

ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

Czech Journal of
ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

3

VOLUME 44
PRAGUE
MARCH 1999
ISSN 1212-1819

An international journal published under the authorization by the Ministry of Agriculture and under the direction of the Czech Academy of Agricultural Sciences

Mezinárodní vědecký časopis vydávaný z pověření Ministerstva zemědělství České republiky a pod gescí České akademie zemědělských věd

EDITORIAL BOARD – REDAKČNÍ RADA

Chairman – Předseda

Ing. Vít Prokop, DrSc. (Výzkumný ústav výživy zvířat, s. r. o., Pohořelice, ČR)

Members – Členové

Prof. Ing. Jozef Bulla, DrSc. (Výzkumný ústav živočišné výroby, Nitra, SR)

Doc. Ing. Josef Čeřovský, DrSc. (Výzkumný ústav živočišné výroby Praha, pracoviště Kostelec nad Orlicí, ČR)

Prof. Dr. hab. Andrzej Filistowicz (Akademia rolnicza, Wrocław, Polska)

Ing. Ján S. Gavora, DrSc. (Centre for Food and Animal Research, Ottawa, Ontario, Canada)

Dr. Alfons Gottschalk (Bayerische Landesanstalt für Tierzucht, Grub, BRD)

Ing. Július Chudý, CSc. (Slovenská poľnohospodárska univerzita, Nitra, SR)

Dr. Ing. Michael Ivan, DSc. (Lethbridge Research Centre, Lethbridge, Alberta, Canada)

Prof. Ing. MVDr. Pavel Jelínek, DrSc. (Mendelova zemědělská a lesnická univerzita, Brno, ČR)

Ing. Jan Kouřil (Výzkumný ústav rybářský a hydrobiologický Jihočeské univerzity, Vodňany, ČR)

Prof. Ing. František Louda, DrSc. (Česká zemědělská univerzita, Praha, ČR)

Prof. Ing. Josef Mácha, DrSc. (Mendelova zemědělská a lesnická univerzita, Brno, ČR)

RNDr. Milan Margetín, CSc. (VÚŽV Nitra, Stanica chovu a šľachtenia oviec a kôz, Trenčín, SR)

Dr. Paul Millar (BRITBREED, Edinburgh, Scotland, Great Britain)

Ing. Ján Poltársky, DrSc. (Výzkumný ústav živočišné výroby, Nitra, SR)

Doc. Ing. Jan Říha, DrSc. (Výzkumný ústav pro chov skotu, s. r. o., Rapotín, ČR)

Ing. Antonín Stratil, DrSc. (Ústav živočišné fyziologie a genetiky AV ČR, Liběchov, ČR)

Ing. Pavel Trefil, CSc. (BIOPHARM, Výzkumný ústav biofarmacie a veterinárních léčiv, a. s., Pohoří-Chotouň, ČR)

Editor-in-Chief – Vedoucí redaktorka

Ing. Marie Černá, CSc.

Aims and scope: The journal publishes scientific papers and reviews dealing with the study of genetics and breeding, physiology, reproduction, nutrition and feeds, technology, ethology and economics of cattle, pig, sheep, goat, poultry, fish and other farm animal management.

The journal is cited in the bibliographical journal *Current Contents – Agriculture, Biology and Environmental Sciences* and abstracted in *Animal Breeding Abstracts*. Abstracts from the journal are comprised in the databases: *Agris*, *CAB Abstracts*, *Current Contents on Diskette – Agriculture, Biology and Environmental Sciences*, *Czech Agricultural Bibliography*, *Toxline Plus*, *WLAS*.

Periodicity: The journal is published monthly (12 issues per year), Volume 44 appearing in 1999.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Marie Černá, CSc., editor-in-chief, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: 02/24 25 34 89, fax: 02/24 25 39 38, e-mail: editor@login.cz.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2. Subscription price for 1999 is 195 USD (Europe), 214 USD (overseas).

Cíl a odborná náplň: Časopis publikuje původní vědecké práce a studie typu review z oblasti genetiky, šlechtění, fyziologie, reprodukce, výživy a krmení, technologie, etologie a ekonomiky chovu skotu, prasat, ovčí, koz, drůbeže, ryb a dalších druhů hospodářských zvířat.

Časopis je citován v bibliografickém časopise *Current Contents – Agriculture, Biology and Environmental Sciences* a v časopise *Animal Breeding Abstracts*. Abstrakty z časopisu jsou zahrnuty v těchto databázích: *Agris*, *CAB Abstracts*, *Current Contents on Diskette – Agriculture, Biology and Environmental Sciences*, *Czech Agricultural Bibliography*, *Toxline Plus*, *WLAS*.

Periodicita: Časopis vychází měsíčně (12x ročně), ročník 44 vychází v roce 1999.

Přijímání rukopisů: Rukopisy ve dvou vyhotoveních je třeba zaslat na adresu redakce: Ing. Marie Černá, CSc., vedoucí redaktorka, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Česká republika, tel.: 02/24 25 34 89, fax: 02/24 25 39 38, e-mail: editor@login.cz.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 1999 je 816 Kč.

MERISTIC STUDIES OF SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT) AND ITS CROSSES WITH GREEN STURGEON (*ACIPENSER MEDIROSTRIS* AYRES)

MERISTICKÉ STUDIUM JESETERA SIBIŘSKÉHO (*ACIPENSER BAERI*
BRANDT) A JEHO HYBRIDŮ S JESETEREM ZELENÝM (*ACIPENSER*
MEDIROSTRIS AYRES)

R. Kolman¹, V. D. Krylova², B. Szczepkowska³, M. Szczepkowski³

¹*Inland Fisheries Institute, Department of Lake Fisheries, Olsztyn-Kortowo, Poland*

²*VNIRO, Moscow, Russia*

³*Experimental Hatchery "Dgal", Pieczarki, Pozezdrze, Poland*

ABSTRACT: Eggs of Siberian sturgeon were fertilised for the first time with milt collected from Sakhalin (green) sturgeon in spring 1995, in the Konakovo Stocking Centre, Russia. A small amount of these hybrid eggs, and eggs of Siberian sturgeon fertilised at the same time, were transported to the Experimental Hatchery "Dgal", Poland, where they were incubated. The hatchlings were reared, and fry of the two groups was produced. Comparative morphometric studies were performed in the course of fry rearing, the objective of which was to define the characters allowing for a reliable distinguishing of the pure species, Siberian sturgeon, from its crosses with green sturgeon. Analysis of the distribution of meristic characters (Figs. 1–6) revealed that the hybrid differed significantly from the maternal species only as regards the number of dorsal and lateral bony plates, the mean numbers of which amounted to 8.9 ± 1.00 and 15.59 ± 2.01 , and 33.47 ± 2.24 and 48.14 ± 3.39 , respectively. Comparison of plastic features, with CD coefficient, showed that major differences between the two groups of fish consisted of relative head dimensions (Figs. 8 and 9). Analysis of the hybridisation index values of meristic characters revealed that the hybrid resembled the green sturgeon with respect to majority of the characters (Fig. 7).

Keywords: sturgeons; *Acipenser baeri*; *A. medirostris*; hybrids; morphometric studies

ABSTRAKT: Jikry jesetera sibiřského byly poprvé oplodněny mlíčem odebraným od jesetera zeleného na jaře roku 1995 v Reprodukčním centru ryb Konakovo v Rusku. Malé množství těchto hybridních jiker a jiker jesetera sibiřského, které byly oplodněny ve stejnou dobu, bylo odvezeno do Experimentální líhně „Dgal“ v Polsku, kde došlo k jejich inkubaci. Vylíhnutý plůdek byl odchováván ve dvou skupinách. V průběhu odchovu plůdku jsme prováděli morfometrické studie, jejichž cílem bylo určení znaků, které umožní spolehlivě rozlišit čistý druh, jesetera sibiřského, od jeho kříženců s jeseterem zeleným. Rozbor rozložení meristických znaků (obr. 1 až 6) ukázal, že se hybrid od mateřského druhu liší významně pouze počtem dorzálních a laterálních kostěných destiček, jejichž průměrný počet činil $8,9 \pm 1,00$ a $15,59 \pm 2,01$, resp. $33,47 \pm 2,24$ a $48,14 \pm 3,39$. Porovnání plastických rysů pomocí koeficientu rozdílu CD naznačilo, že hlavní rozdíly mezi oběma skupinami ryb spočívají v relativní velikosti hlavy (obr. 8 a 9). Analýza hodnot indexu hybridizace pro meristické znaky odhalila, že se hybrid podobá jeseteru zelenému ve většině znaků (obr. 7).

Klíčová slova: jeseteroviti; *Acipenser baeri*; *A. medirostris*; hybridní; morfometrické studie

INTRODUCTION

Preservation of the endangered species of sturgeons depends on the measures used to enhance their natural reproduction, most of all on stocking with the material from artificial spawning, frequently using spawners bred in controlled conditions. Sakhalin (green) sturgeon (*Acipenser medirostris* Ayres) is a typical example. Its distribution range is very wide, comprising west and east coasts of north Pacific (Lindberg, Legeza

1965; Hart, 1973; Scott, Crossman, 1973). Throughout its distribution range this fish is considered to be a rare or a disappearing species. The situation is especially grave at the west Pacific coast, where there is probably only one small population of this fish, which enters the Tumnin River for spawning (Artiukhin, Andronov, 1990; Birstein *et al.*, 1997; Pavlov *et al.*, 1994).

Artificial reproduction of green sturgeon (*Acipenser medirostris* Ayres) was carried out for the first time in 1987 (Artiukhin, Andronov, 1990). Some of the hatch-

lings were transported from the Far East to the Stocking Centre in Konakowo near Moscow, where they were used to obtain a stock of selects. The males attained sexual maturity for the first time in spring 1995, and could have been used to produce an interesting hybrid: eggs of Siberian sturgeon (*Acipenser baeri* Brandt) were fertilised with milt of green sturgeon (*A. medirostris* Ayres). A small part of the hybrid eggs, and eggs of Siberian sturgeon fertilised at the same time, were brought to the Experimental Hatchery "Dgal" of the Inland Fisheries Institute, Poland, in which they were incubated, and then reared to attain the fry stage (Kolman *et al.*, 1997).

Availability of this unique material induced the authors to carry out studies in order to compare morphological characters in the two groups, and to find meristic parameters that might be used to accurately distinguish the pure species, Siberian sturgeon, from its crosses with green sturgeon.

MATERIAL AND METHODS

The two fish groups, i.e. Siberian sturgeon and its hybrid with green sturgeon, were reared in tanks from the stage of eyed egg in a water recirculation system.

Meristic examination was carried out on day 370 of fish rearing, using standard procedures (Krylova, Sokolov, 1981) and live fish collected from the two groups. The sample comprised 22 Siberian sturgeons and 32 hybrids. The following meristic and plastic characters were analysed: W – body weight (g), L – total length (cm), SD – number of dorsal bony plates, SL – number of lateral bony plates, SV – number of ventral bony plates, D – number of rays in dorsal fin, A – number of rays in anal fin, Sp.br. – number of gill rakers on the first arch, l_1 – body length from rostrum tip to the end of middle rays in the caudal fin, l_2 – body length from rostrum tip to the base of middle rays in caudal fin, aD – pre-dorsal length, aV – pre-ventral length, aA – pre-anal length, C – head length, R – rostrum length, op – post-orbital space, o – eye diameter, HC – the biggest head height, hCo – the smallest head height, io – interorbital space. BC – the biggest head breadth, bC – head breadth measured between the upper edges of the operculi, r_c – distance from rostrum end to the base of middle barbs, r_r – distance from rostrum end to cartilaginous snout edge, l_c – external barb length, SR_c – rostrum breadth at barb base, SR_r – rostrum breadth at cartilaginous snout vault, SO – snout breadth, il – breadth of lower lip gap, H – the biggest body breadth, h – the smallest body breadth, pl – length of caudal part, ID – length of dorsal fin base, hD – height of dorsal fin.

To compare Siberian sturgeon with the hybrid, relative values were used of the mentioned plastic characters, expressed as % of body or head length. Significance of the differences between the examined

parameters was tested using the coefficient of difference (CD) (Mayer, 1971):

$$CD = (M_1 - M_2) / (\sigma_1 - \sigma_2) > 1.28 \quad (1)$$

where: M_1, M_2 – mean values of a parameter in the compared groups
 σ_1, σ_2 – standard deviations for this parameter

Based on the means of meristic characters for Siberian sturgeon and the hybrid, as well as for green sturgeon (according to Artiukhin and Andronov, 1990), hybridisation indexes (HI) were calculated, characterising morphological similarities between the hybrid and the parental species (Verigin, Makeeva 1972):

$$HI = \left[\frac{(M_h - M_f) \cdot 100}{M_m - M_f} - 50 \right] \cdot 2 \quad (2)$$

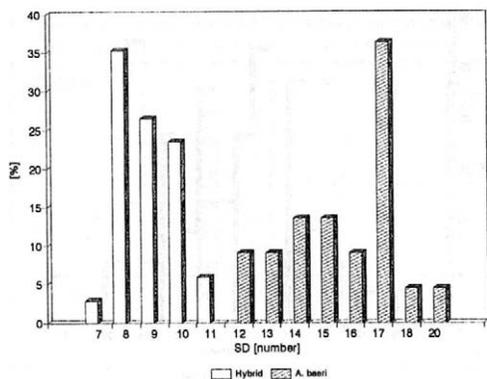
where: M_h – mean value for the hybrid
 M_f – mean value for the species female
 M_m – mean value for the species male

RESULTS AND DISCUSSION

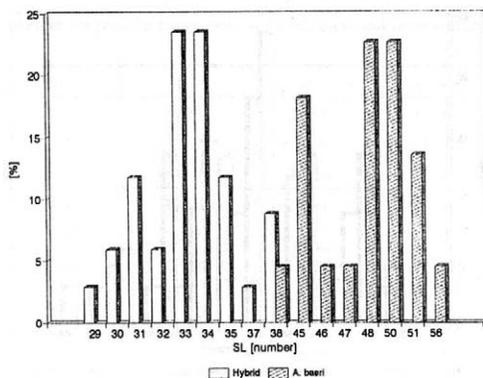
Fish in the two groups were characterised by a relatively uniform body weight and length; the mean values on day 370 were respectively for Siberian sturgeon and hybrid fry: 2432 ± 296 g and 2074 ± 270 g, and 86.0 ± 4.0 cm and 79.5 ± 3.0 cm. Siberian sturgeon was characterised by higher dynamics of body weight increments in relation to length increments than the hybrid, this being reflected in the condition coefficient of Fulton, which amounted to 0.42 ± 0.05 and 0.38 ± 0.03 , respectively.

Fish in the compared groups differed with respect to colouring and body shape. Gray-brownish colouring dominated in Siberian sturgeon. This fish was the darkest over its dorsal part (down to lateral plates), where brownish colour dominated. It gradually passed into greyish-brown and white-greyish over the ventral part. Colouring of bony plates did not differ from skin colouring. Brownish-green colour dominated in the hybrid over the dorsal part, and greyish-olive green over the ventral one. Dorsal and lateral plates were bigger than in Siberian sturgeon. They were lighter in colour, with chocolate shade, and possessed noticeable grooves. The hybrids differed from Siberian sturgeon in the more flat rostrum, and noticeable dorsal-ventral body flattening. The examined crosses of Siberian and green sturgeon resembled green sturgeon as regards body colouring and shape (Scott, Crossman, 1973; Artiukhin, Andronov, 1990).

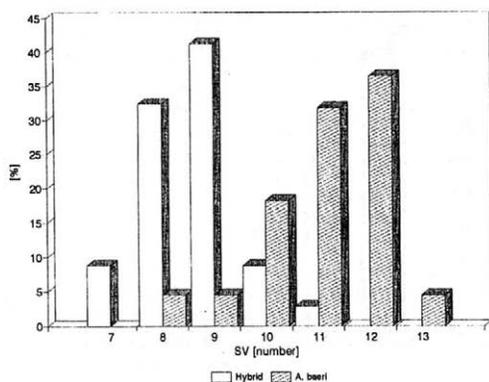
The differences observed between Siberian sturgeon and its crosses with Sakhalin one were confirmed by the examination of meristic and plastic characters. Values of meristic characters showed that the hybrid differed from its maternal species only as regards the number of dorsal (SD) and lateral (SL) plates (Figs. 1 and 2), the mean values of which were 8.9 ± 1.00 and 15.59 ± 2.01 , and 33.47 ± 2.24 and 48.14 ± 3.39 , re-



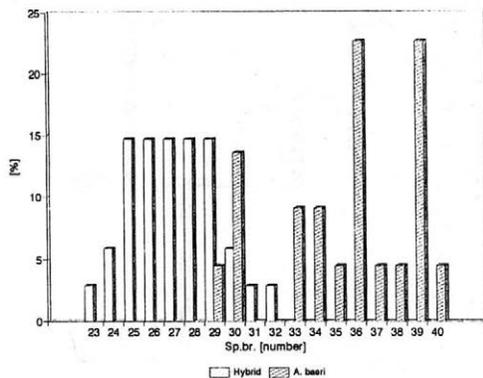
1. Histogram of the frequency distribution of dorsal bony plates (SD) in the hybrid and in Siberian sturgeon



2. Histogram of the frequency distribution of lateral bony plates (SL) in the hybrid and in Siberian sturgeon



3. Histogram of the frequency distribution of ventral bony plates (SV) in the hybrid and in Siberian sturgeon

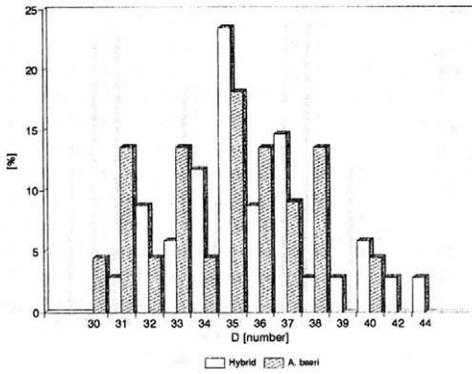


4. Histogram of the frequency distribution of gill rakers on the first arch (Sp.br.) in the hybrid and in Siberian sturgeon

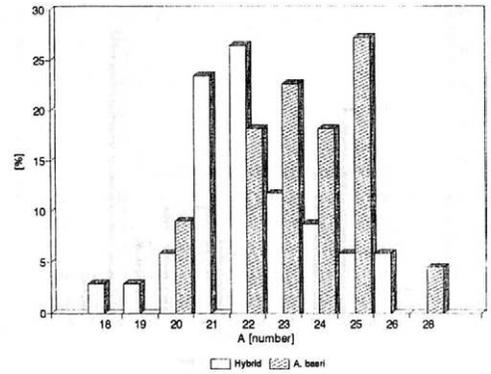
spectively. Values of other meristic characters overlapped either partly (number of ventral plates – SV – and number of gill rakers – sp.br.) (Figs. 3 and 4) or totally (number of rays in dorsal – D – and anal – A – fin) (Figs. 5 and 6), so that they could not be used to distinguish the two fish.

The hybrids had intermediate values of meristic characters compared to the parental species, this being clearly revealed by the analysis of hybridisation indexes (HI). They were calculated from the mean values of meristic parameters for green sturgeon (according to Artiukhin and Andronov, 1990), Siberian sturgeon and the hybrid (Tab. I). It was found from these data that the hybrid resembled green sturgeon as regards majority of the characters (SD, SL, SV and A), while the number of rays in the dorsal fin (D) was close to that in Siberian sturgeon, and sp.br. number was intermediate compared to the parental species (Fig. 7). Analysis of the HI index confirmed the observations on external appearance of Siberian sturgeon and its hybrid with green sturgeon.

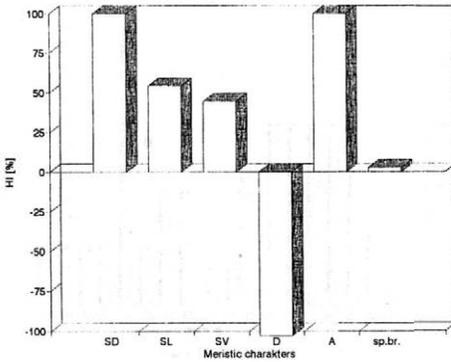
Lack of our own as well as literature data describing plastic characters of Sakhalin sturgeon of a similar age and size range like in this study makes it impossible to calculate the hybridisation indexes. In view of this, it was decided to compare their mean relative values and to assess significance of the differences in order to establish which of them might be used to differentiate between the subspecies ($CD > 1.28$). The following characters were taken into consideration (marked “**” in Figs. 8 and 9): relative head length (C/L), inter-orbital space (io/C), relative rostrum breadth (SR_c/C and SR_r/C) and relative size of the gap in lower lip (il/So). The hybrid, compared to Siberian sturgeon, was characterised by bigger relative head (C/L) and rostrum (R/C) length. The respective values amounted to 24.3 ± 3.8 and 20.6 ± 0.9 , and 53.1 ± 1.4 and 49.6 ± 2.1 . As a consequence, the hybrid had smaller relative head breadth (SR_c/C) than Siberian sturgeon, and smaller inter-orbital space (io/c), the respective values being: 20.0 ± 1.0 and 25.2 ± 1.1 , and 25.0 ± 0.8 and 27.4 ± 1.1 . In addition to this, the hybrid was characterised by



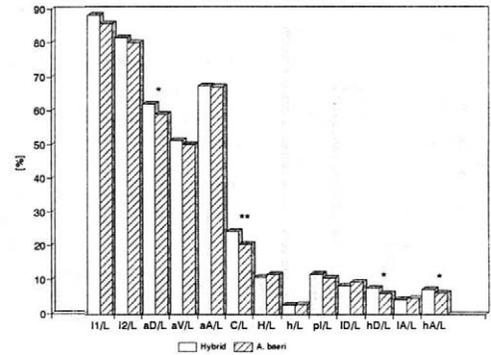
5. Histogram of the frequency distribution of dorsal fin rays (D) in the hybrid and in Siberian sturgeon



6. Histogram of the frequency distribution of anal fin rays (A) in the hybrid and in Siberian sturgeon



7. Hybridisation index values for the meristic characters of the hybrid, Siberian sturgeon and green sturgeon

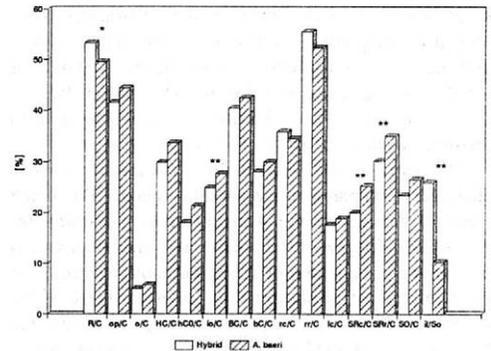


8. Comparison of relative values (related to body length - L) of plastic parameters in the hybrid and in Siberian sturgeon

smaller mouth breadth (SO/C), but a much larger gap in the lower lip (il/SO) compared to Siberian sturgeon, the values of which amounted to: 23.4 ± 1.1 and 26.4 ± 1.4 , and 25.9 ± 3.7 and 10.3 ± 2.2 , respectively.

The results of the examination of plastic characters confirmed dorso-ventral body flattening of the hybrid. The hybrid had lower values related to head and body height than Siberian sturgeon, respectively: H/L 10.7 ± 0.6 and 11.6 ± 0.6 ; h/L 2.8 ± 0.1 and 3.0 ± 0.6 ; HC/C 29.8 ± 1.7 and 33.8 ± 3.1 ; hC_0/C 18.1 ± 0.7 and 21.4 ± 2.8 .

Besides the described morphometric differences, fish in the two groups showed different behavioural patterns. Hybrid fry spent most of the day at the tank bottom, making only slow movements to uptake food pellets, while Siberian sturgeon was an active swimmer penetrating the whole water column. Apart from the heterosis effect characteristic of genetically distant F_1 crosses, hybrid behaviour might have been due to higher growth rate at the same food use (Kolman *et al.*, 1997).



9. Comparison of relative values (related to head length - C) of plastic parameters in the hybrid and in Siberian sturgeon

I. Mean values, standard deviations and ranges of meristic characters of Siberian sturgeon, its hybrid with green sturgeon, and green sturgeon (the latter were taken from Artiukhin and Andronov, 1990)

Character		SD	SL	SV	D	A	Sp.br.
Siberian sturgeon	mean	15.59	48.14	11.05	34.73	23.50	35.36
	SD	± 2.01	± 3.39	± 1.15	± 2.67	± 1.78	± 3.31
	range	12–20	38–56	8–13	30–40	20–20	29–40
Hybrid	mean	8.94	33.47	8.63	35.78	22.16	27.16
	SD	± 1.00	± 2.24	± 0.89	± 2.87	± 1.82	± 2.11
	range	7–11	29–35	7–11	31–44	18–26	23–32
Green sturgeon	mean	9.5	29.1	7.7	34.1	23.0	19.4
	SD	± 0.85	± 2.13	± 1.34	± 4.25	± 2.45	± 0.84
	range	8–11	26–33	5–10	29–44	19–27	18–21

Low activity and keeping to the bottom zone is a characteristic behaviour of green sturgeon fry in small rivers with rapid current (Artjukhin, Andronov, 1990). This type of behaviour, and morphological adaptation to the current (long and flat rostrum, dorso-lateral body flattening), prevent too early fry transportation to sea water. Hence, it can be stated that the hybrid inherited fraternal characters not only as regards morphological parameters, but also the fish behaviour.

CONCLUSIONS

1. Analysis of the distribution of meristic characters (Figs. 1–6) showed that the hybrid differed significantly from Siberian sturgeon only by a smaller number of dorsal (SD) and lateral (SL) bony plates.
2. Analysis of the hybridisation index values of meristic characters revealed that the hybrid resembled the green sturgeon with respect to majority of the characters.
3. Comparison of plastic parameters of the hybrid and Siberian sturgeon, with the CD coefficient, revealed that the differences were at a level characteristic of a subspecies in the case of C/L, io/C, SRc/C, SRr/C and il/S i.e. as regards head morphology.
4. There were morphologic as well as behavioural differences between the hybrid fry and Siberian sturgeon, and many common characters with green sturgeon.

REFERENCES

- Artiukhin E. N., Andronov A. E. (1990): Meristic description of green sturgeon – *Acipenser medirostris* (chondrostei, Acipenseridae) from Tumnin River (Datta) and selected problems of ecology and zoogeography of Acipenseridae. *Zoolog. Zhurn.*, 69: 81–91 (in Russian).
- Birstein V. I., Bemis W. E., Waldman J. R. (1997): The threatened status of acipenseriform species: a summary. *Environ. Biol. Fishes*, 48: 427–435.
- Hart J. L. (1973): Pacific Fishes of Canada. Fishes Research Board of Canada, 180: 82.
- Kolman R., Szczepkowski M., Pyka J. (1997): Evaluation of the Siberian sturgeon (*Acipenser baeri* Brandt) and green sturgeon (*A. medirostris* Ayres) hybrid comparing to the mother species. *Arch. Polish Fisheries*, 5: 51–58.
- Krilova V. D., Sokolov L. I. (1981): Morphobiological Studies of Sturgeons. Moskva, VNIRO, 49 p. (in Russian).
- Lindberg G. U., Legeza M. I. (1965): Fish from Japanese Sea and the neighbouring parts of Okhotskie and Yellow Seas. Part 2. Moskva, Leningrad, Nauka: 33–36 (in Russian).
- Mayer E. (1971): Principles of zoological systematics. Moskva, Mir, 454 p. (in Russian).
- Pavlov D. S., Savvaitova K. A., Sokolov L. I., Alekseev S. S., (1994): Rare and disappearing animals. Fishes. Moskva, Vysshaia Shkola: 73–88 (in Russian).
- Scott W. B., Crossman E. J. (1973): Freshwater fishes of Canada. Fishes Research Board of Canada, 183: 80–82.
- Verigin B. V., Makeeva A. P. (1972): Hybridization of common carp with bighead carp. *Genetika*, 8: 55–64 (in Russian).

