CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

2

VOLUME 47
PRAGUE 2002
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

Mezinárodní vědecký časopis vydávaný pod záštitou České akademie zemědělských věd a s finanční podporou Ministerstva zemědělství České republiky

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.
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Periodicity: The journal is published monthly (12 issues per year). Volume 47 appearing in 2002.

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 2 27 01 03 52, fax: + 420 2 27 01 01 16, 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 2002 is 214 USD.

Cíl a odborná náplň: Časopis publikuje původní vědecké práce a studie typu review z oblasti genetiky, šlechtění, fyziologie, reprodukce, výživy a krmení, technologie, etologie a ekonomiky chovu skotu, prasat, ovcí, koz, drůbeže, ryb a dalších druhů hospodářských zvířat.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences a v časopise Animal Breeding Abstracts. Abstrakty z časopisu jsou zahrnuty v těchto databázích: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts,Toxline Plus.

Periodicita: Časopis vychází měsíčně (12× ročně), ročník 47 vychází v roce 2002.

Přijímání rukopisů: Rukopisy ve dvou kopiích je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Česká republika, tel.: + 420 2 27 01 03 52, fax: + 420 2 27 01 01 16, e-mail: edit@uzpi.cz.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 2002 je 1176 Kč.

F 4886

Integration and expression of the WAP-hPC gene in three generations of transgenic rabbits

Integrácia a expresia WAP-hPC génu v troch generáciach transgénnych králikov

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ABSTRACT: The aim of the present study was to obtain transgenic rabbits expressing human protein C in their mammary gland. A fusion construct which consisted of 4.2 kb long mouse whey acidic protein (WAP) promoter and 9.4 kb genomic human protein C (hPC) was microinjected into rabbit zygotes. Born animals were subjected to PCR amplification to detect the integration of the injected construct into their genome. As examined during three generations, the founders transmitted transgenic allele in a Mendelian fashion. Western blot analysis demonstrated the presence of hPC in the milk of lactating transgenic females. The concentration of the recombinant hPC, as determined by ELISA, ranged between 0.24–0.56 μg/ml. This relatively low expression rate might be caused by the animal species used or might be a subject of position effects of the integration of the heterologous construct.

Keywords: WAP-hPC; transgenic rabbits; integration; expression

ABSTRAKT: Cieľom práce bolo získať transgénne králiky s expresiou ľudského proteínu C v ich mliečnej žľaze. Génová konštrukcia pozostávajúca z promótora – myšieho srvátkového proteinu (WAP) o veľkosti 4,2 kb a génu ľudského proteinu C o veľkosti 9,4 kb bola mikroinjektovaná do králičích zygot. Integrácia injektovaného konštruktu v génome narodených jedincov bola analyzovaná PCR metódou. Transgénna alela bola v nasledovných troch generáciach prenašaná v rámci Mendelových pravidiel. Western blot analýzy ukázali prítomnosť hPC v mlieku laktujúcich transgénnych králičích samíc. Koncentrácia rekombinantného hPC, na základe ELISA testu, bola zistená v rozpätí 0,24–0,56 μg/ml. Táto relatívne nízka úroveň expresie mohla byť spôsobená živočíšnym druhom alebo mohla byť zapríčinená miestom integrácie heterológneho konštruktu.

Kľúčová slova: WAP-hPC; transgénne králiky; integrácia; expresia

Protein C (PC) is a key enzyme in the blood anticoagulation pathway. In humans it is synthesized predominantly in the liver. The hPC consists of a heavy chain (41 kDa) and light chain (21 kDa) linked by a disulfide bond (Kisiel, 1979). In plasma, PC is present at concentrations 10 μg/ml. Deficiency of hPC is inherited as an autosomal dominant disease and it is associated with serious thromboembolic complications. Replacement therapy using a concentrate of hPC was shown to be effective in preventing syndromes related to this disease (Regnault *et al.*, 1991). However the isolation of hPC from human plasma is very difficult (Velander *et al.*, 1989). Therefore the interest in the development of alternative means for the hPC production, especially in the last decade, has been arising.

Transgenic animals represent an alternative way to produce biologically active proteins playing role in the blood coagulation (Lubon and Paleyanda, 1997). Production of recombinant human protein C (rhPC) was accomplished in the mammary gland of transgenic pigs (Velander *et al.*, 1992; Lee *et al.*, 1995; Van Cott *et al.*, 1997) and to a lesser extent in the mammary gland of mice (Velander *et al.*, 1991; Drohan *et al.*, 1994). Recently rabbits were shown to be a suitable model for production of the recombinant proteins in their mammary gland (e.g. Strömqvist *et al.*, 1997). Although rabbits are not conventional dairy stock, the short generation cycle and multiple offspring per litter offer advantages for establishment of a herd-producer of important therapeutic recombinant proteins. This species alleviates some disadvantages of large animals, such as pigs and sheep, and small animals, such as mice. Therefore in the present study, we established transgenic rabbits expressing protein C in their mammary gland. Since production of recombinant proteins requires genetic stability of transgene, we investigated hPC expression in three generations of founder's offspring.

MATERIAL AND METHODS

Generation of transgenic rabbits

Transgenic rabbits were generated by the microinjection of the WAP-hPC gene constructs which consisted of the 4.2 kb WAP promoter and 9.4 kb hPC gene (Drohan *et al.*, 1994) into the pronuclei of fertilized eggs (Brem *et al.*, 1985) from superovulated New Zealand White female rabbits. Microinjected embryos were transferred to the oviducts through the infundibulum of pseudopregnant recipients.

Detection of the WAP-hPC gene integration

Integration of the injected construct into the rabbit genome was analyzed by PCR amplification on the DNA isolated either from blood or from ears (Sambrook *et al.*, 1989) or from the embryos (Chrenek *et al.*, 1998). Firstly, for PCR amplification hPC specific primers: 5'-CAG CAC AGC CTC CCC TAC TCA AA-3' and 5'-CTC CGC CCC CTC AAG ACT CAT TC-3' (Chrenek *et al.*, 1997) at the annealing temperature 68°C in 35 cycles were used. Positive samples were re-analyzed using additional hPC specific primers: 5'-CAG TCA CTT GCC TGA CAC CGG TAC-3' and 5'-GCC AGT GTG CAT TTG AGT AGG GGA-3' (Drohan *et al.*, 1994) at the annealing temperature 58°C in 35 cycles. PCR products were visualized using 1.5% agarose gel (Serva, Heidelberg, Germany).

Milk collection

Milk of the transgenic females or from the control non-transgenic rabbits was collected on the 20th day of lactation. In F_1 generation, milk sample of one transgenic female was collected during the second, third and fourth lactation. In F_2 generation, milk samples of 9 transgenic females were collected and subsequently analyzed. In order to stimulate milk letdown, intramuscular injection of 5 IU of oxytocin (Léčiva, Prague, Czech Republic) was applied 15 min before the milk collection. Thereafter, obtained milk was immediately centrifuged at 12 000 g for 10 min and the upper lipid layer was removed. The samples were either subjected to further analyses or stored in liquid nitrogen.

Western blotting

The defatted milk samples of transgenic and non-transgenic females were separated by SDS-PAGE under non-reduced conditions according to Laemmli (1970). Proteins were then transferred by semi-dry trans-blot (Bio-Rad, Vienna, Austria) onto the ECL Hybond membrane (Amersham Pharmacia Biotec, Uppsala, Sweden). The intrinsic peroxidase activity was quenched by the incubation of the membrane in 3% H_2O_2 for 15 min followed by the incubation in 5% BSA dissolved in TTBS (Tris-buffered PBS – Tween 20, pH 7.5) for 1 h at room temperature. Thereafter, the membrane was treated with sheep anti human protein C primary antibody and subsequently with peroxidase-conjugated rabbit anti sheep IgG secondary antibody (Dako A/S, Glostrup, Denmark). Signals in the membrane were visualized using ECL detecting reagents (Amersham Pharmacia Biotec, Uppsala, Sweden).

ELISA

Detection of the rhPC concentrations in rabbit milk were accomplished by the polyclonal Asserachrom Protein C ELISA kit (Diagnostica Stago, Ansieres, France). Shortly, 200 µl of milk samples were incubated for 2 h into 96-well Immulon plates pre-coated with rabbit anti-human protein C antibody. After a washing step, 200 µl of rabbit anti-human protein C antibody coupled with peroxidase was added and incubated for 2 h at room temperature. All samples were washed again and thereafter incubated with 200 µl OPD/H_2O_2 substrate for 3 min. The reaction was stopped by addition of 50 µl of 3M H_2SO_4 and the absorbancies determined at 492 nm were compared with the established standard curve.

Assay for the biological activity

The biological activity of the rhPC in the milk samples was measured using an activated partial thromboplastin time (APTT) assay (Velander *et al.*, 1991). The APTT reagent included Protac (Agkistrodon contortix venom) from Diagnostica Stago, Ansieres, France to specifically activate hPC (or rhPC derived from milk samples added at different dilutions) which inhibits factors Va and VIIIa prior to the addition of $CaCl_2$ to initiate coagulation. Thus the activation of human protein C results in an increase in the APTT.

RESULTS

Generation of transgenic rabbits and detection of the WAP-hPC gene integration

Altogether 140 zygotes obtained from donor rabbits were injected with the WAP-hPC gene construct into the male pronuclei. The microinjected eggs were transferred into the oviducts of 10 recipients and 30 offspring were born after the transfer.

PCR analysis of the genomic DNA isolated either from blood or from ears (Figure 1) revealed integration of the heterologous gene in one male rabbit born after the transfer (F_0). This male founder was bred with wild-type (wt) females and transmitted the heterologous WAP-hPC gene (Table 1) into F_1 (4 rabbits – one male and three females of 16 born being transgenic). In F_2 generation, 25 transgenic rabbits of 47 rabbits were born (9 females and 16 males). Several transgenic rabbits were further crossed with non-transgenic counterparts to obtain F_3. In F_3, the integration of the heterologous construct was evaluated either by analyzing born rabbits (53 rabbits of 93 rabbits born being transgenic) or by PCR amplification accomplished on the embryos at the blastocyst stage obtained from anesthetized females (46% rabbit embryos being transgenic).

In summary, the transgenic rabbits were apparently normal and crossing of transgenic rabbits with non-transgenic counterparts yielded litters of normal size (7 ± 0.27) with 45% representation of the transgenic allele in 156 analyzed offspring, thus proving its Mendelian genetic distribution. No disturbances in the lactation of transgenic rabbits were observed, however, about 10% of the rabbits in F_1 and F_2 died before reaching their adult age.

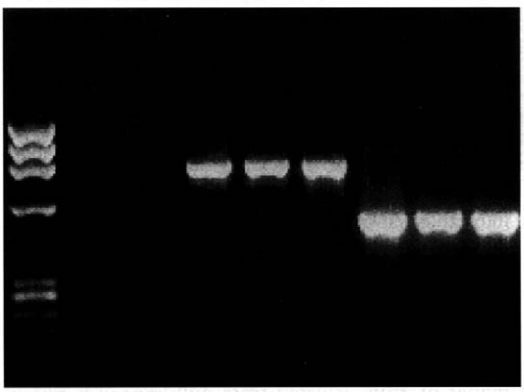

Figure 1. Detection of the hPC gene integration by PCR analyses. The size of the PCR amplified product using primers as described in Material and Methods was 811 bp (lanes 4, 5, 6; lane 2 as a negative control from non-transgenic rabbit) and 501 bp (lanes 7, 8, 9; lane 3 – negative control from non-transgenic rabbit), respectively. Migration of the DNA marker (PhiX 174/ HaeIII) is shown in lane 1

Table 1. Generations of transgenic rabbits

Generation	No. of newborn rabbits	No. of transgenic rabbits	Sex of transgenic rabbits
F_0	30	1/30 (3%)	1♂
F_1	16	4/16 (25%)	1♂, 3♀
F_2	47	25/47 (53%)	16♂, 9♀
F_3	93	53/93 (57%)	33♂, 20♀

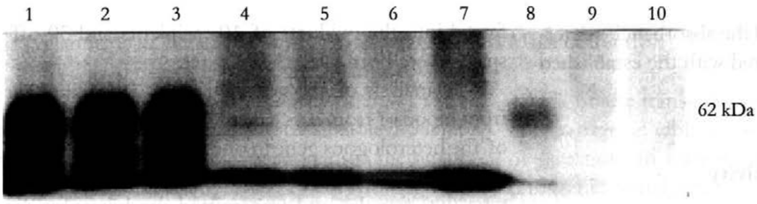

Figure 2. Western blot at non-reduced conditions using sheep anti human protein C antibody and rabbit anti sheep IgG antibody. Detected band of approximate size of 62 kD corresponded to the expected size of the hPC. Lanes 1, 2 and 3 – hPC (2.5 μg, 1.25 μg and 0.62 μg of hPC), lanes 4 – milk from transgenic rabbit of F_1 generation (dilution 1 : 10), lanes 5, 6, 7 – milk from transgenic rabbits of F_2 generation (dilution 1 : 10), lane 8 – human plasma (dilution 1 : 40), lane 9 – milk from non-transgenic rabbit (dilution 1 : 10), lane 10 – milk from transgenic female No. 8 (dilution 1 : 10) incubated with pre-immune serum only

Detection of the rhPC expression

The presence of rhPC in rabbit milk samples was tested in non-reducing conditions using a sheep anti human protein C antibody followed by incubation with a rabbit anti sheep IgG antibody. In milk samples of transgenic females (Figure 2, lanes 4 to 7) a band of similar size (approximately 62 kDa) as detected in human plasma (Figure 2, lane 8) was observed. The milk sample derived from non-transgenic females (negative control – Figure 2, lane 9) and the milk sample of transgenic female which was incubated with pre-immune serum only (Figure 2, lane 10) showed no specific signal.

The concentrations of rhPC in the milk of lactating transgenic female rabbits determined by ELISA varied between 0.24 and 0.56 μg/ml. In the assay for biological activity the addition of milk from transgenic rabbits resulted in increased APTT by 1.5%.

DISCUSSION

Capability of transgene transmission

One of the requirements for transgenic animals used for the production of therapeutic proteins is the stability of transgene transmission being accompanied by a steady secretion of the recombinant protein. In the present study using transgenic rabbits as a model, we demonstrated the transmission of the integrated heterologous construct and the presence of rhPC in the milk of transgenic rabbits during multiple lactation in F_1 generation and in F_2 generations. This is consistent with the report of Van Cott *et al.* (1997), who showed a stable secretion of rhPC secreted in the mammary gland of multiple lines of transgenic pigs over multiple lactations, being transmitted to offspring in a Mendelian fashion.

hPC gene expression

In order to establish transgenic rabbits, we used a fusion construct consisting of a 4.2 kb long WAP mouse promoter and a 9.4 kb genomic clone of human protein C gene which was previously used for establishment of transgenic mice and pigs expressing rhPC in their mammary gland. In mice, the use of this construct resulted in the rhPC secretion at values up to 0.7 mg/ml (Drohan *et al.*, 1994) that are much higher than those (0.03 mg/ml) obtained with the WAP-hPC cDNA construct (Velander *et al.*, 1991). In established transgenic pigs this construct provided rhPC expression ranging between 0.1 and 1.8 mg/ml (Van Cott *et al.*, 1997). These expression rates were comparable with those obtained with a 2.6 kb WAP-hPC cDNA construct in trangenic pigs (Van Cott *et al.*, 1997). In our study, the expression rates of rhPC in established transgenic rabbits ranged between 0.24 and 0.56 μg/ml. This relatively low expression rate might be influenced by the used animal species or might be the subject of position effects of the integrated construct.

