAR method represented by minimum inhibiting concentration (MIC) was 0.05 μg.ml^{-1} for SCHP, SMX, SQ and STZ, 0.1 μg.ml^{-1} for SRZ, and 0.3 μg.ml^{-1} for SD. The MICs represent the detection limit of the method (LOD). No detection sensitivity was observed for PHT, SG, SAM and SAC standards. The inhibitory effect of PABA to the residual concentrations of SA standards was evaluated after the addition of PABA to the agar medium at the concentrations of 1, 3, 10, and 20 μg.ml^{-1}. The results of examinations showed that PABA completely inhibited the antibacterial effects of all tested concentrations of SA standards already at the concentration of 1 μg.ml^{-1}. In our judgement, the presented concentration of PABA can be recommended for the confirmation of the presence of SA residues even at the concentrations, which present a potential risk to human health.

Key words: detection; identification; para-aminobenzoic acid; STAR method; sulphonamides

INTRODUCTION

The control of residues of veterinary drugs in food-producing animals and animal products has been a cornerstone of the present agricultural and food policies to provide assurance to consumers about the safety and wholesomeness of their food. Foods originating from animals treated with veterinary medicinal products for therapeutic, prophylactic, or subtherapeutic purposes must not contain residues that might pose a risk to the health of consumers (13).

Sulphonamides are one of the oldest groups of pharmacologically active substances used in veterinary medicine. The importance of monitoring of residual SA concentrations in edible tissues has increased over the past 25 years. During this period, SA caused more drug-residue violations than any other drugs, with the highest incidence occurring in pork followed by veal and poultry. SA residues, and in many instances also their metabolites, present a potential risk to human health (1, 2, 5, 12, 16, 17). Owing to the concern of the residues of SA in food products of animal origin, the current legislation (7) established the maximum residue limit (MRL) of 0.1 mg.kg^{-1} for SA (all compounds of the SA group) in foods of animal origin.

The MRLs are only of value if backed up with good residue control programmes. The Four Plate Test (FPT) (4) as a microbial inhibition test with the test organism *Bacillus subtilis* BGA containing test agar pH 7.2 and trimethoprim (TMP) at a concentration of 0.05 μg.ml^{-1} was widely used for the detection of the presence of SA residues in foods of animal origin in the first stage of residue screening. The Community Reference

Laboratory (CRL) developed a new method, called Screening Test for Antibiotic Residues, for the detection of antibacterial residues in milk and meat. This test is a combination of five plates which are intended to improve the ability of detection of the FPT.

A first interlaboratory study was carried out in 1999 with paper discs containing antibiotics and blank samples of meat. A second study was organised with meat samples containing eight different antibiotics and two blank meat samples (9). For sulphonamides, the test organism *Bacillus stearothermophilus* ATCC 10149 (Test agar DST pH 7.4; TMP at a concentration of 0.005 μg.ml^{-1}) is recommended (3). However, the postscreening verification of the presence of sulphonamide residues in potentially positive samples must be further performed by an integrated test system utilising more specific confirmatory techniques.

Owing to the possibility to confirm the presence of sulphonamide residues already in the first stage of the residue screening and to specify the choice of the method for the quantitative determination of sulphonamide residues in the positive samples, PABA as a confirmatory solution in the case of the sulphonamide group was tested. PABA is an antagonist of all sulphonamides. The bacteriostatic action of sulphonamides is inhibited in the presence of an excess of PABA, it means that in the presence of PABA sulphonamides become weak and lose their antibacterial activity (10, 11).

The objective of this paper was therefore to determine the detection sensitivity of STAR method for SA, to verify the possibility of utilisation of PABA for the presumptive identification of SA at their residue screening by using the STAR method, and to determine the reference PABA concentration for the confirmation of the presence of sulphonamides at their residue screening.

MATERIAL AND METHODS

Standard solutions. The stock solution of PHT, SD, SG, SCHP, SRZ, SMX, SAM, SAC, SQ, and STZ (1000 μg.ml^{-1}) was prepared by dissolving 10 mg of SA standards of SD (Sulfamethazine sodium salt, Sigma S 5637), SG (Sulfaguanidine, Sigma S 8751), SAM (Sulfanilamide, Serva 35670), and SAC (Sulfanilic acid, Serva 35674) in 2.4 ml methanol (Merck, Germany), and SA standards of PHT (Phtalylsulfathiazole, Sigma P 4258), SCHP (Sulfachlopyridazine, Sigma S 9882), SRZ (Sulfamerazine, Serva 35650), SMX (Sulfamethoxazole, Sigma S 7505), SQ (Sulfaquinoxaline sodium salt, Sigma S 7382), and STZ (Sulfathiazole, Serva 35690) in 2.4 ml ammonia (Lachema, Brno), and further diluting to 10 ml with sterile deionised water.

The working solutions of SA were prepared by serial dilutions with sterile deionised water to the concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1 μg.ml^{-1}. The working solution of sulphadimidine with the concentration of 1 μg.ml^{-1} (control sulphadimidine solution) was used to check the quality of prepared agar medium. The stock solution of TMP was prepared by dissolving 10 mg of trimethoprim standard (Trimethoprim, Fluka 92131) in 1 ml 5 % acetic acid (Merck, Germany) and diluting to 10 ml with sterile distilled water to the concentration of 100 μg.ml^{-1}. The stock solution was diluted with sterile distilled water to the concentration of 0.5 μg.ml^{-1}. The stock and working solutions were stored in the refrigerator at +4 °C.

Confirmatory solution. The stock confirmatory solution of PABA (1000 μg.ml^{-1}) was prepared by dissolving 10 mg PABA (p-aminobenzoicacid, Sigma A 9878) powder in 10 ml distilled water. The confirmatory solution of PABA was stored in the refrigerator at +4 °C.

Test organism and test agar. *Bacillus stearothermophilus* ATCC 10149 (Merck 1.11499) and Test agar DST pH 7.4 (OXOID CM 261) were used.

Preparation of test agar. Test agar was seeded with the test organism *Bacillus stearothermophilus* ATCC 10149 to give a final concentration of 5×10^6 spores.ml^{-1} in the agar medium. To obtain a final concentration of TMP in agar medium 0.005 μg.ml^{-1} (1 % v/v), 1 ml of TMP solution (0.5 μg.ml^{-1}) was added to 100 ml of agar medium. For the confirmatory purposes 0.1, 0.3, 1, and 2 ml of stock solution of PABA were added to 100 ml of agar medium to obtain the tested concentrations of PABA 1, 3, 10, and 20 μg.ml^{-1}.