Received for publication on September 16, 1998

Accepted for publication on November 10, 1998

Contact Address:

Doc. Dr. Ryszard Kolman, Inland Fisheries Institute, Department of Lake Fisheries, ul. Oczapowskiego 10, 10-957 Olsztyn-Kortowo, Poland

Oznamujeme čtenářům a autorům našeho časopisu,

že v návaznosti na časopis *Scientia agriculturae bohemoslovaca*, který až do roku 1992 vycházel v Ústavu vědeckotechnických informací Praha, vydává od roku 1994

Česká zemědělská univerzita v Praze

časopis

SCIENTIA AGRICULTURAE BOHEMICA

Časopis si zachovává původní koncepci reprezentace naší vědy (zemědělství, lesnictví, potravinářství) v zahraničí a jeho obsahem jsou původní vědecké práce uveřejňované v angličtině s rozšířenými souhrny v češtině.

Časopis je otevřen nejširší vědecké veřejnosti a redakční rada nabízí možnost publikace pracovníkům vysokých škol, výzkumných ústavů a dalších institucí vědecké základny.

Příspěvky do časopisu (v angličtině, popř. v češtině či slovenštině) posílejte na adresu:

Česká zemědělská univerzita v Praze
Redakce časopisu *Scientia agriculturae bohemica*
165 21 Praha 6-Suchdol

THE INFLUENCE OF CHLORINE ON THE ACTIVITIES OF LACTATE DEHYDROGENASE AND ITS ISOENZYMES IN PLASMA OF LAMBS*

VPLYV CHLÓRU NA AKTIVITU LAKTÁTDEHYDROGENÁZY A JEJ IZOENZÝMOV V KRVNEJ PLAZME JAHNIAT

I. Šutiaková¹, E. Bekeová¹, V. Šutiak²

¹Research Institute of Experimental Veterinary Medicine, Košice, Slovak Republic

²University of Veterinary Medicine, Košice, Slovak Republic

ABSTRACT: The influence of chlorine doses in drinking water on the activities of lactate dehydrogenase and its isoenzymes in blood plasma of ewe lambs was studied after short-term and long-term chlorine action. Drinking water was treated with chlorine gas and adjusted with preparation Savo-Super in such a way that it contained 0.3 mg active chlorine/l at the time of administration. We also used a medication of chlortetracycline-chloride (preparation Aureovit 12 C 80 plv. a. u. v.), added to the feed mixture to ensure consumption of the preparation 0.35 g/kg l.w./day. The short-term action of chlorine concentrations showed no significant differences in the total LD activity and LD isoenzymes between the control and the experimental groups. However, significant difference in the activity of isoenzyme LD1 ($P < 0.05$) was observed at one sampling in the experimental group treated with Aureovit after the 3-months exposure.

Keywords: ewe hogs; bioindicators; lactate dehydrogenase isoenzymes; drinking water; chlorination

ABSTRAKT: Sledovali sme vplyv koncentrácií chlóru v pitnej vode na aktivitu laktátdehydrogenázy a jej izoenzýmov v krvnej plazme jahničiek po krátkodobej a dlhodobej aplikácii. Použili sme pri tom dezinfekčný prípravok plyný chlór a Savo-Super, a to tak, aby pri podaní voda obsahovala 0,3 mg aktívneho chlóru na liter vody, a liečivo chlortetracyklín-chlorid (prípravok Aureovit 12 C 80 plv. a. u. v.) tak, aby každé jahňa skonzumovalo 0,35 g prípravku na kg ž. h. a deň po primiešaní do kŕmnej zmesi. Počas krátkodobého pôsobenia nízkych koncentrácií chlóru neboli zistené významné odchýlky v celkovej aktivite LD a v izoenzýmoch LD medzi kontrolnou a pokusnými skupinami. Zaznamenali sme však významné rozdiely v aktivite izoenzýmu LD1 ($P < 0,05$) v pokusnej skupine s Aureovitom, iba raz po trojmesačnom pôsobení.

Kľúčové slová: jahničky; bioindikátory; izoenzýmy laktátdehydrogenázy; pitná voda; chlorácia

INTRODUCTION

Drinking water is one of the components essential for life, and its disinfection by chlorination belongs to prevention of various viral, bacterial, protozoan and parasitic diseases. On a worldwide scale there are several alternatives to chlorination of drinking water, however, there is a danger of formation of so called by-products which can have carcinogenic or mutagenic effects (Bull *et al.*, 1995). The formation of by-products in drinking water depends on its chlorine treatments. The toxicological research on disinfection by-products has focused on the carcinogenic and mutagenic activity of the halogenated organic disinfection by-products (Gonzales-Leon *et al.*, 1997; Giller *et al.*, 1997). Therefore we face a dilemma whether or not to

continue to use different types of chlorination because even low concentrations of by-products, when they act for longer period of time, can seriously affect the health (Gauthier *et al.*, 1995).

Enzyme studies are still an important part of investigations of the effect of various xenobiotics on cellular systems and tissues (Poráčová *et al.*, 1992; Braun *et al.*, 1993; Šutiaková *et al.*, 1995). Lactate dehydrogenase (E.C.1.1.1.27, LD hereafter) plays an important role in the glycolytic cycle and is therefore crucially important for muscle physiology. It is a cytosol biomarker enzyme, and it occurs in isoenzyme forms which can be utilised as indicator of damage to tissues or organs (Kopperchläger, Kirchberger, 1996).

The present study investigated the effects of acute and chronic exposure to two concentrations of chlorine,

* This work was supported by grant No. 95/53 05/490 of the Ministry of Agriculture of the Slovak Republic.

based on the disinfectants chlorine gas and Savo Super in drinking water and the therapeutic chlortetracycline-chloride (preparation Aureovit 12 C 80 plv. a. u. v.), in feed on the activities of lactate dehydrogenase and its isoenzymes in blood plasma of lambs.

MATERIAL AND METHODS

Two months old lambs (18 animals) of Slovak Merino breed, weighing 11.0–14.5 kg, were used in the experiments. The lambs were obtained from location N.B., from a private breeder. Prior to their inclusion in the experiments, they were nursed by mothers in such a way that they had limited access to drinking water and received no additional feed. Two weeks before the experiment the lambs were adapted to experimental housing conditions as follows: all animals had access to spring non-chlorinated water (*ad libitum*) obtained from the plant Východoslovenské vodárne a kanalizácie, Košice. They also had free access to the feed mixture ČOJ 2 and meadow hay. Lambs were supplied with standard feed rations, i. e. 35 dkg meadow hay/head/day and 25–30 dkg feed mixture ČOJ 2/head/day.

EXPERIMENTAL SCHEME

Two series of experiments were carried out, the first of shorter duration (1 month) and the second longer-lasting (3 months). Each of the experimental series consisted of one control group and two groups of experimental lambs. The control group in acute exposure experiments consisted of 6 ewe lambs and that in the chronic experiments of 3 ewe lambs. The control animals were supplied with spring water without any additions, obtained from the supplier mentioned above in the same quantity and the system as that mentioned in the characterisation of the preliminary period. In the first series of experiments, the ewe lambs were supplied with chlorinated water which contained 0.3 mg active chlorine/l. The water supplied was first treated with chlorine gas and then adjusted with the preparation Savo-Super (Biochimie a.s., Bohumín, 950ml bottles, sodium hypochloride concentration min. 47.2 g/l at filling). The first experimental group of animals was supplied with water with the same concentration of free chlorine and the same system of consumption in both series of experiments. Animals of both series were offered feed in the same way described for the pre-treatment period.

The second experimental group was supplied with water in the same way and quality as the control group in both series of experiments. In addition to that, the ČOJ 2 mixture was supplemented with chlortetracycline-chloride (preparation Aureovit 12 C 80 plv. a. u. v., containing 8% of the active ingredient, 0.5 kg packaging) in such a way that each lamb consumed 0.35 g of the preparation per kg live weight and day, added to

the feed mixture. The total quantity of the feed mixture and meadow hay supplied was the same as in the other groups.

Blood samples were withdrawn from *vena jugularis* into heparin (15 i.u./ml) in the morning, in the set time intervals. The blood plasma was obtained by centrifugation for 15–20 min at 190 g. The total LD activity was determined spectrophotometrically by means of Seva-test LD (Imuna). Measurements were made in triplicate (the total LD activity) and duplicate (activities LD isoenzymes). The LD isoenzymes were separated in 5.5% polyacrylamide gel according to Dietz and Lubrano (1967). Quantitative evaluation of isoenzymes was carried out by means of a densitometer DS 90 equipped with control and evaluation software Denzito (Mikro LaAp Ltd, Co.) Results were evaluated statistically using Student's *t*-test.

RESULTS

The results of the total activity of LD and its isoenzymes, obtained during the acute exposure to 2 different types of chlorine, are presented in Tab. I. During the short-term action of these chlorine concentrations no significant differences in the total LD activity and individual isoenzymes were observed between the control and the two experimental groups. Tab. II shows the results of 3-month action of chlorine on the activity of polymorphic isoenzyme system of lactate dehydrogenase. Significant changes were recorded only in the activity of isoenzyme LD1 ($P < 0,05$) in the second experimental group at sampling on June 18, 1996. The activities of individual isoenzymes in the remaining experimental groups showed no significant changes.

DISCUSSION

LD is a cytosol enzyme present in almost all organ systems in mammals. Therefore the determination of total activity of LD in the serum may be a sensitive but not specific bioindicator of organ damage. However, the function of specific indicators is performed by LD isoenzymes which allow us to identify the damage either to tissues or at least to organs (e.g. during dehydration, action of bacterial toxins, some xenobiotic, etc.) (Drent *et al.*, 1996 Huijigen *et al.*, 1997). On the other hand, there are some interspecies differences in the activity of LD isoenzymes which can be affected by physiological factors such as age, sex, breed and so on (Heinová *et al.*, 1996; Avallone *et al.*, 1996; Šutiaková *et al.*, 1996; Mattová *et al.*, 1997). No significant differences in the activities of LD isoenzymes were observed in the experimental groups of ewe lambs after the short-term exposure to chlorine concentrations. Two ewe lambs (in the control and the second experimental group) exhibited higher activity of the LD5 isoenzyme, the reasons should be analysed. For

I. Activities of lactate dehydrogenase and its isoenzymes in the acute lamb experiments exposed to two chlorine compounds

Experiments	Groups of animals	Statistical values	Blood plasma					
			TA	LD1	LD2	LD3	LD4	LD5
			$\mu\text{kat.l}^{-1}$	%	%	%	%	%
0. experiment 23. 4. 1996	control group	\bar{x}	7.08	72.40	11.74	11.04	3.00	1.78
		$\pm s$	1.34	8.60	5.70	4.30	1.58	1.20
		n	6	6	6	6	6	6
	1. experimental group (SAVO)	\bar{x}	7.56	79.30	6.60	7.41	1.74	4.96
		$\pm s$	1.62	3.48	1.72	2.84	0.90	2.60
		n	6	6	6	6	6	6
	2. experimental group (AUREOVIT)	\bar{x}	7.92	69.10	7.70	8.50	4.96	11.06
		$\pm s$	1.02	16.20	3.47	2.93	5.60	9.50
		n	6	6	6	6	6	6
1st experiment 7. 5. 1996	control group	\bar{x}	3.38	71.50	12.80	7.57	3.07	5.03
		$\pm s$	1.61	4.40	4.80	2.32	1.09	2.31
		n	6	6	6	6	6	6
	1. experimental group (SAVO)	\bar{x}	3.77	68.90	11.42	11.90	3.30	4.47
		$\pm s$	1.47	5.40	2.40	6.30	1.31	2.06
		n	6	6	6	6	6	6
	2. experimental group (AUREOVIT)	\bar{x}	4.62	67.70	10.70	7.01	2.59	12.03
		$\pm s$	0.92	15.90	3.98	2.85	1.78	17.90
		n	6	6	6	6	6	6
2nd experiment 21. 5. 1996	control group	\bar{x}	5.3	64.29	5.60	8.12	8.30	13.70
		$\pm s$	2.72	19.50	1.85	3.40	7.50	18.50
		n	6	6	6	6	6	6
	1. experimental group (SAVO)	\bar{x}	4.42	64.01	4.62	4.92	3.93	22.52
		$\pm s$	1.14	23.90	1.99	2.32	2.10	26.04
		n	6	6	6	6	6	6
	2. experimental group (AUREOVIT)	\bar{x}	5.32	77.48	5.40	5.66	2.81	8.64
		$\pm s$	1.29	6.90	3.95	2.58	1.70	2.35
		n	6	6	6	6	6	6
		P	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	

TA – total activity

example, in the case of liver disorders the activity of the LD1 isoenzyme in plasma with the highest electrophoretic mobility increases in horses, sheep, pigs and cows and, on the contrary, that of the isoenzyme LD5 with the lowest electrophoretic mobility increases more frequently in rats, dogs and cats (Bláhovec *et al.*, 1992). The differences in the distribution of LD isoenzymes in tissues are caused by the differences in their metabolic activity. Cathodal isoenzymes LD4 and LD5 enable rapid accumulation of lactate and occur in the tissues with anaerobic glycolysis (Beatty, Doxey, 1983). During the 3-month action of the preparation mentioned in the LD isoenzyme system in blood

plasma, a significant difference was recorded in one case only, for isoenzyme LD1 in the experimental group, treated with Aureovit. Sparrow *et al.* (1994) investigated the influence of chlorinated herbicide 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD) also on the activities of LD isoenzymes in liver in male mice of the strain C 57 BL/6J.

A dose-dependent reduction of hepatic pyruvate carboxylase levels and activity occurs in C 57 BL/6J male mice given TCDD i.p. in a corn oil carrier. Their data presented the evidence that in C 57 BL/6J male mice treated with less than lethal doses of TCDD exhibit a substantial dose-dependent reduction in hepatic pyru-

II. Activities of lactate dehydrogenase and its isoenzymes in the lamb experiments exposed to two chlorine compounds for the period of 3 months

Continuation of the chronic exposure	Groups of animals	Statistical values	Blood plasma					
			TA $\mu\text{kat.l}^{-1}$	LD1 %	LD2 %	LD3 %	LD4 %	LD5 %
1st experiment 4. 6. 1996	control group	\bar{x}	5.73	65.86	5.78	3.67	6.56	18.13
		$\pm s$	0.38	9.57	1.98	0.45	4.55	13.90
		<i>n</i>	3	3	3	3	3	3
	1. experimental group (SAVO)	\bar{x}	5.18	75.60	5.70	6.10	6.01	6.70
		$\pm s$	0.14	9.30	1.60	3.90	3.62	4.70
		<i>n</i>	6	3	3	3	3	3
		<i>P</i>	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
	2. experimental group (AUREOVIT)	\bar{x}	5.73	64.51	5.13	4.90	9.84	15.65
		$\pm s$	0.70	10.10	1.40	3.90	3.95	9.87
<i>n</i>		3	3	3	3	3	3	
<i>P</i>		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	
2nd experiment 18. 6. 1996	control group	\bar{x}	5.18	93.13	1.60	2.80	1.08	1.40
		$\pm s$	2.49	0.68	0.40	1.30	0.64	1.20
		<i>n</i>	3	3	3	3	3	3
	1. experimental group (SAVO)	\bar{x}	5.76	91.40	2.98	3.11	1.12	1.54
		$\pm s$	1.27	4.70	0.69	0.74	0.22	0.93
		<i>n</i>	3	3	3	3	3	3
		<i>P</i>	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
	2. experimental group (AUREOVIT)	\bar{x}	5.89	95.90	2.60	2.80	1.44	0.30
		$\pm s$	0.79	2.20	1.20	0.40	0.96	0.18
<i>n</i>		3	3	3	3	3	3	
<i>P</i>		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	
3rd experiment 2. 7. 1996	control group	\bar{x}	5.63	91.83	2.35	3.40	0.09	2.40
		$\pm s$	0.15	5.54	1.84	0.80	0.06	2.94
		<i>n</i>	3	3	3	3	3	3
	1. experimental group (SAVO)	\bar{x}	4.2	92.80	2.43	3.85	0.85	0.41
		$\pm s$	0.14	0.23	0.73	0.035	0.045	0.14
		<i>n</i>	2	2	2	2	2	2
		<i>P</i>	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
	2. experimental group (AUREOVIT)	\bar{x}	5.63	88.54	3.80	6.70	0.86	0.14
		$\pm s$	0.15	1.80	1.06	2.60	0.28	0.08
<i>n</i>		3	3	3	3	3	3	
<i>P</i>		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	
4th experiment 16. 7. 1996	control group	\bar{x}	4.67	92.62	3.71	3.16	0.71	0.18
		$\pm s$	0.86	0.41	0.43	0.51	0.23	0.13
		<i>n</i>	3	3	3	3	3	3
	1. experimental group (SAVO)	\bar{x}	5.85	93.52	2.57	2.53	0.80	0.09
		$\pm s$	0.78	0.56	0.06	0.31	0.28	0.07
		<i>n</i>	2	2	2	2	2	2
		<i>P</i>	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
	2. experimental group (AUREOVIT)	\bar{x}	4.13	91.10	4.50	3.40	1.50	0.44
		$\pm s$	0.87	2.70	1.80	1.20	0.32	0.31
<i>n</i>		3	3	3	3	3	3	
<i>P</i>		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	

vate carboxylase levels and activity as well as changes in LD isoenzyme activity. This is accompanied by an increase in blood lactic acid levels. Normal liver LD is composed of M subunits. They noted that it is necessary to gain the knowledge about the mechanism of action of TCDD on the system mentioned. Gupta *et al.* (1991) studied the changes in the profile of total LD and characteristics LD isoenzymes patterns in the serum and tissue of rats by acute carbofuran intoxication. The patterns of LD isoenzymes in serum revealed a significant ($P < 0.01$) increase in all the isoenzymes except LD4 (64% decrease). Analysis of each tissue revealed specific changes in LD isoenzyme patterns indicating organ-specific tissue damage.

The studies mentioned could bring some interesting results by examining the activities of LD isoenzymes also in some tissues in relation to the preservation of health in animals and human.

REFERENCES

Avallone L., Lombardi P., Florio S., D'Angelo A., Bogin E. (1996): Age-dependent variations of lactate dehydrogenase and creatine kinase activities in water buffalo calf serum. *Eur. J. Clin. Chem. Clin. Biochem.*, 34: 961-964.

Beatty E. M., Doney D. L. (1983): Lactate dehydrogenase and creatine kinase isoenzyme levels in the tissues and serum of normal lambs. *Res. Vet. Sci.*, 35: 325-330.

Bláhovec J., Šlesárová L., Rosival I. (1992): Sérové enzýmy v diagnostike chorôb domácich zvierat. *Veterinárství*, 42 (5): 174-176.

Braun J. P., Aktas M., Lefelwre H., Rico A. G., Toutain P. L. (1993): Clinical enzymology for the assessment of organ damage: Interspecific differences. *Comp. Haematol. Int.*, 3: 27-32.

Bull J. R., Birnbaum L. S., Cantor K. P., Rose J. B., Butterworth B. E., Pegram R., Tuomisto J. (1995): Water chlorination: Essential process or cancer hazard? *Fundam. Appl. Toxicol.*, 28: 155-166.

Dietz A. A., Lubrano T. (1967): Separation and quantitation of lactic dehydrogenase isoenzymes by disc electrophoresis. *Anal. Biochem.*, 20: 246-257.

Drent M., Cobben N. A. M., Hendersson R. F., Wouters E. F. M., van Diejen-Visser M. (1996): Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. *Eur. Respir. J.*, 9: 1736-1742.

Gauthier F., Emard J. F., Thouze J. P., Gauvredu D. (1995): Possible links between the physicochemical characteristics of drinking water and dementia of the Alzheimer

type in a region of Quebec, Canada. In: Majumdar S. K., Miller E. W., Brenner F. J. (Eds.): *Environmental contaminants, ecosystems and human health*. Pennsylvania Acad. Sci.: 343-394.

Giller S., Le Curieux F., Erb F., Marzin D. (1997): Comparative genotoxicity of halogenated acetic acids found in drinking water. *Mutagenesis*, 12: 321-328.

Gonzales-Leon A., Schultz J. R., Xu G., Bull J. (1997): Pharmacokinetics and metabolism of dichloroacetate in the F 344 rat after prior administration in drinking water. *Toxicol. Appl. Pharmacol.*, 146: 189-195.

Gupta R. C., Gvad J. T., Kadel W. L. (1991): *In vivo* alterations in lactate dehydrogenase (LDH) and LDH isoenzymes patterns by acute carbofuran intoxication. *Arch. Environ. Contam. Toxicol.*, 21: 263-269.

Heinová D., Bláhovec J., Rosival I. (1996): Lactate dehydrogenase isoenzyme patterns in bird, carp and mammalian sera. *Eur. J. Clin. Chem. Clin. Biochem.*, 34: 91-95.

Huijigen H. J., Sanders T. B., Koster R. W., Vreeken J., Bossuzt M. M. (1997): The clinical value of lactate dehydrogenase in serum. *Quantit. Rev.*, 35: 569-579.

Kopperschlager G., Kirchberger J. (1996): Review of methods for the separations of lactate dehydrogenase and clinical significance of the enzyme. *J. Chromatogr., B*, 684: 25-49.

Matková J., Buleca J., Šutiaková I. (1997): Variability of lactate dehydrogenase isoenzyme spectrum in phylogenetically different pig breeds and crossbreeds. *Živoč. Vyr.*, 42: 299-302.

Poráčová J., Dudriková E., Legáth J., Kačmár P. (1992): The toxic effect of pyrethroid insecticide supermethrin on the LDH activity and percent abundance of its isoenzymes in sheep exposed to 6-weeks intoxication. *Folia veter.*, 36: 91-107.

Sparrow B. R., Thompson C. S., Ryu B. W., Selevonchick D. P., Schaup W. (1994): 2,3,7,8-tetrachlorodibenzo-p-dioxin induced alternations of pyruvate carboxylase levels and lactate dehydrogenase isoenzyme shifts in C57BL/6J male mice. *Biochem. Toxicol.*, 9: 329-335.

Šutiaková I., Bekeová E., Šutiak V. (1995): Efekt karbimazolu na izoenzýmy laktátdehydrogenázy a koncentrácie tyroideálnych hormónov u oviec. *Vet. Med. - Czech*, 40: 341-344.

Šutiaková I., Hendrichovský V., Šutiak V. (1996): Early postnatal age and sexual dynamics of plasma lactate dehydrogenase isoenzymes in lambs. *Živoč. Vyr.*, 41: 441-444.

Received for publication on May 12, 1998

Accepted for publication on November 10, 1998

Contact Address:

RNDr. Irena Šutiaková, CSc., Ústav experimentálnej veterinárnej medicíny, Oddelenie teriogenológie a genetiky zdravia, Škultétyho 3, 040 01 Košice, Slovenská republika, tel.: 62 200 74, fax: 63 318 53

**Nejčerstvější informace o časopiseckých člancích
poskytuje automatizovaný systém**

Current Contents

na disketách

Ústřední zemědělská a lesnická knihovna odebírá časopis „**Current Contents**“ řadu „**Agriculture, Biology and Environmental Sciences**“ a řadu „**Life Sciences**“ na disketách. Řada „Agriculture, Biology and Environmental Sciences“ je od roku 1994 k dispozici i s abstrakty. Obě tyto řady vycházejí 52krát ročně a zahrnují všechny významné časopisy a pokračovací sborníky z uvedených oborů.

Uložení informací z Current Contents na disketách umožňuje nejrozmanitější referenční služby z prakticky nejčerstvějších literárních pramenů, neboť báze dat je **doplňována každý týden** a neprodleně expedována odběratelům. V systému si lze nejen prohlížet jednotlivá čísla Current Contents, ale po přesném nadefinování sledovaného profilu je možné adresně vyhledávat informace, tisknout je nebo kopírovat na disketu s možností dalšího zpracování na vlastním počítači. Systém umožňuje i tisk žádánek o separát apod. Kumulované vyhledávání v šesti číslech Current Contents najednou velice urychluje rešeršní práci.

Přístup k informacím Current Contents je umožněn dvojím způsobem:

- 1) Zakázkový přístup** – po vyplnění příslušného zakázkového listu (objednávky) je vhodný především pro mimopražské zájemce.
Finanční podmínky: – použití PC – 15 Kč za každou započatou půlhodinu
– odborná obsluha – 10 Kč za 10 minut práce
– vytištění rešerše – 1,50 Kč za 1 stranu A4
– žádanky o separát – 1 Kč za 1 kus
– poštovné + režijní poplatek 15 %
- 2) „Self-service“** – samoobslužná práce na osobním počítači v ÚZLK.
Finanční podmínky jsou obdobné. Vzhledem k tomu, že si uživatel zpracovává rešerši sám, je to maximálně úsporné. (Do kalkulace cen nezapočítáváme cenu programu a databáze Current Contents.)

V případě Vašeho zájmu o tyto služby se obraťte na adresu:

Ústřední zemědělská a lesnická knihovna

Dr. Bartošová

Slezská 7

120 56 Praha 2

Tel.: 02/24 25 79 39, l. 520, fax: 02/24 25 39 38

Na této adrese obdržíte bližší informace a získáte formuláře pro objednávku zakázkové služby. V případě „self-servisu“ je vhodné se předem telefonicky objednat. V případě zájmu je možné si objednat i průběžné sledování profilu (cena se podle složitosti zadání pohybuje čtvrtletně kolem 100 až 150 Kč).

DYNAMIKA ZMĚN CHOLESTEROLU V KREVŇÍ PLAZMĚ SLEPIC V PRŮBĚHU SNÁŠKOVÉHO OBDOBÍ

VARIATIONS IN CHOLESTEROL CONCENTRATIONS IN THE BLOOD PLASMA OF HENS THROUGHOUT THE LAYING PERIOD

P. Suchý, E. Straková, A. Hrubý

Veterinary and Pharmaceutic Faculty, Brno, Czech Republic

ABSTRACT: The objective of the paper was to study variations in cholesterol concentrations in the blood plasma of hens throughout the laying period. A total of 30 clinically healthy hens of laying hybrid MORAVIA BSL were used. Hens were kept individually in cages and received complete feed mixtures for laying hens with these nutrient contents in 100% dry matter: proteins 162.5 g/kg, fat 52.4 g/kg, fiber 31.7 g/kg, Ca 46.3 g/kg, P 10.4 g/kg, Mg 5.6 g/kg, ash 160.2 g/kg and with declared minimum energy content of 10.5 MJ/kg. Cholesterol concentrations in the blood plasma of hens were regularly determined in monthly intervals for ten months throughout the laying period. Blood was sampled by the puncture of *vena basilica* and heparin stabilized. Plasma cholesterol was determined photometrically using commercial test kits Bio-Lachema-Test. Ten blood collections in all 30 hens were made in the laying period: 300 individual values of plasma cholesterol of hens were obtained. Great variations in cholesterol concentrations in the blood plasma of hens are evident from experimental data. Individual concentrations of plasma cholesterol ranged from 1.24 to 10.92 mmol/l with average value of 4.51 mmol/l in the experimental set of hens throughout the laying period. It is interesting that cholesterol concentrations in the blood plasma of hens are characterized by typical curves in the laying period. Tab. 1 shows average values of cholesterol concentrations determined in the separate months of laying. Average cholesterol concentrations in blood plasma throughout the laying period ranged from 2.72 to 6.18 mmol/l showing typical curves: their low values ranged from 2.72 to 3.66 mmol/l within the first part of laying period, i.e. by the fifth month. A statistically highly significant ($P \leq 0.1$) increase to 5.45 mmol/l was observed in the second part of the laying period, i.e. since the 6th month, with maximum value of 6.18 mmol/l in the 7th month of laying. From the 7th month to the end of observation (10th month), differences in average values determined in the next months were small and statistically insignificant. Variations in cholesterolemia dynamics throughout the laying period are to be related to laying intensity because higher laying intensity requires higher cholesterol output to produce yolk matter, and this can result in lower plasma cholesterol concentrations. On the other hand, lower laying intensity, decreasing from the 6th and 7th month of laying, is accompanied by lower cholesterol output for egg production, and it can be the reason for its increase in the blood plasma of hens.

