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OCCURRENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCI IN HENS IN THE CENTRAL REGION OF MORAVIA *

VÝSKYT VANKOMYCIN-REZISTENTNÍCH ENTEROKOKŮ U SLEPIC NA STŘEDNÍ MORAVĚ

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ABSTRACT: Occurrence of vancomycin-resistant enterococci (VRE) in animals in the central part of Moravia is described. It is the first finding of VRE in animals in the Czech Republic. A total number of 109 strains of the genus *Enterococcus* were isolated from various biological material of different animal species (561 samples) in the period of January to April 1999. One *Enterococcus faecium* strain isolated from hens was found to be resistant to vancomycin. On the basis of this result targeted monitoring of poultry breeding was performed and 112 smears from the cloaca and 8 from pathological material taken during routine autopsies were investigated. From these 120 specimens, 48 strains of *Enterococcus* sp. were obtained and three of them were identified as vancomycin-resistant enterococci. The percentages of VRE isolated thus reached 2.5% in the group of all examined specimens and 6.3% in the group of all enterococci. The frequency of multiresistance was determined as 72.9% of all enterococcal strains from poultry breeding in the farm P. On the whole, four VRE strains were isolated while three of them were identified as *Enterococcus faecium* phenotype VanA and one as *Enterococcus* sp. group III, phenotype VanB.

enterococci; resistance to vancomycin; animals; hens

ABSTRAKT: Enterokoky patří v současné době mezi důležité bakteriální patogeny pro jejich stoupající rezistenci k antimikrobním preparátům (Dever aj., 1995; Facklam a Sahn, 1995; Zeckel, 1997). Tyto bakteriální kmeny se přirozeně vyskytují v trávicím traktu savců, ptáků, plazů i hmyzu a rovněž mohou být izolovány z prostředí (voda, rostliny) (Devriese aj., 1992; Leclerc aj., 1996; Švec a Sedláček, 1999). Významný problém v současné době představují enterokoky rezistentní k vancomycinu (VRE). Tyto kmeny lze izolovat jak z prostředí, včetně zvířecího trusu a lidské potravy živočišného původu, tak z hospitalizovaných pacientů. Jejich výskyt u zvířat mohl být podmíněn rozšířením používáním avoparcinu jako růstového promotoru ve zvířecích chovech (Goossens, 1998). Důležitou otázkou je vztah mezi výskytem VRE u lidí a používáním antimikrobních preparátů nejen v humánní medicíně, ale i ve veterinární oblasti (zvláště ve formě růstových promotorů). Lze předpokládat, že výskyt těchto kmenů u nehospitalizovaných jedinců může mít původ právě v potravě živočišného původu (Cookson, 1998). Výskyt VRE u zvířat nebyl zatím v České republice publikován. Cílem naší studie byla analýza výskytu VRE u zvířat na střední Moravě. Vyšetřovaný materiál byl rozdělen do dvou skupin. V první skupině byly izolovány enterokoky z různých zvířat (tab. I), ve druhé skupině byly vyšetřovány enterokoky izolované při cílené depistáži chovu slepic na farmě P. V první skupině bylo vyšetřeno celkem 561 vzorků různé provenience a izolováno 109 kmenů *Enterococcus* sp. Z tohoto počtu byl identifikován jeden kmen rezistentní k vancomycinu. Na základě tohoto nálezu byla provedena cílená depistáž a v uvedeném chovu bylo získáno dalších 120 vzorků (hlavně kloakálních výtěrů). Z tohoto počtu vzorků bylo kultivováno 48 enterokokových kmenů a tři z nich byly identifikovány jako VRE. Celkem byly určeny tři VRE kmeny jako *Enterococcus faecium* a jeden VRE kmen jako *Enterococcus* sp. skupina III. Podle stanovení minimálních inhibičních koncentrací vancomycinu a teikoplaninu lze kmeny *Enterococcus faecium* charakterizovat jako VRE fenotypu VanA, zatímco kmen *Enterococcus* sp. skupina III. jako VRE fenotypu VanB (tab. II). Frekvence multirezistentních enterokoků ve sledovaném chovu slepic činila 72,9 % (tab. III). Naše sdělení je prvním popisem VRE u zvířat v České republice. Tyto nebezpečné kmeny byly izolovány u 2,5 % všech vyšetřených vzorků odebraných od slepic z farmy P. a jejich výskyt mezi izolovanými kmeny enterokoků dosáhl 6,3 %. VRE byly většinou diagnostikovány jako *Enterococcus faecium* fenotyp VanA (75 %).

enterokoky; rezistence k vancomycinu; zvířata; slepice

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INTRODUCTION

The recent interest in enterococci has been spurred on by increasing of their antibiotic resistance, which can cause important problems in antibiotherapy of serious enterococcal infections (Dever et al., 1995; Facklam and Sahn, 1995; Zeckel, 1997). Enterococci naturally occur in large numbers in the intestines of mammals, birds, reptiles and insects, and can be also found in environment (water, plants) (Devriese et al., 1992; Leclere et al., 1996; Švec and Sedláček, 1999).

Enterococci are important nosocomial pathogens characterized by their natural resistance to a variety of antimicrobials. At present, the increasing occurrence of vancomycin-resistant enterococci (VRE) is a great problem. VRE, first reported in Europe in 1988, are emerging as a global threat for public health (Uttley et al., 1988; Weber and Rutala, 1997). The incidence of VRE among hospitalized patients has increased rapidly during the last decade, especially in Northern America. The proportion of VRE, reported by the system of the National Nosocomial Infections Surveillance in USA, increased 20-fold from 1989 to 1993 (Center for Disease Control and Prevention, 1993). The chief risk factor for the appearance of VRE in hospitalized patients is excessive administration of glycopeptides (Heath et al., 1996; Martone, 1998).

Many reports from Europe suggest that VRE exist in environment, including animal faeces and human foods of animal origin (Aarestrup, 1995; Aarestrup et al., 1996; Bates et al., 1993, 1994; Bogaard et al., 1996; Klare et al., 1993). Their occurrence might be explained by the extended use of the glycopeptide antibiotic avoparcin as a growth promotor for farm animals (Goossens, 1998). The transmission of VRE to persons in contact with these sources results in an increasing of human reservoir (Bates et al., 1993, 1994; Bogaard et al., 1996; Kruse and Rorvik, 1996).

There are 3 types of vancomycin resistance identified in enterococci. The VanA phenotype with high level inducible resistance to vancomycin and teicoplanin (a result of the acquisition of the *vanA* resistance gene cluster on Tn 1546 or related elements), the VanB phenotype with a lower level of inducible resistance to vancomycin and susceptibility to teicoplanin, and the VanC phenotype due to the chromosomal *vanC* resistance gene in certain species (*Enterococcus gallinarum*, *E. casseliflavus*). Glycopeptides act by binding to the terminal D-Ala-D-Ala moiety of bacterial peptidoglycan and thus interfere with normal cell wall growth. However, using transposon encoded *vanA* or *vanB* gene clusters, VRE have been able to alter the terminal D-Ala to D-Lac resulting in a reduced binding of vancomycin and teicoplanin (Arthur et al., 1996; Gin and Zhanel, 1996; Zeckel, 1997).

The occurrence of VRE in the Czech Republic was for the first time described in 1997 in the Hemato-oncology Clinic of the University Hospital in Olomouc (Kolář et al., 1997). Within one year other reports men-

tioned the occurrence of such strains in hospitalized patients in this country (Bergerová and Turková, 1997; Urbášková, 1997a). The occurrence of VRE in animals has not been described yet in the Czech Republic.

A very important problem is the relationship between the occurrence of VRE in humans and antibiotic usage, especially the glycopeptide growth promotors in animal husbandry. A limited number of studies has indicated that some non-hospitalized individuals have only exceptionally VRE in their faecal flora and it is assumed, but not proven, that these come from ingested food of animal origin (Cookson, 1998). Our investigation is focused on the occurrence of VRE in animals from the central part of Moravia, where avoparcin was used in the feed in the poultry farms but only for broilers.

MATERIAL AND METHODS

At the National Veterinary Institute in Olomouc (NVI) biological material obtained from animals was evaluated in the period from January to April 1999. This material was divided into two groups. The first group included samples of pathological material taken during routine autopsies and samples of clinical material (especially dermal and cloaca smears) from mammals, birds and reptiles, which were delivered to NVI. The latter involved specimens from cadavers and clinical materials (cloaca smears) of hens (layers with approximate age from 8 to 10 months), which were taken during targeted monitoring of poultry breeding in the farm P.

Enterococci were isolated in Mueller-Hinton broth with 6.5% NaCl (without vancomycin) and identified according to their growth characteristics on blood agar (Blood Agar Base, Oxoid) and by their biochemical activities: inability of catalase production, detection of pyrrolidonylarylamidase (Lachema Brno, Czech Republic), growth in nutrient broth with 6.5% NaCl, acid production from arabinose, lactose and mannitol and hydrolysis of arginine (Oxoid). All strains were positive for the streptococcal group D antigen (Oxoid).

Detection of susceptibility to antibiotics was performed by the microdilution method (MIC) in accordance with the NCCLS guideline (National Committee for Clinical Laboratory Standards, 1995). The following eight drugs and their breakpoints were used: 8 mg/l for teicoplanin (TEI) and ampicillin (AMP), 4 mg/l for vancomycin (VAN) and chloramphenicol (CMP), 2 mg/l for tetracycline (TET) and ofloxacin (OFL), 0.5 mg/l for erythromycin (ERY) and 32 mg/l for nitrofurantoin (FUR).

RESULTS

The survey of species and numbers of investigated animals, including numbers of *Enterococcus* sp. and VRE strains is given in Tab. I. Within the first period, 561 samples were investigated. From this group 109 *Enterococcus* sp. strains were isolated and 1 strain from hen in the breeding farm P. was identified as VRE. On

I. Survey of species of examined animals, numbers of isolated enterococcal strains and VRE in two observed groups

Group 1	Results without targeted screening			
	number of examined animals	number of farms or owners	number of <i>Enterococcus</i> sp. strains isolated	number of VRE strains isolated
Cattle	39	12	14	0
Pigs	73	37	16	0
Sheep	1	1	0	0
Poultry	251	31	38	1
Water fowl	2	2	1	0
Cats	5	5	1	0
Dogs	29	25	11	0
Other carnivores	37	7	3	0
Domesticated rabbit	8	8	0	0
Hare	8	8	0	0
Black deer	6	5	1	0
Red deer	5	3	3	0
Fish	41	19	1	0
Exotic mammals	8	2	3	0
Exotic birds	23	2	4	0
Exotic reptiles	17	5	11	0
Laboratory animals	8	4	2	0
Total	561	~	109	1
Group 2	Targeted screening in hen breeding with first VRE strain isolated			
Hens	120	1	48	3

the basis of this finding, the targeted monitoring embracing 112 smears from the cloaca and 8 from pathological material taken during routine autopsies was performed in the second period. On the whole, 48 *Enterococcus* sp. strains were obtained and three of them were diagnosed as resistant to vancomycin. The occurrence of VRE reached 2.5% in the group of all examined hen specimens and 6.3% in the group of all isolated enterococcal strains.

The VRE isolates were diagnosed as *Enterococcus faecium* (3 strains) and 1 strain as *Enterococcus* sp. group III (involving *E. durans*, *E. hirae*, *E. dispar* and *E. faecalis* (var. strains). According to their MICs for vancomycin and teicoplanin it can be concluded that all three *Enterococcus faecium* strains could be classified as phenotype VanA, while one *Enterococcus* sp. group III strain as phenotype VanB (Tab. II).

As determined by MICs, the resistance to tested antibiotics in the *Enterococcus* sp. isolates from hens was found in 6.3% strains to ampicillin, 81.3% to chloramphenicol, 60.4% to tetracycline, 85.4% to erythromycin, 8.3% to vancomycin, 6.3% to teicoplanin, 33.3% to nitrofurantoin and 35.4% to ofloxacin. The frequency of multiresistant enterococci in observed poultry breeding in farm P. was determined in 72.9% from all enterococcal strains in second group (Tab. III).

DISCUSSION

VRE were first reported outside the health-care setting in 1993 when vancomycin-resistant *Enterococcus*

faecium was recovered from waste water samples (Bates et al., 1993). In the following year Bates et al. (1994) recovered VRE from livestock faeces and from uncooked chicken samples purchased from retail outlets. The relationship between VRE colonization of animals used in food production and VRE colonization of humans was first suggested by Bates et al. (1993, 1994), who recovered VRE with identical ribotypes from retail chicken carcasses and humans.

Our report is the first description of occurrence of VRE in animals in the Czech Republic. The VRE were isolated from 2.5% of examined hens of the breeding farm P. and their occurrence among all collected enterococcal strains reached 6.3%. In this farm (layers only) avoparcin was not used in the feed as a glycopeptide growth promotor. VRE were diagnosed mostly as *Enterococcus faecium* phenotype VanA (75%). These results are similar to the occurrence of VRE in hemat oncology patients at the University Hospital in Olomouc, where their frequency reached 3.7%, and 74.0% of them were identified as *Enterococcus faecium* phenotype VanA (Kolář et al., 1999). It can be suggested that VRE from these animals might play a role in human colonization and serve as a source of VanA resistance genes transferable by transposon Tn1546 on other enterococcal strains in the intestinal tract (Taylor, 1999; Aarestrup et al., 1996).

