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The new monoclonal antibodies against the bovine sperm

Nové monoklonové protilátky proti spermiám hovädzieho dobytka

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ABSTRACT: Monoclonal antibodies (mAbs) recognising the antigens of spermatozoa could be efficiently used for the study of immunology processes during the spermatogenesis and fertilisation. In this study mAbs showing different reaction patterns on bull and boar sperms have been prepared. BALB/c mice were immunised with whole bull sperm, and their spleen cells were fused with SP2/0 myeloma cells in the fusion experiment, resulting in the generation of fifteen mAbs. The antibodies tested in indirect immunofluorescence assay were specific for two antigens localised at different parts of sperm surface. Five mAbs are reactive on the whole sperm surface, 3 mAbs recognise antigenic sites on the proximal part of sperm head, 3 mAbs on acrosome, 2 mAbs on the tail of sperm (principal piece), 1 mAb on the distal region of the head and 1 mAb was bound to the head and middle part of tail.

Keywords: sperm; monoclonal antibodies; antigens; bull

ABSTRAKT: Monoklonové protilátky (mAbs) rozpoznávajúce antigény spermií sa môžu využiť pre štúdium imunologických procesov v priebehu spermiogenézy a počas fertilizácie. Myši kmeňa BALB/c boli imunizované celými spermiami a ich slezinné bunky sa potom fúzovali s SP2/0 myelómovými bunkami. V experimente sme získali 15 hybridómov produkujúcich monoklonové protilátky. Získané protilátky testované nepriamym imunofluorescenčným testom boli špecifické pre antigény lokalizované v rozdielnych častiach spermií. Päť mAbs reagovalo s celým povrchom spermií, 3 mAbs rozpoznávali antigény na proximálnej časti hlavičiek spermií, 3 mAbs na akrozóme, 2 mAbs na bičíku spermií (hlavný oddiel bičíka), 1 mAb na distálnej časti hlavičky a jedna protilátka sa viazala na hlavičku a spojovací oddiel bičíka.

Kľúčové slová: spermie; monoklonové protilátky; antigény; býk

INTRODUCTION

Spermatogenesis is a complex process of germ cell differentiation in testes. Sperm membrane is developed during the spermatogenesis and further modified in composition at the time of epididymal maturation, ejaculation and the course of capacitation in the female's genital tract. During these processes the molecules (antigens) integrating at the sperm plasma membrane or secondarily bound to the sperm surface may be involved in different steps of fertilisation (Calvete *et al.*, 1996; Töpfer-Petersen *et al.*, 1998).

Monoclonal antibodies (mAbs) against the surface and subsurface antigens of sperm are valuable tools for identification and characterisation of these molecules and understanding of the reproductive mechanisms (Bellve and Moss, 1983). In recent years, a set of mAbs has been generated against sperm antigens of several species. Similarly to other species, in cattle mAbs specific for sperm surface and seminal plasma antigens have been prepared (Chakraborty *et al.*, 1985; Ambrose *et al.*, 1996; Bellin *et al.*, 1996). Moreover, to study the sperm antigens of bull, cross-reactive mAbs originally produced against human or pig sperm have been used (Coonrod *et al.*, 1994, 1996; Rajamahendran *et al.*, 1994; Pěknicová and Moos, 1998; Čapková *et al.*, 2000).

Because mAbs specifically directed against bull sperm available at present time could detect only a small portion of sperm antigens, the topic of this work was to prepare new mAbs recognising well defined or unknown antigens of sperm or seminal fluids. In this communication, we re-

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port on the generation and partial characterisation of mAbs directed against bull spermatozoa.

MATERIAL AND METHODS

Sperm and seminal fluids

Ejaculate or frozen sperm from healthy and reproductive active bulls was a generous gift of Slovak Biological Servis, Bernolákovo and boar ejaculate of the INSEMA Rybničky. Testes and epididymis were obtained from random bulls in a local slaughterhouse. Mouse epididymal sperm was obtained from BALB/c mice maintained in the Institute of Animal Biochemistry and Genetics, SAS by excision and flushing of the epididymis into phosphate buffer saline.

Preparation of the monoclonal antibodies

Sperms from ejaculated semen samples were separated from seminal fluid by centrifugation at 300 g for 10 minutes and washed three times with phosphate buffer saline (PBS pH 7.4). Concentration of spermatozoa was adjusted to 1×10^7 sperms/ml and frozen for the purposes of immunisation and hybridoma screening. BALB/c mice were immunised three times intraperitoneally with frozen-thawed sperm suspension (0.2 ml of 1×10^7 sperms/ml). Two times at the interval of two weeks and the third boost, injection was applied 3 days before fusion. Spleen cells from immunised mice obtained by manual dispersion of the gland were washed, fused with myeloma cells SP2/0 at a ratio of 1 : 1 (3×10^7 cells of both types) in polyethylene glycol 6000 (SERVA) according to (Lane, 1985). Immediately after cell fusion, the mixture of cells was cultured in Dulbecco's modified Eagle medium (DMEM) (Sigma) supplemented with foetal calf serum (10%), hypoxantine (1×10^{-4} M), aminopterin (4×10^{-7} M) and thymidine (1.6×10^{-5} M) in 96-well plates. Several days after cell fusion the supernatant of each well with distinct colonies of hybridoma cells were collected and screened by immunoflourescence test for the presence of the anti-sperm monoclonal antibodies. Positive hybridomas were cloned by limiting dilution and cultured for antibodies production.

Indirect immunofluorescence assay

Indirect immunofluorescence assay described by Boucheix *at al.* (1983) was used to screen for the presence of specific antibodies to bull, boar and mouse sperm in culture supernatants. Briefly, sperm cells separated from seminal plasma, frozen-thawed sperm or mouse epididymis were washed three times with PBS plus 1% bovine serum albumin and 0.1% sodium azid. The sperm suspensions were incubated with monoclonal antibodies in round-bottomed 96-well polystyrene plates for 45 minutes at a room temperature. The cells were washed 3 times and treated with diluted (1 : 30–40) fluorescein isothiocyanate (FITC)-conjugated swine anti-mouse immunoglobulin (SEVAC Praha), for 45 minutes at a room temperature. After further washing the sperm suspension was placed on slide and examined under epifluorescence microscope (Jenalumar).

Enzyme-linked immunoabsorbent assay (ELISA)

ELISA was performed as previously described (Kennett, 1983). The wells in Terasaki microtiter plates were coated with seminal plasma diluted in carbonate buffer, pH 9.6 and left overnight at 4°C. Wells were washed with 0.25% Tween 20 in saline and twice with saline, then open sites of plate were blocked with 0.1% gelatine in PBS for 1 hour at 37°C. After washing with saline-Tween and saline the aliquots of mAbs were added to each well and the plates were incubated for 2 hours at a room temperature. The plates were washed again with saline-Tween and saline. Swine anti-mouse second antibody conjugated to horseradish peroxidase (1 : 500, SEVAC Prague) was added and incubated for 1 hour at 37°C. The plates were finally washed with saline-Tween and saline. The peroxidase activity was detected using *o*-phenylenediamine (5 mg/10 ml substrate solution).

Isotyping for immunoglobuline class of monoclonal antibodies

Hybridoma culture media representing the produced mAbs were used to determine the immunoglobulin classes of each mAb. Sub-Isotyp-Kit, Mouse (Calbiochem, USA) was used for the determination of immunoglobuline isotypes. Test was performed according to the manufacturer's instruction manual. At the end of assay, the reactions were developed with chromafor.

Western blotting

The sperm membrane antigens were solubilized from washed ejaculated bull sperm. Spermatozoa were extracted in 1% NP40 in (20 mM TRIS HCl pH 7.6, 150 mM NaCl, 2 mM PMSF) at 4°C, 30 minutes. Sperm extracts were centrifuged at 10 000 g for 30 min at 4°C. Clear supernatant was separated by SDS-PAGE method described by Laemmli (1970). After electrophoresis, separated proteins were transferred to nitrocellulose sheets by Bjerrum a Schafer-Nielsen (1986). After incubation of membrane in the blocking solution (5% milk), strips of blots were incubated separately for 2 hours with mAbs and after washing they were treated with horseradish peroxidase-conjugated swine anti-mouse immunoglobulin (SEVAC Prague). Reactions were developed with solution of 0.3% 4-chlor-1-naftol + 0.2% diaminobenzidine. The molecular weight of separated proteins was estimated by comparison with the kit of protein standards (Boehringer, Mannheim) run in parallel.