Keywords: hens; blood plasma; cholesterol

ABSTRAKT: Cílem předložené práce bylo studium dynamiky cholesterolu v krevní plazmě slepic. Hladiny plazmatického cholesterolu byly sledovány v měsíčních intervalech v průběhu snáškového období. Do sledování bylo zařazeno 30 klinicky zdravých slepic. Celkem bylo provedeno 300 vyšetření a byla stanovena průměrná hodnota cholesterolu 4.51 mmol.l^{-1} ($s_{n-1} = 1.973$) v krevní plazmě vyšetřovaných slepic. Zajímavé výsledky byly dosaženy při sledování dynamiky hladiny cholesterolu v krevní plazmě slepic v jednotlivých měsících snášky. Průměrné hodnoty plazmatického cholesterolu se pohybovaly v rozmezí od 2,73 do 6,18 mmol.l^{-1} . Charakteristická změna byla pozorována v polovině snáškového období, kdy od 6. měsíce snášky byl zaznamenán statisticky vysoce průkazný ($P \leq 0,01$) vzestup hladiny cholesterolu v krevní plazmě slepic. Obecně lze charakterizovat úroveň cholesterolu jako nízkou – hladiny cholesterolu se pohybovaly v rozmezí průměrných hodnot 2,73–3,66 mmol.l^{-1} . Ve druhé polovině snášky (od 6. měsíce) byla hladina cholesterolu vysoká – průměrné hodnoty se pohybovaly v rozmezí od 5,45 do 6,18 mmol.l^{-1} . Změny hladin cholesterolu v krevní plazmě slepic dáváme do souvislosti s intenzitou snášky.

Klíčová slova: slepice; krevní plazma; cholesterol

ÚVOD

Hladina cholesterolu v krevní plazmě je jedním z významných diagnostických ukazatelů při bioche-

mickém vyšetření zvířete. Většina údajů o cholesterolemii v krevní plazmě drůbeže pochází z vědeckých sdělení zabývajících se úrovní metabolického profilu u jednotlivých druhů a věkových kategorií drůbeže.

Sledováním cholesterolu v krvi slepic se zabývala řada autorů. Za významné lze považovat zejména práce autorů Furuse *et al.* (1990), Poyraz (1988) a Salageanu (1989). Většina autorů uvádí průměrné hodnoty krevního cholesterolu v rozmezí od 1,93 do 3,43 mmol.l⁻¹ s tendencí vzrůstu hladiny cholesterolu v průběhu snášky (Salageanu, 1989). Na obdobné změny v dynamice cholesterolu v průběhu snášky upozorňuje i Poyraz (1988), který stanovil v krevním séru slepic ve snáškovém období průměrnou hodnotu cholesterolu 121,31 mg/100 ml (3,14 mmol.l⁻¹). Z našich autorů se vztahem mezi úrovní hladiny cholesterolu v krevní plazmě slepic a vejčích zabývali Suchý *et al.* (1995). Uvádějí, že hladina individuálních hodnot cholesterolu v krevní plazmě 60 vyšetřených slepic se pohybovala v poměrně širokém rozmezí – od 3,55 do 10,25 mmol.l⁻¹.

Z literárních údajů vyplývá, že je třeba upřesnit a doplnit stávající poznatky o dynamice hladiny cholesterolu v krevní plazmě slepic v krátkých časových intervalech v průběhu celého snáškového období a dále prověřit, zda intenzita snášky může ovlivnit i molární koncentraci cholesterolu v krevní plazmě slepic v průběhu snáškového období.

MATERIÁL A METODY

Cílem práce bylo sledovat dynamiku hladiny cholesterolu v krevní plazmě slepic v průběhu snáškového období od měsíce ledna do října. Za tímto účelem bylo vybráno 30 klinicky zdravých slepic Moravia BSL, kterým byla v průběhu snášky v jednoměsíčních intervalech odebírána krev. Slepice byly chovány v klecové technologii v experimentální stáji Ústavu výživy, dietetiky a hygieny vegetabilní VFU v Brně.

Mikroklimatické parametry pokusné stáje byly: průměrná denní teplota vzduchu 13–18 °C, průměrná relativní vlhkost vzduchu 70–75 %, světelný režim ve věku 24 týdnů 10 hodin, 27 týdnů 11 hodin, 29 týdnů 12 hodin, 31 týdnů 13 hodin a od 42. týdne 14 hodin.

Krmení bylo zajištěno kompletní krmnou směsí pro užitkové nosnice s obsahem živin ve 100% sušině: NL 162,5 g.kg⁻¹, tuk 52,4 g.kg⁻¹, vláknina 31,7 g.kg⁻¹, Ca 46,3 g.kg⁻¹, P 10,4 g.kg⁻¹, Mg 5,6 g.kg⁻¹, popel 160,2 g.kg⁻¹ a s minimálním obsahem deklarované ME = 10,5 MJ.kg⁻¹ krmné směsi. Tato směs byla zkrmována po celé období sledování, čímž jsme vyloučili vliv krmiva na kolísání hladiny cholesterolu v krevní plazmě slepic.

Krev pro laboratorní účely byla odebírána punkcí *vena basilica* a stabilizována heparinem.

Součástí laboratorního zpracování odebrané krve bylo z hlediska průběžného objektivního posouzení zdravotního stavu slepic provedeno i základní hematologické a biochemické vyšetření. Protože sledované hematologické i biochemické ukazatele se pohybovaly ve fyziologických mezích a jejich sledování nebylo cílem této práce, uvádíme pouze hladiny cholesterolu. Cho-

lesterol byl stanoven v krevní plazmě fotometricky pomocí komerčně vyráběných testacích souprav Bio-Lachema-Test. Bylo provedeno 10 vyšetření na 30 slepicích. Celkem bylo získáno 300 údajů o molární koncentraci cholesterolu v krevní plazmě slepic v průběhu snáškového období.

Statistické zpracování bylo provedeno metodou podle autorů Venčíků a Venčíkov (1977). Z jednotlivých statistických ukazatelů byly vypočítány: aritmetický průměr (\bar{x}), směrodatná odchylka (s_{n-1}), střední chyba aritmetického průměru ($s_{\bar{x}}$) a variační koeficient (v). Pro posouzení průkaznosti rozdílů mezi průměrnými hodnotami bylo použito Studentova testu s pravděpodobností $P \leq 0,05$ a $P \leq 0,01$.

VÝSLEDKY

Výsledky sledování cholesterolémie byly získány od klinicky zdravých slepic, které byly umístěny individuálně v klecové technologii a u kterých byl průběžně na základě klinického, hematologického a biochemického vyšetření sledován jejich zdravotní stav. Slepice byly chovány za standardních mikroklimatických podmínek a při konstantní úrovni výživy.

Průměrná hladina cholesterolu v krevní plazmě slepic za celé období snášky byla 4,51 mmol.l⁻¹ s rozmezím individuálních hodnot od 1,24 do 10,92 mmol.l⁻¹. Dynamika průměrných hodnot hladin cholesterolu v krevní plazmě slepic se v průběhu snáškového období pohybovala v rozmezí od 2,73 do 6,18 mmol.l⁻¹.

Z průměrných hodnot stanovených při vyšetření slepic v jednotlivých měsících snášky (tab. I) je zřejmé, že je rozdílná úroveň cholesterolémie v první polovině snášky, tj. od 1. do 5. měsíce, a v druhé polovině snášky, tj. od 6. do 10. měsíce, s dramatickým zvratem mezi 5. a 7. měsícem snášky.

V první polovině snášky kolísaly průměrné hladiny cholesterolu v krevní plazmě od 2,73 do 3,66 mmol.l⁻¹. Nejnižší průměrná hodnota cholesterolu 2,73 mmol.l⁻¹ byla zjištěna v 1. měsíci snášky, ve 2. měsíci nastal statisticky vysoce průkazný ($P \leq 0,01$) vzestup na 3,57 mmol.l⁻¹, ve 3. měsíci snášky byl zachycen neprůkazný pokles na průměrnou hodnotu 3,07 mmol.l⁻¹, ve 4. měsíci snášky průkazný vzestup ($P \leq 0,05$) na 3,66 mmol.l⁻¹ s následným statisticky průkazným ($P \leq 0,05$) poklesem na 3,13 mmol.l⁻¹ v 5. měsíci snášky. Dynamický vzestup cholesterolémie byl zaznamenán od 5. měsíce snášky, kdy průměrná hladina cholesterolu vysoce průkazně vzrostla ($P \leq 0,01$) z 3,13 mmol.l⁻¹ v 5. měsíci na 5,45 mmol.l⁻¹ v 6. měsíci snášky a dále bylo zaznamenáno průkazné zvýšení ($P \leq 0,05$) až na 6,18 mmol.l⁻¹ v 7. měsíci snášky, kdy byla dosažena nejvyšší průměrná hladina cholesterolu v krevní plazmě slepic za celé snáškové období. V následujícím období byl zaznamenán neprůkazný pokles na 5,69 mmol.l⁻¹ v 8. měsíci snášky. V dalších měsících kolísaly průměrné hladiny cholesterolu jen minimálně. a to v 9. měsíci byla průměrná hladina cholesterolu

I. Průměrné hladiny cholesterolu v krevní plazmě slepic v jednotlivých měsících snášky – Average cholesterol concentrations in the blood plasma of hens in the separate months of laying

Měsíc ¹	n	\bar{x}	s_{n-1}	$s_{\bar{x}}$	v	P
1.	30	2,73	0,595	0,109	21,80	
2.	30	3,57	0,836	0,153	23,43	**
3.	30	3,07	1,125	0,205	36,64	
4.	30	3,66	0,804	0,147	21,96	*
5.	30	3,13	1,045	0,191	33,38	*
6.	30	5,45	1,615	0,295	29,64	**
7.	30	6,18	2,004	0,366	32,43	*
8.	30	5,69	1,688	0,308	29,67	
9.	30	5,76	1,974	0,360	34,27	
10.	30	5,80	2,251	0,411	38,82	

* $P \leq 0,05$; ** $P \leq 0,01$

¹ month

5,76 mmol.l⁻¹ a v posledním sledovaném 10. měsíci snášky 5,80 mmol.l⁻¹.

DISKUSE

Celková průměrná hodnota hladiny cholesterolu v krevní plazmě slepic 4,51 mmol.l⁻¹ je téměř shodná s výsledky autora Poyraz (1988), který stanovil hodnotu 121,31 mg/100 ml, tj. 3,14 mmol.l⁻¹.

Za významné z hlediska posouzení dynamiky hladiny cholesterolu v krevní plazmě slepic lze pokládat zjištění, že v průběhu snášky dochází k charakteristickým změnám v úrovni cholesterolémie.

Na obdobné změny v hodnotách cholesterolu, jako jsme zjistili v našem sledování, upozorňují i Furuse *et al.* (1990), Poyraz (1988) a Salageanu (1989), kteří rovněž uvádějí výraznou tendenci vzrůstu hladiny cholesterolu v průběhu snášky.

Při vyšší intenzitě snášky se tedy dá předpokládat i vyšší množství uloženého cholesterolu ve vyprodukované hmotě žloutku, což se může projevit i nižšími hladinami cholesterolu v plazmě slepic.

Naopak při nižší intenzitě snášky se ve vyprodukovaných vejcích ukládá i méně cholesterolu, což může být i jednou z příčin zvýšené hladiny plazmatického cholesterolu. Tento předpoklad potvrzuje i měsíční intenzita snášky, která byla nejvyšší ve 3. měsíci (90,08 %), 4. měsíci (89,70 %) a 5. měsíci (88,78 %). Od 6. do 10. měsíce snášky byl již pozorován výrazný pokles intenzity snášky, a to na 83,17 % (6. měsíc), 81,96 % (7. měsíc), 77,14 % (8. měsíc), 72,00 % (9. měsíc) a 67,92 % (10. měsíc). Výsledky sledování intenzity snášky korespondují s výsledky cholesterolémie v krevní plazmě slepic. Tuto myšlenku potvrzují i závěry práce autorů Suchý *et al.* (1995), kteří uvádějí, že vylučování cholesterolu do žloutku je řízeno aktivním geneticky limitovaným transportem. Proto ani tito autoři neprokázali korelační vztah mezi koncentrací cholesterolu v krevní plazmě slepic a ve vaječném žloutku.

LITERATURA

- Furuse M., Nakajima S. I., Nakagawa J., Shimizu T., Okumura J. I. (1990): Regulation of lipid metabolism by dietary sorbose in laying. *Poult. Sci.*, 69: 1508–1512.
- Poyraz O. (1988): Study of plasma glucose, cholesterol and protein values in chickens, quail and their hybrids. *Lalahan Hayvancılık Arastırma Enstitüsü Dergisi*, 28. (1–4): 24–35.
- Salageanu G. (1989): Blood lipid dynamics in fowls raised for eggs. *Lucrari Stiintifice, Institutul Agronomic Nicolae Balcescu, Bucuresti, Seria C, Medicina Veterinara*, 32 (1): 33–39.
- Suchý P., Ingr I., Stráková E. (1995): Vztah mezi hladinami cholesterolu ve vejcích a krevní plazmě slepic. *Živoč. Vyr.*, 40: 11–14.
- Venčík A. I., Venčík V. A. (1977): Základní metody statistického zpracování dat ve fyziologii. Praha, Avicenum.

Došlo 13. 7. 1998

Přijato k publikování 10. 11. 1998

Kontakní adresa:

Doc. MVDr. Ing. Pavel Suchý, CSc., Veterinární a farmaceutická univerzita, Fakulta veterinární hygieny a ekologie, Ústav výživy, dietetiky a hygieny vegetabilii, Palackého 1/3, 612 42 Brno, Česká republika, tel.: 05/41 56 25 41, fax: 05/41 56 20 12

ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

Ústřední zemědělská a lesnická knihovna (ÚZLK)

Slezská 7, 120 56 Praha 2, tel.: 02/24 25 79 39, fax: 02/24 25 39 38

Máte zájem o pravidelné sledování nejčerstvějších informací ze zahraničních odborných časopisů?

Tento požadavek Vám rádi splníme, objednáte-li si naši informační reprografickou službu „Obsahy zahraničních časopisů“ a články typu „Current Contents“.

Vyberete-li si z každoročně aktualizovaného **Seznamu časopisů objednaných do fondu ÚZLK** sledování nejzajímavějších časopisů z Vašeho oboru, zašleme Vám nejprve kopie obsahů nejčerstvějších čísel časopisů a na základě výběru kopie požadovaných článků.

Chtěli bychom Vás také upozornit na další reprografickou službu ÚZLK, a to na poskytování kopií článků z knih a časopisů, které jsou ve fondu ÚZLK. Požadavky na tyto kopie můžete uplatňovat v průběhu celého roku na formulářích „Objednávka reprografické práce“, které si můžete objednat v Technickém ústředí knihoven, Solniční 12, 601 74 Brno, pod katalog. č. TÚK 138-0.

Veškeré další informace a objednávky na reprografické služby včetně Vašich připomínek Vám poskytneme na adrese:

Ústřední zemědělská a lesnická knihovna – ÚZPI

Odd. reproslužeb

Slezská 7, 120 56 Praha 2

Poštovní schránka 39

Telefonické dotazy: 02/24 25 79 39, linka 329, 421 nebo 306

VPLYV SKRMOVANIA MLIEČNYCH FERMENTOVANÝCH PRODUKTOV NA SPOTREBU ŽIVÍN U TELIAT DO VEKU 90 DNÍ

THE EFFECT OF FEEDING LACTIC FERMENTED PRODUCTS ON NUTRIENT CONSUMPTION IN CALVES UNTIL 90 DAYS OF AGE

M. Svetlanská¹, A. Sommer¹, P. Petrikovič¹, V. Boros²

¹Research Institute of Animal Production, Nitra, Slovak Republic

²Dairy Industry Research Institute, Žilina, Slovak Republic

ABSTRACT: A feeding trial was conducted on calves of Black-Pied breed managed under an individual housing and feeding system between 12 and 90 days of age; the calves that received lactic culture in powder form containing more than 10^6 of active microflora of the bacteria *Bifidobacterium* and *Lactobacillus* showed the average daily weight gain of 0.746 kg (+ 5.4%) while the weight gain of control group of calves was 0.708 kg. The average values of differences in daily weight gains of calves determined over the whole period were nonsignificant. The calf health was not negatively influenced by the tested product. The calves of experimental group had higher consumption of supplemental feed mixture (55.78 kg + 19%) and alfalfa hay (70.24 kg + 24%) than the calves of control group ($P < 0.001$), 46.70 kg, 56.74 kg. The product applications influenced ($P < 0.001$) the consumption of dry matter, crude protein, fiber, NEL, Ca and P in weekly intervals while the largest differences between the groups of calves were determined between 29th and 77th day of calf age. The differences of the nutrient conversion per 1 kg of weight gain were not significant over the experimental period. The consumption in the calves of experimental group was 655 g of crude protein, 330 g PDI and 22.43 MJ NEL per 1 kg weight gain. The respective values in the calves of control group were lower: 596 g of proteins, 303 g PDI and 21.35 MJ NEL per 1 kg of weight gain.

Key words: calves; bifidobacteria; lactobacilli; feed consumption; dry matter; crude protein; fiber; NEL

ABSTRAKT: V individuálnom kŕmnom pokuse v období 12. až 90. dňa veku sme u teliat čiernostrakatého plemena sledovali vplyv skrmovania mliečného fermentovaného produktu, ktorý v suchom stave obsahoval viac ako 10^6 aktívnej mikroflóry baktérií rodov *Bifidobacterium* a *Lactobacillus*. Teľatá pokusnej skupiny ($n = 11$) dosiahli za celé sledované obdobie priemerný denný prírastok živej hmotnosti 0,746 kg (+ 5,4 %) a kontrolnej skupiny ($n = 11$) 0,708 kg. U teliat pokusnej skupiny sme zistili vyššiu spotrebu doplnkovej kŕmnej zmesi (55,78 kg + 19 %) a lucernového sena (70,24 kg + 24 %) oproti teľatám kontrolnej skupiny ($P < 0,001$) – 46,70 kg, 56,74 kg. Skrmovanie preparátu ovplyvnilo ($P < 0,001$) spotrebu sušiny, N-látok, vlákniny, NEL, Ca a P v týždňových intervaloch, keď najväčšie rozdiely medzi skupinami teliat boli v období 29. až 77. dňa veku teliat.

Kľúčové slová: teľatá; bifidobaktérie; laktobacily; spotreba kŕmív; sušina; N-látky; vláknina; NEL

ÚVOD

Profylaktický a terapeutický účinok bifidobaktérií a laktobacilov sa odvíja od ich schopnosti adherovať k intestinálnemu povrchu a osídľovať zažívacie trakt mláďat hospodárskych zvierat a takto súťažiť o priestor a živiny s ostatnou mikroflórou. Tieto kmene mikroorganizmov tvoria prirodzenú súčasť črevnej mikroflóry cicavcov a prostredníctvom vytvorených metabolitov sa výrazne podieľajú na potláčaní nežiadúcej mikroflóry v zažívacom trakte. V praktickom kŕmení to umožňuje zefektívniť využitie živín v období, keď postnatálny vývoj teliat z nutričného hľadiska predstavuje kritické ob-

dobie adaptácie prechodu z mliečnej výživy a slezového trávenia na rastlinnú výživu a bachorové trávenie. Podľa autorov Burgstaller *et al.* (1984), Kirchgessner (1987), Svozil *et al.* (1987), Roth *et al.* (1992), Wallace, Newbold (1992) a Kampf, Flachowsky (1997) mliečne fermentované produkty zvyšujú prírastky živej hmotnosti, zlepšujú konverziu živín kŕmiva a uplatňujú sa na prevenciu hnačkových ochorení teliat (Bomba *et al.*, 1996 a inf).

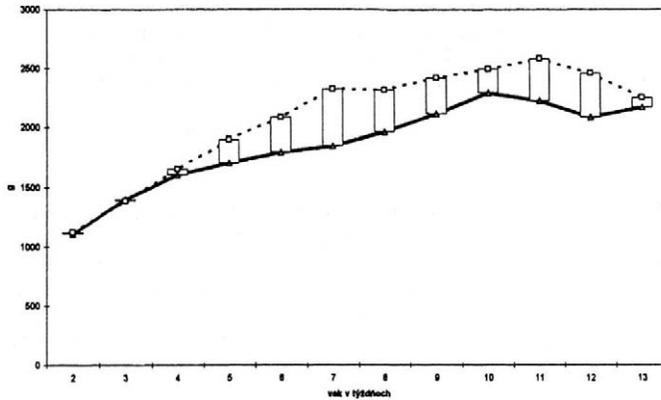
Cieľom práce bolo sledovať spotrebu živín u teliat do veku 90 dní pri skrmovaní mliečného fermentovaného produktu v suchom stave s obsahom viac ako $10^6 \cdot g^{-1}$ aktívnej mikroflóry baktérií rodov *Bifidobacterium* a *Lactobacillus*.

MATERIÁL A METÓDA

Do kŕmneho pokusu (VÚŽV Nitra) sme zaradili 22 teliat čiernostrakateho plemena v druhom týždni po narodení (12 ± 5 dní). Tefatá sme ustajnilí individuálne v búdach a rozdelili do dvoch skupín (pokusná – P, kontrolná – K) podľa živej hmotnosti, veku a pohlavia. Obidve skupiny zvierat sme napájali mliečnou kŕmnu zmesou MKZ (pôvodná sušina 949 g) tohto zloženia (na kg sušiny): NL 213 g, tuk 37 g, PDI 94 g, NEL 9 MJ, bielkoviny 196 g, škrob 86 g, celkové cukry 156 g, Ca 5,2 g, P 8,5 g s tým, že pokusným tefatám sme pridávali mliečny fermentovaný produkt MFP (sušina 930 g) tohto zloženia (na kg sušiny): NL 350 g, tuk 3 g, PDI 107 g, NEL 9 MJ, bielkoviny 337 g, škrob 74 g, celkové cukry 316 g, Ca 11 g, P 7 g v dávkach odporučených podľa VÚM Žilina, kde 100 g MFP nahrádzalo 1 liter MKZ. Množstvo nápoja u oboch skupín teliat bolo rovnaké. Riedenie MFP a MKZ bolo 1 : 9. Tefatá sme napájali trikrát denne. Od 14. do 56. dňa veku dostávali 8 litrov nápoja, od 57. do 70. dňa 6 l a od 71. dňa 5 l. Odstav od tekutej výživy bol v 12. týždni veku teliat (78–84 dní). Metodicky sme zvolili „tradičný“ odchov teliat, aby sa prejavil vplyv použitia testovaného prípravku v čo najdlhšom časovom období. Lucernové seno (sušina 872 g; na kg sušiny: NL 197 g, PDI 96 g, vláknina 336 g, NEL 5 MJ) a doplnkovú kŕmnu zmes na odchov a výkrm teliat TKŠ (sušina 871 g; na kg sušiny: NL 189 g, PDI 124 g, vláknina 87 g, NEL 7 MJ) sme podávali oboch skupinám *ad libitum*. Spotrebu kŕmiv a živín sme sledovali individuálne denne u každého zvieraťa a prírastky živej hmotnosti v dvojtýždňových intervaloch. Priemerné vzorky kŕmiv sme analyzovali podľa STN 46 7092 Analytické metódy skúšania kŕmiv, Ca na AA spektrofotometri (Umicam 939), P fotometricky (Spekol 11) raz mesačne. Na základe získaných údajov obsahu živín v sledovaných kŕmivách sme urobili prepočty výživnej hodnoty kŕmiv (Sommer *et al.*, 1994). Pokus sme ukončili v 90. dni veku teliat. Na spracovanie štatistických charakteristík sme použili PC-program Microsoft Excel 7.

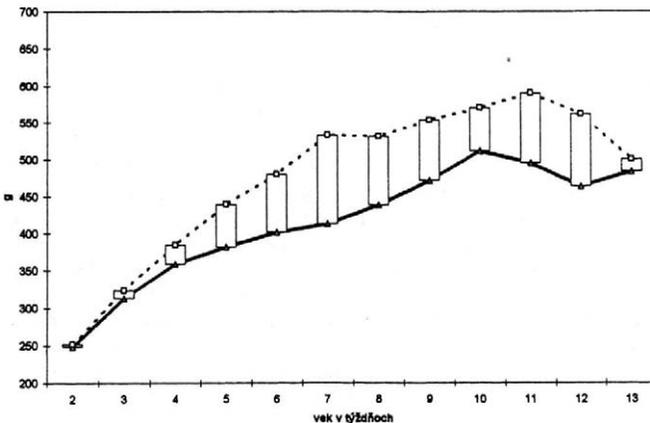
VÝSLEDKY A DISKUSIA

Za obdobie 12. až 90. dňa veku dosiahla pokusná skupina teliat pri skrmovaní mliečného fermentovaného produktu priemerný denný prírastok živej hmotnosti



1. Priemerný príjem sušiny v gramoch za sledované obdobie (kus a deň) – Average uptake of dry matter in grams over the experimental period (head and day)

For Figs. 1–5: kontrola = control, pokus = experiment, vek v týždňoch = age in weeks



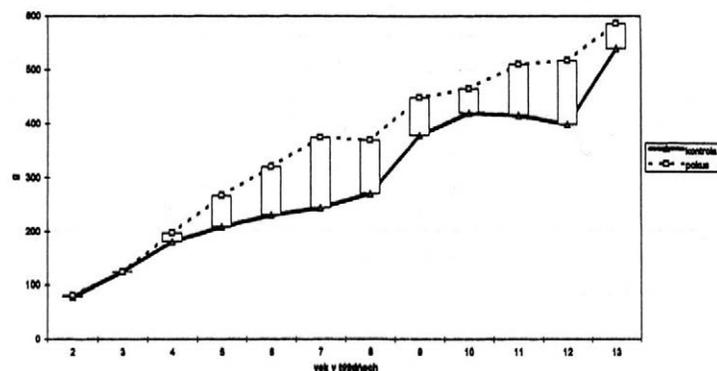
2. Priemerný príjem N-látok v gramoch za sledované obdobie (kus a deň) – Average uptake of proteins in grams over the experimental period (head and day)

vyšší o 5 % ($P = 0,746$ kg, $K = 0,708$ kg). Do 56. dňa veku teliat sme zistili u pokusnej skupiny priemerný denný prírastok živej hmotnosti 0,605 g a od 57. do 90. dňa 0,933 g (kontrolná skupina teliat – 0,587 g, t.j. o 3 % menej, resp. 0,889 g, t.j. o 5 % menej). Zistené rozdiely v priemerných denných prírastkoch živej hmotnosti teliat za obdobia nie sú štatisticky preukazné ($P > 0,05$) a korešpondujú s výsledkami autorov Svozil *et al.* (1987) a Skřivanová, Machaňová (1990).

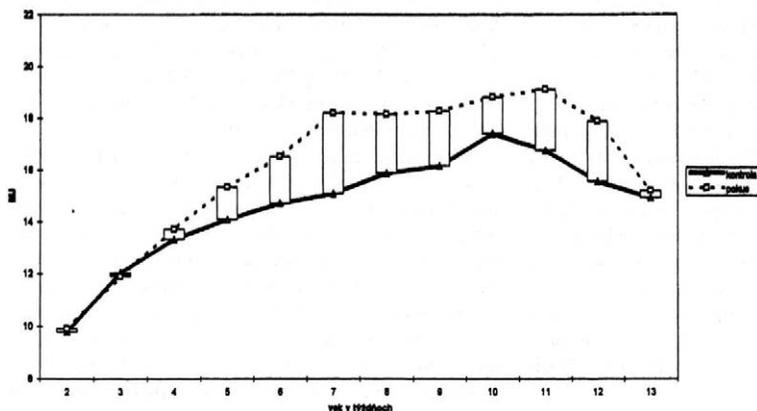
Za celé obdobie pokusu sme u teliat nezaznamenali ani jeden úhyn. Tetať pokusnej aj kontrolnej skupiny boli zdravé, bez zjavných klinických príznakov. Výsledky sú zrovnateľné s údajmi uvádzanými pri aplikácii mikrobiálnych kultúr vo výžive teliat (Burgstaller *et al.*, 1984; Kirchgessner, 1987 a iní).