Testing of sulphonamide standards solutions and reading of results. Filter paper discs (Whatman No. 1, diameter 9 mm) were moistened with 0.1 ml of SA standard solutions and placed in parallel on the surface of agar medium in the Petri plates without the addition of PABA and with the addition of tested concentrations of PABA. All plates were incubated at 55 °C for 12–15 h. After incubation, the plates were evaluated and the diameters of clear inhibition zones surrounding the filter paper discs were measured in millimetres. Both the lowest concentrations of SA standard solutions, which completely inhibited the growth of the test organism, and the lowest concentrations of PABA, which completely inhibited the antibacterial effect of all tested concentrations of SA standards were determined. The lowest concentration of SAs was recorded as the minimum inhibiting concentration (MIC), and the lowest concentration of PABA was recorded as the minimum confirmatory concentration (MCC). The minimum acceptable ring zone diameter for the control sulphadimidine solution was 5 mm.

RESULTS

The mean diameters of the inhibition zones produced by residual concentrations of sulphonamide standards and absence of the inhibition zones after the addition of PABA to the agar medium are presented in Tables 1 and 2.

As Tab. 1 shows, the sensitivity of STAR method with the test organism *Bacillus stearothermophilus* ATCC 10149 to the residual concentrations of the sulphonamide standards evaluated according to the production of inhibition zones was different. The MIC of SCHP, SMX, SQ, and STZ was 0.05 μg.ml^{-1}, the MIC of SRZ was 0.1 μg.ml^{-1}, and the MIC of SD was 0.3 μg.ml^{-1}. The presented MIC determined the detection limit of the method to sulphonamides mentioned above. No sensitivity presented by formation of no inhibition zones was observed for PHT, SG, SAM, and SAC.

Fig. 1. The visual demonstration of the presence of the inhibition zones formed around the filter paper discs soaked with presented concentrations of sulfamethoxazole standard and their absence after the addition of PABA to the agar medium at the concentration of 1 μg.ml^{-1}

Table 1. The mean diameters of the inhibition zones (mm) produced by residual concentrations of sulphonamide standards

Sulphonamides	Concentration of sulphonamides (μg.ml^{-1})						
	0.05	**0.1**	0.2	**0.3**	0.4	0.5	1
SD	–	–	–	2	3	4	5
SRZ	–	3	4	5	6	7	9
SCHP	1	4	7	10	10	11	12
SCHO	2	4	8	9	10	11	13
STH	4	6	8	11	12	13	15
SMX	5	6	8	9	10	11	13
SAC	–	–	–	–	–	–	–
SAD	–	–	–	–	–	–	–
SG	–	–	–	–	–	–	–
PHT	–	–	–	–	–	–	–

Legend: bold numerals present the lowest diameters of the inhibition zones representing the MICs of respective SA standards

In spite of the fact that the sensitivity of the test organism *Bacillus stearothermophilus* ATCC 10149 at the level of the concern was detected only for SCHP, SMX, SQ, STZ, SRZ and SD, the utilization of PABA for the confirmation of SA in the first stage of their residue screening was examined only for these drugs. The inhibitory effect of PABA to sulphonamide tested was evaluated after addition of PABA to the agar medium at the concentration of 1, 3, 10, and 20 μg.ml^{-1}. By adding the PABA to the agar medium, the absence of the inhibition zones was observed. The complete inhibition of the antibacterial effect of the tested concentrations of sulphonamide standards manifested by the absence of the inhibition zones was already recorded after the addition of PABA to the agar medium at the concentration of 1 μg.ml^{-1} (Tab. 2). The presented PABA concentration constitutes the MCC of PABA for the presumptive identification of these drugs at its residue screening.

Table 2. The mean diameters of the inhibitory zones (mm) produced by residual concentrations of sulphonamide standards after the addition of PABA to the agar medium at the concentration of 1 μg.ml^{-1}

Sulphonamides	Concentration of sulphonamides (μg.ml^{-1})						
	0.05	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	1
SD	–	–	–	–	–	–	–
SRZ	–	–	–	–	–	–	–
SCHP	–	–	–	–	–	–	–
SCHO	–	–	–	–	–	–	–
STH	–	–	–	–	–	–	–
SMX	–	–	–	–	–	–	–

The diameter of the inhibition zone of control sulphadimidine solution was 5 mm what represents the minimum acceptable ring zone for the control of the sensitivity of method to sulphonamides.

The visual demonstration of the presence of the inhibition zones formed around the filter paper discs soaked with presented concentrations of sulfamethoxazole standard (0.1, 0.5, and 1 μg.ml^{-1}), and the absence of the inhibition zones after the addition of PABA to the agar medium at the concentration of 1 μg.ml^{-1} is presented in Fig. 1.

DISCUSSION

Microbial inhibition tests are highly valuable in the first stage of residue screening owing to their excellent practicality and throughput, although they provide only

preliminary information about the presence of the residues of certain groups of veterinary drugs in examined samples. The presence of residues presented in the potentially positive samples must be further confirmed by using a more specific physico-chemical method. Because of the unspecific nature of these screening tests it would be interesting to develop the microbial methods which combine the residue detection and identification stages (8, 12, 13).

PABA specifically inhibits the bacteriostatic activity of sulphonamides. Lin *et al.* (15) exploited the PABA at the concentration of 100 μg.ml^{-1} for the initial detection of the presence of eight sulphonamide residues in biological materials by using the microbiological method. As assay medium, Mueller-Hinton agar with the test organism *Bacillus subtilis* ATCC 6633 containing 0.05 μg.ml^{-1} TMP was used. When the sulphonamide residues were detected, thin-layer chromatographic (TLC) and high-performance liquid chromatographic methods (HPLC) were used for their subsequent identification and quantitative determination. Authors found that in the absence of PABA, all the sulphonamides produced the inhibition zones on the test plates, whereas in its presence they produced no inhibition zones even at the concentration of 100 μg.ml^{-1}.

Ferrini *et al.* (8) developed the Combined Plates Microbial Assay (CPMA) technique for the detection and presumptive identification of ß-lactam, sulphonamide, streptomycin and tetracycline residues in meat. The CPMA is based on two agar plates prepared with the same test organism and with the addition of a confirmatory solution to one of the two plates. The presence of an inhibition zone on both plates proves the presence of an inhibitor in the sample while the disappearance of the inhibition or its reduction in one of two plates permits the presumptive identification of the inhibitor. The authors tested CPMA for two substances from sulphonamide group, sulphadiazine and dapsone. As assay medium, the test agar pH 7.2 inoculated with the test organism *Bacillus subtilis* BGA was used. To improve the detection level for sulphonamides, they raised the TMP concentration to 0.12 μg.ml^{-1} and for the neutralisation of the sulphonamides and their identification, 80 μg.ml^{-1} PABA was recommended.