RESULTS

Over 50 hybridoma clones were picked for culture from the cell fusion experiment. During culturing, some clones failed to continue secretion of mAb. So that at the end of screening process 15 hybridoma clones were identified to be secreting mAbs specific to discrete antigens localised in different parts of bull sperm. Moreover, from each clone subclones have been derived. Specificity of mAbs produced by subclones does not differ from the originally developed clones, therefore in this communication the characterisation of 15 hybridomas are presented.

The binding of monoclonal antibodies to bull sperm tested by indirect immunofluorescence have shown six binding patterns (Table 1). The most frequent reaction pattern (5 out of 15) was observed when the whole spermatozoa were stained. Reactivity of remaining mAbs on the spermatozoa was more restricted. Three mAbs recognise antigenic sites on the proximal part of sperm head, three on the acrosome, two on the tail, one on the distal part of head and one mAb is reactive with the head and middle piece of tail. Portions of mAbs are cross-reactive with boar sperm but no clearcut reaction was observed with mouse sperm. The last data are not shown in the Table 1. The cross-reactivity is characteristic feature of the acrosome specific mAbs because all of them (IVA 519, IVA 520 and IVA 545) are cross-reactive with boar sperm. Majority of the studied mAbs belong to the IgG class and the subclass IgG1, whereas five mAbs represented the IgM isotype (Table 1).

To study the expression of detected antigens in seminal fluids, the mAbs were tested with seminal plasma and seminal vesicle fluid (Table 2). Monoclonal antibodies determining antigens on whole sperm, were reactive (out of IVA 524) with seminal plasma but not with seminal vesicle fluid of bull. No reaction was recorded in the fluids with the sperm head region specific mAbs. Antigen recognised by the mAb IVA 520 has been present in all tested fluids.

Table 1. Reactivity of the anti-bull sperm mAbs with bull and boar sperm determined by by indirect immunofluorescence

MAb No.	Staining pattern	Bull sperm	Boar sperm	Isotype of mAbs
IVA 502	WS	+++	–	IgG1
IVA 518	WS	+++	–	IgG1
IVA 524	WS	++	–	IgG3
IVA 537	WS	+++	+	IgG1
IVA 543	WS	++	+	IgG1
IVA 534	H	++	–	IgG2
IVA 508	H	+++	++	IgG1
IVA 527	H	+	–	IgM
IVA 519	A	+++	++	IgG1
IVA 520	A	++	++	IgG2
IVA 545	A	+++	++	IgM
IVA 513	D	+++	++	IgM
IVA 510	HM	++	–	IgG
IVA 517	P	++	–	IgM
IVA 526	P	+	–	IgM

Staining pattern:
WS = whole sperm
H = proximal part of the head
A = acrosome
D = distal region of the head
HM = head and middle piece of tail
P = principal (posterior) piece of tail

Fluorescence intensity:
+++ intense
++ moderate
+ weak

Table 2. Reactivity of the anti-bull sperm mAbs with seminal fluids of bull and bore in ELISA test

MAb No.	Staining pattern on the bull sperm	Seminal plasma		Seminal vesicle fluids of bull
		bull	boar	
IVA 502	WS	+	–	–
IVA 518	WS	+	–	–
IVA 524	WS	–	–	–
IVA 537	WS	+	+	–
IVA 543	WS	+	+++	–
IVA 534	H	–	–	–
IVA 508	H	–	–	–
IVA 527	H	–	–	–
IVA 519	A	–	–	–
IVA 520	A	+++	+++	+++
IVA 545	A	–	–	–
IVA 513	D	–	–	–
IVA 510	HM	+++	–	+++
IVA 517	P	–	–	–
IVA 526	P	–	–	–

Staining pattern:
WS = whole sperm
H = proximal part of the head
A = acrosome
D = distal region of the head
HM = head and middle piece of tail
P = principal (posterior) piece of tail

Intensity of ELISA staining:
+++ intense
++ moderate
\+ weak

Table 3. Molecular size analysis of the antigens determined by mAbs

MAb No.	Protein (kDa) – Under conditions	
	reducing	non–reducing
IVA 502	14	10
IVA 518	14	11
IVA 524	14	11
IVA 537	14	11 10
IVA 543	14	–
IVA 534	114 99	114 94 83
IVA 508	–	–
IVA 527	32	–
IVA 519	–	–
IVA 520	–	–
IVA 545	24 20	–
IVA 513	49 30 19	–
IVA 510	15	88
IVA 517	–	–
IVA 526	–	–

Western blot analysis of sperm plasma membrane extracts obtained with 1% NP 40 lysis of bull spermatozoa was used to determine the relative molecular weight under reducing and non-reducing conditions (Table 3). Five mAbs detecting molecules on the whole sperm identified one uniform band with molecular size 14 kDa under reducing conditions and one band with molecular size 10 or 11 kDa under non-reducing conditions. Molecular weights recognised by remaining mAbs were more dispersed, with molecular size ranges of 15–114 kDa. Four mAbs (IVA 537, IVA 534, IVA 545, IVA 513) identified more than one distinct protein band under reducing or non-reducing conditions and some mAbs apparently did not recognise any protein in Western blot.

DISCUSSION

Surface molecules exposed at sperm plasma membrane are important for physiology of sperm and their ability to interact with the zona pellucida during the fertilisation process. In the fusion experiment 15 hybridomas producing mAbs have been prepared with different reaction pattern on bull sperm. Some of them are also reactive with seminal plasma and seminal vesicle fluid. The most frequent mAbs are reactive on whole sperm surface. Three mAbs recognise antigenic sites on proximal part of sperm head, 3 mAbs on the acrosome, 2 mAbs on the sperm tail, 1 mAb on the distal region of the head, whereas 1 mAb was bound on two sites of sperm surface, the head and middle part of tail. Four mAbs generated from the bull sperm immunisation were reactive in the middle piece of ejaculated sperm (Chakraborty *et al.*, 1985). Ambrose *et al.* (1996) similarly to this study, prepared mAbs against bull sperm reacting with five different parts of sperm (the principal acrosomal pattern, the apical crescent pattern, the whole head pattern, the equatorial band pattern and the posterior tail pattern). Two reaction patterns (acrosome and posterior tail) seem to be shared by the two reaction types of some mAbs presented in this study. However, the relative weight of the molecule detected by the specific mAb on spermatozoa and described by Ambrose *et al.* (1996) is higher (70–200 kDa) than the molecular weigh of sperm protein recognised by IVA 545 mAb. These 24 kDa proteins are specific for acrosome. Furthermore, their mAbs detect intra-acrosomal antigens to opposite of mAbs presented here which are binding to the acrosome surface of intact sperm. Higher molecular weight protein (28 kDa) was detected also on bull sperm acrosome by the cross-reactive monoclonal antibody against pig intra-acrosomal sperm protein by Pěknicová and Moos (1998). Unfortunately, the remaining two mAbs of the acrosome specific group (IVA 519 and IVA 520) as well as the tail specific mAbs IVA 517 and IVA 526 have not developed band(s) in Western blot.

Data of the indirect immunofluorescence test and the Western blotting did not reveal clear similarity between newly generated monoclonal antibodies and the mAbs described earlier, including the mAb M1 detecting the heparin binding proteins of sperm and seminal fluid of bull (Bellin *et al.*, 1996) as well as the cross-reactive mAbs with bull sperm (Coonrod *et al.*, 1994, 1996; Rajamahendran *et al.*, 1994). The fusion experiment was performed by spleen cells from mice immunised with whole bull sperm. The mice humoral immune response was directed against random immunogen occurring on the cell surface of sperm. Consequently, mAbs of different specificity were generated. To identify the target molecules determined by the produced mAbs needs a detail biochemical and functional analysis. The group of five mAbs reacting with the whole sperm surface and seminal fluids, in the Western blot analysis uniformly recognises one band with molecular weight 14 kDa under reducing conditions and 10–11 kDa under non-reducing conditions. This size of molecule is characteristic to the spermadhesin molecules. Spermadhesins are new family of secretory antigens in the genital tract of pig, horse and bull. There is a suggestion that the spermadhesins are involved in different steps of fertilisation (Töpfer-Petersen *et al.*, 1998).