Tetať pokusnej skupiny spotrebovali viac ($P < 0,001$) TKŠ – 55,78 kg (+ 9 kg, resp. + 19 %) a lucernového sena – 70,24 kg (+ 14 kg, resp. + 24 %) ako tetať kontrolnej skupiny. Skrmovanie sušenej mliečnej kultúry bifidobaktérií ovplyvnilo príjem krmív, čo je v súlade s výsledkami autorov Czister *et al.* (1997), ktorí zistili štatisticky preukazné ($P < 0,05$) vyšší príjem jadrového krmiva (okrem 6. týždňa veku) a sena pri experimentálnom podávaní mikrobiálnych kultúr.

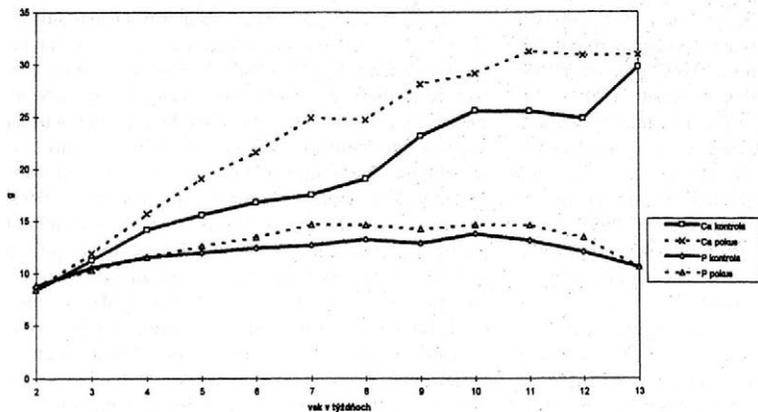
Na obr. 1 až 4 uvádzame priemerný príjem sušiny, N-látok, vlákniny a energie u teliat za sledované obdobie v týždenných intervaloch. Štatisticky vysoko preukazné rozdiely ($P < 0,001$) sme zaznamenali v období medzi 29. až 77. dňom veku teliat, keď tetať pokusnej skupiny spotrebovali o 200 až 400 g viac sušiny ako tetať kontrolnej skupiny. V priemere za celé sledované obdobie tetať pokusnej skupiny spotrebovali 2 079 g sušiny a tetať kontrolnej skupiny 1 858 g. Rozdiel bol štatisticky preukazný ($P < 0,05$). Pri sledovaní príjmu N-látok v týždenných intervaloch sme zaznamenali štatisticky preukazné rozdiely ($P < 0,001$) medzi skupinami teliat vo všetkých týždňoch (okrem prvého a posledného týždňa sledovania). Tetať pokusnej skupiny prijali viac N-látok ako tetať kontrolnej skupiny. V priemere za celé sledované obdobie spotrebovali tetať pokusnej skupiny 475 g N-látok a tetať kontrolnej skupiny 415 g. Podobnú tendenciu sme zistili aj v spotrebe vlákniny (obr. 3). V priemere za celé sledované obdobie prijali tetať pokusnej skupiny o 64 g vlákniny viac ako tetať kontrolnej skupiny. Rozdiel bol štatisticky významný ($P < 0,05$). V prijíme NEL bol medzi 29. až 84. dňom veku teliat štatisticky významný rozdiel ($P < 0,001$, resp. $P < 0,05$) medzi skupinami teliat.



3. Priemerný príjem vlákniny v gramoch za sledované obdobie (kus a deň) – Average uptake of fiber in grams over the experimental period (head and day)



4. Priemerný príjem NEL v MJ za sledované obdobie (kus a deň) – Average uptake of NEL in MJ over the experimental period (head and day)



5. Priemerný príjem Ca a P v gramoch za sledované obdobie (kus a deň) – Average uptake of Ca and P in grams over the experimental period (head and day)

I. Štatistické spracovanie priemerného príjmu živín v týždňových intervaloch – Statistical processing of data on average nutrient uptake in weekly intervals

Vek (deň) ¹	Sušina ²	N-látky ³	Vláknina ⁴	Organická hmota ⁵	NEL	PDIN	Škrob ⁶	Cukry ⁷	Ca	P
8.–14.	–	–	–	–	–	–	–	–	–	–
15.–21.	–	+	–	–	–	+	–	+++	–	+
22.–28.	–	++	+	+	–	++	–	+++	++	–
29.–35.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
36.–42.	++	+++	++	++	+	+++	–	+++	+++	–
43.–49.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
50.–56.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
57.–63.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
64.–70.	+++	+++	++	+++	+++	+++	+++	+++	+++	++
71.–77.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
78.–84.	+	+	–	+	+	–	+++	+++	+	–
85.–90.	–	–	+	–	–	–	+	–	–	–

Štatistická preukaznosť – Statistical significance: + $P < 0.05$; ++ $P < 0.01$; +++ $P < 0.001$

¹age (day), ²dry matter, ³proteins, ⁴fiber, ⁵organic matter, ⁶starch, ⁷sugars

Telatá pokusnej skupiny prijali v tomto období o 1,24 až 2,64 MJ viac NEL ako telatá kontrolnej skupiny. V priemere za sledované obdobie prijali telatá pokusnej skupiny 16,05 MJ NEL a telatá kontrolnej skupiny 14,64 MJ NEL. Rozdiel bol štatisticky významný ($P < 0,05$). Pri sledovaní priemerného príjmu vápnika (obr. 5) sme zistili štatisticky významné rozdiely medzi telatami oboch skupín ($P < 0,001$) v období medzi 22. až 77. dňom veku a u fosforu v období 29. až 77. dňa veku teliat. Telatá pokusnej skupiny prijali za celé obdobie v priemere 23 g Ca a 13 g P a telatá kontrolnej skupiny 19 g Ca a 12 g P.

Skrmovanie sušenej mliečnej kultúry bifidobaktérií štatisticky preukazne ($P < 0,001$) ovplyvnilo spotrebu živín v týždňových intervaloch, keď najväčšie rozdiely medzi skupinami teliat boli v období 29. až 77. dňa veku (tab. I). Účinok mliečného fermentovaného produktu sa prejavil v spotrebe živín až po dvoch týždňoch jeho skrmovania.

Pri prepočte spotreby živín na 1 kg prírastku živej hmotnosti sme zistili štatisticky nepreukazné ($P > 0,05$) rozdiely medzi skupinami teliat. Pokusná skupina spotrebovala na 1 kg prírastku ž.h. 655 g N-látok, 330 g PDI a 22,43 MJ NEL, zatiaľ čo kontrolná skupina teliat 596 g N-látok, 303 g PDI a 21,35 MJ NEL. Rozdiel medzi porovnávanými skupinami bol v spotrebe N-látok 9,9 %, v PDI 8,3 % a v NEL 5,1 %.

Skrmovanie mliečného koncentráту v suchom stave s obsahom viac ako 10^6 aktívnej mikroflóry baktérií rodov *Bifidobacterium* a *Lactobacillus* u teliat do veku 90 dní pozitívne ovplyvnilo prírastky živej hmotnosti teliat o 5 %. Výrazne zvýšilo spotrebu najmä objemových krmív, keď do 56. dňa veku teliat sa príjem lucernového sena zvýšil o 33 % a za celé sledované obdobie o 24 %, čo má vplyv na rýchlejší rozvoj predžalúdkov a pozitívny vplyv na vývoj a vývin teliat. Telatá sa takto rýchlejšie pripravujú na zvýšený príjem objemových

krmív, čo sa pozitívne prejaví v neskoršom období ich výkrmu.

LITERATÚRA

- Bomba A., Kravjanský I., Kaštel R., Herich R., Juhásová Z., Čížek M., Kapitančík B. (1996): Inhibitory effects of *Lactobacillus casei* upon the adhesion of enterotoxigenic *Escherichia coli* K99 to the intestinal mucosa in gnotobiotic lambs. *Small Ruminant Res.*, 23: 199–206.
- Burgstaller G., Ferstl R., Alps H. (1984): Zum Zusatz von Milchsäurebakterien (*Streptococcus faecium* SF-68) im Milchaustauschfuttermittel für Mastkälber. *Züchtungskunde*, 56: 156–162.
- Czister L. T., Jurcoane S., Pamfil M., Vamanu A. (1997): The influence of bactoprolact on growth performance and health of calves. In: 48th Annual Meet. of the EAAP, Vienna, August.
- Kampf D., Flachowsky G. (1997): Untersuchungen zum Einsatz von *Saccharomyces cerevisiae* in der Milch Milchviehfütterung. In: 48th Annual Meet. of the EAAP, Vienna, August.
- Kirchgesner M. (1987): Tierernährung. DLG-Verlag, 523 s.
- Roth F. X., Kirchgesner M., Eidelsburger U., Gedek B. (1992): Zur nutritiven Wirksamkeit von *Bacillus cereus* als Probiotikum in der Kalbermast. I. Einfluss auf Wachstumsparameter, Schlachtleistung und mikrobielle Metaboliten im Dunndarm. *Agribiol. Res.*, 45: 294–302.
- Skřivanová V., Machaňová L. (1990): Vliv probiotika *Lactobacillus acidophilus* na užítkovost a parametry bachorové tekutiny telat. *Živoč. Vyr.*, 35: 87–94.
- Sommer A., Čerešňáková Z., Frydrych Z., Pajtáš M., Petrikovič P., Krása A., Zeman L. (1994): Potreba živín a výživná hodnota krmív pre hovädzí dobytok, ovce a kozy. I. vyd. Nitra, VÚŽV, 113 s.
- Svozil B., Daněk P., Kumprecht I., Zobač P. (1987): Účinnost odstupňovaných hladin baktérií *Streptococcus faecium* M-74 ve výživě telat. *Živoč. Vyr.*, 32: 265–271.
- Wallace R. J., Newbold C. J. (1992): Probiotics for ruminants. In: Fuller R. (ed.): *Probiotics the Scientific Basis*. London, Chapman and Hall: 317–353.
- STN 46 7092 (1988): Analytické metódy skúšania krmív. 1988.

Došlo 7. 4. 1998

Prijaté k publikovaniu 7. 9. 1998

Kontaktná adresa:

Ing. Mária Svetlanská, PhD., Výskumný ústav živočíšnej výroby, Hlohovská 2, 949 92 Nitra, Slovenská republika, e-mail: svetlan@vuzv.sk

ÚSTŘEDNÍ ZEMĚDĚLSKÁ A LESNICKÁ KNIHOVNA, PRAHA 2, SLEZSKÁ 7

Ústřední zemědělská a lesnická knihovna v Praze (dále jen ÚZLK), která je jednou z největších zemědělských knihoven na světě, byla založena v roce 1926. Již od počátku šlo o knihovnu veřejnou. Knihovna v současné době obsahuje více než jeden milion svazků knih, cestovních zpráv, dizertací, literatury FAO, svázaných ročníků časopisů z oblasti zemědělství, lesnictví, veterinární medicíny, ekologie a dalších oborů. Knihovna odebírá 750 titulů domácích a zahraničních časopisů. Informační prameny získané do fondu jsou v ÚZLK zpracovávány do systému katalogů – je budován jmenný katalog a předmětový katalog jako základní katalogy knihovny a dále různé speciální katalogy a kartotéky. Počátkem roku 1994 přistoupila ÚZLK k automatizovanému zpracování knihovního fondu v systému CDS/ISIS.

Pro informaci uživatelů o nových informačních pramenech ve fondech ÚZLK zpracovává a vydává knihovna následující publikace: Přehled novinek ve fondu ÚZLK, Seznam časopisů objednaných ÚZLK, Přehled rešerší a tematických bibliografií z oboru zemědělství, lesnictví a potravinářství, AGROFIRM – zpravodaj o přírůstcích firemní literatury (je distribuován na disketách), AGROVIDEO – katalog videokazet ÚZLK.

V oblasti mezinárodní výměny publikací knihovna spolupracuje s 800 partnery ze 45 zemí světa. Knihovna je členem IAALD – mezinárodní asociace zemědělských knihovníků. Od září 1991 je členem mezinárodní sítě zemědělských knihoven AGLINET a od 1. 1. 1994 je depozitní knihovnou materiálů FAO pro Českou republiku.

Knihovna poskytuje svým uživatelům následující služby:

Výpůjční služby

Výpůjční služby jsou poskytovány všem uživatelům po zaplacení ročního registračního poplatku. Mimopražští uživatelé mohou využít možností meziknihovní výpůjční služby. Vzácné publikace a časopisy se však půjčují pouze prezenčně.

Reprografické služby

Knihovna zabezpečuje pro své uživatele zhotovování kopií obsahů časopisů a následně kopie vybraných článků. Na počkání jsou zhotovovány kopie na přání uživatelů. Pro pražské a mimopražské uživatele jsou zabezpečovány tzv. individuální reproslužby.

Služby z automatizovaného systému firemní literatury

Jsou poskytovány z databáze firemní literatury, která obsahuje téměř 13 000 záznamů 1 700 firem.

Referenční služby

Knihovna poskytuje referenční služby z vlastních databází knižních novinek, odebíraných časopisů, rešerší a tematických bibliografií, vědeckotechnických akcí, firemní literatury, videotéky, dále z databází převzatých – Celostátní evidence zahraničních časopisů, bibliografických databází CAB a Current Contents. Cílem je podat informace nejen o informačních pramenech ve fondech ÚZLK, ale i jiné informace zajímavé zemědělskou veřejnost.

Půjčování videokazet

V AGROVIDEU ÚZLK jsou k dispozici videokazety s tematikou zemědělství, ochrany životního prostředí a příbuzných oborů. Videokazety zasílá AGROVIDEO mimopražským zájemcům poštou.

Uživatelům knihovny slouží dvě studovny – všeobecná studovna a studovna časopisů. Obě studovny jsou vybaveny příručkovou literaturou. Čtenáři zde mají volný přístup k novinkám přírůstků knihovního fondu ÚZLK.

Adresa knihovny:

Ústřední zemědělská a lesnická knihovna
Slezská 7
120 56 Praha 2

Výpůjční doba:

pondělí, úterý, čtvrtek	9.00–16.30
středa	9.00–18.00
pátek	9.00–13.00

Telefonické informace:

vedoucí:	24 25 50 74, e-mail: IHOCH@uzpi.agrec.cz
referenční služby:	24 25 79 39/linka 520
časopisy:	24 25 66 10
výpůjční služby:	24 25 79 39/linka 415
meziknihovní výpůjční služby:	24 25 79 39/linka 304
fax:	24 25 39 38
e-mail:	ÚZLK@uzpi.agrec.cz

THE EFFECT OF TARGETED COMBINATION OF ADDITIVES TO PRESTARTER ON NUTRITION PARAMETERS AND PERFORMANCE OF PIGLETS*

VLIV CÍLENÉ KOMBINACE ADITIV V PRESTARTÉRU NA NUTRIČNÍ UKAZATELE A UŽITKOVOST SELAT

V. Prokop¹, J. Heger², M. Kumprecht², L. Klapil¹

¹Research Institute of Animal Nutrition, s.r.o., Pohořelice, Czech Republic

²BIOFAKTORY Praha, spol. s r.o., Prague, Czech Republic

ABSTRACT: A comparative breeding and feeding trial was conducted on 235 piglets from 24 litters to study the effect of the combination of olaquinox additives, *Enterococcus faecium* Cernelle 68 probiotic, xylanase and protease enzymes (group and prestarter 3), olaquinox supplement only (group and prestarter 2) and control without additives (group and prestarter 1), on some breeding and nutrition parameters. The trial lasted from piglet birth to 56 days of age, piglets were weighed at birth, weaning and 56 days of age. Prestarters were used until 40 days of age, a single starter between 41 and 56 days of age. All feeds were in granular form. The highest live weight gain by 7–10% was determined in piglets of groups 2 and 3 in the period from birth to 56 days of age. The lowest mortality of piglets was recorded in group 3 (4.3% only), but it was significantly higher in groups 1 and 2 (10% and 14.5%, resp.). Prestarter consumption until weaning was significantly highest in piglets of group 3, total prestarter consumption was obviously higher in groups 2 and 3 than in group 1. Feed conversion from weaning to 56 days of age was better by 16% and 15%, respectively, in piglets of group 3 than in piglets of groups 1 and 2. Apparent digestibility of nutrients and amino acids LYS, THR, MET, CYS, and N balance of prestarters 1 and 3 in two balance periods were determined in a metabolic trial on nine piglets. All experimental parameters were higher in prestarter 3: digestibility of proteins by 3%, amino acids by 3–5% ($P < 0.01$), fiber by 17% ($P < 0.01$), N utilization from N uptake by 10% ($P < 0.01$), and protein deposition per piglet/day by 9% ($P < 0.01$).

Keywords: piglets; prestarter; growth stimulator; probiotic; enzymes; growth; feed conversion; nutrient digestibility; N balance

ABSTRAKT: Ve srovnávacím chovatelsko-krmném pokusu s 235 selaty z 24 vrhů byl sledován vliv kombinace aditiv olachindoxu, probiotika *Enterococcus faecium* Cernelle 68, enzymů xylanázy a proteázy (skupina a prestartér 3), doplňku pouze olachindoxu (skupina a prestartér 2) a kontroly bez aditiv (skupina a prestartér 1) na některé chovatelské a nutriční ukazatele. Pokus probíhal od narození selat do 56. dne věku. Selata byla vážena při narození, při odstavu a v 56. dni věku. Prestartéry byly zkrmovány do 40. dne věku, jednotný startér od 41. do 56. dne věku. Všechny směsi byly granulovány. Nejvyššího přírůstku živé hmotnosti bylo dosaženo u selat skupin 2 a 3, a to od narození do 56. dne věku o 7–10 %. Nejnížší úhyn selat byl evidován ve skupině 3 (pouze 4,3 %), ve skupinách 1 a 2 byl významně vyšší (10 %, resp. 14,5 %). Spotřeba prestartéru do odstavu byla významně nejvyšší u selat skupiny 3, celková spotřeba prestartéru byla ve skupinách 2 a 3 zřetelně vyšší než ve skupině 1. Konverze směsí od odstavu do 56. dne věku byla u selat skupiny 3 o 16 %, resp. 15 % lepší než u selat skupiny 1, resp. skupiny 2. V bilančním pokusu s 9 selaty byla stanovena zdánlivá stravitelnost živin a aminokyselin LYS, THR, MET, CYS a bilance dusíku prestartérů 1 a 3 ve dvou bilančních obdobích. Všechny sledované ukazatele byly vyšší u prestartéru 3 – stravitelnost dusíku o 3 %, aminokyselin o 3–5 % ($P < 0,01$), vlákniny o 17 % ($P < 0,01$), využití dusíku z přijatého dusíku o 10 % ($P < 0,01$) a ukládání N-látek na sele a den o 9 % ($P < 0,01$).

Klíčová slova: selata; prestartér; stimulator růstu; probiotikum; enzymy; růst; konverze směsí; stravitelnost živin; bilance dusíku

* This study was supported by the National Agency for Agricultural Research of the Ministry of Agriculture of CR (project No. 7015), by the company BIOFAKTORY Praha, spol. s r.o., and by the Research Institute of Animal Nutrition, s.r.o., Pohořelice.

INTRODUCTION

Applications of feed additives are justified particularly in the young of farm animals, mainly in piglets. Feed additives are crucial for good health, influenced particularly by balanced microbial colonization of the digestive tract (eubiosis), as a precondition of high performance and survival. Feed additives are nutritive, pronutritive, bioregulating, growth-stimulating and auxiliary. As nutritive additives (minerals, trace elements, vitamins, synthetic amino acids) are components of the necessary nutrient complex and auxiliary additives adjust physical properties of feeds (binders, sorbents), pronutritive, bioregulating and growth-stimulating additives are currently highlighted. Since the desirable state of eubiosis in piglets in the population, herds and groups of these animals is accompanied by latency periods (disturbed eubiosis manifested by moderate health and dietetic problems) and crisis periods (expressive disbalance of microbial colonization of the digestive tract manifested by severe dietetic problems, diarrheas, dehydration and mortality), the combined use of additives of all the three groups is very urgent while it is expected to produce additive or synergetic effects.

The particular preparations of the above groups of additives are produced by many renowned world companies that usually declare separately positive effects of their products, but for competition reasons neither are they willing nor capable to solve the problems arising when combinations of compatible additives of different and targeted effects are applied. Many foreign producers of premixes for prestarters and starters for piglets use combinations of additives but their formulations are empirical or influenced by commercial interests of producers of the particular preparations.

Information on separate effects of the particular additives is sufficient, and effects of some simple combinations are also evident. Lists of compatibility are available for most preparations. Hence it is possible to expect that the dietetic and productive effects of prestarters and starters will be improved by applications of targeted combinations of pronutritive, bioregulating and growth-stimulating additives.

Effective additives have to be used in feed mixtures for piglets in the present conditions of piglet production, mainly in prestarters. Effects of the separate pronutritive, growth-stimulating and bioregulating additives are generally well-known (Prokop, 1996), hence compatible effective combinations of these additives are searched for. Possible combinations are often discussed and partly tested (Anonymous, 1994; Zolotnitskaya, 1994). Literary sources dealing with this problem are scarce, and they often refer to the theoretical existence of different combinations (Balta, 1994). Information on a definite group of additives to be applied to piglets has been published more frequently, as reported by Morlacchini (1994) and Dong-Kesu-Xiao-Zhenduo (1994) in the case of probiotics.

A book published by Kreuzer (1994) provides more specific information; he studied parallel effects of probiotics and antibiotics on intestinal fermentation and characteristics of the intestinal chyme while experimental results were substantially influenced by the rate of compatibility of the additives of both groups.

The objective of this study is to compare the nutritive and breeding effects of prestarters applied different additives and their targeted combination.

I. Formulations of experimental feed mixtures (%) and their nutritive values

Ingredient	Prestarters			Starter	
	1	2	3		
Wheat	-	-	-	29.0	
Barley	-	-	-	-	
Corn	-	-	-	23.6	
Wheat bran	-	-	-	3.0	
Hulled oats	-	-	-	4.0	
Linseed	-	-	-	2.0	
Extruded wheat	28.3	28.3	28.3	-	
Extruded barley	14.1	14.1	14.1	-	
Extruded corn	10.3	10.3	10.3	-	
Extruded wheat bran	3.8	3.8	3.8	-	
Extruded hulled oats	5.1	5.1	5.1	-	
Extruded linseed	2.6	2.6	2.6	-	
Soybean extrudate	15.0	15.0	15.0	14.0	
Soybean meal	-	-	-	5.0	
Fish meal	6.0	6.0	6.0	3.0	
Specilac 10	8.0	8.0	8.0	9.0	
Meat-bone meal	-	-	-	4.0	
Acid pak 4 way	-	-	-	0.2	
Premix Lys HCl (50%)	0.5	0.5	0.5	0.2	
Premix Met (50%)	0.2	0.2	0.2	-	
Premix Thr (20%)	0.7	0.7	0.7	0.3	
Calcite	1.1	1.1	1.1	1.5	
Dicalcium phosphate	1.1	1.1	1.1	0.5	
Feeding salt	0.2	0.2	0.2	0.2	
Rapeseed oil	1.5	1.5	1.5	-	
Aminovitan COS Special 1	1.5	-	-	-	
Aminovitan COS Special 2	-	1.5	-	-	
Aminovitan COS Special 3	-	-	1.5	-	
Aminovitan COS plus	-	-	-	0.5	
	100	100	100	100	
Crude protein	g/kg	242	242	242	222
MEp	MJ/kg	14.0	14.0	14.0	13.8
Lys	g/kg	14.5	14.5	14.5	13.7
Thr	g/kg	9.3	9.3	9.3	8.5
Met + Cys	g/kg	8.3	8.3	8.3	7.1
Fiber	g/kg	34	34	34	32
Ca	g/kg	9.9	9.9	9.9	9.7
P	g/kg	7.8	7.8	7.8	7.5

MATERIAL AND METHODS

Twenty-four litters with 235 piglets in total were included in a comparative breeding and feeding trial. The trial was conducted in three groups; Table III shows the number of litters and piglets in the separate groups. Group 1 comprised 9 litters, sows were at the 4.1th litter on average, 7 sows were LW x L crossings, 2 sows were Seghers hybrids. The sows in Group 2 were at the 6th litter on average, 7 sows were LW x L crossings, one sow was a Seghers hybrid. Group 3 comprised 7 litters, sows were at the 5.8th litter on average, 5 sows were LW x L crossings, 2 sows were Seghers hybrids. The litter size in the particular groups was from 9.5 to 10.0 piglets per litter.

The three experimental groups received different contents of additives in prestarters in agreement with the trial objective (the only difference between their diets). Tabs. I and II show experimental treatments. The prestarter for Group 1 did not contain any pronutritive, bioregulating or growth-stimulating additives (zero control), the prestarter for Group 2 contained 50 mg of olaquinox per 1 kg of feed while the prestarter for group 3 was added the same dose of olaquinox as Group 2, and *Enterococcus faecium* 68 in form of LBC ME-10 at a dose of 1.4 g per 1 kg of feed as a bioregulator, and the enzymes xylanase (at least 5,000 units per 1 g) and protease (at least 500 units per 1 g) in form of the preparation Porzyme 8300 at a dose of 1 g per 1 kg feed as pronutritive additives.

II. Contents of active ingredients per 1 kg of Aminovitan supplements

Ingredients	Unit	Aminovitan ČOS supplements			
		Special 1	Special 2	Special 3	Plus
Vitamin A	i.u.	1 330 000	1 330 000	1 330 000	2 000 000
Vitamin D ₃	i.u.	150 000	150 000	150 000	400 000
Vitamin E	mg	6 670	6 670	6 670	6 000
Vitamin K ₃	mg	120	120	120	300
Vitamin B ₁	mg	130	130	130	200
Vitamin B ₂	mg	510	510	510	900
Vitamin B ₆	mg	510	510	510	400
Vitamin B ₁₂	mg	3.3	3.3	3.3	4
Niacin	mg	2 670	2 670	2 670	4 000
Calcium pantothenate	mg	1 000	1 000	1 000	2 000
Biotin	mg	10	10	10	20
Folic acid	mg	67	67	67	-
Cholin chlorid	mg	33 350	33 350	33 350	80 000
Vitamin C	mg	8 340	8 340	8 340	8 000
Antioxidant	mg	10 000	10 000	10 000	20 000
Saccharin	mg	10 000	10 000	10 000	-
Cobalt	mg	43	43	43	115
Copper	mg	11 130	11 130	11 130	27 000
Iron	mg	21 350	21 350	21 350	20 700
Iodine	mg	75	75	75	185
Manganese	mg	4 000	4 000	4 000	9 170
Zinc	mg	11 700	11 700	11 700	23 300
Selenium	mg	26	26	26	48
Magnesium	mg	33 300	33 300	33 300	-
Lys HCl	mg	312 500	312 500	312 500	250 000
Thr	mg	107 000	107 000	107 000	-
Met	mg	47 000	47 000	47 000	-
Trp	mg	10 000	10 000	10 000	-
Pig flavor F	mg	16 670	16 670	16 670	-
olaquinox	mg	-	3 300	3 300	-
LBC ME 10	mg	-	-	93 300	-
Porzyme 8300	mg	-	-	66 660	-
Feeding wheat flour		ad 1 kg	ad 1 kg	ad 1 kg	ad 1 kg

The trial lasted from piglet birth to 56 days of their age. Piglets were weighed at birth, at weaning and at 56 days of age. Prestarter consumption was recorded until weaning (31 days of age), from weaning to 40 days of age, and subsequently, starter consumption was recorded between 41 and 56 days of age. The diets did not differ since 41 days of age any longer, and all groups received the same starter; its formulation is shown in Tab. I. All feeds were granulated on a 2.5mm matrix. The health of sows and piglets was examined during the trial. The sows with a diagnosis of MMA syndrome were not included in the trial.