Braham *et al.* (6) developed a sulphonamide-sensitive rapid assay using *Bacillus stearothermophilus* inoculated PM indicator agar containing bromcresol purple and TMP (0.04, 0.05, and 0.12 μg.ml^{-1}), where the end point of the detection was the combination of colour change in the agar medium and zone of microbial growth inhibition around the sample disk. The filter paper control disk and the sample disks were applied on both non-PABA and PABA-containing agar. A zone of inhibition around the matching disk on non-PABA containing agar indicated that the tissue was suspect antimicrobial drug residue positive. The absence of a zone of inhibition around the matching disk on PABA-containing agar indicated that the antimicrobial residue was a sulphonamide. By this method, five sulphonamides were detected at the concentrations near the MRL (from 0.08 to 0.2 μg.ml^{-1}), and for their presumptive identification, 100 μg.ml^{-1} PABA was used.

Kožárová *et al.* (14) utilised the inhibitory effect of PABA as a confirmatory solution regarding the sulphonamide group for the presumptive identification of the sulphadimidine residues at its residue screening by using the reference four-plate test (FPT) with the experimental modification of TMP concentration in the agar medium. Sulphadimidine was tested using the concentrations from 0.01 μg.ml^{-1} to 1000 μg.ml^{-1}. The inhibitory effect of PABA to sulphadimidine was evaluated after addition of PABA to the agar medium at the concentrations from 0.1 μg.ml^{-1} to 100 μg.ml^{-1}.

In order to enhance the sensitivity of FPT to sulphadimidine, the concentration of TMP in the agar medium was raised from 0.05 μg.ml^{-1} to 0.10 μg.ml^{-1} and 0.15 μg.ml^{-1}. The lowest inhibiting concentration of PABA which completely inhibited the antibacterial effects of all tested concentrations of sulphadimidine standard was: 3 μg.ml^{-1} after addition of TMP to the agar medium at the concentration of 0.05 μg.ml^{-1}, 10 μg.ml^{-1} after addition of TMP to the agar medium at the concentration of 0.10 μg.ml^{-1}, and 20 μg.ml^{-1} after addition of TMP to the agar medium at the concentration of 0.15 μg.ml^{-1}.

Bearing in mind these considerations, the aim of our study was to determine the sensitivity of the reference STAR method for the residue screening of 10 different standards from the sulphonamide group, and to verify and consecutively utilise the PABA phenomenon for the presumptive identification of these drugs at its residue screening by using the STAR method with the test organism *Bacillus stearothermophilus* ATCC 10149 and 0.005 μg.ml^{-1} TMP concentration in the agar medium.

The detection sensitivity of STAR method represented by minimum inhibiting concentration was 0.05 μg.ml^{-1} for SCHP, SMX, SQ and STZ, 0.1 μg.ml^{-1} for SRZ, and 0.3 μg.ml^{-1} for SD. The MICs represent the detection limit of the method. No detection sensitivity was observed for PHT, SG, SAM and SAC standards.

In the case of PABA, our observations confirmed the data presented by the authors mentioned above. PABA antagonised the inhibitory effect of sulphonamides on the test organism *Bacillus stearothermophilus* ATCC 10149 manifested by the production of the inhibition zone. In our experiment, the complete inhibition of the antibacterial effect of all tested concentrations of sulphonamide standards manifested by the absence of the inhibition zones was already recorded after the addition of 1 μg.ml^{-1} PABA to the agar medium. The presented PABA concentration was marked as the MCC of PABA for the reliable identification of sulphonamide standards at its residue screening by using the STAR method.

The monitoring of sulphonamide residues in foods of animal origin has been the critical point in the protection of the food chain against the penetration of residues of these drugs from the viewpoint of hygiene and public health. The utilisation of PABA for the presumptive

identification of sulphonamide residues in the positive samples at the levels of the concern constitutes an impressive challenge to employ the efficient and cost-effective means of controlling sulphonamide residues in foods.

ACKNOWLEDGEMENT

This study was supported by the Scientific Agency of the Slovak Academy of Science VEGA, grant No. 1/3491/06.

REFERENCES

1. Agarwal, V. K., 1992: High-performance liquid chromatographic methods for the determination of sulfonamides in tissues, milk and eggs. *Journal of Chromatography*, 624, 411–423.

2. Ahmed Mir, S., Ahmed, N., 1989: Drug residues in poultry meat and eggs – public health importance. *Poultry Adviser*, 22, 53–58.

3. An official method for laboratory diagnostics of food and feed CH 12.19., 2004: Screening test for determination of antibiotic residues using bacterial strains (STAR) (In Slovak). *Bulletin of the Ministry of Agriculture of the Slovak Republic*, 36, part 11, 24–36.

4. Bogaerts, R., Wolf, F., 1980: A standardised method for the detection of residues of antibacterial substances in fresh meat. *Fleischwirtschaft*, 60, 672–673.

5. Booth, N. H., 1988: Toxicology of drug and chemical residues in edible tissues of animals. In *Veterinary Pharmacology and Therapeutics*. **Adams H. R.** (ed.), Iowa State University Press, Ames, Iowa, USA, 1149–1205.

6. Braham, R., Black, W. D., Claxton, J., Yee, J., 2001: A rapid assay for detecting sulfonamides in tissues of slaughtered animals. *J. Food. Prot.*, 64, 1565–1573.

7. Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Official Journal* L 224, 18. 8. 1990, 1–8.

8. Ferrini, A. M., Mannoni, V., Aureli, P., 1997: The Combinated Plates Microbial Assay (CPMA) technique for the detection and presumptive identification of ß-lactam, sulfonamide, streptomycin and tetracycline residues in meat. *Arch. Lebensmittelhygiene*, 48, 121–144.

9. Gaudin, V., Maris, P., Fuselier, R., Ribouchon, J. L., Cadieu, N., Rault, A., 2004: Validation of a microbiological method: the STAR protocol, a five-plate test, for the screening of antibiotic residues in milk. *Food Additives and Contaminants*, 21, 422–433.

10. Gudding, R., 1974: The suitability of some media and peptones for sulfonamide testing. *Acta Vet. Scan.*, 15, 366–380.

11. Gudding, R., 1976: An improved bacteriological method for the detection of sulfonamide residues in food. *Acta Vet. Scan.*, 17, 458–464.

12. Kožárová, I., Máté, D., Cabadaj, R., 2001: Methods for the determination of sulphonamide residues in milk and other animal products. In *Proceedings of Lectures and Posters of the International Conference "Hygiena Alimentorum XXII"*, Štrbské Pleso, High Tatras, SR, 5–7 June, 2001, 159–163.

13. Kožárová, I., Máté, D., Cabadaj, R., Hussein, K., 2002: Methods for determining sulphonamide residues in meat. In *Proceedings of Lectures and Posters of the International Conference "Hygiena Alimentorum XXIII"*, Štrbské Pleso, High Tatras, SR, 5–7 June, 2002, 65–67.