In established transgenic pigs the anticoagulant activities of rhPC isolated from milk ranged between 70% and 150% compared with the anticoagulant activity of hPC derived from human blood (Velander *et al.*, 1992; Lee *et al.*, 1996), thus indicating that the expressed rhPC is active. The accomplished assay in our study indicates that rhPC expressed in the milk of transgenic rabbits might be active and a relatively slight increase in APTT might be correlated with low rhPC concentration in the milk of transgenic rabbits.

In conclusion, by microinjection of the WAP-hPC gene into the male pronucleus we have produced transgenic rabbits expressing recombinant human protein C in their mammary gland. Our study confirms that rhPC can be steadily secreted over multiple generations and lactations. As the secretion level of the obtained rhPC is very low, expedience of rabbit as a source for the production of human protein C still remains under consideration.

Acknowledgments

We would like to thank Dr. Ivankova from National Center of Haemostasis and Thrombosis, Faculty Hospital, Martin, Slovakia for accomplishment of ELISA tests and determination of biological activities of protein C in the milk of transgenic rabbits and Margaret Griffiths from Technoclone, Vienna, Austria for her constructive advice.

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Received: 01–08–23
Accepted after corrections: 02–01–1

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Degradability and intestinal digestibility of crude protein and amino acids of extracted rapeseed meal

Degradovatelnost a střevní stravitelnost dusíkatých látek a aminokyselin u řepkového extrahovaného šrotu

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ABSTRACT: Samples of extracted rapeseed meal (RM) were incubated using the "nylon bag" method in two steers fitted with rumen cannulas in intervals of 0, 2, 4, 8, 16, 24, and 48 hours. The effective degradability of crude protein (CP) and amino acids (AA) in the feed was calculated at the outflow rate of 0.05 per hour. Correction of microbial contamination was not performed. Effective degradability (DEG) of CP in RM was determined to amount to 68.1% and that of AA in the following sequence: Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys a Arg was 73.8%, 67.2%, 69.5%, 65.9%, 65.4%, 77.0%, 70.1%, 72.4%, 69.8%, 70.8%, 69.8%, 73.1%, 68.8%, 66.4%, 69.8%, 71.1% and 72.6%, respectively. Intestinal digestibility (DSI) of CP in the diet was determined by the "mobile bag" method. The calculated value of DSI was 65.9%. The results were compared with accessible literature sources. We can state that during the comparison of DEG values in RM with literature data good coincidences were attained. Determined DSI values were lower than those detected by most authors. Henceforth, it is necessary to standardise the above-mentioned DEG and DSI assessment methods.

Keywords: extracted rapeseed meal; crude protein; amino acids; effective degradability; intestinal digestibility

ABSTRAKT: Vzorky řepkového extrahovaného šrotu (RM) byly inkubovány metodou „nylon bags" na dvou volech opatřených ruminální kanylou v časových intervalech 0, 2, 4, 8, 16, 24 a 48 hodin. Efektivní degradovatelnost (DEG) byla vypočítána pro dusíkaté látky (CP) a jednotlivé aminokyseliny (AA) při výtokové rychlosti 0,05 h. Korekce na mikrobiální kontaminaci nebyla prováděna. Efektivní degradovatelnost (DEG) dusíkatých látek u RM byla stanovena 68,1 % a DEG AMK v pořadí Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys a Arg byla 73,8 %, 67,2 %, 69,5 %, 65,9 %, 65,4 %, 77,0 %, 70,1 %, 72,4 %, 69,8 %, 70,8 %, 69,8 %, 73,1 %, 68,8 %, 66,4 %, 69,8 %, 71,1 % a 72,6 %. Střevní stravitelnost (DSI) byla stanovena metodou „mobile bags". Zjištěná hodnota DSI byla 65,9 %. Výsledky byly srovnávány s dostupnými literárními zdroji. Můžeme konstatovat, že bylo dosaženo dobré shody při stanovení DEG u RM s literárními údaji. Námi zjištěná hodnota DSI byla nižší, než stanovení většiny autorů. Nadále je nutné standardizovat postupy výše uvedených metod stanovení DEG a DSI.

Klíčová slova: řepkový extrahovaný šrot; dusíkaté látky; aminokyseliny; efektivní degradovatelnost; střevní stravitelnost

A new system of crude protein (CP) evaluation in ruminants, based on the French system "Protéines vraies réellement Digestibles dans l'Intestin grêle – PDI" INRA (1987) and Vencl *et al.* (1991), has been adopted in the Czech Republic. Determination of effective degradability (DEG) of crude protein and amino acids (AA) in the rumen and actual digestibility of undegraded crude protein in the small intestine (DSI) is one of the basic parameters in the evaluation system (Sommer *et al.*, 1994).

Partially supported by the Ministry of Agriculture of the Czech Republic (Project No. QD 0211).

Extracted rapeseed meal (RM) is currently a common feed component in cattle and sheep nutrition (in Europe). Lactating cows can be fed this meal, after gradual adaptation, to a maximum of 1.5 kg per day (Labuda *et al.*, 1982). Zeman (1990) reported that RM can represent 15% of feed intake of high-yielding dairy cows while a rapeseed "00" meal (with low glucosinolate content) can exceed this amount. Cattle may tolerate a high intake of RM (20–30% of dietary DM = dry matter) without any adverse effect provided that RM is of the "00" quality (Kalač and Míka, 1997).

Canola, as one of the rape cultivars (*Brassica* sp.), is an important oil crop in western Canada. This variety contains a low level of glucosinolate and erucic acid. Moreover, it has more than 40% of crude protein in the dry matter (DM), therefore it is added as a protein supplement to the feed of ruminants (Kendall *et al.*, 1991).

Canola meal is used as a protein supplement in the diet of lactating cows. The percentage of essential AA in canola meal is nearly equivalent to that of milk protein. In canola meal, the limiting essential amino acids Ile, Val, Lys, and Leu contain 54–73% of the corresponding content of these AA in the milk protein. In samples of canola meal incubated for 16 hours, the primary limiting AA was Ile followed by Lys, Val, His and Leu, which accounts for 20 to 25% of corresponding AA in the milk protein (Moshtaghi Nia and Ingalls, 1995).

Based on degradability characteristics, Fox *et al.* (1990) divided feed protein into five fractions: 1) non-protein nitrogen considered to be a sub-fraction of soluble protein, 2) rapidly degraded soluble true protein, 3) intermediately degraded pure protein, 4) slowly degraded pure protein, and 5) unavailable protein.

The objective of the present study was to estimate the value of effective degradability of CP and AA of rapeseed meal by the "nylon bag" method and to determine the value of intestinal digestibility of rapeseed meal by "mobile bag" method and to compare these values with the literature data.

MATERIAL AND METHODS

The contents of dry matter (DM), CP, fat, ash and AA in RM are shown in Table 1. Degradability of RM was measured by the "nylon bag" method (Orskov and McDonald, 1979). CP and AA degradability were determined in two steers of Czech Pied breed. The animals were fitted with permanent rumen cannula and given a daily ration of 6 kg hay, 1 kg wheat, mineral and vitamin supplements and *ad libitum* barley straw. The animals were fed twice daily with free access to water. CP and AA disappearance was measured in 0, 2, 4, 8, 16, 24 and 48 hours. The weight of original material in individual bags was 2 g. The active surface of the bags was 15 mg dry matter of the feed/cm^2 surface (pore size of 42 microns). Before rumen incubation, the bags were immersed in warm water for 10 minutes to determine the zero incubation interval. Rumen incubation was carried out using a cylindrical carrier (Třináctý *et al.*, 1996). After incubation, except the zero incubation interval, all bags were rinsed in water to remove the coarse content of the rumen from the bag surface, and subsequently washed 3 times in a washing machine (with no spinning). In each animal, three bags of the same sample were incubated during each incubation interval. To determine the CP disappearance, six values were considered in each incubation interval. After incubating and drying at the temperature 60°C, the content of each bag was analysed to determine the CP content. For the analysis of AA six bags were also incubated at each time of interval but only two samples for each interval were analysed. Each of them was made by the mixing of three bags from each time interval. The disappearance, means ($\bar{x}$) and standard deviations (s.d.) of crude protein (CP) and AA were subsequently calculated for all incubation intervals. For the calculation the 6.1 version of STATGRAPHICS programme (Manugistics, Statgraphics, Inc. Rockville, Maryland, USA) was used.

The measured values were not corrected for bacterial nitrogen content. CP and AA degradability was estimated using the equation according to Orskov and McDonald (1979).

Table 1. Content of crude protein (CP), fat, ash and AA in extracted rapeseed meal (g/kg DM)

Extracted rapeseed meal (n = 2)			
CP	372.40	Gly	21.73
Fat	25.60	Ala	18.54
Ash	75.90	Val	24.24
Cys	10.18	Ile	18.17
Met	9.13	Leu	29.63
Asp	32.73	Tyr	8.81
Thr	17.76	Phe	16.92
Ser	15.27	His	13.92
Glu	68.10	Lys	24.88
Pro	23.41	Arg	28.02

Intestinal digestibility of rumen undegraded CP was determined by the"mobile bag" method (Frydrych, 1992 and Homolka *et al.*, 1996).

For the assessment of DSI two Black Pied cows (weight 790 and 770 kg) fitted with simple duodenum T-cannula were used. The feed ration was composed of 4 kg of lucerne hay, 10 kg of maize silage and 1 kg of barley.

Samples of rapeseed meal were incubated in the rumen for 16 hours. Dried at the temperature 50°C and homogenised 0.8 g of RM were put into 4 cm × 4 cm nylon bags (pore size of 42 microns). The bags were sealed and incubated in a solution of 0.1 N HCl and pepsin for 2.5 hours at 39°C under continual stirring. The bags were inserted throughout the cannula into duodenums of cows (twenty bags at a time – 10 bags in each animal at intervals of 10 min).

The bags were collected for a period of 24 hours – since the insertion into the intestine, rinsed with tap water for 2 hours and weighed after drying at the temperature 50°C. After calculating CP disappearance, the intestinal digestibility was determined, 13 bags were recovered and evaluated.

According to Kacerovský *et al.* (1990) the chemical score (CHS) for feed and undegradable residues of essential amino acids (except tryptophan) was calculated according to the equation:

$$CHS = \frac{AA_t}{AA_s} \times 100$$

where: AA_t – content of the limiting amino acid in tested protein

AA_s – content of the same amino acid in standard protein

According to Labuda *et al.* (1982) the Essential Amino Acid Index (EAAI) was calculated according to the equation:

$$EAAI = \sqrt[n]{\frac{100a \times 100b \ldots 100j}{a_v \times b_v \ldots j_v}}$$

where: a, b …..j = amino acids in tested protein (%)

$a_v, b_v \ldots j_v$ = content of the same amino acids in standard protein (%)

Casein was used as standard protein (the values according to Zeman *et al.*, 1995)

Analytical evaluation of DM, CP, fat, ash and AA content was carried out according to Anonym (1996a, b, c).

Crude protein was determined by Kjeldahl method – apparatus Kjeltec System 1002 Distilling Unit. The amino acids cystine and methionine were hydrolysed by oxidative hydrolysis (formic acid) and all samples of AA were hydrolysed by acidolysis (6 N HCL). OSTION LG ANB ionex was used for the partitioning of AA after acidolysis.

I. pH = 2.95; 0.3 M Na^+ t = 55°C; 37minutes
II. pH = 4.25; 0.3 M Na^+ t = 55°C; 19 minutes
III. pH = 7.9; 0.3 M Na^+ t = 65°C; 44 minutes

For assessment of AA containing sulphur, ionex pH = 2.95 was used; 0.3 M Na^+; t = 55°C; 35 minutes.

The analysis of samples for amino acid content did not include an assessment of tryptophan. The assessment was performed by T 339 Analyzer M of aminoacids.

RESULTS AND DISCUSSION

The contents of dry matter, CP, fat, ash and AA content of RM (Table 1) are in agreement with the characteristics of this feed given by Zeman *et al.* (1995), Boila and Ingalls (1994), Kendall *et al.* (1991). Masoero *et al.* (1994) reported that the fat content of the RM sample was 82.2 g/kg of dry matter (DM); it means that their sample was not RM. For example the paper of Zedník (1999) mentioned the fat content to amount to 19.9 g/kg of dry matter (DM).

Degradability of crude protein

The values of effective degradability of crude protein (CP) and amino acids (AA) in RM, calculated on the basis of an outflow rate (k) of 0.05 h, are presented in Table 2. CP degradability in our study was 68.1%. Similarly, Vencl *et al.* (1991) and Zeman *et al.* (1995) reported degradability of CP 69%. Sommer *et al.* (1995) found CP effective degradability of RM to amount to 65% at an outflow rate of 0.06 h conducted in 3, 6, 9, 16 and 24 hour incubation intervals. CP degradability of canola meal was 67.3% in the experiment of Boila and Ingalls (1992) with incubation intervals of 0, 1, 4, 8, 12 and 36 hours at an outflow rate of 0.05 h. The degradation parameters of Boila and Ingalls (1992) were as follows: soluble fraction 28.2%, non-soluble potentially degradable fraction 68.4, lag time 0.1 h. In our study we found the following degradation parameters: soluble fraction 18.5%, non-soluble but potentially degradable fraction 77.5%, lag time 0.8 h. Degradability of CP reported by the above mentioned authors is in agreement with our results. Homolka *et al.* (1996) also gave a similar value of CP effective degradability in RM: 73%. Masoero *et al.*

Table 2. Values of effective degradability of CP and AA (%) calculated at an outflow rate of 0.05 h

Effective degradability of extracted rapeseed meal (n = 6)			
		min.	max.
CP	68.1	65.9	69.8
Cys	73.8	71.3	75.3
Met	67.2	65.0	69.8
Asp	69.5	67.3	72.6
Thr	65.9	62.3	68.4
Ser	65.4	62.5	67.1
Glu	77.0	74.9	78.8
Pro	70.1	67.4	72.8
Gly	72.4	69.5	74.5
Ala	69.8	67.4	73.0
Val	70.8	68.3	73.9
Ile	69.8	67.8	73.2
Leu	73.1	71.1	76.1
Tyr	68.8	65.7	71.7
Phe	66.4	64.1	69.5
His	69.8	66.7	72.5
Lys	71.1	68.9	73.9
Arg	72.6	69.8	74.6

(1994) reported lower values of effective degradability of two RM samples (48.35% and 57.69%, respectively) in comparison with our results. These discrepancies could be explained by various outflow rates, different feed particle sizes. Kendall *et al.* (1991) suggested that feed processing at a high temperature could increase the amount of nitrogen permanently fixed with the fibre of feeds of plant origin, which decreases the content of available nitrogen for rumen micro-organisms. The above authors using incubation intervals of 0, 4, 8, 12, 16 and 30 hours at an outflow rate of 0.05 h determined the effective degradability of CP in five canola meal samples in the range from 44.3% to 59.0%. In this case, the average effective degradability was 51.5%, a lower value than we have found (68.1%). The correction for microbial contamination made by the authors could be the grounds for lower detected degradability. Some authors do not present effective degradability of feed, but disappearance of CP and/AA in a particular incubation interval. Disappearances of CP and AA determined in our work are shown in Table 3. Rokke (1985) determined CP disappearance in RM within an 8-hour interval to be 40%, which is less in comparison with our result (58%). A fairly low value of CP of canola meal disappearance in the 12-hour interval rumen incubation was found by Boila and Ingalls (1994) – 63.4%. In the present paper this figure was 67.9%, as an average of two values obtained within 8- and 16-hour incubation interval. According to Dakowski *et al.* (1996) CP disappearance of extracted rapeseed meal "00" was 76.4% (Danish product) and 67.9% (Polish product). This is in agreement with our results.