A metabolic trial was conducted on 9 piglets-barrows to study the effect of a combination of additives (prestarter 3) on N balance and apparent digestibility of nutrients and amino acids. Control group consisted of 5 piglets, experimental group of 4 piglets, average live weight of experimental piglets was 9.74 kg. Tab. I

shows feed formulations (control – prestarter 1, experimental diet – prestarter 3). The metabolic trial had 2 successive five-day balance periods, every experimental treatment and parameter were documented by 8–10 data. The data were processed by analysis of variance.

Classical procedures on Tecator apparatuses were used for nutrient analysis, amino acids were determined by gradient elution column ionex chromatography on an AAA 400 apparatus.

RESULTS AND DISCUSSION

Tab. III and IV show the results of comparative breeding and feeding trial. The effects of additives were reflected in the growth of piglet live weight while live weight gains were higher in Group 2 (olaquinox)

III. Results of the comparative trial

Characteristic	Group – diet		
	1	2	3
Litter number	9	8	7
Number of born piglets	90	76	69
Number of weaned piglets	83	69	67
Number of piglets at 56 days of age	81	65	66
Piglet age at weaning (days)	31	31	31
Average live weight of piglets at birth (kg)	1.45	1.69	1.38
Average live weight of piglets at weaning (kg)	5.71	7.52	6.50
Average live weight of piglets 10 at 56 (kg) days of age	14.42	15.98	15.20
Total mortality (number of piglets)	9	11	3
Mortality rate (%)	10.0	14.5	4.3
Total weight gain from birth to 56 days (kg)	12.97	14.29	13.82
Index	100	110	107
Total weight gain from weaning to 56 days (kg)	8.71	8.46	8.70
Index	100	97	100
Average daily gain from birth to 56 days (kg)	236	260	251
Index	100	110	106
Average daily gain from birth to weaning (kg)	137	188	165
Index	100	137	120
Average daily gain from weaning to 56 days (kg)	348	338	348
Index	100	97	100

IV. Feed consumption and conversion

Characteristic	Group – diet		
	1	2	3
Prestarter consumption per 1 piglet until weaning (g)	113	151	258
From weaning to 40 days of age (g)	2 952	3 551	3 448
Starter consumption – 41st to 56th days of age (g)	10 037	8 308	7 485
Feed consumption from weaning to 56th day of age (g)	12 989	11 859	10 933
Feed conversion from weaning to 56th day of age (kg/kg)	1.524	1.510	1.278
Index	100	99	84

V. N balance and nutrient digestibility

Characteristic		Group diet				index (K = 100)
		1 (n = 10)		3 (n = 8)		
		\bar{x}	$s_{\bar{x}}$	\bar{x}	$s_{\bar{x}}$	
N digestibility	%	87.27	2.65	89.92	1.60	103
Fat digestibility	%	88.49 ^A	1.85	91.32 ^B	1.80	103
Fiber digestibility	%	64.84 ^A	8.75	75.98 ^B	3.27	117
Nitrogen-free extract digestibility	%	93.37	0.97	94.23	0.98	101
N utilization of N uptake	%	62.33 ^A	2.89	68.60 ^B	3.11	110
N utilization of N digested	%	71.46 ^A	3.46	76.28 ^B	2.87	107
Protein deposition per head/day	g	44.30 ^a	1.42	48.17 ^b	1.40	109
Feed conversion in metabolic trial	(kg/kg)	1.06	x	1.01	x	95

A, B - $P < 0.01$; a, b - $P < 0.05$

VI. Amino acid digestibility

Characteristic		Group diet				index (K = 100)
		1 (n = 10)		3 (n = 8)		
		\bar{x}	$s_{\bar{x}}$	\bar{x}	$s_{\bar{x}}$	
Digestibility of LYS	%	89.97 ^A	0.55	92.96 ^B	0.61	103
Digestibility of THR	%	85.29 ^A	0.72	88.93 ^B	0.81	104
Digestibility of MET	%	85.75 ^A	0.78	89.77 ^B	0.87	105
Digestibility of CYS	%	86.07 ^A	0.60	89.45 ^B	0.68	104

A, B - $P < 0.01$

than in Group 3 (combination of additives). Different live weights of piglets at birth could influence the difference to some extent. This can be documented by relative growth rate (RGR); it is a value calculated from the equation: $q = (y_t - y_0)/y_0 \cdot 100$ (y_t = weight at a given age, y_0 = weight at the beginning). The respective values for groups 1, 2, 3 are 294, 345, 371 at weaning and 894, 845 and 1001 at 56 days of age. Even though both cases are respected, Group 3 is clearly dominant. Piglet mortality showed significant differences. The respective mortality rates of Groups 1 and 2 were 10.0% and 14.5% while the mortality rate of Group 3 was 4.3% only. This effect can be ascribed not only to the growth-stimulating additive but also especially to the bioregulating additive (*E. faecium*).

Prestarter consumption until weaning is an important indicator of prestarter quality, and consequently of successful piglet production. The consumption per piglet was 1.34 times and 2.28 times higher in Groups 2 and 3, respectively, than in Group 1. Total prestarter consumption per piglet until 40 days of piglet age was by 637 g and 641 g higher in Groups 2 and 3, resp., than in Group 1. The data document positive effects of applied additives, particularly of their targeted combination. A compensation was observed between 41 and 56 days of age, when the single starter was used in all groups and when starter consumption decreased in groups 1-3 in this order. Total feed conversion from weaning to 56 days of age was better by 15-16% in

Group 3 due to the effect of probiotic and enzymatic applications if compared with Groups 1 and 2.

The results of N balance and nutrient digestibility indicate positive effects of the applied enzymes, and also of the probiotic *E. faecium*; its positive effect on cellulase activation was determined previously. It is documented by fiber digestibility higher by 17% in the piglets of experimental group in our trial. Protease effects are also evident, illustrated by 10% higher N utilization in the piglets of experimental group. The effect on N digestibility was not high (by 3% higher in the experimental group), similarly like the digestibility of the main amino acids (LYS, THR, MET, CYS) was higher by 3-5% in the experimental group. Tabs. V and VI show the results of metabolic trial.

REFERENCES

Anonymous (1994): In der Ferkelfütterung: Wirkstoffe kombinieren? Ein Weg zur Stabilisierung der Gesundheit und zur Qualitätssicherung. Schweinewelt, 19: 11-13.
 Balta J. (1994): La alimentación animal: suplementos y aditivos. Nuestra-Cabana, 259: 47-48.
 Dong-Kesu-Xiao-Zhenduo (1994): Observations of the effects of an organic acid-producing probiotics on villi of small intestine in newborn piglets. J. Jilin Agric. Univ., 16: 93-96.

- Kreuzer M. (1994): Wechselwirkungen zwischen Probiotika und Antibiotika in Leistung, intestiler Fermentation und Guellecharakteristik von Ferkeln beim Einsatz eines Bacillus-Präparats und Carbadox. *Agribiol. Res.*, 47: 13–23.
- Morlacchini M. (1994): Probiotici nei mangimi sottoscrofa. *Inform. Zootec.*, 41: 37–39.
- Prokop V. (1996): Principles of categorization of feeding additive effects, particularly in the nutrition of growing piglets. In: *Proc. Pig Nutrition 96 Conf.*, RIAN Pohořelice: 1–5.
- Zolotnitskaya V. J. (1994): Stimulirujuscije kormovyje dobavki v racionach porosjat. *Zootehnija*, 12: 14–16.

Received for publication on September 8, 1998
Accepted for publication on November 10, 1998

Contact Address:

Ing. Vit Prokop, DrSc., Výzkumný ústav výživy zvířat, s. r. o., 691 23 Pohořelice, Česká republika, tel.: 0626/42 45 41, fax: 0626/42 43 66

THE EFFICACY OF DIETARY SUPPLEMENTS OF BENTONITE AND SULPHUR PLUS MOLYBDENUM TO ALLEVIATE CHRONIC COPPER TOXICITY IN SHEEP FED PALM KERNEL CAKE

VPLYV PRÍDAVKOV BENTONITU, SÍRY A MOLYBDÉNU DO KRMIVA NA ZNÍŽENIE CHRONICKEJ TOXICITY MEĎOU U OVIEC KŔMENÝCH PALMOJADROVÝMI VÝLISKAMI

M. Ivan^{*}, M. Rusihan, A. R. Alimon, M. Hair-Bejo, Z. A. Jelan, S. Jalaludin

Department of Animal Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

ABSTRACT: Malin-Dorset crossbred sheep of approximately 16 kg live weight were used to study the effects of dietary supplements of bentonite (16 g/kg dry matter – DM) (treatment BE), sulphur (600 mg/kg DM) plus molybdenum (23 mg/kg DM) (treatment SM), and the combination of bentonite and sulphur plus molybdenum (treatment BE+SM) on the hepatic and kidney concentrations of copper and iron in sheep fed a palm kernel cake (PKC) – based control diet (CD). Treatments CD, BE and SM were assigned five sheep each, whereas treatment BE+SM was assigned four sheep. The sheep were fed the assigned PKC diet plus 10% grass on a DM basis. After 26 weeks on the experiment the surviving sheep were killed and the liver and kidney removed for chemical analysis. The bentonite supplement decreased ($P < 0.01$) the hepatic and kidney copper concentration, but did not prevent mortality due to high copper in PKC. The supplement of sulphur plus molybdenum decreased ($P < 0.001$) the hepatic copper and iron concentrations to normal levels, and none of the sheep showed signs of copper or molybdenum toxicity. The combination of bentonite and sulphur plus molybdenum supplements produced an interaction ($P < 0.05$) which appeared to lower the efficacy of the sulphur plus molybdenum supplement to decrease the hepatic copper concentration. The results showed that sulphur plus molybdenum supplements can be used to prevent copper poisoning and detrimental effects of high iron in sheep fed high levels of PKC.

Keywords: sheep; bentonite; sulphur; molybdenum; copper poisoning; liver; kidney

ABSTRAKT: V pokuse zameranom na štúdium vplyvu prídavkov bentonitu (16 g/kg suchej hmoty, BE), síry (600 mg/kg suchej hmoty) a molybdénu (23 mg/kg suchej hmoty, MM) a v kombinácii bentonitu spolu so sírou a molybdénom (BE+MM) na koncentráciu medi a železa v pečeni a obličkách boli použité 16kg ovce (kríženci plemien malin x dorset) kŕmené základnou kontrolnou diétou, ktorá obsahovala hlavne palmojadrové výlisky (KD). V kontrolnej a v pokusných skupinách s bentonitom a so sírou spolu s molybdénom bolo v každej skupine 5 oviec. V pokusnej skupine s prídavkom bentonitu a síry s molybdénom boli 4 ovce. Ovce boli kŕmené základnou diétou (KD) spolu s prídavkom 10 % trávy. Po 26 týždňoch trvania pokusu boli zvieratá, ktoré prežili, odporazené a pečeň a obličky boli vybraté pre chemické analýzy. Prídavok bentonitu znížil ($P < 0,01$) koncentráciu medi v obličkách a v pečeni, ale neznižil mortalitu spôsobenú vysokou koncentráciou medi v palmojadrových výliskoch. Prídavok síry s molybdénom ($P < 0,001$) znížil koncentráciu medi a železa v pečeni na normálne hladiny a žiadna z oviec nevykazovala toxicitu spôsobenú meďou alebo molybdénom. Kombinácia bentonitu a síry s molybdénom spôsobila zníženie očakávaného vplyvu ($P < 0,05$) medi a molybdénu na koncentráciu medi v pečeni. Výsledky ukazujú, že prídavky síry spolu s molybdénom môžu byť použité ako prevencia proti otrave meďou a nežiadúcemu vplyvu zvýšenej hladiny železa pri skrmovaní diét s vysokým obsahom palmojadrových výliskov.

Kľúčové slová: ovce; bentonit; síra; molybdén; otrava meďou; pečeň; obličky

* Present Address: Lethbridge Research Centre, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1

Palm kernel cake (PKC) is a by-product of extraction of oil from the kernel of the palm fruit. Malaysia is the world's largest producer of palm oil and, consequently, of PKC which is a potentially useful feed for ruminants (Jalaludin, 1995). The product is largely exported, mainly to the Netherlands and Germany for use in dairy and beef cattle diets. The PKC with its content of 14 to 16% crude protein and 9.5 to 10.5 MJ/kg metabolizable energy is the only feedstuff for ruminants produced in sufficient quantities within Malaysia that is available for use in diets for dairy cattle and feedlot beef cattle. The use of PKC in diets for sheep is restricted to a maximum of 50% of the dietary dry matter (DM). This is due to high contents of copper (Cu) in PKC (11 to 55 mg/kg DM; Rahman *et al.*, 1989; Jalaludin *et al.*, 1991) and the relatively high sensitivity of sheep in comparison with cattle to high dietary Cu (Underwood, 1966). Dietary inclusions of over 50% PKC may result in chronic Cu toxicity of sheep as early as 4 to 6 weeks thereafter (Rahman *et al.*, 1989; Hair-Bejo, Alimon, 1992). However, in spite of the restriction of the amount of PKC in the diet to only 50% of DM, the sheep fed such diets may accumulate Cu in the liver to concentrations of over 800 mg/kg DM and develop hypercuprosis and stress induced haemolytic crisis (Bostwick, 1982). Such a problem is the main obstacle in efforts to develop an efficient PKC-based feeding system for the modern sheep production industry within Malaysia. Although cattle, buffaloes and goats are relatively resistant to toxic effects of Cu in PKC the hepatic Cu contents in these animals when fed PKC were found to be increased significantly (Hair-Bejo *et al.*, 1993, 1994). Such an increase may not be desirable because the internal organs are widely consumed by humans. It is, therefore, important to develop means by which high liver Cu contents could be rapidly decreased and then maintained at normal levels in all ruminants, but especially in sheep.

Supplements of sulphur (S) and molybdenum (Mo) were shown to be effective to control an outbreak of chronic Cu toxicity in sheep (Hidiroglou *et al.*, 1984). More recently Ivan *et al.* (1992) reported that a dietary supplement of bentonite (BE) decreased the accumulation of Cu in the liver of sheep by 29%. Consequently, the present experiment was designed to study the efficacy of dietary supplements of BE, S plus Mo (S-Mo) and the combination of BE and S-Mo to prevent hepatic Cu accumulation and Cu toxicity in sheep fed a high level of PKC in the diet. In addition to high Cu, the contents of iron (Fe) in PKC are also high (800 to 6000 mg/kg DM). Excessive dietary concentrations of Fe may result in bent legs of lambs (Hidiroglou *et al.*, 1978) and decreased productivity of dairy cows (Coup, Campbell, 1964). Consequently, the effects of the above dietary supplements on the accumulation of Fe and other elements in the liver and kidney were also measured in the present experiment.

Twenty-three Malin-Dorset crossbred sheep, approximately 3.5 months old and 16 kg of live weight, were purchased from the Gurthrie Farm, Seremban, Negri Sembilan and used in the present study. The sheep were given corn during a 2-week adaptation period. Thereafter, four sheep were selected at random and killed. Livers and kidneys were removed to establish baseline concentrations of Cu and other mineral elements. The rest of the sheep were divided according to body weight into four groups of approximately equal average body weight and each group was assigned to one of four diets. The sheep were placed in individual pens with wooden slatted flooring and automatic water drinking nipples. Group 1 was fed a PKC-based control diet (CD), while groups 2 to 4 were given the same diet supplemented with BE (treatment BE), S-Mo (treatment SM), or combinations of BE and S-Mo (treatment BE+SM), respectively. Groups 1 to 3 were assigned five lambs each, whereas group 4 was assigned four lambs. At the beginning of the experiment there were no statistical differences in the average body weight among treatment means ($P > 0.05$). The lambs were fed the assigned diets *ad libitum* and had continuous access to drinking water. Each sheep was offered grass at approximately 10% of the intake of the experimental diet (DM basis). The control diet (CD) consisted (DM basis) of 96.2% PKC, 1% cobalt-iodized salt and 2.8% limestone (34% Ca). Vitamins A, E and D were added to supply 600, 15 and 1100 IU per kg of the diet, respectively. Additional experimental diets were formulated by adding to the control diet (per kg DM) 16 g sodium bentonite (NATIONAL[®] Standard Bentonite, mesh 200; Bentonite Performance Minerals, Baroid Division of Dresser Industries, Inc., Denver, CO, USA) (diet BE); 600 mg S (as sodium sulphate) plus 23 mg Mo (as ammonium molybdate) (diet SM); and 16 g sodium bentonite and 600 mg S plus 23 mg Mo (diet BE+SM). On the average the grass used contained (by analysis) 17.3% DM, 6.4% ash, 9.5% crude protein, 2.1% fat, 47.7% acid detergent fibre, 12 mg/kg Cu, 71 mg/kg Zn and 350 mg/kg Fe. PKC contained 96.9% DM, 7.4% ash, 15.4% crude protein, 3.7% fat, 45.7% acid detergent fibre, 23 mg/kg Cu, 0.83 mg/kg Mo, 60 mg/kg Zn, 840 mg/kg Fe and 2.2 g/kg S. Individual intakes of grass and the assigned diet were recorded daily. Weighing was performed every two weeks. The experiment lasted for 26 weeks.

The lambs were monitored daily for any Cu or Mo poisoning-related signs. Post-mortem was performed on each dead sheep and the day of death was recorded. The livers and kidneys of dead sheep were examined, weighed, sampled, and the samples dried and stored for chemical analysis. At the end of the experiment all the sheep were weighed and then one sheep from each treatment group was killed each consecutive day. The livers and kidneys were removed, weighed, sampled, and samples dried and stored for chemical analysis.

Feed and tissue samples were dried at 60 °C for 48 hours, followed by determination of analytical DM and proximate analyses. Small amounts of samples (approximately 2 g) were ashed at 600 °C in a muffle furnace and mineral elements were determined by atomic absorption spectrophotometer (Varian Spectra 400) after digesting the ash in hydrochloric acid (Association of Official Analytical Chemists, 1984). The results were analysed statistically as 2 x 2 factorial design and the differences were considered significant at $P < 0.05$.

RESULTS

Mortalities during the experiment (Tab. I) occurred in the group of sheep fed unsupplemented PKC (treatment CD) and in those sheep fed PKC supplemented with BE. There was no mortality in the groups of sheep fed the diets containing the S-Mo supplement (treatments SM and BE+SM). The mortality was higher for the BE treatment (four sheep out of five) than for CD (three sheep out of five), and on the average deaths occurred in 126 and 161 days, respectively. For most cases, mortality occurred one to two days after the animal showed anorexia, restlessness and recumbence. Post-mortem findings showed yellowish serous and

mucous surfaces, enlargement and yellowing of the liver, kidney necrosis and the bladder filled with blood-stained urine, which are indicative of copper toxicity. The average daily gain in the surviving sheep ranged between 108 g (treatment CD) and 140 g (treatment BE), while the average daily feed DM intake ranged between 795 g (treatment CD) and 941 g (treatment BE). The average feed/gain ratio was lowest for the SM treatment (6.1) and highest for the CD treatment (7.7).

The concentrations of Cu, Fe, manganese (Mn), Zn, calcium (Ca) and magnesium (Mg) in the liver of sheep are summarized in Tab. II. In the group of sheep killed at the beginning of the experiment the average concentration of Cu was 1101 mg/kg DM. The concentration increased to between 1491 and 2192 mg/kg DM during the experiment in sheep of treatments BE and CD, respectively. However, the concentration decreased in both treatments containing the S-Mo supplement (treatments SM and BE+SM). This resulted in a highly significant effect of SM treatment on the concentration of Cu in the liver. Also, there was a significant interaction between BE and SM treatments. The average concentrations of Fe in the liver increased during the experiment in sheep of treatments CD and BE, and decreased in sheep of treatment SM. For treatment BE+SM, the concentration during the experiment remained almost constant. There was a highly significant effect of SM

I. Mortality, live weight gain (g/day), feed intake (g dry matter/day) and feed/gain ratio (g/g) in surviving sheep fed a palm kernel cake-based control diet (CD) supplemented with bentonite (BE), sulphur plus molybdenum (SM), or BE+SM

Treatment	Initial number of sheep	Surviving number of sheep	Days to mortality		Live weight gain		Feed intake		Feed/gain ratio	
			mean	SE	mean	SE	mean	SE	mean	SE
CD	5	2	161	21.0	108	26.7	795	36.0	7.7	2.29
BE	5	1	126	20.1	140	-	941	-	6.7	-
SM	5	5	-	-	137	2.8	841	25.0	6.1	0.18
BE+SM	4	4	-	-	117	8.6	799	25.1	6.9	0.63

II. Effects of dietary supplements of bentonite (BE) and sulphur plus molybdenum (SM) to a palm kernel cake-based control diet (CD) on the concentrations (mg/kg dry matter) of mineral elements in the liver of sheep

Treatment	Number ^a	Copper		Iron		Manganese		Zinc		Calcium		Magnesium	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
	(4) ^b	1101	116.4	250	22.1	6	0.6	134	10.5	465	47.7	449	16.7
CD	5	2192	256.3	501	109.8	13	2.3	133	35.8	545	58.2	590	15.3
BE	5	1491	317.1	406	87.4	20	6.6	120	16.9	568	105.6	550	17.3
SM	5	451	136.0	184	15.1	17	2.6	121	8.1	396	19.7	635	38.9
BE+SM	4	852	189.9	251	30.4	15	0.6	165	34.6	435	39.1	611	16.9
Effects													
BE		NS ^c		NS		NS		NS		NS		NS	
SM		***		***		NS		NS		***		***	
Interaction		*		NS		NS		NS		NS		NS	

^a Includes livers of sheep dying prior to the end of the experiment

^b Sheep killed at the beginning of the experiment

^c not significant ($P > 0.05$)

* $P < 0.05$; *** $P < 0.001$

treatment on the liver concentration of Fe, but the interaction was not significant. The average liver concentrations of Mn and Zn were not affected ($P > 0.05$) by any treatments. However, similar to Fe, the SM treatment had a highly significant effect on the liver concentrations of Ca and Mg. The S-Mo supplement decreased ($P < 0.001$) the liver Ca concentration. The concentrations of Mg were higher ($P < 0.001$) for treatments containing S-Mo supplement than for other treatments, and all concentrations appeared to be higher at the end than at the beginning of the experiment.

The concentrations of Cu, Fe, Mn, Zn, Ca and Mg in the kidney cortex of sheep are summarised in Tab. III. In comparison with the beginning the kidney cortex concentration of Cu was elevated dramatically during the experiment in sheep on CD treatment (39 vs. 658 mg/kg DM). In comparison with the CD treatment the concentration was lower for all other treatments. The effects were significant for both BE ($P < 0.01$) and SM ($P < 0.001$) treatments, but the SM treatment was the most effective in decreasing the concentration of Cu in the kidney cortex. Similar to Cu, the Fe and Zn concentrations in the kidney cortex also increased during the experiment in sheep on the CD treatment. In comparison with CD, only the S-Mo supplement (treatments SM and BE+SM) decreased the Fe and the Zn concentrations significantly. Consequently, the concentrations of both elements were lower in sheep on SM and BE+SM treatments than in sheep on other treatments (CD and BE). There were no significant effects of treatments or interaction on the kidney cortex concentrations of Mn, Ca and Mg.

DISCUSSION

In sheep liver, Cu concentrations of up to 500 mg/kg DM are considered to be normal, but over 800 mg/kg

DM hypercuprosis and stress induced haemolytic crisis may develop (Bostwick, 1982). During the latter part of the prehaemolytic stage and the haemolytic stage of Cu toxicity, Cu concentrations may reach 1000–3000 mg/kg DM (Bostwick, 1982). In a reported Cu poisoning in a sheep flock (Hidiroglou *et al.*, 1984) the average monthly hepatic Cu concentrations (745–1251 mg/kg DM) in sheep that died of copper toxicity were similar to our pre-experimental sheep group (1101 mg/kg DM). Therefore, Cu concentrations in the livers of sheep killed at the beginning of the present experiment indicated a possibility of approaching haemolytic stage. However, Cu concentrations in the kidneys were relatively normal, indicating that there was no liver damage at this point. To rapidly reduce death losses due to chronic Cu poisoning, Hidiroglou *et al.* (1984) used a treatment of dietary supplements of 0.1 g of ammonium molybdate and 1g of sodium sulphate per sheep per day, for approximately 4 months. However, after the end of the treatment there were still dead sheep with high hepatic Cu concentrations, while 500 mg/kg DM of ammonium molybdate for five months eliminated completely deaths due to Cu toxicity (Hair-Bejo, Alimon, 1992). It would appear, therefore, that a daily supplement of 0.1 g of ammonium molybdate (approximately 20 mg Mo/kg DM) was not sufficient to totally prevent Cu poisoning in sheep. In contrast, dietary Mo supplements between 4 and 16 mg/kg DM were reported as effective to reduce hepatic Cu concentration to normal levels (Kline *et al.*, 1971; Suttle, 1977; Van der Schee *et al.*, 1980; Suttle, 1983). We used a somewhat higher level of supplemental Mo (23 mg/kg DM) than was used by Hidiroglou *et al.* (1984), but much lower than that used by Hair-Bejo, Alimon (1992). However the effectiveness of dietary Mo supplement may depend on the initial Cu status of the animal (Gray, Daniel, 1964) and on the dietary Cu concentration and its biological availability. The rumen

III. Effects of dietary supplements of bentonite (BE) and sulphur plus molybdenum (SM) to a palm kernel cake-based control diet (CD) on the concentrations (mg/kg dry matter) of mineral elements in the kidney cortex of sheep

Treatment	Number ^a	Copper		Iron		Manganese		Zinc		Calcium		Magnesium	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
	(4) ^b	39	7.7	94	25.2	10	1.1	130	7.9	798	42.2	599	117.2
CD	5	658	186.2	1015	335.1	20	3.8	200	7.8	884	212.8	551	8.7
BE	5	290	105.7	877	408.3	22	8.4	184	34.5	653	132.6	531	46.5
SM	5	79	16.1	153	13.2	20	2.1	164	19.6	502	20.7	615	24.4
BE+SM	4	125	53.8	146	10.7	19	2.5	148	16.6	663	41.4	727	29.8
Effects													
BE		**		NS ^c		NS		NS		NS		NS	
SM		***		***		NS		*		NS		NS	
Interaction		NS		NS		NS		NS		NS		NS	

^a Includes kidney cortex of sheep dying prior to the end of the experiment

^b Sheep killed at the beginning of the experiment

^c not significant ($P > 0.05$)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

solubility and biological availability of Cu in PKC may be quite high. Indeed, 78% of the 21 mg Cu/kg DM in PKC was released in the rumen of goats (Jin *et al.*, 1995).

It is obvious that before the beginning of the present experiment the sheep accumulated large amounts of Cu in the liver, with the concentration being at the threshold of chronic Cu toxicity. In both the liver and the kidney, Cu and Fe concentrations further increased (2-fold and 17-fold, respectively) during the experiment in sheep fed the PKC control diet (CD), resulting in three mortalities out of five sheep. It would appear, therefore, that the biological availability of Cu in the PKC used in the present experiment was high. It is interesting to note that in comparison with CD, both dietary supplements and their combination decreased notably (between 32 and 79%) the liver Cu concentration during the experiment. However, only the effect of S-Mo supplement was significant statistically. There was also significant interaction that appeared to result in decreased efficacy of the S-Mo supplement. Similarly, the concentrations of Cu in the kidney were decreased by the dietary supplements between 56 and 88%, but in contrast to the liver, both S-Mo and BE effects, but not the interaction, were significant statistically. The largest relative decreases in Cu concentrations in both liver and kidney were obtained when S-Mo was supplemented alone or in combination with BE, and it was accompanied with a zero sheep mortality for these treatments. It is evident from the results that there was no additive, but rather, negative effect on the liver Cu concentration when the supplements were combined. Such an effect was not expected. Therefore, S-Mo was the sole supplement that provided total protection from Cu poisoning in sheep. It also maintained significantly lower Fe concentrations in the liver and kidney.

