CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

10

VOLUME 48
PRAGUE 2003
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

EDITORIAL BOARD

Chairman

Prof. Ing. JAN ŘÍHA, DrSc., Research Institute of Cattle Breeding, Ltd., Rapotín, Czech Republic

Members

Doc. MVDr. Ing. JIŘÍ BROŽ, PhD., Rheinfelden, Switzerland
Prof. Ing. JOZEF BULLA, DrSc., Research Institute of Animal Production, Nitra, Slovak Republic
Doc. Ing. JOSEF ČEŘOVSKÝ, DrSc., Research Institute of Animal Production, Prague – workplace Kostelec nad Orlicí, Czech Republic
Prof. Dr. hab. ANDRZEJ FILISTOWICZ, Agricultural University, Wrocław, Poland
Prof. RNDr. KAREL HALA, CSc., University of Innsbruck, Innsbruck, Austria
Dr. Ing. OTO HANUŠ, Research Institute of Cattle Breeding, Ltd., Rapotín, Czech Republic
Prof. Ing. MVDr. PAVEL JELÍNEK, DrSc., Mendel University of Agriculture and Forestry, Brno, Czech Republic
Prof. MVDr. FRANTIŠEK JÍLEK, DrSc., Czech University of Agriculture, Prague, Czech Republic
Ing. JAN KOUŘIL, PhD., University of Southern Bohemia, České Budějovice – Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic
Prof. Ing. ALOJZ KÚBEK, CSc., Slovak University of Agriculture, Nitra, Slovak Republic
Dr. CLAUS LEIDING, Besamungsverein Neustadt a.d. Aisch e.V., Neustadt a.d. Aisch, Deutschland
RNDr. MILAN MARGETÍN, CSc., Research Institute of Animal Production, Nitra – workplace Trenčianska Teplá, Slovak Republic
Prof. Ing. VÁCLAV MATOUŠEK, CSc., University of Southern Bohemia, České Budějovice, Czech Republic
Prof. Ing. ŠTEFAN MIHINA, PhD., Research Institute of Animal Production, Nitra, Slovak Republic
Dr. DUŠAN NOSÁL, Swiss Research Institute for Agricultural Economics and Engineering, Tänikon b. Aadorf, Switzerland
Doc. Ing. JAROSLAV PETR, DrSc., Research Institute of Animal Production, Prague, Czech Republic
Doc. Ing. ANTONÍN STRATIL, DrSc., Institute of Animal Physiololgy and Genetics of the Academy of Sciences of the Czech Republic, Liběchov, Czech Republic
Doc. Ing. EVA TŮMOVÁ, CSc., Czech University of Agriculture, Prague, Czech Republic
Prof. Ing. LADISLAV ZEMAN, CSc., Mendel University of Agriculture and Forestry, Brno, Czech Republic

Editor-in-Chief

Ing. ZDEŇKA RADOŠOVÁ

For information on Czech J. Anim. Sci. and full papers from Vol. 47 visit http://www.cazv.cz

Aim 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 Envi-ronmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts, Toxline Plus.

Periodicity: The journal is published monthly (12 issues per year). Volume 48 appearing in 2003.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: +420 227 010 352, fax: +420 227 010 116, e-mail: edit@uzpi.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, Czech Republic. Subscription price for 2003 is 1176 CZK or 214 USD.

© Institute of Agricultural and Food Information, Prague 2003 MK ČR E 4886

CZECH JOURNAL OF ANIMAL SCIENCE

Volume 48, No. 10 October 2003

CONTENTS

OBSAH

POKYNY PRO AUTORY

Časopis uveřejňuje původní vědecké práce, výběrově krátká sdělení, aktuální literární přehledy i knižní recenze. Práce jsou publikovány v angličtině. Rukopisy musí být doplněny anglickým a českým (slovenským) abstraktem včetně klíčových slov. Autor je plně odpovědný za původnost práce a za její věcnou i formální správnost. K rukopisu musí být přiloženo prohlášení autora (i spoluautorů) o tom, že práce nebyla publikována jinde. O uveřejnění článku rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a kvalitě rukopisu. Rozsah rukopisu nemá přesáhnout 15 normovaných stran včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI. Rukopis se odevzdává ve dvou úplných kopiích s přiloženou, řádně označenou disketou s identickým obsahem včetně grafické dokumentace, nebo lze rukopis zaslat v e-mailové příloze.

Copyright. Časopis je chráněn autorskými právy, kterými disponuje vydavatel od přijetí rukopisu do tisku. Korespondující autor přebírá odpovědnost za všechny autory ohledně převodu práv. Žádná část této publikace nesmí být jakkoli reprodukována, uchovávána ani šířena bez písemného souhlasu vydavatele.

Vlastní úprava rukopisu. Rukopis se vyhotoví ve formátu A4 s velikostí písma 12 mm, mezi řádky dvojité mezery. Text musí být vypracován v textovém editoru Word a řádně označen. Tabulky, grafy a ostatní dokumenty se dodávají zvlášť. Každý uvedený dokument musí být vytištěn na zvláštní straně s výstižným názvem a přesným popisem, včetně platných jednotek. Tabulky se zpracují v programovém editoru Word, každou položku je třeba umístit do zvláštní buňky. Tabulky se číslují arabskými číslicemi v pořadí, ve kterém jsou odkazovány v textu. Grafy se pořizují v programu Excel a je třeba je uložit s původními daty (velikost a typ použitého písma by měly korespondovat s formátem časopisu a s případným zmenšením grafu). Autotypie se dodávají v černobílém provedení, nejlépe ve formátu TIF, JPGE nebo PDF. Všechny obrázky se číslují průběžně podle pořadí uvedeného v textu rovněž arabskými číslicemi. Všechny ostatní doplňky musí být dodány v digitalizované podobě ve velmi dobrém rozlišení a v černobílém provedení. Barevné obrázky či mapy je možno publikovat po předchozí domluvě, výlučně však na náklady autorů. Na všechny přílohy musí být odkazy v textu. Pokud autor používá v práci zkratky jakéhokoliv druhu, je nutné, aby byly při prvním použití v textu řádně vysvětleny. V názvu práce a v abstraktu je vhodné zkratky nepoužívat.

Název práce má být stručný, srozumitelný a nemá přesáhnout 85 úhozů. Jsou vyloučeny podtitulky článků.

Abstrakt je informačním výběrem obsahu a závěru článku, nikoliv však jeho pouhým popisem. Má vyjádřit vše podstatné, co je obsaženo ve vědecké práci. Má obsahovat základní číselné údaje včetně statistického hodnocení. Musí obsahovat klíčová slova. Jeho rozsah nemá překročit 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě. Abstrakt je důležitou součástí článku, protože je uveřejňován a citován v celosvětových databázích. Domácí autoři musí dodat název a abstrakt článku v angličtině a češtině (slovenštině), u zahraničních přispěvatelů postačí abstrakt v angličtině.

Úvod má stručně nastínit hlavní důvody, proč byla práce realizována, jaký cíl si autoři vytyčili a jaký je současný stav znalostí daného problému. Ten by měl být vyjádřen stručným literárním přehledem, sestaveným především z ektorovaných periodik, majících úzký vztah k danému tématu.

Materiál a metody. V této části se detailně popíše pokusný materiál, prováděné experimenty, jejich rozsah, podmínky a průběh. Uvádějí se všechny originální postupy, kterých bylo využito při zpracování experimentálního materiálu, a veškeré analytické postupy potřebné k hodnocení. U použitých metod je nutné doplnit i údaje ověřující kvalitu získaných dat. Celá metoda se popisuje pouze tehdy je-li původní, jinak postačuje citace autora metody s vysvětlením případných odchylek. V této části se uvedou i statistické metody hodnocení, včetně použitého softwaru.

Výsledky a diskuse. Tato kapitola dává autorovi prostor ke grafickému či tabulkovému vyjádření získaných výsledků, včetně jejich statistického vyhodnocení a vlastního komentáře. Dílčí výsledky autor konfrontuje s publikovanými údaji ostatních autorů, jejichž jména a rok vydání publikace uvádí přímo [Novák (2002)] či nepřímo [(Novák and Dvořáková, 2002), (Novák *et al.*, 2002)] do textu. Na závěr této části se doporučuje provést stručné hodnocení, jak byl splněn záměr práce.

Literatura má být sestavena hlavně z lektorovaných periodik a řadí se abecedně podle příjmení prvních autorů. Po úplném výčtu všech autorů se do závorky uvede rok vydání publikace, její originální název, název periodika s využitím oficiálních zkratek, volume a stránky. Citace více prací jednoho roku se v textu i seznamu literatury odlišují písmeny a, b, c ... (2002a,b). U knihy nebo sborníku se po názvu uvádí vydavatel a místo vydání. Je-li citovaný zdroj přeložen do angličtiny, připojí se do závorek na konec citace jazyk, ve kterém byl materiál publikován. Do seznamu literatury se zařadí jen práce citované v textu.

Příklady:

Brown J. (1995): Estradiol determination in post-partum sows. J. Endocrinol., *198*, 155–169.

Green K.L., Grey M. (1996): Hormones in milk. J. Anim. Res., *29*, 1559–1571.

Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): Lactation in Cattle. Academic Press, London. 876–888.

Adresa autorů. Na zvláštním listu dodá autor plné jméno (i spoluautorů), akademické, vědecké a pedagogické tituly a podrobnou adresu pracoviště s PSČ, číslo telefonu, faxu a e-mail. V autorském kolektivu je nezbytné řádně označit korespondujícího autora.

Separáty. Autor obdrží zdarma deset separátních výtisků práce a elektronickou poštou „elektronický separát" ve formě pdf.

Uvedené pokyny jsou závazné pro všechny autory. V případě, že nebude rukopis po formální stránce odpovídat uvedeným požadavkům, nebude redakcí přijat k oponentnímu řízení.

Factors affecting the shape of lactation curves of Holstein cows in the Czech Republic

L. Dědková, E. Němcová

Research Institute of Animal Production, Prague-Uhříněves, Czech Republic

ABSTRACT: The effects of year and season of calving, days open and age at calving on the parameters of lactation curve were analysed using 166 140 first lactations of Czech Holstein cows. Wilmink's function was used for the analysis. Persistency was expressed as the difference of milk yield from 60th to 280th day in milk from the population average. The peak of milk yield in kg and the peak of milk yield in days were analysed. The highest influence on the shape of lactation curve was found for year of calving, month of calving and days open. Grouping for fixed regression in the test-day model was suggested according to the results of analysis.

Keywords: lactation curve; persistency; environmental effects; dairy cattle

The shape of lactation curve in dairy cattle is influenced by miscellaneous factors such as year and season of calving, days open and age at calving (Danell, 1982; Strandberg and Lundberg, 1999; Kučera *et al.*, 1999; Emmerling *et al.*, 2000; Tekerli *et al.*, 2000). Therefore cows must be grouped according to the shape of lactation curve when employing test-day models (Ptak and Schaeffer, 1993) for the genetic evaluation of dairy cattle. The lactation curve of cows belonging to the same subclass is modelled by one joint fixed regression and additional random regressions describing the genetically based deviations of individual cows from the average lactation curve of the subclass (Schaeffer and Deckers, 1998).

The aim of this study was to analyse the effects of season and year of calving, age at calving and days open on the shape of lactation curve and the index of persistency in Holstein cows in the Czech Republic. On the basis of this analysis the main factors influencing the shape of lactation curve should be identified and the way of grouping for fixed regression in the test-day model for genetic evaluation of Holstein cattle should be suggested.

MATERIAL AND METHODS

The data consisted of 1 552 626 test-day records belonging to 166 140 first lactations of Holstein cows calved in 1995 to 1999. Only cows with 8 to 10 test days were considered. The lactation curve was described using Wilmink's function (Wilmink, 1987):

$$y_t = w_0 + w_1 t + w_2 e^{-0.05t}$$

where: t = days in milk
y_t = milk yield in kg
w_0 = is associated with the level of production
w_1 = with production decrease after peak yield
w_2 = with production increase toward peak yield

The parameters of the lactation function were estimated by linear regression using the procedure REG of the statistical program package SAS® (SAS, 1988).

The index of persistency was calculated for each lactation according to Schaeffer *et al.* (1997):

Supported by the Ministry of Agriculture of the Czech Republic (Project No. QC 1235).

$p_1 = [(\bar{y}_{60} - \bar{y}_{280}) - (y_{60} - y_{280})]*100$ (milk yield in kg)

where: y_{60} and y_{280} are test-day milk yields on days 60 and 280 in milk, respectively, with the average values $\bar{y}_{60}$ = 21.86 and $\bar{y}_{280}$ = 13.69.

The yield from 7th to 305th day of lactation was calculated using the estimated parameters of Wilmink's function. A production peak was determined in days (DIMPEAK) and in kg of milk (MLKPEAK). Some basic data are summarised in Table 1.

The effects of season of calving, year of calving, age at calving and days open on the analysed dependent variables were determined on the basis of the following linear model:

$$y_{ijklm} = Month_i + Year_j + L\_DO_k + L\_AGE_l + e_{ijklm}$$

where: y_{ijklm} = the value of the considered trait or parameter (parameters of Wilmink's function, index of persistency according to Schaeffer *et al.*, 1997, production peak in milk yield, production peak in days in milk, milk yield in the whole lactation) of lactation record *m*

$Month_i$ = fixed effect of the *i*th month of calving

$Year_j$ = fixed effect of the *j*th year of calving

L_AGE_l = effect of the *k*th level of age at calving

L_DO = effect of the *l*th level of days open

e_{ijklm} = random residual effect for the *m*th lactation

The levels for age at calving and days open are defined in Table 2, for two different models employed. In Model II the season of calving was included instead of the month of calving. The calculations were carried out using the procedure GLM of SAS® (SAS, 1988).

RESULTS

The correlations between the analysed parameters are presented in Table 3. Positive correlations were found between the parameters connected with the production level and between the parameters describing persistency. The parameter of the lactation function w_0 was negatively correlated with the other parameters of Wilmink's function and with the parameter of persistency p_1. A high negative correlation occurred between the number of days in milk at the production peak and the parameter w_2.

In both variants of the model, each effect significantly ($P < 0.01$) influenced the analysed traits with the exception of season of calving and age at calving in Model II. The season of calving did not influence the lactation milk yield. Age at calving affected the production peak in milk yield only on a probability level of $P < 0.05$. In both analyses, the year of calving was suggested to be the most significant factor. On the contrary, age at calving was the least influential factor.

Table 1. Basic statistical data

Variable	No.	Mean	SD	Minimum	Maximum
Milk yield (kg)	1 552 626	18.4	5.94	4	53
Days in milk (day)	1 552 626	152.4	83.97	7	305
w_0	166 140	24.47	6.636	–0.13	63.99
w_1	166 140	–0.038	0.023	0.19	0.08
w_2	166 140	–6.036	15.316	–81.71	58.97
mlk7_305	166 140	5 433	1 363.1	1 795	11 679.81
mlk60	166 140	21.86	5.345	4.75	49.99
mlk280	166 140	13.69	5.001	2.00	38.59
p_1	166 140	–27.16	512.227	–3 295	2 646
MLKPEAK	166 140	24.71	6.408	8.79	71.62
DIMPEAK	166 140	37.42	24.887	8.00	113.00

SD = standard deviation, w0, w1 and w2 = parameters of Wilmink's function, mlk7_305 = milk yield from 7th to 305th day of lactation, mlk60 and mlk280 = milk yield at 60 or 280 days in milk, respectively, p1 = index of persistency, MLKPEAK = production peak in kg of milk, DIMPEAK = production peak in days in milk

Table 2. Used models

Effect	Model I	Model II
Season of calving in months of calving	12 seasons per year (each month is treated as a season)	Season I: December to May Season II: June to November
Year of calving	1995–1999	1995–1999
Days open (definition of levels)	1 42–59 2 60–89 3 90–119 4 >120 5 missing	1 42–59 2 60–89 3 90–119 4 >120 and missing
Age of calving (in days) (definition of levels)	1 660–709 2 710–769 3 770–839 4 840–919 5 920–1 000	1 660–774 2 775–883 3 884–1 000

Table 3. Pearson's correlation coefficients between lactation function parameters, production traits and index of persistency

	MLKPEAK	mlk7_305	w_0	w_1	w_2	p_1
DIMPEAK	–0.20	0.29	0.45	–0.30	**–0.84**	–0.19
MLKPEAK	1	**0.67**	**0.52**	–0.17	0.24	–0.22
mlk7_305		1	**0.80**	–0.15	–0.28	–0.12
w_0			1	–0.71	–0.61	–0.67
w_1				1	**0.57**	**0.99**
w_2					1	**0.45**

w_0, w_1 and w_2 = parameters of Wilmink's function, mlk7_305 = milk yield from 7th to 305th day of lactation, p_1 = index of persistency, MLKPEAK = production peak in kg of milk, DIMPEAK = production peak in days in milk

Table 4. Least-squares estimates for months (season) of calving and year of calving

Level[1]	No.	p_1 (kg)	w_0 (kg/day)	w_1 (kg/day)	w_2 (kg/day)	DIMPEAK (day)	MILK PEAK (kg)	milk7_305 (kg)
Months (Season) of calving								
Model I								
Jan	14 437	–114.59	24.73	–0.043	–8.71	40.60	23.83	5 276
Feb	12 577	–177.89	25.17	–0.045	–9.13	40.43	23.92	5 276
Mar	14 593	–201.30	25.13	–0.046	–8.69	39.84	24.10	5 229
Apr	14 274	–182.34	24.70	–0.045	–6.40	36.14	24.50	5 195
May	13 964	–105.59	23.86	–0.041	–4.57	33.93	24.41	5 137
Jun	12 844	–20.91	23.02	–0.038	–3.32	33.14	24.17	5 081
Jul	13 614	53.88	22.55	–0.034	–3.10	33.65	23.93	5 090
Aug	14 255	109.26	22.38	–0.032	–2.90	33.78	23.90	5 150
Sep	12 755	117.29	22.88	–0.032	–3.85	35.51	24.13	5 291
Oct	14 086	80.68	23.86	–0.034	–5.63	38.01	24.36	5 470
Nov	14 933	5.05	24.81	–0.038	–7.60	40.13	24.52	5 558
Dec	13 808	–63.48	25.42	–0.041	–8.81	41.43	24.87	5 579
Model II								
I	83 653	–185.10	25.21	–0.046	–8.15	38.80	24.42	5 298
II	82 487	12.75	23.64	–0.037	–4.92	35.89	24.30	5 290
Year of calving								
Model I								
1995	25 606	0.30	21.95	–0.037	–3.98	34.19	22.76	4 785
1996	29 531	–43.53	22.66	–0.039	–4.78	34.82	23.14	4 894
1997	33 898	8.14	23.04	–0.037	–4.89	35.74	23.52	5 101
1998	38 586	–75.30	25.50	–0.041	–7.41	39.27	25.26	5 615
1999	38 519	–97.93	27.06	–0.042	–9.22	42.05	26.41	5 993
Model I								
1995	25 606	–40.10	22.38	–0.039	–4.41	34.29	22.99	4 826
1996	29 531	–87.24	23.06	–0.041	–5.30	35.03	23.28	4 914
1997	33 898	–36.26	23.44	–0.039	–5.41	35.92	23.68	5 123
1998	38 586	–119.46	25.91	–0.043	–7.94	39.48	25.41	5 638
1999	38 519	–147.81	27.34	–0.044	–9.61	41.99	26.45	5 971

No. = number of records, w_0, w_1 and w_2 = parameters of Wilmink's function, mlk7_305 = milk yield from 7th to 305th day of lactation, p_1 = index of persistency, MLKPEAK = production peak in kg of milk, DIMPEAK = production peak in days in milk

[1]see Table 2

For month at calving (Table 4), the lowest persistency occurred for cows calved during February to April. The best persistency was found for calving in August and September, whereas in the remaining periods (from October to January and from May to July) the persistency changed gradually.