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The immunomodulatory activity of soluble β-1,3-D-glucan derivatives in piglets after weaning

Imunomodulační aktivita derivátů rozpustného β-1,3-D-glukanu u selat po odstavu

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ABSTRACT: The effect of intramuscularly administered soluble derivatives of β-1,3-D-glucan, obtained from the culture filtrate of the yeast *Saccharomyces cerevisiae*, on the immunological activity of neutrophils and monocytes in piglets after weaning was examined. The single intramuscular administration of carboxymethylglucan at doses 0.5 and 5.0 mg/kg b.w. and sulfoethylglucan at a dose of 0.5 mg/kg b.w. enhanced the reductase activity of neutrophils. The greatest enhancing effect was observed at 0.5 mg/kg b.w. of carboxymethylglucan. Bactericidal activity of peripheral phagocytes and LPS- or PHA-induced proliferation of peripheral blood monocytes were also enhanced after the administration of carboxymethylglucan and sulfoethylglucan by the intramuscular route. These results demonstrate that immunocompetent cells were activated after a single intramuscular administration of all soluble derivatives of β-1,3-D-glucan tested. It is probable that an adverse influence of stress on immune functions in animals after weaning may be reduced by the administration of soluble derivatives of β-1,3-D-glucan.

Keywords: piglet; carboxymethylglucan; sulfoethylglucan; neutrophil; bactericidal activity; proliferation of peripheral blood monocytes

ABSTRAKT: Pomocí imunologických testů byl hodnocen vliv intramuskulárně podaných rozpustných derivátů β-1,3-D glukanu, izolovaného z kvasinky *Saccharomyces cerevisiae*, na imunokompetentní buňky selat zatížených stresem vyvolaným odstavem, změnou krmné technologie a manipulací při aplikaci testovaných látek a odběrech krve. Jednorázová intramuskulární aplikace karboxymethylglukanu v dávkách 0,5 a 5,0 mg/kg stejně jako dávka 0,5 mg/kg sulfoethylglukanu zvyšovaly v porovnání s kontrolní skupinou reduktázovou aktivitu neutrofilních granulocytů selat. Rozpustné deriváty β-1,3-D-glukanu rovněž ovlivňovaly baktericidní aktivitu plné krve selat po odstavu stejně jako proliferační odpověď monocytů na *in vitro* přidané stimuly (LPS nebo PHA). Je tedy pravděpodobné, že aplikací rozpustných forem β-1,3-D-glukanu lze snížit negativní vliv stresu na imunitní funkce u zvířat po odstavu.

Klíčová slova: sele; karboxymethylglukan; sulfoethylglukan; neutrofilní granulocyt; baktericidní aktivita plné krve; proliferace periferních monocytů

INTRODUCTION

Various types of biological response modifiers (BRM) have been developed for therapy of microbial infections and cancer in animals and humans. However, most BRM are classified as non-specific because their exact mode of action is unknown. β-glucan BRM are primarily derived from fungi and have backbone structures of β-1,3-linked D-glucose (β-1,3-D-glucan) with β-1,6-linked side chains of β-1,3-D-glucan of varying sizes and frequencies along the backbone (Bohn *et al.*,1995). Glucan polymers exist in a non-branched or branched form, as single polymer strands with a helical conformation or as a stable of 3 polymer strands forming a triple helix.

β-glucan BRM rank among anti-allergic, non-toxic substances. These very stable polymers can stimulate a hematopoietic response (Hofer *et al.*, 1995), activate the blood coagulation system and the complement system (Alban and Franz, 1994), decrease cholesterol levels (Behall *et al.*, 1997). The β-1,3-D-glucan exerts a therapeutic effect on variety of experimental bacterial, viral and fungal diseases (Williams *et al.*, 1983; Browder *et al.*, 1990). Specifically, the β-1,3-D-glucan isolated from *Saccharomyces cerevisiae* is a water-insoluble, micro-particulate (1–2 μm) polymer upon initial isolation. While topical or intralesional administration of a micro-particulate glucan induces no toxicity (Browder *et al.*, 1988), the systemic administration of the micro-particulate form is associated with hepatosplenomegaly, granuloma formation, microembolization (Patchen *et al.*, 1986) and enhanced endotoxin sensitivity.

Soluble derivatives are free of side effects. These derivatives can be prepared from corpuscular glucan either by hydrolysis or by chemical derivatization (Williams *et al.*, 1991). However, the biological activity of soluble derivatives mostly decreases with increasing solubility and a degree of substitution (DS). Clearly, there is a need for development of a process for converting water-insoluble β-1,3-D-glucans to safe, effective, water-soluble forms.

The aim of the present study was to examine the effects of two β-1,3-D-glucan soluble derivatives, both carboxymethylglucan and sulfoethylglucan, on the immune response in piglets after a single intramuscular administration.

MATERIAL AND METHODS

Experimental animals

Twelve healthy piglets after weaning (Czech Large White Breed), 21–25 days of age, were used throughout this study. Piglets were housed conventionally. They were fed a standard diet (ČOS, Zdice near Beroun, Czech Republic). Temperature and humidity were monitored and recorded.

Test polysaccharides and their administration

The initial substance of particular β-1,3-D-glucan was isolated from cell walls of the yeast *Saccharomyces cerevisiae* after disintegration in alkaline solution. Soluble β-1,3-D-glucan derivatives carboxymethylglucan (CMG) with DS 0.67 and sulfoethylglucan (SEG) with DS 0.12 were prepared by the firm Contipro (Ústí nad Orlicí, Czech Republic). The preparation of CMG was carried out in an anhydrous medium by reaction of free –OH groups with a halogen derivative of acetic acid (monochloroacetate). SEG was prepared in an alkaline medium by reaction with chlorosulfonic acid ($ClSO_3$). Optimum degrees of substitution 0.68 and 0.12 for CMG and SEG, respectively, were obtained proceeding from our previous experiments. The tested compounds were dissolved in a sterile saline and administered only once intramuscularly, dosed 0.5 mg/kg b.w. or 5 mg/kg b.w, to piglets at the age of 21–25 days (day 0 of this experiment).

Chemicals

Reagents and manufacturers were as follows: RPMI 1640, H-MEM, D-MEM (Sevac, Czech Republic), balanced salt solution (BSS), gentamycin, fetal calf serum (FCS), Histopaque, trypan blue, phytohemagglutinin (PHA), lipopolysaccharide from *Salmonella typhimurium* (LPS), 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium-chloride (INT), dimethyl sulfoxide (DMSO), zymosan (Sigma Chemical Co. St. Louis, MO), β-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-kit (Boehringer Mannheim Biochemicals, Indianapolis, IN), trypticase soy broth (TSB), Endo agar plate (Oxoid, England) and heparin (Léčiva, a. s., Czech Republic).

Bacterial strain

E. coli strain CNCTC 408/81 (Brno, Czech Republic)

Blood samples

Approximately 10 ml of blood samples were collected from the *vena cava* to sterilized and heparinized tubes. The samples of blood were collected on days 0, 1, 3, 6 and 10 of this experiment.

Histopaque gradient separation of neutrophils and monocytes

Samples of heparinized blood were layered on Histopaque medium. After centrifugation a fraction of erythrocytes in pellet was lysed by exposure to hypotonic shock. Neutrophils were washed twice in BSS and finally suspended in H-MEM at a concentration 1×10^7 cells/ml.

Monocytes concentrated in a layer (inter-phase) were washed three times in D-MEM medium and re-suspended in RPMI 1640 culture medium with 10% fetal bovine serum and gentamycin at a concentration 4×10^6 cells/ml. Cell viability was determined by trypan blue exclusion.

The oxidative burst of neutrophils after *in vitro* stimulation by particular glucan or zymosan (INT test)

100 μl aliquots of the final concentration suspensions of neutrophils harvested from different experimental animals were inoculated into quadruplicate flat-bottomed wells of a 96-well tissue culture plate (5×10^5 cells/well). The optimal concentrations of zymosan or particular glucan (10 mg/ml) were added to the wells for *in vitro* stimulation. Cultures were incubated for 1 hour. The amount of reduced INT (the formazan crystals) was measured after

solubilization in DMSO. The resulting colored solution was quantified using a scanning multi-well spectrophotometer at 450/650 nm.

Results were expressed as an index of stimulation (SI), which was calculated as differences between an INT reduction triggered by zymosan or particular glucan and a spontaneous INT reduction.