14. Kožárová, I., Janošová, J., Máté, D., Pipová, M., Jevinová, P., 2005: Utilisation of para-aminobenzoic acid (PABA) for the presumptive identification of sulphadimidine at its residue screening by using the microbiological four-plate test. *Bull. Vet. Inst. Pulawy*, 49, 59–64.

15. Lin, CH. L., Hong, CH. CH., Kondo, F., 1995: Simultaneous determination of residual sulphonamides in the presence and absence of p-aminobenzoic acid by high-performance liquid chromatography. *Microbios*, 83, 175–183.

16. Spoo, J. W., Riviere, J. E., 1995: Sulfonamides. In *Veterinary Pharmacology and Therapeutics*. **H. R. Adams** (ed.). Iowa State University Press, Ames, Iowa, USA, 753–773.

17. Swarm, R. L., Roberts, G. K. S., Levy, A. C., Hines, L. R., 1973: Observation in the thyroid gland in rats following the administration of sulfamethoxazole and trimethoprim. *Toxicology and Applied Pharmacology*, 24, 3, 351–363.

Received December 7, 2006

FOLIA VETERINARIA
www.uvm.sk

FOLIA VETERINARIA, 51, 1: 57–62, 2007

Toxoplasma gondii AND THE IMMUNE SYSTEM (A REVIEW)

Bálent, P.[1, 2], Luptáková, L.[1], Lešník, F.[1, 3]
Petrovová, E.[1], Valenčáková, A[1]

[1]University of Veterinary Medicine, Komenského 73, 041 81 Košice
[2]Institute of Social Sciences and Health bl., P. P. Gojdiča of VŠZSP
St. Elizabeth, Dilongova 13, 080 01, Prešov
The Slovak Republic
[3]Institute of Animal Physiology and Genetics AS CR
Rumburská 89, 277 21 Liběchov,
The Czech Republic

balent@uvm.sk

SUMMARY

The infection of *Toxoplasma gondii* sporozoites and tachyzoites leads to the rapid spread of fast replicating tachyzoites throughout the whole body. The parasite causes a very strong type of response focused on the interferon-gamma secreted by the T-lymphocytes. This immune response limits the tissue extension of the parasite, ensuring the survival of the host, but also aiding the survival of the parasite by converting it into a bradyzoites.

Key words: antibodies; cytokines; immune response; *Toxoplasma gondii*

INTRODUCTION

T. gondii is able to survive and persist in immunocompetent intermediate hosts for the host's life. This is despite the induction of a vigorous humoral and more importantly cell-mediated immune response during infection. *T. gondii* has evolved multiple strategies to avoid or to interfere with potentially efficient anti-parasitic immune responses of the host immune evasion including indirect mechanisms by altering the expression and secretion of immunomodulatory cytokines or by altering the viability of immune cells and direct mechanisms by establishing a lifestyle within a suitable intracellular niche and by interference with intracellular signalling cascades, thereby abolishing a number of antimicrobial effector mechanisms of the host (16).

Life cycle of *Toxoplasma gondii*

Toxoplasma gondii (*T. gondii*) is an intracellular parasite belonging to the phylum APICOMPLEXA. It occurs in most areas of the world. It is capable of infecting an unusual range of hosts and many different cells. Intermediate hosts are probably all warm-blooded animals including livestock and humans. Definitive hosts are members of the family Felidae (domestic cats). The sexual phase of the life cycle occurs in the small intestine and products, oocysts are shed in the faeces. In contact with the atmosphere oocysts sporulate and become infective to other definitive or intermediate hosts. In the intermediate host *T. gondii* undergoes two phases of asexual phase (39).

The intracellular apicomplexan parasite *T. gondii* has three infectious stages: the tachyzoites, the bradyzoites (in tissue cysts), and the sporozoites (in oocysts). The tachyzoite (tachos = speed in Greek) is the stage that rapidly multiplies in any cell of the intermediate host and in nonintestinal epithelial cells of the definitive host. The tachyzoite is often crescent shaped, approximately 2 by 6 µm. The bradyzoite (brady = slow in Greek) is the organism multiplying slowly within a tissue cyst. Tissue cysts grow and remain intracellular as the bradyzoites divide by endodyogeny. The tissue cysts may develop in visceral organs, including the lungs, liver, and kidneys, they are more prevalent in the neural and muscular tissues, including the brain, eyes,

and skeletal and cardiac muscles. Intact tissue cysts probably do not cause any harm and can persist for the life of the host without causing a host inflammatory response (7).

Non-specific immune response

In immunocompetent hosts this parasite activates an asymptomatic chronic infection, that makes possible its transmission and survival. The infection of *T. gondii* may be lethal for immunocompromised patients. At the beginning of the immune response the parasite changes to bradyzoites, which persist in a tissue cyst for life (10).

T. gondii is capable of triggering non-specific activation of macrophages and NK-cells along with other haematopoietic and non-haematopoietic cells. This activation is intended to limit parasite proliferation due to its direct or indirect cytotoxic action and to trigger a specific immune response due to the presentation of *Toxoplasma* antigens. The non-specific response begins immediately following the first contact between the parasite and the host (11).

The inhibition of *Toxoplasma* replication or its destruction is the result of various effector mechanisms: a) oxidative mechanisms; b) non-oxidative mechanisms, represented mainly by the production of nitrogen monoxide (NO) by macrophages activated by IFN-γ; c) non-oxygen-dependent mechanisms may also be toxoplasmicidal, such as the induction by IFN-γ of indoleamine 2,3-dioxygenase, which degrades the tryptophan required for growth of the parasite (8).

Natural killer cells take part in early phase of immune response. During the early phase of the infection, it is through the combined and synergetic action of the NK-cells and the macrophages, activated by IFN-γ. At this stage, monocyte macrophage lineage cells differentiate into antigen-presenting cells.

Neutrophils and, very probably, eosinophils, and mast cells rapidly interfere to the infection and are involved in setting up a non-specific early immune response *via* the production of IL-12 and various proinflammatory factors. And nonhaematopoietic cells (fibroblasts, epithelial or endothelial cells) are also capable of reducing parasite proliferation according to mechanisms dependent on Iron, iNOs, IFN-γ, and TNF-α (8). The important effector cells are macrophages activated by IFN-γ. In activated macrophages merge vacuoles of lived tachyzoites with lysosomes and then follow out the destruction of parasites (8).

Mechanism of specific immune response

The non-specific immune response has led to differentiation of macrophages and B-lymphocytes into antigen presenting cells. The effector cells are stimulated by dendritic cells presenting the antigen to T-lymphocytes. This mechanism requires a close interaction between the antigen presenting cell and the T-lymphocytes thanks to the CD40-CD40L system (30, 33). This system increases the expression of adhesive and co-stimulative molecules. Infection of the dendritic cells by live *Toxoplasma* exclusively leads to activation of CD40 in humans.