Degradability of amino acids

Effective degradability of: Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg, in RM was 73.8; 67.2; 69.5; 65.9; 65.4; 77.0; 70.1; 72.4; 69.8; 70.8; 69.8; 73.1; 68.8; 66.4; 69.8; 71.1 and 72.6%, respectively.

DEG of Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg in the paper of Boila and Ingalls (1992) was 72.0; 65.8; 63.2; 62.5; 62.9; 77.3; 72.0; 66.0; 63.,5; 61.7; 62.2; 64.5; 58.9; 60.8; 68.7; 67.1 and 68.6 %, respectively, which is in agreement with our results. The content of lysine, histidine and arginine after degradation was not different as compared with unincubated samples. In some samples Boila and Ingalls (1992) found a higher content of amino acids after incubation than before incubation, in the sequence: tyrosine (+27%), phenylalanine (+24%), valine (+20%), isoleucine (+17%), threonine (+17%), leucine (+10%) and methionine (+6%). A high concentration of easily degradable glutamic acid in the original diet apparently increased the content of other amino acids that were recovered from undegraded residues. Tyrosine was the least degradable amino acid in the diet due to soluble fraction, low rate of degradation and extended lag time. The values of degradability were not corrected for microbial contamination. Rokke (1985) determined the disappearance of Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg in RM to be 0.0; +33.3; +38.1; +27.3; +36.5; +16.9; +29.8; +3.9; +24.5; +30.6; +23.2; +50.0; +34.2; 0.0; +10.3 and +5.2 %, respectively, after 8 hours of incubation in the rumen. The presence of micro-organisms adhering to the diet residue that escape degradation could be another factor influencing the above-mentioned variations. The disappearances (after 12 hours of rumen incubation, canola meal) of Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys and Arg: 72.9, 63.3, 56.6, 57.7, 58.2, 79.0, 70.4, 62.2, 58.2, 55.7, 54.6, 59.5, 51.6, 56.6, 67, 64.1 and 66.2% respectively, were mentioned in the paper of Boila and Ingalls (1994). Our data based on average values from incubation intervals 8 and 16 hours

Table 3. Extracted rapeseed meal – disappearance of CP and AA in incubation intervals of 0, 2, 4, 8, 16, 24, and 48 h (n = 6)

	Time of incubation (hours)													
	0		2		4		8		16		24		48	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
Disappearance of CP (%)	**18.46**	**1.19**	**28.56**	**3.13**	**38.21**	**4.00**	**57.99**	**5.73**	**77.86**	**3.25**	**91.74**	**0.61**	**93.50**	**0.57**
Disappearance of AA (%)														
Cys	15.88	1.18	37.35	5.58	50.24	6.52	72.35	5.82	81.89	2.26	92.09	0.26	92.91	0.34
Met	15.08	1.25	23.11	9.59	34.16	9.39	61.47	7.67	73.99	4.05	92.99	0.44	95.59	0.13
Asp	30.13	1.29	34.87	1.66	37.37	9.07	63.71	7.43	73.17	5.21	91.85	0.31	93.78	0.12
Thr	11.69	1.44	21.36	3.37	29.45	8.71	61.74	8.06	73.51	4.71	90.95	0.28	92.56	0.19
Ser	3.16	1.57	17.82	2.83	27.54	9.09	63.61	7.73	74.20	4.08	91.21	0.29	91.65	0.23
Glu	29.21	1.12	44.50	2.84	53.41	6.01	73.66	5.63	82.93	3.14	95.13	0.16	96.67	0.08
Pro	17.29	1.32	35.13	6.08	41.54	6.83	65.70	7.09	76.88	3.47	90.70	0.28	92.74	0.21
Gly	25.15	1.44	36.69	3.26	45.98	6.58	67.29	6.98	77.70	3.99	92.73	0.24	94.69	0.18
Ala	18.19	1.71	31.12	3.38	40.14	7.20	64.17	7.68	75.59	4.33	92.76	0.25	95.09	0.13
Val	26.45	1.44	33.73	3.00	43.05	7.02	64.42	7.39	75.84	5.38	92.42	0.25	94.60	0.13
Ile	25.87	3.06	34.27	1.93	41.97	8.07	62.49	7.73	73.97	5.16	92.14	0.26	94.74	0.11
Leu	28.94	1.04	36.97	2.45	49.58	6.35	66.70	6.97	78.18	4.22	93.78	0.22	95.41	0.11
Tyr	23.17	2.68	29.99	2.30	32.03	9.12	62.23	7.90	74.84	4.74	93.64	0.47	95.16	0.11
Phe	14.94	1.69	26.33	2.53	35.22	8.81	61.37	8.02	71.43	5.95	91.83	0.30	93.45	0.14
His	16.38	1.41	32.53	5.00	38.38	7.51	65.27	7.33	75.52	4.10	92.31	0.26	95.37	0.13
Lys	23.36	0.79	35.96	2.45	44.44	7.78	64.73	7.17	76.08	5.03	93.03	0.25	95.47	0.10
Arg	18.63	1.73	34.83	3.96	44.71	6.49	68.17	6.89	78.92	4.02	94.31	0.18	96.18	0.12
Mean of disappearance of AA (%)	20.21		32.15		40.54		65.24		76.16		92.58		94.47	

are in good agreement with the above-mentioned results of Boila and Ingalls (1994). According to Dakowski *et al.* (1996) the disappearances of Cys, Met, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Ile, Leu, Tyr, Phe, His, Lys a Arg in RM of Danish product (sample a) and Polish product (sample b) after 16 hours of rumen incubation were: 79.3; 75.0; 72.4; 68.9; 69.9; 81.9; 73.6; 74.2; 73.0; 70.4; 70.8; 72.0; 66.7; 69.9; 75.7; 71.7 and 76.9 (sample a), and 74.7; 66.6; 63.4; 61.1; 62.3; 75.7; 65.9; 66.7; 64.1; 61.3; 63.0; 64.9; 56.5; 62.3; 69.5; 65.4 and 69.6 (sample b), respectively. Our data based on 16-hour incubation was in agreement with the above-mentioned results, particularly with those of Danish product.

The values of AA disappearances determined in our work are in agreement with those of Dakowski *et al.* (1996) regarding sample a. Moshtaghi Nia and Ingalls (1995) found the disappearance of AA in canola meal within an incubation interval of 1, 8, 16, and 24 hours to amount to 27.4%, 50.8%, 63.8% and 80.3%, respectively. However, our results for the corresponding incubation intervals were higher in comparison with the above data of Moshtaghi Nia and Ingalls (1995).

The chemical composition/score (CHS) of essential amino acids was calculated for feed and non-degradable residues. Based on the CHS values the sequence of the first three limiting amino acids for original RM was assessed, namely for methionine, leucine and lysine (CHS for Met, Thr, Val, Ile, Leu, Phe, His, Lys a Arg was 30.54; 42.18; 36.24; 34.62; 30.80; 33.90; 48.33; 33.35 and 91.57). After 8-hour incubation of RM in the rumen (the value of effective degradability CP in RM was calculated closest to this time of incubation) the sequence of the first three essential AA was assessed on the basis of calculated CHS, namely for leucine, methionine and lysine (CHS for Met, Thr, Val, Ile, Leu, Phe, His, Lys a Arg was 11.71; 16.15; 12.86; 12.95; 10.29; 13.03; 16.67; 11.80 and 29.08).

Essential Amino Acid Index was calculated for primary sample of RM (39.76%), after 8 hours of incubation in the rumen (14.24%) and after 16 hours of incubation in the rumen (9.81%).

Intestinal digestibility of rumen undegraded crude protein

We found CP intestinal digestibility (DSI) in RM to be 65.9%, which is consistent with the data of Homolka *et al.* (1996) 63%. The value of intestinal digestibility of rumen undegraded CP is presented in Table 4.

However, the results of Vencl *et al.* (1991) or Zeman *et al.* (1995) are somewhat higher: 75.0%. Dakowski *et al.* (1996) found DSI 70.2% in sample (a), and 74.1% in sample (b). The author used bags with porosity 9 μm and the active area 6 × 6 cm. Rokke (1985) using a 2.5 × 6 cm bag with porosity of 10 μm reported DSI in RM to be 75.6%. The weight/surface ratio and porosity of the bag are known to influence degradability measurements. It is however questionable whether different results obtained by Rokke (1985) and Dakowski *et al.* (1996) in comparison with our results could be attributed only to the above-mentioned conditions. Masoero *et al.* (1994) recorded higher values of intestinal digestibility of two RM samples 78.04% and 78.09%, in our case it was 65.9%. Kendall *et al.* (1991) determined DSI in five samples of canola meal and the values ranged from 82.9% to 90.7%. The average intestinal digestibility was 87.1%. Because of the DSI determination the author did not describe the applied procedure. Boila and Ingalls (1994) determined DSI in canola meal to amount to 90%, however incubation in the rumen was performed in a 12-hour interval.

Table 4. Values of intestinal digestibility of rumen undegraded CP (%)

	Extracted rapeseed meal
Intestinal digestibility (DSI)	65.9
n	13
Standard deviation	0.8

CONCLUSION

CP and AA disappearance and effective degradability of extracted rapeseed meal determined in our experiment were in good agreement with the literature data, similarly like the determined values of CP and AA degradability. The determined value of intestinal digestibility of rumen undegraded CP was lower than the values reported by most authors.

Further specification of the above-mentioned methods of DEG and DSI assessment is recommended.

Acknowledgements

The authors thank all staff of the Central Institute for Supervising and Testing in Agriculture for their assistance in research.

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Received: 01–08–21
Accepted after corrections: 02–02–26

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A comparison of selected quality parameters of the meat of Czech Pied and Montbéliard bulls

Porovnání vybraných jakostních parametrů masa býků českého strakatého a montbéliardského plemene

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ABSTRACT: Selected quality parameters of two milked dual-purpose breeds (Montbéliard and Czech Pied) were compared. Samples of "pure" sirloin *(musculus longissimus pars thoracis)* taken on the level of the 9th–11th thoracic vertebra were subjected to chemical and physical analyses. The bulls were fattened under the same conditions of management and to the same age, i.e. 524–527 days. At the end of the experiment the slaughter weight of the Montbéliard bulls was 538 kg and the meat yield was 76.74%. In the control group of Czech Pied cattle these values were insignificantly higher, i.e. 557 kg and 77.58%, respectively. In the majority of nutritional and technological characteristics of beef quality the differences between the control group of Czech Pied cattle and Montbéliard cattle were not significant. More significant are the differences in the content of intra-muscular fat, energy value of beef and the content of hydroxyproline, which is directly correlated with the tenderness of meat, and that were lower in the Montbéliard breed ($P < 0.05$). Investigations of the technological quality parameters of beef revealed a significant difference only in the content of muscle pigments, which indicates a tendency of the Montbéliard breed to produce lighter meat. The higher average reflectance confirms this conclusion in the group of bulls. The imported French breed showed a statistically insignificantly increased cut area of the muscle *(musculus longissimus pars thoracis)* on the level of the 9th thoracic vertebra and a decrease in the average thickness of muscle fibres. The overall evaluation of quality parameters of beef of the Montbéliard breed indicates that the imported milked breed did not negatively affect the characteristics of beef on the domestic market if the bulls were fattened to a slaughter weight of 550 kg.

Keywords: cattle; Montbéliard; beef; nutritional quality; technological quality

ABSTRAKT: V práci jsme provedli porovnání zvolených jakostních parametrů masa dvou dojených plemen s kombinovanou užitkovostí (montbéliard, české strakaté). Chemicko-fyzikální analýzy byly provedeny u vzorků „čisté" svaloviny roštěnce *(musculus longissimus pars thoracis)* odebraných na úrovni 9. až 11. hrudního obratle. Býci byly vykrmováni ve stejných chovatelských podmínkách do shodného věku (524–527 dnů). Na konci pokusu dosáhli býci montbéliardského plemene porážkové hmotnosti 538 kg, s výtěžností masa 76,74 %. U kontrolní skupiny českého strakatého skotu byly hodnoty uvedených ukazatelů nevýznamně vyšší (557 kg, 77,58 %). U většiny nutričních a technologických charakteristik jakosti hovězího masa jsme nezjistili signifikantní diference mezi kontrolní skupinou českého strakatého skotu a plemenem montbéliard. Za významnější rozdíly ($P < 0.05$) ve prospěch plemene montbéliard lze považovat nižší hodnoty obsahu vnitrosvalového tuku, energetické hodnoty masa a obsahu hydroxyprolinu, který má přímý vztah k jeho křehkosti. Při sledování technologických ukazatelů jakosti masa byl stanoven signifikantní rozdíl pouze v obsahu svalových pigmentů, který naznačuje tendenci k produkci světlejšího masa montbéliardským plemenem. Tento závěr v souboru býků potvrzuje i vyšší průměrná hodnota remise. U importovaného francouzského plemene jsou statisticky nevýznamné tendence ke zvýšení plochy řezu svalovinou *(musculus longissimus pars thoracis)* na úrovni 9. hrudního obratle a ke snížení průměrné síly svalových vláken. Z celkového hodnocení jakostních parametrů masa plemene montbéliard vyplývá, že importované dojené plemeno při výkrmu býků do porážkové hmotnosti 550 kg negativně neovlivní jakostní charakteristiku hovězího masa na domácím trhu.

Klíčová slova: skot; montbéliard; maso; nutriční ukazatele; technologické ukazatele

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 4321 0001).

In the past decade, in connection with the increased import of breeding animals to the Czech Republic, the breeding of the dual-purpose breed – Montbéliard of French provenance increased. This breed is phylogenetically related to the domestic population of Czech Pied cattle. The Montbéliard breed has lately been bred as purebred cattle and in the framework of improvement of Czech Pied cattle the breeding bulls and insemination doses have been used for more than 25 years. The breeding of these animals is focused on milk production and the bulls not included into breeding are fattened and contribute to the production of slaughter cattle and beef. Due to increasing requirements for the nutritional and technological quality of beef, a comparative analysis of the results of selected quality parameters of beef of the Czech Pied and Montbéliard bulls is given in the present paper.

Meat is an important foodstuff and one of the most expensive components of human nutrition; therefore the quality parameters predetermining the method and form of its processing and culinary use have been evaluated. Due to its high content of biologically valuable nutrients and its ready and versatile use meat is among the most customary foods in the food chain. The trends in the consumption of the different kinds of meat are largely influenced particularly by health, breeding and processing factors that immediately affect the criteria of meat quality coming to the fore of consumers' attention.