Determination of a lymphoproliferative response (proliferation assay)

100 µl aliquot of the final concentration suspension of peripheral blood monocytes (PBMC) was inoculated into quadruplicate flat-bottomed wells of a 96-well tissue culture plate (4×10^5cells/well). The optimal mitogen concentrations (PHA 5 µg/ml, LPS 10 µg/ml) were used for *in vitro* stimulation. Cultures were incubated for 67 hours in an atmosphere of 5% CO_2 with 100% relative humidity. Ten microliters of the MTT labeling reagent (final concentration 0.5 mg/ml) were added to each well after termination of the incubation period. The culture plates were incubated for 4 hrs in a humidified atmosphere (37°C, 5% CO_2). The produced formazan crystals were solubilized and quantified using a scanning multi-well spectrophotometer (Dynatech) at 550–600/650 nm.

Results were presented as the proliferation index (m.a. is mean absorbance):

$$= \frac{\text{m.a. (experimental conditions)} - \text{m.a. (medium control)}}{\text{m.a. (medium control)}}$$

Bactericidal activity

An 18-hour TSB culture of *E. coli* strain CNCTC 408/81 was prepared. Two concentrations of bacterial suspensions (5×10^9 colony forming unit (CFU/ml and 5×10^8 CFU per ml) were used. Each suspension (50 µl) was inoculated into fresh heparinized blood (500 µl) of piglets so that two ratios (1 : 10 and 1 : 1) of bacterial cells and phagocytes were reached. Both vials were mixed and cultivated while shaking at 37°C. 100 µl samples were taken at following intervals: 0, 30, 60 and 180 minutes. Each sample was diluted and placed onto an Endo agar plate so that the total colony count after incubation was between 30 and 300 CFU per plate. Real counts of bacteria per ml of blood were calculated.

Statistical analysis

The data were expressed as mean values ± S.D. Significant differences between groups were determined by Student's *t*-test. The value of $P < 0.05$ was considered significant.

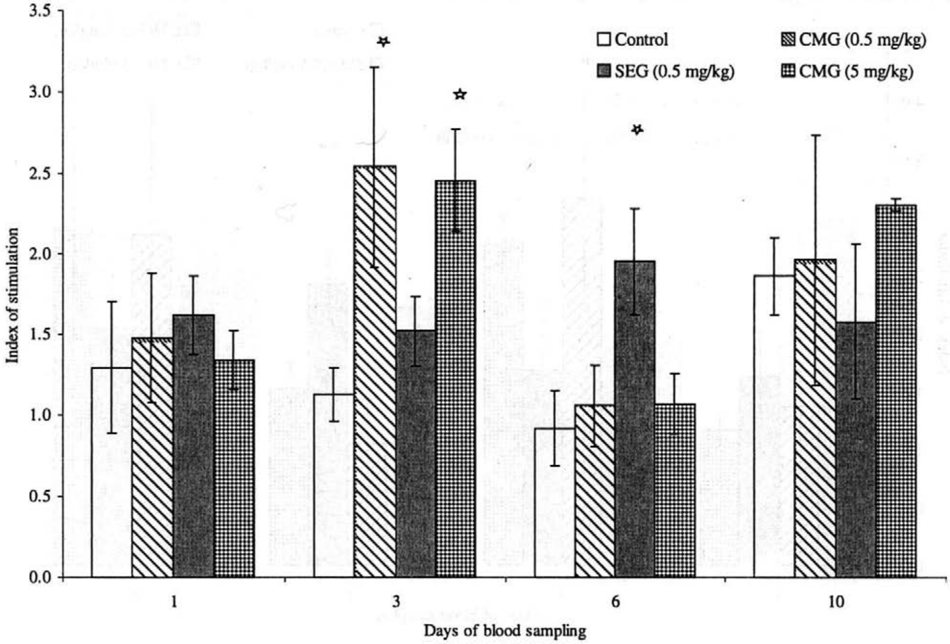

Figure 1. The oxidative burst of *ex vivo* obtained piglet neutrophils after *in vitro* stimulation by particular glucan. Piglet groups treated were administered *i.m.* CMG dosed 0.5 and 5.0 mg/kg b.w., and SEG dosed 0.5 mg/kg b.w. Neutrophils harvested from different experimental piglets were *in vitro* cultivated for 1 hour with an optimum concentration (10 mg/ml) of particular glucan. Data are expressed as the stimulation index, evaluated from quadruplicate cultures. Values are given as the mean stimulation index ± S.D. Significantly different from the control group: *$P < 0.05$, **$P < 0.01$

RESULTS

The oxidative burst of neutrophils after *in vitro* stimulation by particular glucan or zymosan

The ability of neutrophils to generate an oxidative burst in response to *in vitro* added particular glucan (Figure 1) or zymosan (Figure 2) was tested on piglets treated with a single dose of carboxymethylglucan or sulfoethylglucan. On day 3 of this experiment, significant enhancing of neutrophil metabolic activity was detected at 0.5 mg/kg b.w. and 5 mg/kg b.w. of carboxymethylglucan in comparison with the control group. An effect of sulfoethylglucan at 0.5 mg/kg b.w. on enhancing the metabolic activity in piglet neutrophils after *in vitro* stimulation was detected from the sixth day.

Modulation of PHA or LPS -induced mono-cells proliferation by soluble derivatives of β-1,3-D-glucan

In order to investigate whether the mitogenic activity of intramuscularly administered soluble glucans interfered with cell proliferation induced by known mitogens, *ex vivo* isolated peripheral blood monocytes were activated *in vitro* with appropriate mitogens. When monocytes were *in vitro* stimulated with LPS, the proliferation increased from the third day at 0.5 mg/kg b.w. of carboxymethylglucan and/or from the sixth day at 0.5 mg/kg b.w. of sulfoethylglucan in comparison with the control group. These results indicate that LPS and the soluble derivatives of β-1,3-D-glucan partly synergize in the induction of mono-cells proliferation (Figure 3).

When monocytes were *in vitro* stimulated with PHA, the proliferation increased from day ten in all groups as compared with the control group, except for a positive response on day 3, at 0.5 mg/kg b.w. of carboxymethylglucan (Figure 4).

Modulation of whole blood bactericidal activity

Relative CFU (%) values of the control group, the sulfoethylglucan group (dosed 0.5 mg/kg b.w.), the carboxymethylglucan group (dosed 0.5 mg/kg b.w.), and the CMG group (dosed 5 mg/kg b.w.) are presented in Figures 5, 6, 7, and 8, respectively.

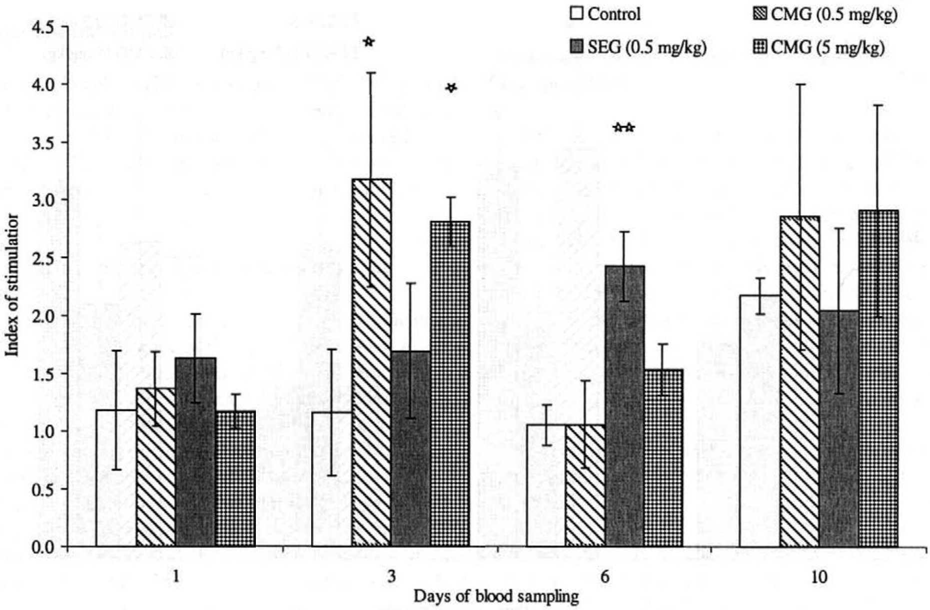

Figure 2. The oxidative burst of *ex vivo* obtained piglet neutrophils after *in vitro* stimulation by zymosan. Piglet groups treated were administered . CMG dosed 0.5 and 5.0 mg/kg b.w., and SEG dosed 0.5 mg/kg b.w. Neutrophils harvested from different experimental piglets were *in vitro* cultivated for 1 hour with an optimum concentration (10 mg/ml) of zymosan. Data are expressed as the stimulation index, evaluated from quadruplicate cultures. Values are given as the mean stimulation index ± S.D. Significantly different from the control group: $*P < 0.05$, $**P < 0.01$