Table 5. Least-squares estimates for days open and age at calving

Level[1]	No.	p_1 (kg)	w_0 (kg/day)	w_1 (kg/day	w_2 (kg/day)	DIMPEAK (day)	MILK PEAK (kg)	milk7_305 (kg)
Days open								
Model I								
1	17 745	–229.51	24.71	–0.047	–7.85	37.43	23.91	5 071
2	36 115	–145.43	24.64	–0.044	–7.63	38.27	24.09	5 217
3	28 030	–37.15	24.31	–0.039	–6.34	37.75	24.37	5 358
4	65 469	107.43	23.84	–0.033	–4.23	36.29	24.93	5 548
5	18 781	96.35	22.72	–0.033	–4.25	36.35	23.80	5 194
Model II								
1	17 745	–236.81	24.81	–0.048	–7.85	37.32	24.00	5 085
2	36 115	–154.73	24.75	–0.044	–7.64	38.15	24.18	5 230
3	28 030	–47.38	24.44	–0.039	–6.38	37.68	24.47	5 376
4	84 250	94.24	23.71	–0.033	–4.27	36.22	24.78	5 486
Age at calving								
Model I								
1	4 921	14.25	23.61	–0.037	–6.30	38.45	23.75	5 249
2	31 642	–38.23	24.41	–0.039	–6.98	38.96	24.30	5 371
3	57 681	–58.31	24.33	–0.040	–6.40	37.63	24.38	5 323
4	47 426	–62.54	24.06	–0.040	–5.68	36.19	24.31	5 252
5	24 470	–63.48	23.81	–0.040	–4.93	34.84	24.35	5 193
Model II								
1	40 611	–67.69	24.59	–0.040	–7.33	39.07	24.30	5 358
2	83 195	–95.18	24.53	–0.041	–6.65	37.42	24.40	5 305
3	42 334	–95.65	24.16	–0.041	–5.62	35.54	24.39	5 219

w_0, w_1 and w_2 = parameters of Wilmink's function, mlk7_305 = milk yield from 7th to 305th day of lactation, p_1 = index of persistency, MLKPEAK = production peak in kg of milk, DIMPEAK = production peak in days in milk
[1]see Table 2

Persistency decreased from October to January and went up from May to July. The slope of lactation curve (w_2) to production peak was steeper for winter calving and for calving at the beginning of spring. Similarly the parameter w_0 connected with level of production was higher for the lactations that started during the winter and spring and lower for those that started in the summer and autumn. The lactation yield was lowest for the cows calved in June and July and highest for those that calved from October to December. Summer calving was connected with an earlier attainment of production peak.

Two seasons of calving (Table 4) were created according the results from the Model I analysis with emphasis on the parameter w_1. The seasons differed most of all in the persistency of lactation (p_1) and in the shape of lactation curve (w_1, w_2), while for the

parameters describing the production level (w_0, milk7_305, MLKPEAK) the differences between seasons were low.

The analysis for year of calving (see Table 4) showed that there were changes in the population of Holstein cattle during the years 1995 to 1999. It was shown that with increasing production level (w_0, MLKPEAK, milk7_305) the lactation curve went down more rapidly (p_1, w_1). The production peak was attained later in lactation.

The days open influenced mainly the parameters describing the shape of lactation curve (p_1, w_1, w_2) (Table 5). Persistency improved with the number of days open. At the same time, the cows with the longer days open period had a higher lactation yield. The best persistency was suggested for levels 4 and 5 (days open longer than 120 days or missing, respectively). Lactation yield in level 5 that involved cows with missing information on days open was lower than in level 4. Similar results were found for the parameter w_0 and production peak in kg of milk. The number of days in milk at production peak declined from level 2 to level 5. The differences due to days open were lower for the parameters connected with milk production that for those describing the shape of lactation curve.

Because of similarity in persistency, levels 4 and 5 for days open were joined into one level. The analysis by Model I showed that the estimates for all parameters were on the average (Table 5). It was most evident for the lactation yield.

The level for the lowest age at calving (Table 5) appeared as the most distinguishing. It showed the best persistency (p_1, w_1) and a trend to a low lactation yield. A slowly growing slope (w_2) to production peak was found for cows with the highest age at calving. The lactation yield was lowest for level 5.

In Model II, the levels for age at calving (Table 5) were created according to quantiles 25%, 50% and 75%. It was found that the youngest cows had the best persistency (p_1, w_1) but a steeper lactation curve (w_2) at the beginning of lactation.

DISCUSSION

Positive correlations between parameters p_1 and w_1 confirm the validity of the description of persistency by parameter p_1 (Schaeffer *et al.*, 1997). Because of the high negative correlation between parameter w_2 and days in milk at production peak, the earlier the production peak, the slower the increase in production towards peak.

The same trends of persistency caused by months of calving were found by Tekerli *et al.* (2000). Strandberg and Lundberg (1991) presented a similar development of milk production between months of calving and in days in milk at the production peak. The maximum production of milk for cows that calved from December to March and the lowest production for those that calved from June to September were reported by Kučera *et al.* (1999).

The results found for the influence of persistency correspond with the analysis of Strandberg and Lundberg (1991). They showed a decrease in milk production from the 160th day of gestation period. In our analysis the pregnancy appears to influence milk production earlier in comparison with the results published by Louca and Legates (1968), Olds *et al.* (1979), Danell (1982). Differences in persistency were also observed for the group of cows that conceived from 90th to 119th day of lactation.

In test-day models, different strategies are proposed for the effect of days open (pregnancy). In the Canadian estimation procedure, this effect is completely ignored (Schaeffer *et al.*, 2000) while in Finland the variable "days carried calf" is included in the model (Lidauer *et al.*, 2000). Emmerling *et al.* (2000) did not recommend an inclusion of days open classes in the model because of positive correlation between days open and yield variables. It seems to be the same case in the present analysis because the level of days open influenced the parameters describing the production level (w_0, milk7_305, MLKPEAK). Instead of days open classes they proposed to integrate the model by a third order polynomial of "days carried calf".

The influence of age at calving on the shape of lactation curve was lower than the effects of month of calving and the other analysed effects. But the calving age groups for fixed regression are regular components of test-day models (Swalve, 2000). The different lactation curves for the subclasses according to age at calving were presented by Jamrozik *et al.* (1997). Wilmink (1987) showed the effect of age at calving on test-day milk yield. The explanation of less significant effects of age at calving found in this study could be relative small differences in calving age between the cows.

CONCLUSIONS

The shape of the lactation curve of Holstein cattle is influenced by all analysed factors, i.e. season and year of calving, days open and age at calving. The assignment of month of calving into two seasons of calving seems to be a poor solution because there are differences between months within the season. It could be possible to create four seasons with low, increasing, high and decreasing persistency. Involving days open classes into the test-day model seems to be doubtful, therefore further research in this area is proposed.

Acknowledgement

The authors gratefully acknowledge Dr. Jochen Wolf for helpful discussion.

REFERENCES

Danell B. (1982): Studies on lactation yield and individual test-day yields of Swedish dairy cows. I. Environmental influence and development of adjustment factors. Acta Agric. Scand., *32*, 65–81.

Emmerling R., Dempfle L., Götz K.-U. (2000): Validation of fixed effects in a test day model. In: Proc. 51rd Annual Meeting of the European Association for Animal Production, Den Haag, The Netherlands, August 21–24.

Jamrozik J., Schaeffer L.R., Deckers J.C.M. (1997): Genetic evaluation of dairy cattle using test yields and random regression model. J. Dairy Sci., *80*, 1217–1226.

Kučera J., Hyánek J., Mikšík J., Čermák V. (1999): The influence of the season of parturition on milk performance in the Czech Pied cattle (in Czech). Czech J. Anim. Sci., *44*, 343–350.

Lidauer M., Mäntisaari E., Strandèn I., Pösö J. (2000): Multiple-trait random regression test-day model for all lactations. In: Proc. Interbull Meeting, Vienna, Austria, Int. Bull Evaluation Service, Bulletin No.16, 75–79.

Louca A., Legates J.E. (1968): Production losses in dairy cattle due to days open. J. Dairy Sci., *51*, 576–583.

Olds D., Cooper T., Thriff F.A. (1979): Relationship between milk yield and fertility in dairy cattle. J. Dairy Sci., *62*, 1140–1144.

Ptak E., Schaeffer L.R. (1993): Use of test day yields for genetic evaluation of dairy sires and cows. Livest. Prod. Sci., *34*, 23–34.

SAS (1988): SAS-STAT. User's Guide, Release 6. 03 Ed. SAS Institute, Inc., Carry, NC. 1028 pp.

Schaeffer L.R., Deckers J.C.M. (1998): Random regressions in animal models for test-day production in dairy cattle. In: Proc. 5th World Cong. Genet. Appl Livest. Prod., Guelph, ON, Canada XVIII: 443.

Schaeffer L.R., Jamrozik J., Lazenby D. (1997): Canadian MTP Procedure for Calculating Lactation Totals. Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario,

Schaeffer L.R., Jamrozik J., Kistemaker G., Van Doormaal B. (2000): Experience with a test-day model. J. Dairy Sci., *83*, 1135–1144.

Strandberg E., Lundberg C. (1991): A note on the estimation of environmental effects on lactation curves. Anim. Prod., *53*, 399–402.

Swalve H.H. (2000): Theoretical basis and computational methods for different test-day genetic evaluation methods. J. Dairy Sci., *83*, 1115–1124.

Tekerli M., Akinci Z., Dogan I., Akcan A. (2000): Factors affecting the shape of lactation curves of Holstein cows from the Balikesir province of Turkey. J. Dairy Sci., *83*, 1381–1386.

Wilmink J.B.M. (1987): Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. Livest. Prod. Sci., *16*, 335–348.

Received: 03–03–24
Accepted after corrections: 03–10–01

ABSTRAKT

Faktory ovlivňující průběh laktační křivky u holštýnských krav v České republice

Byly analyzovány vlivy (období otelení, rok otelení, service perioda, věk při otelení) na parametry laktační křivky u prvních laktací (166 140) holštýnského skotu v České republice. K popisu průběhu laktační křivky byla použita

Wilminkova funkce. Persistence byla popsána jako rozdíl od průměrné užitkovosti mezi 60. a 280. dnem laktace. Bylo analyzováno maximum užitkovosti a počet dnů laktace při dosažení tohoto maxima. Tvar křivky byl ovlivněn především rokem otelení, měsícem otelení a service periodou. Závěrem bylo navrženo vytvoření skupin rozdílných ve tvaru laktační křivky pro pevnou regresi v test-day modelu.

Klíčová slova: laktační křivka; persistence; vlivy prostředí; dojený skot

Corresponding Author

Ing. Ludmila Dědková, CSc., Výzkumný ústav živočišné výroby, Přátelství 815, P.O.Box 1,
104 01 Praha 10-Uhříněves, Česká republika
Tel. +420 267 009 574, fax +420 267 710 779, e-mail: dedkova@vuzv.cz

Metabolic and hormonal profiles of potentiated cold stress in lambs during early postnatal period

J. Doubek[1], S. Šlosárková[1], P. Fleischer[2], G. Malá[3], M. Skřivánek[1]

[1]Department of Physiology, [2]Clinic of Diseases of Ruminants, Veterinary and Pharmaceutical University, Brno, Czech Republic

[3]Research Institute of Animal Production, Prague-Uhříněves, Czech Republic

ABSTRACT: Metabolic and hormonal blood profiles under the influence of cold stress potentiated by a 20-minute cold water shower were studied in 2- to 3-day old lambs of Merinolandschaf (Group I, $n = 8$) and Romney Marsh (Group II, $n = 11$) breeds. The metabolic profile included parameters such as total proteins (TP), urea, glucose, nonesterified fatty acids (NEFA) and triacylglycerols (TGL). The concentrations of NEFA and TGL were found to increase significantly ($P < 0.05$) in lambs of Merinolandschaf breed following the potentiated cold stress. Comparing the breeds subjected to the potentiated cold stress, there were no significant differences in the metabolic profile. The hormonal profile was evaluated using cortisol, triiodothyronine (T_3) and thyroxine (T_4). A significant increase ($P < 0.05$) in both cortisol and T_3, T_4 levels was observed in lambs of both breeds following the potentiated cold stress. Comparing the breeds subjected to the potentiated cold stress, the level of cortisol was significantly higher in the Romney Marsh breed ($P < 0.05$), while, on the other hand, the levels of T_3 and T_4 were significantly higher ($P < 0.05$) in lambs of Merinolandschaf breed. The stronger adrenocortical response of Romney Marsh breed was probably due to higher sensitivity of lambs to emotional components of stress evidenced by more intensive changes of behaviour. The weaker thyroidal response of lambs of Romney Marsh breed as compared to the Merinolandschaf ones could also be associated with a higher level of physical thermoregulation (i.e. better thermo-isolation qualities of their fleece). The activation of the thyrotropic axis together with shivering thermogenesis resulted in a significant increase in the rectal temperature in lambs of both breeds immediately after the potentiation of cold stress.

Keywords: lamb; cold stress; total proteins; urea; glucose; NEFA; triacylglycerols; cortisol; triiodothyronine; thyroxine

The economic efficiency of sheep raising in the Czech Republic is based on the sufficient production of lambs for slaughter. Specifically, it means that it is necessary to obtain 1.6 to 1.7 lambs per ewe and lambing period and to decrease losses considerably during rearing. The most serious losses occur within 5 days of birth. This fact is promoted by the so-called Anglo-Saxon system of sheep raising with lambing in spring months (i.e. March to May). Losses are significantly due to the exposure to meteorotropic factors such as low temperatures in particular. Sensory perception of cold is that of a physical somatic stressor. Some authors, e.g. Moberg (1987), argue about the non-specificity of the stress reaction as presented by Selye (1936, 1946, 1981). The response of organisms to stressful stimuli is promoted by the neuroendocrine system – hypothalamic hormones and structures forming the sympathoadrenal system (Chrousos and Gold, 1992). Somatotropic, thyrotropic, gonadotropic and other functions may be activated in this way. Therefore the multidimensional character of stress reactions and their development on different levels in the organism is more and more often accentuated (Weybrew, 1992). Species-specific behavioural reactions may also influence the resulting reaction

Supported by the Ministry of Agriculture of the Czech Republic (Grant No. QD 1236).

of the organism (Wingfield and Ramenofsky, 1999). It is understandable that in practice various physiological phenomena are less considered than the stressor-mediated depression of yield as well as any stress diseases (Marschang, 1989).

The cold stress-induced hypothermia results in hypoxia, hypoglycaemia, metabolic acidosis and alterations in the metabolism of water and electrolytes. As the energy intake is used mainly for thermoregulation, it is possible to observe a depression of yield as well as an increase in mortality, in particular due to the development of the respiratory distress syndrome. It is understandable that there are breed differences in resistance to cold (Slee *et al.*, 1980). Stable heat balance results in the equilibrium of production and output of heat. It is controlled by the hypothalamus processing signals from the periphery and central heat receptors in order to activate effector functions of thermoregulation (Schmidt and Chan, 1992).

There are some peculiarities of heat management in neonates and individuals in the early postnatal period such as immature CNS, higher value of the ratio of body surface to body weight as compared to adults, inadequate responses to cold, etc. That is why there is a specific mechanism of thermogenesis in young animals. Subjecting such an organism to cold results in an increase in the activity of the sympathoadrenal system (SAS) as well as in the metabolic rate. It is due in particular to non-shivering thermogenesis in the brown fatty tissue (lipolytic thermogenesis). Apart from catecholamines (adrenaline and noradrenalin), thyroidal hormones (triiodothyronine and thyroxin) play also a role in non-shivering thermogenesis.

Upon a considerable drop in the surrounding temperature, lipolytic thermoregulation in the newborn is not sufficient and therefore shivering thermogenesis is initiated. However, the mechanism of shivering thermogenesis in neonates during the early postnatal period is not so developed as in adults. Shivering thermogenesis (which is less advantageous because of higher oxygen consumption) begins as a consequence of excitation of the motor centre of shivering in the hypothalamus by peripheral stimuli. Impulses from the centre then stimulate motor neurons which, at first, increase the muscle tone (muscle rigidity) and later on in dependence on the synchronisation of discharges activate individual muscle units and result in muscle shivering (Symonds *et al.*, 1992; Ganong, 1993; Mayes, 2000). The output of heat is due to physical processes (radiation, conduction, convection and evaporation) and forms a complex of physical thermoregulation.

The aim of this study was to evaluate the metabolic and hormonal response of lambs of Merinolandschaf and Romney Marsh breeds to a potentiated cold stress during the early postnatal period and to assess their resistance to cold which in practice is an important factor limiting the losses of lambs.

MATERIAL AND METHODS

A total of 8 lambs of Merinolandschaf breed (5 ram and 3 ewe lambs; 1 male and 1 female single born) with the average birth weight of 4.88 ± 0.85 kg were included in Group I of the experiment. Group II comprised a total of 11 lambs of Romney Marsh breed (5 ram and 6 ewe lambs; 1 male and 1 female single born) with the average birth weight of 4.18 ± 0.65 kg. In both groups the lambs were kept from birth together with their mothers in air-conditioned stables on deep litter. During the experiment the lambs were fed only mother's milk. The ewes of Merinolandschaf breed were provided with hay *ad libitum* and grain in doses gradually increasing to amount to 0.5 kg on the day of lambing. The ewes of Romney Marsh breed also received hay *ad libitum,* maize silage in the dose of 2.5 kg per individual and day and grain in doses gradually increasing to amount to 0.5 kg on the day of lambing.

The air-conditioning equipment maintained stable microclimatic conditions throughout the day. Microclimatic conditions in Group I and II could be characterised by the following values: temperature 2.9 ± 0.5°C and 2.5 ± 0.5°C, relative air humidity 60.1 ± 1.7% and 59.9 ± 6.9%, respectively. Such values were selected because they corresponded to the conditions which lambs are born into during the spring lambing period and which they have to survive.

The animals were examined clinically prior to and three weeks after the experiment. The examination was aimed at the general condition including behaviour, skin, lymph nodes, mucous membranes, respiratory, gastrointestinal, locomotor and nervous systems. Only clinically healthy and vital lambs were included in the experiment. The behaviour of lambs was monitored by cameras during the cold stress.

Experimental protocol

On day 2 or 3 after birth the lambs were individually subjected to a shower of cold water of 5.7 ± 0.7°C of temperature which potentiated the cold stress. Lambs were showered from a distance of 30 cm using the flow of 1.5 l/min through the nozzle. The air temperature, relative air humidity and air flow in the stable amounted to 3.0 ± 2.0°C, 68.5 ± 5.3% and 0.14 ± 0.11 m/s, respectively. Rectal temperatures were taken prior to and after showering.

Blood was collected from lambs one hour prior to and after cooling by the shower using the puncture of *v. jugularis*. The animals were handled at the same daytime in order to eliminate differences in the levels of hormones, i.e. in particular cortisol, due to circadian variations. The collected blood was used to obtain plasma and serum samples. Samples of plasma were used to determine the levels of glucose, while sera were employed to examine the concentrations of total proteins (TP), urea, nonesterified fatty acids (NEFA), triacylglycerols (TGL) and hormones such as cortisol, triiodothyronine (T_3) and thyroxin (T_4). Analyses were performed using the automatic analyser Cobas Mira employing specific assays: TP (Total protein, Biuret, Cat. No. 12751, Bio Vendor), urea (Urea UV KIN 4x50, Cat. No. 1307017, Lachema), glucose (Glucosa, Cat. No. 11601, Randox), NEFA (NEFA, Cat. No. FA 115, Randox), TGL (TGL 4×100, Cat. No. 312983, Lachema). The levels of cortisol (LKCO1, Bio Vendor), triiodothyronine (LKT35T_3, Bio Vendor) and thyroxin (LKT45TT_4, Bio Vendor) were determined by immunofluorescence using the Immulite apparatus.

The results were statistically evaluated using paired Student's *t*-test (Microsoft® Excel® 7.0). Following characteristics are presented: arithmetic mean ($\bar{x}$), standard deviation (SD) and *t*-value. The sign* means $P < 0.05$ hereinafter in the text.

The study was performed in accordance with Act No. 246/1992 on the Protection of Animals against Cruelties.