Vancomycin-resistant enterococci, often multiresistant to many other antibiotics, are a great problem in therapy of nosocomial infections caused by these microorganisms. It is necessary to stress the need of con-

II. MICs of tested antibiotics in enterococci isolated from hens of poultry farm P. with occurrence of VRE

Strain	MICs of tested antibiotics							
	AMP	CMP	TET	ERY	VAN	TEI	FUR	OFL
1	16	8	0.25	8	512	32	64	16
2	2	16	4	32	1	0.5	64	2
3	2	8	8	8	1	0.5	32	4
4	1	8	4	8	1	0.5	32	8
5	2	8	4	16	1	0.5	32	2
6	2	16	16	8	1	0.5	64	2
7	1	8	32	8	1	0.5	32	2
8	1	8	4	64	1	0.5	32	2
9	1	32	4	32	1	0.5	64	2
10	2	32	4	8	1	0.5	16	2
11	1	8	16	8	1	0.5	32	8
12	2	16	8	16	1	0.5	32	2
13	1	32	4	8	1	0.5	32	2
14	2	16	4	32	0.5	0.5	128	2
15	1	8	16	8	1	0.5	64	2
16	1	8	32	16	1	0.5	32	2
17	2	8	4	8	1	0.5	32	2
18	0.25	16	4	0.06	0.5	0.5	128	16
19	1	64	4	2	1	0.5	16	2
20	2	32	4	1	1	0.5	8	2
21	16	8	0.25	8	512	32	32	16
22	0.125	8	1	1	0.25	0.5	16	0.25
23	2	8	4	8	0.5	0.5	64	4
24	0.25	8	2	0.5	4	0.5	8	4
25	2	8	2	8	1	0.5	32	2
26	0.25	8	2	8	1	0.5	8	1
27	2	8	2	8	1	0.5	8	2
28	1	16	16	32	1	0.5	64	8
29	2	32	2	16	0.5	0.5	32	4
30	2	32	0.25	0.5	1	0.5	8	1
31	4	4	2	16	0.5	0.5	64	4
32	8	16	4	16	0.5	0.5	64	1
33	0.5	32	2	16	1	0.5	8	2
34	1	4	0.25	0.5	1	0.5	8	1
35	4	4	4	8	0.5	0.5	128	4
36	16	32	16	32	512	32	32	8
37	4	4	4	8	0.5	0.5	32	4
38	0.5	4	2	8	0.5	0.5	32	4
39	0.5	16	4	32	1	0.5	8	2
40	0.5	16	2	8	512	2	64	1
41	4	4	4	8	1	0.5	32	8
42	2	16	2	8	1	0.5	32	2
43	1	16	8	32	1	0.5	64	2
44	2	16	32	32	1	0.5	64	2
45	1	8	2	8	0.5	0.25	64	16
46	1	2	1	0.25	0.125	0.125	16	2
47	2	4	2	0.25	0.125	0.125	16	1
48	1	2	2	0.5	0.25	0.25	32	2
No. of S	45	9	19	7	44	45	32	31
No. of R	3	39	29	41	4	3	16	17
% of R	6.3	81.3	60.4	85.4	8.3	6.3	33.3	35.4

III. Multiresistance to antibiotics in enterococci from hens of poultry farm P.

VAN-susceptible									
AMP	CMP	TET	ERY	VAN	TEI	FUR	OFL	No. of strains	SUM
S	R	R	R	S	S	S	S	11	31
S	R	R	R	S	S	R	S	8	
S	R	R	R	S	S	S	R	3	
S	R	R	R	S	S	R	R	2	
S	S	R	R	S	S	S	R	2	
S	R	S	R	S	S	R	R	1	
S	R	R	S	S	S	R	R	1	
S	R	S	R	S	S	S	R	1	
S	S	S	R	S	S	R	R	1	
S	S	R	R	S	S	R	R	1	
SUM								35 (72.9%)	

VAN-resistant									
R	R	S	R	R	R	R	R	2	4
R	R	R	R	R	R	R	R	1	
S	R	S	R	R	S	R	S	1	
SUM								35 (72.9%)	

sistent and continual monitoring of bacterial resistance to antimicrobials as an integral part of antibiotic policy (Kolář and Látal, 1996). If community transmission is important in the global spread of VRE, factors leading to its emergence in hospitals must be examined. Of course, measures must be taken to the control of their transmission also in veterinary setting. Among these methods at least rough identification of enterococci belongs on the basis of morphological features, production of catalase and pyrrolidonylamidase and determination of their sensitivity to vancomycin by using disc diffusion method (event. by using nutrient agar with concentration of vancomycin 6 mg/l as a screening test) (Urbášková, 1997b). When VRE is detected, it is necessary to identify the strain precisely and to verify resistance to vancomycin by the microdilution method. Further steps should be then focused on the following solutions: restricted use of antibiotics and improved infection controls in hospitals, the monitoring of occurrence of VRE in animal breedings and consequential controls of animal husbandry and food production industry. It is very important that both medical and non-medical uses of antibiotics have been reduced. It must be emphasized that close cooperation between human and veterinary microbiologists is necessary. More scientific data are needed to address issues related to antibiotic application in food of animals, including elucidating the human health impact, e.g. the percentage of resistance genes or resistant organisms occurring in animals.

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ULTRASTRUCTURE OF THE TRACHEAL EPITHELIUM AFTER ORAL ADMINISTRATION OF SALBUTAMOL*

ULTRAŠTRUKTURA EPITELU TRACHEY PO PERORÁLNÍM PODÁNÍ SALBUTAMOLU

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ABSTRACT: The ultrastructure of the rabbit tracheal epithelium was studied 15 min and 30 min after oral administration of 5 ml of Ventolin sirup (2 mg of salbutamol). 15 min post exposure, the injury to the tracheal epithelium due to the treatment with a single therapeutic dose of an oral bronchospasmolytic drug that belongs to the family of the selective β_2 adrenergic agonists was considered moderate. Morphological signs of impaired self-cleaning ability were revealed especially in the later phase of the experiment. 30 min after administration of salbutamol, signs of regeneration of the injury prevailed in the epithelium.

airways; Ventolin; β_2 adrenergic agonist; electron microscopy

ABSTRAKT: Studovali jsme ultrastrukturu tracheálního epitelu králíků 15 a 30 minut po perorální aplikaci 5 ml preparátu Ventolin sirup (2 mg salbutamolu). 15 minut po podání jedné terapeutické dávky perorálního bronchospasmolytika patřícího do skupiny 2 sympatomimetik jsme hodnotili poškození epitelu jako středně závažné. Morfologické známky poruchy samočisticí schopnosti epitelu dýchacích cest jsme zaznamenali především v pozdější fázi experimentu. 30 minut po aplikaci salbutamolu již v epitelu převažovaly známky regenerace vyvolaných změn.

dýchací cesty; Ventolin; β_2 sympatomimetikum; elektronová mikroskopie

INTRODUCTION

In our previous studies, the effect of intratracheal administration of two adrenergic agonists and a cholinergic antagonist on the ultrastructure of the airway epithelium was studied (Konrádová et al., 1997, 1998). We demonstrated that two puffs of a β_2 adrenergic agonist – salbutamol- caused moderate to severe damage to the tracheal epithelium accompanied by changes in the proportion of sialylated and sulphated glycoconjugates produced by the goblet cells (Vajner, 1998). In our present study, we decided to investigate also the effect of oral administration of a similar dose of this drug.

MATERIAL AND METHODS

The same group of three healthy rabbits used in our previous study (Konrádová et al., 1997) served as un-

treated controls. Two groups of three healthy rabbits (body weight 2 000 g – 2 500 g) were treated with single therapeutic doses of Ventolin oral preparation. Each animal received 5 ml of Ventolin sirup (Glaxo Group Ltd., Greenford, England) containing 2 mg of salbutamol sulphate. Under general anaesthesia induced by *i.m.* administration of a mixture of ketamine and xylazine (Konrádová et al., 1997), material for the electron microscopic examination was collected 15 min and 30 min post exposure. Tiny fragments of the tracheal mucous membrane were processed using the same methods as described in our previous paper (Konrádová et al., 1997).

For quantitative estimation, in controls and 15 min and 30 min after administration of 5 ml of Ventolin, 1 058 μm^2 , 1 848.5 μm^2 and 1 321 μm^2 of ciliary border with 10 252, 11 492 and 9 131 kinocilia were evaluated, respectively. In those experimental groups also a total of 186, 138 and 390 goblet cells were studied, respectively. The goblet cells were classified into

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I. Quantitative evaluation of goblet cells (GC) and ciliary border in the trachea of rabbits 15 and 30 min after oral administration of 5 ml of Ventolin sirup (2 mg of salbutamol)

		Control rabbits	Ventolin 5 ml <i>p.o.</i> 15 min	Ventolin 5 ml <i>p.o.</i> 30 min
Nonstimulated GC	(%)	96.8 ± 1.5	*22.5 ± 2.4	99.4 ± 0.7
Mucus-discharging GC	(%)	3.2 ± 1.5	*29.9 ± 3.5	*0.3 ± 0.8
Degenerated GC	(%)	0	*47.6 ± 2.3	0.3 ± 0.4
Stimulated GC total	(%)	3.2 ± 1.5	*77.5 ± 2.4	*0.6 ± 0.6
GC arranged in groups	(%)	5.9 ± 3.1	9.5 ± 0.5	*34.9 ± 4.3
Number of cilia per 1 m ² of ciliary border		9.7 ± 0.3	*6.2 ± 0.3	*6.9 ± 0.3
Intact cilia	(%)	98.8 ± 0.1	*97.4 ± 1.2	98.9 ± 0.5
Pathological cilia	(%)	0.5 ± 0.2	*1.3 ± 1.0	0.3 ± 0.3
Degenerating cilia	(%)	0.3 ± 0.1	*0.9 ± 0.6	0.4 ± 0.4
Malformed cilia	(%)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1
Altered cilia total	(%)	1.2 ± 0.1	*2.6 ± 1.2	1.1 ± 0.5

n = 3, mean ± SD, values designated * differ significantly ($\alpha = 0.01$) from controls, values connected by a line differ significantly ($\alpha = 0.01$) from each other

three categories: mucus-filled, mucus-discharging and degenerated ones. Kinocilia were classified into four categories: intact 9+2 cilia, slightly damaged pathological cilia with local swellings of the ciliary membrane or with tiny vacuoles situated in their shafts, degenerating cilia, represented by axonemes incorporated into the cytoplasmic blebs or by isolated axonemes, and malformed cilia with either abnormal arrangement or number of microtubules in their axonemes.

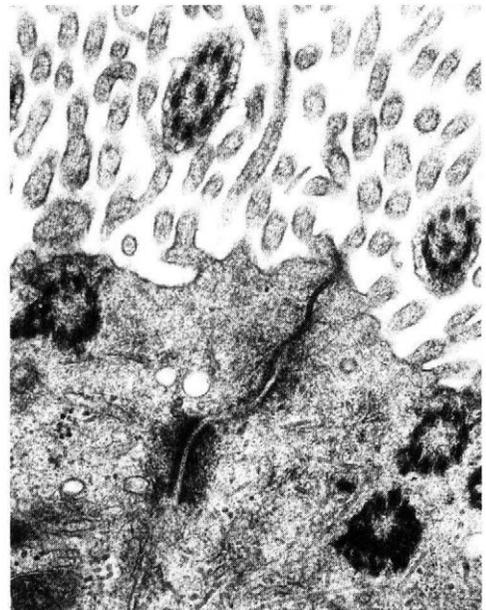
For statistical evaluation, relative values of the 3 categories of goblet cells and 4 categories of cilia were evaluated by the chi-square test of homogeneity in frequency tables. To specify categories causing deflections from the hypothesis of homogeneity, adjusted standardised deviations were used. Means of cilia/ μm^2 were compared by the one-way analysis of variance (ANOVA) and by a non-parametric analogy of the ANOVA – Kruskal-Wallis test. The differences between groups were assessed by the Tukey's test for multiple comparison. The Leven's test of homogeneity of variance was also performed.

RESULTS

The ultrastructure of the tracheal epithelium of control rabbits was described in detail in our previous paper (Konrádová et al., 1997) and the quantitative data are given in Tab. I.

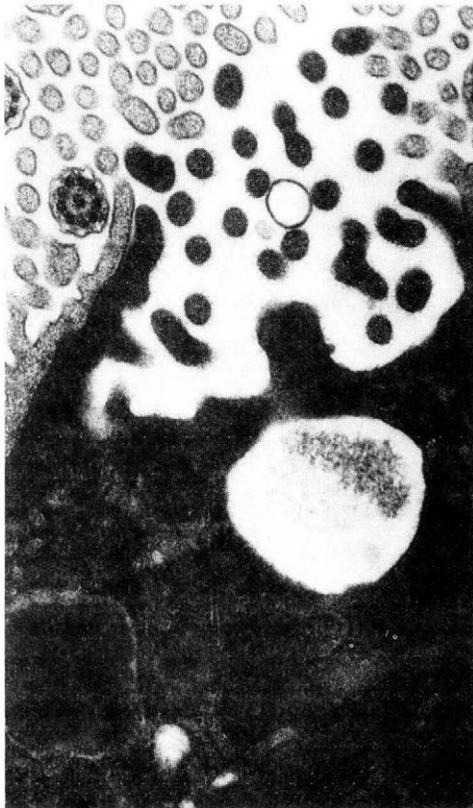
15 min post exposure, rabbits' tracheae were lined with an altered pseudostratified columnar ciliated epithelium with narrow intercellular spaces and intact apical junctional complexes (Fig. 1).

The ciliated cells revealed only slight signs of pathological alteration. In the deeper portions of their cytoplasm, a slight increase in the number of small vacuoles was observed. The goblet cells were found as isolated elements among the ciliated ones. Only $9.5 \pm 0.5\%$ of them were arranged in tiny groups, $21.0 \pm 0.6\%$ of goblet cells were filled with large light mucous granules and $29.9 \pm 3.5\%$ with discharged mucus (Tab. I). The



I. Apical junctional complex (rabbit tracheal epithelium 15 min after oral administration of 5 ml of Ventolin sirup); 50 000x

stimulated cells communicated widely with the lumen of the respiratory passages. Mucus was evacuated simultaneously from apical mucous granules. Packets of mucous granules were also detached from the goblet cells. In some stimulated goblet cells, chain fusion of the adjacent mucous granule membranes was noticed. The completely exhausted degenerated secretory cells amounted to $47.6 \pm 2.3\%$ (Fig. 2). After losing their contacts with the basal lamina, the exhausted elements were mostly encountered in the apical portion of the epithelium and after sloughing off, remnants of their electron-dense



2. Apical portion of an exhausted degenerated goblet cell (rabbit tracheal epithelium 15 min after oral administration of 5 ml of Ventolin sirup); 50 000x

degenerated cytoplasm were observed in the area of the ciliary border.