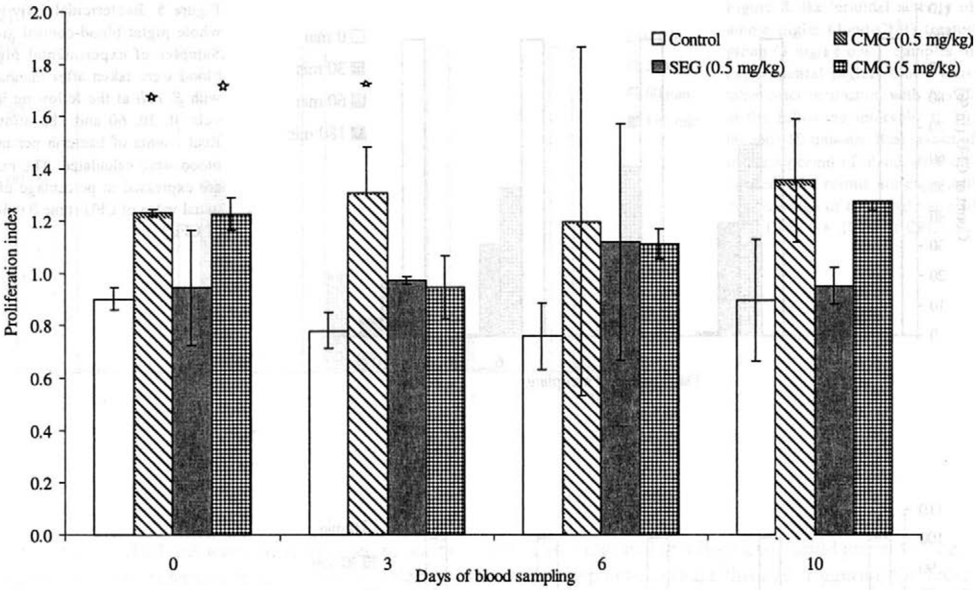

Figure 3. Influence of parenteral administration of soluble derivatives of 1,3-β-D-glucan on LPS-induced peripheral blood monocytes proliferation. Piglet groups treated were administered *i.m.* CMG dosed 0.5 and 5.0 mg/kg b.w., and SEG dosed 0.5 mg/kg b.w. Peripheral monocytes were *in vitro* stimulated with a dose of LPS (10 μg/ml). Proliferation was measured by MTT assay. Data are expressed as the stimulation index, evaluated from quadruplicate cultures. Values are given as the mean stimulation index ± S.D. Significantly different from the control group: $^*P < 0.05$, $^{**}P < 0.01$

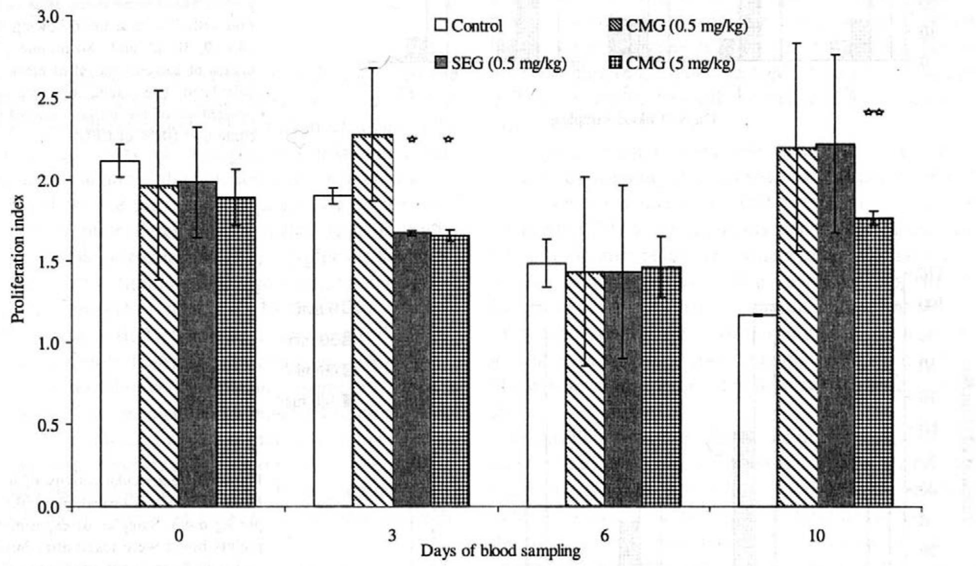

Figure 4. Influence of parenteral administration of soluble derivatives of 1,3-β-D-glucan on PHA-induced peripheral blood monocytes proliferation. Piglet groups treated were administered *i.m.* CMG dosed 0.5 and 5.0 mg/kg b.w., and SEG dosed 0.5 mg/kg b.w. Peripheral monocytes were *in vitro* stimulated with a dose of PHA (5 μg/ml). Proliferation was measured by MTT assay. Data are expressed as the stimulation index, evaluated from quadruplicate cultures. Values are given as the mean stimulation index ± S.D. Significantly different from the control group: $^*P < 0.05$, $^{**}P < 0.01$

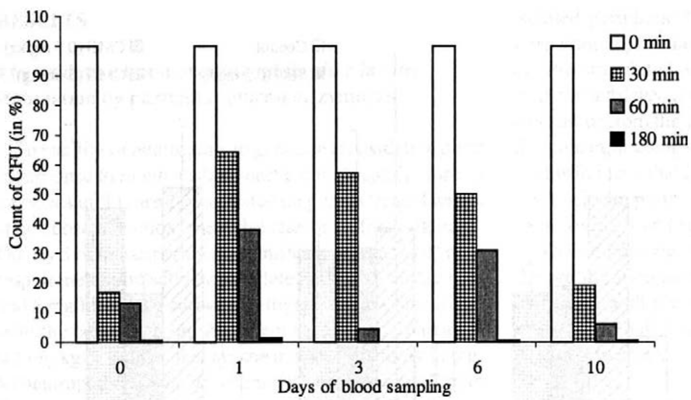

Figure 5. Bactericidal activity of whole piglet blood-control group. Samples of experimental piglets blood were taken after incubation with *E. coli* at the following intervals: 0, 30, 60 and 180 minutes. Real counts of bacteria per ml of blood were calculated. The results are expressed in percentage of the initial value of CFU (time 0 = 100% of CFU)

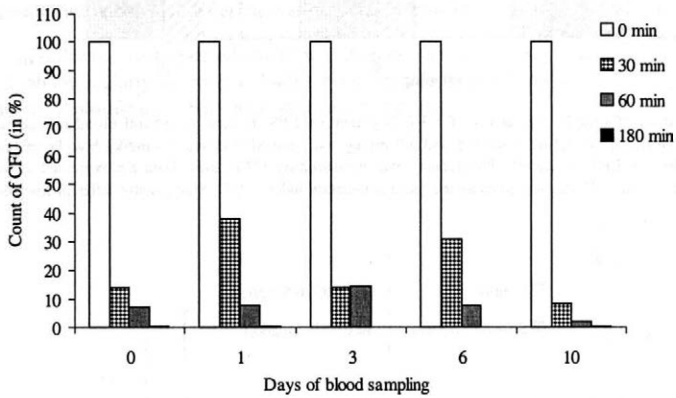

Figure 6. Bactericidal activity of whole piglet blood-SEG treated group (0.5 mg per kg b.w.). Samples of experimental piglets blood were taken after incubation with *E. coli* at the following intervals: 0, 30, 60 and 180 minutes. Real counts of bacteria per ml of blood were calculated. The results are expressed in percentage of the initial value of CFU (time 0 = 100% of CFU)