The selection of commercial type and breed, age and weight at the end of fattening, technique and technology of fattening and the standard of nutrition are among the most important breeding factors that immediately affect the quality parameters of beef intended for processing or cutting. Many papers published in foreign countries and in the Czech Republic were focused on analyses of the quality parameters of beef from various aspects of production. However, only a few works published the resulting differences in evaluations of some quality parameters of the carcass and partly also meat of the Montbéliard breed and its crosses (Makarenko *et al.*, 1988; Šubrt and Župka, 1988; Golda *et al.*, 1989; Warzecha *et al.*, 1995; Mikšík *et al.*, 1996; Šubrt *et al.*, 1997; Aumaitre, 1999).

Considerably fewer analyses have been focused on evaluations of the quality parameters of meat of purebred Montbéliard bulls. Meat such as skeletal muscles with inter-muscular and intra-muscular fat is meat of the highest quality parameters important both for the consumer and the processor. For the consumer the most important is the nutritional quality expressed by the content of dry matter, proteins and fat, amino acid and fatty acid profile, energy value and content of macro and micro elements – ash. Important properties that influence the immediate quality of meat and attract the consumers' interest are the colour and sensory properties (taste, aroma, juiciness, tenderness) and whether the meat is suitable for cooking specific meals (Jedlička, 1988; Ingr, 1996; Pipek, 1997). Golda *et al.* (1989) discovered that the values of dry matter in the *musculus longissimus pars thoracis* and prime rib of the Czech Pied and Montbéliard crosses were by 0.46% and 3.04%, respectively, higher compared with the Czech Pied cattle. The protein content in the *musculus longissimus pars thoracis* of the crosses was 20.49%. Out of the technological parameters of quality the authors reported losses of the water absorption capacity, 22.87% in the crosses and 23.35% in C. The water absorption capacity was significantly higher in the crosses than in the Czech Pied cattle (i.e. 22.87% and 13.18%, respectively).

MATERIAL AND METHODS

Parallel experimental fattening was conducted with 29 purebred Montbéliard bulls and 23 Czech Pied bulls. The Montbéliard bulls were the progeny of 9 breeding bulls and the Czech Pied bulls were the progeny of 6 breeding bulls. Fattening was terminated basically at the same age, i.e. between 524 and 527 days. The feed ration of both groups consisted of maize silage, clover-grass hay with a supplement of dry roughage and concentrates. The feed ration was modified according to the weight of the animals in both groups. Selected parameters of the nutritional and technological quality of meat (the *musculus longissimus pars thoracis* – MLT) taken on the level of the 9th to 11th thoracic vertebrae were analysed. Chemical analyses were based on classical methods of analysis. Kjehdahl's method was used to determine the content of total N, the extraction method was applied to define the content of intramuscular fat using the petroleum ether solvent. For the parameters of technological quality we defined the pH value of the muscles 48 hours *post mortem*, the colour was evaluated on the basis of reflectance per cent (wave length 542 nm) and content of muscle pigments, for the water absorption capacity we used the press method and the content of fibrous proteins was determined by means of the content of hydroxyproline. The analyses were supplemented with an analysis of the average diameter of muscle fibres in the MLT determined from

150 measurements of each sample and the MLT area was determined planimetrically on the level of the 9th thoracic vertebra.

Mathematical and statistical tests of the average data in the groups were carried out using the statistical UNISTAT 5.0 package and two selective *t*-tests, with a 95 and 99% level of significance.

RESULTS AND DISCUSSION

Table 1 gives the basic characteristics of the meat efficiency of Czech Pied bulls (control group) and Montbéliard bulls. The difference in the weight of bulls before slaughter in the experimental groups was insignificant, only 19 kg in favour of the Czech Pied slaughter bulls. The difference in the slaughter weight is a result of different life-long growth intensity expressed by the average daily gain (–40 g/day). At the end of the fattening period the bulls weighed 557 and 538 kg, respectively. The weight is relatively low due to the fact that the experimental fattening was adapted to the methods of testing the progeny of breeding bulls. If commercial fattening is applied, the slaughter weight may reach as much as 700 kg (Reeb, 1988) and the daily gain over the entire period of fattening is mostly around 1 000 kg (Mikšík *et al.*, 1996). The dressing percentage of Montbéliard bulls was also insignificantly lower (57.62% and 58.51%, respectively) and was significantly ($P < 0.05$) reflected in the lower carcass gain (–34 g/day of life). The resulting dressing percentage corresponds to the values of Czech Pied, Montbéliard and other genotypes bred in the Czech Republic and published by Golda *et al.* (1989) and Teslík *et al.* (1995). In the dressed carcasses of the control group (Czech Pied cattle) the losses caused by cooling were found to be significantly higher (+0.26%). The meat yield from the dressed carcass and the meat/bone ratio in the control group were insignificantly higher, i.e. 77.58% and 76.74%, respectively, and 3.96 and 3.76, respectively. Golda *et al.* (1989) published a significantly more favourable meat/bone ratio (i.e. 5.07) in the Czech Pied and Montbéliard crosses. In the Czech Pied cattle Šubrt *et al.* (1995) and Teslík *et al.* (1995) reported lower values of this indicator characterising the meatiness of the carcass, i.e. 4.14 and 4.05, respectively.

In the sirloin with intra-muscular fat the present authors determined an insignificantly lower dry matter content in Montbéliard slaughter bulls (–0.11%) due to the significantly lower ($P < 0.05$) content of intra-muscular fat (–0.28%), Table 2. In their conclusions Golda *et al.* (1989) reported an increased content of dry matter in the crosses of Montbéliard breed with Czech Pied breed. This conclusion was not confirmed by the analyses of meat of purebred animals carried out in the present work and the conclusions of evaluations of meat quality conducted in muscle samples suggest that the content of intra-muscular fat was significantly lower in the Montbéliard breed. The production of intra-muscular fat in the studied breeds is in accordance with the conclusions of Makarenko *et al.* (1988) and Warzecha *et al.* (1995). Insignificant differences in the nutritional quality of meat were found between the breeds in terms of the content of total protein (Montbéliard = +0.10%) and ash (Montbéliard = +0.04%). Significant ($P < 0.05$) differences were found between the control and experimental group (i.e. 0.27 and 0.23 mg) in the average values of the content of the fibrous amino acid hydroxyproline, which monitors the amount of collagen in the fibrous tissue and is indirectly correlated with the consistency of meat. The analysed energy values of meat (5.33 and 5.16 MJ/kg) correspond largely to the content of intra-muscular fat and the difference in the average values of the groups was not significant. With regard to the lower content of intra-muscular fat and lower energy value, from the medical point of view the meat of Montbéliard bulls slaughtered at the above given weight and age is considered to be superior.

Evaluations of the technological quality of meat were based on 6 parameters (Table 3). From the concentrations of hydrogen ions 48 hours *post mortem* (pH_{48}) we estimated the course of glycolysis as a part of the early *post mortem* changes in the meat. The average pH_{48} values of the meat in both groups of bulls did not exceed the critical value (6.2) limiting the incidence of the DFD syndrome although the maximal individual values of 3 animals in both groups were above the limit. Jedlička (1988) reported that the resulting value of pH 24–48 hours after slaughter was around 5.5 and he stated that the optimal pH value at this *post mortem* stage should not be higher than 5.7. The values of water absorption, which characterizes the technological and culinary use of meat, and of reflectance, which is a parameter of the superficial richness of the red colour of meat, were relatively balanced (Table 3). The meat of Montbéliard bulls is lighter and is based on the significantly ($P < 0.05$) lower count of muscle pigments (3.11 mg and 2.74 mg, respectively). The reflectance values are the same as in the Black Pied cattle (Ingr *et al.*, 1996). The range of the content of muscle pigments in the meat of commercial animals is relatively wide (Ingr, 1996; Pipek, 1997). Our results are in the lower half of the published ranges. The area

Table 1. Growth and carcass traits of bulls

Parameter	Statistical indicator	Breed	
		Czech Pied $n = 23$	Montbéliard $n = 29$
Slaughter weight (kg)	x	557	538
	s	53.72	47.79
	$V_{\%}$	9.64	8.89
	max.	675	630
	min.	460	435
Slaughter age (days)	x	524	527
	s	17.02	15.97
	$V_{\%}$	3.25	3.03
	max.	571	565
	min.	480	486
Average life-long daily gain (kg)	x	1.04	1.00
	s	0.10	0.09
	$V_{\%}$	9.62	9.00
	max.	1.29	1.11
	min.	0.86	0.79
Carcass gain (g)*	x	0.622	0.588
	s	0.06	0.05
	$V_{\%}$	8.85	9.16
	max.	0.741	0.670
	min.	0.522	0.449
Dressing percentage (%)	x	58.51	57.62
	s	1.50	1.72
	$V_{\%}$	2.56	2.99
	max.	61.85	61.57
	min.	54.56	54.25
Weight losses by cooling (%/24 hours)*	x	1.29	1.03
	s	0.83	0.57
	$V_{\%}$	64.34	55.34
	max.	3.41	2.24
	min.	0.00	0.00
Meat yield (%)	x	77.58	76.74
	s	1.80	1.98
	$V_{\%}$	2.32	2.58
	max.	80.82	80.77
	min.	73.98	73.20
Meat/bone ratio	x	3.96	3.76
	s	0.46	0.44
	$V_{\%}$	11.62	11.70
	max.	4.82	4.84
	min.	3.08	3.09

*$P < 0.05$

of the *musculus longissimus pars thoracis* on the level of the 9th thoracic vertebra is a supplementary and important quality parameter of the technological and culinary value of meat. Although the resulting difference in the values was insignificant, the Montbéliard cattle showed a tendency towards a larger cut area (64.70 and 67.83 cm^3, respectively). An insignificant tendency towards a smaller diameter of muscle fibres was observed in the French breed (i.e. 37.46 and 35.92 μm, respectively) and this is significantly associated with the tenderness and consistency of meat.

The establishment and interpretation of control fattening of bulls is expected to contribute to the distribution of information in the area of meat efficiency of the purebred population of Montbéliard cattle. No significant differences in the majority of nutritional and technological characters of beef quality were discovered between the control group of Czech Pied cattle

Table 2. Nutritional value of meat of bulls

Parameter	Statistical indicator	Breed	
		Czech Pied $n = 23$	Montbéliard $n = 29$
Dry matter (%)	x	24.27	24.16
	s	0.98	0.61
	$V_{\%}$	4.04	2.52
	max.	26.47	25.10
	min.	20.98	22.59
Total protein (%)	x	21.48	21.58
	s	0.61	0.51
	$V_{\%}$	2.84	2.31
	max.	22.72	22.20
	min.	19.95	20.32
Hydroxyproline (mg/100 mg of muscle)*	x	0.27	0.23
	s	0.07	0.06
	$V_{\%v}$	25.93	26.06
	max.	0.41	0.34
	min.	0.15	0.13
Fat (%)*	x	1.43	1.15
	s	0.52	0.37
	$V_{\%}$	36.36	32.17
	max.	2.70	1.90
	min.	0.86	0.21
Ash (%)	x	1.10	1.14
	s	0.09	0.05
	$V_{\%}$	8.18	4.39
	max.	1.23	1.26
	min.	0.92	1.01
Energy value (MJ/kg of original matter)*	x	5.33	5.16
	s	0.27	0.26
	$V_{\%}$	4.97	5.01
	max.	5.98	5.70
	min.	4.86	4.55

*$P < 0.05$

Table 3. Technological quality of meat of bulls

Parameter	Statistical indicator	Breed Czech Pied $n = 23$	Breed Montbéliard $n = 29$
pH_{48}	x	5.74	5.70
	s	0.29	0.39
	$V_{\%}$	5.05	6.84
	max.	6.40	6.84
	min.	5.20	5.10
Water absorption capacity (%)	x	93.62	93.21
	s	3.23	3.49
	$V_{\%}$	3.45	3.74
	max.	98.00	98.15
	min.	83.03	83.85
Reflectance (%)	x	4.79	5.03
	s	1.81	2.18
	$V_{\%}$	37.79	43.34
	max.	9.60	12.60
	min.	3.10	3.00
Muscle pigments (mg/1 g of muscle)*	x	3.11	2.74
	s	0.60	0.52
	$V_{\%}$	19.29	18.98
	max.	4.46	3.91
	min.	2.06	1.80
MLT area (cm^2)	x	64.70	67.83
	s	12.49	9.86
	$V_{\%}$	19.30	14.54
	max.	99.00	92.00
	min.	68.00	69.00
Average diameter of muscle fibres (μm)	x	37.46	35.92
	s	5.09	3.26
	$V_{\%}$	13.59	9.08
	max.	47.80	40.81
	min.	30.12	28.17

*$P < 0.05$

and the Montbéliard breed. We can consider the lower content of intra-muscular fat, energy value of meat and content of hydroxyproline, which is directly connected with the tenderness of meat, in favour of the Montbéliard breed as being more significant ($P < 0.05$). In terms of the technological parameters of meat quality only the content of muscle pigments was found to be significantly different indicating that the Montbéliard breed tends to produce lighter meat. The higher average reflectance value also confirms this conclusion. In the imported French breed a statistically insignificant tendency to an increased cut area of the *musculus longissimus pars thoracis* on the level of the 9th thoracic vertebra and decreased average thickness of muscular fibres was observed. We can conclude from the overall evaluations of the quality parameters of meat of the Montbéliard breed that the meat quality is not affected if the bulls are fattened to a weight of 530–550 kg.

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Received: 01–11–12
Accepted after corrections: 02–02–28

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Effect of heat-treated rapeseed cake on fatty acid pattern in meat of fattened bulls

Vliv upravených řepkových výlisků na zastoupení mastných kyselin v mase výkrmových býků

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ABSTRACT: Bulls, Czech Red-Pied × Ayrshire × Red Holstein crossbreds (initial live weight 90 kg), fed the basal diet supplemented with production mixture containing either soybean meal (SBM; control group; 11 animals) or heat-treated rapeseed cake (HRC; experimental group; 12 animals) were fattened to the age of 16 months. The difference between live weight of SBM bulls (546 kg) and HRC bulls (569 kg) at the end of fattening was not significant ($P > 0.05$). Loin (*musculus longissimus lumborum et thoracis*) and tallow samples were taken from each animal after slaughter and 15 fatty acids were determined by gas chromatography after lipid extraction with diethyl ether. No impairment of health status was observed in HRC-bulls as compared with the control group. Lipid contents in the loin of HRC-bulls (4.17%) and control bulls (3.69%) did not differ ($P > 0.05$). The percentage of total saturated fatty acids (SFA; 46.2%) was lower ($P < 0.05$) and the percentage of oleic acid (C18:1; 46.9%) was higher ($P < 0.05$) in loin lipids of HRC-bulls in comparison with SBM-group (49.0% and 48.2%, respectively). SFA/MUFA (monounsaturated fatty acid) ratio and C16/C18 ratio in loin lipids was lower ($P < 0.05$ and $P < 0.01$, respectively) in HRC-group than in the control group. Total polyunsaturated fatty acid (PUFA) percentage in the loin of HRC-bulls was higher ($P < 0.05$) than in bulls receiving SBM (3.3 and 2.8%, respectively). The SFA/PUFA ratio was also more favourable (lower, $P < 0.01$) in the loin of experimental group as compared with SBM-bulls. SFA deposition in the loin of HRC-bulls was lower ($P < 0.001$) and that of MUFAs was higher ($P < 0.001$) than in tallow. It was concluded that from the viewpoint of human nutrition, the following markers of the nutritive value of beef meat are more favourable than in poultry meat (based on the present and previous experiments): arachidonic acid content (<5 mg/100 g of fresh tissue and 53–73 mg/100 g, respectively) and PUFA n-6/PUFA n-3 ratio (4.3 and 10–12, respectively).