Differentiating secretory elements represented $1.5 \pm 0.2\%$. These cells were equipped with short irregular microvilli and they contained a few small secretory granules of various density in their undifferentiated cytoplasm. Their apical portions sometimes bulged above the epithelium. Elements filled with small mucous granules separated by the voluminous cytoplasmic septa were also encountered in the epithelium.

The regular arrangement of the ciliary border was slightly impaired. The mean number of cilia was $6.2 \pm 0.3/\mu\text{m}^2$ (Tab. I). The altered elements represented $2.6 \pm 1.2\%$. The proportions of the individual types of altered cilia are given in Tab. I. In the area among the kinocilia, isolated clumps of inspissated mucus were observed.

30 min after oral administration of salbutamol, the pseudostratified columnar ciliated epithelium lining the tracheae was still only slightly altered and the narrow intercellular spaces were sealed with intact apical junctional complexes.



3. Tiny vacuoles, secondary lysosomes and dilated cisternae of the Golgi complex in the cytoplasm of an altered ciliated cell (rabbit tracheal epithelium 30 min after oral administration of 5 ml of Ventolin sirup); 50 000x

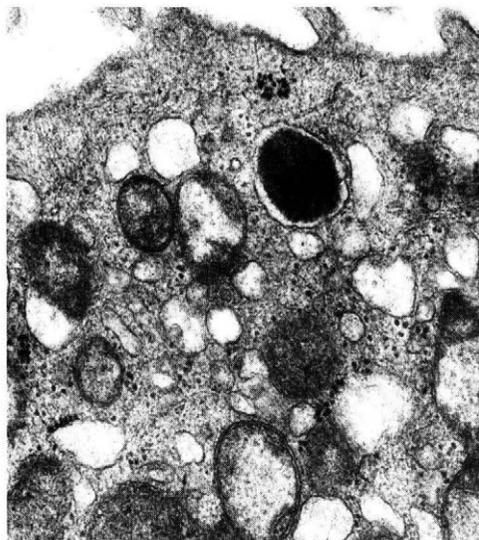
In the ciliated cell cytoplasm, rather numerous vacuoles and lysosomes, dilated cisternae of the granular endoplasmic reticulum and of the Golgi complex were recorded (Fig. 3).

$34.9 \pm 4.3\%$ of goblet cells formed small groups composed of 2 – 3 elements. The goblet cells not revealing signs of secretion amounted to $99.4 \pm 0.7\%$, but only $2.0 \pm 1.6\%$ of them were filled with large light mucous granules. $97.4 \pm 2.3\%$ represented the differentiating secretory elements in various phases of their development (Figs. 4, 5). In their cytoplasm, an increase in the number of tiny vacuoles together with the presence of altered mitochondria was demonstrated. The proportions of the mucus discharging and exhausted degenerated cells are given in Tab. I.

On average, $6.9 \pm 0.3/\mu\text{m}^2$ kinocilia appeared in the slightly altered ciliary border where often clumps and layers of condensed secretion embedded the mostly intact cilia (Fig. 6). The altered kinocilia amounted only to $1.1 \pm 0.5\%$ (Tab. I).

DISCUSSION

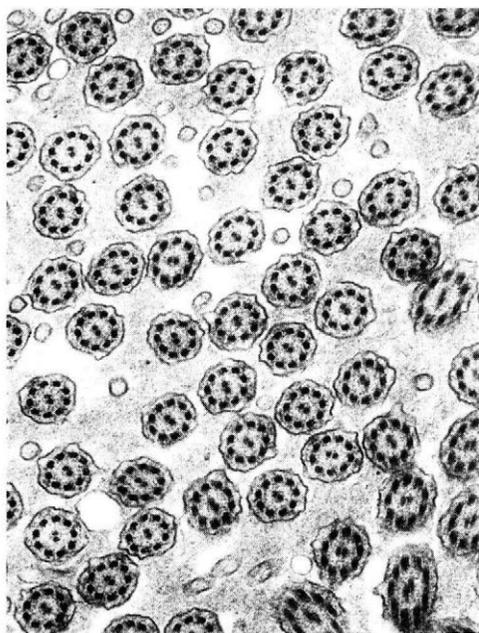
Our results describing the degree of damage to the tracheal epithelium due to oral administration of salbutamol were in accordance with those obtained after in-



4. Apical portion of a differentiating goblet cell containing an isolated, small electron dense secretory granule (rabbit tracheal epithelium 30 min after oral administration of 5 ml of Ventolin sirup): 50 000x



5. Apical portion of a differentiating goblet cell containing several isolated, small secretory granules containing fibrogranular matrix (rabbit tracheal epithelium 30 min after oral administration of 5 ml of Ventolin sirup): 50 000x



6. Layer of condensed secretion in the area of the ciliary border (rabbit tracheal epithelium 30 min after oral administration of 5 ml of Ventolin sirup): 37 500x

halation of aerosol of this drug. Similar results were also described by Spahr-Schopfer and Shorten with their fellow workers studying the effect of salbutamol

on the airway mucous membrane using the light microscope (Spahr-Schopfer et al., 1994; Shorten et al., 1995).

During the whole experiment, the tracheae were lined with altered pseudostratified columnar ciliated epithelium. The ciliated cells were less altered compared to the secretory ones. The cortical portions of their cytoplasm remained intact, the process of apical blebbing was not revealed. The signs of pathological alteration of the deeper portions of their cytoplasm were more pronounced 30 min post exposure. Compared with controls, no increase in the number of differentiating ciliated cells was revealed.

Due to the treatment with Ventolin sirup, the secretory elements were highly affected. 15 min after salbutamol administration, 77% of goblet cells were stimulated to discharge their mucus. The process of mucus evacuation was accelerated and also the mechanism of secretion was influenced. Signs of an apocrine type of secretion were frequently encountered and also compound exocytosis (Neutra and Schaffer, 1977; Specian and Neutra, 1980; Roumagnac and Laboisie, 1987; Specian and Oliver, 1991; Konrádová et al., 1996;

	Control rabbits	Degree of damage			Ventolin 5 ml <i>p.o.</i> 15 min	Ventolin 5 ml <i>p.o.</i> 30 min
		mild	moderate	severe		
Stimulated GC	< 4%	4-50%	50-90%	> 90%	77.5%	0.6%
Ratio $\frac{\text{discharging GC}}{\text{degenerated GC}}$	degenerated GC not found	> 1	0.1-1	< 0.1	0.6	---*
Number of cilia/ μm^2	> 9	7-9	3-7	< 3	6.2	6.9
Altered cilia	<1.2%	1.2-2.0%	2.0-10.0%	> 10.0%	2.6%	1.1%
Signs of impairment of the self-cleaning ability	0	±	+	++	±	+

GC = goblet cells

* determination of this value was not possible because of low absolute values of mucus-discharging and degenerated GC

Newman et al., 1996) representing the most rapid way of mucus discharge was observed in numerous cells.

The oral administration of Ventolin not only over-stimulated but also damaged the secretory elements. After rapid mucus discharge, more than 47% of the exhausted goblet cells degenerated and were gradually sloughed off. On the basis of our previous experiments (Konrádová et al., 1990), we expected massive differentiation of new secretory elements resulting in the appearance of intraepithelial mucous glands to occur in the tracheal epithelium after degeneration of more than a half of the goblet cells. 15 min after oral administration of a single therapeutic dose of salbutamol, only 47% of secretory elements were completely exhausted and degenerated. Nevertheless, an increase in the number of differentiating secretory elements was encountered. 30 min post exposure, the differentiating goblet cells absolutely prevailed in the epithelium.

As the differentiating goblet cells are still able to divide, an increase in the number of these elements is accompanied by the change of their distribution in the epithelium. 15 min after administration of salbutamol, the proportion of goblet cells arranged in groups was slightly higher but did not differ significantly compared with that of healthy control rabbits. 30 min post exposure, almost 35% of goblet cells formed tiny intraepithelial glands.

During the whole experiment, slightly altered ciliary border appeared above the epithelium. In the area of the ciliary border, a significant decrease in the mean number of kinocilia to $6.2/\mu\text{m}^2$ and $6.9/\mu\text{m}^2$ was revealed, respectively. At first, for the decrease of the average number of cilia per $1 \mu\text{m}^2$, the high level of goblet cell stimulation was responsible. The stimulated goblet cells communicated widely with the lumen of the respiratory passages and disturbed the regular arrangement of kinocilia. Later, the decrease in the number of kinocilia was due to the process of massive differentiation of new goblet cells accompanied by the development of tiny intraepithelial mucous glands.

The kinocilia were mostly intact. Only during the first 15 min, a slight increase in the number of altered kinocilia was recorded. 30 min post exposure, the proportion of altered kinocilia did not significantly differ

compared with the findings in healthy control rabbits. The administration of salbutamol did not cause an increase in the proportion of malformed cilia showing thus that the ciliogenesis was not affected in the course of this treatment.

In accordance with other authors who studied the relation of the cilia to the layer of secretion in the respiratory passages (Yoneda, 1976; Sturgess, 1977; Stratmann et al., 1991; Geiser et al., 1997), we consider the appearance of inspissated mucus and bacteria in the area of the ciliary border as morphological signs of impaired self-cleaning ability of the airway epithelium. 15 min after oral administration of salbutamol, first signs of local mucus flow disturbances were observed. 30 min post exposure, clumps or layers of inspissated mucus marked rather severe impairment of the self-cleaning ability of the airway epithelium. On the basis of our previous experiments, a classification of the degree of injury to the airway epithelium was proposed (Konrádová, 1991) (Tab. II). 15 min post exposure, the injury to the tracheal epithelium due to the treatment with an oral therapeutic dose of Ventolin sirup was considered moderate according to this classification. 30 min after administration, signs of regeneration of the injury prevailed in the epithelium.

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3rd ANNUAL KÁBRT DIETETICS DAYS

3. KÁBRTOVY DIETETICKÉ DNY

A FEW WORDS ABOUT THE STATUS AND RESULTS OF THE CONFERENCE

Science workers, university lecturers, veterinarians and other agricultural experts working in the field of nutrition and the nutrition hygiene for agricultural animals met for what is now the third time at this conference to inform each other about the results of their work, exchange their experience and acquire new knowledge in this field.

The conference is named after the most distinguished Czech veterinary dietician, the Emeritus Professor of the Veterinary and Pharmaceutical University in Brno, Prof. MVDr. Jaroslav Kábrt, and this may be considered an appreciation of his life-long work. At the institute which he headed from 1950 to 1970, it was predominantly the technical direction of nourishment which through his efforts and under his leadership was shifted to biological, hygienic and dietary principles based on physiological knowledge and the requirements of individual species and categories of animals. He has continued the development of the field in this spirit right up to the present day.

In the introductory part of the conference, the presentation of Prof. MVDr. Kábrt aroused interest when he, in his unique way, assessed the development of the field, mentioned the fast progress of the discipline in the last few years and, moreover, he shared many of his personal memories with the audience.

After the official part of the conference in which all delegates participated, the expert discussions took place in two sections. Among the active participants of the discussion were veterinary and agricultural experts from universities and research institutes in Poland, Slovenia, Croatia, the Slovak and Czech Republics, and also the representatives of feed manufacturers. 23 presentations were given, and 25 other pieces of research were presented in the form of posters in the sections. The discussion covered a wide area, such as soil, including plants, feed modification, the harmlessness of feed to health, the nutrition and dietetics of animals, the influence of feed on the productive health of animals and the quality of food and its safety.

The conference not only made it possible for experts from many areas to meet, allowed for the exchange and confrontation of expert ideas and brought new views and incentives for interdisciplinary solutions to complex problems raised by today's agricultural practice but it also fulfilled the expectations of the organizers. The conference confirmed that the Dietetics Days are becoming a tradition and if the interest of the expert public continues they will remain a significant professional and social event. At the same time, however, it would be useful to consider the range of contents and specialization which would, in my opinion, contribute to the enhancement of the conference's reputation in future years.

The publication of chosen works in the form of short communications or abstracts in the scientific journal *Veterinary Medicine* should also assist the popularization of the discussed problems and the results presented at this conference held on 2nd September 1999 in Brno.

NĚKOLIK SLOV K VÝZNAMU A VÝSLEDKŮM KONFERENCE

Již potřeby se sešli vědečtí pracovníci, vysokoškolští učitelé, veterinární lékaři a další zemědělské odborníci pracující v oblasti výživy a hygieny výživy hospodářských zvířat na konferenci, aby se vzájemně informovali o výsledcích své práce, vyměnili si své zkušenosti a získali nové poznatky z této oblasti.

Konference nese jméno nestora českých veterinárních dietetiků, emeritního profesora Veterinární a farmaceutické univerzity v Brně, prof. MVDr. Jaroslava Kábrta, což lze považovat za ocenění jeho celoživotního díla. Jeho zásluhou a pod jeho vedením se na půdě ústavu, který v letech 1950 až 1970 vedl, přetvářel převážně technický směr výživy na principy biologické, hygienické a dietetické, založené na fyziologických znalostech a požadavcích jednotlivých druhů a kategorií zvířat. V těchto intencích pokračoval rozvoj oboru až do současnosti.