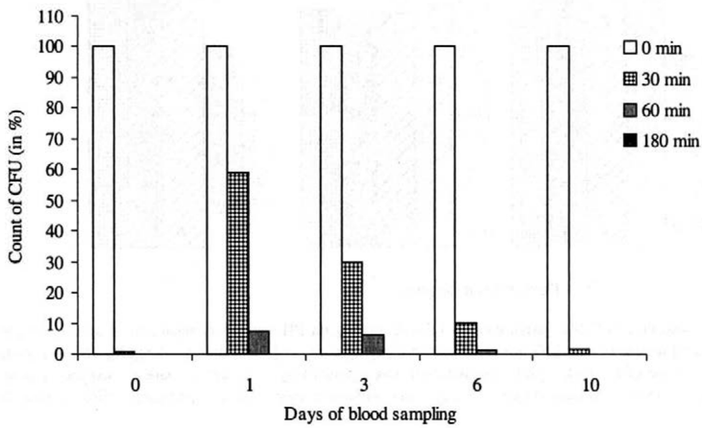

Figure 7. Bactericidal activity of whole piglet blood-CMG treated group (0.5 mg per kg b.w.). Samples of experimental piglets blood were taken after incubation with *E. coli* at the following intervals: 0, 30, 60 and 180 minutes. Real counts of bacteria per ml of blood were calculated. The results are expressed in percentage of the initial value of CFU (time 0 = 100% of CFU)

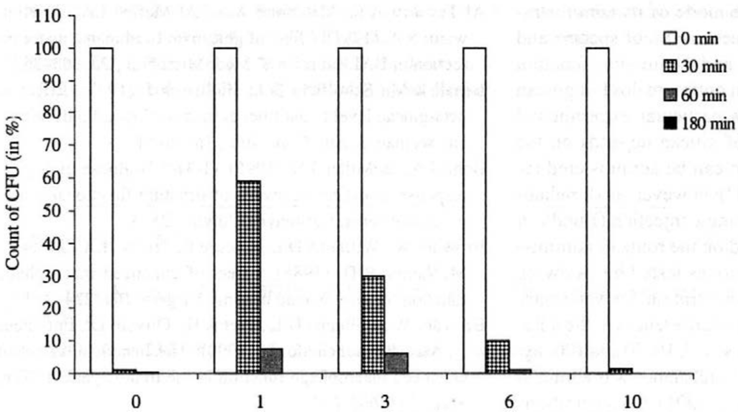

Figure 8. Bactericidal activity of whole piglet blood-CMG treated group (5 mg/kg b.w.). Samples of experimental piglets blood were taken after incubation with *E. coli* at the following intervals: 0, 30, 60 and 180 minutes. Real counts of bacteria per ml of blood were calculated. The results are expressed in percentage of the initial value of CFU (time 0 = 100% of CFU)

There was a marked influence of stress factors on the bactericidal activity dynamics in all groups. The depression was most conspicuous on the first day after weaning in 30-minute samples. Better bactericidal activity of piglet blood was revealed from the sixth day after application of all tested compounds in comparison with the control group.

DISCUSSION

β-1,3-D-glucans are studied for their ability to activate host defense mechanisms against tumors, microbial, fungal and parasitic infections. The exact mechanism of the immunomodulatory effect of glucan is unknown. It may include stimulation of the functional and metabolic activity of cells of the mononuclear phagocytic system and that of neutrophils (Ferenčík *et al.*, 1988). High molecular weight soluble or particulate β-1,3-D-glucans have also been shown to stimulate neutrophil degranulation and respiratory burst (Větvička *et al.*, 1996) and the secretion of interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) from macrophages (Doita *et al.*, 1991). Polymorphonuclear cells play an important role in the body defense mechanisms against invading microorganisms. A number of events is involved in this elimination process, opsonization, recognition, chemotaxis, phagocytosis, intracellular killing (by degranulation and formation of peroxides and free radicals), and finally digestion.

Macrophages are probably the most important targets of the β-glucan activity, nevertheless only some of their functions seem to be mediated directly. Other modulating effects of β-glucan evidently depend on the cooperation with other cell populations, namely T-lymphocytes. Some data also suggested that β-glucans could promote T cell-specific response, perhaps through triggering the secretion of interferon-γ (IFN-γ), IL-6, IL-8, IL-12 from macrophages, neutrophils and natural killer (NK) cells (Ross *et al.*, 1999).

In case of stress loads, many defense mechanisms are weakened or altered and susceptibility of organisms in respect of pathogenic agents is enhanced. This study was to verify to what extent a single parenteral administration of soluble glucan modification is able to affect the health status in piglets subjected to many stress factors after weaning (e.g. change in feed, change in frequency of feéding, loss of the mother, transfer to a new environment).

Early stabilization of immune responses after the stress induced by weaning reduces risks of diseases in animal youngsters. It is interesting that after administration of soluble β-1,3-D-glucan derivatives to piglets, the restoration of immunity response markers to their initial values was earlier in comparison with the control group. The stabilization or enhancement of immunocompetent cell activity after administration of the soluble forms of tested glucan have indicated their potential utilization in prophylaxis or in the treatment of diseases in animal youngsters.

In correlation with results of the neutrophil metabolic activity test, there was an increase in phagocyte bactericidal activity in the piglet whole blood. Both the values of neutrophil oxidative burst and bactericidal values were the same on day 10 as on day 0 (before the weaning). These results correspond to the presumption that activation of professional phagocytes by glucans is mediated through specific β-D-glucan surface receptors (Czop *et al.*, 1986).

Obviously, it depends on the mode of its administration, properties of the used glucan, type of species and strain of experimental animals, and the immune function which should be influenced. An optimum dose of glucan should be determined for each particular experimental model. Obviously, the effect of glucan depends on the route of administration. Glucan can be administered repeatedly (Al Tuwaijri *et al.*, 1987); however, its stimulatory effect occurs usually after a single injection (Buddle *et al.*, 1988). This fact has decided on the route of administration of soluble glucan derivatives tested by us owing to advantages of a single administration for veterinary practice. For example, only minor differences in the adjuvant activity of various glucan doses (5, 10, 50 and 100 mg/kg) in mice were found when this substance was administered *i.v.* or *s.c.* (Wagnerová *et al.*, 1991). Concentrations of soluble β-1,3-D-glucan derivatives used in our study were selected based on results obtained in the model of a single intramuscular administration of the test substance in mice (data obtained at our workplace and not published so far). It is evident from these results that lower doses of the immunomodulator (0.5 mg/kg b.w. and 0.1 mg/kg b.w.) exhibited a better modulating effect in respect of corresponding cells.

The parenteral administration of carbohydrate immunomodulators is frequently associated with a biphasic stimulatory effect (Williams *et al.*, 1992). It is, therefore, recommended to prolong the testing schedule up to 21 to 28 days in order to characterize more precisely the kinetics of stimulation of the immune response. This fact must be taken into account in subsequent studies.

It is not quite clear what contributes to an enhancement or reduction of a lymphoproliferative response after glucan activity, whether lymphocytes responding to individual mitogens rank among primary targets of the immunoregulating interference, or whether the enhanced DNA synthesis is a reflection of the activity of accessory cells which regulate, directly or indirectly, the cells capable of reaction to individual mitogens. It is possible that the reduced proliferative response to mitogens may be a consequence of the regulatory activity which inhibits a polyclonal response anytime when a parallel stimulation of the immune system takes place (Macela, 1998).

It is evident from numerous publications that glucans – thanks to their ability to modify functions of mononuclear phagocytes – may affect almost all phases of an induced immune response. Owing to their broad-spectrum activity and efficacy in combination with other therapeutic agents, they are worth further comprehensive study.