RESULTS

Rectal temperatures at the end of showering significantly increased from 39.65 ± 0.30°C

Table 1. Concentrations of selected metabolic parameters prior to and after the potentiation of cold stress in lambs of Merinolandschaf breed (Group I)

Group I (n = 8)	TP (g/l)		Urea (mmol/l)		Glucose (mmol/l)		NEFA (mmol/l)		TGL (mmol/l)	
	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.
$\bar{x}$	66.7	61.3	6.36	6.56	7.26*	7.72*	0.58*	0.66*	0.48*	0.96*
SD	14.3	15.1	2.63	2.85	0.72	1.11	0.16	0.14	0.36	0.48

TP = total proteins, TGL = triacylglycerols, NEFA = nonesterified fatty acids, potnt. = stress potentiation
*$P < 0.05$

Table 2. Concentrations of selected metabolic parameters prior to and after the potentiation of cold stress in lambs of Romney Marsh breed (Group II)

Group II (n = 11)	TP (g/l)		Urea (mmol/l)		Glucose (mmol/l)		NEFA (mmol/l)		TGL (mmol/l)	
	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.
$\bar{x}$	64.4	63.0	9.48	9.14	6.27	6.65	0.73	0.87	1.30	1.31
SD	11.1	10.5	3.23	3.03	0.78	1.12	0.20	0.32	0.65	0.63

TP = total proteins, TGL = triacylglycerols, NEFA = nonesterified fatty acids, potnt. = stress potentiation

to 40.28 ± 0.23°C and from 39.56 ± 0.24°C to 40.41 ± 0.14°C in the Merinolandschaf and Romney Marsh breeds, respectively. Comparing the breeds after the potentiation of cold stress by showering, rectal temperatures in the lambs of Romney Marsh bred were higher, but the difference was not significant. The lambs of Romney Marsh breed showed stronger signs of fear (frightened expression, intensive body activities and searching for the mother, etc.). Muscle rigidity, shrinking with cold and then muscle shivering were observed in lambs of both breeds. During the whole period of study, i.e. within three weeks of life, clinical examinations did not reveal any changes in the physiological state of health in the lambs. The results of concentrations of selected metabolic parameters and hormones are summarised in Tables 1 to 6.

Studying the effects of potentiated cold stress on the metabolic rate of nitrogen, carbohydrates and lipids as expressed by concentrations of TP, urea, glucose, TGL and NEFA we found considerably higher levels of glucose, TGL and NEFA in the lambs of Merinolandschaf breed (Table 1), while in Romney Marsh breed the differences were not

Table 3. Concentrations of selected metabolic parameters after the potentiation of cold stress in lambs of Merinolandschaf and Romney Marsh breeds (Group I and II)

Group		TP (g/l)	Urea (mmol/l)	Glucose (mmol/l)	NEFA (mmol/l)	TGL (mmol/l)
				after potnt.		
I (n = 8)	$\bar{x}$	61.3	6.56	7.72	0.66	0.96
	SD	15.1	2.85	1.11	0.14	0.48
II (n = 11)	$\bar{x}$	63.0	9.14	6.65	0.87	1.31
	SD	10.5	3.03	1.12	0.32	0.63

TP = total proteins, TGL = triacylglycerols, NEFA = nonesterified fatty acids, potnt. = stress potentiation

Table 4. Concentrations of selected hormones prior to and after the potentiation of cold stress in lambs of Merinolandschaf breed (Group I)

Group I	Cortisol (nmol/l)		T_3 (nmol/l)		T_4 (nmol/l)	
(n = 8)	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.
$\bar{x}$	38.3*	114.9*	7.6*	8.7*	147.1*	174.5*
SD	14.5	24.6	1.3	0.5	24.0	26.5

potnt. = stress potentiation
*P < 0.05

Table 5. Concentrations of selected hormones prior to and after the potentiation of cold stress in lambs of Romney Marsh breed (Group II)

Group II	Cortisol (nmol/l)		T_3 (nmol/l)		T_4 (nmol/l)	
(n = 11)	prior to potnt.	after potnt.	prior to potnt.	after potnt.	prior to potnt.	after potnt.
$\bar{x}$	104.0*	175.1*	5.5*	5.5*	128.0*	144.8*
SD	37.4	50.4	1.1	0.5	25.5	31.9

potnt. = stress potentiation
*P < 0.05

Table 6. Concentrations of selected hormones after the potentiation of cold stress in lambs of Merinolandschaf and Romney Marsh breeds (Group I and II)

Group		Cortisol (nmol/l)	T_3 (nmol/l)	T_4 (nmol/l)
		after potnt.		
I (n = 8)	$\bar{x}$	114.9*	8.7*	174.5*
	SD	24.6	0.5	26.5
II (n = 11)	$\bar{x}$	175.1*	5.9*	144.8*
	SD	50.4	0.9	31.9

potnt. = stress potentiation
*$P < 0.05$

statistically significant (Table 2). Comparing the breeds subjected to the potentiated cold stress, no significant differences were found out in the abovementioned parameters (Table 3).

Evaluating the endocrine response to the effect of the potentiated cold stress, a significant increase ($P < 0.05$) in cortisol, triiodothyronine (T_3) and thyroxin (T_4) levels was observed in lambs of both breeds (Tables 4 and 5). Comparing the breeds subjected to the potentiated cold stress, the level of cortisol was significantly higher in Romney Marsh breed ($P < 0.05$), while, on the other hand, the levels of thyroidal hormones were significantly higher ($P < 0.05$) in the lambs of Merinolandschaf breed (Table 6).

DISCUSSION

Thermoregulation in the early postnatal period is imperfect. Various mechanisms aimed at maintaining the body core temperature participate in the reaction of the organism following the exposure of the neonate to cold stress during the early postnatal period (i.e. when the temperature drops below the so-called thermoneutral zone).

Resistance to cold can be evaluated by measurements of rectal temperature, surface temperature, oxygen consumption and characteristics of hair coat (Forman *et al.*, 1986; Muller and McCutcheon, 1991; Symonds *et al.*, 1992; Knížková *et al.*, 2002). The response of the organism to cold has an acute and an adaptive form (Young *et al.*, 1989). We were engaged in the evaluation of the acute response of the organism to cold. As the animal is getting cold, the blood flow through the brown fatty tissue increases together with the temperature of this tissue. Therefore it would be possible to evaluate the response of the organism to cold by measuring the temperature in the areas of large fatty tissue bodies. The temperature of these areas may even be higher than in the rectum. However, measurements of surface temperature in the areas of large fatty tissue bodies in hair-covered animals are of no informative value. In clinical practice the rectal temperature is considered to be the central one, even though not representing exactly the temperature of the body core, because it is somewhat lower (Robinson, 2002). The determined values of rectal temperatures were proved to significantly increase after the potentiation of cold stress in lambs of both breeds; comparisons of breeds, however, resulted in insignificant differences. The increase in rectal temperatures, however, did not exceed the reference values. Greater behavioural changes after the potentiation of cold stress were found in the lambs of Romney Marsh breed. It is the breed having rougher fleece poorly absorbing water. The Romney Marsh breed, contrary to the Merinolandschaf breed, is designed to be kept on pasture enclosures without housing or even permanent presence of tenders all the year round. The fact of putting the animals of this breed into a restricted stable area and permanent contact with tenders might be responsible for the association of stressors. The higher sensitivity of lambs of Romney Marsh breed could therefore be due to this stress association. The increased locomotion of these lambs could be considered as a sign of highly efficient behavioural changes in order to increase heat production. The difference in the body weight at birth in lambs of Romney Marsh (4.18 ± 0.65) and Merinolandschaf (4.88 ± 0.85)

breeds was not significant (t = 0.0711) enough to play a role in the differences of heat production. In contrast with Knížková *et al.* (2002) we did not find any differences in the intensity and duration of thermogenesis by shivering. The above-mentioned authors, however, evaluated only a 5-minute cold stress potentiation. We took great care to differentiate the signs of shivering thermogenesis (increased muscle tone, muscle tremor) from mere behavioural changes (fear). The question to what extent the increase in temperature could be due to the cold-induced redistribution of blood will be the subject of further studies.

The parameter of total proteins (TP) was selected as a marker of nitrogen metabolism that during a short period of cold stress can show signs of malnutrition due to behavioural causes such as impaired colostrum intake. Thus starvation in association with another stressor could have an additive effect. The intake of colostrum was not impaired in our case of a short-time exposure to cold. There was a drop in the concentration of TP following the potentiation of cold stress in both breeds; however, the difference was insignificant. Comparisons of breeds did not result in any significant differences either (Tables 1 to 3). There were not enough reasons to examine other proteins for example of the electrophoretic type in our study. The concentration of urea, a final metabolite of nitrogen from proteins and amino acids, respectively, may be higher under stress due to the increased production of glucocorticoids enhancing its synthesis. Although a significant increase in the concentration of cortisol following the potentiation of cold stress was confirmed (see below), there were no significant differences in the concentration of urea (Tables 1 to 3). Even though the ureasynthetic action of glucocorticoids takes a longer time to produce an effect, we can find some associations between the high levels of cortisol and urea in lambs of Group II, both prior to and after the potentiation of cold stress. Glucose is an important marker of carbohydrate metabolism. We can expect changes in the glucose level during stress. In lambs of both breeds we observed increases in the glucose level following cold stress and a significant increase (Table 1) in Group I (Merinolandschaf). A great many authors (e.g. Baum *et al.*, 1968; Porte, 1969) put the effect of hyperglycaemia during hypothermia in association with a drop of insulin activity and a glycogenolytic effect of catecholamines. Thyroidal hormones activated during stress were shown to have anti-insular effects. It is clear that the increase in the concentration of glucose in our study could be caused by a gluconeogenetic effect of cortisol, i.e. by the activation of the hypothalamic-pituitary-adrenocortical axis (Steffens and de Boer, 1999). The increase in the glucose level corresponds with a short duration of the stressor action. On the other hand, in chronic stress cases we can expect utilisation of glucose, depletion of glycogen reserves and hypoglycaemia. Comparing both breeds following the potentiation of cold stress we found no significant differences in the concentration of glucose (Table 3). In both groups under study, however, the concentrations of TP, urea and glucose did not fall outside the range of reference values for Merino lambs (Bickhardt *et al.*, 1999). The reference values of Romney Marsh breed are not available. Concentrations of NEFA and glycerol, respectively, can be used as a biochemical marker of heat production (Aulie *et al.*, 1971). Concentrations of NEFA following the potentiation of cold stress were higher; however, of significant increase only in the Merinolandschaf breed (Tables 1 to 3).

Increased metabolic rates in the brown fatty tissue can be considered as a source of higher concentrations of NEFA. This metabolism is influenced by thyroidal hormones and catecholamines. Thyroxin directly acts upon the metabolism of the brown fatty tissue or enhances the action of noradrenalin. Catecholamines act through α_1-adrenoreceptors upon 5-deiodinase in a positive way, which transforms T_4 to T_3 in the cells of the brown fatty tissue, and increases the activity of lipase splitting triacylglycerols to glycerol and NEFA through the action of β_3-adrenoreceptors and cAMP. Moreover, poorer utilisation during stress may also participate in the process. Glycerol is metabolised in the liver, fatty acids are metabolised in the brown fatty tissue subjected to β-oxidation and the acetyl-CoA enters the citrate cycle. The energy, released during the transport of electrons from their high potential in fatty acids and temporarily stored in the form of a proton electrochemical gradient, gets free due to the specific membrane protein thermogenin as heat. This process is stimulated by triiodothyronine (Mayes, 2000). It is clear from the concentrations of TGL that there is a higher intensity of lipolytic thermogenesis in the lambs of Merinolandschaf breed. While the increase in the lambs of Romney Marsh breed was negligible, in the Merinolandschaf breed it was significant (Table 1 to 3).

SAS and other functions get activated during stress (Chrousos and Gold, 1992). In our study, direct evaluations of the activity of SAS, i.e. on the basis of measurements of the levels of catecholamines and their metabolites, respectively, or the density of adrenergic receptors in tissues, were not performed due to the demanding character of analyses. Therefore we proceeded from the fact that SAS is in a close functional relation with the adrenal cortex (Tilders *et al.*, 1982). It is known that these main axes interact in the maintenance of homeostasis of the organism exposed to the action of various stressors (Axelrod and Reisine, 1984). The stress reaction of the adrenal cortex is based on the stimulation of secretion of the corticotropin releasing hormone (CRH) with a subsequent response in the form of the adrenocorticotropin release (ACTH) (Hodges and Mitchley, 1970; Rivier and Plotsky, 1986). Glucocorticoids, in particular, and to a lesser extent mineralocorticoids, are released during stress in this way. Cortisol as the main glucocorticoid significantly increased following the 20-minute period of cold stress potentiation in both breeds. Comparing both breeds it is clear that the increase was significantly higher in Romney Marsh breed. It is necessary to mention the fact that the concentration of cortisol opposite to thyroidal hormones was significantly higher in Romney Marsh breed even prior to the potentiation of cold stress. Lambs were subjected to cold stress even prior to showering. In ruminant neonates the higher concentration of cortisol may also be associated with delivery (Hoyer *et al.*, 1989; Stanko *et al.*, 1991). In our case it can be supposed that these facts might be influenced by other behavioural (emotional) factors such as fear, signs of which we noted in Romney Marsh breed in a more intensive form. Fear could enhance the stress reaction. For example, Thornton and Parrott (1989) reported that even blood collection might result in an increase in the cortisol concentration under certain circumstances. As far as thyroidal hormones are concerned, we are not aware of such a fact being reported. The association of a higher adrenocortical reaction and the action of emotional factors could be explained by the functional interrelation of the adrenal cortex and SAS (Tilders *et al.*, 1982). Thyroidal reactions to the action of cold were more pronounced in Merinolandschaf breed. Significantly higher concentrations of TGL and NEFA following the potentiation of cold stress corresponded to the above-mentioned facts. These findings may be in association with the quality of fleece because the cooling effect of water depends on the thermo-isolation characteristics of the hair coat, and it can be supposed that in the animals the output of heat as well as the heat production were influenced. In Romney Marsh breed, in particular, wool is coarser and of lesser absorption capabilities. The output of heat is thus less intensive. Immediately after the potentiation of cold stress the activation of the thyrotropic axis was not sufficient enough to maintain the rectal temperature within the range just prior to the stress. However, together with shivering thermogenesis the activation of the thyrotropic axis was able to maintain rectal temperatures within reference ranges.

The experiment did not result in any deterioration of the health state of lambs as evidenced by clinical examinations throughout a three-week period following the episode of cold stress.

REFERENCES

Aulie A., Astrup H.N., Nedkvitne J.J., Velle W. (1971): Serum non-esterified fatty acids and plasma glycerol as indicators of fat mobilisation in pregnant sheep subjected to cold stress. Acta Vet. Scand., *12*, 496–503.

Axelrod J., Reisine T.D. (1984): Stress hormones: their interaction and regulation. Science, *224*, 452–459.

Baum D., Dillard D.H., Porte D. Jr. (1968): Inhibition of insulin of release in infants undergoing deep hypothermic cardiovascular surgery. New Engl. J. Med., *279*, 1309–1314.

Bickhardt K., Dudziak D., Ganter M., Henze P. (1999): Untersuchungen zur Altersabhängigkeit hämatologischer und blutchemischer Messgrössen bei gesunden Schaflämmern – ein Beitrag zur Definition von Referenzwerten beim Schaf. Dtsch. Tierärztl. Wschr., *106*, 445–451.

Chrousos G.P., Gold P.S. (1992): The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. J. Am. Med. Assoc., *267*, 1244–1252.

Forman D.J., Bhutani V.K., Tran N., Shaffer T.H. (1986): A new approach to induced hypothermia. J. Surg. Res., *40*, 36–42.

Ganong W.F. (1993): Review of Medical Physiology. Appleton and Lange, Los Altos. 774 pp.

Hodges J.R., Mitchley S. (1970): The effect of "training" on the release of corticotrophin in response to minor stressful procedures in the rat. J. Endocrinol., *47*, 253–254.

Hoyer C., Grunert E., Benesi F. (1989): Veränderungen der Glukokortikoidwerte und des weissen Blutbildes bei neugeborenen Kälbern in den ersten 72 Lebensstunden als Ausdruck des Stressgeschehens. In: Tag. Fachgr. Rinderkrankh. Giessen: Dt. Vet.-Med. Geselsch., 79–83.

Knížková I., Kunc P., Mátlová V., Knížek J., Malá G. (2002): Thermal responses of newborn lambs (Romney Marsh, Merinolandschaf) exposed to cold environmental temperatures and rain. In: Proc. Actual Questions of Animal Bioclimatology 2002. Brno, 39–42.

Marschang F. (1989): Faktoren, die Stressoren sind. Tierärztl. Umsch., *44*, 217, 220, 223–224.

Mayes P.A. (2000): Lipid transport and storage. In: Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W. (eds.): Harper's Biochemistry. 25th ed. Appleton and Lange, New York. 168–284.

Moberg G.P. (1987): Problems in defining stress and distress in animals. J. Am. Vet. Med. Assoc., *191*, 1207–1211.

Muller S., McCutcheon S.N. (1991): Comparative aspects of resistance to body cooling in newborn lambs and kids. Anim. Prod., *52*, part 2, 301–301.

Porte D. Jr. (1969): Sympathetic regulation of insulin secretion. Its relation to diabetes mellitus. Arch. Int. Med., *123*, 252–260.

Rivier C.L., Plotsky P.M. (1986): Modification by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. Ann. Rev. Physiol., *48*, 475–494.

Robinson N.E. (2002): Thermoregulation. In: Cunningham J.G. (ed.): Textbook of Veterinary Physiology. 3rd ed. W.B. Saunders, Philadelphia. 533–544.

Schmidt K.D., Chan C.W. (1992): Thermoregulation and fever in normal persons and in those with spinal cord injuries. Mayo Clin. Proc., *67*, 469–475.

Selye H. (1936): A syndrome produced by diverse nocuous agents. Nature, *138*, 32.

Selye H. (1946): The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., *6*, 117–130.

Selye H. (1981): Geschichte und Grundzüge des Stresskonzepts. In: Nitsch J.R. (Hrsg.): Stress, Theorien, Untersuchungen, Massnahmen. Huber, Bern. 163–197.

Slee J., Griffiths R.G., Samson D.E. (1980): Hypothermia in newborn lambs induced by experimental immersion in a water bath and by natural exposure outdoor. Res. Vet. Sci., *28*, 275–280.

Stanko R.L., Guthrie M.J., Randel R.D. (1991): Response to environmental temperatures in Brahman calves during the first compared to the second day after birth. J. Anim. Sci., *69*, 4419–4427.

Steffens A.B., Boer de S.F. (1999): Impact of stress on animal intermediate metabolism. In: Balm P.H.M. (ed.): Stress Physiology in Animals. Sheffield Academic Press, Sheffield. 108–129.

Symonds M.F., Bryant M.J., Clarke L., Darby C.J., Lomax M.A. (1992): Effect of maternal cold-exposure on brown tissue and thermogenesis in the neonatal lamb. J. Physiol.-London, *455*, 487–502.

Thornton S.N., Parrott R.F. (1989): Naloxone affects the release of cortisol, but not of vasopressin or oxytocin, in dehydrated sheep. Acta Endocrinol. (Copenhagen), *120*, 50–54.

Tilders F.J.H., Berkenbosch F., Smelik P.G. (1982): Adrenergic mechanism involved in the control of pituitary-adrenal activity in the rat: a β-adrenergic stimulatory mechanism. Endocrinology, *110*, 114–120.

Weybrew B.B. (1992): The ABC of Stress. Praeger, Westport. 220 pp.

Wingfield J.C., Ramenofsky M. (1999): Hormones and the behavioral ecology of stress. In: Balm P.H.M. (ed.): Stress Physiology in Animals. Sheffield Academic Press, Sheffield. 1–51.

Young B.A., Walker B., Dixon A.E., Walker V.A. (1989): Physiological adaptation to the environment. J. Anim. Sci., *67*, 2426–2432.