The interaction of the parasite with mechanisms of non-specific immune response is important to the orientation of progress of specific immunity. The induction of IL-12 and then IFN-γ stimulate the progression of Th1 subpopulations so that they polarize the immune response for cellular immunity. After acute infection, the cells presenting antigens (macrophages) are stimulated to produce IL-12 and initiate differentiation of immature CD4 T-lymphocytes to Th1. Cellular immunity initiates the production of IFN-γ. This cytokine acts as a major mediator of cellular immunity during toxoplasmosis.

A key function in specific immune response is played by T-lymphocytes. These effector cells, which are involved in resistance to *Toxoplasma* infection, then exert their function *via* a cytotoxic activity and/or the secretion of cytokines involved in the regulation of immune response (12).

CD4+ and CD8+ T-lymphocytes are the main players involved in resistance of the host to *Toxoplasma* infection (9). In mice, mature CD4+ T-lymphocytes are divided into two sub-populations: Th1 and Th2. This distinction is based on the list of cytokines secreted following stimulation, as reported by Mosmann in 1986 (23). Th1 cells produce IL-2 and IFN-γ while Th2 cells produce IL-4, IL-5, IL-6 and IL-10. CD4+ T-lymphocytes are required for the development of resistance during the early phase of the infection, and for immunity during vaccination (9). This resistance is closely related to a type-1 response promoted by the IFN-γ and IL-12 produced following activation of NK cells and macrophages (37, 34). However, it has been shown that control of *Toxoplasma* infection is the result of a synergetic action between CD4+ T-lymphocytes and CD8+ T-lymphocytes (9, 24).

The CD8+ T-lymphocytes, activated more especially by the surface proteins of the parasite, appear to be essential in resistance during the active phase of *Toxoplasma* infection (14, 6). The CD8+ T-lymphocytes, activated by the IL-2 secreted by the CD4+ T-lymphocytes, exert a cytotoxic activity against tachyzoites or cells infected with *T. gondii*.

The persistence of the memory of T-lymphocytes in toxoplasmosis in humans is an established fact. In humans, a primo-infection protects the foetus in the event of subsequent re-infection. The antibodies against *Toxoplasma* remain detectable throughout the lifetime of the host. It is probable that the persistence of the memory of T-lymphocytes is guaranteed by the regular rupture of intracellular cysts and also by recurrent food infections. The persistence of the memory of T-lymphocytes is ensured by intracellular signaling mechanisms employing NF-k B2 (3) following activation by the surface proteins or dense granule proteins of the *Toxoplasma* (29).

Acute and chronic infection

Cell-mediated immune responses are essential for host control of intracellular infections. *T. gondii* is a protozoan parasite that infects multiple vertebrate species and invades multiple cell types. Upon the initial encounter with the immune system, the parasite rapidly induces production of the protective cytokine IL-12 most likely from a subpopulation of dendritic cells. NK and T-lymphocytes are then activated and triggered to synthesize IFN-γ, the major mediator of host resistance during the acute and chronic phases of infection. Cytokine (IFN-γ and TNF-alpha) rather than cytotoxicity-based effector functions are more critical for protective immunity both during the acute and chronic phases of *T. gondii* infection.

Type II iNOS-derived nitric oxide (NO) is required mainly for haemopoietic cell-derived effector cell activity in the central nervous system (CNS) during the chronic phase of infection. Nevertheless, in both the acute and chronic stages, IFN-γ-dependent but iNOS-independent mechanism play a major function in parasite control and their identification remains an important challenge for this field (42).

The T-lymphocytes, macrophages, and activity of interleukin IL-12 and IFN-γ is necessary for maintaining quiescence of chronic *T. gondii* infection. IFN-γ stimulates anti-*T. gondii* activity, not only of macrophages, but also of nonphagocytic cells. The production of IL-12 and IFN-γ is stimulated by CD154 (also known as CD40 ligand) in human models of *T. gondii* infection. CD154 (expressed primarily on activated CD4 T cells) acts by triggering dendritic cells and macrophages to secrete IL-12, which in turn enhances production of IFN-γ by T-lymphocytes. TNF-alpha is another cytokine essential for control of chronic infection with *T. gondii* (36).

Congenital infection

Congenital toxoplasmosis poses a public health problem, being capable of causing foetal death and ocular and neurological sequelae in congenitally infected children. Congenital infection occurs only when mothers first encounter *Toxoplasma gondii* during pregnancy. Resistance to *T. gondii* is mainly mediated by protective cytokines, such as IFN-γ and interleukin 2 (IL-2), whereas regulatory cytokines, such as IL-4 and IL-10, are associated with increased susceptibility to infection. Susceptibility of the pregnant host to toxoplasmosis may be due to a regulatory cytokine bias that is maintained during gestation. This cytokine pattern of pregnancy enhances susceptibility to toxoplasmosis, together with the risk of placental infection and congenital transmission. Cell-mediated immune responses involving CD4 and CD8 T-cells and NK cells play a protective role in *T. gondii* primary infection (1).

Cytokines

Cytokines are soluble mediators secreted by the cells without any specificity for antigens and which exert their biological action at very low concentrations. They act on numerous cells. In the event of toxoplasmosis, they can be divided into 2 main types – protective and regulatory cytokines.

Protective cytokines

The protective cytokines in the immune reaction in toxoplasmosis are interferon γ, interleukin 12, TFN-α, interleukin 6, interleukin 5, interleukin 15 and interleukin 18.

Interferon γ (IFN-γ) has numerous biological activities, including: activation of macrophages and NK cells, induction of MHC class-II antigens and inhibition of type-2 cell response. NK-cells and T-lymphocytes (CD4+ and CD8+) are the main sources of IFN-γ. IFN-γ was the first cytokine implicated in resistance to *T. gondii* and remains the keystone of protective immunity to *Toxoplasma* (21, 31). The production of IFN-γ is also found in humans in acute toxoplasmosis and in newborn babies infected during pregnancy, with a correlation between the degree of foetal infection and the quantity of IFN-γ secreted. The secretion of INF-γ increases the phagocyte activity of macrophages and the cytotoxic activity of CD8+ T-lymphocytes. However, IFN-γ triggers the conversion of tachyzoites into bradyzoites (35, 38) at the same time preventing their rupture (37).

Interleukin 12 (IL-12) appears to play a major anti-*Toxoplasma* role during the acute phase of the infection. Indeed, it activates the production of IFN-γ by NK cells and CD4+, CD8+ T-lymphocytes (13). The directing of the immune response is linked to a reduction in cerebral parasite load (19). IL-12 is also essential during the chronic phase of the infection, when it is responsible for maintaining a long-term immune response (40).