Keywords: rapeseed cake; fatty acids; arachidonic acid; meat quality; bulls

ABSTRAKT: Pokusná (12 kusů) a kontrolní (11 kusů) skupina býků, kříženců plemen české strakaté × ayrshirské × holštýnské červené, byla krmena základní krmnou dávkou na bázi sena, senáže a kukuřičné siláže, doplněnou produkční krmnou směsí se sojovým extrahovaným šrotem (kontrolní skupina, SBM), resp. tepelně upravenými řepkovými výlisky (pokusná skupina, HRC). Býci (počáteční živá hmotnost 90 kg) byli vykrmováni do věku 16 měsíců, kdy dosáhli živé hmotnosti 546 kg (SBM), resp. 569 kg (HRC). Rozdíl v živé hmotnosti nebyl průkazný ($P > 0,05$). Po poražení byly odebrány vzorky roštěnce a břišního loje a po extrakci lipidů dietyléterem bylo ve vzorcích stanoveno 15 mastných kyselin plynovou chromatografií. Ukazatele zdravotního stavu pokusné skupiny se nelišily od kontroly. Rozdíl v obsahu lipidů roštěnce pokusné (4,17 %) a kontrolní skupiny (3,69 %) byl neprůkazný ($P > 0,05$) v důsledku vysoké variability hodnot v rámci dané skupiny. Zastoupení sumy nasycených mastných kyselin (SFA) v roštěnci pokusné skupiny (46,2 %) bylo nižší, zastoupení kyseliny olejové (C18:1; 46,9 %) naopak vyšší ($P < 0,05$) ve srovnání se skupinou SBM (49,0 %, resp. 48,2 %). Poměr SFA/MUFA (mononenasycené mastné kyseliny) a poměr celkových C16 a C18 mastných kyselin v lipidech roštěnce byl u HRC býků nižší ($P < 0,05$, resp. $P < 0,01$) ve srovnání s kontrolou. Zastoupení sumy

The experiments were carried out within research project of Mendel University of Agriculture and Forestry Brno (No. MSM 43210 0001) and of the University of Veterinary and Pharmaceutical Sciences Brno (No. MSMT 16170 0002).

polynenasycených mastných kyselin (PUFA) v roštěnci HRC býků bylo vyšší ($P < 0,05$) než u kontroly (3,3, resp. 2,8 %). Také poměr SFA/PUFA v lipidech roštěnce pokusné skupiny byl příznivější (nižší, $P < 0,01$) ve srovnání s kontrolou. V rámci HRC skupiny bylo zjištěno nižší ukládání SFA ($P < 0,001$) a vyšší ukládání MUFA ($P < 0,001$) v roštěnci ve srovnání s lojem. Obdobný výsledek byl zjištěn i v rámci kontrolní skupiny. Na základě výsledků předkládané práce a předchozího pokusu lze vyvodit, že z hlediska lidské výživy má hovězí maso ve srovnání s drůbežím lepší parametry (dosahuje podstatně nižších hodnot) pokud jde o obsah kyseliny arachidonové a poměr PUFA n-6/PUFA n-3 (4,3, resp. 10–12).

Klíčová slova: řepkové výlisky; mastné kyseliny; arachidonová kyselina; jakost masa; býci

Rapeseed is currently considered to be an adequate substitution of soya in cattle diets in some European countries because rapeseed cake is cheaper than soya or cereal concentrates, and feeding costs are lower (Amman, 1996). Moreover, rapeseed has a high content of oleic acid that lowers total plasma cholesterol and low-density lipoprotein cholesterol in humans (Rule *et al.*, 1989), and a relatively low ratio of n-6 and n-3 polyunsaturated fatty acids (PUFA).

Therefore, apart from economic reasons, the relatively favourable fatty acid spectrum of rapeseed supports a possibility of using this component in diets for beef cattle also from the viewpoint of human nutrition. Meat, fish, fish oils and eggs are the only important sources of n-3 PUFA. Since the fish consumption is relatively low (in the Czech Republic very low), meat is an important source of PUFA n-3 despite the lower concentration of these fatty acids compared with oily fish (Scollan *et al.*, 2001). In ruminants, a general goal is to retain n-6/n-3 PUFA found in cattle (and sheep) fed forage diets, and simultaneously to increase the polyunsaturates : saturates ratio (Wood and Enser, 1997).

Data regarding the use of rapeseed in diets for ruminant fattening are sparse in the available literature in comparison with milk production. Rule *et al.* (1994) compared canola with soybean as protein supplements for slaughter bulls and steers with the objective to evaluate the fatty acid composition of meat, kidney and liver. Ponnampalam *et al.* (2001) recently used either unprotected rapeseed or protected canola (among other lipid supplements) with the aim to manipulate the PUFA content in lamb meat.

The objective of the present study was not to manipulate the fatty acid spectrum of beef meat, but simply to compare, from the viewpoint of this qualitative aspect, beef meat (muscle and adipose tissue, respectively) produced using the diet with soybean meal, and the diet where a substantial part of soybean was replaced by heat-treated rapeseed cake.

MATERIAL AND METHODS

Animals and diets

An experiment was carried out at the co-operative farm Žichlínek, Czech Republic. Twenty-four bulls (Czech Red-Pied × Ayrshire × Red Holstein crossbreds) with the initial average live weight 90 kg were divided into two groups by twelve using the system of analogous pairs. Stanchion housed bulls were fed the same basal diet supplemented with either control production mixture with soybean meal (SBM) or experimental production mixture where a substantial part of soybean meal was replaced by heat-treated rapeseed cake (HRC). The basal diet comprised meadow hay (1.8 to 0.4 kg per day according to the stage of fattening), grass-clover haylage (4.5 to zero kg/day) and maize silage (4.5 to 19.5 kg/day). The daily ration of experimental or control production mixture increased from 1.5 kg (until 4 months after the beginning of the experiment) to 3 kg (until 12 months) and consequently

Table 1. Composition of production mixtures for bulls

Component (%)	Production mixture	
	HRC[1)]	SBM[2)]
Wheat	9.2	15.0
Oat	45.5	48.2
Wheat bran	2.5	10.0
Soybean meal	5.0	22.0
Heat-treated rapeseed cake	35.0	–
Feed salt	1.0	1.5
Ground limestone	0.8	0.8
MgO	–	0.2
Supplementary minerals	0.5	1.8
Premix of biofactors	0.5	0.5

[1)] with heat-treated rapeseed cake
[2)] with soybean meal

Table 2. Nutrient content of production mixtures, including the quantitatively most important fatty acids and glucosinolates

Nutrient (g/kg of fresh matter)	Production mixture HRC[1)]	SBM[2)]
Crude protein	186	189
Crude fat	60	24
Crude fibre	90	77
Crude ash	87	68
Nitrogen free extract	469	523
Organic matter	805	813
Oleic acid[3)]	39.9	20.8
Linoleic acid[3)]	34.8	44.5
α-linolenic acid[3)]	10.8	13.7
Dry matter (%)	89	88
Glucosinolates (mmol/kg)	1.19	<0.05

[1)] with heat-treated rapeseed cake
[2)] with soybean meal
[3)] % of total determined fatty acids

decreased to 2.5 kg in the time period till the end of the experiment. The composition of both production mixtures is presented in Table 1, their nutritive value follows from Table 2 (parameters determined according to ČSN 46 7092).

Rapeseed cake was treated using the combination of heat, pressure, chemical substances (citric acid, calcium hydroxide) and water. Details of the procedure are protected by Certificate No. 6297 on the registration of the utility patent Feed for Farm Animals Based on Rapeseed, issued by the Office for Industrial Property, Prague, Czech Republic. Rapeseed cake after treatment (HRC) contained per kg of fresh matter (with dry matter content 91.1%): 267 g of crude protein, 66 g of crude fibre, 141 g of crude fat, 130 g of crude ash and 309 g of nitrogen free extract, and the percentage of the main fatty acids in total fatty acids was as follows: palmitic acid 8.0, oleic acid 55.4, linoleic acid 20.6 and α-linolenic acid 6.1%.

Sample preparation and determination of fatty acids

Bulls were fattened until 16 months of age. Twelve animals from the experimental group and eleven animals from the control group (one bull was culled during the experiment due to the injury on a pelvic extremity) were slaughtered at a commercial abattoir. 500 g sample of *musculus longissimus lumborum et thoracis* at the level of the 8th rib and 500 g sample of tallow of the flank at the same level were taken in all carcasses.

Meat samples were homogenized in a Moulinex blender (model D56, Moulinex, France), put into dark glass powder bottles, frozen and stored at –20°C until analyses. Total lipid fractions of loin and tallow samples, respectively, were obtained after extraction with diethyl ether under reflux for 5 hours (ČSN 57 0185). Saponification, preparation of fatty acid methyl esters (FAMEs) and FAME determination are described in detail in the paper of Komprda *et al.* (2000). The only modification in the present study in comparison with the quoted paper was an addition of internal standard (C15:0; Supelco) to the reaction flask for the FAME preparation and use of different external standards: PUFA No. 2 (Animal Source; Supelco) and Oil Reference Standard No. 6 (Sigma). The following fatty acids were determined in the loin and tallow of steers: C14:0, C16:0, C18:0, C16:1, C18:1, C20:1, C18:2 n-6, C18:3 n-6, C20:4 n-6, C22:4 n-6, C22:5 n-6, C18:3 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3. The content of the fatty acid was expressed as mg/100 of fresh tissue according to Komprda *et al.* (2001) and subsequently in per cent of all determined fatty acids.

Statistical evaluation

Basic statistical characteristics were determined and the differences between the experimental and control group in total lipid content, percentage (% of total determined fatty acids) or content (mg/100 g of fresh tissue) of individual fatty acids or group of fatty acids were evaluated using one-way classification of the variance-ratio test. The Unistat package, version 4.53 (Unistat Ltd., London, England) was used.

RESULTS AND DISCUSSION

Growth and metabolic characteristics

Although the rapeseed cake treated by the combination of heat, pressure, chemical substances and water contained a minimum amount of antinutritive substances (glucosinolates; Table 2), we considered a possible effect of rapeseed on the growth characteristics and metabolic status of steers. The mean of live weight in the experimental and control group was 569 and 546 kg at the end of fattening, live weight gain was 1 109 and 1 075 g/day, respectively. These values did not differ significantly ($P > 0.05$). Similarly, Scollan *et al.* (2001) found no effect of lipid source (high in either palmitic or α-linolenic or eicosapentaenoic + docosahexaenoic acid) in the diet for beef cattle on the growth rate.

In a model experiment with mice Du *et al.* (2001) observed higher hepatic triacylglycerol (TAG) accumulation in animals fed rapeseed oil in comparison with other diets. We found higher ($P < 0.05$) plasma TAG levels (2.55 vs. 2.30 g/l) in the experimental group in comparison with control group due to the higher total lipid content of experimental feed mixture (Table 1).

We did not find any differences ($P > 0.05$) in the plasma levels of thyroid hormones (triiodothyronine and thyroxine) between the experimental (3.44 ± 0.67 and 84.74 ± 20.66 nmol/l) and control group (3.55 ± 0.63 and 87.77 ± 20.70 nmol/l), which implies no impairment of thyroid gland. Neither macroscopic nor histological examination revealed any changes in the structure, consistency or activity of thyroid gland in experimental bulls in comparison with control animals. Similarly, no increase in the levels of enzymes suggesting the overloading of liver was observed in bulls fed rapeseed cake.

Fatty acid spectrum in loin

Total lipid content in the loin of bulls receiving SBM tended to be higher (4.17 ± 2.38%) in comparison with bulls fed the diet with rapeseed cake (3.69 ± 1.81) in the present experiment. This is in accordance with our previous results (Komprda *et al.*, 2000) concerning milk of dairy cows fed the diet with either SBM or HRC (higher fat content of SBM milk). However, the difference was not significant in the present experiment ($P > 0.05$) due to the high variability of total lipid content in each group of animals. The range of the measured values was from 1.1 to 7.4 and from 1.7 to 9.7% in the HRC and SBM group of bulls, respectively. This variability is difficult to explain because the groups of animals were selected to be as homogeneous as possible. On the other hand, Scollan *et al.* (2001) reported no differences in carcass fatness of beef cattle fed the diets with either palm oil or linseed or fish oil.

The differences in the ratio of particular fatty acids or groups of fatty acids in the loin of bulls receiving HRC or SBM are presented in Table 3. Total percentage of saturated fatty acids in total lipids of the loin was lower ($P < 0.05$) in the experimental (HRC) group as compared with the control group, due to the lower ($P < 0.001$) ratio of palmitic acid. Rule *et al.* (1994) found lower percentage of palmitic acid (similarly to our results) but higher percentage of stearic acid (contrary to our results) in cattle fed extruded full-fat canola in comparison with cattle fed soybean meal.

Although the percentage of the MUFA sum only tended to be higher ($P > 0.05$) in experimental bulls in our experiment, the content of oleic acid in total lipids of the loin of HRC-bulls was significantly higher ($P < 0.05$). It is not surprising because the oleic acid content in the production mixture with rapeseed cake was substantially higher in comparison with the mixture based on soybean meal (Table 2). Differences in both SFA percentage and oleic acid percentage in the meat of HRC and SBM bulls fully confirm our previous results regarding milk produced using a production mixture based on HRC or SBM (Komprda *et al.*, 2000).

Consequently, the SFA/MUFA ratio was lower ($P < 0.05$), and therefore more favourable from the viewpoint of healthy human nutrition, in the meat of bulls fed rapeseed cake. The ratio of total C16 and total C18 fatty acids was also lower (more favourable; $P < 0.001$) in the HRC group in comparison with the control group. This latter ratio is currently considered to be an important marker of the nutritive value due to a suggestion (Kennely and Glimm, 1998) that saturated stearic acid (C18:0) affects the plasma cholesterol level in humans analogously to monounsaturated oleic acid (C18:1). Similarly, according to Flachowsky *et al.* (1994), rapeseed supplementation of the diet for fattening bulls decreased C16 fatty acids and increased C18 fatty acids both in depot and in muscle (*musculus longissimus lumborum et thoracis*) fat.

Both total PUFA n-6 and total PUFA n-3 percentage in the loin of bulls fed rapeseed cake was significantly higher ($P < 0.05$) in comparison with bulls fed soybean meal in the present experiment, despite the fact that the production mixture based on HRC had a lower percentage of both linoleic acid and α-linolenic acid than the mixture with SBM (Table 2). As a consequence, total PUFA percentage was higher ($P < 0.05$) in the loin of HRC bulls. This is a confirmation of our previous results obtained in dairy cows (Komprda *et al.*, 2000). Scheeder *et al.* (2001) reported an increase in the proportions of some nutritionally favourable fatty acids in the patties produced from steers fed the diet containing full-fat rapeseed.