Received: 03–03–31
Acepted after corrections: 03–09–30

ABSTRAKT

Metabolický a hormonální profil při potencovaném chladovém stresu u jehňat v časném postnatálním období

Cílem práce bylo zhodnotit úroveň chladové odolnosti u dvou plemen jehňat, která je v podmínkách praxe významným faktorem limitujícím výši ztrát jehňat. U dvou- až třídenních jehňat plemene merinolandschaf (skupina I, n = 8) a romney marsh (skupina II, n = 11) byl sledován vliv chladové zátěže potencované 20minutovou sprchou

chladnou vodou na metabolický a hormonální profil krve. Metabolický profil byl vyjádřen parametry: celková bílkovina (TP), urea, glukóza, neesterifikované mastné kyseliny (NEFA) a triacylglyceroly (TGL). Byl zjištěn signifikantně vyšší vzestup ($P < 0,05$) koncentrací NEFA a TGL u jehňat plemene merinolanschaf po potencované chladové zátěži. Při meziplemenném srovnání po potencované chladové zátěži nebyly zjištěné rozdíly signifikantní. Hormonální profil byl vyjádřen parametry: kortizol, trijotyronin (T_3) a tyroxin (T_4). U jehňat obou plemen došlo po potencované chladové zátěži k signifikantnímu vzestupu ($P < 0,05$) koncentrací jak kortizolu, tak T_3 a T_4. Při meziplemenném srovnání po potencované chladové zátěži byla koncentrace kortizolu u plemene romney marsh signifikantně vyšší ($P < 0,05$), koncentrace T_3 a T_4 byly naopak signifikantně vyšší ($P < 0,05$) u jehňat plemene merinolandschaf. Silnější adrenokortikální odezva u plemene romney marsh byla patrně důsledkem větší senzitivity jehňat na emocionální komponentu stresu, což bylo patrné i na intenzivnějších změnách chování. Menší tyroidální odezva u jehňat plemene romney marsh oproti jehňatům plemene merinolandschaf mohla rovněž souviset s vyšší úrovní fyzikální termoregulace (lepšími tepelně izolačními vlastnostmi jejich rouna). Aktivace tyreotropní osy vedla společně s třesovou termogenezí u jehňat obou plemen k signifikantnímu vzestupu rektální teploty těsně po potenciaci chladového stresu. Meziplemenné rozdíly však nebyly signifikantní.

Klíčová slova: jehně; chladový stress; krevní sérum/plazma; celková bílkovina; urea; glukóza; NEFA; triacylglyceroly; kortizol; trijodtyronin; tyroxin

Corresponding Author

Doc. MVDr. Jaroslav Doubek, CSc., Ústav fyziologie, Fakulta veterinárního lékařství, Veterinární a farmaceutická univerzita Brno, Palackého 1–3, 612 42 Brno, Česká republika
Tel. +420 541 562 301, e-mail: doubekj@vfu.cz

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 120 56 Prague 2, Czech Republic
Tel.: + 420 227 010 111, Fax: + 420 227 010 116, E-mail: redakce@uzpi.cz

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 English with abstracts in Czech.

Journal	Number of issues per year	Yearly subscription in USD
Plant, Soil and Environment	12	285
Czech Journal of Animal Science (Živočišná výroba)	12	285
Agricultural Economics (Zemědělská ekonomika)	12	285
Journal of Forest Science	12	285
Veterinární medicína (Veterinary Medicine – Czech)	12	222
Czech Journal of Food Sciences	6	129
Plant Protection Science	4	85
Czech Journal of Genetics and Plant Breeding	4	85
Horticultural Science (Zahradnictví)	4	85
Research in Agricultural Engineering	4	85

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

Genetic parameters of the traits recorded in the performance test of dual-purpose bulls

J. Bouška[1], M. Štípková[1], J. Frelich[2], J. Zedníková[2], L. Bartoň[1]

[1]Research Institute of Animal Production, Prague-Uhříněves, Czech Republic
[2]University of South Bohemia, České Budějovice, Czech Republic

ABSTRACT: The data on 1 144 performance tested Czech Pied bulls born from 1991 to 1997 were analysed. The test started at 111 days of bulls' age and lasted until the age of 420 days. Simulation analysis was carried out for the age of bulls at the end of the test 210, 300, 330, 365, and 420 days. Genetic parameters were estimated for different test periods and the relationships between the periods were analysed. The heritability coefficients for live weights of young bulls recorded at different age ranged from h^2 = 0.20 to h^2 = 0.46. For average daily gains in different periods according to the simulated termination of the test, the estimated heritability coefficients ranged from h^2 = 0.20 to h^2 = 0.59. The genetic correlations between the test termination at 300 days of age and the termination at 330, 365 or 420 days ranged from r_g = 0.88 to r_g = 0.99 for live weight and from r_g = 0.61 to r_g = 0.91 for average daily gain. These results support the idea to reduce considerably the duration of performance test in young bulls.

Keywords: Czech Pied cattle; bulls; performance test; genetic parameters

The first proof of the quality of Czech Pied bulls is obtained on the basis of the station test of their own performance. Growth ability and body development are analysed and the first selection is based upon the results. A similar approach is used in other European dual-purpose breeds of cattle as well. The results obtained from performance testing are of great practical importance but the system is also rather expensive. The tests performed in various countries differ in their duration and age of bulls at the beginning and end of the test. It is due to the effort to find the balance between genetic benefits of the test for breeding of the particular breed and its economic cost.

Different systems of bull performance testing in Germany were compared by Wassmuth and Alps (1995). The reported duration of tests used for dual-purpose breeds ranged from 200 to 300 days and the tests were terminated at the age of young bulls ranging from 312 to 420 days. The age of bulls entering the test in various countries ranges from 42 to 165 days and the tests are terminated at the age of 225 to 420 days.

The effort to reduce the age of bulls at the end of performance test is substantiated by the requirement to shorten the generation interval as a factor significantly influencing the level of genetic gain in the population. The second reason why to shorten the test is the necessity to reduce its economic cost while the accuracy of the test should be maintained.

Estimates of genetic parameters for growth traits in young bulls of different breeds were reported by Clabbers and Trappmann (1992), Schaefer *et al.* (1998) and Eriksson *et al.* (2000). The estimated heritability for average daily gain ranged from h^2 = 0.30 to h^2 = 0.50. Genetic parameters for growth and feed conversion in performance tested bulls were documented by Jensen *et al.* (1991), Potthast *et al.* (2000) and Jakobsen *et al.* (2000a,b). The

Supported by the Ministry of Agriculture of the Czech Republic (Project M02-99-03) and Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 122200002).

authors reported a very close relationship between daily gain and feed conversion.

Jakobsen *et al.* (2000a,b) analysed the relationships between different traits measured during the performance test of bulls and their daughters' milk yield. They concluded that average daily weight gain in the test is a better predictor for milk yield than the other recorded traits associated with feed intake and conversion. These findings may be regarded as highly significant as they demonstrate a possibility to reduce the number of recorded traits with the average daily gain of bulls in test being the primary selection criterion.

MATERIAL AND METHODS

The results of performance tested Czech Pied bulls born from 1991 to 1997 were analysed. The bulls were tested in the period from 111 to 420 days of age at one test station. Simulation analysis was performed for the age of bulls at the end of the test 210, 300, 330 and 365 days. The following traits were recorded during the test:

- live weights of bulls at 110, 210, 300, 330, 365 and 420 days of age
- average daily gains in different test periods
- average daily gains in the preparatory period, i.e. from birth to 110 days of age and in the following test periods: 111 to 210 days, 111 to 300 days, 111 to 330 days, 111 to 365 days, 111 to 420 days of age

To estimate the genetic parameters, data on 1 144 bulls in total – the sons of 85 Czech Pied sires – were analysed. Statistic programmes SAS v. 6.12 (1996) and Harvey (1987) were used for calculations. The following model was used:

$$Y_{ijk} = \mu + A_{ij} + b_k + e_{ijk}$$

where: μ = overall mean
A_{ij} = fixed effect of year and season of bull's birth
b_k = random effect of sire
e_{ijk} = random error

As the analysed test results originated from one station only, the effect of station was not included in the used model.

To determine the relationship between average daily gain of bulls in the test and milk yield of their daughters, the analysis was carried out of data on 658 bulls positively selected on the basis of their performance test results and used for insemination.

RESULTS AND DISCUSSION

Basic statistic parameters of the analysed traits are given in Table 1. The observed live weights in individual test periods are up to the growth standard of Czech Pied bulls. The bulls reached average live weights 273.71, 392.45, 474.88 and 535.09 kg at 210, 300, 365, and at the end of the test, respectively. When the average daily gains in different test periods were compared, the lowest value (887 g) was found for the period from birth to 110 days of age. The highest growth intensity was observed in the period from 211 to 300 days of age with the average daily gain 1 321 g. After 330 days of age the intensity of growth gradually decreased and in the last test period (from 366 to 420 days of age) the average daily gain of bulls was 1 093 g.

Average daily gains exceeding 1 250 g were observed while evaluating individual model alternatives differing in test duration. The highest growth intensity was recorded for the test duration from 111 to 300 and from 111 to 330 days of age when the average daily gains were 1 300 and 1 299 g, respectively.

Heritability of the particular trait is very important for selection efficiency. The heritability coefficients (h^2) for live weight and average daily gain estimated for different age at the test termination are shown in Table 2 and Table 3. It is quite logical that the longer test duration resulted in higher heritability coefficients. The heritability coefficients for live weight 0.35, 0.36, and 0.46 were calculated for the weights recorded at 330, 365, and 420 days of age, respectively (Table 2).

The estimated heritability coefficients for average daily gain in test, which is the major trait of the bulls' performance test, ranged from 0.20 to 0.59 (Table 3). The coefficients for the tests lasting from 111 to 210 and from 111 to 300 days of age were $h^2 = 0.20$ and $h^2 = 0.38$, respectively. The heritability coefficients $h^2 = 0.46$ and $h^2 = 0.48$ were estimated for the tests terminated at 330 or 365 days of age, respectively. The highest heritability for average daily gain $h^2 = 0.59$ was estimated for the longest period of the test, i.e. from 111 to 420 days. Similar results were also reported by other authors. The heritability coefficients $h^2 = 0.33$ and $h^2 = 0.48$ for average daily gain in test were estimated for

Table 1. Basic statistic parameters of the analysed traits (n = 1 189)

Trait		Mean	Standard deviation
Live weight (kg)	Age of bulls (days)		
	110	145.57	23.02
	210	273.71	32.39
	300	392.48	36.99
	330	431.40	37.54
	365	474.88	39.40
	420	535.09	40.09
Average daily live weight gain (g)	Test period (days of age)		
	birth – 110	887	209
	111–210	1 281	219
	211–300	1 320	183
	301–330	1 297	286
	331–365	1 245	331
	366–420	1 093	283
Average daily live weight gain (g)	Test duration (days of age)		
	111–210	1 281	219
	111–300	1 300	146
	111–330	1 299	132
	111–365	1 292	123
	111–420	1 257	106

Deutsche Schwarzbunte and Deutsche Rotbunte bulls, respectively (Clabbers and Trappman, 1992). Eriksson *et al.* (2000) calculated the coefficients for growth of bulls at test stations in the range from h^2 = 0.30 to h^2 = 0.50. Potthast *et al.* (2000) estimated the heritability coefficient of average daily gain in test (1 302 g) at the level of h^2 = 0.31. The estimated heritability coefficients for average daily gain in dual-purpose bulls h^2 = 0.35 and h^2 = 0.37 were reported for the test periods from 28 days of age to live weight of 200 kg and from 200 to 470 kg live weight, respectively (Jensen *et*

Table 2. Estimates of heritability for live weight of bulls in the test (n = 1 144)

Age (days)	Heritability (h^2)	Standard error (s_{h2})
110	0.20	0.07
210	0.20	0.07
300	0.29	0.09
330	0.35	0.09
365	0.36	0.09
420	0.46	0.10

Table 3. Estimates of heritability for average daily gain in dependence on the test duration (n = 1 144)

Test duration (days of age)	Heritability (h^2)	Standard error (s_{h2})
111–210	0.20	0.07
111–300	0.38	0.09
111–330	0.46	0.10
111–365	0.48	0.10
111–420	0.59	0.11

Table 4. Phenotypic (r_p – above diagonal) and genetic (r_g – below diagonal) correlations between live weights recorded in different periods of the test

Age of bulls (days)	Age of bulls (days)					
	110	210	300	330	365	420
110		0.79	0.68	0.65	0.62	0.59
210	0.75		0.88	0.84	0.79	0.74
300	0.56	0.91		0.97	0.91	0.84
330	0.48	0.89	0.99		0.96	0.89
365	0.42	0.87	0.97	0.99		0.92
420	0.40	0.87	0.88	0.93	0.97	

Table 5. Phenotypic (r_p – above diagonal) and genetic (r_g – below diagonal) correlations between average daily gains recorded for different duration of the test

Test period	Test period (days of age)				
	111–210	111–300	111–330	111–365	111–420
111–210		0.81	0.75	0.66	0.56
111–300	0.76		0.96	0.86	0.75
111–330	0.64	0.91		0.94	0.83
111–365	0.57	0.85	0.96		0.89
111–420	0.45	0.61	0.77	0.91	

al., 1991). The analysis of the bulls' performance test from 42 to 336 days of age was described by Jakobsen *et al.* (2000a,b). The estimated heritability for average daily gain in all the tested dairy breeds in Denmark was $h^2 = 0.43$.

The estimated genetic (r_g) and phenotypic (r_p) correlations are shown in Table 4. The results indicate that there is a close relationship between the live weight recorded as early as at 300 days of age and that recorded at the other intervals (330, 365, and 420 days) with the estimated genetic correlations ranging from $r_g = 0.88$ to $r_g = 0.99$. The relationship between the live weight measured at 330 days and the weights at 365 or 420 days can be characterised by high values of genetic and phenotypic correlations $r_g = 0.93$ to $r_g = 0.99$ and $r_p = 0.89$ to $r_p = 0.96$, respectively.

The estimated genetic correlations between average daily gains in alternatively terminated tests according to the age of bulls are the major factor for the evaluation of a possibility to reduce the duration of performance test used for Czech Pied bulls. The results are given in Table 5. If the test was terminated at 210 days of age, the genetic and phenotypic correlations to the other time intervals ranged from $r_g = 0.45$ to $r_g = 0.76$ and $r_p = 0.56$ to $r_p = 0.81$, respectively. The estimated genetic correlations between the results of the test terminated at 300 days and at the age of 330, 365, and 420 days were $r_g = 0.91$, $r_g = 0.85$, and $r_g = 0.61$, respectively. When the test period was extended to the interval from 111 to 330 days, the estimated genetic correlations to the other two intervals 365 and 420 days were $r_g = 0.96$ and $r_g = 0.77$, respectively, and the phenotypic correlations were $r_p = 0.94$ and $r_p = 0.83$, respectively. The correlations estimated for the relationship between the results obtained from the tests terminated at 365 and 420 days were $r_g = 0.91$ and $r_p = 0.89$. The correlations reported by Jensen *et al.* (1991) for average daily gain in the period up to the live weight 200 kg and from 201 to 470 kg were $r_g = 0.47$ and $r_p = 0.19$, respectively.

The relationship between average daily gain of bulls in the performance test and milk production

Table 6. Genetic correlations (r_g) between average daily gain of bulls in the test and milk production traits of their daughters

Trait	Average daily gain in the test (r_g)
Milk yield (kg)	0.12
Fat yield (kg)	0.44
Protein yield (kg)	0.21

of their daughters can be considered as significant. Genetic correlations between these two traits are shown in Table 6. The estimated correlations between average daily gain and milk, fat and protein yields were r_g = 0.12, r_g = 0.44 and r_g = 0.21, respectively. The genetic correlations reported by Jakobsen *et al.* (2000a,b) between average daily gain of performance tested bulls and milk production in the Danish Red breed were r_g = 0.42, r_g = 0.39 and r_g = 0.44 for milk, fat and protein yields, respectively. For Black and White cattle, these correlations ranged from 0.05 to 0.19.

CONCLUSIONS

The test of bulls' own performance is the fundamental part of selection programme in the Czech Pied breed. Based on our results it can be recommended to reduce the duration of the test to the period from 111 to 300 or 330 days of bulls' age. This reduction will result in a significant decrease in the economic cost of the test and, at the same time, in the shortening of the overall generation interval in the population. The results also confirmed the positive relationship between the basic selection criterion for bulls, which is average daily gain, and milk production of their daughters.

REFERENCES

Clabbers W., Trappmann W. (1992): Zuchtwertschätzung auf Fleischleistung beim Zweinutzrind. Züchtungskunde, *64*, 101–110.

Eriksson S., Näsholm A., Johansson K., Philipsson J. (2000): Genetic analysis of station performance testing and field recording for Swedish beef cattle. In: EAAP – 51st Annual Meeting, The Hague.

Jakobsen J.H., Madsen P., Jenden J., Petersen P.H., Pedersen G.A. (2000a): Genetic correlations between beef traits measured on young performance test bulls and daughter milk yield. Acta Agric. Scand. Sect. A, Anim. Sci., *50*, 39–46.

Jakobsen J.H., Madsen P., Jensen J., Pedersen G.A., Petersen P.H. (2000b): Genetic parameters for average daily gain, area of *m. longissimus dorsi*, feed efficiency and feed intake capacity in young bulls of dairy populations. Acta Agric. Scand. Sect. A, Anim. Sci., *50*, 146–152.

Jensen J., Mao I.L., Bech Andersen B., Madsen P. (1991): Genetic parameters of growth, feed intake, feed conversion and carcass composition of dual-purpose bulls in performance testing. J. Anim. Sci., *69*, 931–939.

Potthast J., Tholen E., Trappmann W. (2000): Untersuchungen zur Integration des Merkmals Futteraufnahmekapazität wachsender Bullen in Besamungszuchtprogramme bei Milchrindern. 1. Mitteilung: Schätzung von genetischen Parametern. Züchtungskunde, *72*, 88–101.

Schafer C.S., Tholen E., Trappmann W. (1998): Development of a breeding programme for beef cattle based on the case of the Fleischrinder – Herdbuch Bonn e. V. 1 st communication: Breeding programme, breeding goal, performance testing, estimation of breeding values. Züchtungskunde, *70*, 157–171.

Wassmuth R., Alps H. (1995): Stand der stationären Fleischlestungsprüfung beim Rind in Deutschland. Züchtungskunde, *67*, 185–205.

Received: 03–08–27
Accepted after corrections: 03–09–30

ABSTRAKT

Odhad genetických parametrů pro ukazatele testu vlastní výkonnosti plemeníků kombinovaného užitkového typu skotu

Byla provedena analýza testu vlastní výkonnosti 1 144 býčků českého strakatého skotu narozených v letech 1991 až 1997. Vlastní test byl uskutečněn od 111. do 420. dne věku plemeníků. Výsledky analýzy byly modelově

vyhodnoceny se simulací ukončení testu ve 210, 300, 330, 365 a 420 dnech věku býčků. Byly odhadnuty genetické parametry pro jednotlivé fáze testu a analyzován vztah mezi hodnocenými časovými úseky. U živé hmotnosti mladých plemeníků byly pro jednotlivé časové horizonty ukončení testu odhadnuty koeficienty dědivosti v rozmezí h^2 = 0,20–0,46. Pro průměrné denní přírůstky odhadnutými v modelově ukončených testech podle věku plemeníků byly odhadnuty koeficienty dědivosti v rozmezí od h^2 = 0,20 do h^2 = 0,59. Genetické korelace při ukončení testu ve 300 dnech věku plemeníka k ukončení ve 330, 365 resp. 420 dnech byly odhadnuty pro živou hmotnost v rozmezí r_g = 0,88–0,99 a u průměrného denního přírůstku na úrovni r_g = 0,61–0,91. Uvedené výsledky dávají možnost výrazně zkrátit délku testu růstové schopnosti mladých plemeníků.

Klíčová slova: český strakatý skot; býci; test vlastní výkonnosti; genetické parametry

Corresponding Author

Ing. Josef Bouška, CSc., Výzkumný ústav živočišné výroby, Přátelství 815, P.O. Box 1,
104 01 Praha 10-Uhříněves, Česká republika
Tel. +420 267 710 869, e-mail: bouska@vuzv.cz

Development of NIR calibration valid for two different grass sample collections

V. Míka[1], J. Pozdíšek[2], P. Tillmann[3], P. Nerušil[1], K. Buchgraber[4], L. Gruber[4]

[1]Research Institute of Crop Production, Prague – Research Station of Grassland Ecosystems, Jevíčko, Czech Republic

[2]Research Institute of Cattle Breeding, Ltd., Rapotín, Czech Republic

[3]VDLUFA, Kassel, Germany

[4]Bundesanstalt für alpenländische Landwirtschaft, Gumpenstein, Irdning, Austria

ABSTRACT: The prediction equation of parameters of forage nutritive value, derived entirely from Czech samples, was not suitable for sufficiently accurate evaluation of the nutritive quality of Austrian samples. However, when it was extended by randomly selected 171 Austrian samples, it became more robust and the standard error of prediction decreased significantly (e.g. from 0.826 to 0.321 MJ NEL/kg DM). It can be used for samples from larger geographical and climatic areas or under different grassland management practices with various proportions of grasses, legumes and other herbs. There was not any consistent improvement when NEL values were calculated from partial prediction of constituents in comparison with direct prediction of NEL by NIRS. The *r* values were 0.866 vs. 0.876 in the case of Czech validation set and 0.852 vs. 0.811 for the Austrian validation set.