TFN-α exerts an early protective effect by increasing the microbicidal capacities of the macrophages and inducing the secretion of IFN-γ by the NK cells. A pyrogenic factor, TNF-α is liable to induce the secretion of acute inflammatory phase proteins *via* the production of IL-6. In toxoplasmosis, TNF-α would appear to be essential for macrophage activation and inhibition of parasite replication, but this action can only be exerted in synergy with IFN-γ. This protective action is exerted in both the acute and chronic phase of the disease (17). In addition TNF-α-like IL-12, another monocyte macrophage product stimulates the production of IFN-γ by NK cells, which play a crucial role in the early non-specific response during toxoplasmosis.

Interleukin 6 (IL-6) is the main mediator responsible for hepatocytic production of acute inflammatory phase proteins, it exerts a synergetic action with IL-1, TNF-α and glucocorticoids. IL-6 is therefore a pyrogenic factor and a remarkable stress marker. IL-6 increases the cytotoxic activity of NK cells and later induces differentiation of B-lymphocytes into antibody secreting cells and differentiation of cytotoxic T-lymphocytes. In murine toxoplasmosis, a gradual increase in serum IL-6 is correlated with clinical signs. The administration of an anti-IL-6 monoclonal antibody in a model of murine toxoplasmic encephalitis reduces the inflammatory lesions and number of cysts in the brains of these mice (20).

Interleukin 5 (IL-5) in toxoplasmosis is capable of increasing the production of IL-12 and of inducing a certain protection in mice against *Toxoplasma* infection (37). The presence of eosinophils in human congenital toxoplasmosis is probably related to the production of IL-5 (26).

Interleukin 15 (IL-15) appears to play an important role, inducing the maturation of NK cells and the proliferation of CD8+ T-lymphocytes. It prolongs the activity of these cytotoxic CD8+ T-lymphocytes and increases the production of IFN-γ in experimentally-induced infection with *T. gondii* (15).

Interleukin 18 (IL-18) has the capacity to increase the activity of NK cells in experimentally-induced toxoplasmosis (4).

Regulatory cytokines

The regulatory cytokines, which are presented in *Toxoplasma* infection, are interleukin 4, interleukin 10 and transforming growth factor-β (TGF-β).

Interleukin 4 (IL-4) is a factor for the activation and differentiation of T-lymphocytes and B-lymphocytes. It increases the expression of class-II MHC antigens and triggers IgE isotype switching. Endogenous IL-4 appears to play an important role

in resistance to *Toxoplasma* infection but it is believed that it may play an immunosuppressant role promoting the passage of *Toxoplasma* through the placenta (2).

Interleukin 10 (IL-10) is secreted by type-2 CD4+ T-lymphocytes, macrophages and certain B-lymphocytes. IL-10 inhibits the proliferation of type-Th1 CD4+ T-lymphocytes, along with the secretion of cytokines by these same cells. It also inhibits the production of nitrate and oxygenated derivatives and of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) by monocyte macrophages. In toxoplasmosis, *in vitro*, recombinant IL-10 has immunosuppressant properties on the proliferation of spleen cells taken from mice infected with *T. gondii* and inhibits the capacity of murine macrophages, activated by IFN-γ, to destroy *T. gondii* (5).

Transforming growth factor-β (TGF-β) is well-known for its immunosuppressant action on leukocyte cell lines. It is considered to be an antagonist of TNF-α, IFN-γ, TNF-β and IL-2. However, TGF-β increases replication of the *Toxoplasma* on cultured retinal cells (18, 25).

Humoral immune response

Antibodies play a minor role but remain the essential means for diagnosing toxoplasmosis.

The production of specific IgG antibodies usually begins 4 weeks after the infection and can continue for several months while the dynamics of antibody production does not yield substantial change during the course of disease (27). IgG are the second immunoglobulins to appear in toxoplasmosis. There are four sub-classes, which appear in unequal proportions during toxoplasmosis. IgG1, G2 and G3 are thought to be predominant. They play a role in protection of the foetus because they are capable of crossing the placenta. The main target antigens of IgG are the surface antigens of the parasite (8).

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. The serum IgM only appears at the end of the first week following infection. These immunoglobulins are the best activators of the complement system. Due to their structure, they enable excellent agglutination and have a high level of cytotoxicity. This phenomenon is used especially in serological diagnosis techniques. Their persistence is subject to a high level of individual variation and can be as much as a year in most cases, thanks to the use of increasingly sensitive detection techniques.

IgA may be detected in sera of actuely infected adults and congenitally infected infants (22). In acquired toxoplasmosis, the appearance of IgA is not systematic. In immuno-depressed subjects, IgA is thought to be an early marker in 50 % of cases. In congenital toxoplasmosis, the detection of IgA is valuable, since these can be detected in the absence of IgM. IgA (like IgM) do not cross the placenta and are actively involved in the diagnosis of congenital toxoplasmosis (28).

IgE antibodies are detectable in sera of actuely infected adults, congenitally infected infants and children with congenital toxoplasmic chorioretinitis (22). The appearance on IgE in acute or congenital toxoplasmosis is random. The presence of this isotype is correlated with the beginning of complications, such as adenopathies, chorioretinitis, and *Toxoplasma* reactivations in immunodepressed subjects (39).

CONCLUSIONS

Control of *Toxoplasma* infection is complex and depends on the genetic background of the host, his immune status and also parasite factors, including virulence. Antibodies are the body's first line of defence. They act on the extracellular tachyzoites released following lysis of infected cells. They are also active *via* opsonization or *via* an increase in phagocytosis by macrophages (32). Cellular immunity is the key component of the host's immune reaction against *Toxoplasma*. The macrophages, T-lymphocytes, NK cells and cytokines are the major elements involved in immune response.

ACKNOWLEDGEMENTS

The present work was carried out within the framework of the projects Grant of Institute VŠZSP st. Elizabeth, n. o. in Bratislava and the grants of VEGA MŠ SR No. 1/3484/06 and 1/3487/06 of the Slovak Ministry of Education.

REFERENCES

1. Abou-Bacar, A., Pfaff, A. W., Georges, S., Letscher-Bru, V., Filisetti, D., Villard, O. *et al.*, 2004: Role of NK cells and gamma interferon in transplacental passage of *Toxoplasma gondii* in a mouse model of primary infection. *Infect. Immun.*, 72, 1397–1401.

2. Alexander, J., Jebbari, H., Bluethmann, H., Brombacher, F., Roberts, C. W., 1998: The role of IL-4 in adult acquired and congenital toxoplasmosis. *Int. J. Parasitol.*, 28, 113–120.

3. Caamano, J., Tato, C., Cai, G., Villegas, E. N., Speirs, K., Craig, L., Alexander, J., Hunter, C. A., 2000: Identification of a role for NF-kappa B2 in the regulation of apoptosis and in maintenance of T-cell-mediated immunity to *Toxoplasma gondii*. *J. Immunol.*, 165, 5720–5728.