V úvodní části konference vyvolalo pozornost vystoupení prof. MVDr. Jaroslava Kábrta, který svým osobitým způsobem zhodnotil vývoj vědního oboru, připomněl prudký rozvoj disciplíny v posledních několika letech a posluchačům sdělil i řadu svých osobních vzpomínek.

Po ukončení společně, oficiální části konference, proběhla odborná jednání ve dvou sekcích. Aktivními účastníky jednání byli veterinární a zemědělské odborníci vysokých škol, výzkumných ústavů z Polska, Slovinska, Chorvatska, Slovenské a České republiky, ale i zástupci krmivářských firem. V sekcích bylo předneseno 23 přednášek, dalších 25 sdělení bylo prezentováno formou posterů. Jednání postihlo širokou oblast od půdy, přes rostliny,

úpravu krmív, zdravotní nezávadnost krmív, výživu a dietetiku zvířat, vliv krmív na produkční zdraví zvířat až po kvalitu potravin a jejich bezpečnost.

Konference umožnila setkání odborníků z řady oblastí, poskytla prostor pro výměnu a konfrontaci odborných názorů, přinesla nové pohledy a podněty pro interdisciplinární řešení složitých problémů nastolovaných dnešní zemědělskou praxí, ale naplnila i očekávání organizátorů. Potvrdilo se, že Dietetické dny se stávají tradicí a při pokračujícím zájmu odborné veřejnosti zůstanou významnou pracovní i společenskou událostí. Zároveň však by bylo vhodné uvažovat o obsahové profilaci a specializaci, což by podle mého názoru přispělo ke zvýšení prestiže dalších ročníků konference.

K popularizaci projednávané problematiky a výsledků přednesených na konferenci, která se uskutečnila 2. září 1999 v Brně, má sloužit i zveřejnění vybraných prací – krátkého sdělení, resp. abstraktů ve vědeckém časopise Veterinární medicína.

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EFFECTS OF ENTEROCOCCUS FAECIUM ON THE GROWTH RATE AND CONTENT OF INTESTINAL MICROFLORA IN SHEAT FISH (*SILURUS GLANIS*)^{*}

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ABSTRACT: Effect of the probiotic preparation PDFM on sheat fish yield and intestinal microflora content has been investigated in this paper. The experimental fish group was fed pelleted diet containing 40.06% of proteins with additional 50 g of probiotic per 100 kg of diet composed of 2×10^8 g of *Enterococcus faecium* bacteria. After 58 feeding days the experimental fish group achieved higher gain by 10.85% ($P < 0.01$) compared to the control one. Results of bacteriological analyses of intestinal microflora of sheat fish fry indicate that the lactic-acidic bacterium *E. faecium* affected reduction of harmful bacteria *Staphylococcus aureus*, *Escherichia coli*, as well as bacterium from the family Enterobacteriaceae and complete elimination of *Clostridium* spp. after two week feeding.

Silurus glanis; probiotics; fish nutrition; microflora; *Enterococcus faecium*

INTRODUCTION

Probiotic preparations are common complete diet ingredients in intensive monogastric animal breeding. There they serve as a preventive factor against intestinal diseases and as a positive factor for eubiosis establishing. Faster growth, better feed conversion and lower mortality were determined by the former investigations of probiotics application in fish feeding (Párova et al., 1987; Peñáz et al., 1988; Hamáčková et al., 1992a, b; Adámek et al., 1996). However, intestinal microflora content was not investigated. Taking into account the above mentioned facts, the aim of this paper was to investigate the effect of the probiotic preparation PDFM containing lyophilized, stabilized and incapsulated bacteria of the strain *E. faecium* on intestinal microflora content and sheat fish yield.

MATERIAL AND METHODS

An experiment on the bacterium *E. faecium* effect on yield and intestinal microflora was carried out from 4 July to 30 August in 1997. Probiotic PDFM contains live natural strains of the bacterial species *E. faecium*. PDFM strains were derived from American collection of microbial cultures (American Type Culture Collection). PDFM granule contains 2×10^9 g of *E. faecium*. The investigations were conducted in 6 cages where a hundred sheat fish individuals with average weight of 84.52 g (from the beginning of the second growing year) were spawned. The fish from cages 1, 2 and 3 were fed a control feed (C) whereas sheat fish fry from cages 4, 5 and 6 were fed an experimental feed (E) which contained an addition of the bacterium *E. faecium* in a dose

of 50 g per 100 kg of feed. One gram of probiotic PDFM contained 2×10^8 colonies of forming units (cfu).

Sheat fish intestine samples were analyzed five times during the experiment. Three samples from the control group and three from the experimental one were analyzed. Each sample was analyzed in 4 replications. The below mentioned microorganism groups of intestinal content were detected by bacteriological analyses:

1. Enterobacteriaceae – to EE Broth Mosel and VRBG agar, 24 – 30 h, 37 °C, oxidase-negative colonies;
2. *Escherichia coli* – to peptonin water and MacConkey Agar, 24 h, 30 °C, fluorescence, indole positive test;
3. *Staphylococcus aureus* – to Giolitti Cantoni broth and Baird Parker agar base, 48 h, 34 °C, coagulase-positive black colonies;
4. *Bacillus* spp. – to Plate-count agar, pasteurization 10 minutes at 70 °C, 72 h, 30 °C, catalase-positive rods;
5. *Clostridium* spp. – DRGM agar + overlay, pasteurization 10 minutes at 70 °C, 48 h, 37 °C, anaerobic, black colonies.

Bacteria colonies were identified, counted and expressed as average number of live bacteria per gram of intestinal content (cfu/g). The number of live cells was determined by multiplication of counted colonies with dilution factor. Dilution factor is a reciprocal value of dilution exponent. Such a value is expressed as cfu (colony forming units), i.e. units that form colonies.

RESULTS AND DISCUSSION

Average individual weight of sheat fish fry was 84.56 g in the control group and 84.46 g in the experimental one at the beginning of the experiment. At the

* Short communication presented at the 3rd Annual Kabrt Dietetics Days.

I. Values of individual biomass at stocking, fishing out and weight gain

Group	Stocked		Fished out		Mean gain \pm S.D. (g)
	number of fish	mean weight (g)	number of fish	mean weight (g)	
Control	300	84.56	285	326.23	241.67 \pm 4.007
Experimental	300	84.46	298	355.53	271.07 \pm 4.312**

** $P < 0.01$

II. Comparative representation of microorganisms in the control and experimental Sheat fish groups (cfu/g of intestinal content)

Microorganisms	Sheat fish – control					Sheat fish – <i>Enterococcus faecium</i>				
	4.07	19.07	3.08	18.08	30.08	4.07	19.07	3.08	18.08	30.08
<i>Escherichia coli</i>	4.7 ⁴	3.7 ⁴	3.1 ⁴	2.5 ⁴	3.1 ⁴	4.0 ⁴	2.9 ⁴	2.9 ⁴	1.0 ⁴	1.1 ³
Enterobacteriaceae	1.2 ⁶	2.3 ⁶	3.1 ⁶	2.7 ⁶	3.0 ⁶	1.2 ⁶	2.1 ⁵	3.0 ⁴	3.2 ⁴	1.9 ⁴
<i>Staphylococcus aureus</i>	5.1 ⁵	5.9 ⁵	3.8 ⁵	2.9 ⁵	4.7 ⁵	6.9 ⁵	2.7 ⁵	–	–	1.4 ⁵
<i>Bacillus</i> sp.	3.5 ⁸	1.0 ⁷	2.5 ⁶	6.1 ⁶	6.0 ⁶	5.1 ⁵	2.0 ⁴	1.0 ⁵	3.7 ⁵	5.6 ⁶
<i>Clostridium</i> sp.	5.2 ¹	1.1 ²	1.7 ²	–	2.1 ²	2.2 ²	1.2 ²	–	–	–

end of the investigations that lasted for 58 feeding days it was shown that fish fed a diet with addition of the bacterium *E. faecium* had body weight gains higher by 29.4 g, i.e. 10.84%. Highly significant differences in body weight gains between the control and experimental group were determined by the statistical analysis (Tab. I). Somewhat better results regarding body weight gains were reported by Hamáčková et al. (1992a, b) where probiotic Ascogen was added to sheat fish diet.

The probiotic PDFM which contained 2×10^8 /g of stable lactic bacteria *E. faecium* affected differently the qualitative and quantitative content of microflora in the sheat fish fry intestinal tract (Tab. II).

Numerical values of *E. coli* ranged within the limits of 2.5×10^4 – 4.7×10^4 cfu/g of intestinal content in the sheat fish control group during the investigation. Results of bacteriological analyses of the sheat fish experimental group intestinal content indicate *E. coli* gradual reduction by 97%. Identical results were obtained by Bogut et al. (1998) using probiotic bacteria in the carp experiments as well as Deprez et al. (1989) and Kumprecht et al. (1983, 1994) in the experiments with warm-blooded animals.

Isolated bacteria of the family Enterobacteriaceae in the control group fluctuated from initial 1.2×10^6 to 3.0×10^6 cfu/g during the investigations. Continuous application of *E. faecium* in pelleted diet of the sheat fish experimental group resulted in a highly significant enterobacteria reduction during the six week investigations from 1.2×10^6 to 1.9×10^4 cfu/g of intestinal content. Considerably higher effects of *E. faecium* on the viability of pathogenic bacterium *Staphylococcus aureus* were observed. Their density was 1.4×10^5 cfu/g, i.e. by 70% less compared to the same period of sheat fish control group, after fifty-eight feeding days. Sporogenic bacteria of the genus *Bacillus* spp. showed an outstanding resistance to probiotic *E. faecium* effi-

ciency. Their amount per gram of intestinal content fluctuated in both the control and experimental group of sheat fish during the research. Total number of surviving bacteria of the genus *Clostridium* spp. ranged from 0 – 2.1×10^2 cfu/g in the control group whereas they were completely eliminated in the experimental group fifteen days after the probiotic *E. faecium* had been consumed.

Results of bacteriological analyses of the sheat fish fry intestinal microflora indicate that most of autochthonous bacteria are dominant in the period of their intensive growth. All the factors stated by other authors (Sugita et al., 1985, 1987; Mitsuoka, 1983) affected their fluctuation during the investigated period. The results indicate that the lactic-acidic bacterium *E. faecium* consumed by the fish through pelleted diet possesses high adhesive ability in the digestive tract epithelium. It resulted in a reduction of harmful bacteria of the families Enterobacteriaceae, *Staphylococcus aureus*, *Escherichia coli* as well as *Clostridium* spp. complete elimination two weeks after the fish received pelleted diet with probiotic addition. Thus better body weight results were achieved.

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PROBIOTIC AND ENZYME PREPARATIONS – ALTERNATIVES TO ANTIBIOTIC AND CHEMICAL ADDITIVES TO DIETS FOR MONOGASTRIC ANIMALS*

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ABSTRACT: The additives with probiotic effects include: organic acids, bioplexes, mannanoligosaccharides, bacteria, live yeasts. According to their specific effects on the intestinal microflora the above additives can be divided into three subgroups (PPP):

1. Probiotics – preparations containing stabilised cultures of exactly defined microorganisms as active ingredients
2. Prebiotics – biological additives facilitating fast growth of favourable intestinal microflora (protein hydrolysates from fish and yeasts, fructooligosaccharides, etc.)

3. Paraprobiotics – additives protecting the intestinal mucosa and stimulating the immune system (glutamine)

Nowadays, active ingredients of many probiotic preparations are bacterial strains *Enterococcus faecium* M-74 or C-68. As a result of continuous supplementation of these bacteria to chicken broiler diets, significantly ($P < 0.05$) higher body weight (by 4–5%) and lower feed consumption (by 5%) were observed in comparison with controls. Up to 2.5 times higher cellulase activity in caecal contents at $P < 0.01$ was observed in broilers that were fed diets supplemented with these bacteria.

Similar results were recorded in piglets, lambs, calves and fish.

Apart from lactic acid bacteria, sporulating bacteria of the genera *Bacillus subtilis* and *B. cereus* are also active ingredients of probiotic preparations. The advantage of these bacteria is high resistance against extreme internal and external conditions. Experiments with the strains *Bacillus* C.I.P. 5832 and *Bacillus toyoi* in chicks and swine proved a significant ($P < 0.05$) stimulating effect on growth, feed consumption as well as on nitrogen metabolism parameters. In the Czech Republic, the strain *Bacillus subtilis* CCM 2216 is used for the production of probiotics.

Selected strains of yeasts *Saccharomyces cerevisiae* (S.c.) such as *S. cerevisiae* var. *elipsoideus*, *S. cerevisiae* 1026 and *S. cerevisiae* 47 are also active ingredients of probiotic preparations that exert significantly positive effects on growth and feed consumption in monogastric animals. The effects of the mentioned yeast strains were the most pronounced if administered in diets with lower levels of B vitamins; final body weight of chicks and feed conversion ratio were significantly ($P < 0.01$) improved. The isolation of mannanoligosaccharides from cell walls of *Saccharomyces cerevisiae* meant an important progress in the development of biological preparations. Diets which were fed to broilers and piglets and which were supplemented with preparations based on mannanoligosaccharides by 6–8%, achieved better final performance results than those fed to control groups. Diets which were fed to poultry and piglets and which were supplemented with combinations of either *Saccharomyces cerevisiae* + *Enterococcus faecium* or mannanoligosaccharides + *Enterococcus faecium* showed up to 10% higher production performance ($P < 0.05$) than unsupplemented control groups. The fact that a reduction in faecal nitrogen output can also be achieved with appropriate combinations of probiotic preparations, is an important finding.

In this decade, the importance of enzyme preparations has been fully appreciated.