The PUFA n-6/PUFA n-3 ratio was not different between groups of bulls in the present experiment (Table 3), both values being substantially lower as compared to *musculus longissimus dorsi* of bulls fed concentrates in the experiment of Enser *et al.*, 1998 (the ratio 8.4). On the other hand, the above values (Table 3) are still too high in comparison with corresponding values

Table 3. Differences in fatty acid pattern of the loin of bulls receiving a production mixture with HRC[1)] or SBM[2)]. One-way classification of the variance ratio test. Mean ± standard deviation

Fatty acid (% of total measured fatty acids)	HRC ($n = 12$)	SBM ($n = 11$)	P
C14:0	2.3 ± 0.44	2.6 ± 0.35	0.08
C16:0	24.9 ± 1.03	27.4 ± 0.97	0.00
C18:0	18.9 ± 1.94	19.0 ± 2.29	0.97
Σ SFA[3)]	46.2 ± 2.64	49.0 ± 2.69	0.02
C16:1	3.2 ± 0.55	3.3 ± 0.55	0.67
C18:1	46.9 ± 2.49	44.6 ± 2.64	0.04
C20:1	0.4 ± 0.05	0.3 ± 0.06	0.04
Σ MUFA[4)]	50.5 ± 2.72	48.2 ± 3.01	0.07
C18:2 n-6	2.4 ± 0. 40	2.0 ± 0.43	0.04
C18:3 n-6	0.2 ± 0.02	0.1 ± 0.02	0.00
C20:4 n-6	0.1 ± 0.06	0.1 ± 0.05	0.77
C22:4 n-6	0.0 ± 0.01	0.0 ± 0.01	0.49
C22:5 n-6	0.0 ± 0.00	0.0 ± 0.00	0.17
Σ PUFA n-6	2.7 ± 0.46	2.3 ± 0.47	0.04
C18:3 n-3	0.5 ± 0.06	0.5 ± 0.07	0.01
C20:5 n-3	0.0 ± 0.01	0.0 ± 0.01	0.51
C22:5 n-3	0.0 ± 0.02	0.0 ± 0.02	0.74
C22:6 n-3	0.0 ± 0.01	0.0 ± 0.01	0.37
ΣPUFA n-3	0.6 ± 0.08	0.5 ± 0.09	0.02
ΣPUFA[5)]	3.3 ± 0.53	2.8 ± 0.55	0.04
PUFA n-6/PUFA n-3	4.3 ± 0.28	4.2 ± 0.39	0.47
SFA/MUFA	0.9 ± 0.10	1.0 ± 0.12	0.03
SFA/PUFA	14.2 ± 1.87	18.0 ± 3.02	0.00
MUFA/PUFA	15.6 ± 2.37	18.0 ± 4.04	0.10
ΣC16/ΣC18	0.4 ± 0.03	0.5 ± 0.03	0.00

[1)] heat-treated rapeseed cake; [2)] soybean meal; [3)] saturated fatty acids; [4)] monounsaturated fatty acids; [5)] polyunsaturated fatty acids

reported by Enser *et al.* (1998) for steers fed grass (1.25). At any rate, our results are rather different from the findings of Ponnampalam *et al.* (2001) that feeding of protected canola seed to lambs increased the PUFAn-6/PUFAn-3 ratio in the *longissimus* muscle. A likely reason of different results is the protection of canola in the quoted paper (however, the method of protection is not mentioned). On the other hand, Rule *et al.* (1994) reported higher C18:3 in bulls fed extruded canola as compared to SBM fed bulls.

The SFA/PUFA ratio was more favourable (lower, $P < 0.01$) in the loin of experimental group in comparison with the control group of bulls in the present experiment. However, in the experiment of Scollan *et al.* (2001), an increase in PUFA n-3 in the meat of steers fed the diet with linseed or fish oil in comparison with animals receiving palm oil had a low effect on the SFA/PUFA ratio.

Fatty acid percentage in tallow

The above-mentioned lower deposition of palmitic acid and total SFA, and higher deposition of oleic acid and total MUFA in the loin of bulls fed the production mixture with rapeseed cake in comparison with bulls fed soybean meal was also confirmed in tallow (in all four cases $P < 0.01$; Table 4). Similarly, Rule *et al.* (1989) reported a lower content of palmitic acid and a higher content of stearic acid in the subcutaneous adipose tissue from steers fed canola seeds as compared to soybean meal.

The SFA/MUFA ratio was substantially lower ($P < 0.01$) in the tallow of HRC bulls than in SBM bulls in our experiment. The same was true about the C16/C18 ratio ($P < 0.001$).

As regards the percentage of both PUFA n-6 and PUFA n-3 and total PUFA in tallow, we found no significant differences ($P > 0.05$) between the experimental group and control group of bulls. However, the SFA/PUFA ratio was lower ($P < 0.01$) in the tallow of HRC bulls, similarly to the loin. Contrary to our findings, Rule *et al.* (1989) reported a higher percentage of 18 : 3 in the adipose tissue of steers fed canola seeds in comparison with steers on the soybean meal diet.

Comparison of tissues

We tested the deposition of fatty acids in loin and tallow in the group of bulls receiving a production mixture with heat-treated rapeseed cake. The results for the three basic groups of fatty acids are presented in Figure 1. SFA deposition in loin was much lower ($P < 0.001$) and on the other hand, MUFA deposition in loin was substantially higher ($P < 0.001$) than in tallow. Within the SFA group the largest difference was found in the stearic acid ratio (46.2 ± 2.64 and 57.0 ± 3.55% of total fatty acids in loin and tallow, respectively; $P < 0.001$), within the group of MUFAs in the oleic acid ratio (46.9 ± 2.49 and 37.7 ± 3.36%, respectively; $P < 0.001$). It can be of some interest that the C16/C18 ratio was less favourable in loin (0.41 ± 0.03) than in tallow (0.36 ± 0.04; $P < 0.001$).

However, we found no difference between loin and tallow in the deposition of PUFAs (3.32 ± 0.53 and 3.32 ± 0.31; $P > 0.05$). This is contrary to the results of Flachowsky *et al.* (1994), who found not only more MUFA but also more PUFA in muscle fat than in depot fat in bulls with rapeseed supplementation of the diet.

No difference in PUFA deposition in loin and tallow in our experiment applies to PUFA n-6 and PUFA n-3, and the PUFA n-6/PUFA n-3 ratio. One exception in the PUFA group was arachidonic acid (AA; C20:4 n-6). The percentage of this fatty acid in loin was several times higher than in tallow ($P < 0.01$). This corresponds with the findings of Wattkins *et al.* (2000) that

Table 4. Differences in fatty acid pattern of the tallow of bulls receiving a production mixture with HRC[1)] or SBM[2)]. One-way classification of the variance ratio test. Mean ± standard deviation

Fatty acid (% of total measured fatty acids)	HRC (n = 12)	SBM (n = 11)	P
C14: 0	2.6 ± 0.47	3.1 ± 0.40	0.01
C16: 0	23.7 ± 1.75	27.2 ± 1.69	0.00
C18:0	30.8 ± 3.32	31.6 ± 2.48	0.50
Σ SFA[3)]	57.0 ± 3.55	61.8 ± 2.22	0.00
C16: 1	1.7 ± 0.28	1.7 ± 0.16	0.77
C18:1	37.7 ± 3.36	33.2 ± 2.29	0.00
C20:1	0.3 ± 0.05	0.2 ± 0.04	0.00
Σ MUFA[4)]	39.7 ± 3.58	35.1 ± 2.41	0.00
C18:2 n-6	2.3 ± 0.15	2.1 ± 0.29	0.04
C18:3 n-6	0.2 ± 0.03	0.2 ± 0.03	0.29
C20:4 n-6	0.0 ± 0.02	0.0 ± 0.01	0.58
C22:4 n-6	0.1 ± 0.09	0.1 ± 0.06	0.58
C22:5 n-6	0.1 ± 0.13	0.1 ± 0.10	0.89
Σ PUFA n-6	2.7 ± 0.26	2.5 ± 0.35	0.12
C18:3 n-3	0.5 ± 0.05	0.5 ± 0.08	0.11
C20:5 n-3	0.0 ± 0.00	0.0 ± 0.00	0.60
C22:5 n-3	0.0 ± 0.03	0.0 ± 0.02	0.87
C22:6 n-3	0.1 ± 0.04	0.1 ± 0.03	0.61
Σ PUFA n-3	0.6 ± 0.08	0.6 ± 0.06	0.07
Σ PUFA[5)]	3.3 ± 0.31	3.1 ± 0.40	0.09
PUFA n-6/PUFA n-3	4.3 ± 0.56	4.3 ± 0.43	0.93
SFA/MUFA	1.5 ± 0.22	1.8 ± 0.19	0.00
SFA/PUFA	17.3 ± 1.87	20.6 ± 2.97	0.00
MUFA/PUFA	12.1 ± 1.62	11.8 ± 2.23	0.71
Σ C16/Σ C18	0.36 ± 0.04	0.43 ± 0.04	0.00

[1)] heat-treated rapeseed cake; [2)] soybean meal; [3)] saturated fatty acids; [4)] monounsaturated fatty acids; [5)] polyunsaturated fatty acids

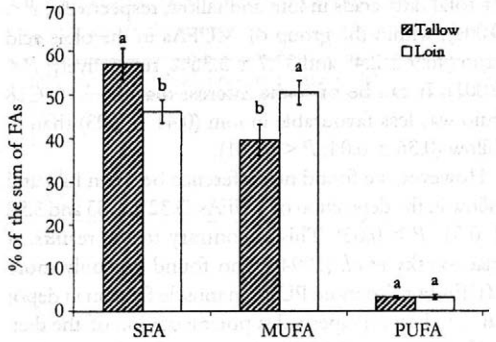

Figure 1. The deposition of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the loin and tallow of bulls fed a diet with heat-treated rapeseed cake

AA deposition is higher in polar lipids than in neutral lipids. It should be mentioned in this context that a mixture of polar and non-polar solvents is recommended for extraction of phospholipids and neutral lipids (Komprda *et al.*, 2001). Therefore some results of the content of higher unsaturated fatty acids could be biased to some extent due to the use of simpler Soxhlet extraction in the present experiment instead of e.g. hexane/2-propanol mixture.

We focused our attention on AA because this most important metabolite of dietary linoleic acid as a precursor of so called pro-inflammatory eicosanoids can modulate cell differentiation, cell proliferation, gene expression and signal transduction, and is linked to serious chronic diseases of man (Okuyama *et al.*, 1997). It follows from the above mentioned that the knowledge of AA content in foods of animal origin is very important. Hence it is interesting to compare arachidonic acid content in the loin of bulls (present experiment) with our previous findings regarding AA content in poultry meat (Komprda *et al.*, 2001). This comparison is presented in Figure 2. From the viewpoint of healthy human nutrition, poultry meat is usually considered more favourable in comparison with beef, especially as far as the content of SFA, MUFA and PUFA is concerned. However, as follows from Figure 2, the content of AA in beef meat was nearly by one order lower (and therefore much more favourable) than in poultry meat. Our results are in accordance with the data of Taber *et al.* (1998), who found nearly 2.5 times higher content of AA in chicken thigh meat (106 mg/100 g of raw tissue) in comparison with rib meat of bulls (46 mg/100 g). The relatively too low AA content in beef in our experiment as compared to the

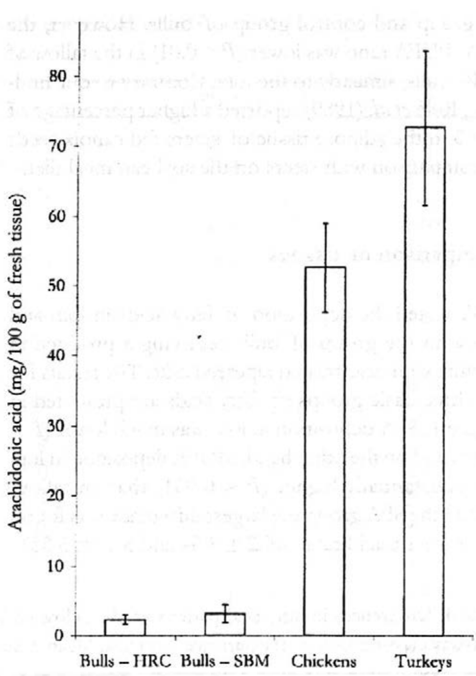

Figure 2. Comparison of arachidonic acid content in beef (present experiment; loin of bulls fed heated-treated rapeseed cake HRC and soybean meal SBM, respectively) and poultry meat (previous experiment; thigh meat of chickens and turkeys, respectively, fed a standard commercial feed mixture; Komprda *et al.*, 2001)

quoted paper could be due to the above-mentioned insufficient extraction of the membrane phospholipids using diethyl ether extraction.

If we continue to compare the nutritive value of beef with poultry meat, the same conclusion as in the case of arachidonic acid can be drawn as far as the PUFA n-6/PUFA n-3 ratio is concerned. We found this ratio to be 4.3 both in the loin and tallow of bulls fed both production mixtures in the present experiment (Table 3 and 4). This is much more favourable in comparison with poultry meat (the ratio 10–12; Komprda *et al.*, 2001), considering that this ratio is recommended to be as near to 1 as possible (Okuyama *et al.*, 1997).

CONCLUSIONS

It follows from the results of the present experiment that heat-treated rapeseed cake administered at an amount of more than 3% of the total daily ration is safe from the viewpoint of animal health.

As far as the nutritive value of meat is concerned, the loin of bulls fed rapeseed cake was superior to the corresponding product of fattening with soybean meal in the following traits important for healthy human nutrition: saturates/monounsaturates, saturates/polyunsaturates and total C16/total C18 ratio. Moreover, the PUFA n-6/PUFA n-3 ratio in the loin of bulls fed rapeseed cake was not higher ($P > 0.05$) than in bulls fed soybean meal, both values being substantially lower as compared to the corresponding muscle of bulls fed concentrate-based diet in the experiment of Enser *et al.* (1998).

Further, based on the results of the present experiment and our previous experiment in poultry (Komprda *et al.*, 2001), beef produced using heat-treated rapeseed cake (and also soybean meal) seems superior to poultry meat in the PUFA n-6/PUFA n-3 ratio and arachidonic acid content.

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Received: 01–10–11
Accepted after corrections: 02–02–14

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Effects of different amounts and types of fat on fatty acid composition of fat deposit in lambs

Vliv různého množství a druhu tuku na složení mastných kyselin v depotním tuku u jehňat

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ABSTRACT: Interest in the fatty acid composition of a product received from ruminants has increased in the last decade as a result of health benefits of some of them related to its consumption. In the present experiment the effect of three fat supplements on the fatty acid composition of subcutaneous and perirenal adipose tissues and in the muscles of lambs was measured. 24 fattened lambs (average BW = 22 ± 2 kg) were used during the experiment. The animals were divided into four groups and fed control (without fat addition) and experimental (6% of linseed oil, rapeseed oil or hydrogenated rapeseed oil in dry matter) diets. The results confirmed a significant ($P < 0.05$ and $P < 0.01$) influence of diet and source of added fat on the fatty acid composition of products received from ruminants, mostly in muscles and subcutaneous fat. Addition of hydrogenated rapeseed oil increased the level of saturated fatty acids and as a consequence decreased the level of monounsaturated fatty acids in muscles and subcutaneous fat. Linseed oil increased the level of n-3 polyunsaturated fatty acids in muscles and in subcutaneous fat. Rapeseed oil did not change the fatty acid composition either in muscles or in subcutaneous fat. There was no influence of added fats on the fatty acid composition in perirenal fat.