The fact that dietary Mo in the presence of adequate S can reduce the concentration of Cu in the liver (Dick, 1954; Suttle, 1974) or Cu storage in the liver (Ross, 1966, 1970; Hogan *et al.*, 1968) has been established decades ago. Later Suttle (1974, 1975) reported that S in either organic or inorganic form could directly affect the absorption of Cu, but to a limited degree only. Our results clearly showed that the amounts of the S and Mo supplements used in the present experiment were adequate to decrease the hepatic Cu concentrations to a normal range and to prevent Cu toxicity. It is, however, reasonable to suggest that lower amounts of Mo supplement in PKC-based diets would be required to maintain a normal range of the hepatic Cu concentration for an extended time period. Work is underway to establish an optimal level of dietary Mo for this purpose, and the necessity for addition of S to diets based entirely on PKC.

It has been reported that the dietary supplementation with bentonite decreased the hepatic Cu accumulation by 29% (Ivan *et al.*, 1992). This is in agreement with the present results. However the 32% decrease in the

hepatic copper concentration obtained in the present experiment was not sufficient to prevent Cu poisoning in sheep fed the bentonite supplemented diet, resulting in four mortalities out of five sheep within an average of 126 days. It is also apparent that the supplement with bentonite significantly affected the efficacy of the S-Mo supplement to lower the hepatic Cu concentration. This is probably due to the adsorptive property of bentonite, which may have rendered part of the S-Mo supplement from interacting with the dietary Cu.

The present results clearly show that the dietary S-Mo supplement is the only one that showed sufficient efficacy to prevent Cu poisoning in sheep when fed PKC-based diets. In addition to decreased hepatic Cu, Fe and Ca concentrations, the S-Mo supplement decreased also the concentrations of Cu, Fe and Zn in the kidney. The decreases in the Fe concentrations in the liver and the kidney is especially significant because deleterious health effects of both high Cu and high Fe in PKC when fed to sheep may be alleviated by using the same dietary supplement (S-Mo). However, more research is required to determine the exact levels of the supplement required to prevent Cu poisoning, without creating Cu deficiency and/or molybdenosis, in sheep when fed PKC diets with variable Cu contents, and for prolonged periods of time.

Acknowledgment

The authors wish to thank the BENTONITE Performance Minerals, Baroid Division of Dresser Industries, Inc., Denver, Colorado, USA for donation of the bentonite product used in the present experiment.

REFERENCES

- Association of Official Analytical Chemists. 1984. Official methods of analysis. AOAC, Arlington, VA, USA.
- Bostwick J. L. (1982): Copper toxicosis in sheep. *J. Am. Vet. Med. Assoc.*, 180: 386-387.
- Coup M. R., Campbell A. G. (1964): The effect of excessive iron intake upon the health and production of dairy cows. *N.Z. J. Agric. Res.*, 7: 624-638.
- Dick A. T. (1954): Studies on the assimilation and storage of copper in crossbred sheep. *Aust. J. Agric. Res.*, 5: 511-544.
- Gray L. F., Daniel L. J. (1964): Effect of the copper status of the rat on the copper-molybdenum-sulfate interaction. *J. Nutr.*, 84: 31-37.
- Hair-Bejo M., Alimon A. R. (1992): Hepatic damages and the protective role of zinc and molybdate in palm kernel cake (PKC) toxicity in sheep. In: Proc. 15th MSAP Conf., Malaysia: 93-95.
- Hair-Bejo M., Alimon A. R., Yusof M. (1993): Copper tolerance in goats: the potential toxic effect of copper in palm cake and copper supplemented diets. In: Proc. 5th Vet. Assoc. Mal. Cong., Malaysia: 63-64.
- Hair-Bejo M., Liang J. B., Alimon A. R. (1994): Copper tolerance in cattle: the potential toxic effect of copper in

- cattle fed palm kernel cake. In: Proc. Int. Cong. Qual. Vet. Serv. 21st Cent., Malaysia: 262.
- Hidiroglou M., Dukes T. W., Ho S. K., Heaney D. P. (1978): Bent-limb syndrome in lambs raised in total confinement. *J. Am. Vet. Assoc.*, 173: 1571-1574.
- Hidiroglou M., Heaney D. P., Hartin K. E. (1984): Copper poisoning in a flock of sheep. Copper excretion patterns after treatment with molybdenum and sulfur or penicillamine. *Can. Vet. J.*, 25: 377-382.
- Hogan K. G., Money D. F. L., Blayney A. (1968): The effect of a molybdate and sulphate supplement on the accumulation of copper in the livers of penned sheep. *N.Z. J. Agric. Res.*, 11: 435-444.
- Ivan M., Dayrell M. de S., Hidiroglou M. (1992): Effects of bentonite and monensin on selected elements in the stomach and liver of fauna-free and faunated sheep. *J. Dairy Sci.*, 75: 201-208.
- Jalaludin S. (1995): Feeding systems based on palm oil products and by-products. In: Ivan M. (ed.): *Animal Science and Development: Moving Toward a New Century*. Ottawa, Canada: 295-306.
- Jalaludin S., Jelani Z. A., Abdulah N., Ho Y.W. (1991): Recent developments in the oil palm by-product based ruminant feeding system. In: Proc. 3rd Int. Symp. Nutr. Herb., Malaysia: 35-44.
- Jin L. Z., Alimon A. R., Abdullah N., Ho Y. W., Jalaludin S. (1995): Minerals released from palm kernel cake (PKC) and corn-soybean concentrate + copper in the rumen of goats. *Mal. J. Anim. Sci.*, 1: 41-44.
- Kline R. D., Hays V. W., Cromwel G. L. (1971): Effects of copper, molybdenum and sulfate on performance, hematology and copper stores of pigs and lambs. *J. Anim. Sci.*, 33: 771-779.
- Rahman A. M. Y., Wong H. K., Zaini H., Sharif H. (1989): Preliminary observation on the alleviation of copper in sheep fed with palm kernel meal based diet. In: Proc. 12th MSAP Conf., Malaysia: 75-78.
- Ross D. B. (1966): The diagnosis, prevention and treatment of chronic copper poisoning in housed lambs. *Brit. Vet. J.*, 122: 279-284.
- Ross D. B. (1970): The effect of oral ammonium molybdate and sodium sulphate given to lambs with high liver copper concentrations. *Res. Vet. Sci.*, 11: 295-297.
- Suttle N. F. (1974): Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. *Brit. J. Nutr.*, 32: 559-568.
- Suttle N. F. (1975): The role of organic sulphur in the copper-molybdenum-S interrelationship in ruminant nutrition. *Brit. J. Nutr.*, 34: 411-420.
- Suttle N. F. (1977): Reducing the potential copper toxicity of concentrates to sheep by the use of molybdenum and sulphur supplements. *Anim. Feed Sci. Technol.*, 2: 235-246.
- Suttle N. F. (1983): Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. *J. Agric. Sci., Camb.*, 100: 652-656.
- Underwood E. J. (1966): *The Mineral Nutrition of Livestock*. Aberdeen, Great Britain, The Central Press Ltd.
- Van der Schee W., Garretsen J. W., Van der Berg R. (1980): Effect of zinc and molybdenum supplementation of the feed concentrate on the storage of copper in the liver of lambs. *Vet. Quart.*, 2: 82-89.

Received for publication on September 29, 1998

Accepted for publication on November 10, 1998

Contact Address:

Dr. A. R. Alimon, Universiti Putra Malaysia, Department of Animal Science, 43400 UPM Serdang, Selangor, Malaysia, tel.: 603/9486101, fax: 603/9432954, e-mail: ralimon@vet.upm.edu.my

VLIV DOJENÍ S RŮZNÝM PODTLAKEM A STRUKOVÝMI NÁVLEČKAMI RŮZNÉHO TVARU NA ZMĚNU TEPLoty POVRCHU STRUKU*

THE INFLUENCE OF MILKING WITH DIFFERENT VACUUM AND DIFFERENT DESIGN OF LINER ON THE CHANGE OF TEAT SURFACE TEMPERATURE

P. Kunc¹, I. Knížková¹, M. Koubková²

¹Research Institute of Animal Production, Prague-Uhřetěves, Czech Republic

²Czech University of Agriculture, Faculty of Agronomy, Prague, Czech Republic

ABSTRACT: The objective of this study was to investigate the dynamics of surface teat temperature changes in dependence on vacuum changes and changes of design of rubber liner by means of thermography. Two experiments were conducted in an autotandem milking parlour with 5 milking boxes. The teats of 9 (or 7) dairy cows (H x C, milk yield 5,650 l) were measured in 2 milking boxes by thermovision set AGA 880 in 3 series (2 days): series A (evening milking): vacuum 45 kPa; series B (morning milking): vacuum 40 kPa; series C (evening milking): vacuum 45 kPa. In the first experiment rubber liners with circular section (55° Shore) were located as follows: box 2: all 4 teats – left fore teat (LP), right fore teat (PP), left hind teat (LZ) and right hind teat (PZ). In the second experiment rubber liners with triangular section were located as follows: box 1: only 2 teats – left fore teat (LP) and right fore teat (PP). The thermograms of measured teats were obtained in each experiment: 1. before milking (A), 2. immediately after milking (B), 3. two minutes after milking (2B). The differences between B : A, 2B : B and 2B : A were calculated. CM-SOFT computer program was used for data and thermogram analyses. The thermographic study of teat temperature responses to machine milking showed that milking causes considerable temperature changes in the teats. These teats are in direct contact with milking machine and they are strained during milking. However, after evaluation of all thermograms with a combination design of liners with circular section – vacuum 45 kPa vs. a combination design of liners with circular section – vacuum 40 kPa it was found that lower vacuum with these liners acted positively. The changes of teat surface temperature after milking with 40 kPa were lower (Tab. I) and the traumatization of mammary gland decreased. The use of liners with triangular section did not reduce traumatization of teats by means of the changes of vacuum from 45 to 40 kPa

Keywords: dairy cows; teats; machine milking; vacuum; rubber liners; teat temperature; thermography

ABSTRAKT: Pomocí termografické metody byla sledována dynamika teplotních změn struků dojnice v závislosti na změně podtlaku a tvaru strukových návleček. Pokus byl uskutečněn v autotandemové dojrně. Struky 14 vybraných dojnic byly měřeny termograficky ve třech sériích: série A (večerní dojení) – podtlak 45 kPa; série B (ranní dojení) – podtlak 40 kPa; série C (večerní dojení) – podtlak 45 kPa. V každé sérii byly použity gumové strukové návlečky trojúhelníkovitého a kruhového průřezu. U každé dojnice byly vytvořeny termogramy struků: 1. před dojením, 2. bezprostředně po dojení, 3. dvě minuty po dojení. Porovnáním termogramů s dojením kombinací kruhové návlečky – podtlak 45 kPa vs. kruhové návlečky – podtlak 40 kPa bylo zjištěno, že při použití těchto návleček se projevily pozitivně nižší podtlak – zjištěné změny teplot struků po dojení byly významně nižší než při dojení s 45 kPa. Při použití strukových návleček trojúhelníkového průřezu nebylo změnou podtlaku z 45 na 40 kPa dosaženo snížení traumatizace struků.

Klíčová slova: dojnice; strojní dojení; podtlak; strukové návlečky; teplota struku; termografie

ÚVOD

Během dojení se dojící stroj dostává do těsného kontaktu s velmi senzitivním orgánem dojnice – mléčnou žlázou. Strojní dojení má za úkol získat hygienicky

nezávadné mléko a přitom zajistit dobrý zdravotní stav mléčné žlázy. Dojení nesmí být pro dojnici nepříjemné a bolestivé a je třeba ho přizpůsobit anatomii vemene a fyziologii uvolňování mléka (Kejík, Mašková, 1989). Prokázalo se, že se zvyšujícím se podtlakem klesá podíl

* Řešení bylo uskutečněno v rámci grantu NAZV č. EP 0960006313.

zdravých čtvrtí. Rovněž tak nevhodné strukové gummy či zvýšený počet pulzů jsou významnými traumatizujícími činiteli (Štros, 1998). Avšak i dojení v případech, kdy technické parametry charakterizující proces dojení jsou v souladu s normou, může způsobovat traumatizaci mléčné žlázy. Vznikají traumatizační zóny, které se projevují zvýšenou teplotou zejména na strucích a nelze je detekovat zrakem ani podchytit kontaktním teploměrem. Průběh těchto teplotních změn na vemeni a strucích lze objektivně zjišťovat pomocí termografické metody, která podává ucelený obraz o teplotní odezvě organismu na dané podmínky. Hamann (1985) zjistil termografickým měřením, že běžné dojící systémy zvyšují povrchovou teplotu struků během dojení o 2 °C. Mašková (1991), která pomocí termografie hodnotila nový tvar strukové gummy, zjistila, že hrot struku není novým tvarem významně namáhán. Kunc a Knížková (1997a) monitorovali teplotní reakce vemene zdravých dojnic v běžných provozních podmínkách v autotandemové dojárně, kde veškeré technické parametry odpovídaly normě. Z porovnání všech získaných termografických snímků vyplynulo, že během procesu dojení docházelo k výrazným změnám zejména na strucích, které jsou během dojení v přímém kontaktu s dojícím strojem a jsou značně namáhány. Rozdíl teplot před dojením a po něm zde činil až 5 °C. Teplotní změny ve vemeni nepřekročily 1 °C. Obdobně sledovali Kunc a Knížková (1997b) teplotní reakce vemene na úpravu dojícího stroje, resp. na výměnu strukových návleček. Na základě zhodnocení teplotních reakcí mléčné žlázy konstatovali, že dynamika změn teplot vemene a struků byla výměnou strukových návleček ovlivněna. Po výměně strukových návleček bylo zaznamenáno delší přetrvávání vyšších teplot struků oproti teplotním stavům před výměnou.

Cílem práce bylo sledovat pomocí termografické metody dynamiku teplotních změn struků vemene dojnic v závislosti na změnách fyzikálních parametrů dojícího zařízení. Ke sledování byla vybrána změna podtlaku a změna tvaru strukových návleček.

MATERIÁL A METODA

Byly uskutečněny dva souběžné experimenty v provozních podmínkách, v autotandemové dojárně tvaru Π s pěti dojícími stánkami. Měřené dojnice (křížky H x C s průměrnou užitkovostí 5 650 l), resp. vemena byla snímána v dojících boxech č. 1 a č. 2 termovizní soupravou AGA 880.

Termovizní snímání v obou pokusech se uskutečnilo ve třech snímacích sériích ve dvou po sobě následujících dnech:

Série A (večerní dojení): podtlak běžně používaný – 45 kPa

Série B (ranní dojení): podtlak byl snížen na 40 kPa

Série C (večerní dojení): podtlak navrácen na 45 kPa.

Pokus č. 1 — Vliv změny podtlaku: počet měřených dojnic $n = 9$; box č. 2: návlečky kruhového průřezu (tvrdost gummy = 55° Shore) na na všech čtyřech

strucích – levý přední (LP), pravý přední (PP), levý zadní (LZ), pravý zadní (PZ).

Pokus č. 2 – Vliv změny podtlaku a změny strukových návleček: počet měřených dojnic $n = 7$; box č. 1: návlečky trojúhelníkového průřezu (tvrdost gummy výrobcem neuvedena) na levém předním struku (LP) a pravém předním struku (PP), na levém zadním struku (LZ) a pravém zadním struku (PZ) návlečky kruhového průřezu (55° Shore).

V obou pokusech u každé dojnice byla z termozáznamů vytvořena tato teplotní zobrazení: 1) stav před dojením, 2) stav bezprostředně po sejmutí strukových násadců, 3) stav dvě minuty po skončeném dojení. Na základě změn teploty struků v těchto časových intervalech byla posouzena intenzita traumatizace.

Souběžně s termovizním snímáním byly po dobu experimentů měřeny tyto veličiny: teplota vzduchu a relativní vlhkost pomocí záznamového termohygrografu Commeter, a to na třech stanovištích v dojárně (u dveří, střed a zadní část dojírny), dále proudění vzduchu a zchlazovací hodnota pomocí Hillova katateploměru (třikrát po dobu termovizního měření) a také nádoj mléka.

Vyhodnocení všech nasnímaných termogramů a získaných dat bylo uskutečněno pomocí speciálního počítačového programu CM SOFT.

VÝSLEDKY A DISKUSE

Mikroklimatické podmínky v dojárně během měření

Série A (odpolední dojení): Teplota vzduchu se po dobu měření pohybovala od 12,8 do 15,5 °C, u relativní vlhkosti bylo zaznamenáno rozpětí 61–68 %. Proudění vzduchu bylo v rozmezí od 0,1 do 0,2 m.s⁻¹, zchlazovací hodnota byla zjištěna v rozmezí 209–215 W.m⁻².

Série B (ranní dojení): Teplota vzduchu se pohybovala během měření od 14,4 do 16,7 °C, relativní vlhkost od 64 do 77 %. Proudění vzduchu dosáhlo rozpětí 0,1–0,25 m.s⁻¹, zchlazovací hodnota se vyskytovala v rozmezí od 207 do 382 W.m⁻².

Série C (odpolední dojení): Teplota vzduchu byla zaznamenána v rozpětí od 14,0 do 16,5 °C, relativní vlhkost se pohybovala od 68 do 76 %. Proudění vzduchu bylo zjištěno v rozmezí 0,1–0,25 m.s⁻¹, zchlazovací hodnota pak v rozmezí 205–383 W.m⁻².

Průměrný nádoj mléka během měření

Série A (odpolední dojení): Průměrný nádoj ve sledovaném boxu č. 1 byl 7 litrů, ve sledovaném boxu č. 2 7,9 litrů mléka.

Série B (ranní dojení): Průměrný nádoj ve sledovaném boxu č. 1 byl 7,4 litrů, ve sledovaném boxu č. 2 8,2 litrů mléka.

Série C (odpolední dojení): Průměrný nádoj ve sledovaném boxu č. 1 byl 7,4 litrů, ve sledovaném boxu č. 2 6,8 litrů mléka.

Zhodnocení vlivu změny fyzikálních parametrů na teplotní reakce struků

Pokus č. 1: Vliv změny podtlaku

Srovnání teplotních změn na strucích během dojení s běžně používanými návlečkami kruhového průřezu a se změnou podtlaku ze 45 na 40 kPa je uvedeno v tab. I. Teplotní změny jsou uváděny pro jednotlivé struky. Byly vypočteny rozdíly v teplotách struků bezprostředně po dojení a před dojením (B : A), dále teplotní stav 2 minuty po dojení a bezprostředně po dojení (2B : B) a rozdíl mezi stavem 2 minuty po dojení a počátečním teplotním stavem struků před dojením (2B : A). V tomto případě lze hodnotit všechny čtyři struky: levý přední (LP), pravý přední (PP), levý zadní (LZ) a pravý zadní (PZ).

LP: Ze srovnání stavu B : A vyplynulo, že podtlak 40 kPa v kombinaci s běžnými návlečkami výrazně snížil povrchovou teplotu struku, a to o 1,63 °C, u srovnání 2B : B byla zaznamenána rovněž nižší teplota (rozdíl činil 1,07 °C). Na základě porovnání stavu 2B : A lze konstatovat, že i v tomto případě byla teplota u podtlaku 40 kPa nižší (rozdíl 0,59 °C).

PP: U rozdílu B : A byla zaznamenána nižší teplota struku bezprostředně po dojení o 2,15 °C ve prospěch podtlaku 40 kPa, u srovnání 2B : B byla rovněž zaznamenána nižší teplota, a to o 0,13 °C, u stavu 2B : A byla zjištěna stejná teplotní reakce (o 2,02 °C nižší oproti 45 kPa).

LZ: Rozdíl B : A činil 3,72 °C pro podtlak 40 kPa, u stavu 2B : B byl zjištěn rozdíl 0,7 °C a u 2B : A pak rozdíl 3,01 °C opět ve prospěch sníženého podtlaku.

PZ: V případě porovnání A : B byla teplota struku při dojení 40 kPa nižší o 1,78 °C, v případě 2B : B však byla tato teplota na struku nepatrně vyšší, rozdíl oproti 45 kPa byl 0,39 °C. Stav 2B : B opět vykázal nižší teplotu struku pro 40 kPa (rozdíl 2,17 °C).

Celkově lze konstatovat, že snížení podtlaku z 45 na 40 kPa s použitím běžných návleček kruhového průřezu se projevilo výrazným snížením povrchových teplot struků, a to zejména v porovnání stavu bezprostředně před dojením a bezprostředně po něm. Tyto zaznamenané teplotní změny byly v případě B : A u LZ statisticky významné ($P < 0,01$). Rovněž tak v případě 2B : A byl zjištěn u LZ statisticky významný rozdíl mezi teplotami struků při 45 a 40 kPa ($P < 0,01$). I když ostatní hodnoty nebyly statisticky průkazné, v mnoha případech se průkaznosti limitně blížily.

Pokus č. 2: Vliv změny strukových návleček a změny podtlaku

Srovnání teplotních změn na strucích během dojení s návlečkami trojúhelníkového průřezu a změnou podtlaku ze 45 na 40 kPa je uvedeno v tab. II. Teplotní změny jsou uváděny pro jednotlivé struky. Byly vypočteny rozdíly v teplotách struků bezprostředně po dojení a před dojením (B : A), dále teplotní stav 2 minuty po dojení a bezprostředně po dojení (2B : B) a rozdíl mezi

I. Srovnání teplotních změn na strucích během dojení podtlakem 45 a 40 kPa (návlečky s kruhovým průřezem) – Comparison of teat surface temperature changes during milking with 45 and 40 kPa vacuum (design of liners with circular section)

Rozdíl mezi ¹	LP			PP			LZ			PZ		
	B : A °C	B : 2B °C	2B : A °C	B : A °C	B : 2B °C	2B : A °C	B : A °C	B : 2B °C	2B : A °C	B : A °C	B : 2B °C	2B : A °C
45 kPa	3,09	-1,12	2,00	4,59	-0,48	4,11	5,64	-1,13	4,51	4,23	-0,31	3,92
40 kPa	1,46	-0,05	1,41	2,44	-0,35	2,09	1,92	-0,43	1,50	2,45	-0,70	1,75
Rozdíl ²	1,63	-1,07	0,59	2,15	-0,13	2,02	3,72*	-0,70	3,01*	1,78	0,39	2,17

* $P < 0,01$

A = před dojením – pre-milking; B = po dojení – post-milking; 2B = 2 minuty po dojení – 2 minutes after milking

¹difference between, ²difference

II. Srovnání teplotních změn na strucích během dojení podtlakem 45 a 40 kPa (návlečky s trojúhelníkovým průřezem) – Comparison of teat surface temperature changes during milking with 45 and 40 kPa vacuum (design of liners with triangular section)

Rozdíl mezi ¹	LP			PP		
	B : A °C	B : 2B °C	2B : A °C	B : A °C	B : 2B °C	2B : A °C
45 kPa	-0,39	0,43	0,04	2,34	-0,19	2,16
40 kPa	2,10	0,20	2,30	2,62	-0,20	2,42
Rozdíl ²	-2,49*	0,23	-2,26*	-0,28	0,01	-0,26

* $P < 0,01$

A = před dojením – pre-milking; B = po dojení – post-milking; 2B = 2 minuty po dojení – 2 minutes after milking

¹difference between, ²difference

stavem 2 minuty po dojení a počátečním teplotním stavem struků před dojením (2B : A). V tomto případě lze hodnotit pouze struky levý přední (LP) a pravý přední (PP), na kterých byly použity nové návlečky.

LP: Ze srovnání stavu B : A vyplynulo, že u souběžného použití návleček trojúhelníkového průřezu a sníženého podtlaku 40 kPa byla zaznamenána výrazně vyšší teplota tohoto struku po dojení v porovnání s podtlakem 45 kPa, a to o 2,1 °C. U srovnání 2B : B však byla zjištěna teplota struku nižší pro 40 kPa (rozdíl činil 0,23 °C). U stavu 2B : A lze opět zaznamenat výraznější přetrvávání vyšší teploty struku u 40 kPa, rozdíl oproti 45 kPa činil 2,26 °C.

PP: U rozdílu B : A bylo zaznamenáno zvýšení teploty tohoto struku o 0,28 °C ve srovnání s 45 kPa, u 2B : B byly zjištěny prakticky shodné teploty struků jak při 45 kPa, tak při 40 kPa. Rozdíl činil 0,01 °C, avšak po porovnání rozdílu 2B : A přetrvávala opět u podtlaku 40 kPa na struku vyšší teplota (rozdíl 0,26 °C).

Na základě zjištěných hodnot, resp. rozdílů lze konstatovat, že u kombinace souběžného použití návleček trojúhelníkového průřezu a snížení podtlaku z 45 na 40 kPa byl zaznamenán negativní vliv této kombinace na teplotní změny na obou strucích, a to zejména na levém předním struku, kde teplotní změny byly statisticky významné v případech B : A a 2B : A ($P < 0,01$).

Ze sledování teplotních reakcí struků dojníc na strojní dojení pomocí termovizního měření vyplynulo, že povrchová teplota struků bezprostředně po dojení je zvýšená, což koresponduje se zjištěními autorů Hamann (1985), Kejík, Mašková (1989), Malík *et al.* (1989), avšak i Caruolo *et al.* (1989), kteří stejné teplotní změny na strucích zjistili i po strojním dojení koz. Avšak na základě porovnání kombinace kruhové návlečky – podtlak 45 kPa vs. kruhové návlečky – podtlak 40 kPa, bylo zjištěno, že nižší podtlak s těmito návlečkami se projevil v daných podmínkách pozitivně. Zjištěné změny povrchových teplot struků, po dojení byly nižší než při dojení s 45 kPa, a byla tak snížena traumatizace mléčné žlázy.

Při použití strukových návleček trojúhelníkového průřezu nebylo dosaženo změnou podtlaku ze 45 kPa na 40 kPa snížení traumatizace struků. Výsledky naznačují, že pro daný typ strukové návlečky je vhodnější vyšší hladina podtlaku.

LITERATURA

- Caruolo E. V., Jarman R. F., Dickey D. A. (1989): Milk temperature in the claw piece of the milking machine and mammary surface temperature are predictors of internal mammary temperature in goats. *J. Vet. Med.*, 37: 61–67.
- Hamann J. (1985): Infection rate as affected by teat tissue reactions due to conventional and non-conventional milking systems. *Kieler Milchwirtsch. Forsch.-Ber.*, 37: 426–430.
- Kejík C., Mašková A. (1989): Termovizní měření povrchových teplot vemene v průběhu strojního dojení. *Živoč. Vyr.*, 35: 225–230.
- Kunc P., Knížková I. (1997a): Reakce vemene dojnice na úpravu dojícího stroje – termografická studie. In: Aktuální otázky šlechtění, zdraví, růstu a produkce skotu, České Budějovice: 355.
- Kunc P., Knížková I. (1997b): Temperature responses of the cow udder to machine milking. *ISAH '97, Helsinki, Finsko*: 107–108.
- Malík K., Mašková A., Vévoda J. (1989): A thermovision study of the rubber teat cup function. *Plastics and Rubber – Special Issue*: 49–51.
- Mašková A. (1991): Termovizní měření povrchových teplot vemene v průběhu dojení. [Technická zpráva.] Brno, VŠZ. 50 s.
- Štros J. (1998): Mastitidy – sekreční poruchy neinfekční povahy. *Farmář* (2): 70–71.