At present, mainly preparations containing cellulase which can significantly increase fibre digestibility and enable utilisation of less digestible vegetable components as energy sources are used. Through supplementing broiler starter and production diets of standard formulation with enzyme preparations Bio Feed and Bio Feed Plus containing a complex of cellulase and amylases, we have achieved a significant increase in the growth and a reduction of feed consumption. Supplementation with Bio Feed Plus permitted the inclusion of barley in broiler production diets due to increased digestibility. The inclusion of phytase in diets for monogastric animals permits the liberation and utilisation of phytate phosphorus contained in cereals, therefore contributing to a reduction of faecal phosphorus output. Feeding diets BR1 and BR2 supplemented with the enzyme preparation Natuphos had a significant positive influence on chicken body weight while maintaining the same value of FCR. The digestibility of P and C increased by up to 13% and P output in droppings significantly decreased ($P < 0.01$). Reduced faecal P excretion was also observed in growing pigs. As part of an extensive research, the effects of the preparation Fytáza, made in the Czech Republic, were also investigated. The preparation was isolated from the strain *Aspergillus niger* 921. In trials with broilers, piglets and layers, we demonstrated that the enzyme Fytáza exerted a stimulating effect on growth, permitted a decrease of the amount of inorganic phosphorus supplemented to diets as well as the role of the preparation as an important additive decreasing phosphorus output in faeces. In monogastric animal nutrition, soybean and rape seed meals make up to 30% of the diet and they are important protein sources. Digestibility and availability of vegetable protein from these components are lower than these of protein from animal origin sources. The inclusion of enzyme preparations based on proteases can markedly increase vegetable protein digestibility. The results we have achieved with the preparation Vegpro (Alltech, USA) showed that the preparation can favourably influence not only digestibility of protein in feed but also the growth and feed consumption.

bacteria; yeasts; mannanoligosaccharides; cellulases; amylases; proteases; phytase; body weight; feed consumption; digestibility of nutrients; faecal N and P output

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CALCIUM FORMATE AND FORMIC ACID AS FEED ADDITIVES – NUTRITIVE AND PREVENTIVE EFFECTS

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ABSTRACT: This paper summarises the results of experiments observing the influence of adding formic acid and calcium formate to feed mixture on milk nutrition of calves and lambs and on the feeding and fattening of hogs and chickens.

Milk nutrition of calves by acidified milk replacer (MR) in experimental and field conditions has a significant biological influence on digestion and feeding technology. The treatment of milk by adding 2 to 3 ml of 85% formic acid ensures the storage life of the drink for 10 to 14 days. A significant decrease of abomasum pH ($P < 0.01$) was proven in fistulated calves fed on acidified MR. Values of pH 4 increase after 2 hours in these calves, whereas when fed on sweet MR the values increase 5 hours after feeding with a marked effect on GIT colonisation. Being fed this way, potentiation of lactobacilli growth and reduction of coliform germs were proven. Feeding on acidified MR affected the acid-base homeostasis with a tendency towards acidification of the inside environment, particularly in casein type of MR. According to health and productive indices, reduction in the occurrence of morbidity in calves on the farm was proven during a 2-year observation period with morbidity incidence 24–31% when fed on acidified MR, compared with 59–62% when fed on sweet MR. Total losses decreased from 11.5% or 14.9% when fed on sweet MR, to 4.6% or 7.5% when a regimen of feeding on acidified MR was applied. When fed on acidified MR, the growth intensity increased by 7.2 kg up to the 5th week, and 8–12 kg was achieved when weaned by digestion potentiation as well as by absorption increase by means of GIT colonisation in the course of feeding on acidified MR.

During the intensive fattening of lambs by acidified MR and intake *ad libitum*, growth intensity with weight gains of 180–230 g per day was achieved when 1.15–1.5 kg of MR per 1 kg of weight gain was used, and a final weight of 12–14 kg was achieved in 6th–9th week depending on the breed, weight at birth and state of health.

In the feeding and fattening of hogs, the nutritional and dietetic effect of calcium formate was proven in a concentration of 1.5% in feed mixture. In experimental conditions in equal entries, a higher conversion of feed was obtained with a consumption reduction of 360 g per 1 kg of weight gain and higher weight gains of 60 g daily and a final weight which was 7.5 kg higher in the group of pigs fed on added calcium formate. In field conditions for the fattening of hogs from private producers concentrated in large-scale breeding facilities and in stationary, the fact that increased morbidity occurrence was equal to nutritional entries proved the effect of the calcium formate additive (1.5%) on health, production and feed conversion. The experimental group at the farm (541 head in the hall) had a loss-rate of 5%, compared to 18% in the control group (447 head in the hall), average weight gains of 630 g or 590 g daily, and a feed consumption rate of 3.81 or 4.03 kg per 1 kg of weight gain.

In the fattening of chickens, experiments were carried out to verify the possibilities of a biological application for the addition of calcium formate and dried whey in nutritional prevention and digestion in chickens. We proved: a) an acidifying effect of calcium formate in feed components which was stimulated by the addition of dried whey, b) a positive effect of 0.4% calcium formate on the rate of elimination of salmonellae from the digestive tract of infected chickens and on the prevalence of lactobacilli and enterococci in the intestine of experimental chicks, c) 0.8% calcium formate failed to affect the elimination of salmonella from the chicken caecum.

calves; lambs; hogs; chickens; acidified milk replacer; calcium formate; digestion; nutritive and preventive effects

SOIL FERTILITY AT THE SCHOOL-FARM IN NOVÝ JIČÍN AND ITS EFFECT ON NUTRITION AND HEALTH OF ANIMALS

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ABSTRACT: The use of fertilisers in the Czech Republic has been declined since 1991. This may lead to the depletion of essential nutrients in the soil, and changes in soil reaction, acidity, and structure. Thus, renewal or maintenance of soil composition will be possible only by the addition of certain nutrients, especially clover. Different reasons may be given to the low application of fertilisers, for instance, significant decrease of livestock animals affected the production of fertilisers at the farm and thus influencing the production of organic fertilisers. Moreover, the financial crisis in the sphere of agriculture reduced the use of calcium by about 10% compared to 1989. Similar problems occur with the concentrations of phosphorous, potassium and magnesium. The consequence is, therefore, an increase in soil acidity and a depression of soil nutrients. The study was conducted at a school-farm (owned by the Veterinary and Pharmaceutical University) in Nový Jičín, an area lying among three districts with different weather conditions (mainly district MT 3 and MT 4, and partly MCH: the Beskydy Mountains).

The aim of this study was to assess soil reaction and agrochemical characteristics, and hence the level of fertilisation and amount of calcium since 1990 along with the production of basic crops. For the final evaluation, different forms of evidence and statistical results from the school-farm and results of analysis from Central Control and Testing Institute for Agriculture (Opava, Czech Republic) were being used.

The comparison of production parameters was conducted according to the following formula:

$$K_s = Y_s/Y_{prod}$$

Assuming that Y_s is equal to the dry matter-yield of crops, beet roots, straw, and beet tops, Y_{prod} is the yield, and K_s is the coefficient for calculating dry matter, when the harvest of dry matter is $Y_s = Y_{prod} \cdot K_s$ (the ratio was set based on long-term results of statistics).

The aim of the study was also to evaluate the balance of active carbon present in the structure of crops by means of the carbon balance method. The principle is that the volume of the active carbon calculated by means of the coefficient is equal to the volume of the dry matter of beet roots or potatoes tubers and the volume of dry matter of perennial fodder crops on arable land, ground and underground matter like rhizomes, annual fodder crops, and cereal straws.

Soil reaction: The decreased application of calcium, i.e. from 25.7% (2.65 t CaO per ha) to 14.5% (1.5 t CaO per ha) affected the soil reaction in such a way that area of lands with pH > 6.5 decreased from 48.8% to 29.6% in favour of lands with pH 5.6–6.5. Those with the pH of ≤ 5.5 remained unchanged. Even though the lower application of commercial fertilisers had a positive effect on the soil reaction, it is still important to consider the negative consequence on the soil structure and its biological activity.

According to the above table, the content of phosphorus in the soil (1989–1997) was basically low, which indicated the necessity for its correction. Regarding the concentration of potassium, the results were similar to those of phosphorus, therefore, requiring its correction. As far as magnesium was concerned, its concentration between 1989–1997 was unfavourable, although its dynamics was the least affected by fertilisation. Accordingly, to stabilise the concentrations of phosphorus and potassium, it was necessary to add 40–60 kg of P₂O₅ per ha and 60–80 kg of K₂O per ha, respectively, every year.

The above table indicates a total decrease of dry matter production, and especially its low stability and significant differences among the various lands. It is also apparent that the production of dry matter was affected by the climatic conditions of the years, which characterises the decrease of soil fertility. This was, in fact, proved by the negative balance of active carbon present in the structure of crops, which was recorded as 2 t per 1 ha of land between 1991–1995, and 0.4 t per 1 ha of land between 1996–1998 (after its correction).

The aim of the analysis was also to investigate the concentration of Ca, P and Mg in roughage and to compare the results between 1990–1998. Therefore, hay of VLP, clover and lash-grass together with corn silage and lash-grass were examined. The phosphorous concentration in individual feeds remained almost at the same level, with the exception of corn silage, where it was decreased by about 18.8%. Basically, the quality of clover hay (nutrient content) was intact, whereas the concentration of Ca and Mg in the other feeds decreased by about 20%.

Conclusion

The commencement of adverse signs indicates calcium deficiency in soil and deterioration of soil structure. This may lead to low hygienic and ecotoxicological function of the soil in the future.

Phosphorous deficiency is increasing.

The concentration of potassium is on the border-line.

It is necessary to solve the problem of Mg concentration in the soil.

The stability and production capacity of the soil is declining.

The concentrations of Ca, Mg, and partly P in feeds have been decreased.

The need of a balanced diet for animals increased the demand of incorporating supplements in feeds, and thus augmenting financial expenses.

Possible occurrence of different diseases caused by unbalanced diet.

soil fertility; soil reaction; deficiency of essential nutrients; biological soil activity; production of dry matter; balance of active carbon

REPLACEMENT OF EXTRACTED SOYBEAN MEAL BY CURED RAPECAKE IN LACTATING COWS*

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ABSTRACT: The aim of the study was to investigate the effect of feeding lactating cows with cured rape cake (CR) on lactation, selected biochemical parameters and the rumen flora. Cows were divided into two groups based on their number of lactations, season of parturition and milk yield in the first sixty days after parturition. Each group consisted of twelve cows. The experiment was conducted in three periods, of which each period was made up of 30 days. Besides basic rations (corn silage 19 kg, clover silage 2 kg and lash-grass 2 kg), the control group (C) was fed 35% of extracted soybean meal (ESM), and the experimental cows (E) 35% of cured rape cake (CR). For every litre of milk produced above 10 l/day, the milking cows received a ration of 0.4 kg/l of milk.

In the first sixty days of lactation, the feed composition was as follows (%): wheat (29), oat (12), wheat bran (20), extracted soybean meal (15), rape cake — 12% fat (20), salt (1), and mineral additives (3). At the end of the sixty days of lactation, samples of rumen fluid, blood, urine and milk were collected. Cured rape cake and extracted soybean meal were incorporated into the feed.

The metabolic parameters of the rumen in both the experimental and control cows were in the range of reference values. Although the pH in the experimental cows was slightly higher, the difference from the control group was not statistically significant (E: 6.68 ± 0.24 , C: 6.56 ± 0.12). Lower level of ammonia was detected in the rumen fluid of the experimental cows (E: 7.54 ± 1.86 , C: 8.35 ± 1.86 mmol/l). The difference between the groups was statistically significant ($P < 0.01$). This finding proved the low degradability of protein present in the cured rape cake. In each period, the level of butyric acid in the experimental cows was slightly lower. However, the difference between the groups was not statistically significant (E: 14.2 ± 1.50 , C: 15.1 ± 1.44 mol/100 mol). Metabolic parameters of the rumen fluid indicated that the fermentation process in both the experimental and control cows was very good.

The urea concentration in blood plasma and milk of the experimental cows was low compared to that of the control group (statistically very significant), both during individual periods and at the end of the experiment. This was due to the low amount of crude protein degraded in the rumen (E: 5.2 ± 1.24 , C: 7.2 ± 0.88 mmol/l, $P < 0.01$).

During each period of the experiment as well as at the end of the investigation (ninety days), the total blood lipid level was increased (statistically very significant) to 5.44 ± 0.86 and 4.13 ± 0.7 g/l in the experimental and control groups, respectively. This was attributed to the high oil content in the cured rape cake (12.8%). Similarly, the blood calcium concentration in the experimental animals was statistically very significant (E: 3.8 ± 0.79 , C: 3.1 ± 0.48 mmol/l, $P < 0.01$) during the first and second period, and also at the end of the experiment. This justified the good biological accessibility of calcium in the form of calcium salts of fatty acids available in the rape cake. Regarding the level of magnesium in the blood plasma, a statistically significant difference was noted during the third period. Low milk acidity (SH) during the II and III period ($P < 0.01$), and after the ninety-day experiment ($P < 0.05$), indicated the alkalinization of the rape cake during its processing. This was in accordance with the high pH (statistically significant) of the rumen fluid in the experimental cows.

Concerning the milk-yield, no statistical difference was observed between the experimental and control groups (E: 21.39 ± 3.98 , C: 21.99 ± 4.37 kg/day). In the first experimental period, the group which was fed SEM showed statistically very significant high milk-protein content ($3.5 \pm 0.18\%$) when compared to those that were fed CR ($3.30 \pm 0.07\%$). In fact, this was confirmed by the high milk-protein (statistically very significant) in the control group during the third period ($3.52 \pm 0.20\%$) compared to the experimental animals ($3.21 \pm 0.15\%$). Similarly, the milk-fat level in the control group (SEM) was statistically very significant ($4.41 \pm 0.58\%$) compared with the experimental one ($3.73 \pm 0.34\%$). On the other hand, no statistically significant difference in the monitored parameters was noted between the groups in the second period. The final comparison of parameters between both groups was in correlation with the results obtained during individual periods.

lactating cows; soybean extracted meal; cured rape cake; replacement; metabolism; production

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THE EFFICIENCY OF AN AFTER-CALVING DRINK ON THE DM INTAKE, MILK YIELD AND PARAMETERS OF RUMEN LIQUID AND BLOOD IN DAIRY COWS*

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ABSTRACT: The after-calving time and good nutrition during this time are very important in dairy cows. Insufficient nutrition at this time (which could, among other effects, further deepen the negative energetic balance), could be the cause of excessive utilisation of body reserves. If the physiological limits of such utilisation are exceeded, health problems, lower performance or other problems often follow. For these reasons, it is very important to reduce the impact of the negative energetic balance to minimum. Recently, after-calving drinks, aimed at improving the health condition of animals and, consequently, also at enhancing of performance, have been used.