Keywords: forage nutritive value; near infra-red spectroscopy (NIRS); quality evaluation methods; net energy of feedstuffs

Diffuse reflectance spectroscopy in the near-infrared region (1 100–2 500 nm), NIR, is increasingly used for simultaneous estimation of basic parameters of the nutritive value of feedstuffs. It is an extremely quick, non-destructive method convenient for measurement of large series of samples of equal character. If this method is used sensibly, it is accurate enough. Nevertheless it is a secondary method, that means you cannot expect more accurate results than reference methods (conventional chemical analyses). It is a method with quite a short history whose methodology develops in connection with further advances in hardware and software tools.

The normal processing of NIR spectroscopic data relies on the development of specific calibrations. This has been a serious restriction of NIRS application because of the high cost of development (Davies and Grant, 1987) and procedure pitfalls, esp. if the sample collection is not sufficiently large, homogeneous, precise, and typical. For example, if the local calibration, derived only from a Czech collection of grasses harvested in conditions typical of the local farming practice, was used for prediction of Austrian samples, harvested on their floristically rich grasslands in a significantly earlier growth stage, the result was generally unsatisfactory (Buchgraber and Hrabě, 2001). Fundamentally it is not possible to extrapolate the values outside our model and to expect more precision than we put in (Murray, 1994).

This study analyses possible reasons of disproportion and specifies pitfalls of enlarging the domain of the calibration equation for herbage samples from different geographical and climatic areas or different grassland management practices.

Supported by the Ministry of Agriculture of the Czech Republic (Project No. M01-01-01).

MATERIAL AND METHODS

For the development of local calibration equation (calibration equation I) samples of grassland herbage were used that were selected from the collection of many thousands of samples measured in Jevíčko until 1995 on NIRSystems 6500 instrument. Using the program ISI 3.0 "centre" and "select" 82 samples with characteristic spectra were selected for calibration. Reference values were taken from these samples (19 parameters of forage quality) by standard chemical methods in the RICB laboratory at Rapotín (ČSN 46 7007, Sommer *et al.*, 1994). The calibration equation was developed for all 19 constituents by standard procedure. The accuracy of prediction was evaluated on the basis of a validation set of samples from the AKTION project 1998–1999 from Czech (n = 115) and Austrian sites (n = 511) against laboratory methods, set for these samples in the BAL Gumpenstein laboratory (according to interior BAL methodology). The most significant results are summarised in Table 1.

In 2000 the calibration set was extended by 520 samples from Czech-Austrian programs KONTAKT and AKTION; from these 129 pure grass and leguminous samples and 391 grassland herbage samples originated equally from trials conducted at MZLU Brno, Czech Republic, and at BAL Gumpenstein, Austria, from harvests in 1998–2000 (Table 2). Until then the original Czech and Austrian collections were divided by random sampling (according to the programme ISI 3.01 "centre" and "select") of each i-th sample into calibration set and validation set. Calibration models were created by PLS algorithms and neuron net (back-propagation) from spectra values in the NIR region, while the values of correlation coefficients (RSQ) and standard difference (SD) were determined. PLS algorithm was used for OMD and PDIN constituents and ANN algorithm for the others. This extended equation is next referred to as calibration equation II.

The prediction accuracy according to this equation was verified on two independent validity collections of samples: they were (1) from trials on arable land by Pozdíšek at RICB Rapotín, Czech Republic (n = 28) and (2) from pasture trials by Gruber at BAL Gumpenstein, Austria (n = 27). The results are demonstrated in Table 3 and Table 4.

The reference values for calibration set and both validation sets of Czech samples were established at RICB laboratory at Rapotín, of Austrian sam-

Table 1. Prediction accuracy of selected parameters of forage nutritional quality (on dry matter laboratory basis) using calibration equation I and validation set with a majority of samples of Austrian origin

Constituent	n	Mean ± S.D.	SEP	SEP (C)	Bias	r^2	Slope
Crude protein (g/kg)	342	187.75 ± 36.73	20.444	19.007*	7.599*	0.737	1.086
Fat (g/kg)	342	20.90 ± 3.36	3.833	3.838*	0.057	0.488*	0.440
Fibre (g/kg)	342	251.19 ± 25.58	46.993	19.453	42.791*	0.439*	0.836
Ash (g/kg)	382	108.37 ± 19.34	20.943	16.155*	–13.354*	0.369*	0.702
ME (MJ/kg)	382	9.82 ± 0.94	1.205	0.947*	0.747*	0.113*	0.493
NEL (MJ/kg)	382	5.80 ± 0.69	0.826	0.675*	0.478*	0.124*	0.551

Table 2. Some characteristics of the Czech (CZ) and Austrian (A) trial sites

Characteristic	Jevíčko (CZ)	Gumpenstein (A)	Pieber (A)
Altitude (m a.s.l.)	330	700	450
Annual temperature average (°C)	7.5	6.8	7.9
Annual long-term precipitation average (mm)	629	1 015	937
Snow cover (days per year)	8	100	59
Soil	Fluvisol	Cambisol	Cambisol
pH/KCl	6.5	5.3	6.4

Table 3. Prediction accuracy of selected parameters of forage nutritional quality (on dry matter laboratory basis) using extended calibration equation (calibration equation II) and Czech validation set

Constituent	*n*	Mean ± S.D.	SEP	SEP (C)	Bias	r^2	Slope
Crude protein (g/kg)	28	151.26 ± 62.52	8.141	7.550	3.363	0.990	1.075
Fat (g/kg)	28	26.70 ± 6.42	2.566	2.277*	0.428	0.860	1.188
Fibre (g/kg)	28	241.26 ± 69.48	19.648	17.805	–8.965*	0.953	1.161
Ash (g/kg)	28	111.84 ± 26.86	9.398	9.365	1.940	0.917	1.259
OMD (%)	28	73.11 ± 8.48	5.232	4.979	–1.861	0.712	0.780
PDIN (g/kg)	28	96.22 ± 38.54	10.006	9.026	4.643	0.960	1.144
PDIE (g/kg)	28	88.20 ± 11.98	5.439	4.705	2.870*	0.850	0.934
ME (MJ/kg)	28	9.83 ± 0.97	0.452	0.460	0.016	0.810	0.832
NEL (MJ/kg)	28	5.88 ± 0.70	0.371	0.378	–0.001	0.751	0.808

Table 4. Prediction accuracy of selected parameters of forage nutritional quality (on dry matter laboratory basis) using extended calibration equation (calibration equation II) and Austrian validation set

Constituent	*n*	Mean ± S.D.	SEP	SEP (C)	Bias	r^2	Slope
Crude protein (g/kg)	27	133.99 ± 29.20	7.346	7.403	–1.090	0.936	0.995
Fat (g/kg)	27	22.48 ± 3.82	1.682	1.713	0.068	0.800	1.033
Fibre (g/kg)	27	282.30 ± 38.81	15.426	13.958	–7.095	0.873	1.061
Ash (g/kg)	27	115.16 ± 22.17	8.927	8.887	–1.907	0.842	0.944
OMD (%)	27	66.87 ± 6.56	4.028	3.701	1.741	0.698	0.867
PDIN (g/kg)	27	86.69 ± 18.17	8.242	7.920	2.745	0.810	1.024
PDIE (g/kg)	27	83.53 ± 7.57	3.351	3.378	–0.490	0.801	1.014
ME (MJ/kg)	27	8.88 ± 0.89	0.464	0.465	–0.086	0.737	0.884
NEL (MJ/kg)	27	5.20 ± 0.62	0.365	0.338	–0.153	0.726	0.841

ples at BAL laboratory Gumpenstein, using the above-mentioned methods. The calculation of ME and NEL concentrations was carried out at RICB Rapotín (Sommer *et al.*, 1994) and the results of laboratory analyses from both laboratories were used.

NIRS scanning

The spectra of grass samples were scanned at Jevíčko on a NIRSystems 6500 instrument equipped with a spinning sample module, in reflectance range 1 100–2 500 nm, band width 2 nm, measured in small ring cups, in 2 × 2 replications (i.e. 2 fillings of each sample, double scanning of each cup filled), using 3.01 software.

To calculate the concentration of energy from Weende analysis these equations were used:

GE = 0.0239 CP + 0.0397 F + 0.020 CF + 0.0174 NfE

ME = 0.01517 OMD

NEL = ME (0.463 + /0.24 q/)

where: GE = gross energy (MJ/kg DM) according to Petrikovič *et al.* (2000), derived from Schiemann *et al.* (1971)
ME = metabolisable energy (MJ/kg DM) according to AFRC (1993)
NEL = net energy for lactation (MJ/kg DM) according to Sommer (1994)
q = ME/BE
OMD = g digestible organic matter in 1 000 g DM
CP = g crude protein in 1 000 g DM
F = g fat in 1000 g DM
CF = g crude fibre in 1 000 g DM
NfE = g nitrogen-free extract in 1 000 g DM

Error terms used in NIR analysis (Murray, 1994):

The standard error of the laboratory (SEL) can be estimated from the standard error of the difference between blind duplicate measurements L_1 and L_2 using the reference method:

$$SEL = \sqrt{\frac{\sum(L_1 - L_2)}{n}}$$

where: n = number of samples

If the s tandard deviation of the population (SD_{pop}) is known, the maximum explainable variance (r^2) can be calculated from the error/spread ratio:

$$\frac{SEL}{SD_{pop}} = \sqrt{\frac{n(1-r^2)}{n-2}}$$

where: n = number of samples
r^2 = coefficient of determination

In the above equation, SEL may be replaced by the standard error of NIR calibration (SEC) which is defined as:

$$SEC = \sqrt{\frac{\sum(L-M)^2}{n-1-p}}$$

where: L = laboratory reference
M = NIR measured value
n = number of samples
p = number of terms in the model

Where the calibration model is validated on a validation sample set, the standard error of performance (SEP) is obtained from:

$$SEP = \sqrt{\frac{\sum(L-M)^2}{n}}$$

The SEP contains a systematic component known as BIAS.

$$BIAS = \bar{L} - \bar{M} = \frac{\sum(\bar{L} - \bar{M})}{n}$$

where: $\bar{M}$ = mean of laboratory measurement
$\bar{L}$ = mean of NIR measurement

and a random unexplained error known as the standard error of performance corrected for bias [SEP(C)]

$$SEP(C) = \sqrt{\frac{\sum(L-M)^2 - (\sum L - \sum M)^2 / n}{n-1}}$$

It is important to note that variances are additive but not standard errors. Thus the SEC or SEP(C) consists of errors from the laboratory (SEL) and from the NIR instrument and modelling process (SE_{NIR}):

$$SEC \text{ or } SEP(C) = \sqrt{SEL^2 + SE_{NIR}^2}$$

If SEL = SE_{NIR}, then SEC is 1.41 SEL
If SEL = 2 SE_{NIR}, then SEC is 1.12 SEL
If SEL = 0.5 SE_{NIR}, then SEC is 2.24 SEL

Standard errors of performance are frequently twice the magnitude of the standard error of the laboratory in successful NIR calibrations. In spite of this the repeatability of NIR measurement is almost always better than the repeatability of the reference procedure.

RESULTS AND DISCUSSION

When the constituents in Czech samples of meadow forage were predicted according to local calibration (calibration equation I), the prediction accuracy was from good to very good (Míka *et al.*, 1997). When this equation was used to predict the constituents in samples of pure grasses and legume species of Czech and Austrian origin, and also in samples from Austrian meadows and pastures, the accuracy was not good. Prediction of crude protein (CP) was generally satisfactory (r = 0.75, n = 511), however, with the content ≤150 g CP in kg DM NIRS methodology slightly overrated the CP content (Buchgarber and Hrabě, 2001). Prediction of crude fibre (CF) was even less accurate (Table 1) when with the content ≤200 g CF in kg DM its content was overrated, with the content ≥300 g CF in kg DM its content was underrated (on average by 70 g). Similarly like CP, CF also demonstrated a significant bias (in Table 1 signed by *), that means the value describing a systematic difference between the analyses carried out by laboratory methods and the NIRS predictions. Prediction of organic matter digestibility (OMD) of forage samples harvested at the stage of heading till the start of flowering (on average 70–60 OMD) demonstrated a satisfactory coefficient of determination (r^2 = 0.69), however in the OMD range 70–75 the prediction gave higher values. The relevant values were also overrated with <60 OMD (by about 10%). In the case of metabolisable energy (ME) and net energy of lactation (NEL) the

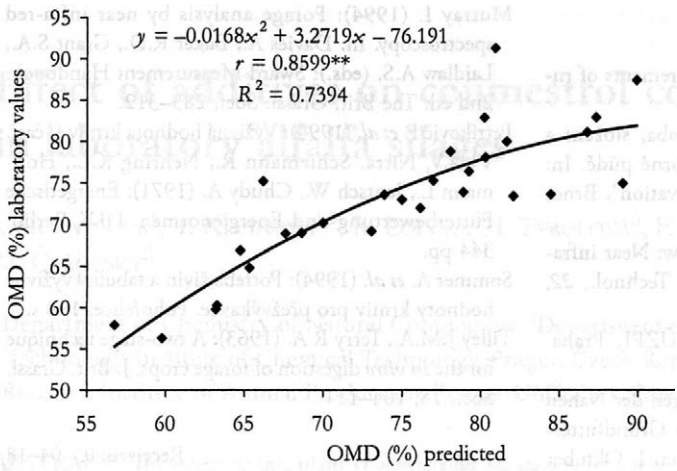

Figure 1. Comparison of organic matter digestibility (OMD) predicted in grass samples of Czech origin versus laboratory (Tilley and Terry, 1963) *in vitro* values

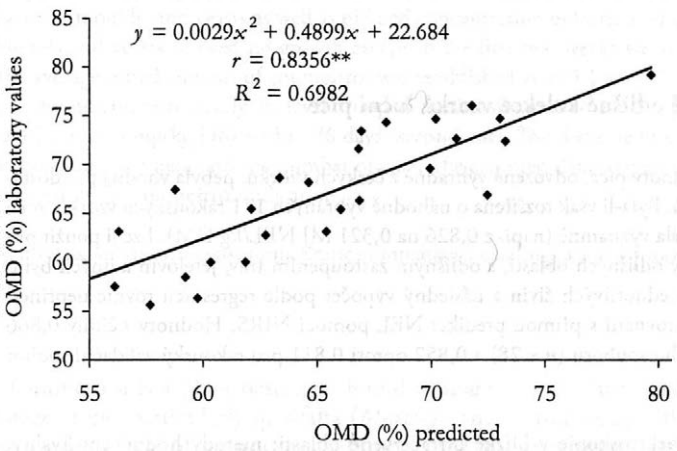

Figure 2. Comparison of organic matter digestibility (OMD) predicted in grass samples of Austrian origin versus laboratory (Tilley and Terry, 1963) *in vitro* values

prediction was absolutely unsatisfactory (Table 1, also Buchgraber and Hrabě, 2001). This leads to a conclusion that the equation derived from the sample population close by quality to the forage used in Czech farming practice is suitable for samples of similar character, but it is not robust enough to suit the grasses from outside (Murray, 1985).

When this equation was extended by a sufficient number of samples from other populations, close by character to samples also utilised by it (calibration equation II), the accuracy of prediction of forage nutrition quality parameters did not worsen compared to calibration equation I (Table 3, Figure 1). It is similarly suitable for the samples of the same character by which it was extended (Table 4, Figure 2). It was definitely affected in a positive way by a sufficient range that enables to optimise the potential of calibration by neuron nets. The quality of calibration is shown not only by relatively low values of standard error of prediction (SEP), high *r*, but also by mostly insignificant bias, and the slope of regression line near to 1 (Tables 3 and 4). This calibration equation can be used for the prediction of parameters of nutritional quality of both Czech and Austrian samples.

There was not any consistent improvement when NEL values were calculated from partial prediction of constituents in comparison with direct prediction of NEL by NIRS (Tables 3 and 4). The *r* values were 0.866 vs. 0.876 in the case of Czech validation set and 0.852 vs. 0.811 for the Austrian validation set, respectively.

REFERENCES

AFRC (1993): Energy and protein requirements of ruminants. Wallingford, UK. 159 pp.

Buchgraber K., Hrabě F. (2001): Výroba, složení a kvalita trvalých travních porostů na orné půdě. In: Proc. 10th Int. Symp. "Forage conservation", Brno, 3–18.

Davies A.M.C., Grant A. (1987): Review: Near infrared analysis of food. Int. J. Food Sci. Technol., *22*, 191–207.

Míka V. *et al.* (1997): Kvalita píce. ÚZPI, Praha. 227 pp.

Murray I. (1985): Grenzen und Leistungen der Nahen Infrarot Rexlexions (NIR) – Analyse in Grundfuttermitteln. Kurzfassungen der Vorträge vom 1. Oktober 1985. In: NIR – Anwenderseminar, FAL Braunschweig, 3–8.

Murray I. (1994): Forage analysis by near infra-red spectroscopy. In: Davies A., Baker R.D., Grant S.A., Laidlaw A.S. (eds.): Sward Measurement Handbook. 2nd ed. The Brit. Grassl. Soc., 285–312.

Petrikovič P. *et al.* (1994): Výživná hodnota krmív I. časť. VÚŽV, Nitra. Schiemann R., Nehring K.L., Hoffmann L., Jentsch W., Chudy A. (1971): Energetische Futterbewertung und Energienormen. DLV, Berlin. 344 pp.

Sommer A. *et al.* (1994): Potřeba živin a tabulky výživné hodnoty krmiv pro přežvýkavce. Pohořelice, 198 s.

Tilley J.M.A., Terry R.A. (1963): A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassl. Soc., *18*, 104–111.

Received: 03–04–18
Accepted after corrections: 03–09–26

ABSTRAKT

Vývoj kalibrace NIR platné pro dvě odlišné kolekce vzorků luční píce

Predikční rovnice parametrů nutriční hodnoty píce, odvozená výhradně z českých vzorků, nebyla vhodná pro dostatečně přesnou predikci rakouských vzorků. Byla-li však rozšířena o náhodně vybraných 171 rakouských vzorků, stala se robustnější a chyba predikce (SEP) klesla významně (např. z 0,826 na 0,321 MJ NEL/kg DM). Lze ji použít pro vzorky ze širších geograficky a klimaticky odlišných oblastí, s odlišným zastoupením trav, jetelovin a jiných bylin a úrovní agrotechniky. Predikce obsahů jednotlivých živin a následný výpočet podle regresních rovnic nepřinesl jednoznačné zlepšení hodnoty NEL ve srovnání s přímou predikcí NEL pomocí NIRS. Hodnoty r činily 0,866 oproti 0,876 v případě českého validačního souboru (n = 28) a 0,852 oproti 0,811 pro rakouský validační soubor (n = 27).

Klíčová slova: nutriční hodnota píce; spektroskopie v blízké infračervené oblasti; metody hodnocení kvality; nettoenergie krmiv

Corresponding Author

Ing. Václav Míka, DrSc., Výzkumný ústav rostlinné výroby, Praha-Ruzyně, Výzkumná stanice travních ekosystémů, K. H. Borovského 461, 569 43 Jevíčko, Česká republika
Tel./fax +420 461 234 814, e-mail: vste@seznam.cz

Effect of additives on coumestrol content in laboratory alfalfa silages

J. Moravcová[1], T. Kleinová[1,3], R. Loučka[3], I. Tyrolová[3], F. Kvasnička[2], M. Dušek[2], M. Čeřovský[2]

[1]Department of Chemistry of Natural Compounds, [2]Department of Food Preservation and Meat Technology, Institute of Chemical Technology, Prague, Czech Republic
[3]Research Institute of Animal Production, Prague-Uhříněves, Czech Republic

ABSTRACT: The effect of inoculant (*Lactobacillus plantarum* or a mixture of *Enterococcus faecium* with *Lactobacillus rhamnosus*), sucrose and formic acid on coumestrol content in laboratory alfalfa silages at high moisture content (30% dry matter, DM) was investigated. Alfalfa (Morava cultivar) was harvested in May and June 2001, chopped to the length of 30 mm and conserved with additives or without them (control). The number of lactic bacteria, moulds, and yeasts as well as pH and concentration of lactic acid were indicative of adequate preservation. Moulds and yeasts showed no growth except in the first two weeks when slightly aerobic deterioration occurred. The average initial content of coumestrol was established at 213.1 ± 29.2 and 207.6 ± 7.7 mg/kg DM for the first and second cut, respectively. Its level dropped continually to 20.6 ± 9.5 mg/kg DM within 110 days (first cut) and to 44.1 ± 10.3 mg/kg DM within 76 days (second cut). The decrease in coumestrol concentration was unaffected by both the treatment and the number of cuts. The acquired data suggest that the residual enzymic activity can be responsible for coumestrol utilization.