Keywords: sheep; fat deposits; fatty acids; rapeseed oil; linseed oil; hydrogenated rapeseed oil; meat

ABSTRAKT: V uplynulém desetiletí se zvýšil zájem o složení mastných kyselin obsažených v produktu původem z přežvýkavců v důsledku některých jejich zdravotních přínosů plynoucích z jeho spotřeby. V tomto pokuse jsme sledovali vliv tří tukových doplňků na složení mastných kyselin v podkožních a obledvinových tukových tkáních u jehňat. Během pokusu jsme použili 24 jehňat ve výkrmu (průměrná hmotnost těla = 22 ± 2 kg). Zvířata, která byla rozdělena do čtyř skupin, dostávala kontrolní (bez přídavku tuku) a pokusnou krmnou dávku (6 % lněného oleje, řepkového oleje nebo hydrogenovaného řepkového oleje v sušině). Výsledky potvrdily významný vliv krmné dávky a zdroje doplňkového tuku na složení mastných kyselin v produktech původem od přežvýkavců, zejména ve svalovině a podkožním tuku. Doplněk hydrogenovaného řepkového oleje zvýšil hladinu nasycených mastných kyselin a v důsledku toho snížil hladinu mononenasycených mastných kyselin ve svalovině a podkožním tuku. Lněný olej zvýšil hladinu n-3 polynenasycených mastných kyselin ve svalovině a podkožním tuku. Řepkový olej nezměnil složení mastných kyselin ani ve svalovině ani v podkožním tuku. Tukový doplněk neovlivnil složení mastných kyselin v obledvinovém tuku.

Klíčová slova: ovce; depotní tuk; řepkový olej; lněný olej; hydrogenovaný řepkový olej; maso

High producing animals are usually in negative energy balance. To avoid mobilization of adipose fatty acids, high amounts of grain as a source of fermentable starch can be fed to animals. But at higher intake of grain (usually > 50% of dietary dry matter), increasing starch intake depresses milk fat percentage and causes metabolic disorders (Palmquist *et al.*, 1993). Additional fat can be an alternative of increasing the energy level of the diet, and incorporated into received products (milk and meat) it can also be a source of potential anticarcinogenic components including conjugated linoleic acid, sphingomyelin, butyric acid and ether lipids (Parodi, 1997). Hence, interest in the fatty acid composition of a product received from ruminants has increased in the last decade as a result of health benefits of some of them related to its consumption. According to Hansen Petrik *et al.* (2000a) western-style diets characterized by high intakes of energy and fat (mostly saturated ones) are strongly linked with an increased risk of colorectal cancer. Because of the numerous human health benefits especially of mono- and polyunsaturated fatty acids (C18:1, C18:2, C18:3), scientists have developed an intense interest in increasing their content in food derived from animals. The amount and type of dietary fat consumed is of particular importance (Hansen Petrik *et al.*, 2000b). Not only milk fat but also meat composition can readily be modified by changing the feeding regimen. Bas and Morand-Fehr (2000) underlined difficulties in understanding the diet effects on the fatty acid composition of fat deposits and muscles without taking into account feeding and other management aspects.

In the present experiment the effect of three fat supplements on the fatty acid composition of subcutaneous and perirenal adipose tissues and of lambs' muscles was measured.

MATERIAL AND METHODS

During the experiment 24 fattened lambs (average BW = 22 ± 2 kg) were used. The animals were divided into four groups and fed control (without fat addition) and experimental (6% of linseed oil – LSO, rapeseed oil – RSO or hydrogenated rapeseed oil – HRSO in dry matter) diets. Lambs were fattened until 40 kg. The composition of a concentrate used in the experiment is shown in Table 1.

Table 1 Composition of the concentrate (%)

Components	Level of added protected fat 0%	6%
Wheat meal	60	60
Rapeseed meal	18	18
Wheat bran	20	14
Minerals	2	2
Protected fat	0	6

Water, concentrate and meadow hay were available *ad libitum*. Total fatty acid content and composition of subcutaneous and perirenal adipose tissues and muscles (*longissimus dorsi*) of lambs were determined according to the procedures of Heinig *et al.* (1998), modified by Czauderna *et al.* (2001). All data were analyzed using SAS procedures (User's Guide, 1990).

RESULTS

Treatment effects on the fatty acid composition of meat, subcutaneous and perirenal fat are shown in Tables 2, 3 and 4, respectively. The results confirmed a significant influence of diet on the fatty acid composition of products received from ruminants. Addition of hydrogenated rapeseed oil, a source of saturated fatty acids (SFA), to sheep rations statistically significantly increased the level of SFA both in muscles and in subcutaneous fat. Linseed oil addition increased the level of SFA in muscles, whereas rapeseed oil did not change the fatty acid composition of muscle, subcutaneous fat and perirenal fat. As a consequence of SFA increase in muscle and subcutaneous fat, HRSO and LSO decreased ($P < 0.05$) the level of monounsaturated fatty acids (MUFA) in those fat deposits. Hydrogenated

Table 2. Results of fat addition on the fatty acid composition of lamb fat deposits – muscle (%)

	Control	Rapeseed oil	Hydrogenated rapeseed oil	Linseed oil
Total SFA	46.19^{bc}	49.40^{a}	63.96^{ab}	58.86^{c}
Total MUFA	36.01^{ab}	32.70^{c}	25.42^{bc}	27.96^{a}
PUFA n-3	1.63^{c}	1.64^{b}	1.03^{abc}	1.76
PUFA n-6	16.16	16.24	9.58	11.41
Total PUFA	17.80	17.88	10.62	13.18

Means in rows with the same letter differ statistically significant a, b, c – $P \leq 0.05$

Table 3. Results of fat addition on the fatty acid composition of lamb fat deposits – subcutaneous fat (%)

	Control	Rapeseed oil	Hydrogenated rapeseed oil	Linseed oil
Total SFA	37.06	47.58	51.71[a]	47.51[a]
Total MUFA	57.27	47.15	44.39	46.27
PUFA n-3	1.92[a]	1.56[b]	1.01[abc]	1.34[c]
PUFA n-6	3.75[A]	3.71[BC]	2.88[ABC]	4.87[C]
Total PUFA	5.67[ABC]	5.26[ADE]	3.89[CE]	6.21[BD]

Means in rows with the same letter differ statistically significant A, B, C, D, E – $P \leq 0.01$;
a, b, c – $P \leq 0.05$

Table 4. Results of fat addition on the fatty acid composition of lamb fat deposits – perirenal fat (%)

	Control	Rapeseed oil	Hydrogenated rapeseed oil	Linseed oil
Total SFA	44.84	44.98	51.98	41.75
Total MUFA	49.18	50.02	44.39	54.21
PUFA n-3	1.36	1.21	0.82	1.16
PUFA n-6	4.61	3.79	2.80	2.86
Total PUFA	5.97	5.00	3.62	4.02

rapeseed oil, as an additional source of energy for ruminants, decreased the level of PUFA n-3 in muscle and also the levels of PUFA n-3, PUFA n-6 and total PUFA in subcutaneous fat. The results indicate that the PUFA n-3 in muscle, PUFA n-6 and total PUFA in subcutaneous fat can be increased by feeding a linseed oil supplement. Whereas a decreased level of PUFA n-3 in subcutaneous fat was observed when LSO was added to the diet. Diet manipulation had no influence on the fatty acid composition in perirenal fat.

DISCUSSION

The use of dietary fat in high producing animals increases the energy density of the diet and improves energetic efficiency (McNamara *et al.*, 1995) but also changes the body fat composition. Casey and Webb (1995) suggested that dietary factors do not change the body fat composition in ruminants as much as in monogastric animals, but past and recent research has indicated possible diet-induced changes in the fat composition of ruminants. According to Ponnampalam *et al.* (2001) the alteration in the essential fatty acid classes of intramuscular fat of lambs may improve the nutritional quality of lamb meat and offer an alternative choice of meat products available to nutrition- and health-conscious meat eaters. Eating quality of lambs is limited to the long-chain fatty acids. To reach consumer acceptability the concentration of saturated and monounsaturated fatty acids mostly in subcutaneous adipose tissue should be limited. The results of experiments suggest that the proportions of fatty acids in lamb fat deposits may be influenced by the energy source of the diet. It appears that feeding linseed oil in a sheep ration can result in a higher level of preferable fatty acids, mostly PUFA n-3. Increasing dietary canola seeds in lambs Stanford *et al.* (1999) reduced saturated fatty acids and increased polyunsaturated fatty acids in meat. His study demonstrated that the addition of high concentrations of canola screenings to lamb diets changes the fatty acid composition, with potential benefits to human health. Rapeseed oil used in our experiment had no influence on lambs' deposits, whereas in their study Solomon *et al.* (1991) increased the total level of SFA by adding 6.5% whole rapeseed to lamb diets. Hydrogenated rapeseed oil as a source of mostly saturated fatty acids did not increase consumer acceptable fatty acids – muscle long-chain n-3 fatty acids.

According to Ponnampalam *et al.* (2001) further research is needed to investigate the optimum level of muscle long-chain n-3 fatty acids enrichment with respect to fat deposition and eating quality of lamb meat.

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Received: 01–11–12

Accepted after correlations: 02–02–27

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Polymorphism of the growth hormone gene in Limousine cattle

Polymorfismus genu pro růstový hormon u limousinského skotu

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ABSTRACT: The frequencies of alleles and genotypes of the growth hormone gene (*GH* gene) of cattle – deletion $^{-35}$AAG^{-33} in the promoter region and Leu/Val polymorphism in exon 5 of the *GH* gene were determined. The polymorphism located in the promoter part of the *GH* gene was tested by means of the PCR-RFLP technique. The following primer sequences were designed: PGH1 5'-GACATGACCCCAGAGAAGGA-3' and PGH2 5'-GCCTAGGGAGAGACCAGGAG-3'. A 493 bp fragment of the *GH* gene was amplified. The restriction analysis of the PCR products (with the use of *Mbo*II enzyme) confirmed the restriction fragments length polymorphism (RFLP). The following frequencies of genotypes and alleles were obtained: *LL* – 0.461, *LV* – 0.363, *VV* – 0.176, GH^L – 0.642, GH^V – 0.358 for *GH-Alu*I polymorphism and *AA* – 0.726, *AB* – 0.235, *BB* – 0.039 and GH^A – 0.843, GH^B – 0.157 for *GH-Mbo*II polymorphism.

Keywords: cattle; DNA; genetic polymorphism

ABSTRAKT: Stanovili jsme frekvence alel a genotypů genu růstového hormonu (genu *GH*) u skotu – deleci $^{-35}$ AAG^{-33} v oblasti stimulátoru a polymorfismus Leu/Val v exonu 5 genu *GH*. K ověření polymorfismu lokalizovaného ve stimulační části genu *GH* byla použita metoda PCR-RFLP. Byly zkonstruovány tyto sekvence primerů: PGH1 5'-GACATGACCCCAGAGAAGGA-3' a PGH2 5'-GCCTAGGGAGAGACCAGGAG-3'. Došlo k amplifikaci fragmentu 493 bp genu *GH*. Restrikční analýza produktů PCR (při použití enzymu *Mbo*II) potvrdila polymorfismus délky restrikčních fragmentů (RFLP). Získané frekvence genotypů a alel byly následující: *LL* – 0,461, *LV* – 0,363, *VV* – 0,176, GH^L – 0,642, GH^V – 0,358 pro polymorfismus *GH-Alu*I a *AA* – 0,726, *AB* – 0,235, *BB* – 0,39 a GH^A – 0,843, GH^B – 0,157 pro polymorfismus *GH-Mbo*II.

Klíčová slova: skot; DNA; genový polymorfismus

The study of genetic polymorphism on the basis of nucleotide sequences is a promising way to improve utility characters of farm animals. It is possible to identify genotypes of certain interest to farmers before the information on the performance of an animal is obtained. It seems reasonable to use such information along with traditional methods of the selection of animals (Falaki *et al.*, 1997).

The cattle growth hormone (somatotropin) is a protein hormone secreted by a glandular part of the hypophysis. It is made of 190 or 191 amino acids and alanine or phenylalanine at the N-terminus (Wood *et al.*, 1989). Due to the allele dependent variability, leucine or valine may appear in position 126 bST (Seavey *et al.*, 1971).

The *GH* gene was mapped to chromosome 19 in cattle (Hediger *et al.*, 1990). It consists of 5 exons and (separated by) four introns (Gordon *et al.*, 1983). Six polymorphic sites were detected in the promoter region of the *GH* gene. These are mainly point mutations in the DNA. Some of them overlap potential places of binding the transcription factors involved in the *GH* gene expression (Hetch and Geldermann, 1996). Furthermore, the deletion of 3 bp ($^{-35}$AAG^{-33}) was detected in the promoter region (situated 9 nucleotides upstream TATAAA box). The polymorphism as described above was found only in the beef cattle (Rodrigues *et al.*, 1998; Dybus *et al.*, 2001). The 5' region of the *GH* gene includes regulation sequences that control the gene expression and interact with a number of transcription factors (Crone *et al.*, 1990). The change in the affinity of binding the transcription factors through slight changes in DNA nucleotide sequences in the 5' region may affect the gene transcription and consequently the concentration of *GH* in the blood (Yao *et al.*, 1996).

Lucy *et al.* (1991) detected a polymorphic site located in exon 5 of the *GH* gene (transversion C → G in position 2141 of the gene) and consequently the substitution of Leu by Val in the protein product (Lucy *et al.*, 1993). The study made by Yao *et al.* (1996) also reported the polymorphism in exon 5 of the *GH* gene, in position 2241 (transversion A → C). The substitution concerned does not cause any change of amino acid in the protein product (the new triplet codes the same amino acid, arginine). This mutation changes the recognition sequence (C/TNAG) of the restriction enzyme *Dde*I. Chikuni *et al.* (1994) detected another polymorphic site in the *GH* gene (point mutation C → T in the codon 172) in the Japanese Black and Brown cattle and consequently threonine was changed to methionine in the protein chain.

Higher frequencies of the GH^L allele were usually found in dairy breeds of a larger body size (e.g. Holstein-Friesian breed) compared to breeds of a smaller body size, e.g. Jersey breed (Lucy *et al.*, 1993). The GH^L allele is likely to be treated as an indicator of high milk yield and the GH^V is connected with meat yield (Zwierzchowski *et al.*, 1995).

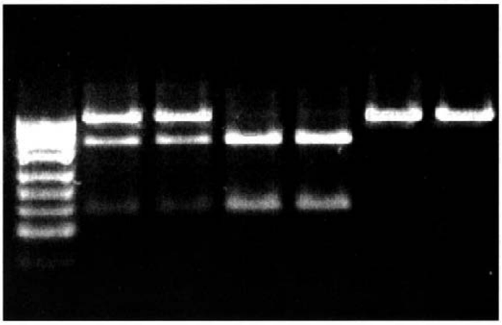

Figure 1. Representative results of *GH-Mbo*II analysis detected by agarose gel electrophoresis

M – DNA marker (pUC19/*Msp*I)

The purpose of this study was to find the frequencies of genotypes and alleles of the *GH* gene in the Limousine cattle.

MATERIAL AND METHOD

The study involved 102 cows of the Limousine breed kept in South-Eastern Poland. The isolation of DNA was carried out with the use of reagents of Master-Pure™ (Epicentre Technologies). The PCR-RFLP method was applied to study DNA polymorphism of selected genes.