Došlo 26. 6. 1998

Přijato k publikování 10. 11. 1998

Kontaktní adresa:

Ing. Petr Kunc, Výzkumný ústav živočišné výroby, 104 00 Praha 10-Uhřetěves, Česká republika, tel.: 02/67 71 17 47, fax: 02/67 71 07 79, e-mail: kunc@novell.vuzv.cz

NUTRITIONAL FACTORS AFFECTING EGGSHELL QUALITY*

NUTRIČNÍ FAKTORY OVLIVŇUJÍCÍ KVALITU SKOŘÁPKY

Y. Nys

INRA, Station de Recherches Avicoles, Tours, Nouzilly, France

ABSTRACT: The hen's eggshell has a perfectly ordered structure, sequentially elaborated during a defined period in the isthmus and the uterus. It is composed of calcium carbonate pervaded by a small amount of organic components which are believed to influence calcite nucleation and growth, and, consequently, the biomechanical properties of the eggshell. Eggshell quality therefore depends on the supply of ionic material, calcium and carbonate but also on the organisation of eggshell deposition. At the production level, eggshell quality is influenced by management, nutritional and environmental factors. Adequate dietary levels of calcium have to be provided during the rearing, transition and laying periods but further increasing the level of calcium or the use of Ca step-up phase feeding system is poorly efficient. Factors favorising the supply of calcium during eggshell formation, i.e. during the night, are more important. Midnight feeding can improve eggshell quality. The use of coarse particle size of calcium improves eggshell quality especially in old hens compared to young ones and in hens suffering from high temperatures. The origin of the calcium source little affects the response. Particle size has to be between 1 and 4 mm. Pelleting process (crumble feed) decreases calcium particle size and could therefore reduce the positive effect of Ca particle size. An elevated consumption of saturated fatty acids leads to the formation of calcium soaps and decreases calcium and fat retention. There are no negative effect on shell quality when dietary fat is lower than 6%. High levels of dietary phosphorus are detrimental to eggshell quality and 0.3% available phosphorus allows normal performance and bone integrity. Dietary Na (minimum 0.12%) and K levels have a low effect on eggshell quality but elevated chloride (> 0.2 %) is unfavourable. Mg is largely supplied by the vegetal feed (1500 mg/kg) and can depress eggshell quality when in large excess (> 5000 mg/kg). Synthetic zeolites (0.75%) with high cationic-exchange capacity slightly improve shell quality but they are solubilized in the intestine and provide aluminium. Other natural clays have a low effect on shell quality. Increasing vitamin D dietary level has no influence on eggshell quality and dietary supplementation with its active metabolite has a low effect. High temperatures decrease eggshell quality as a consequence of the reduced Ca consumption and the respiratory alkalosis induced by hyperthermia. The positive effects of nutritional factors (coarse calcium particle, zeolites) are more pronounced in hot environments, and additional factors may also be useful: carbonated water, bicarbonate, vitamin C. An adequate supply of nutrients will limit the incidence of eggshell breakage, which remains elevated despite considerable efforts in manipulating the nutritional, environmental or genetic factors.

Keywords: poultry; egg; eggshell quality; nutrition

ABSTRAKT: Skořápka slepičoho vejce má dokonale organizovanou strukturu, která vzniká během jeho průchodu vejcovodem z istmu do dělohy. Tato skořápka se skládá z uhlíčitánu vápenatého, který je prostoupen malým množstvím organických složek, u nichž se předpokládá, že ovlivňují nukleaci kalcitů a růst, a tím také biochemické vlastnosti skořápky. Kvalita skořápky tudíž závisí jednak na přísunu iontového materiálu, vápníku a uhlíčitánu a jednak na organizaci jejich ukládání ve skořápce. Na produkční úrovni je kvalita vaječné skořápky ovlivněna ošetřováním a nutričními a environmentálními faktory. Během odchovu, přechodného období i snášky je třeba zajistit adekvátní dietetickou hladinu vápníku; další zvyšování hladiny vápníku nebo použití krmného systému s fází zvýšeného přísunu Ca (Ca step-up phase feeding system) však je jen málo účinné. Mnohem důležitější jsou faktory podporující přísun vápníku v období tvorby vaječné skořápky, tj. během noci. Krmení o půlnoci může kvalitu skořápky zlepšit. Použití hrubě namletých částic s obsahem vápníku zlepšuje kvalitu vaječných skořápek více u starších slepic než u nosnic mladých nebo žijících v podmínkách s vysokými teplotami. Zdroj vápníku má na odezvu u zvířat jen malý vliv. Velikost podávaných částic se má pohybovat v rozmezí od 1 do 4 mm. Granulace (expandované krmivo) zmenšuje velikost částic Ca a může tudíž redukovat zjištěné pozitivní účinky této velikosti. Zvýšený příjem

* Part of this work was prepared with the support of the ECOS-CONICYT (France – Chile).

nasyčených mastných kyselin vede k tvorbě mýdelných sloučenin obsahujících vápno a snižuje jak retenci vápníku, tak také retenci tuků. Při dietetické hladině tuků pod 6 % nebyl pozorován žádný nepříznivý vliv na kvalitu skořápky. Vysoké hladiny dietetického fosforu mají na kvalitu skořápky škodlivý účinek; dosažení normální užitkovosti a zachování integrity kostí zaručuje přísun 0,3 % dostupného fosforu. Dietetické hladiny Na (minimálně 0,12 %) a K mají na kvalitu vaječné skořápky jen malý vliv, zvýšená hladina chloridů (> 0,2 %) však působí nepříznivě. Mg je z větší části dodáván v rostlinné složce potravy (1 500 mg/kg) a při značném nadbytku (> 5 000 mg/kg) může kvalitu vaječné skořápky zhoršit. Syntetické zeolity (0,75 %) s vysokou kapacitou výměny kationtů kvalitu skořápky mírně zlepšují, ve střevě se však rozpouštějí a uvolňují hliník. Ostatní přírodní jíly mají na kvalitu skořápky jen malý vliv. Zvýšená dietetická hladina vitamínu D nemá na kvalitu skořápky žádný vliv a doplňování krmné dávky jeho aktivními metabolity je jen málo účinné. Vysoké teploty kvalitu skořápky zhoršují v důsledku sníženého příjmu Ca a respiračních alkalóz, jež jsou hypertermií vyvolávány. Pozitivní účinky nutričních faktorů (tj. vání hrubých částic s obsahem Ca a zeolitů) jsou zřetelnější v prostředí se zvýšenou teplotou, užitečné však mohou být i další faktory, například karbonátová voda, bikarbonát či vitamin C. Dostatečný přísun živin omezuje výskyt poškození skořápky (křepů), který zatím zůstává poměrně vysoký i přes značné úsilí, které je věnováno kontrole nutričních, environmentálních či genetických faktorů.

Klíčová slova: drůbež; vejce; kvalita skořápky; výživa

INTRODUCTION

The calcareous eggshell protects the contents of the egg from the microbial environment and controls the exchange of water and gases through the pores during the extrauterine development of chick embryo. Eggshell quality is therefore essential for the reproduction of the species and for egg commercialisation. For the last 20 years, the number of eggs produced per hen has largely increased (+70) without being associated with a change in the percentage of cracked eggs (about 8% in Europe) because of the numerous efforts in genetics, nutrition and in the control of environmental factors. 90% of downgraded eggs result from breakage of the eggshell and, therefore, control of the eggshell quality still has a part to play. A better understanding of eggshell formation and of the role of the organic matrix in eggshell mineralisation may help in the future to lower the incidence of cracks in market shell eggs but, presently, variation in eggshell quality and downgraded eggs between producers can be mainly alleviated by a better control of quality factors such as nutrition and environmental variables. After a brief description of eggshell structure, the present review summarises information on nutritional factors, mainly minerals which can affect the eggshell quality in normal or hot environment.

STRUCTURE AND FORMATION OF THE EGGSHELL

The avian eggshell is a highly ordered calcareous structure which is divided into six layers. The innermost two layers are the inner and outer shell membranes made up of a network of fibres which envelop the yolk and albumen. The calcified portion of the shell is anchored on the outer membrane fibres, and forms irregular cones corresponding to the mammillary knob layer. The palisade layer extends for 200 μm beyond the base of the cone and ends in a thin vertical crystal

layer aligned perpendicularly to the shell surface. The cuticle, an organic layer, is laid on the surface of the egg and contains a large part (2/3) of superficial pigments (Nys *et al.*, 1997).

The membranes are produced 3.5 hr after the ovulation of the yolk, in the distal part of the oviduct, the isthmus. Spherulitic crystal growth is initiated caudally to this region, in the transition of the red isthmus and the uterus, by deposition of calcium carbonate (calcite) on specific aggregates, the precursors of the mammillary knobs, which have previously been laid on the outer shell membranes. The bulk of calcite is deposited thereafter, from 10 to 22 hrs post ovulation in the uterus giving rise to the cone and palisade layers. Shell formation is therefore a sequential process, the phases of which follow a definite duration after the ovulation of the ovum. The ovulation of the yolk is synchronised with the oviposition of the preceding egg. In a 14–10 light dark lighting programme, the majority of oviposition occurs 2–4 hrs after lights on and therefore, eggshell deposition is initiated a few hours before lights off and mainly occurs during the night.

The eggshell is formed in the uterus which secretes a hypersaturated milieu relative to calcite solubility during the phase of eggshell formation. The uterine fluid also contains organic molecules which are precursors of a matrix and pervade the calcified layer. The matrix is composed of a complex array of proteins, among them osteoleidin-17 (OC17), a specific uterine protein and osteopontin, a bone matrix protein. Serum albumin, ovalbumin, lysozyme and ovotransferrin are also present in hen eggshell. Eggshell extracts or uterine fluid contain components which are able *in vitro* to delay, from a metastable solution of calcium and carbonate, the rate of precipitation of calcium carbonate and to modify the size and crystal morphology of the resulting calcite. Proteoglycans, primarily keratan- and dermatan-sulfate are also found in the eggshell matrix, and they also modify crystal precipitation and morphology. These observations suggest that these macromolecules influence the organisation of crystal growth by

controlling size, shape and orientation of calcite crystals. The analysis of their biochemical and functional properties may be useful in the future for the development of new approaches to control eggshell quality. Further research is needed to quantify the importance of this factor. In contrast, it is established that eggshell quality depends on the amount of eggshell deposited in the uterus and on the proportion of shell material relative to egg weight. Numerous efforts in nutrition have been carried out to optimise the uterine supply of mineral and to alleviate the decrease in eggshell quality observed in old hens.

CONTROL OF EGG WEIGHT

One of the main concerns about eggshell quality is the increase in egg breakage with increasing age of hens. The increase in egg weight with a concomitant decrease in percent eggshell partially explains the impairment in shell quality with age (Washburn, 1982). However, attempts to limit the increase in egg weight with age by decreasing the dietary protein level or the methionine intake were unsuccessful as it can reduce hen performance. Similarly, many reports indicate no response of egg weight to change in energy level of the diet because hens adapt their level of food consumption to the energy content of the diet (Summers and Robinson, 1995). In contrast, adding fat to the laying diet increases egg size. The positive effect of vegetal oil and especially linoleic acid on egg size is well established. The substitution of vegetal oil by tallow reduces the egg weight (March and MacMillan, 1990). That may be used toward the end of the laying year for improving eggshell quality as the lowering of egg weight occurs without change in eggshell weight (Joly, 1994).

CALCIUM NUTRITION

Dietary calcium level

During the growing period of the pullets, the calcium requirement of bird is 0.9–1.1% calcium between 8 and 16 weeks of age and excessive dietary calcium during this period depresses the growth performance of pullets, causes kidney damages and increases mortality (Sauveur, 1987). The level of calcium has to be increased 2–3 weeks before the onset of egg production to facilitate the development of the medullary bone. A delay in the introduction of a higher calcium diet negatively affects the eggshell quality of the first eggs but is also detrimental for the whole laying period (Leeson *et al.*, 1986). It is therefore recommended to introduce a higher calcium diet (2.5%) two weeks before the expected period of the first egg. Coarse particles of calcium in free choice could be used and it is observed that the consumption of calcium increases in parallel with that of egg production (Mongin, Sauveur, 1979).

During the laying period, hens export daily 2.2 g calcium and the laying hen diet should supply at least 4.5 g calcium. A low level of dietary calcium (< 2.5%) increases hen's mortality and depresses egg production (Hartel, 1990). In order to alleviate the loss due to egg breakage, the level of calcium in the diet tends to be increased with hen age but evaluation of high calcium level in layer diet demonstrated the low efficiency of such an increase on shell quality (Hartel, 1990). The increase from 3.5 to 4.5% of calcium in hens fed 0.27 to 0.47% available phosphorus does not affect shell breaking strength or thickness, but tends to decrease slightly food intake and egg mass (Hartel, 1990). In hens of 80 weeks at age, a high level of dietary calcium (5%) affects little eggshell quality (Keshavarz, 1986). A step-up phase feeding system (3.5, 4.5, 5.5% Ca) is also without effect on eggshell quality (Keshavarz, Nakajima, 1993).

Coarse particles of calcium

The daily kinetics of intestinal calcium absorption is of great importance because of the lack of coincidence between the deposition of calcium for shell formation in the uterus during the night and the calcium intake during the day. The specific appetite for calcium in hens and storage of food in the crop compensate partly for this gap and it is well established that the use of coarse calcium particles favours the expression of this physiological property of hens. However, the positive effect of calcium particles is inconsistently observed and it is of interest to know the origin of the factors which modify the eggshell response to calcium particles.

The sources of calcium carbonate obtained from quarries or of marine origin (oyster shells or other species of bivalves) are available under various particle sizes and correspond to various chemico-physical characteristics. Chemical analysis shows that calcium and trace mineral levels largely vary (Reid, Weber, 1976; Guinotte, 1992) but the crystal types of calcium carbonate (calcite, aragonite, amorphous) have no influence on eggshell quality in laying hens (Brister *et al.*, 1981). Physical characteristics mainly depend on particle size (ground or coarse) and to a smaller extent on the origin of the sources. The review of the literature since 1927 (320 experimental comparisons; Guinotte, Nys, 1993) allows the evaluation of various factors influencing the response of calcium source and size on eggshell quality and bone response. In 51% of the trials a significant increase is demonstrated in eggshell quality in hens fed coarse particles of calcium compared to those fed ground calcium. The marine origin is favourable in 13% of the trials compared to limestone whatever the size of the particle. The percent of positive responses is higher (51 vs 19%) when particles are larger than 1 mm compared with smaller particles (0.2–1 mm, or inferior to 0.2 mm). It is slightly increased (53 vs 41) when half of the calcium carbonate is sup-

plied as coarse particles in lieu of 33 or 100% or distributed completely separated from the diet. Use of coarse calcium source is particularly efficient under hot climate environment (78 vs 43%) and when distributed to hens toward the end of the laying period (62 vs 30%). Feeding low to medium levels of dietary calcium increases also the frequency of positive responses. The use of coarse calcium carbonate particles induces in most of the experiments (83%) an increase in bone mineralization but the origin of the sources has no effect.

It will be very convenient to predict by an *in vitro* test the efficiency of a calcium source with a defined size. *In vitro* measurement of calcium salt solubility may be of importance because calcium carbonate solubilization is a prerequisite to calcium absorption in the digestive tract. Solubility of various calcium sources mainly decreases in proportion to the size of the particles and depends also on the origin of the source. Moreover, technological treatments modify solubility of a source. The treatment of marine shells by phosphoric acid (Guinotte, 1992) and of oyster shells by high voltage current (Fujita *et al.*, 1988) increases their solubility. Tentatives to optimise particle size of a source for improving eggshell quality from its solubility are inconclusive due to interactions between origin of the source and size of the particle (Nys, 1995). Nevertheless, a coarse particle with a very low solubility will be deleterious for eggshell quality when introduced as the only calcium supplement in the diet.

Coarse calcium particles are retained longer in the crop and gizzard than other feed particles because of their large size (Meyer *et al.*, 1973; Gonalons, Moretto, 1989). Particles have to be superior to 0.8 mm for selective retention in the gizzard (Rao, Roland 1990). They increase also the enzyme activity responsible for gastric acid secretion in laying hens by 20% (Guinotte *et al.*, 1993) due to dilatation of the crop (Ruoff, Sewing, 1971; Lee *et al.*, 1988). The amount of intestinal calcium solubilized during the night (i.e. the period of eggshell calcification when there is no food intake) increases with coarse particles. As a consequence, the intestinal supply of calcium is synchronised with eggshell calcification, favouring eggshell quality and bone mineralization.

The use of coarse calcium particles completely separated from the food requires an additional silo of calcium carbonate to distribute calcium a few hours before extinction of lights. However, coarse particles of calcium carbonate could be incorporated into the diet as laying hens preferentially eat the calcium at the onset of shell formation due to a specific appetite for calcium. Pelleting of diets and the use of crumbs have been suggested to overcome insufficient feed consumption, which could be observed at the onset of egg production or in hens submitted to high temperature. Pelleting the diet reduces the size of the calcium carbonate particles (Guinotte, 1993) and, therefore, coarse calcium should be mixed after pelleting and crumbling rather than incorporated early in the diet.

An additional means to synchronise calcium intake and eggshell formation is the introduction of midnight feeding. Eggshell quality is improved when intermittent lighting supplement (2 h) was introduced in the middle of the night (Grizzle *et al.*, 1992). Combination of midnight feeding and food distribution with an automatic feeder can improve eggshell quality especially for the egg laid after light on (Harms *et al.*, 1996).

Fat incorporation and calcium utilization in hens

In the digestive tract, fatty acids can associate with calcium and magnesium and form insoluble soaps particularly when fats are enriched with saturated fatty acids. In laying hens, an increase in calcium level or the use of coarse particles of calcium and the consumption of a large amount of fat or saturated fatty acids lead to the formation of calcium soaps in the digestive tract (Atteh, Leeson, 1985) which are excreted (March, Amin, 1981). Calcium retention is lower in hens fed a high level (10%) of saturated fat (Atteh, Leeson, 1985) and fat retention decreases when calcium level increases in the diet. However, calcium and fat absorption are not affected by the formation of soaps when the level of fat in the diet is inferior to 6%. Eggshell quality and bone mineralization are not, therefore, modified (Atteh, Leeson, 1985). Various hypotheses may explain the limited effect of fat on calcium absorption in hens. Firstly, intestinal calcium absorption takes place before fat absorption and calcium soaps form from the nonabsorbed calcium and free fatty acids in the distal part of the digestive tract (Guéguen, 1992). Secondly, bile secretion which lowers soaps formation (Krogdahl, 1985) is higher in adult birds than that of young birds. Finally, gastric emptying and digestive transit time is longer when fat is added to the diet (Krogdahl, 1985) facilitating calcium solubilization and its absorption which may compensate the adverse effect of soap formation.

PHOSPHORUS

Numerous studies have demonstrated that eggshell quality decreases in the presence of high levels of available phosphorus (Miles, 1980; Vogt, 1982; Roland, 1989a; Keshavarz, Austic, 1990). In young hens, however, levels of available phosphorus lower than 0.25% have negative effects on egg production and bone quality (Rao *et al.*, 1995). As a consequence, the European mineral group of the WPSA recommends that phosphorus should be incorporated in the diet at a constant level of 0.28% available phosphorus throughout the laying period (Sauveur, 1992). Phosphorus requirements decrease with age and production in laying hens. Phase-feeding experiments (Roland, 1989a) show that step-down available phosphorus phase feeding regimens of 0.35, 0.25 and 0.15% aP at 20–35, 35–50, 50–70 wks of age maintain laying hens performance

(Rodriguez *et al.*, 1984) especially when hens are fed with calcium carbonate particles (Kerhavarz, Nakajima, 1993). Incorporation of particular calcium decreases mobilisation of bone reserves and permits lower dietary phosphorus levels (Sauveur, 1992). It is noteworthy that the addition of mineral phosphorus could be reduced by 0.1% when diets contain at least 300 UP vegetal (Sauveur, 1989) or microbial phytase (Aubert, Bouvarel, 1995; Usayran, Balnave, 1995).

There is evidence that the phosphorus requirement of laying hens slightly increases in hot environments. aP levels below 0.25% increase mortality in hens exposed to high temperatures (Garlich *et al.*, 1978). These authors recommended 350 mg/day/hen to sustain skeletal mineralization. Scheideler, Sell (1986) and Usayran, Balnave (1995) have demonstrated a reduction in phosphorus retention at high temperatures. A low dietary phosphorus level (0.3% aP) associated with low calcium intake, as is frequently observed at high temperatures, causes weak bone (Frost, Roland, 1991) and may facilitate the appearance of cage fatigue in layers.

VITAMIN D

Vitamin D controls calcium metabolism and intestinal absorption of calcium (Norman *et al.*, 1992; Ameenuddin *et al.*, 1985). There have been abundant researches in this area but it is beyond the scope of this review to summarise this work (Soares *et al.*, 1995; Sauveur, 1981). Briefly, vitamin D₃ is essential for egg production and eggshell quality and the requirement is 400 IU for normal performance (Whitehead, 1986; 300 IU/kg NRC, 1994). In practise, higher levels are used but there is little evidence that it improves shell quality (Tsang, Grunder, 1993; Keshavarz, Nakajima, 1993). 25(OH) D₃ shows a higher biological activity than vitamin D₃ and can be substituted to vitamin D₃ (Soares *et al.*, 1995) but again when large dietary levels are used, no improvement in eggshell quality is observed. Supplementation of hens with calcitriol, the active metabolite of vitamin D improves calcium retention but effects on eggshell quality are inconsistent, possibly because of the small difference between the optimal dietary level and toxic level (Tsang, Grunder, 1993). Moreover, when supplemented in addition to dietary vitamin D, the calcitriol has no influence on specific gravity (Harms *et al.*, 1990). These data are in agreement with the observation that calcitriol stimulates calcium absorption but is unable to improve calcium transfer at the uterine level (Nys, 1993). In 84 weeks old hens, the combination of vitamin D with 2 µg 1,25(OH)₂ D₃ tends however to reduce the percent of broken eggs and the presence of vitamin C reinforces the positive effect (Völker, Weiser, 1993).

MAGNESIUM

The concentration of magnesium in the eggshell is similar to that of phosphorus (0.9% total mineral).

A deficiency in Mg (< 0.021%) reduces egg production and the deposition of eggshell (Waddell *et al.*, 1989). Use of high dietary phosphorus increases the severity of magnesium deficiency (Edwards, Nugara, 1968). However, there is no routine supplementation of Mg in the diet because of high Mg level in the raw vegetal material. A layer diet (corn-soya) contains about 0.16% magnesium, i.e four times the requirement of laying hens (0.04 %; Vogt *et al.*, 1984). This level can be higher when foodstuffs (bran, sunflower, rapeseed, meat meal or dolomitic limestone) are used and the risk for eggshell quality is therefore that of an excess of magnesium. However magnesium incorporated into layer diet up to 0.52% (Anonymous, 1996) or 0.77% (Atteh, Leeson, 1983) has no detrimental effect on egg specific gravity or eggshell deformation even if the concentration of magnesium in the eggshell increases with the level of Mg supplementation. Increasing magnesium increases water consumption and has a cathartic effect at high level (> 0.6%) and therefore could increase the number of dirty eggs.

DIETARY ELECTROLYTES

Electrolyte balance of the diet influences the acid-base homeostasis which is also markedly modified by eggshell formation (Mongin, 1978). Attempts to establish the optimum dietary ratio between Na, K and Cl have been inconclusive. Changes in Na + K/Cl ratio from 0.4 to 7.7 induced by modifying the levels of Na and Cl, markedly modify blood pH and ionic concentrations but have no consequences on eggshell quality (Hamilton, Thompson, 1980) in agreement with the observations of Sauveur and Mongin (1978).

Numerous observations have shown that an excess of chloride has detrimental effects on eggshell quality. Weaker shells are observed when 0.75% (Austic, 1984), 0.8 (Keshavarz, Austic, 1990) or higher levels are used (Hamilton, Thompson, 1980). However, at a level lower than 0.3% of chloride, eggshell quality is not affected (Vogt, 1977; Hess and Britton, 1989). High sodium levels (0.35 and 0.45%) combined with high (0.47%) or low (0.12%) chloride levels also depress shell quality (Hughes, 1988). Sodium chloride when present in drinking water (2 g/l NaCl) reduces eggshell quality but the incidence of eggshell defects can be lowered by supplementing dietary zinc (0.1 g/kg, Balnave, Zhang, 1993). On the other hand, deficiencies in sodium (< 0.1, Sauveur, Mongin, 1978) and chloride (< 0.11%, Vogt, 1977; 0.14%, Sauveur, Mongin, 1978) decrease performance or eggshell quality.

ALUMINOSILICATES (CLAYS) AND EGG QUALITY

Numerous natural and synthetic clays are available. Their ion-exchange capability and adsorption properties have been tentatively exploited in animal nutrition

as binding agents in pelletized foodstuffs, to improve feed efficiency or to control moisture and ammoniac content of manure and their malodour.

In laying hens, sodium aluminosilicates (zeolites) have received much attention because of their ability to complex with calcium and their potential to improve shell quality. Sodium zeolite is a synthetic crystalline material with a three dimensional structure, a high ion-exchange capacity and high selectivity for calcium. It has the formula $\text{Na}_{12}((\text{AlO}_2)_{12}(\text{SiO}_2)_{12}) \cdot 27\text{H}_2\text{O}$ and contains 13% Na, 15% Al and 18.6% Si.

Most of the trials (77% of the 35 assays) show a beneficial effect of Na zeolite on shell quality as measured by egg specific gravity. The positive effect is observed in all cases when the level of calcium in the diet is marginal (2.75%, Roland *et al.*, 1985; Roland, 1989b) and seems greatest in elevated environmental temperature (Keshavarz, McCormick, 1991). The frequency of positive responses is not affected by the level of sodium zeolite (77% and 74% for levels of 0.75 and 1.5% zeolite in the diet, respectively).

Sodium and chloride adjustment has been carried out to correct for the increase in dropping moisture observed after the addition of Na zeolite (Roland, 1989b). Correcting for Na has no influence on the hen response to sodium zeolite and is ineffective in restoring the dropping moisture to normal (Keshavarz, McCormick, 1991) which may be affected, consequently, by the supply of aluminium (0.15%) or silicon (0.18%) following the supplementation of 1% zeolite. The combination of sodium zeolite with particulate calcium maintains a positive response suggesting that they may affect shell quality by different ways. The lack of effect of sodium zeolite on bone mineralization or on the plasma level of phosphorus during the laying cycle in opposite to the changes induced by particulate calcium confirms this hypothesis.

It is noteworthy that the attribute of zeolite which is responsible for its positive effect is still controversial. It may exert its effect by its ion-exchange capacity as supported by the disappearance of the beneficial effect when zeolites of similar formula but little cation-exchange capacity are used. However, Roland *et al.* (1993) show that sodium zeolite is solubilized in the intestine and consequently loses its physical properties. Aluminium salt (0.15%) has no influence on shell quality but decreases the phosphorus availability which may explain the negative effect of sodium zeolite on hen performance when the level of phosphorus is marginal in the diet. In the opposite, this antagonism may influence positively shell quality at high levels of phosphorus (0.7%) which were used in part of the first trials. Moreover, aluminium is known to be toxic to hens: egg production decreases in quail when 0.15% Al is added to the diet and is interrupted at a level of 0.3% (Hussein *et al.*, 1988). This observation and the intestinal absorption of 10 to 25% of aluminium and 40% of silicon from sodium zeolite in laying hens (Roland *et al.*, 1993) may limit the use of such an additive.

Natural zeolite has the composition $\text{Ca Na}_4\text{K}_4(\text{AlO}_2)_6(\text{SiO}_2)_{30} \cdot 24\text{H}_2\text{O}$. It has no ion-exchange capacity and is more stable than synthetic zeolite which may explain the lack of positive effect of natural zeolite, on shell quality.