Keywords: phytoestrogens; fermentation; inoculant; ensiling; animal production

Coumestrol is a plant oestrogen found in many forage crops, particularly in alfalfa (*Medicago sativa*). A high level of oestrogenic substances fed to cattle or sheep may result in several adverse biological effects, including increased teat length, gestation time, and uterine weight, as well as prolapsed vagina. In contrast, beneficial effects of alfalfa silage have also been reported; an increased rate of growth and milk production can serve as good examples (Vagnoni and Broderick, 1997; Broderick *et al.*, 1999). Although it has been known for a long time that alfalfa haylage containing 37 ppm of coumestrol or more caused clinical signs of oestrogenic stimulation in cattle (Lookhart, 1980), data on coumestrol content in alfalfa silage are surprisingly sporadic. In contrast, a great attention has been paid to the quality of silages from the aspect of water activity, dry matter content, application of inoculants, and length of chopped forage (Whiter and Kung, 2001; Broderick *et al.*, 2002) as these conditions are important for the proper fermentation.

The objective of the present study was to investigate the effect of several additives (inoculant, sucrose, formic acid, and their combination) on the coumestrol level in alfalfa silages made of herbage from the first and second cut.

MATERIAL AND METHODS

Fresh forage and silages

The experiments were conducted from May to June in 2001. Alfalfa (*Medicago sativa*), Morava cultivar, was grown in an experimental field at the

Supported by the Grant Agency of the Czech Republic (Grant No. 523/00/0567).

Research Institute of Animal Production in Prague-Uhříněves (sugar beet production region, 280 m above sea level). Alfalfa was planted at a seeding rate of 18 kg/ha in 1999 with oats as a foregoing crop and legume-cereal mixture as a cover crop. After wilting in the swath to ca. 30% DM, forage was chopped by a conventional forage harvester to a theoretical length of 30 mm. Forage was harvested and chopped on sunny days without rain and tedding was applied about 24 h after mowing.

Chopped forage was divided into four 3-kg piles and treated with: (1) the inoculant applied either in deionized water at 4 g/tonne (*Lactobacillus plantarum* – DSM 12771) or solid at 0.5 kg/tonne (a mixture of *Enterococcus faecium* – NCIMB 30 122 with *Lactobacillus rhamnosus* – NCIMB 30 121), (2) sucrose diluted in deionized water at 15 kg/tonne, (3) a combination of sucrose and the inoculant at concentrations given above, and (4) formic acid at 5 l/tonne. Water containing the inoculant, sucrose solution and formic acid was applied with a hand-sprayer while mixing the forage samples. The dry inoculant formulation was sprinkled by hand onto the forage mass while the sample was mixed. After treatment, forages (ca. 250 g) were packed into plastic packs made of polyethylene covered with aluminium foil (PETP12/PE50). After sealing in vacuum, packs were stored at a temperature between 18 to 23°C. Packing and sealing of bags was completed within 1 to 2 h after chopping. Two bags for each experiment were opened at each sampling time. The followed labelling of trials is kept throughout this study: M5 – harvested on May 21; M6 – harvested on June 25; 0 – no treatment; B – treated with *Lactobacillus plantarum* for M5 or with *Enterococcus faecium* and *Lactobacillus rhamnosus* for M6, respectively; S – treated with sucrose; BS – treated with both sucrose and inoculant; F – treated with formic acid.

Chemical and microbiological analyses of forages

The DM content of fresh forage and silages was determined by drying at 60°C in a forced-draft oven. Sample of silage (50 g) was homogenized in 250 ml of water for 5 min and filtered through Whatman filter paper. The pH values of homogenized water extracts were determined with a microprocessor pH meter WTW 537 (WTW GmbH, Weilheim, Germany).

For the determination of lactic, acetic, propionic and butyric acids, a portion of silage (5 g) was stored in a mixture of 20 ml of ethanol with 0.5 ml of chloroform at –18°C until analyzed. Prior to analysis, the sample was completely transferred into a vessel and 200 ml of water was added. A mixture was then homogenized with a hand mixer for 3 minutes. Solids were removed by filtration and clear filtrate was injected into a separation capillary after dilution. Capillary zone electrophoresis was carried out on an EA 100 instrument (Villa-Labeco, Slovak Republic) equipped with FEP capillary (160 × 0.3 mm I.D.) and a conductivity detector. Working electrolyte consisted of 6 m*M* morpholine ethanesulphonic acid, 4 m*M* L-histidine and 0.1% hydroxypropylmethylcellulose, and the driving current was 20 μA. Under these conditions, calibration curves were strictly linear in a broad concentration range giving the detection limit of 3 μ*M*.

Coumestrol was determined according to the described method (Moravcova *et al.*, 2002). Ten replicate analyses of alfalfa containing 203 mg/kg DM of coumestrol gave the relative standard deviation 4.34%.

For microbiological analyses, samples of fresh silages (20–30 g) were homogenized in 100 ml of sterile solution containing 1 g of peptone, 8.5 g of sodium chloride, 1 g of Tween 80 and water up to 1 000 ml volume. A laboratory homogenizer Stomacher was used for 2 minutes. After filtration, water extracts were serially diluted (10-fold) for the enumeration of LAB, yeasts, and moulds. The numbers of lactic acid bacteria (LAB) were determined in duplicate by pour plating on MRS agar (Merck KgaA, Darmstadt, Germany). Yeasts and moulds were determined in duplicate by pour plating on malt extract agar containing glucose and chloramphenicol (Merck, Darmstadt, Germany) according to CSN ISO 7954. Plates were incubated at 30°C for 48 h (LAB) and 72 h (yeasts and moulds), respectively. All microbial data expressed as cfu/g were transformed to $\log_{10}$. Chemical data are presented on a DM basis.

Statistical analysis

The content of coumestrol was analyzed by ANOVA and a significant ($P < 0.05$ or $P < 0.01$) *F*-test was detected. Friedmann test was also evaluated.

RESULTS AND DISCUSSION

The alfalfa forage (Morava cultivar) was harvested in May and June as this time is kept for the first and second cut in the Czech Republic. The DM content of forage was consistent throughout the whole ensiling period (Tables 1 and 2) and ranged from 31.60 up to 36.00%. Fresh forage from the first cut had the pH value about 6.0 (Table 1), which dropped to at least 4.4 within 15 days. The initial pH corresponding to the second cut was slightly below 5.9 (Table 2) and it decreased more slowly to 4.6 or 5.3 after 14 days. The treatment with formic acid resulted in low pH 5.2 in both trials (Tables 1

Table 1. DM content and pH for silages, first cut, May 2001

Day	M5-0		M5-B		M5-S		M5-BS		M5-F	
	DM (%)	pH	DM (%)	pH	DM (%)	pH	DM (%)	pH	DM (%)	pH
0	33.3	5.90	32.32	5.93	34.48	5.90	32.9	5.94	31.64	5.05
15	30.44	4.42	30.17	4.46	31.44	4.18	32.08	4.22	31.05	4.29
35	30.75	4.42	29.89	4.46	30.62	4.07	32.09	4.08	30.84	4.20
48	30.1	4.50	30.25	4.49	31.93	4.17	31.71	4.22	31.17	4.24
110	31.6	4.45	29.87	4.6	32.14	4.10	31.88	4.16	–	–

Table 2. DM content and pH for silages, second cut, June 2001

Day	M6-0		M6-B		M6-S		M6-BS		M6-F	
	DM (%)	pH	DM (%)	pH	DM (%)	pH	DM (%)	pH	DM (%)	pH
0	30.7	5.78	34.43	5.83	35.29	5.86	35.41	5.87	36.00	5.16
14	31.54	4.58	32.81	4.60	33.9	5.31	33.35	5.31	36.06	4.40
29	31.09	5.20	31.55	5.24	33.42	4.70	32.06	4.55	35.08	4.26
76	33.64	5.10	34.12	5.00	34.84	4.56	33.83	4.52	35.19	4.17

Table 3. The concentrations of lactic, acetic and butyric acid in silages, first cut

Trial	Acid (mg/g DM)	Days of ensiling			
		15	35	48	110
M5-0[1]	lactic acid	78.3	86.8	112.1	89.4
	acetic acid	4.0	15.7	40.6	46.6
M5-B[2]	lactic acid	84.9	116.7	114.9	69.8
	acetic acid	6.8	14.2	16.5	66.7
M5-S	lactic acid	103.5	141.1	133.3	133.0
	acetic acid	3.0	10.9	14.4	21.1
M5-BS[3]	lactic acid	96.8	129.8	126.9	136.4
	acetic acid	3.8	9.6	14.4	27.8
M5-F	lactic acid	50.5	77.6	69.0	64.4
	acetic acid	0	0	3.0	7.5

[1]butyric acid, 2.3 mg/g after 48 days; [2]butyric acid, 2.7 mg/g after 110 days; [3]butyric acid, 7.1 and 7.3 mg/g after 15 and 35 days, respectively

and 2). The rapid drop in pH that occurred after 15 d coincided with lactic acid production. Lactic acid prevailed in all silages but it was always accompanied by a minor amount of acetic acid while butyric acid was found rarely (Tables 3 and 4). The initial number of lactic bacteria (LAB) ranged from 4.1 to 5.6 log cfu/g. During ensiling, the number of LAB was dependent on the treatment of forage and the lowest LAB count was determined for silages treated with formic acid (Tables 5 and 6). Furthermore, the improving microbial activity of all silages with time was observed as indicated by the yeast and mould count. Our results are in good agreement with the published data (Nadeau *et al.*, 2000; Whiter and Kung, 2001; Broderick *et al.*, 2002) and demonstrate that laboratory silage was well preserved as indicated by a rapid drop in pH and production of lactic acid.

Averaged across all M5 and M6 trials, the initial coumestrol content of 213.1 ± 29.2 and

Table 4. The concentration of lactic and acetic acid in silages, second cut

Trial	Acid (mg/g DM)	Days of ensiling		
		14	29	76
M6-0	lactic acid	61.3	106.4	89.9
	acetic acid	16.1	10.5	53.1
M6-B	lactic acid	72.3	74.6	102.0
	acetic acid	30.2	52.5	68.5
M6-S	lactic acid	106.1	113.2	120.0
	acetic acid	23.9	46.3	44.5
M6-BS	lactic acid	105.9	135.5	116.4
	acetic acid	24.5	45.5	44.0
M6-F	lactic acid	86.5	92.9	129.5
	acetic acid	11.3	16.9	20.9

Table 5. Number of lactic acid bacteria (LAB), yeasts and moulds (log cfu/g) in silages, first cut

Trial		Days of ensiling			
		15	35	48	110
M5-0	LAB	8.75	8.66	8.66	9.26
	yeasts	–	–	0.45	–
	moulds	0.43	<0.40	0.45	<0.40
M5-B	LAB	9.34	8.66	8.72	8.65
	yeasts	–	–	0.41	–
	moulds	<0.40	<0.40	0.41	<0.40
M5-S	LAB	8.45	8.70	8.28	6.40
	yeasts	–	–	0.36	–
	moulds	5.71	<0.40	<0.40	<0.40
M5-BS	LAB	8.46	8.72	8.53	6.40
	yeasts	–	–	0.46	–
	moulds	2.15	<0.40	0.41	<0.40
M5-F	LAB	8.48	8.26	8.36	8.59
	yeasts	–	–	0.42	–
	moulds	2.18	<0.40	0.46	<0.40

Table 6. Number of lactic acid bacteria (LAB), yeasts and moulds (log cfu/g) in silages, second cut

Trial		Days of ensiling		
		14	29	76
M6-0	LAB	7.92	>10	8.85
	yeasts	–	1.96	–
	moulds	1.20	1.99	1.04
M6-B	LAB	8.23	9.81	8.74
	yeasts	–	1.15	–
	moulds	1.60	1.40	0.79
M6-S	LAB	>10	9.68	8.85
	yeasts	–	1.38	–
	moulds	1.10	<0.40	<0.40
M6-BS	LAB	8.26	9.68	8.74
	yeasts	–	<0.40	–
	moulds	0.60	0.68	<0.40
M6-F	LAB	6.15	9.64	6.88
	yeasts	–	0.40	–
	moulds	0.83	0.40	<0.40

207.6 ± 7.7 mg/kg DM was determined in forage from the first and second cut, respectively. The level of coumestrol decreased rapidly in all silages within 15 d of ensiling (Figures 1 and 2). Moreover, the rate of coumestrol decomposition was approximately of the same magnitude in all trials giving the half-time of ca. 11.5 days. The dependence of coumestrol concentration on the day of ensiling for M5 silages differed from that estimated for M6 trials only in the occurrence of a local maximum after 35 days. The averaged final content of coumestrol was lower in M5 silages (after 110 d:

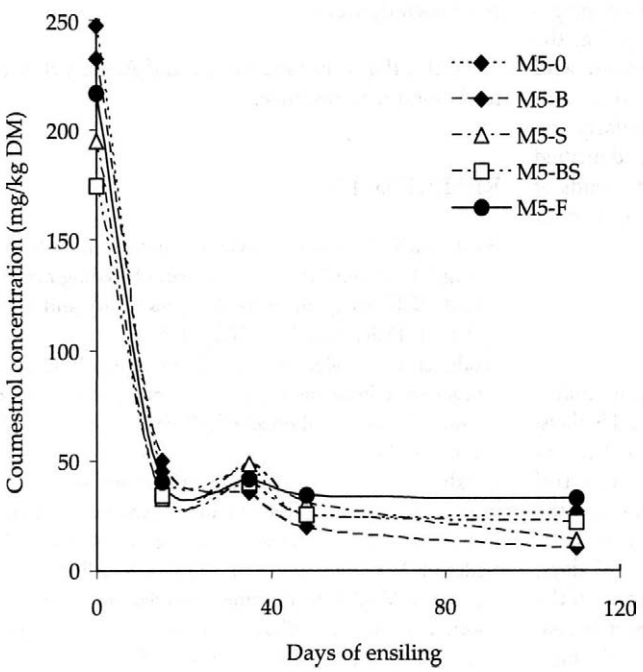

Figure 1. Coumestrol concentrations in alfalfa silages made from the first cut

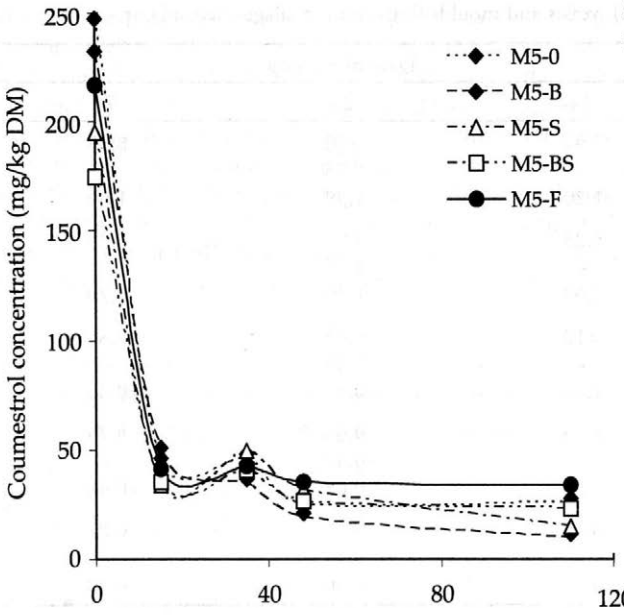

Figure 2. Coumestrol concentrations in alfalfa silages made from the second cut

20.6 ± 9.5 mg/kg DM) than that in M6 silages (after 76 d: 44.1 ± 10.3 mg/kg DM). Nevertheless, this concentration level could cause a symptom of oestrogenic stimulation in cattle fed alfalfa silage as a main constituent of diet (Lookhart, 1980). No significant differences ($P < 0.05$) were found between coumestrol concentrations when silages of the same cut were compared. It means that the treatment with inoculant, sucrose or formic acid displayed no effect on the coumestrol history in comparison with untreated silage. Similarly, no significant differences ($P < 0.05$) were identified between the first and second cut as both trends of time-dependent coumestrol content were similar ($P < 0.05$).

CONCLUSIONS

Coumestrol content was found to decrease markedly in laboratory alfalfa silages within 15 days. After that time its concentration remained rather unchanged. Interestingly, the rate of coumestrol decomposition seems to be constant whether the silage was treated or not. The half-time of the reaction was estimated to be approximately 11.5 days. This is the reason why we suppose the impact of the residual enzymic machinery of plant. Nevertheless, the final level of coumestrol seems to be still high enough to cause oestrogenic stimulation in cattle. To our best knowledge, this is the first proof suggesting that coumestrol can survive the ensiling process.

Acknowledgments

We also thank Tatiana Vrbska and Alena Vyborna for laboratory assistance.

REFERENCES

Broderick G.A., Koegel R.G., Mauries M.J., Schneeberger E., Kraus T.J. (1999): Effect of feeding macerated alfalfa silage on nutrient digestibility and milk yield. J. Dairy Sci., *82*, 2472–2485.

Broderick G.A., Mertens D.R., Simons R. (2002): Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. J. Dairy Sci., *85*, 1767–1776.

Lookhart G.L. (1980): Analysis of coumestrol, a plant estrogen, in animal feeds by high-performance liquid chromatography. J. Agric. Food Chem., *28*, 666–667.

Nadeau E.M.G., Buxton D.R., Russell J.R., Allison M.J., Young J.W. (2000): Enzyme, bacterial inoculant, and formic acid effect on silage composition of orchard grass and alfalfa. J. Dairy Sci., *83*, 1487–1502.

Moravcova J., Kleinova T., Loucka R. (2002): The determination of coumestrol in alfalfa (*Medicago sativa*) by capillary electrophoresis. Czech Plant Prod., *48*, 224–229.

Vagnoni D.B., Broderick G.A. (1997): Effects of supplementation of energy or ruminally undegraded protein to lactating cows fed alfalfa hay or silage. J. Dairy Sci., *80*, 1703–1712.

Whiter A.G., Kung L. Jr. (2001): The effect of a dry or liquid application of *Lactobacillus plantarum* MTD1 on the fermentation of alfalfa silage. J. Dairy Sci., *84*, 2195–2202.

Received: 03–02–10
Accepted after corrections: 03–09–30

ABSTRAKT

Vliv aditiv na obsah kumestrolu při laboratorním silážování vojtěšky

Byl sledován obsah kumestrolu v laboratorních silážích vojtěšky odrůdy Morava, která byla sklízena ve dvou sečích (květen a červen 2001) a po zavadnutí na pokose na obsah sušiny 30 % byla rozřezána na velikost řezanky 30 mm. Siláže z 250 g řezanky byly založeny vždy dvě vedle sebe, a to v pěti paralelních pokusech: kontrolní, po ošetření inokulantem bakterií mléčného kvašení (*Lactobacillus plantarum* nebo směs *Enterococcus faecium* a *Lactobacillus rhamnosus*), s přídavkem sacharózy, s inokulantem a sacharózou, a s konzervovací kyselinou mravenčí. Výsledek fermentačního procesu byl charakterizován pomocí pH vodného výluhu, obsahem kyseliny mléčné, octové a máselné a rovněž počtem bakterií mléčného kvašení, plísní a kvasinek. Všechny tyto charakteristiky byly v souladu s údaji z odborné literatury a dokazovaly správný průběh fermentace. Kumestrol byl stanoven pomocí vysokoúčinné kapilární elektroforézy po extrakci a kyselé hydrolýze. Počáteční průměrná koncentrace kumestrolu v řezance spočítaná na sušinu byla 213,1 ± 29,2 pro první seč a 207,6 ± 7,7 pro druhou seč. Koncentrace kumestrolu klesla pro siláže založené z první seče až na 20,6 ± 9,5 mg/kg po 110 dnech silážování a na 44,1 ± 10,3 mg/kg po 76 dnech silážování řezanky ze druhé seče. Nebyly nalezeny žádné statisticky významné rozdíly mezi koncentrací kumestrolu v siláži a způsobem ošetření siláže nebo počtem sečí. Z výsledků vyplývá, že hlavním důvodem snižování koncentrace kumestrolu během silážování je pravděpodobně zbytková aktivita enzymového aparátu původně v rostlině přítomného.