The study focused on two polymorphic sites of the *GH* gene that were localized in exon 5 (Leu/Val) and a promoter region (deletion $^{-35}$AAG^{-33}). The procedure developed by Schlee *et al.* (1994) was used to determine the polymorphism in exon 5 of the *GH* gene (*GH-Alu*I). The PCR-RFLP method was used for the polymorphism located in the promoter region of the *GH* gene. The following primer sequences were designed on the basis of the nucleotide sequence of the *GH* gene (Gordon *et al.*, 1983) and Primer3 software (http://www.genome.wi.mit.edu/egibin/primer/primer3)

PGH1 - 5'-GACATGACCCCAGAGAAGGA-3'

PGH2 - 5'-GCCTAGGGAGAGACCAGGAG-3'

The PCR reaction was carried out in the mixture of 20 µl. The mixture contained 15 pmol each primer, 200 µM dNTP, 10× PCR buffer, 1.5 mM $MgCl_2$, 0.5 units *Taq* polymerase and about 100 ng DNA. The following cycles were applied: denaturation – 94°C per 5 min, followed by 30 cycles – 94°C/40 s, primer annealing – 61°C/40 s, PCR products synthesis – 72°C/40 s, and final synthesis – 72°C/5 min. A 493 bp fragment was amplified. Restriction analysis of the

Table 1. Frequencies of genotypes and alleles of the *GH* gene in Limousine cattle

Polymorphism	Genotypes			χ^2 test	Alleles	
*GH-Alu*I	*LL*	*LV*	*VV*		GH^L	GH^V
	0.461 (n = 47)	0.363 (n = 37)	0.176 (n = 18)	0.1712	0.642	0.358
*GH-Mbo*II	*AA*	*AB*	*BB*		GH^A	GH^B
	0.726 (n = 74)	0.235 (n = 24)	0.039 (n = 4)	0.0145	0.843	0.157

PCR products showed that the amplified region of DNA was a part of the *GH* gene (169 bp of the promoter region, exon 1, intron 1 and 2 bp of exon 2). The polymorphic site located in the promoter region (deletion AAG) was detected by digesting the PCR products with *Mbo*II restriction enzyme ($GAAGA_{(N)8}$). The restriction fragments were separated in 2% agarose gels (Gibco-BRL).

RESULTS AND DISCUSSION

The following DNA restriction fragments were obtained in the case of *GH-Alu*I polymorphism: 171 and 52 bp for the *LL* genotype, 223, 171 and 52 bp for the *LV* and 223 bp (no digestion) for the *VV*.

The *LL* genotype was the most frequent in the population under study – Table 1.

The higher frequency of GH^L in the Limousine cattle was observed by Zhang *et al.* (1993) – 0.76 and Zwierzchowski *et al.* (2001) – 0.78.

The following DNA restriction fragments were obtained in the case of *GH-Mbo*II polymorphism: 351 and 142 bp for the *AA* genotype, 493, 490, 351 and 142 bp for the *AB* and 490 bp (no digestion, deletion allele) for the *BB* (Figure 1).

The *AA* genotype was the most frequent in the population under study (Table 1). The obtained frequency of GH^A, i.e. 0.843, was slightly higher than frequencies previously obtained for beef cattle. Rodrigues *et al.* (1998) obtained the frequency of 0.85 for the GH^A allele in the Nelore breed and 0.91 in the Chianina breed.

The higher frequencies of the GH^L allele are usually observed in breeds of a larger body size, (e.g. the Holstein-Friesian) compared to those of a smaller body size, e.g. the Jersey breed, (Lucy *et al.*, 1993). The lack of the GH^B allele in dairy cattle (it was found only in the beef cattle) might be assumed to regard the GH^B allele as a characteristic for beef cattle. The influence of GH^B allele of the *GH* gene on the production traits of beef cattle should be verified in further studies.

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Received: 01–10–23
Accepted after corrections: 02–02–07

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Influence of stage of lactation on the chemical composition and physical properties of sheep milk

Vliv laktační fáze na chemické složení a fyzikální vlastnosti ovčího mléka

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ABSTRACT: The aim of research was to determine the influence of stages of lactation on the chemical composition and physical properties of sheep milk. The analysis involved a total of 202 milk samples in various stages of lactation (beginning, midpoint, end). Milk analyses included determination of the contents of dry matter, fat, protein, lactose, solids-non-fat, degree of acidity, pH values and freezing point. Data was statistically processed in accordance with the SAS statistics package (1996). Sheep milk contained on average 19.11% total solids, 7.52% fat, 5.90% protein, 4.55% lactose, 11.45% solids-non-fat. Titratable acidity of milk amounted to 9.30°SH, ionometric 6.78, and freezing point was –0.566°C. In the middle and at the end of the lactation period the contents of total solids, fat, protein, and pH value were significantly higher ($P < 0.01$) than at the beginning. In the same period lactose content and titratable acidity values were significantly lower ($P < 0.01$).

Keywords: sheep milk; stage of lactation; milk quality; chemical composition; physical properties

ABSTRAKT: Cílem výzkumu bylo szjištění vlivu jednotlivých laktačních fází na chemické složení a fyzikální vlastnosti ovčího mléka. Bylo analyzováno celkem 202 vzorků mléka z různých fází laktace (začátek, střed, konec). Stanovil se obsah sušiny, tuku, bílkovin, laktózy, tukuprosté sušiny, stupeň kyselosti, hodnota pH a bod mrznutí. Pro statistické zpracování dat jsme použili statistický balík SAS (1996). Ovčí mléko obsahovalo v průměru 19,11 % celkové sušiny, 7,52 % tuku, 5,90 % bílkovin, 4,55 % laktózy, 11,45 % tukuprosté sušiny. Titrační kyselost mléka činila 9,30 °SH, iontometrický bod 6,78 a bod mrznutí –0,566 °C. Obsah celkové sušiny, tuku, bílkovin a hodnota pH byly významně vyšší ($P < 0,01$) uprostřed a na konci laktačního období než na jeho začátku. Obsah laktózy a hodnoty titrační kyselosti byly ve stejném období významně nižší ($P < 0,01$).

Klíčová slova: ovčí mléko; laktační fáze; kvalita mléka; chemické složení; fyzikální vlastnosti

An interest in the production and processing of sheep milk has increased in the Republic of Croatia in the last few years. Milk production is predominantly based on different breeds with local characteristics, adapted to meagre vegetation, varied climatic conditions, shallow soil and lack of precipitation (Mioč *et al.*, 2000). The quantity of produced milk, its chemical composition and physical properties are influenced by numerous factors: genetic (breed and genotype), physiological (age, lambing, body weight, number of lambs, stage and number of lactation), management (feeding regime) and method of milking (Bencini and Pulina, 1997; Antunac and Havranek, 1999). The stage of lactation has a significant influence on the chemical composition of produced milk (Gonzalo *et al.*, 1994; Fuertes *et al.*, 1998; Fenyvessy and Javor, 1999). The physical and chemical properties of milk vary as depending on production conditions and on the individual characteristics of particular animals. Large quantities of sheep milk produced in family households are processed into cheese. The uneven quality of milk also reflects on cheese quality. Therefore the aim of this paper was to establish the influence of the stage of lactation on the variability of chemical composition and physical properties of milk.

MATERIAL AND METHODS

Individual samples of milk for chemical analyses were taken from Travnik sheep at monthly intervals during the second lactation period. The sheep, owned by a family holding in the continental part of Croatia, were kept in the same conditions with regard to housing and feeding. The winter-feeding regime consisted of field hay and of 200 g of corn grain per head/day. With the commencement of the vegetation period (April) to the end of lactation (September) sheep were put out to pasture and received no additional feed in the form of a concentrate. In the course of the 240-day lactation period (from 20th February to 20th September) a total of 202 milk samples were collected. In the first three months of lactation, lambs suckled and were separated from their mothers 12 hours prior to milking. The beginning of the lactation period covered the first 60 days, the midpoint stage lasted from day 61 to day 180, and the final stage lasted from day 181 to day 240. The animals were milked manually on the day of the controls, and a proportional sample of milk was taken from each animal from both morning (7 a.m.) and evening (7 p.m.) milking. Milk samples were not conserved but they were kept in a refrigerator at a temperature of +4°C by the time of analyses.

The following methods were used for determining the chemical composition of milk: total solids (% w/v), milk fat (% w/v), crude protein (% w/v) and lactose anhydrite (% w/v) were determined by the infrared spectrometric method (FIL-IDF, 1990), using a Bentley 150 instrument, which was calibrated against the known sample standards (10 samples) of sheep milk. In standard samples the total solids content was determined by drying at 102°C (FIL-IDF, 1987), milk fat content by Gerber method (FIL-IDF, 1981), protein content by Kjedahl method (FIL-IDF, 1993), lactose content by enzymatic method – Boehringer Mannheim kit (FIL-IDF, 1991b). The content of solids-non-fat was arrived at through calculation from the difference between total solids and milk fat content. At each sample collection a total daily sample was taken, its milk ash content being determined according to the reference method (AOAC, 1995). Corrections of dry solids and non-fat solids at the beginning, in the middle and at the end of lactation period were based on the following established average quantities: 1.008%, 0.875%, and 0.905%. The given values were corrected according to location. Titratable acidity (°SH) was established by Soxhlet-Henkel method (AOAC, 1995), and pH values with a pH-meter (Mettler-Toledo). The freezing point of milk was measured with a thermistor cryoscope CRYO-STAR, Funke Gerber, by a cryoscopic method (FIL-IDF, 1991a). The results were processed in accordance with the SAS (1996) statistics package.

RESULTS AND DISCUSSION

Milk samples from Travnik sheep contained on average 19.11% total solids, 7.52% fat, 5.90% protein, 4.55% lactose and 11.45% solids-non-fat (Table 1). Dozet (1964) arrived at a considerably lower content of total solids (17.74%) and fat (5.53%) for the same breed, and a distinctly higher protein content (6.15%). The given results are also higher than those reported by Jančić (1967) for milk obtained from Lika sheep. A significant influence of the breed on the chemical composition of sheep milk was established by Mavrogenis and Louca (1980), Anifantakis (1986), Voutsinas *et al.* (1988), Bencini and Pulina (1997), Bedö *et al.* (1999). It was found that the stage of lactation had a significant ($P < 0.01$) influence on all analysed parameters, which corresponds with the information provided by Gonzalo *et al.* (1994) and Fuertes *et al.* (1998). At the beginning of the lactation period the

Table 1. Chemical composition of sheep milk in different stages of lactation period

Components (%)	Lactation period											
	beginning			midpoint			end			average		
	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV
Total solids	17.57^a	0.31	12.09	19.58^b	0.20	11.17	20.21^b	0.48	14.65	19.11	0.18	13.28
Fat	5.40^a	0.23	29.36	8.28^b	0.20	24.59	8.76^b	0.38	26.85	7.52	0.17	32.07
Protein	5.47^a	0.08	10.89	5.94^b	0.05	8.21	6.46^c	0.14	13.51	5.90	0.05	11.79
Lactose	4.97^a	0.03	3.81	4.48^b	0.04	8.57	4.09^c	0.09	14.19	4.55	0.04	10.93
Solids-non-fat	11.69^a	0.14	8.40	11.30^b	0.06	6.09	11.46	0.16	8.51	11.45	0.06	7.01

$^{a, b, c}$ = means in the row with no common superscript letters differ ($P < 0.01$)

Table 2. Physical properties of sheep milk in different stages of lactation

Components (%)	Lactation period											
	beginning			midpoint			end			average		
	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV	$\bar{x}$	SE	CV
Acidity (°SH)	10.01^{a}	0.20	15.48	9.30^{b}	0.16	17.59	8.39^{c}	0.30	21.82	9.29	0.13	19.89
pH	6.66^{a}	0.01	1.48	6.81^{b}	0.01	2.04	6.89^{c}	0.03	2.37	6.77	0.01	2.34
Freezing point (°C)	–0.564^{a}	0.001	1.64	–0.566^{a}	0.001	1.53	–0.570^{b}	0.001	1.17	–0.566	0.001	1.64

^{a,b,c} = means in the row with no common superscript letters differ ($P < 0.01$)

contents of total solids, fat and protein were significantly lower ($P < 0.01$) with regard to the midpoint and end stages. The milk component most prone to variability was milk fat, which is also confirmed by the values of coefficients of variation (from 24.59 to 29.36%) that correspond almost exactly to data presented by Gonzalo *et al.* (1993). In relation to the starting stage, protein content in the middle and end lactation stages was higher by 8.59 and 18.09%, respectively. Manfredini *et al.* (1993) concluded that sheep milk contains a significantly lower protein content at the start of the lactation period than at the end (5.38 and 7.11%). Quite a contrary trend was established for lactose: its content was highest at the beginning (4.97%) and lowest at the end (4.09%) of lactation period. Dario *et al.* (1996) also reported a higher lactose content at the beginning (5.32%) in relation to the end (4.93%) of lactation period for milk taken from Leccese sheep.

In the course of the lactation period, milk obtained from Travnik sheep showed on average a higher titratable acidity 9.3°SH, pH value 6.77, freezing point being –0.566°C (Table 2). Since published data on the physical properties of sheep milk are few, they are often compared with those for cow's milk. Alichanidis and Polychroniadou (1995) gave a slightly higher acidity value for sheep milk in relation to cow's milk, due to its higher protein content. Milk acidity is also influenced by hygienic and climatic conditions (temperature) to a great extent. The high variability of titratable acidity during the course of lactation period is an indication of poor hygienic conditions during milking. Average acidity values and freezing point of milk were significantly higher ($P < 0.01$) at the beginning and in the middle of lactation period than at the end whereas the pH values showed an opposite trend. Significant ($P < 0.001$) correlation was established between titratable acidity and pH value (Table 3). Manfredini *et al.* (1993) reported lower pH values for sheep milk at the beginning of lactation period in relation to the end stage (6.57 : 7.01) while acidity showed an opposite trend (8.78°SH : 5.97°SH).

The freezing point of sheep milk compared to cow's milk is lower due to a higher content of solids-non-fat (Alichanidis and Polychroniadou, 1995). The freezing point value of sheep milk at the end of lactation period

Table 3. Coefficients of correlation

$n = 202$	Fat	Protein	Lactose	Solids-non-fat	Acidity	pH value	Freezing point
Total solids	0.96***	0.70**	–0.31**	0.55**	–0.28**	0.23**	0.31**
Fat		0.62**	–0.47**	0.33**	–0.10	0.08	0.09
Protein			–0.36**	0.71**	0.06	0.01	–0.03
Lactose				0.09	0.30**	–0.002	–0.25**
Solids-non-fat					–0.12	0.24**	0.13
Acidity						–0.66**	–0.97***
pH value							0.65**

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

was significantly lower ($P < 0.01$) in relation to the beginning and midpoint stages.

Significant correlations ($P < 0.01$ and $P < 0.001$) were established between total solids and fat content (0.96), protein (0.70), solids-non-fat (0.55), lactose (–0.31) and the freezing point of milk (0.31). The correlation between fat and protein content was also significant ($P < 0.01$), which was also claimed by Gut *et al.* (1996) and Ubertalle *et al.* (1996). Significant and negative correlations were established between the content of lactose and fat (–0.47), and between lactose and protein (–0.36), which coincides with the results obtained by Bufano *et al.* (1996), Gut *et al.* (1996) and Ubertalle *et al.* (1996). The correlation between freezing point and acidity was also significant ($P < 0.001$).

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Received: 01–01–29
Accepted after corrections: 02–02–05

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CZECH JOURNAL OF ANIMAL SCIENCE

Volume 47, No. 2 February 2002

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