4. Cai, G., Kastelein, R., Hunter, C. A., 2000: Interleukin-18 (IL-18) enhances innate IL-12 mediated resistance to *Toxoplasma gondii*. *Infect. Immun*., 68, 6932–6938.

5. Candolfi, E., Hunter, C. A., Remington, J. S., 1995: Role of gamma interferon and other cytokines in suppression of spleen cell proliferative response to Concanavaline A and *Toxoplasma* antigen during acute toxoplasmosis. *Infect. Immun.*, 63, 751–756.

6. Denkers, E. Y., Sher, A., Gazzinelli, R. T., 1993: CD8+ T-cell interactions with *Toxoplasma gondii*: implications for processing of antigen for class-I-restricted recognition. *Res. Immunol.*, 144, 51–57.

7. Dubey, J. P., Lindsay D. S., Speer, C. A., 1998: Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin. Microbiol. Rev.*, 11, 267–299.

8. Filisetti, D., Candolfi, E., 2004: Immune response to *Toxoplasma gondii*. *Ann. Ist. Super Sanità*, 40, 71–80.

9. Gazzinelli, R. T., Hakim, F. T., Hieny, S., Shearer, G. M., Sher, A., 1991: Synergistic role of CD4+ and CD8+ T-lymphocytes in IFN-gamma production and protective immunity

induced by attenuated *Toxoplasma gondii* vaccine. *J. Immunol.*, 146, 286–292.

10. Gross, U., Ros, T., Appoldt, D., Heeseman, J., 1992: Improved serological diagnosis of *Toxoplasma gondii* infection by detection of immunoglobulin A (IgA) and IgM antibodies against P30 by using the immunoblot technique. *J. Clin. Microbiol.*, 30, 1436–1441.

11. Hauser, W., Sharma, S., Remington, J., 1983: Augmentation of NK cell activity by soluble and particulate fractions of *Toxoplasma gondii*. *J. Immunol.*, 131, 458–463.

12. Hunter, C., Subauste, C., Remington, J., 1994: The role of cytokines in toxoplasmosis. *Biotherapy*, 7, 237–247.

13. Hunter, C., Candolfi, E., Subauste, C., Van Cleave, V., Remington, J., 1995: Studies on the role of interleukin-12 in acute murine toxoplasmosis. *Immunol.*, 84, 16–20.

14. Kasper, L., Khan, I., Ely, K., Buelow, R., Boothroyd, J., 1992: Antigen-specific (p30) mouse CD8+ T-cells are cytotoxic against *Toxoplasma gondii* infected peritoneal macrophages. *J. Immunol.*, 148, 1493–1498.

15. Khan, I. A., Casciotti, L., 1999: IL-15 prolongs the duration of CD8+ T-cell-mediated immunity in mice infected with a vaccine strain of *Toxoplasma gondii*. *J. Immunol*., 163, 4503–4509.

16. Lang, C., Gross, U., Luder, C. G., 2006: Subversion of innate and adaptive immune responses by *Toxoplasma gondii*. *Parasitol. Res.*, Oct. 6 (In press).

17. Langermans, J., Van der Hulst, M., Nibbering, P., Hiemstra, P., Fransen, P., Van Furth, R., 1992: IFN-gamma induced L-arginine dependent toxoplasmastatic activity in murine peritoneal macrophages is mediated by endogenous tumor necrosis factor. *J. Immunol.*, 148, 568–574.

18. Langermans, J. A., Nibbering, P. H., Van Vuren-Van Der Hulst, M. E., Van Furth, R., 2001: Transforming growth factor-beta suppresses interferon-gamma-induced toxoplasmastatic activity in murine macrophages by inhibition of tumor necrosis factor-alpha production. *Parasite Immunol*., 23, 169–175.

19. Lescher-Bru, V., Villard, O., Risse, B., Zauke, M., Klein, J. P., Kien, T. T., 1998: Protective effect of vaccination with combination of recombinant surface antigen 1 and interleukin-12 against toxoplasmosis in mice. *Infect. Immun.*, 66, 4503–4506.

20. Lyons, R. E., Anthony, J. P., Ferguson, D. J., Byrne, N., Alexander, J., Roberts, F., Roberts, C. W., 2001: Immunological studies of chronic ocular toxoplasmosis: up-regulation of major histocompatibility complex class I and transforming growth factor-beta and protective role for interleukin-6. *Infect. Immun.*, 69, 2589–2595.

21. McCabe, R. E., Luft, B. J., Remington, J. S., 1984: Effect of murine interferon gamma on murine toxoplasmosis. *J. Infect. Dis.*, 150, 961–962.

22. Montoya, J. G., 2002: Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J. Infect. Dis.*, 185, Suppl. 1, 73–82.

23. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., Coifman, R. L., 1986: Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.*, 136, 2348–2357.

24. Nagasawa, H., Manabe, T., Maekawa, Y., Oka, M., Himeno, K., 1991: Role of L3T4+ and Lyt-2+ T-cell subsets in protective immune responses of mice against infection with a low or high virulent strain of *Toxoplasma gondii*. *Microbiol. Immunol*., 35, 215–222.

25. Nagineni, C. N., Detrick, B., Hooks, J. J., 2002: Transforming growth factor-beta expression in human retinal pigment epithelial cells is enhanced by *Toxoplasma gondii*: a possible role in the immunopathogenesis of retinochoroiditis. *Clin. Exp. Immunol.*, 1282, 372–378.

26. Nakazaki, S., Saeki, N., Itoh, S., Osato, K., Watanabe, O., Hamada, N. *et al.*, 2000: Toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome – four case reports. *Neurol. Med. Chir.* (Tokyo), 40, 120–123.

27. Ondriska, F., Jalili, N. A., Catar, G., 2000: The diagnostic procedures in toxoplasmosis. *Bratisl. Lek., Listy*, 101, 294–301.

28. Pinon, J. M., Dumon, H., Chemla, C., Franck, J., Petersen, E., Lebech, M. *et al.*, 2001: Strategy of diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M and A antibodies. *J. Clin. Microbiol.*, 39, 2267–2271.

29. Prigione, I., Facchetti, P., Lecordier, L., Deslee, D., Chiesa, S., Cesbron-Delauw, M. F., Pistoia, V., 2000: T-cell clones raised from chronically infected healthy humans by stimulation with *Toxoplasma gondii* excretory-secretory antigens cross-react with live tachyzoites: characterization of fine antigenic specifity of the clones and implications for vaccine development. *J. Immunol.*, 164, 3741–3748.

30. Reichmann, G., Walker, W., Villegas, E. N., Craig, L., Cai, G., Alexander, J., Hunter, C. A., 2000: The CD40/CD40 ligand interaction is required for resistance to toxoplasmic encephalitis. *Infect. Immun.*, 68, 1312–1318.