Klíčová slova: rostlinné estrogeny; fermentace; inokulant; silážování; živočišná výroba

Corresponding Author

Doc. Ing. Jitka Moravcová, CSc., Ústav chemie přírodních látek, Vysoká škola chemicko-technologická Praha, Technická 5, 166 28 Praha, Česká republika
Tel. +420 224 354 283, fax +420 224 311 082, e-mail: jitka.moravcova@vscht.cz

Effect of supplementation of copper in copper sulphate and Cu-glycine on fatty acid profile in meat of broiler chickens, cholesterol content and oxidation stability of fat

S. Ševčíková[1], M. Skřivan[1,2], V. Skřivanová[1], E. Tůmová[2], M. Koucký[1]

[1]Research Institute of Animal Production, Prague-Uhříněves, Czech Republic
[2]Czech Agricultural University, Prague-Suchdol, Czech Republic

ABSTRACT: An experiment was conducted to study the effect of a diet consisting of soybean, wheat and maize with 5% of rapeseed oil and different supplements of copper in the form of $CuSO_4.5H_2O$ or bioplex on cholesterol content and fatty acid composition in breast meat of broiler cockerels. The experiment was conducted on 600 straight-run broiler cockerels randomly divided into 4 groups according to the type of diet: group I – control, no Cu supplement; experimental groups II – 35 mg Cu/kg and III – 175 mg Cu/kg in the form of $CuSO_4.5H_2O$; IV – 175 mg Cu/kg in the form of bioplex. Chickens were sacrificed at 42 days of age. No statistically significant differences were determined in productive parameters except for mortality that was lowest in group IV receiving copper in the Cu-glycine. Cu supplementation decreased ($P < 0.05$) cholesterol content by 24.9% in group IV (493 mg/kg) compared to the control group (656 mg/kg) and significantly decreased the concentration of polyenic fatty acids (PUFA) in groups III and IV (25.90 and 26.02%) in comparison with control group (27.73%) and group II (27.55%). The content of total saturated fatty acids (SFA) increased significantly in group II (27.01%) against the control group (25.41%). The contents of total monoenic fatty acids (MUFA) were significantly higher in groups III and IV (44.01% and 43.14%) than in group II (40.66%).

Keywords: broilers; copper sulphate; Cu-glycine; mortality; breast meat; TBA; fatty acids; cholesterol

The composition of fatty acids in poultry products (meat and eggs) can be influenced by modification of their dietary content (Kinsella *et al.*, 1990; Nam *et al.*, 1997; Eder *et al.*, 1998). The application of 6% and 5% of rapeseed oil and 50 mg/kg vitamin E resulted in higher content of PUFA, reduced n-6/n-3 ratio and lower cholesterol concentration in breast meat of broiler chickens. The effect of vegetable oil was higher compared to lard (Skřivan *et al.*, 1999). A higher amount of rapeseed oil (5%) and a supplement of 50 mg/kg vitamin E in broiler diets increased the content of docosahexaenoic acid (DHA: C22:6 n-3) in breast meat of chickens (Skřivan *et al.*, 2000a,b). Dietary content of DHA (dietary source of DHA) can be determinative for its adequate concentration in membranes because the transformation of alpha-linolenic acid (LNA: 18:3 n-3) to DHA in the human organism is negligible as discovered by Burdge *et al.* (2002). Unsaturated fatty acids are rather susceptible to oxidation and copper is an oxidation-supporting factor. Nevertheless, after supplementation of 250 mg copper Amer and Elliot (1973) found out a significant decrease in palmitic acid in adipose tissues of pigs and simultaneously a lower melting point of fat and increased proportion of phospholipids while the content of neutral fats was lower. The soil in the Czech Republic is deficient in copper, and Illek *et*

Supported by the Ministry of Agriculture of the Czech Republic (Project No. M02-99-04).

al. (1999) demonstrated copper deficiency in cattle and its low concentration in bulk and concentrated feeds and in total mixed rations for high-productive dairy cows. Ruiz *et al.* (2000) reported that copper stimulated poultry growth and increased its performance while Nys (2001) stated that copper promoted the growth of chickens weakly if it was administered at high doses. It was also confirmed by Chiou *et al.* (1999), who did not find any significant effect of copper supplementation in the form of copper sulphate pentahydrate (250 mg/kg). Using a copper sulphate pentahydrate supplement Skřivan *et al.* (1999, 2000a,b) recorded higher live weight of pullets (by 8.5%) and cockerels (2.8%). Dove (1995) observed a 250 mg copper × 5% dietary animal fat interaction for weight gains, feed consumption and nutrient digestibility in pigs. Copper supplementation in the form of copper sulphate to feed for chicken broilers decreased the content of total lipids and cholesterol in meat but it did not influence the profile of fatty acids (Skřivan *et al.*, 2002). On the contrary, the dietary copper supplement administered to pigs (Lauridsen *et al.*, 1999; Armstrong *et al.*, 2001) did not affect plasma cholesterol concentration nor fatty acid composition. Various inorganic and organic compounds (bioplexes – trace elements are bound to amino acids and peptides) were tested as copper sources in poultry; copper citrate was found the most efficient compound (Pesti and Bakalli, 1996; Ewing *et al.*, 1998) compared to copper sulphate and oxychloride. Compared to copper sulphate, higher efficiency of copper in the chelate form was reflected in higher weight gains and feed intake in pigs and chickens (Paik *et al.*, 2001). Oxidation stability of poultry meat highly correlates with the tissue content of alpha-tocopherol as the most efficient form of vitamin E, which acts in membranes as an antioxidant of protection from free radicals causing lipid oxidation (Whitehead, 1986; Schaefer *et al.*, 1995; Coetzee and Hoffman, 2002). Maurice and Lightsey (1996) reported the highest feed conversion and performance after dietary supplementation of 100 mg vitamin E. Surai and Sparks (2000) demonstrated the effect of dietary PUFA n-3 on DHA content and vitamin E accumulation in meat of cockerels and protective effect of vitamin E supplement against lipid peroxidation in tissues.

If there is little information about the effect of dietary copper supplementation on the content and composition of fatty acids in adipose tissues of farm animals, such information on the use of copper chelate is absolutely missing. The objective of our experiment was to evaluate the effect of different dietary sources and amounts of copper on chicken mortality, contents of fatty acids and cholesterol and on oxidation stability of intramuscular fat in breast meat of broiler chickens receiving a diet with 5% of rapeseed oil as a source of fatty acids and 50 mg/kg vitamin E.

MATERIAL AND METHODS

Diets and husbandry

An experiment was conducted on 600 broiler cockerels of hybrid combination ROSS 208. Straight-run cockerels were randomly divided into 12 subgroups and 4 experimental groups; each subgroup comprised 50 chickens (150 chickens per group). Broiler cockerels received a loose feed mixture consisting of wheat, maize and soybean, supplemented with 5% of rapeseed oil and 50 mg/kg vitamin E, 35 mg Cu/kg in the form of copper sulphate pentahydrate (group II), 175 mg Cu/kg in the form of copper sulphate pentahydrate (group III) and 175 mg Cu/kg in the form of Cu-glycine (group IV). Control group (group I) was not supplemented with copper. Vitamin E and copper supplements were included in the premix. Table 1 shows the formulation and chemical composition of feed mixture. Feed consumption, live weight and mortality were examined during the experiment. *Ad libitum* feeding regime and automatic drinkers were used. When the experiment terminated at 42 days of age, 8 broilers of average live weight of the group were selected from each group and sacrificed to carry out slaughter analysis and breast meat analyses.

Analyses

Total lipids of raw breast meat were extracted with 2 : 1 chloroform-methanol according to the method of Folch *et al.* (1957). Meat samples were finely ground before the analysis. Fatty acids were isolated from a homogenised sample (30 g). Alkaline transmethylations of fatty acids were carried out in accordance with ISO 5509. Gas chromatographic analysis of methyl esters was performed on a Hewlett-Packard 5890 gas chromatograph equipped with programmed HP-Innowax

Table 1. Composition of the diets

	Group			
	I	II	III	IV
Ingredients (g/kg)				
Wheat	280	280	280	280
Maize	290	290	290	290
Soybean meal	325	325	325	325
Fish meal	20	20	20	20
Rapeseed oil	50	50	50	50
Limestone	15	15	15	15
Dicalcium phosphate	12	12	12	12
Vitamin-mineral mix*	5	5	5	5
Sodium chloride	2	2	2	2
DL-methionine	1	1	1	1
$CuSO_4.5H_2O$	–	0.14	0.70	–
Cu-glycine	–	–	–	1.75
Composition by analysis (g/kg)				
Dry matter	888			
Crude protein	220			
Fat	76			
Fibre	41			
Ash	51			
Ca	9.2			
P	5.6			
ME by calculation (MJ/kg)	12.77			

*The vitamin-mineral premix provided per kg of diets: vitamin A = 12 000 i.u., vitamin D_3 = 500 i.u., vitamin E = 55 mg, vitamin K_3 = 3 mg, vitamin B_1 = 3 mg, vitamin B_2 = 5 mg, vitamin B_6 = 4 mg, vitamin B_{12} = 0.04 mg, niacinamide =40 mg, Ca pantothenate = 12 mg, biotin = 0.15 mg, folic acid = 1.5 mg, choline-Cl = 250 mg, ethoxyquin = 100 mg, Mn = 80 mg, Zn = 60 mg, Fe = 50 mg, I = 1 mg, Se = 0.25 mg

capillary column (180–240°C; polyethylene glycol – 30 m × 0.25 mm × 0.25 μm):
sample: dissolved in chloroform
carrier: nitrogen, 15 psi, 1.0 ml/min constant flow
oven: 100°C for 1 min; 100°C–210°C at 5°C/min; 210°C–240°C at 2°C/min; 240°C for 12 min
injection: split (30 : 1), 1.0 μl, inlet 185°C
detector: FID, 240°C

The contents of the particular fatty acids were expressed as per cent of the sum of all analysed fatty acids. The following fatty acids were determined in breast meat:

- the total of saturated fatty acids (SFA), total of monounsaturated fatty acids (MUFA), total of polyunsaturated fatty acids (n-6 series – PUFA n-6 and n-3 series – PUFA n-3)
- the total of saturated fatty acids (SFA) included: myristic acid (C 14:0), palmitic acid (C 16:0) and stearic acid (C 18:0)
- the total of monounsaturated fatty acids (MUFA) included: palmitoleic acid (C 16:1), oleic acid (C 18:1) and eicosaenoic acid (C 20:1)
- polyunsaturated fatty acids of the n-6 series (PUFA n-6) included: linoleic acid (LA; C 18:2),

linolenic gamma acid (LNA-gamma; C 18:3) and arachidonic acid (AA; C 20:4)
– polyunsaturated fatty acids of the n-3 series (PUFA n-3) included: linolenic alpha acid (LNA-α; C 18:3), eisosapentaenoic acid (EPA; C 20:5) and docosahexaenoic acid (DHA; C 22:6).

To determine cholesterol, lipids were saponified and the unsaponified matter was extracted according to Nollet (1996). Sylil derivatives were separated and quantified on a gas chromatograph equipped with SAC-5 capillary column (Supelco; 5% diphenyl, 95% dimethylsiloxane – 15 m × 0.25 mm × 0.25 μm), operated isothermally at 285°C:
sample: dissolved in a silanisation agent in pyridine
carrier: nitrogen, 20 psi, 2.0 ml/min constant flow
oven: 285°C for 20 min
injection: split (50 : 1), 1.0 μl, inlet 285°C
detector: FID, 300°C

The susceptibility of breast meat to copper-induced lipid oxidation in raw meat (TBA-0) and in meat stored for 8 days with freezing point 4°C (TBA-8) was determined by measuring thiobarbituric acid-reactive substances (TBARS) using a distillation method (Tarladgis *et al.*, 1960). 10 g of meat were homogenised for 2 min with 97.5 ml of distilled water and 2.5 ml of 4N HCl solution. The blend was distilled until 50 ml were obtained. Five ml of distillate and 5 ml of TBA reagent (0.02 M of the solution of 2-thiobarbituric acid in acetic acid) were blended and heated in a boiling water bath for 35 minutes. After cooling under running tap water for 10 min the absorbance was measured at 538 nm against a blank (a Backman DU spectrophotometer was used). TBARS values were calculated by multiplying optical density by 7.843. Lipid oxidation products were quantified as mg of malonaldehyde per kg of sample.

Copper was determined by atomic absorption spectrometry (Perkin Elmer, model 5000). All analyses were carried out in the Research Institute of Animal Production at Prague-Uhříněves.

Statistical analyses

The data were analysed by one-way ANOVA. Significant treatment effects were detected by Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$. The results were expressed as means with their standard errors.

RESULTS AND DISCUSSION

As for the productive parameters, no significant differences were determined between the groups in live weight gain and feed intake per 1 kg weight gain. The results of our experiment did not confirm the results presented by other authors (Pesti and Bakalli, 1996; Ewing *et al.*, 1998) who reported a positive effect of copper supplementation on broiler performance. But the effect of pharmacological doses of copper was reflected in the improved health condition of chickens significantly ($P \leq 0.05$) because mortality was lower in group IV receiving copper supplement in the form of bioplex. A marked decrease in mortality as a result of the administration of pharmacological copper doses was also observed in rabbits (Skřivanová *et al.*, 2001).

Table 2. Intramuscular fat, copper in breast muscle and liver and TBA (mean ± SE)

Parameter		Group			
		I	II	III	IV
Intramuscular fat	(mg/kg)	7.03 ± 0.40	7.40 ± 0.42	8.19 ± 0.28	7.66 ± 0.44
Copper in breast muscle	(mg/kg)	0.49^{c} ± 0.04	0.58^{bc} ± 0.03	0.71^{a} ± 0.03	0.66^{ab} ± 0.03
Copper in liver	(mg/kg)	2.92^{ab} ±0.07	2.93^{ab} ± 0.17	2.74^{b} ± 0.07	3.14^{a} ± 0.16
TBA-0^{x}		40^{a} ± 0.28	39^{a} ± 0.37	27^{b} ± 0.14	29^{b} ± 0.13
TBA-8^{xx}		45^{b} ± 0.28	38^{b} ± 0.11	42^{b} ± 0.18	57^{a} ± 0.34

^{a,b,c}means with different superscripts in lines differ at $P < 0.05$; ^{x}in raw meat; ^{xx}in meat stored for 8 days with freezing point 4°C

Table 2 shows the content of intramuscular fat, copper content in breast meat and liver and TBARS values for meat. There were no significant differences in the intramuscular fat content between groups and its decreased values like in the experiment of Skřivan *et al.* (2002a,b) were not achieved. All experimental groups had higher contents of copper in meat; the highest values ($P \leq 0.05$) were measured in group III (0.71 mg/kg) and IV (0.66 mg/kg) receiving higher amounts of copper in the form of sulphate and bioplex compared to control group (0.49 mg/kg). Group IV had the highest ($P \leq 0.05$) content of copper in liver (3.14 mg/kg) compared to experimental group III, which had the lowest value (2.74 mg/kg). The measured values were lower than the hygienic limit of copper content in meat and liver. The TBARS number indicating fat susceptibility to oxidation was lowest ($P \leq 0.05$) in raw breast meat in group III (27 mg/kg), which received 0.7 g $CuSO_4.5H_2O$, and in group IV (29 mg/kg), whose chickens were administered 1.75 g bioplex, compared to the control (40 mg/kg) and group II (39 mg/kg). The values increased against the initial values after 8-day storage in chickens with the higher copper dose while the increase was higher for chelates. It was highest ($P \leq 0.05$) in group IV (57 mg/kg) compared to experimental groups II, III and control group (38 mg/kg, 42 mg/kg and 45 mg/kg). Higher susceptibility of fat to oxidation after meat storage in group IV was likely a consequence of the higher copper supplementation in combination with a low anti-oxidation effect of dietary vitamin E.

Table 3 documents cholesterol values (mg/kg) and composition of fatty acids (g/100 g total fatty acids) in breast meat. Cholesterol content in tissues

Table 3. The content of cholesterol (mg/kg) and fatty acid pattern (g/100 g of total fatty acids) of breast meat samples (mean ± SE)

Compound	Groups			
	I (a)	II (b)	III (c)	IV (d)
	control no Cu added	0.14 g $CuSO_4.5H_2O$/kg	0.70 g $CuSO_4.5H_2O$/kg	1.75 g Cu-glycine/kg
Cholesterol	$656^{a} \pm 28.21$	$595^{a} \pm 26.63$	$592^{a} \pm 26.95$	$493^{b} \pm 28.90$
14:0	$0.45^{bc} \pm 0.01$	$0.51^{a} \pm 0.03$	$0.46^{ab} \pm 0.02$	$0.41^{c} \pm 0.01$
16:0	$17.90^{b} \pm 0.29$	$19.43^{a} \pm 0.25$	$19.01^{a} \pm 0.50$	$18.99^{a} \pm 0.23$
18:0	7.06 ± 0.25	7.08 ± 0.20	7.11 ± 0.26	7.25 ± 0.19
16:1	2.09 ± 0.08	2.17 ± 0.17	2.26 ± 0.14	2.10 ± 0.17
18:1	$39.14^{bc} \pm 0.58$	$37.83^{c} \pm 0.26$	$41.03^{a} \pm 0.53$	$40.32^{ab} \pm 0.41$
20:1	0.70 ± 0.01	0.67 ± 0.02	0.72 ± 0.04	0.73 ± 0.02
18:2 n-6	$17.86^{a} \pm 0.36$	$17.93^{a} \pm 0.30$	$17.24^{ab} \pm 0.34$	$16.91^{b} \pm 0.19$
18:3 n-6	$0.30^{a} \pm 0.03$	$0.25^{ab} \pm 0.01$	$0.24^{b} \pm 0.01$	$0.24^{b} \pm 0.01$
20:4 n-6	$3.27^{a} \pm 0.20$	$3.51^{a} \pm 0.23$	$2.67^{b} \pm 0.14$	$3.01^{ab} \pm 0.11$
18:3 n-3	2.80 ± 0.15	2.80 ± 0.07	2.84 ± 0.16	2.70 ± 0.11
20:5 n-3	$0.59^{ab} \pm 0.04$	$0.68^{a} \pm 0.03$	$0.52^{b} \pm 0.04$	$0.57^{b} \pm 0.02$
22:6 n-3	$2.90^{a} \pm 0.19$	$2.37^{b} \pm 0.07$	$2.39^{b} \pm 0.17$	$2.60^{ab} \pm 0.15$
Others	4.93	4.80	3.50	4.19
SFA	$25.41^{b} \pm 0.51$	$27.01^{a} \pm 0.20$	$26.59^{ab} \pm 0.69$	$26.65^{ab} \pm 0.29$
MUFA	$41.93^{bc} \pm 0.60$	$40.66^{c} \pm 0.33$	$44.01^{a} \pm 0.55$	$43.14^{ab} \pm 0.37$
PUFA	$27.73^{a} \pm 0.33$	$27.55^{a} \pm 0.49$	$25.90^{b} \pm 0.45$	$26.02^{b} \pm 0.25$
PUFAn-6	$21.43^{a} \pm 0.23$	$21.69^{a} \pm 0.44$	$20.15^{b} \pm 0.36$	$20.15^{b} \pm 0.16$
PUFAn-3	$6.29^{a} \pm 0.18$	$5.86^{ab} \pm 0.10$	$5.75^{b} \pm 0.16$	$5.87^{ab} \pm 0.14$

^{a,b,c}means with different superscripts in lines differ at $P < 0.05$

can be influenced by a fat source and different composition of dietary fatty acids (Ajuyah *et al.*, 1991) or by dietary Cu content (Konjufca *et al.*, 1997). If compared with control group (656 mg/kg) and experimental groups II and III (595 and 592 mg/kg), cholesterol content was lowest ($P < 0.05$) in group IV (493 mg/kg). Copper supplement in the organic form reduced cholesterol content by 24.9%, which confirms similar results of Skřivan's *et al.* (2000a,b) experiments. Konjufca *et al.* (1997) concluded that Cu salts in pharmacological doses decreased the activity of cholesterol 7-α hydroxylase. Komprda *et al.* (1999) stated that cholesterol content and composition of fatty acids in chicken tissues were influenced by growth rate while cholesterol content in breast and shank meat tended to decrease with increasing growth rate.