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Changes in amino acid composition of rabbit (*Oryctolagus cuniculus*) milk within the first 30 days after parturition

Změny aminokyselinového složení králičího (*Oryctolagus cuniculus*) mléka v průběhu 30 dnů po porodu

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ABSTRACT: The variability and changes in amino acid composition of rabbit milk were studied in milk samples collected from 6 nursing female rabbits 2, 4, 11, 14, 17, 21, 24, 27 and 30 days after the parturition. The results documented a marked decrease in concentration from Day 2 to Day 11 after birth and subsequently to Day 30 a gentle decrease in dry matter (from 36.21% to 28.60% and 26.98%, respectively) and crude protein (from 21.09 to 13.80% and 12.89%, respectively). The highest concentrations of all amino acids were observed also on Day 2, changes in the contents from Day 2 to Day 30 were relatively small except Leu (its content dropped from 11.5% on Day 2 to 10% between Day 24 and Day 30) and Glu (a decrease from approx. 18–19% between Day 2 and Day 11 to 15% on Day 30). The average amino acid composition (expressed as percentage of total protein) was as follows: Thr 5.17 ± 0.11, Val 5.07 ± 0.11, Ile 5.47 ± 0.10, Leu 11.02 ± 0.67, Phe 3.93 ± 0.32, His 3.38 ± 0.26, Lys 6.46 ± 0.40, Arg 4.31 ± 0.51, Cys 1.54 ± 0.05, Met 2.55 ± 0.42, Asp 5.29 ± 0.12, Ser 5.98 ± 0.15, Glu 17.63 ± 1.10, Pro 5.51 ± 0.24, Gly 3.04 ± 0.12, Ala 4.86 ± 0.12 and Tyr 4.07 ± 0.47.

Keywords: rabbit; milk; crude protein; amino acid

ABSTRAKT: Variabilita a změny v aminokyselinovém složení králičího mléka byly sledovány u vzorků mlék, odebíraných od šesti kojících ramlic 2., 4., 11., 14., 17., 21., 24., 27. a 30. den po porodu. Výsledky dokumentují výrazný pokles koncentrace od 2. dne do 11. dne a následující mírný pokles do 30. dne u sušiny (z 36,21 na 28,6 a 26,98 %) a hrubých bílkovin (z 21,09 na 13,80 a 12,89 %). Také u všech aminokyselin byly zjištěny nejvyšší koncentrace 2. den, změny mezi 2. a 30. dnem byly relativně malé mimo Leu (z 11,5 % ve 2. dni klesl na 10 % od 24. do 30. dne) a Glu (z úrovně asi 18 až 19 % od 2. do 11. dne následně klesá na 15 % ve 30. dnu). Průměrné obsahy aminokyselin (vyjádřeno jako procenta z celkových bílkovin) byly: Thr 5,17 ± 0,11; Val 5,07 ± 0,11; Ile 5,47 ± 0,10; Leu 11,02 ± 0,67; Phe 3,93 ± 0,32; His 3,38 ± 0,26; Lys 6,46 ± 0,40; Arg 4,31 ± 0,51; Cys 1,54 ± 0,05; Met 2,55 ± 0,42; Asp 5,29 ± 0,12; Ser 5,98 ± 0,15; Glu 17,63 ± 1,10; Pro 5,51 ± 0,24; Gly 3,04 ± 0,12; Ala 4,86 ± 0,12 and Tyr 4,07 ± 0,47.

Klíčová slova: králík; mléko; hrubé bílkoviny; aminokyseliny

INTRODUCTION

Rabbit milk production and composition are the basic factors of successful nursing. From the nutritive point of view, milk protein and its amino acids are important components of rabbit milk. The total dry matter (DM) content of rabbit milk is approx.. 30% (Chawan *et al.*, 1982 report 28.52% on average, Kalugin, 1992 from 14.6 to 14.1/100 ml and Kustos *et al.*, 1999 from 28.9% to 31.7%). Similar values were reported by Laxa (1928), who reviewed the authors from the beginning of the 20th century (31.52% – Abderhalden, 1899 or 30.50% – Pizzi, 1894; Teichert, 1903). Of the total DM in rabbit milk, proteins constitute approximately 50%. In fresh rabbit milk Chawan *et al.* (1982) reported the protein level of about 21% on Day 1 with a gradual decrease to 16.9% on Day 2 and thereafter to 13% on Day 30. Kustos *et al.* (1999) stated that depending on the environmental temperature and feeding conditions, the crude protein content ranged from 14.1% to 14.6%. Kalugin (1992) reported protein content variations on var-

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ious dates of lactation within the range of 13.0 to 16.9%. Similarly, in papers published as early as at the beginning of the 20th century, the mean protein content was reported to be 15.54% (Pizzi, 1894; Teichert, 1903). Abderhalden (1899) reported that average concentrations of casein and albumin in 6 milk samples obtained from 1 female were 8.17% and 2.21%, respectively. Changes in rabbit milk composition including the amino acid content from 3 to 5 nursing rabbits from Day 1 to Day 30 postpartum were reported by Chawan *et al.*, 1982 and the dependence of this parameter on the environmental temperature and restricted feeding was investigated by Kustos *et al.* (1999).

The aim of this study was to assess the variability and changes in dry matter and crude protein contents and in amino acid composition of milk sampled from 6 female rabbits from Day 2 to Day 30 after the parturition.

MATERIAL AND METHODS

Changes in the composition of amino acids in milk of female rabbits were examined in 6 hybrids (New Zealand × Californian) from Day 2 to Day 30 after the parturition in the months of May and June.

During the experiment, the female rabbits were fed a pelleted feeding mixture *ad libitum* (Table 1) that was optimized as recommended by Zeman *et al.* (1995). The diet contained 86.65% of dry matter, 13.73% of crude protein, 1.97% of fat and 13.53% of fiber.

The animals were kept in wire cages. In female rabbits, hairs were clipped around their mammary glands. Milk letdown was induced by subcutaneous injections of 2.5 to 3.5 I.U. of oxytocin (Ferring – Medicaments Co.). After 5–7 minutes, massages were carried out, and milk was drawn from the mammillae and caught into tubes. Milk samples were taken 2, 4, 11, 14, 17, 21, 24, 27 and 30 days

Table 1. Composition of the pelleted feeding mixture

Ingredient	%
Meal wheat and barley (ratio 1 : 1)	41.0
Clover mixture hay	40.0
Soybean meal	7.0
Oat meal	4.5
Molasses	4.0
MIKROS – K (mineral mixtures for rabbits)	2.5
FARVIT LK (biofactor supplement)	1.0
Dry matter	86.65
Crude protein	13.73
Fat	1.96
Fiber	13.53
Lysine	0.641
Cystine + Metionine	0.269
Energy (MJ/kg)	10.13

after birth at amounts of approx. 2 to 3 ml. Tubes containing milk were frozen immediately after sampling and stored at –18°C.

The dry matter content was determined gravimetrically (Davídek *et al.*, 1981). Nitrogen substances were determined by Kjeldahl using the apparatus Kjeltec Auto Analyzer (f. Tecator). Crude protein was calculated as N × factor of 6.38 (Richardson, 1985). Milk samples for amino acid determination were adjusted using oxidative acidic hydrolysis (Davídek *et al.*, 1981). The chromatographic analysis of sample hydrolysates was performed in the analyzer AAA 400 (f. Ingos, Prague) and using Na-citrate buffers and ninhydrin detection. The results were evaluated using the variation statistics (ANOVA) according to Snedecor and Cochran (1967) and mean and relative standard deviations in percent (RSD%) were expressed.

RESULTS AND DISCUSSION

The variability and changes in amino acid composition of rabbit milk were studied in milk samples collected from 6 nursing rabbits 2, 4, 11, 14, 17, 21, 24, 27 and 30 days after birth. The average values (in percent) for total solids (DM) and crude protein (CP) on Day 2 were 36.21 and 21.09%, respectively, and dropped with a small variability to 28.60 and 13.82%, respectively, on Day 11. Thereafter they decreased gradually to 26.98 and 12.83% on Day 30 (Table 2). As compared with the results of other authors, Chawan and Rao (1982) reported for the contents of milk from 3 to 5 nursing rabbits the protein level about 21% on Day 1, 16.9% on Day 2 and 13% on Day 30 while the average content of total solids was 28.52%. Kustos *et al.* (1999) reported the CP content between 14.1 and 14.6 g per 100 ml and DM between 28.9 and 31.3 g/100 ml during the lactation period of Day 3 to Day 27 and Kalugin (1992) mentioned that protein content ranged from 13.0 to 16.9% on various days of lactation; similarly like in publications from the beginning of the 20th century (Laxa, 1928) the mean protein content in rabbit milk is reported to be 15.54%.