NUTRITIONAL MEANS TO IMPROVE EGGSHELL QUALITY IN HENS EXPOSED TO HIGH TEMPERATURES

Optimum ambient temperature for production by laying hens is about 24 °C (Mardsen, Morris, 1987). Heat acclimation of bird occurs mainly by a reduction in heat production as the capacity of the birds to increase heat losses is limited (Geraert, 1991). Consequently, the only means for the birds is to decrease feed consumption. Above 30 °C, feed consumption or metabolizable energy intake and, as a consequence, body weight, egg production and egg weight decrease in hens exposed to constant high temperatures. The decrease in feed intake induced by high temperature reduces calcium intake and partly explains the reduction in eggshell quality induced by high constant temperatures. The decrease in eggshell quality is lower when hens are subjected to cycling daily temperature as compared to constant high temperature (Deaton *et al.*, 1982, De Andrade *et al.*, 1977). This is especially so when the lowest daily temperature is inferior to 25 °C. The decrease in shell quality is, however, greater when heat exposure is during the day than when it is during the night (Wolfenson *et al.*, 1979). Abrupt increases in temperature induce an immediate decline in shell quality (Miller, Sunde, 1975; Wolfenson *et al.*, 1979) and this before any change in feed intake occurs. It is, therefore, likely that physiological modifications induced by hyperthermia are responsible for part of the decline in shell quality. Heat stress induces panting and respiratory alkalosis (Mongin, 1978). The high rate of respiration decreases the carbon dioxide content of the blood and induces a higher blood pH. Bicarbonate ions are, therefore, eliminated by the kidney to restore the normal pH value. The decrease in carbonic anhydrase activity (Goto *et al.*, 1979) and blood flow (-40%, Wolfenson *et al.*, 1981) in the uterus also contribute to reduce deposition of shell material.

The addition of sodium bicarbonate to the diet has been used in attempts to correct the decline in bicarbonate and carbon dioxide blood levels observed at high temperatures but no improvement in shell quality was observed (Sauveur, Picard, 1987; Grizzle *et al.*, 1992). Carbon dioxide enriched air increases shell quality as demonstrated 30 years ago (Franck, Burger, 1965; Mongin, 1968 - cit. Sauveur, Picard, 1987) but could not be used practically because of the need to reduce ventilation. However, drinking of carbonated water during periods of hot weather (35 °C) improves eggshell quality (Odom *et al.*, 1985; Koelkebeck *et al.* 1992 - Koelkebeck *et al.*, 1993) but this finding was

not confirmed in a third experiment (Koelkebeck *et al.*, 1993).

The use of coarse calcium particles restores partly the decline in eggshell quality and could increase the feed consumption in hot environments. Introduction of a midnight lighting may also be favourable by allowing feed and calcium consumption (Grizzle *et al.*, 1992). Na zeolites also improve shell quality at high temperature environments. The positive effects of these nutritional factors are higher in hot environment as compared to normal temperatures.

Vitamin C has been reported to have beneficial effects on eggshell quality in hens exposed to high temperature (Njoku, Nwazota, 1989) but the results in the literature are inconsistent.

REFERENCES

- Ameenuddin S., Sunde M., Cook L. (1985): Essentiality of vitamin D3 and its metabolites in poultry nutrition. *World Poult. Sci. J.*, 41: 52–63.
- Anonymous (1996): Magnesium in layer ration. *Int. Hatchery Pract.*, 10 (8): 40.
- Atteh J. O., Leeson S. (1983): Influence of increasing dietary calcium and magnesium levels on performance, mineral metabolism and egg mineral content of laying hen. *Poult. Sci.*, 62: 1261–1268.
- Aubert C., Bouvarel I. (1995): Influence de l'addition de phytase sur les rejets phosphorés d'un élevage de poules pondeuses. In: Proc. 11^e journées Recherche Avicole, Angers: 68–70.
- Austic R. E. (1984): Excess dietary chloride depresses egg shell quality. *Poult. Sci.*, 63: 1773–1777.
- Balnavé D., Zhang D. (1993): Responses of laying hens on saline drinking water to dietary supplementation with various zinc compounds. *Poult. Sci.*, 72: 603–609.
- Brister R. D., Linton S. S., Creger C. R. (1981): Effects of dietary calcium sources and particle sizes on laying hens performance. *Poult. Sci.*, 60: 2648–2654.
- Deaton J. W., McNaughton J. L., Lott B. D. (1982): Effect of heat stress on laying hens acclimated to cyclic versus constant temperature. *Poult. Sci.*, 61, 1982: 875–878.
- De Andrade A. N., Rogler J. C., Featherston W. R., Alliston C. W. (1977): Interrelationships between diet and elevated temperature (cyclic and constant) on egg production and shell quality. *Poult. Sci.*, 56: 1178–1188.
- Edwards H. M., Nugara D. (1968): Magnesium deficiency in laying hen. *Poult. Sci.*, 47: 963–966.
- Frost T. J., Roland D. A. (1991): The influence of various calcium levels on tibia strength and eggshell quality of pullets during peak production. *Poult. Sci.*, 70: 963–969.
- Fujita T., Fukase M., Nakada M., Koishi M. (1988): Intestinal absorption of oyster shell electrolyte. *Bone Mineral*, 11: 85–91.
- Garlich J. D., Edens F. W., Parkhurst C. R. (1978): The phosphorus requirement of laying hens with special reference to high environmental temperature. In: Proc. XVI World Poult. Sci. Conf.: 598–602.
- Geraert P. A. (1991): Métabolisme énergétique du poulet de chair en climat chaud. *INRA Prod. Anim.*, 4 (3): 257–267.
- Gonalons E., Moreto M. (1989): Intestinal motility and absorption of nutrient in the fowl. In: 7th Europ. Symp. on Poultry Nutrition, Lloret de Mar (Spain): 13–27.
- Goto K., Harris G. C., Waldroup P. W. (1979): Relationship between pimpling of egg shells environmental temperature and carbonic anhydrase activity of certain body tissues. *Poult. Sci.*, 58: 1014.
- Grizzle J., Iheanacho M., Saxton A., Broaen J. (1992): Nutritional and environmental factors involved in egg shell quality of laying hens. *Brit. Poult. Sci.*, 33: 781–794.
- Guéguen L. (1992): Interactions lipides-calcium alimentaires et biodisponibilité du calcium du fromage. *Cah. Nutr. Diét.*, 27: 311–315.
- Guinotte F. (1992): Efficacité biologique de diverses sources de carbonate de calcium chez la poule pondeuse et le poulet en croissance (*Gallus domesticus*). Role de la sécrétion acide du proventricule dans la solubilisation et l'utilisation digestive de ces sources. [PhD Thesis.] University of Paris. 264 p.
- Guinotte F. (1993): Particle size of calcium sources in laying hens crumble feed is affected by pelleting process. In: Proc. V Europ. Symp. on the Quality of Egg and Eggs Products, Tours: 198–204.
- Guinotte F., Nys Y. (1993): Factors affecting the intestinal calcium availability in laying hens: Consequences on shell quality. In Proc. V Europ. Symp. on the Quality of Egg and Eggs products, Tours: 149–162.
- Guinotte F., Gautron J., Soumarmon A., Robert J. C., Nys Y. (1993): Gastric acid secretion in the chicken: Effect of histamine H2 antagonists and H+, K+ ATPase inhibitors on gastro-intestinal pH and of sexual maturity calcium carbonate level and particle size and proventricular H+, K+ ATPase activity. *Comp. Biochem. Physiol.*, 106: 319–327.
- Hamilton R. M. G., Thomson B. K. (1980): Effects of sodium plus potassium to chloride ratio in practical-type diets on blood gas levels in three strains of white leghorn hens and the relationship between the acid-base balance and eggshell strength. *Poult. Sci.*, 59: 1294–1303.
- Harms R. H., Douglas C. R., Sloan D. R. (1996): Midnight feeding of commercial laying hens can improve eggshell quality. *J. Appl. Sci. Res.*, 5: 1–5.
- Harms R. H., Bootwalla S. M., Woodward S. A., Wilson H. R. (1990): Some observations on the influence of vitamin D metabolites when added to the diet of commercial hens. *Poult. Sci.*, 69: 426–432.
- Hartel H. (1990): Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hens. *Brit. Poult. Sci.*, 31: 473–494.
- Hess J. B., Britton W. M. (1989): The effect of dietary chloride or protein changes on eggshell pimpling and shell quality in late-production leghorn hens. *Nut. Rep. Int.*, 40: 1107–1115.
- Hughes R. J. (1988): Inter-relationships between eggshell quality, blood acid-base balance and dietary electrolytes. *World Poult. Sci. J.*, 44: 30–40.
- Hussein A. H., Cantor A. H., Johnson T. H. (1988): Use of high level of dietary aluminum and zinc for inducing pau-

- ses in egg production of Japanese quail. *Poult. Sci.*, 67: 1157-1165.
- Ingram D. R., Kling C. E. (1988): Influence of ethalcal feed component on production parameters of white leghorn hens during high ambient temperature. *Nut. Rep. Int.*, 37: 811-817.
- Joly P. (1994): Qualité de coquille. *Bullet. 10: Isabrown Information*.
- Keshavarz K. (1986): The effect of variation of calcium intake on production performance and shell quality. *Poult. Sci.*, 65: 2120-2125.
- Keshavarz K., Austic R. E. (1990): Effects of dietary minerals on acid-base balance and eggshell quality in chickens. *J. Nutr.*, 120: 1360-1369.
- Keshavarz K., McCormick (1991): Effect of sodium aluminosilicate, oyster shell and their combination on acid-base balance and egg shell quality. *Poult. Sci.*, 70: 313-325.
- Keshavarz K., Nakajima S. (1993): Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. *Poult. Sci.*, 72: 144-153.
- Koelkebeck K. W., Harrison P. C., Madindou T. (1993): Effect of carbonated drinking water on production performance and bone characteristics of laying hens exposed to high environmental temperature. *Poult. Sci.*, 72: 1800-1803.
- Krogdahl A. (1985): Digestion and absorption of lipids in poultry. *J. Nutr.*, 115: 675-685.
- Lee C. M., Dimaline R., Moore S., Vowles L., Dockray G. J. (1988): Reciprocal endocrine interactions between proventriculus and antrum in chickens. *Regulatory Peptides*, 22: 415.
- Leeson S., Julian R. J., Summers, J. D. (1986): Influence of prelay and early-lay dietary calcium concentration on performance and bone integrity of leghorn pullets. *Can. J. Anim. Sci.*, 66: 1087-1095.
- March B. E., Amin C. (1981): Dietary limestone versus dietary oyster shell as calcium supplements to different layer diets. *Poult. Sci.*, 60: 591-597.
- March B. E., MacMillian C. (1990): Linoleic acid as a mediator of egg size. *Poult. Sci.*, 69: 634-639.
- Marsden A., Morris T. R. (1987): Quantitative review of the effects of environmental temperature on food intake, egg output and energy balance in laying pullets. *Brit. Poult. Sci.*, 28: 693-704.
- Meyer R., Baker R. C., Scott M. L. (1973): Effects of hen eggshell and other Ca sources upon eggshell strength and ultrastructure. *Poult. Sci.*, 52: 949-955.
- Miles R. D. (1980): The role of phosphorus in eggshell quality. In: *Proc. Florida Nutr. Conf.*: 95-110.
- Miller P. C., Sunde M. L. (1975): The effects of precise constant and cyclic environments on shell quality and other lay performance factors with leghorn pullets. *Poult. Sci.*, 54: 36-46.
- Mongin P. (1978): Acid-base balance during eggshell formation. In: Piiper J. (ed.): *Respiratory Function of Birds Adult and Embryonic*. Berlin, Springer Verlag: 247-259.
- Mongin P., Sauveur B. (1979): The specific appetite of domestic fowl. In: Boorman K. N, Freeman B. M. (eds.): *Food Intake Regulation in Poultry*. Edinburgh, BPS: 171-189.
- Njoku P. C., Nwazota A. O. U. (1989): Effect of dietary inclusion of ascorbic acid and palm oil on the performance of laying hens in a hot tropical environment. *Brit. Poult. Sci.*, 30: 831-840.
- Norman A. W., Nemere I. et al. (1992): 1,25(OH)₂ D₃, a steroid hormone that produces biologic effect via both genomic and nongenomic pathways. *J. Steroid Biochem. Molec. Biol.*, No 3-8: 231-240.
- Nys Y. (1993): Regulation of plasma 1,25(OH)₂ D₃ of osteocalcin and of intestinal and uterine calbindin in hens. In: *Avian Endocrinology*. Bristol, Sharp: 345-358.
- Nys Y. (1995): Influence of nutritional factors on eggshell quality at high environmental temperature. In: *Proc. VI Europ. Symp. on Egg and Egg Product Quality*, Zaragoza: 209-220.
- Nys Y., Hincke M. T., Arias J., Garcia-Ruiz J. M., Solomon S. E. (1997): Biochemical characterization and *in vitro* functional properties of eggshell matrix extracts and of uterine fluid in hens. In: *Proc. VII Europ. Symp. on Egg and Egg Product Quality*, Poznan: 107-126.
- Odom T. W., Harrison P. C., Darre M. J. (1985): The effects of drinking carbonated water on the egg shell quality of single comb white leghorn hens exposed to high environmental temperature. *Poult. Sci.*, 64: 594-596.
- Rao K. S., Roland D. A. (1990): Retention patterns of various sized limestone particles in gizzard of commercial leghorn hens. *Poult. Sci.*, 69: 185.
- Rao K. S., Roland D. A., Orban J. I., Rabon H. W., Bryant M. M. (1995): Age at sexual maturity influences the response of single white leghorn pullets to marginal and low levels of dietary phosphorus. *J. Nutr.*, 125: 1342-1350.
- Reid B. L., Weber C. W. (1976): Calcium availability and trace mineral composition of feed grade Ca supplements. *Poultry Sci.*, 55: 600-605.
- Rodriguez M., Owings W. J., Sell L. J. (1984): Influence of phase feeding available phosphorus on egg production characteristics carcass phosphorus content and serum inorganic phosphorus levels. *Poult. Sci.*, 63: 1553-1562.
- Roland D. A. (1989a): Phosphorus requirement of commercial leghorns. In: *Proc. Georgia Nutr. Conf.*: 120-139.
- Roland M. L. T. (1989b): Aluminium silicates in poultry nutrition. In: *Proc. Maryland Nutrition Conf. for Feed Manufacturers*, Univ. Maryland, USA: 24-26.
- Roland D. A., Laurent S. M., Orloff H. D. (1985): Shell quality as influenced by zeolite with high ion exchange capability. *Poult. Sci.*, 64: 1177-1187.
- Roland D. A., Rabon H. W., Rao K. S., Smith R. C., Miller J. R., Barnes D. G., Laurent S. M. (1993): Evidence for absorption of silicon and aluminum by hens fed sodium zeolite A. *Poult. Sci.*, 72: 447-455.
- Ruoff H. J., Sewing K. F. (1971): The role of crop in the control of gastric acid secretion in chickens. *Naunyn-Schmiedebergs Archiv für Pharmakologie*, 271: 142-148.
- Sauveur B. (1981): Effect of dietary 25 (OH)D₃ and solanum malacoxilon on shell quality. In: Beuwing G., Scheele C. W., Simmons P. C. M. (eds.): *Quality of Eggs*: 194-202

- Sauveur B. (1987): Effect of prelaying dietary calcium and phosphorus levels on growth and subsequent laying performance of pullets. In: 6th Europ. Symp. WPSA on Poultry Nutrition, Königsutter: E18-E25.
- Sauveur B. (1989): Phosphore phytique et phytases dans l'alimentation des volailles. INRA Prod. Anim., 2: 343-351.
- Sauveur B. (1992): Adaptation des apports alimentaires aux variations journalières des besoins en calcium et phosphore de la poule. INRA Prod. Anim., 5: 19-28.
- Sauveur B., Mongin P. (1978): Interrelationships between dietary concentration of sodium, potassium and chloride in laying hens. Brit. Poult. Sci., 19: 475-485.
- Sauveur B., Picard M. (1987): Environmental effects on egg quality. In: Wells R. G., Beyavin C. G. (eds.): Egg Quality. Current Problems and Recent Advances. London, Butterworths: 219-233
- Soares J. H., Kerr J. M., Gray R. W. (1995): 25 hydroxycholecalciferol in poultry nutrition. Poult. Sci., 74: 1919-1934.
- Scheideler S. E., Sell J. L. (1986): Effects of calcium and phase-feeding phosphorus on production traits and phosphorus retention in two strains of laying hens. Poult. Sci., 65: 2110-2119.
- Summers T. D., Robinson F. E. (1995): Feeding programs for poultry reproduction. In: Hunton P. (ed.): World Animal Science: 319-352.
- Tsang C. P. W., Grunder A. A. (1993): Effect of dietary contents of cholecalciferol 1, 25 dihydroxycholecalciferol and 24,25 dihydroxycholecalciferol on blood concentration of 25, hydroxycholecalciferol, 1,25 dihydroxycholecalciferol, total calcium and eggshell quality. Brit. Poult. Sci.: 1021-1227.
- Usayran N., Balnave D. (1995): Phosphorus requirements of laying hens fed on wheat-based diets. Brit. Poult. Sci., 36: 285-301.
- Völker L., Weiser H. (1993): The relevance of vitamins D3 and C for bone metabolism in poultry. In: Proc. Maryland Nutr. Conf.: 42-54.
- Vogt H. (1982): Phosphorus requirements of laying hens. In: Proc. Vth Ann. Int. Miner. Conf.: 67-101.
- Vogt H. (1977): Versuch über den Chloridbedarf im Legehennenfutter. Arch. Geflügelkde., 41: 124-129.
- Vogt H., Dewar W. A., Sauveur B., Simmons P. C. M. (1984): Mineral requirements and recommendations for adult birds. World Poult. Sci. J., 40: 183.
- Waddel A. L., Board R. G., Scott V. D., Tullet S. G. (1989): Influence of dietary magnesium content in the domestic fowl. Brit. Poult. Sci., 30: 865-876.
- Washburn K. W. (1982): Incidence, cause and prevention of eggshell breakage in commercial production. Poult. Sci., 61: 2005-2012.
- Whitehead C. C. (1986): Requirements for vitamins. In: Proc. Sym. Poult. Sci. 19, Butterworth ed., 1986: 173-189.
- Wolfenson D., Frei Y. F., Snapir N., Berman A. (1979): Effect of diurnal or nocturnal heat stress on egg formation. Brit. Poult. Sci., 20: 167-174.
- Wolfenson D., Frei Y. F., Snapir N., Berman A. (1981): Heat stress effects on capillary blood flow and its redistribution in the laying hen. Pflüger's Arch., 390: 86-93.

Received for publication on September 8, 1998
Accepted for publication on November 10, 1998

Contact Address:

Y. N y s , Station de Recherches Avicoles, centre de Tours, Nouzilly, 37380 France

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 120 56 Praha 2, Czech Republic
Fax: (00422) 24 25 39 38

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences. The periodicals are published in the Czech or Slovak languages with long summaries in English or in English language with summaries in Czech or Slovak.

Subscription to these journals should be sent to the above-mentioned address.

Periodical	Number of issues per year
Rostlinná výroba (Plant Production)	12
Czech Journal of Animal Science (Živočišná výroba)	12
Veterinární medicína (Veterinary Medicine – Czech)	12
Zemědělská ekonomika (Agricultural Economics)	12
Journal of Forest Science	12
Zemědělská technika (Agricultural Engineering)	4
Plant Protection Science (Ochrana rostlin)	4
Czech Journal of Genetics and Plant Breeding (Genetika a šlechtění)	4
Zahradnictví (Horticultural Science)	4
Czech Journal of Food Sciences (Potravinařské vědy)	6

INSTRUCTIONS FOR AUTHORS

Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short and a longer summary (including key words).

The author is fully responsible for the originality of his paper, for its subject and formal correctness. The author shall make a written declaration that his paper has not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

The paper extent shall not exceed 15 typescript pages, including tables, figures and graphs.

Manuscript layout: quarto, 30 lines per page, 60 strokes per line, double-spaced typescript. A PC diskette should be provided with the paper and graphical documentation. Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes.

If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

The title of the paper shall not exceed 85 strokes. Subtitles of the papers are not allowed either.

Abstract is an information selection of the subject and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes, and comprise basic numerical data including statistical data. It must contain key words. It should be submitted in English and if possible also in Czech or Slovak.

Introduction has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form.

Review of literature should be a short section, containing only literary citations with close relation to the treated problem.

Only original method shall be described, in other cases it is sufficient enough to cite the author of the used method and to mention modifications of this method. This section shall also contain a description of experimental material.

In the section **Results** figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

Discussion contains an evaluation of the study, potential shortcomings are discussed, and the results of the study are confronted with previously published results (only those authors whose studies are in closer relation with the published paper should be cited). The sections Results and Discussion may be presented as one section only.

The section **References** should preferably contain reviewed periodicals. The citations are arranged alphabetically according to the surname of the first author. References in the text to these citations comprise the author's name and year of publication. Only the papers cited in the text of the study shall be included in the list of references. All citations shall be referred to in the text of the paper.

The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telephone and fax number or e-mail.

The manuscript will not be accepted to be filed by the editorial office if its formal layout does not comply with the instructions for authors.

POKYNY PRO AUTORY

Časopis uveřejňuje původní vědecké práce, krátká sdělení a výběrově i přehledné referáty, tzn. práce, jejichž podkladem je studium literatury a které shrnují nejnovější poznatky v dané oblasti. Práce jsou uveřejňovány v češtině, slovenštině nebo angličtině. Rukopisy musí být doplněny krátkým a rozšířeným souhrnem (včetně klíčových slov).

Autor je plně odpovědný za původnost práce a za její věcnou i formální správnost. K práci musí být přiloženo prohlášení autora o tom, že práce nebyla publikována jinde.

O uveřejnění práce rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a přínosu a kvalitě práce.

Rozsah vědeckých prací nesmí přesáhnout 15 strojopisných stran včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI (ČSN 01 1300).

Vlastní úprava rukopisu: formát A4, 30 řádek na stránku, 60 úhozů na řádku, mezi řádky dvojité mezery. K rukopisu je třeba přiložit disketu s prací pořízenou na PC a s grafickou dokumentací. Tabulky, grafy a fotografie se dodávají zvlášť, nepodlepují se. Na všechny přílohy musí být odkazy v textu.

Pokud autor používá v práci zkratky jakéhokoliv druhu, je nutné, aby byly alespoň jednou vysvětleny (vypsány), aby se předešlo omylům. V názvu práce a v souhrnu je vhodné zkratky nepoužívat.

Název práce (titul) nemá přesáhnout 85 úhozů. Jsou vyloučeny podtitulky článků.

Krátký souhrn (Abstrakt) je informačním výběrem obsahu a závěru článku, nikoliv však jeho pouhým popisem. Musí vyjádřit všechno podstatné, co je obsaženo ve vědecké práci, a má obsahovat základní číselné údaje včetně statistických hodnot. Musí obsahovat klíčová slova. Nemá překročit rozsah 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě. Je uveřejňován a měl by být dodán ve stejném jazyce jako vědecká práce.

Rozšířený souhrn (Abstract) je uveřejňován v angličtině, měly by v něm být v rozsahu cca 1–2 strojopisných stran komentovány výsledky práce a uvedeny odkazy na tabulky a obrázky, popř. na nejdůležitější literární citace. Je vhodné jej (včetně názvu práce a klíčových slov) dodat v angličtině, popř. v češtině či slovenštině jako podklad pro překlad do angličtiny.

Úvod má obsahovat hlavní důvody, proč byla práce realizována, a velmi stručnou formou má být popsán stav studované otázky.

Literární přehled má být krátký, je třeba uvádět pouze citace mající úzký vztah k problému.

Metoda se popisuje pouze tehdy, je-li původní, jinak postačuje citovat autora metody a uvádět jen případné odchylky. Ve stejné kapitole se popisuje také pokusný materiál.

Výsledky – při jejich popisu se k vyjádření kvantitativních hodnot dává přednost grafům před tabulkami. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce, diskutuje se o možných nedostatcích a práce se konfrontuje s výsledky dříve publikovanými (požaduje se citovat jen ty autory, jejichž práce mají k publikované práci bližší vztah). Je přípustné spojení v jednu kapitolu spolu s výsledky.

Literatura by měla sestávat hlavně z lektorovaných periodik. Citace se řadí abecedně podle jména prvních autorů. Odkazy na literaturu v textu uvádějí jméno autora a rok vydání. Do seznamu se zařadí jen práce citované v textu. Na práce v seznamu literatury musí být odkaz v textu.

Na zvláštním listě uvádí autor plné jméno (i spoluautorů), akademické, vědecké a pedagogické tituly a podrobnou adresu pracoviště s PSČ, číslo telefonu a faxu, popř. e-mail.

Rukopis nebude redakcí přijat k evidenci, nebude-li po formální stránce odpovídat pokynům pro autory.

CONTENTS

Genetics and Breeding

- Kolman R., Krylova V. D., Szczepkowska B., Szczepkowski M.: Meristic studies of Siberian sturgeon (*Acipenser baeri* Brandt) and its crosses with green sturgeon (*Acipenser medirostris* Ayres) (in English)..... 97

Physiology and Reproduction

- Šutiaková I., Bekeová E., Šutiak V.: The influence of chlorine on the activities of lactate dehydrogenase and its isoenzymes in plasma of lambs (in English)..... 103
- Suchý P., Straková E., Hrubý A.: Variations in cholesterol concentrations in the blood plasma of hens throughout the laying period (in Czech)..... 109

Nutrition and Feeding

- Svetlanská M., Sommer A., Petrikovič P., Boroš V.: The effect of feeding lactic fermented products on nutrient consumption in calves until 90 days of age (in Slovak)..... 113
- Prokop V., Heger J., Kumprecht M., Klapil L.: The effect of targeted combination of additives to prestarter on nutrition parameters and performance of piglets (in English)..... 119
- Ivan M., Rusihan M., Alimon A. R., Hair-Bejo M., Jelani Z. A., Jalaludin S.: The efficacy of dietary supplements of bentonite and sulphur plus molybdenum to alleviate chronic copper toxicity in sheep fed palm kernel cake (in English)..... 125

Management Technology and Hygiene

- Kunc P., Knížková I., Koubková M.: The influence of milking with different vacuum and different design of liner on the change of teat surface temperature (in Czech)..... 131

INFORMATION – STUDIES – REPORTS

- Nys Y.: Nutritional factors affecting eggshell quality (in English)..... 135

OBSAH

Genetika a šlechtění

- Kolman R., Krylova V. D., Szczepkowska B., Szczepkowski M.: Meristické studium jesetera sibiřského (*Acipenser baeri* Brandt) a jeho hybridů s jeseterem zeleným (*Acipenser medirostris* Ayres)..... 97

Fyziologie a reprodukce

- Šutiaková I., Bekeová E., Šutiak V.: Vliv chlóru na aktivitu laktátdehydrogenázy a jej izoenzymů v krvnej plazme jahniat..... 103
- Suchý P., Straková E., Hrubý A.: Dynamika změn cholesterolu v krvnej plazmě slepic v průběhu snáškového období..... 109

Výživa a krmění

- Svetlanská M., Sommer A., Petrikovič P., Boroš V.: Vplyv skrmovania mliečnych fermentovaných produktov na spotrebu živín u teliat do veku 90 dní..... 113
- Prokop V., Heger J., Kumprecht M., Klapil L.: Vliv cílené kombinace aditiv v prestartéru na nutriční ukazatele a užítkovost selat..... 119
- Ivan M., Rusihan M., Alimon A. R., Hair-Bejo M., Jelani Z. A., Jalaludin S.: Vplyv prídavkov bentonitu, síry a molybdénu do krmiva na zníženie chronickej toxicity meďou u oviec krmiených palmojadrovými výliskami..... 125

Technologie a hygiena chovu

- Kunc P., Knížková I., Koubková M.: Vliv dojení s různým podtlakem a strukovými návlečkami různého tvaru na změnu teploty povrchu struku..... 131

INFORMACE – STUDIE – SDĚLENÍ

- Nys Y.: Nutriční faktory ovlivňující kvalitu skořápky..... 135