As for the saturated fatty acids (SFA), the values of myristic (C 14:0), palmitic (C 16:0) and stearic acid (C 18:0) were determined. The total of SFA increased in all experimental groups; the highest ($P < 0.05$) content (27.01%) was measured in group II with supplementation of 0.14 g/kg $CuSO_4.5H_2O$ compared to control group (25.41%). But SFA were not influenced by copper supplement in an experiment conducted by Skřivan *et al.* (2002). A higher intake of myristic acid can be hazardous to human health. Experimental group II had higher ($P < 0.05$) values of myristic acid content (0.51%). With the same copper supplement (175 mg Cu) administered to groups III and IV the content of myristic acid decreased ($P < 0.05$) when Cu-bioplex was used (0.41%). On the contrary, Skřivan *et al.* (2000a,b) reported a significant decrease in the content of myristic acid in breast meat of broiler chickens that received 60 g/kg rapeseed oil, 50 mg/kg vitamin E and copper supplement 200 mg/kg in the form of dietary copper sulphate pentahydrate. In another experiment (Skřivan *et al.*, 2002) a significant and insignificant reduction of their contents (palmitoleic and myristic acid, respectively) was observed. The content of palmitic acid was higher ($P < 0.05$) in all experimental groups (19.43%; 19.01%; 18.99%) compared to the group without copper supplementation (17.90%). On the other hand, Skřivan *et al.* (2000a,b) reported only a marginal decrease in palmitic acid content when copper supplement in the sulphate form was used. However, Amer and Elliot (1973) stated that dietary copper supplement (250 ppm) significantly reduced the amount of C 16:0 in depot fat of pigs while the contents of oleic (C 18:1) and linoleic (C 18:2) acid increased at the same time. A similar trend – an increased ($P < 0.05$) level of oleic acid – was recorded in our experiment in group III (41.03%).

The content of total monoenic fatty acids (MUFA) was higher ($P < 0.05$) in groups III (44.01%) and IV (43.14%) compared to the group supplemented with 0.14 g/kg $CuSO_4.5H_2O$ (group II – 40.66%) and to the group without copper supplement (control – 41.93%). The results published by Bonanome *et al.* (1992) indicate that the higher content of MUFA in animal products can be beneficial to human health because independently of the antioxidant content diets enriched with MUFA increase the resistance of plasma LDL (Low Density Lipoproteins) cholesterol to oxidation changes and decrease the atherogenic effects of these lipoproteins.

The dietary amount of 0.70 g/kg $CuSO_4.5H_2O$ for group III and also the dietary content of 1.75 g per kg bioplex for group IV reduced ($P < 0.05$) the concentration of total PUFA (25.90% and 26.02%) and PUFA n-6 (20.15% and 20.15%) compared to the control (27.73% and 21.43%) and group II (27.55% and 21.69%). The administration of copper bioplex ($P < 0.05$) decreased the content of linoleic acid in breast meat (16.91%) in comparison with control group and experimental group II (17.86% and 17.93%). The higher supplement of $CuSO_4.5H_2O$ (group III) influenced the content of linoleic acid only marginally (insignificantly). The reduction in arachidonic acid after supplementation of 0.70 g/kg copper in the form of $CuSO_4.5H_2O$ was more marked ($P < 0.05$) (2.67%) than after bioplex administration (3.01%) compared to control group and experimental group II (3.27% and 3.51%). Previous results (Skřivan *et al.*, 2002) confirm insignificant changes in the content of these acids when copper sulphate was applied. Gamma-linolenic acid was significantly ($P < 0.05$) reduced in group III and IV (0.24% and 0.24%) compared to the control (0.30%).

The application of a higher amount of copper in the inorganic form to group III resulted in a decrease ($P < 0.05$) in total PUFA n-3 (5.75%) compared to the control group without copper supplement (6.29%). Total PUFA n-6 were lower ($P < 0.05$) in group III and IV (20.15% and 20.15%) than in the control and group II (21.43% and 21.69%). The contents of eicosapentaenoic acid (EPA) in group III (0.52%) and IV (0.57%) were lower ($P < 0.05$) compared to group II (0.68%). Reductions in do-

cosahexaenoic acid (DHA) in groups III (2.39%) and II (2.37%) were also significant ($P < 0.05$) in comparison with control group (2.90%), but a decrease in the bioplex-treated group was insignificant (2.60%).

CONCLUSION

The experimental results did not confirm a positive effect of copper on productive parameters but the highest decrease in mortality was observed after the application of copper organic source in the chelate form (bioplex). A reduction in cholesterol content was also highest (by 24.9%) after copper supplementation in the bioplex form. Copper in the bioplex form reduced cholesterol content more efficiently than copper sulphate. The measured low values of desirable polyenic (PUFA) fatty acids and the increased content of saturated (SFA) and monoenic (MUFA) fatty acids can probably be explained by the oxidation effect of copper on fatty acids that was the most intensive when the supplementation of higher doses of the inorganic form of dietary copper was used. Lipid oxidation is catalysed by compounds of transient-valence heavy metals that are oxidised by the reception of one electron; copper is one of these heavy metals (Fe, Mn, Cr ...). Metals act as catalysts directly or indirectly in the initiation, propagation and termination phase of auto-oxidation reaction, and the velocity of the initiation and propagation of auto-oxidation reactions and termination reactions increases (Velíšek, 2002). The presence of copper probably accelerates the oxidation of unsaturated fatty acids. A 50 mg dose of vitamin E was administered in our experiment with the higher amount of rapeseed oil. But it will probably be necessary to supplement at least 100 mg of alpha-tocopherol in order to maintain PUFA content when the dietary content of heavy metals is higher.

REFERENCES

Ajuyah A.O., Lee K.H., Hardin H.T., Sim J.S. (1991): Influence of dietary full-fat seeds and oils on total lipid, cholesterol and fatty acid composition of broiler meats. Can. J. Anim. Sci., *71*, 1011–1019.

Amer M.A., Elliot J.I. (1973): Effects of supplemental dietary copper on glyceride distribution in the backfat of pigs. Can. J. Anim. Sci., *53*, 147–152.

Armstrong T.A., Spears J.W., Engle T.E., See M.T. (2001): Effect of pharmacological concentrations of dietary copper on lipid and cholesterol metabolism in pigs. Nutr. Res., *21*, 1299–1308.

Bonanome A., Pagnan A., Biffanti S., Opportuno A., Sorgato F., Orella M., Maiorino M., Ursini F. (1992): Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low-density lipoproteins to oxidative modification. Arterioscler. Tromb., *12*, 529–533.

Burdge G.C., Jones A.E., Wootton S.A. (2002): Eicosapentaenoic and docosapentaenosic acids are the principal products of alpha-linolenic acid metabolism in young men. Brit. J. Nutr., *88*, 355–363.

Chiou P.V.S., Chen C.L., Chen K.L., Wu C.P. (1999): Effect of high dietary copper on the morphology of gastro-intestinal tract in broiler chickens. Asian-Australasian J. Anim. Sci., *12*, 548–553.

Coetzee G.J.M., Hoffman L.C. (2002): Effect of dietary vitamin E on the performance of broilers and quality of broiler meat during refrigerated and frozen storage. South Afr. J. Anim. Sci.-Suid-Afr. Tydskr. Vir Veekde, *31*, 158–173.

Dove C.R. (1995) : The effect of copper level on nutrient utilization of wearing pigs. J. Anim. Sci., *73*, 166–171.

Eder K., Roth-Maier D.A., Kirchgessner M. (1998): Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed. Arch. Geflügelkd., *62*, 223–228.

Ewing H.P., Pesti G.M., Bakalli R.I., Menten J.F.M. (1998): Studies on the feeding of Cupric Sulfate Pentahydrate, Cupric Citrate, and Copper Oxychloride to broiler chickens. Poultry Sci., *77*, 445–448.

Folch J.M., Lees M., Sloane-Stanley G.H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., *226*, 497–509.

Illek J., Matějíček M., Bečvář (1999): Karence mědi u skotu. Veterinářství, *49*, 143–144.

ISO 5509 (1994): Animal and vegetables fats and oils. Preparation of methyl esters of fatty acids. Czech Standards Institute, Prague.

Kinsella J.E., Lokesh B., Stone R.A. (1990): Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanism. Am. J. Clin. Nutr., *52*, 1–28.

Komprda T., Zelenka J., Tieffova P., Stohandlova M., Foltýn J. (1999): Effect of the growth intensity on cholesterol and fatty acids content in broiler chicken tissues. Arch. Geflügelkde, *63*, 36–43.

Konjufca V.H., Pesti G.M., Bakalli R.I. (1997): Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poultry Sci., *76*, 1264–1271.

Lauridsen G., Højsgaard S., Sørensen M.T. (1999): Influence of dietary rapeseed oil, vitamin E, and copper on the performance and the antioxidative and oxidative status of pigs. J. Anim. Sci., *77*, 906–916.

Maurice D.V., Lightsey S.F. (1996): Immunocompetence of slow and fast growing broiler chickens fed different levels of vitamin E. In: Proc. World's Poultry Congr., Vol. II., 93–98.

Nam K.T., Lee H.A., Min B.S., Kang C.W. (1997): Influence of dietary supplementation with linseed and vitamin E on fatty acids, alpha-tocopherol and lipid peroxidation in muscles of broiler chicks. Anim. Feed Sci. Technol., *66*, 149–158.

Nys Y. (2001): Trace elements as related to growth and health in chickens. Prod. Anim., *14*, 171–180.

Nollet L.M.I. (1996): Handbook of Food Analysis. Vol. 1. Dekker, New York.

Paik I.K. (2001): Application of chelated minerals in animal production. Asian-Australasian J. Anim. Sci., *14*, 191–198.

Pesti G.M, Bakalli R.I. (1996): Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poultry Sci., *75*, 1086–1091.

Ruiz J.A., Pérez-Vendrell A.M., Esteve-Garcia E. (2000): Effect of dietary iron and copper on performance and oxidative stability in broiler leg meat. Brit. Poultry Sci., *41*, 163–167.

Schaefer D.M., Liu Q., Faustman C., Yin M. (1995): Supranutritional administration of vitamins E and C improves oxidative stability of beef. J. Nutr., *125*, 1792S–1798S.

Skřivan M., Skřivanová V., Marounek M. (1999): Mastné kyseliny v mase brojlerových kuřat a telat. In: III. Kábrtovy dietetické dny, mezinárodní konference, 119-123.

Skřivan M., Tůmová E., Skřivanová V., Holoubek J. (2000a): Vliv síranu měďnatého na užitkovost brojlerových kuřat a cholesterolu v mase. In: Sborník mezinárodní konference Výroba drůbežího masa, MZLU Brno, 41–42.

Skřivan M., Skřivanová V., Marounek M., Tůmová E., Wolf J. (2000b): Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat. Brit. Poultry Sci., *41*, 608–614.

Skřivan M., Ševčíková S., Tůmová E., Skřivanová V., Marounek M. (2002): Effect of copper supplementation on performance of broiler chickens, cholesterol content and fatty acid profile of meat. Czech J. Anim. Sci., *47*, 275–280.

Skřivanová V., Skřivan M., Marounek M., Baran M. (2001): Effect of feeding supplemental copper on performance, fatty acid profile and on cholesterol contents and oxidative stability of meat of rabbits. Arch. Anim. Nutr. – Arch. Tierernahr., *54*, 329–339.

Surai P.F., Sparks N.H.C. (2000): Tissue-specific fatty acid and alpha-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. Poultry Sci., *79*, 1132–1142.

Tarladgis B.G., Wats B.M., Younathan M.T., Dugan Jr. L.R. (1960): A distillation method for quantitative determination of malonaldehyde in rancid foods. J. Anim. Oil Chem. Soc., *37*, 44–48.

Velíšek J. (2002): Chemie potravin 1. OSSIS, Tábor. 146–147.

Whitehead C.C.(1986): Requirements for vitamins. In: 19th Poultry Science Symposium, Nutrient requirements of poultry and nutritional research, 173–189.

Received: 03–04–23
Accepted after corrections: 03–09–29

ABSTRAKT

Vliv přídavku mědi v síranu měďnatém a Cu-glycinu na profil mastných kyselin v mase brojlerových kuřat, obsah cholesterolu a oxidační stabilitu tuku

Byl studován vliv zkrmování krmné směsi na bázi soji-pšenice-kukuřice s 5 % řepkového oleje a rozdílného přídavku mědi ve formě $CuSO_4.5H_2O$ nebo bioplexu na obsah cholesterolu a složení mastných kyselin v prsním svalstvu brojlerových kohoutků. Experiment byl uskutečněn na 600 jednodenních brojlerových kohoutcích náhodně rozdělených do čtyř skupin podle typu diety: I – kontrola, bez přídavku Cu; II – přídavek 35 mg Cu/kg a III – 175 mg Cu/kg ve formě $CuSO_4.5H_2O$; IV – přídavek 175 mg Cu/kg ve formě bioplexu. Kuřata byla odporažena ve 42 dnech věku. V ukazatelích užitkovosti nebyly statisticky významné rozdíly s výjimkou úhynu, který byl nejnižší

u IV. skupiny s mědí v organické formě. Doplněk Cu snížil obsah cholesterolu ($P < 0,05$) o 24,9 % u IV. skupiny (493 mg/kg) v porovnání s kontrolní skupinou (656 mg/kg) a významně snížil koncentraci polyenových mastných kyselin (PUFA) u III. a IV. skupiny (25,90 a 26,02 %) ve srovnání s kontrolní skupinou (27,73 %) a skupinou II (27,55 %). Obsah celkových nasycených mastných kyselin (SFA) byl významně zvýšen u skupiny II (27,01 %) oproti kontrolní skupině (25,41 %). Hodnoty obsahu celkových monoenových mastných kyselin (MUFA) byly zjištěny u III. a IV. skupiny (44,01 % a 43,14 %) významně vyšší než u skupiny II (40,66 %).

Klíčová slova: brojleři; síran měďnatý; Cu-glycin; úhyn; prsní svalstvo; TBA; mastné kyseliny; cholesterol

Corresponding Author

Ing. Světlana Ševčíková, Výzkumný ústav živočišné výroby, Přátelství 815, P.O. Box 1,
104 01 Praha 10-Uhříněves, Česká republika
Tel. +420 267 009 620, fax +420 267 711 448, e-mail: sevcikova@vuzv.cz

INSTRUCTIONS TO AUTHORS

The journal publishes original scientific papers, selective short communications and review articles. Papers are published in English. Manuscripts should have English and Czech (Slovak) abstracts (including keywords). The author is fully responsible for the originality of the paper and its subject and formal correctness. The author's declaration that the paper has not been published anywhere else should be enclosed. The Board of Editors decides on the publication of papers, taking into account peer reviews, scientific importance, and manuscript quality. The SI international system of measurement units should be used. The manuscripts should be submitted in duplicate in hard copy and a properly labeled floppy disk with identical contents, including figures should be enclosed. Alternatively, the manuscript can be sent by e-mail as attachment.

Copyright. The journal is protected by copyright held by the publisher after the manuscript has been accepted for publication. As concerns the transfer of rights, the corresponding author takes over responsibility for all authors. No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher.

Manuscript layout. Standard size of paper (A4 format), type size 12 font, double-space lines, 2.5cm margins on each edge of the page. MS Word (Word version must be specified) should be used. Tables, graphs, and other materials are to be submitted separately from the text. Each document should be printed, commencing on a separate sheet of paper, and its title and detailed description including the used measurement units should be indicated. Word editor should be used to create tables, for tables each item should be placed into a separate cell. Tables are to be numbered with Arabic numerals in the order in which they are referred to in the text. Graphs should be provided in Excel and they should be stored with original data (the font and type size should be consistent with the general journal format requirements to be incorporated into the text). Autotypes (black and white ones are preferred) should be submitted in TIF or JPGE format. All graphs and photos should be numbered, continually according to the order in which they are included in the text, using Arabic numerals again. All other materials should be submitted in digital form, in high resolution black and white format. Colored photos or maps can be published following an agreement, but it will be at the authors' own cost. All materials to be included in the paper should be referred to in the text. If any abbreviations are used in the paper, they shall be explained appropriately when they are used in the text for the first time. It is not advisable to use any abbreviations in the paper title or in the abstract.

Paper title should be short and informative, not exceeding 85 characters. No subtitles shall be used.

Abstract should not have more than 150 words. It should contain important information on methods used to solve the problem, clear description of results and their statistical significance, and brief and unambiguous conclusions drawn from the results. References and discussion of results should not be included in the abstract.

Keywords should not repeat nouns used in the title and should describe the studied problem as best as possible.

Introduction section should provide information on the present state of research in the field concerned and on the goal of the study. References to literary sources document such present findings that are used by the authors, not all that have been published until now. References in the text should agree with those in the list of references. It is recommended to include references to papers from peer periodicals only.

Material and Methods. All preliminary material, conducted experiments, their extent, conditions and course should be described in detail in this section. All original procedures that were used for the processing of experimental material and all analytical methods used for evaluation should also be detailed. Data verifying the quality of acquired data should be indicated for the used methods. The whole methodology is to be described only if it is an original one, in other cases it is sufficient to cite the author of the method and to mention any particular differences. Methods of statistical processing including the software used should also be listed in this section.

Results and Discussion. The results obtained from the experiments including their statistical evaluation and any commentary should be presented graphically or in tables in this section. The author should confront partial results with data published by other authors, whose names and year of publication are to be cited by including them in the text directly [Brown (1995)] or indirectly [(Green and Grey, 1996), (Jakl *et al.*, 2002)].

References should be a list of refereed periodicals arranged in alphabetical order according to the surname of the first author. The full title of all authors should be followed by the year of publication cited in brackets, the original title of the paper, the name of the periodical using its official abbreviations, the respective volume and page number, in the case of a book or proceedings the title should be followed by the name of the publisher and the place of publication. Literary sources should be cited in the original language. Only papers cited in the text should be included in the list of references.

Examples of references in the list (abbreviations of periodicals are given in agreement with Science Citation Index of Current Contents):

Brown J. (1995): Estradiol determination in post-partum sows. J. Endocrinol., *198*, 155–169.

Green K.L., Grey M. (1996): Hormones in milk. J. Anim. Res., *29*, 1559–1571.

Papers published in monographs or proceedings should be cited like this:

Kaláb J. (1995): Changes in milk production during the sexual cycle. In: Hekel K. (ed.): Lactation in Cattle. Academic Press, London. 876–888.

The Authors' Address. On a separate page the author should include his or her full name (co-authors' full names), including all academic, scientific and pedagogic titles and detailed address of the institution with postal code, phone and fax numbers and/or e-mail address. The author who is responsible for any correspondence with the journal should be indicated clearly.

Offprints: Ten (10) reprints of each published paper are supplied and a **free "electronic reprint"** in Portable Document Format (pdf) sent via e-mail as an attachment.

Compliance with these instructions is obligatory for all authors. If a manuscript does not comply exactly with the above requirements, the editorial office will not accept it for a consideration and will return it to the authors without reviewing.

An international scientific journal
published under the auspices of the Czech Academy of Agricultural Sciences
and financed by the Ministry of Agriculture of the Czech Republic

Czech Journal of Animal Science (Živočišná výroba) • Published by Czech Academy of Agricultural Sciences – Institute of Agricultural and Food Information • Editor Office: Slezská 7, 120 56 Praha 2, Czech Republic, phone: + 420 227 010 352, fax: + 420 227 010 116, e-mail: edit@uzpi.cz • © Institute of Agricultural and Food Information, Prague 2003

Distribution: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, phone: + 420 227 010 427, fax: + 420 227 010 116, e-mail: redakce@uzpi.cz