To investigate the efficiency of a two-component energetic mineral drink of the trade mark Drench-mineral mixture G, a periodical experiment with 18 dairy cows was carried out. The animals were divided into control (C) and experimental (E) groups according to the method of analogous pairs, and were gradually introduced into each group according to the date of their calving. Three weeks before calving they were given the same feeding ration on the basis of alfalfa and corn silages, in which the contents of concentrates increased (from 2 to 3 kg per head and day) and one week before calving the dairy cows were also given 10 kg of the same feeding ration as was fed after calving. The experiment lasted for the first 42 days of lactation. After calving, the dairy cows were fed *ad libitum* with a total mixed ration (TMR) from troughs standing on tensometric scales regularly twice a day. The feeding ration contained corn and alfalfa silage, meadow hay, fresh draff, silaged and dried sugar beet pulp, corn grain, high-yielding concentrates, soybean extracted meal, fish meal, ground Ca, salt, MgO and vitamin mixture.

The results reached showed a higher DM intake which led to a statistically insignificant ($P > 0.05$) higher consumption, and through this, also to the higher consumption of nutrients in cows of the experimental group, if calculating the consumption of TMR on the basis of consumption of individual kinds of feed. The higher DM intake in group E could have been caused by the YEA-SACC¹⁰²⁶ yeast, which is also contained in the drink. Only two statistically significant parameters ($P < 0.01$) (which are connected with each other) of cows in group E were found: a higher average daily milk production and average content of FCM in milk. The difference of 1.54 kg of milk per day seems to be connected with higher feed consumption in the experimental cows. At the same time, higher content of fat in milk (by 0.45%) was manifested in the higher production of FCM by 3.87 kg (E = 37.35 kg, C = 33.48 kg). Levels of milk parameters, such as protein, lactose, acetone and urea, biochemical indexes of the rumen liquid, such as pH, the contents of volatile fatty acids (VFA) and NH_3 , and also blood parameters, such as glucose, urea and proteins, were approximately the same in both groups or within reach of the frame of physiological limits. During the experiment, there was a reduction in weight of 61.50 kg in group C, while it was 73.85 kg in group E.

By coincidence, digestive problems, which resulted in a lower activity of the rumen and especially in the decrease of the feed intake, occurred in three cows of group E and three cows of group C after calving. While the cows of group C were routinely treated by a veterinarian (unfortunately without any immediate success – they had to be eliminated from the experiment), the health condition of cows in group E, which were treated by the drink repeatedly for three days, improved and they remained in the experiment.

It may be concluded that while using the mineral drink of the trade mark Drench-mineral mixture G in dairy cows after calving, the DM intake was insignificantly higher ($P > 0.05$). It increased by 365.5 g/head/day. Statistically significant ($P < 0.01$) higher milk yield (by 1.54 kg of milk per head and day and by 3.87 kg of FCM per head and day) was stated in the experimental cows. No significant differences have been found either among indexes of the rumen liquid or among indexes of the blood plasma; the values found were mostly within the frame of physiological values.

dairy cows; DM intake; performance; after-calving mineral drink; rumen liquid; blood plasma

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INFLUENCE OF BROWN COAL, HUMIC ACIDS AND THEIR MIXTURE FED TO CALVES ON HEALTH AND FORMATION OF CHOSEN BLOOD INDEXES

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ABSTRACT: This work was aimed at determining the possibility of counteracting metabolic disorders occurring in calves by administering brown coal, humic acids or a mixture of either of these with milk to them.

The research was conducted on 4 groups of calves between 21 and 56 days old. They were kept in the same house in collective bedded pens, 14 calves in each group, and they were fed according to generally accepted standards. The following nutritional agents diluted with mother's milk were administered to the calves for two weeks: humic acids (group I), brown coal (group II), brown coal mixed with humic acids in 1 : 1 proportion (group III), control calves (group IV). The calves received 50 g of these additions daily per calf.

All calves were subject to clinical observations. Their growth was controlled by periodical weighing. The following haematological and biochemical tests were included: Hb, Ht, RCB, WBC with the use of standard methods, total protein (Tp) and its fractions by electrophoresis with the use of blotting-paper Wathman 1 and acid-base balance (ABB) parameters. Blood samples were taken on the first day of the experiment, and after 1, 3 and 6 weeks. ABB parameters were done with Phlapho 4 apparatus. Results were statistically analysed with Statgraphics version 5.0 procedure.

The use of brown coal, humic acids and their mixtures as fodder additions for calves had a beneficial influence on their health. At the beginning of the experiment all calves had diarrhoea. However, in case of calves receiving the above mentioned additions, we observed its gradual alleviation, until total remission after a few days; remission occurred earliest in the group receiving brown coal. As it is known, diarrhoea causes organism emaciation, inhibits the growth and irrespective of the causes, leads to dehydration, electrolyte losses, metabolic acidosis and hypoglycaemia.

Brown coal added to fodder had the most beneficial influence on the calves' growth, since daily body mass growth (0.580 kg) occurred; smaller growth was observed in the calves receiving mixture of brown coal and humic acids (0.515 kg) and the smallest – even smaller than the control group (0.480 kg) – in the calves receiving only humic acids (0.309 kg). Various authors recommend gains within the range from 0.600 to 0.800 kg/day/calf and the ones obtained in our own experiment were close to these only in the group receiving brown coal.

Levels of the determined haematological parameters (Ht, Hb, red and white cell counts) were within the physiological standards range. We did not observe any clear relation of their levels with fodder additions administered to the calves.

Content of total protein in the calves' blood plasma on the first day of the experiment was from 50.07 g/l in group III to 58.33 g/l in group I. During the research period we observed a highly significant ($p \leq 0.01$) increase in the total protein content in blood plasma of the calves in group III which was 19.13, and 11.53 in group II ($p \leq 0.05$). In case of the other groups the level of Tp did not demonstrate any significant changes.

From all protein fractions, only γ -globulin levels in the calves' blood plasma showed a relation to the administered preparations. During the research period γ -globulin level increased in all groups but it was highest (9.05 g/l) and statistically significant ($p \leq 0.01$) in group III. It was also distinct in the remaining groups, I (6.8 g/l) and II (6.22 g/l). The smallest increase was observed in the control group (4.26 g/l). The increase of γ -globulin level in the group receiving humic acids and brown coal (III) may be a testimony to their beneficial influence on the calves' immunological system.

While investigating the data concerning ABB parameters at the beginning of the experiment, we found chronic respiratory acidosis in groups I, II and IV with the most pronounced one in group II; group III had, during the first test, partly compensated metabolic alkalosis. From the fodder additions used, humic acids had the most beneficial influence on ABE blood plasma parameters since the said parameters reached an equilibrium. The addition of brown coal in the mixture of group II acted as a buffer for 3 weeks, that is during the period during which it was administered, but in the fourth test ABB state shifted towards respiratory acidosis. The addition of brown coal and humic acids in group III alleviated metabolic alkalosis in the second test, however, this group demonstrated irregular shifts of the examined ABE parameters in the next stages of the experiment. In group IV, which did not receive any additions, the acidosis intensified further. These results prove efficiency of brown coal and its mixtures with humic acids as the agents alleviating ABE disorders in calves in the postnatal period.

Conclusion:

1. We found diarrhoea symptoms in most of the calves chosen for experiment during clinical observation. All the fodder additions used had anti-diarrhoeic effects and these were most pronounced in case of brown coal and its mixture with humic acids.
2. The fodder additions used for calves did not have a significant influence on the levels of the examined haematological

parameters which demonstrated irregular shifts during the experiment period.

3. We observed increase of T_p level in the calves' blood plasma during the research period; it was highest (19.13 g/l) in the calves receiving mixture of humic acids with brown coal ($p \leq 0.01$) and lowest in the calves receiving only humic acids (1.47 g/l).

4. From all the examined protein fractions the most significant changes occurred in γ -globulin levels; their content in the calves' blood plasma increased. As with T_p, the increase was the greatest in the calves receiving a mixture of humic acids with brown coal (9.05 g/l, $p \leq 0.01$) and the lowest in the control group (4.26 g/l).

5. From all the fodder additions used, the humic acids addition had the most beneficial influence on the ABE parameters since it permanently stabilised their values. The brown coal addition with humic acids and brown coal itself were effective only during the administration period and the acidosis which occurred in the control group was not compensated during the experiment period.

calves; daily gain; brown coal; humic acid; haematological parameters; acid-base balance

THE SPECIFICITY OF ASSESSMENTS IN VEGETATION GRAZED BY SHEEP IN THE MOHELENSKÁ SERPENTINE STEPPE

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ABSTRACT: The study assesses sheep grazing in the Mohelenská Serpentine Steppe with regard to the enlarging biodiversity of the given habitat. On the steppe grazed, the variability of species of steppe vegetation was assessed as related to site conditions. Phytocentological investigations were carried out three times a year during 1997 to 1999 at five selected sites. The feeding (nutritional) value and toxicity of selected species at the sites under study were assessed according to Jurko (1990). In the grazing sheep flock, the behaviour of animals was also assessed in relation to their daily activity and to site conditions. Sheep returned to the Mohelenská Serpentine Steppe on 15th May 1997 and the proper testing includes the period after grazing was renewed at the turn of May to June 1997. During this period, the area grazed included five sites. The evaluation is presented in the Table:

Site	Species abundance (coverability ≥ 1)								partly harmful	allergenic
	in total	nutritional value						*		
		1	2	3	4	5	*			
C5	5	41 ¹⁾	1			1		1 ²⁾	1	2
D8	8	4	1			1		2	1	2
H10	5	4	1					2	2	1
C10	5	3	1			1		1	1	2
B17	10	5	2		1			3	2	1

1) nutritional value 1 – inferior; 2 – low up to medium; 3 – good; 4 – very good; 5 – excellent

2) if variable in certain species, e.g. 1–2, both levels are observed

* – at higher ration (above 3%), it can be unsuitable, harmful up to very harmful

The values presented in the table show that most species assessed are of inferior feeding value regarding the nutrition of animals. It is typical of the area under study that the more valuable species: *Poa pratensis* (L.) – 5, *Arrhenatherum elatius* (L.) – 4, *Poa pratensis angustifolia* (L.) – 4 are not endemic to the habitat assessed in the numbers presented. Their growth suppresses xerothermic species, and therefore they should be reduced by grazing. This should be supported by the fact that sheep select higher-quality vegetation, as confirmed by findings within ethological assessments. Therefore, the grazing in the steppe will be aimed, paradoxically given the animal nutrition, at the decrease of nutritional value in the vegetation grazed or even at the increase of harmful or toxic species. In the set under study, the occurrence of partly harmful species was registered only in six cases as yet.

The specificity of sheep grazing in the Mohelenská Serpentine Steppe is aimed at enlarging the species biodiversity in the area grazed. This can lead to a decrease in nutritional value in the vegetation grazed and to an eventual increase in the occurrence of harmful or toxic species. For these reasons, increased attention has to be paid to grazing in this habitat.

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sheep grazing; protected territories; growth species variability; nutritional value; toxicity; site ethology

THE USE OF MODIFIED RAPE CAKES IN THE FATTENING OF PIGS*

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ABSTRACT: Pigs (Bu x L x H x Pn crosses) with an average weight of 28 kg were included into a 124-day fattening experiment and were divided into one control and three experimental groups of thirty animals in each. In the experimental groups P-1, P-2 and P-3, soya extracted meal in feed mixtures was replaced in 33, 66 and 100% by rape cakes, modified by chemicals and heat, with glucosinolate contents under 1 mmol/kg.

Live weight at the end of the fattening period was higher in pigs of group P-2 and significantly higher ($P \leq 0.05$) in pigs of group P-3 compared with the control group. A similar trend was observed in yield and average daily gains (Tab. I). Feed consumption per 1 kg of weight gain was higher in all the experimental groups compared with the control. The consumption of nitrogen substances per 1 kg of weight gain was lower in the experimental groups compared with the control but consumption of MEp per 1 kg of weight gain was higher in the groups P-2 and P-3 compared with the control (Tab. II).