31. Rytel, M. W., Jones, T. C., 1966: Induction of interferon in mice infected with *Toxoplasma gondii*. *Proc. Soc. Exp. Biol. Med.*, 1966, 123, 859–862.

32. Sayles, P. C., Gibson, G. W., Johnson, L. L., 2000: B-cells are essential for vaccination-induced resistance to virulent *Toxoplasma gondii*. *Infec. Immun.*, 68, 1026–1033.

33. Seguin, R., Kasper, L. H., 1999: Sensitized lymphocytes and CD40 ligation augment interleukin-12 production by human dendritic cells in response to *Toxoplasma gondii*. *J. Infect. Dis.*, 179, 467–474.

34. Sharma, S. D., Hoffin, J. M., Remington, J. S., 1985: *In vivo* recombinant interleukin 2 administration enhances survival against a lethal challenge with *Toxoplasma gondii*. *J. Immunol.*, 135, 4160–4163.

35. Soete, M., Camus, D., Dubremetz, J., 1994: Experimental induction of bradyzoite-specific antigen expression and cyst formation by RH strain of *Toxoplasma gondii in vitro*. *Exp. Parasitol.*, 78, 361–370.

36. Subauste, C. S., 2002: CD154 and type-1 cytokine response: from hyper IgM syndrome to human immunodeficiency virus infection. *J. Infect. Dis.*, 15, Suppl. 1, 83–89.

37. Suzuki, Y., Orellana, M. A., Schreiber, R. D., Remington, J. S., 1988: Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science*, 240, 516–518.

38. Suzuki, Y., Remington, J. S., 1991: Toxoplasmic en-

cephalitis in AIDS patients and experimental model for study of disease and its treatment. *Res. Immunol.*, 144, 66–67.

39. Tenter, A. M., Heckeroth, A. R., Weiss, L. M., 2000: *Toxoplasma gondii*: from animals to humans. *Internat. J. Parasitol.*, 30, 1217–1258.

40. Villena, I. D., Brodard, V., Queureux, C., Leroux, B., Dupouy, D., Remy, G. *et al.*, 1999: Detection of specific immunoglobulin E during maternal, fetal, and congenital toxoplasmosis. *J. Clin. Microbiol.*, 37, 3487–3490.

41. Yap, G., Pesin, M., Sher, A., 2000: Cutting edge: IL-12 is required for maintenance of IFN-gamma production in T-cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J. Immunol.*, 165, 628–631.

42. Yap, G. S., Sher, A., 1999: Cell-mediated immunity to *Toxoplasma gondii*: initiation, regulation and effector function. *Immunobiology*, 201, 240–247.

43. Zhan, Y., Denkers, E. Y., 1999: Protective role for interleukin-5 during chronic *Toxoplasma gondii* infection. *Infect. Immun.*, 67, 4383–4392.

Received October 16, 2006

FOLIA VETERINARIA

www.uvm.sk

FOLIA VETERINARIA, 51, 1: 63–64, 2007

CHRONICLE

PROF. JOZEF VODRÁŽKA, MVD, DSc – PIONEER SLOVAK PHARMACOLOGIST CELEBRATES HIS 85th BIRTHDAY

In connection with the 85th birthday of Prof. Jozef Vodrážka, MVD, DSc, allow us to inform readers of this journal.

University teacher Jozef Vodrážka was born on the 5th February 1922 in Málinec-Hámor, in the district of Lučenec, Slovakia.

Fig. 1. Prof. Jozef Vodrážka, MVD, DSc

He studied the first part of his education at the Veterinary University in Vienna (Austria) and finished his studies in Brno (Bohemia) in 1946. In the years 1946–1947 he worked at the Slovak Ministry of Agriculture and Agricultural Reform in Bratislava. Then in the year 1948 he furthered his studies in Great Britain. In the period 1949–1950 he was a worker at the State Diagnostic Institute in Bratislava, and from the 1st August, 1950 he worked until his retirement at the Veterinary University in Košice. In 1962 he was promoted to the position of docent, then in 1966 he received his DSc and in 1967 he was made professor. From 1975 he was also active as an external worker in The Institute of Experimental Pharmacology, at The Slovak Academy of Science, in Bratislava. Later on he examined at Makarere University (Uganda) and in 1980 he visited Cairo University.

He laid the foundations of veterinary pharmacology at our university and as a pedagogical subject in Slovakia. He elaborated and published two critical methods for testing and evaluating chemical agents in sheep for antihelminthic activity and effectivity (the method of total tracheotomy, see e.g. article in *Veterinary Record*, 1960,72: 404–405), and liver parasites (collateral tubing method for the gall-bladder, see e.g. *Nature*, 1963,199: 96–97). These were widely cited in home and foreign professional and scientific literature (e.g. *Proceedings of the 1st International Conference of the World Association for the Advancement of Veterinary Parasitology*, Hannover 1963, 34–44, 62–73, 129–136; *Proceedings of the 18th World Veterinary Congress*, Paris, 1967, 1: 101–107; *Tijdschr. Dirgen.*, 1967, 92, 977–979, etc.).

He read many papers about his methods at various world conferences, symposia, and congresses; and also demonstrated them in many veterinary colleges, universities, and research institutes in Europe and overseas. He introduced several veterinary antihelminthic preparations and tested their effectivity and tolerance before their industrial production and practical use. He was also one of founders of the University of Veterinary Medicine in Košice (Slovakia) and an organizer of postgraduate studies for veterinary surgeons and pharmacists in Slovakia. He was author of more than 150 professional and scientific reports and articles at home and abroad. In addition, he was also the principal author of the book *"Veterinary Medicine and Pharmacology for Pharmacists"*, (Martin1974), and its two later editions (1982 and 1986) and an important book *"Veterinary Pharmacology"* (Brno 1980).

Furthermore, he was also one of the co-founders and a member of the European Association of Veterinary Physiologists, Pharmacologists and Biochemists. In the period 1967 to 1973 he was vice-chairman of the Slovak

Pharmacological Society and a member of many ad hoc commissions and scientific boards at the ministries (for agriculture; health), as well as at research institutes. In addition, he was a member of committees at our veterinary university and of the Czechoslovak Pharmacopoeia Commission.

Fig. 2. Decoration of Prof. J. Vodrážka, MVD, DSc

He was decorated with the Silver Adami Medal (1969) and with the Golden Adami Medal at the University of Veterinary Medicine in Košice (1977) and recceived other Commemorative Medails from our university and from foreign institutions.

His scholars, co-workers, and co-authors of several articles and reports, wish him good health and satisfaction *"ad multos annos"* on the occasion of his eightieth fifth birthday.

Václav Šutiak and coworkers

Received January 9, 2007