I. Average live weight, yield and average gain of live weight in pigs

	Live weight (kg)		Yield (%)		Daily weight gain (kg)	
	$\bar{x} \pm s$	Index	$\bar{x} \pm s$	Index	$\bar{x} \pm s$	Index
Control	124.1 ± 14.94	100.0	78.2 ± 0.46	100.0	0.811 ± 0.0958	100.0
P-1	116.8 ± 17.57	94.1	78.0 ± 0.59	99.7	0.743 ± 0.1106	91.6
P-2	132.9 ± 17.34*	107.1	78.4 ± 0.38	100.3	0.869 ± 0.1309	107.1
P-3	128.4 ± 16.00	103.5	78.3 ± 0.36	100.1	0.810 ± 0.1293	99.9

II. Consumption of the complete feed mixtures (CFM), feed conversion and usage of nutrients per 1 kg of weight gain

	Consumption of CFM (kg/head/day)		Consumption of CFM (kg/l kg of weight gain)		Consumption of N-substances (g/l kg of weight gain)		Con. of MEp (MJ/l kg of weight gain)	
	\bar{x}	Index	\bar{x}	Index	\bar{x}	Index	\bar{x}	Index
Control	2.69	100.0	3.37	100.0	574.9	100.0	44.75	100.0
P-1	2.42	90.0	3.44	102.1	494.3	85.5	44.00	98.3
P-2	3.04	113.0	3.53	104.7	526.7	91.1	47.05	106.1
P-3	2.83	105.2	3.52	104.4	472.4	81.7	46.78	104.5

III. Weight of the thyroid gland, levels of triiodothyronine (T₃) and thyroxine (T₄) in blood serum

	Weight of thyroid gland (g)		T ₃ (nmol/l)		T ₄ (nmol/l)		T ₃ /T ₄ (x 1 000)	
	\bar{x}	<i>t</i>	\bar{x}	<i>t</i>	\bar{x}	<i>t</i>	\bar{x}	<i>t</i>
Control	9.1	–	1.54	–	58.9	–	26.8	–
P-1	9.2	0.1665	1.39	2.0665	44.4**	4.6792	31.7	3.3240
P-2	11.1	2.8077	1.35	2.5529	48.2	3.2726	28.2	0.8968
P-3	11.9*	3.9750	1.18**	5.0646	67.1	2.6464	17.9**	6.0140

* $P \leq 0.05$; ** $P \leq 0.01$

The average weight of the thyroid gland was higher in pigs of all the experimental groups compared with the control, the difference being highly significant ($P \leq 0.05$) in group P-3. The levels of thyroid gland hormones, especially triiodothyronine

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(T₃), were lower in all the experimental groups compared with the control, the difference being significant ($P \leq 0.01$) in group P-3. Group P-1 had highly statistically significantly ($P \leq 0.01$) lower thyroxine (T₄) level compared with the control (Tab. III).

The use of 10 and 15% of modified rape cakes with low glucosinolate contents in feed mixtures resulted in the increase of the thyroid gland weight by about 25%. Goitrogenic effect of the modified rape cakes is also suggested by the difference in hormone levels.

glucosinolate; triiodothyronine; goitre; thyroid gland; weight gain; feed conversion

THE DEVELOPMENT OF LEGAL RULES CONCERNING VETERINARY COMPETENCE IN ANIMAL NUTRITION

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ABSTRACT: Legal rules in animal nutrition are topical problems in veterinary medicine at present. This reality is documented by many legal rules concerning animal nutrition in the Czech Republic and also in the European Union. Veterinary competence is very important in legal rules concerning animal nutrition. However, veterinary competence is presented only in several legal rules. The analysis of veterinary competence from the point of view of animal health and subsequently human health can outline the position of state veterinary administration in animal nutrition.

The aim of this report is to analyse legal rules concerning veterinary competence in animal nutrition from last century to the present time in Czech Republic.

This report comes from the law of Austro-Hungary, Czechoslovak Republic, Czech-Slovak Republic, Protectorate of Bohemia and Moravia, Republic of Czechoslovakia, Czechoslovak Socialist Republic, Czech and Slovak Federal Republic, and Czech Republic. We especially analysed the act No. 187/1950 Coll., No. 99/1952 Coll., then No. 66/1961 Coll., No. 154/1961 Coll., No. 69/1974 Coll., then No. 87/1987 Coll., No. 108/1987 Coll., No. 117/1987 Coll., No. 118/1987 Coll., No. 121/1987 Coll., and the amendments of veterinary act especially in act No. 79/1997, No. 110/1997. The reason for this report is the proposed new veterinary act of this time (government version).

Acts have been called into question on the basis of content and form, especially concerning veterinary competence in animal nutrition. An advance in legal rules regarding veterinary competence in animal nutrition was noted and then a general drift of legal rules in animal nutrition was deduced from the point of view of veterinary competence.

In the development of legal rules in animal nutrition, we can see a period of no veterinary competence in animal nutrition, a period of veterinary competence in animal nutrition concerning feeding including hygiene and dietetics, a period of veterinary competence in animal nutrition concerning feeding including the hygiene and dietetics and of veterinary competence in feed production, feed processing, stocking and transport of feed, and a period of veterinary competence in animal nutrition in feeding including the hygiene and dietetics and of no veterinary competence in feed production, feed processing, stocking and transport of feed. The trend of reduction in veterinary competence in feed production, feed processing, stocking and transport of feed in the proposed new veterinary act (government version) is not a fully equivalent contribution. Veterinary supervision has to include all controls in animal nutrition including feed production, feed processing, stocking and transport of feed.

legal rules; animal nutrition; veterinary competence; veterinary supervision

CHANGES IN BIOCHEMICAL PARAMETERS DURING DIFFERENT FEEDING PROGRAMS FOR BROILER CHICKENS

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ABSTRACT: The aim of the study was to follow selected biochemical parameters during the feeding of chickens until the highest possible live weight was attained. The experiment was conducted on hybrid chickens Ross 208 for 62 days. Sixty-four chickens (one-day old) were used for the experiment. They were divided into two groups according to their sex (32F + 32M) and followed separately. Each group was subdivided into two subgroups: control (C), experimental (E). Chickens were fed "complete" diets *ad libitum*; i.e. until day 21 with feed mixture (BR 1) and later until day 62 with granulated form (BR 2). The diet for group E was supplemented with a specially manufactured chicken meal.

Chickens were weighed individually. Blood for biochemical examination was collected from the *v. basilica* (by puncture) on days 21, 42, 52 and 62, between 7 and 8 o'clock in the morning.

There was a statistically very significant difference ($P \leq 0.01$) in the total plasma protein between the hens of groups C and E on day 21 (C: 24.71 g/l and E: 26.82 g/l) and 62 (C: 38.74 g/l and E: 43.97 g/l) compared to day 42 (C: 32.66 g/l, E: 33.38 g/l) and 52 (C: 36.32 g/l and E: 38.58 g/l). Similarly, a statistically very significant difference was noted ($P \leq 0.01$) in the plasma glucose level between groups C and E (C: 12.16 mmol/l, E: 13.77 mmol/l; and C: 18.03 mmol/l, E: 16.93 mmol/l) on the 21st and 62nd day of age. On the other hand, the differences detected on day 42 (C: 12.08 mmol/l; E: 12.70 mmol/l) and 52 (C: 16.93 mmol/l; E: 16.91 mmol/l) were not statistically significant. In the case of plasma cholesterol (hens), a statistically very significant difference was observed at the 42nd (C: 2.48 mmol/l, E: 2.14 mmol/l) and 52nd day of age (C: 3.22 mmol/l, E: 2.52 mmol/l). However, the differences noted on the 21st (C: 2.14 mmol/l, E: 2.11 mmol/l) and 62nd day of age (C: 3.50 mmol/l, E: 3.59 mmol/l) were not statistically significant. The difference in AST concentration in hens between groups C and E on day 21 (C: 0.76 μ kat/l, E: 0.82 μ kat/l), 52 (C: 0.89 μ kat/l, E: 1.01 μ kat/l) and 62 (C: 0.93 μ kat/l, E: 1.06 μ kat/l) ranged from statistically significant ($P \leq 0.05$) to very significant ($P \leq 0.01$) value. However, on the 42nd day of age, differences between C and E (C: 0.91 μ kat/l, E: 0.94 μ kat/l) were not statistically significant. The ALT concentration throughout the experiment was in the range of 0.1–0.15 μ kat/l, and no statistically significant differences were noted between groups C and E on day 21, 42, 52, and 62. In hens, the characteristic change was in the plasma calcium level on day 21 (C: 2.3 mmol/l, E: 2.59 mmol/l), 42 (C: 2.47 mmol/l, E: 3.10 mmol/l), and 62 (C: 2.78 mmol/l, E: 2.59 mmol/l). During this period, the differences observed between the groups were statistically significant ($P \leq 0.05$) or even statistically very significant ($P \leq 0.01$). A statistically significant ($P \leq 0.05$) difference on the level of plasma phosphorus was noticed on day 42 (C: 4.50 mmol/l, E: 3.34 mmol/l) and 62 (C: 3.68 mmol/l, E: 4.48 mmol/l). No statistically significant difference was observed in the magnesium concentration between groups C and E.

In the case of cocks, a statistically significant difference ($P \leq 0.05$) on the level of plasma protein was recorded between the groups only on day 21 (C: 25.53 g/l and E: 23.69 g/l). However, on day 42 (C: 32.34 g/l, E: 30.88 g/l), 52 (C: 39.68 g/l, E: 38.54 g/l) and 62 (C: 41.74 g/l, E: 40.29 g/l), the difference between groups C and E was not statistically significant. A statistically very significant ($P \leq 0.01$) difference in the plasma glucose level was only detected on day 62. On day 42 (C: 2.27 mmol/l, E: 2.02 mmol/l), 52 (C: 2.77 mmol/l, E: 3.08 mmol/l) and 62 (C: 3.22 mmol/l, E: 2.78 mmol/l), changes in the plasma cholesterol level were recorded between groups C and E, where the difference was statistically very significant ($P \leq 0.01$). Difference in AST concentration between groups C and E was recorded as statistically very significant on day 42 (C: 0.84 μ kat/l, E: 0.97 μ kat/l) and 52 (C: 0.90 μ kat/l, E: 0.96 μ kat/l). Statistically very significant differences ($P \leq 0.01$) were observed in the ALT concentration on the 21st (C: 0.19 μ kat/l, E: 0.09 μ kat/l) and 52nd day of age (C: 0.12 μ kat/l, E: 0.10 μ kat/l). On day 62, the difference in the plasma calcium level between groups C (2.78 mmol/l) and E (2.64 mmol/l) was statistically significant ($P \leq 0.05$), whereas on day 42, the difference between groups C (2.65 mmol/l) and E (3.11 mmol/l) was statistically very significant ($P \leq 0.01$). Concerning plasma phosphorus level, statistically very significant differences between the groups were recorded on day 52 (C: 3.42 mmol/l, E: 2.35 mmol/l) and 62 (C: 3.63 mmol/l, E: 5.01 mmol/l). A statistically very significant difference was observed in the magnesium level on the 21st (C: 0.99 mmol/l, E: 0.80 mmol/l) and 62nd day of age (C: 0.75 mmol/l, E: 0.83 mmol/l).

Basically, apparent changes in selected biochemical parameters were observed throughout the experiment (until the 62nd day of feeding) between groups C and E in both hens and cocks. The supplement of chicken meal did not markedly influence the ALT and plasma magnesium level in hens (statistically not significant). In cocks, a statistically significant difference ($P \leq 0.05$) in the plasma protein was noted only on the 21st day of age. In both sexes, characteristic changes occurred, especially in Ca, P, and Mg. This may be attributed to the addition of chicken meal.

Thus, it is possible to state that in both sexes with the increase of chicken's age, the concentrations of plasma protein, glucose and cholesterol were also increased.

chicken; feeding; blood; biochemistry

CHANGES IN HEMATOLOGICAL PARAMETERS DURING DIFFERENT FEEDING PROGRAMS OF BROILER CHICKENS

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ABSTRACT: The aim of the study was to follow certain selected haematological parameters during the feeding of broiler chickens until the highest live weight was attained. The experiment was performed on hybrid chickens ROSS 208 for 62 days. Sixty-four chickens, one-day old, were used for the experiment. They were divided into two groups according to their sex (32F + 32M) and followed separately. Each group was divided into two subgroups: control (C), experimental (E). Feeding was *ad libitum* with a "complete" diet. Until day 21, they were given concentrate (BR 1), and later until day 62, granulated form (BR 2). A specially manufactured supplement of chicken meal was added to the feed of group E.

Chickens were weighed individually. Blood samples were collected from the *v. basilica* by puncture on days 21, 42, 52, and 62 for haematological examinations.

Results of haematological examinations showed that there were characteristic changes in haematological parameters both in hens and cocks.

In hens, a statistically very significant ($P \leq 0.01$) difference in the total erythrocyte count was observed between groups C and E only on day 52 (C: 2.20 T/l, E: 1.66 T/l). However, on day 21 (C: 1.79 T/l, E: 2.07 T/l), 42 (C: 1.85 T/l, E: 1.88 T/l) and 62 (C: 1.76 T/l, E: 1.66 T/l), the difference was not statistically significant.

On day 21 and 52, the difference in the haematocrit value between groups C and E was statistically very significant ($P \leq 0.01$) (C: 0.30 l/l and E: 0.28 l/l; C: 0.31 l/l and E: 0.29 l/l). On day 62, the difference was only statistically significant ($P \leq 0.05$) (C: 0.29 l/l and E: 0.27 l/l). On the 42nd day of age, no statistically significant difference was noted between groups C: 0.28 l/l and E: 0.29 l/l.

Regarding the haemoglobin level, a statistically significant difference between groups C (100.63 g/l) and E (88.17 g/l) was observed only on day 52. On day 21 (C: 100.20 g/l, E: 100.48 g/l), 42 (C: 80.40 g/l, E: 89.59 g/l) and 62 (C: 75.93 g/l, E: 79.13 g/l), no statistically significant difference was noticed.

On the 21st day of age, the difference in the total leukocyte count between groups C (12.44 G/l) and E (15.81 G/l) was $P \leq 0.01$, and on day 52, the difference was $P \leq 0.05$ (C: 15.69 G/l and E: 18.88 G/l). However, on day 42 and 62, the differences between the groups were not significant (C: 19.94 G/l and E: 17.88 G/l, C: 18.06 G/l and E: 21.31 G/l).

Results in cocks were different. No statistically significant difference in the total erythrocyte count was observed between groups C and E on day 21 (C: 1.89 T/l and E: 1.83 T/l), 42 (C: 1.68 T/l, E: 1.73 T/l), 52 (C: 1.80 T/l, E: 1.82 T/l), and 62 (C: 1.66 T/l, E: 1.59 T/l).

On day 21, a statistically very significant ($P \leq 0.01$) difference was observed between groups C: 0.30 l/l and E: 0.27 l/l. While on day 42 and 62, the difference was statistically significant ($P \leq 0.05$) (C: 0.30 l/l and E: 0.27 l/l) and (C: 0.28 l/l and E: 0.30 l/l). The 52nd day was exceptional as no difference was recorded (C: 0.29 l/l, E: 0.31 l/l).

With respect to haemoglobin level, a statistically very significant difference between groups C (71.48 g/l) and E (108.56 g/l) was noted only on the 62nd day of age. On the other hand, on day 21 (C: 90.36 g/l, E: 81.91 g/l), 42 (C: 86.52 g/l, E: 89.59 g/l), and 52 (C: 86.57 g/l, E: 94.65 g/l), no statistically significant difference was recorded.

Therefore, in cocks, statistically very significant differences ($P \leq 0.01$) were observed on day 21 and 42 (C: 10.69 G/l and E: 13.38 G/l; C: 14.06 G/l and E: 18.50 G/l), while on day 52 and 62, the differences were not statistically significant (C: 14.75 G/l and E: 15.75 G/l; C: 16.56 G/l and E: 19 G/l).

Significant differences in the parameters of red and white blood cell count were observed between the control and experimental groups. In hens, changes were observed rather at the end of the experiment, especially on day 52, when statistically significant ($P \leq 0.05$) and very significant ($P \leq 0.01$) differences in the total erythrocyte count, haematocrit, and haemoglobin content were recorded between the control and the experimental broilers. On the other hand, in cocks, statistically significant differences in haematocrit and total leukocyte count was noted on day 21, 42 and 62; and in the haemoglobin level on the 62nd day of age.

chickens; feeding; haematology

THE QUALITY OF OSTRICH MEAT

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ABSTRACT: Weight and nutrient contents of meat from ostriches were monitored for 14 and 16 months in blue neck ostriches. The ostriches were fed with a combined type of feed ration, which consisted of volume fodders and growth feed mixture. The composition of the feed ration during the summer feeding: 1.2 kg feed mixture, 0.5 kg wheat, 3 to 4 kg alfalfa, 0.4 kg alfalfa hay. The feed rations used in winter: 1.2 kg feed mixture, 0.5 kg wheat, 3 kg fodder beet, 0.6 kg alfalfa hay.

I. Carcass, weight and relative portion of individual characteristics of ostrich body

Parameter	kg	%
Live weight	89.50	
Carcass	56.45	
Carcass yields		69.47
Heart	1.25	1.43
Liver	1.60	1.78
Abdominal fat	4.15	4.63
Carcass after 24 hours at 0 °C	54.45	
Thin meat	31.15	57.21
Bones	14.25	26.17

II. Chemical composition of ostrich meat

Parameter	Dry matter	Protein	Fat	Ash	Cholesterol	Acid number of fat
	g/100 g				mg/100 g	mg KOH/g
Thigh muscles	25.41	20.66	1.59	1.09	65.9	–
Liver	27.88	20.46	2.12	1.34	1 153.35	–
Heart	22.08	17.79	0.48	1.14	243.99	–
Back fat	93.87	–	92.12	–	1 415.13	14.41
Abdominal fat	93.54	–	91.21	–	1 326.12	16.59

The average live weight of ostriches was 89.50 kg. The carcass weighed 56.45 kg and carcass yields were 69.47%. The ostrich heart weighed 1.25 kg (1.43%) and the liver 1.60 kg (1.78%). Abdominal fat weighed 4.15 kg (4.63%). The carcass reached 54.45 kg after 24 hours at 0 °C. The lean meat from the whole ostrich weighed 31.15 kg and the bones 14.25 kg. 100 g of the thigh muscles contains 25.41 g of dry matter, 20.66 g of proteins, 1.59 g of fat, 1.09 g of ashes and 65.9 mg of cholesterol. 100 g of the liver contains 27.88 g of dry matter, 20.46 g of protein, 2.12 g of fat, 1.34 g of ashes and 1 153.35 mg of cholesterol. 100 g of the heart contains 22.08 g of dry matter, 17.79 g of protein, 0.48 g of fat, 1.14 g of ashes and 243.99 mg of cholesterol. 100 g of the back fat contains 93.87 g of dry matter, 92.12 g of fat, 1 415.13 mg of cholesterol and acid number of fat is 14.41 mg KOH/1 g. 100 g of the abdominal fat contains 93.54 g of dry matter, 91.21 g of fat, 1 326.12 mg of cholesterol and acid number of fat is 16.59 mg KOH/1 g.

ostrich; meat; quality; chemical composition

HOW TO EVALUATE THE RESULTS OF RESEARCH: SEMINARS, CONFERENCES AND CONGRESSES

Information and advice for postgraduate students and new research workers. The importance of active participation in seminars and conferences.

JAK HODNOTIT VÝSLEDKY VÝZKUMU: SEMINÁŘE, KONFERENCE, KONGRESY

Dobře organizovaná a obsazená setkání lidí s vyhraněným zájmem mají velký význam pro každého, kdo přichází předněst svoje výsledky s cílem ověřit si reakci odborné a zasvěcené veřejnosti, poučit se z připomínek diskutujících a vyvolat zájem o spolupráci. Toho může dosáhnout jen ten, kdo se neomezí na svoje vlastní vystoupení, ale kdo je aktivní i při vystoupení kolegů, řešících stejnou problematiku. Nejde o exhibicionismus nebo o diskusí o všem za každou cenu, to na odborníky neplatí. Znalosti, nabídka spolupráce a ochota spolupráci přijmout jsou však dobrou cestou k účinnému využití mezinárodního setkání. Jeho výsledkem může být později publikace, zpracovaná kvalitním mezinárodním týmem, pozvání k pobytu na dobrém pracovišti, získání zájemce o pobyt na pracovišti vlastním, pozvání k přednesení vyžádané přednášky, pozvání k účasti na pořádání příštích kongresů nebo pověření moderováním některé jejich sekce. To vše jsou významná uznání, která nepřicházejí bez zásluhy a která většinou nejsou formální nebo účelová. Měla by to také být dlouhodobá kritéria úspěchu zahraničních cest.

Pouhé vystoupení na zahraniční konferenci nebo vyvěšení plakátového sdělení nelze považovat za využití výsledků výzkumu, rovnocenné jejich publikaci v časopisu. Až na výjimky nejsou přihlášky k účasti nijak tříděny a podrobovány hodnocení kvality. Každý registrovaný účastník je vítaným plátcem, proto pouze vyžádané přednášky s úhradou nejen účastnického poplatku, ale i pobytu a někdy dokonce i cestovního pořadatelem, jsou známkou kvality pozvaného. V tomto případě se však od pozvaných řečníků většinou očekává souborné a kritické shrnutí tématu a nikoliv prezentace vlastních experimentálních výsledků. Zveřejnění výsledků experimentální práce na konferenci je určitým rizikem ztráty priority, pokud publikace není již v pokročilém stadiu přípravy nebo v tisku. Toto riziko je však nejen přijatelné, ale spíše nezbytné, protože konfrontace názorů autorů s názory odborné veřejnosti může být pro správnou interpretaci výsledků velmi důležitá. Co nejrychlejší uveřejnění výsledků v dobře zvoleném časopisu je však vždy nezbytné. Výsledky, které jsou za několik let po přednesení citovány jejich vlastními autory jako abstrakty ve sborníku z konference, stejně

jako výsledky, uvedené pouze ve zprávě o řešení projektu, jsou výsledky ztracené nebo dokonce špatné. Náročný časopis takovou citaci nepřijme, protože by byl ohrožen negativním hodnocením kvality jeho lektorského řízení.

Svoboda pohybu přinesla ohromnou změnu i v možnostech pracovníků výzkumu zúčastnit se konferencí kdekoli na světě a naši řešitelé projektů to plně využívají. Dovedou svoje požadavky většinou velmi přesvědčivě zdůvodnit a cesty jednoho pracovníka za 100 až 250 tisíc korun ročně nejsou příliš vzácné. Skoro bych se bez rizika vsadil, že rekord bude o hodně vyšší a že jeho držitelem nebude pracovník odborné právě nejproduktivnější. Je třeba počítat i s tím, že kromě odborného a pracovního významu, který je při řádném využití všech nabízených příležitostí nesporný, jsou k služebním výjezdům do zahraničí i další subjektivní důvody. Kromě příjemné změny prostředí a nového poznání je možno při trošce skromnosti z diet něco málo ušetřit. Pro naše většinou mizerně placené lidi ve státní službě to je nepochybně významné. Pokud však svoji spořivost přeženou, omezí si možnosti využití příležitosti, kterou jim cesta poskytuje. Spolupráce se navazuje nejen na samotných jednáních, ale i při jiných příležitostech, za které se mnohdy musí platit. Bez rizika není ani přijetí pozvání na sklenici piva nebo na večeři. Obvykle se očekává reciprocita a ta něco stojí. Takže chudý nebo spořivý účastník kongresu, který se po přednáškách trochu projde kolem hotelu a tráví večery v hotelovém pokoji u kufru s proviantem, má menší šanci uspět v navazování kontaktů. Přitom mnoho z žadatelů o souhlas s účastí na zahraničním kongresu ve vztahu k vysílající organizaci nevyvíká mimořádnou skromností, která by odrážela ekonomickou situaci jejich pracovišť nebo jejich úsilí, dosáhnout s dostupnými prostředky co nejvíce nových poznatků. Často není žádná cílová země dost daleká a žádný účastnický poplatek nepřiměřeně vysoký. Naopak předpisy, umožňující vyplacení kapesného, bývají prostudovány a uplatňovány velmi dobře. Pozornost však většinou není věnována studiu informací, na jejichž základu by bylo možno v navštívené zemi získat co nejvíce poznatků i mimo konferenci a které by nadřazeného nebo vysílající agen-

туру přesvědčily o oprávněném požadavku na krátké prodloužení pobytu nebo o zajištění do jiného města k návštěvě důležitého partnera.

Tak jako se nelze při zdůvodňování nedostatku publikací vymlouvat na politickou diskriminaci za deset let po odstranění překážek v zaslání rukopisů do mezinárodních časopisů (i tyto překážky však postihly jen některé a zdaleka ne všechny pracovníky výzkumu), nelze využívat řadu let možnost cestovat po celém světě a zůstat u mírně obměňovaného plakátového sdělení a abstraktu ve sborníku. Bez publikací v časopisech, bez snahy o získání zahraničních grantů a bez zřetelných výsledků v navazování kontaktů, vedoucích k významnému zlepšování schopností řešit problémy a získávat finanční prostředky, jsou náklady na zahraniční cesty výdajem, který by pracoviště měla důkladně zvažovat.

K povinnostem účastníka zahraniční služební cesty patří (nebo by patřit měla) i dobrá příprava, která musí, kromě pečlivě připraveného plakátového sdělení nebo referátu s kvalitními diapozitivy nebo obrázků a souborů k projekci z počítače, zahrnovat i oživení znalostí základních údajů o navštívené zemi a o pracovištích, která by stálo za to navštívit. Dnes jsou většinou tyto informace snadno dostupné na domácích stránkách v internetu a každý je může využít a předem si zajistit rozšíření programu pobytu tak, aby mu přinesl co nejvíce poznatků. Každý, kdo se připravuje na cestu do zahraničí, by měl využít také poznatky svých kolegů, kteří byli ve stejné zemi před ním. K tomu může nejlépe posloužit dobře zpracovaná a dostupná zpráva o služební cestě do zahraničí, jejíž předložení musí patřit k povinnostem účastníka cesty. Průběh cesty, přednesená sdělení a reakce na ně, důležité poznatky o vývoji oboru a poznatky z návštěvy pracovišť, případně kladné i záporné připomínky k organizaci akce by měly být ve zprávě stručně a přehledně shrnuty. Jako součást

zprávy by v knihovně pracoviště měly být uchovávány i kopie navštívenek lidí, s nimiž byla vedena významnější jednání a získané informační materiály, výroční zprávy a propagační materiály navštívených pracovišť. Účast na kongresu nebo jiná zahraniční služební cesta není soukromou záležitostí, ale reprezentací sebe, svého pracoviště a České republiky. Na uskutečněnou návštěvu mnohdy mohou navazovat další jednání, která musí zajistit vedení ústavu nebo jiní pracovníci, protože autor zprávy třeba již na pracovišti nepůsobí. Často však zpráva poslouží i jejímu autorovi, až si nebude moci vzpomenout, koho před rokem vlastně navštívil a kde má jeho navštívenku. Nejde o sepisování podrobné informace podle požadavků někdejších kádrováků a estébáků, jako byla jména všech emigrantů, s nimiž mohl být spatřen při nástupu do stejného městského autobusu nebo čestné prohlášení, že po dobu cesty nebyly porušeny normy socialistického chování. Pro mladé, kteří to nezažili a sotva si to dovedou představit nutno připomenout, že to znamenalo že se účastník cesty na recepci neopil a nepomlouval politické zřízení ve své vlasti nebo že nepodepsal spolupráci s nepřátelskou rozvědkou. Samozřejmě že nečekali, že se k opilosti a k následně ztrátě paměti velikosti výkladní skříně Marks & Spencer někdo ve zprávě přizná. Pokud to však na někoho později prasklo, mohlo se prohlášení o bezúhonnosti náramně hodit k jeho potopení, nebo naopak k získání pro spolupráci.

K nákladům na zahraniční cestu, která většinou trvá nejméně týden, je třeba přidat dva až tři týdny přípravy a několik dní opětovné pracovní aklimatizace po návratu, kdy je významně narušen běžný pracovní program. A jsme tam, kde jsme začali: pokud není zahraniční služební cesta opakovaně dobře využita a navíc přednášené výsledky ani nebyly publikovány, nejsou finanční prostředky na řešení projektu vynakládány účelně.

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