Similarly like in the milk of other animal species (cow milk, goat milk, sheep milk and human milk – Velíšek *et al.*, 1999) the highest content of all amino acids present in rabbit milk (expressed as percentage of total protein) was recorded for Leu (11.5% on Day 2 dropped to 10% from Day 24 to Day 30) and Glu (a decrease from approx. 18 to 19% between Day 2 and Day 11 to 15% on Day 30). Changes in the contents of other amino acids in the period of Day 2 to Day 30 were relatively small (Table 3). Variability of their contents in the milk of individual animal species was also relatively low and a high variability was observed only in the case of Tyr (from 6.0 to 13.5% RSD) and Met (from 3.4 to 13.7% RSD). The average amino acid composition (expressed as percentage of total protein) was as follows: Thr 5.17 ± 0.11, Val 5.07 ± 0.11, Ile 5.47 ± 0.10, Leu 11.02 ± 0.67, Phe 3.93 ± 0.32, His 3.38 ± 0.26, Lys 6.46 ± 0.40, Arg 4.31 ± 0.51, Cys 1.54 ± 0.05, Met 2.55 ± 0.42, Asp 5.29 ± 0.12, Ser 5.98 ± 0.15, Glu 17.63 ± 1.10, Pro 5.51 ± 0.24, Gly 3.04 ± 0.12, Ala 4.86 ± 0.12 and Tyr 4.07 ± 0.47.

Variability in the contents of Σ EAA and Σ NEAA (Table 2) is low and on Day 2 and Day 21 the measured levels were approx. 45 and 47–48%, respectively. Subsequently, they dropped by Day 30 to 38.5% in Σ EAA and to 40.6% in Σ NEAA. Larger differences than in our analysis of amino acid contents were reported by Chawan and Rao

Table 2. Quantitative values of the sums of essential, non-essential amino acids and crude protein and dry matter (g/100 g) of rabbit milk on days 2–30 after parturition (mean, RSD in %)

	Σ EAA		Σ NEAA		CP		DM	
Day	mean	RSD	mean	RSD	mean	RSD	mean	RSD
2	45.96	0.5	48.37	1.3	21.09	0.8	36.21	1.1
4	45.69	0.7	48.25	2.2	16.62	0.9	33.20	1.7
8	45.55	0.7	48.06	1.9	15.49	0.6	30.20	1.5
11	45.89	2.1	48.03	0.3	13.82	1.2	28.64	1.4
14	45.22	0.7	47.46	1.0	13.71	2.6	28.41	1.5
17	45.07	0.6	47.74	2.3	13.43	0.7	28.13	1.8
21	44.47	0.7	46.95	1.5	13.40	3.3	27.82	1.7
24	41.13	0.5	45.29	2.0	13.27	0.9	27.43	1.4
27	40.66	0.5	43.12	1.9	13.12	0.7	27.27	1.3
30	38.52	0.3	40.62	0.3	12.83	0.4	26.98	1.9

Table 3. Quantitative values of essential and non-essential amino acids of rabbit milk (g/16 g N) on days 2–30 after parturition (mean, RSD in %)

	Thr		Val		Ile		Leu		Phe		His		Lys		Arg	
Day	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD
2	5.18	1.5	5.16	0.7	4.48	2.5	11.57	3.2	4.21	0.4	3.57	0.2	6.97	1.1	4.81	3.6
4	5.12	2.8	5.07	1.6	4.50	5.8	11.56	1.5	4.19	1.4	3.52	1.1	6.92	1.0	4.81	3.4
8	5.07	0.8	5.08	1.2	4.59	6.4	11.55	1.6	4.14	0.9	3.52	0.7	6.80	0.7	4.80	3.3
11	5.07	4.5	4.94	1.5	4.46	2.0	11.47	1.0	4.16	0.7	3.55	0.6	6.95	0.7	5.29	4.3
14	5.20	1.5	5.15	2.2	4.62	4.2	11.49	5.2	4.23	0.9	3.59	0.6	6.56	3.6	4.36	4.5
17	5.23	1.5	5.11	1.7	4.48	4.2	11.58	1.0	4.22	0.9	3.62	0.6	6.52	0.7	4.32	2.8
21	5.27	2.3	5.23	2.8	4.62	3.9	11.30	1.3	3.79	0.7	3.59	0.8	6.44	1.5	4.23	2.6
24	5.40	1.2	5.22	1.9	4.30	4.5	9.53	3.9	3.18	0.9	2.89	0.6	6.48	0.5	4.14	2.5
27	5.28	1.0	5.08	1.8	4.53	2.7	10.51	1.1	3.65	1.3	2.83	0.8	5.57	1.1	3.20	2.8
30	4.84	1.9	4.67	1.1	4.16	3.8	9.70	1.2	3.50	0.6	3.13	0.7	5.38	0.9	3.14	2.5

	Cys		Met		Asp		Ser		Glu		Pro		Gly		Ala		Tyr	
Day	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD	mean	RSD
2	1.61	0.7	2.98	10.4	5.24	1.5	6.10	1.6	18.93	1.0	5.69	1.2	3.02	10.0	4.85	2.1	4.54	5.28
4	1.60	0.9	2.93	6.5	5.22	1.6	6.11	3.4	18.94	0.8	5.71	1.0	2.92	7.2	4.82	2.4	4.52	11.12
8	1.61	1.6	2.94	11.4	5.26	2.8	6.03	2.7	18.77	0.8	5.67	1.7	2.98	9.3	4.81	2.6	4.54	11.13
11	1.59	2.1	2.62	10.9	5.24	1.1	6.03	3.6	18.78	0.5	5.69	1.6	2.93	6.8	4.83	2.9	4.53	9.87
14	1.51	1.7	2.94	3.4	5.38	1.5	6.11	1.2	18.00	1.1	5.64	1.2	3.13	3.5	5.01	3.1	4.20	13.56
17	1.53	4.6	2.84	5.4	5.39	1.7	6.12	2.4	17.90	0.8	5.73	1.8	3.17	5.1	4.96	2.3	4.47	6.27
21	1.53	3.2	2.75	6.4	5.41	3.9	6.04	1.8	17.57	2.5	5.59	1.8	3.28	7.3	5.14	4.9	3.93	7.23
24	1.55	1.5	1.98	13.7	5.60	1.6	6.09	2.2	16.58	1.9	5.59	1.5	3.16	6.7	4.82	2.2	3.45	6.22
27	1.53	1.5	1.83	13.1	5.30	1.5	5.88	2.5	15.79	0.9	4.82	1.9	3.09	6.7	4.89	6.0	3.34	9.01
30	1.32	2.2	1.72	8.9	4.86	1.8	5.32	2.2	15.08	3.2	5.00	1.7	2.72	6.1	4.43	1.7	3.21	9.85

in Σ NEAA. Larger differences than in our analysis of amino acid contents were reported by Chawan and Rao (1982) and Kustos *et al.* (1999) for Arg (4.4, 4.0 and 8.4% resp.), Lys (6.46, 7.3 and 11.1% resp.), Val (5.07, 5.0 and 10.5% resp.) and Pro (5.8, 2.6 and unpublished, resp.).

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Phenotypic parameters of meat production traits of crossbred bulls

Fenotypové parametry znaků produkce masa u býků kříženců

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ABSTRACT: The objective of the experiment was to determine an extent of phenotypic correlations between the basic fattening performance traits and post-slaughter parameters in the slaughter value assessment of crossbred bulls, produced from Black × White Landrace cows mated by bulls of the beef breeds most frequently used for commercial crossing in Poland. Diverse relationships were determined between the growth rate traits and some slaughter value parameters of the bulls obtained by commercial crossing. The highest correlation coefficient for the weight and meat content of 5 cuts was observed relative to the energy consumption per 1 kg body weight gain of bulls. The weight and fat content of 5 cuts was most highly correlated with the round weight. The weight of 5 cuts turned out to be the most precise indicator of the meat content in 5 prime cuts.

Keywords: bulls; crossbreeding; slaughter value; correlations

ABSTRAKT: Cílem pokusu bylo stanovení rozsahu fenotypových korelací mezi základními znaky výkrmnosti a ukazateli získanými po porážce během hodnocení jatečné hodnoty býků-kříženců matek plemene černobílé landrace a býků masných plemen, která se v Polsku používají nejčastěji k užitkovému křížení. U býků pocházejících z užitkového křížení byly zjištěny rozdílné závislosti mezi znaky rychlosti růstu a některými ukazateli jatečné hodnoty. Nejvyšší korelační koeficient byl zjištěn mezi hmotností a podílem masa v pěti částech jatečného trupu na jedné straně a spotřebou energie na 1 kg přírůstku tělesné hmotnosti býků na druhé straně. Hmotnost a obsah tuku pěti částí jatečného trupu vykazovaly nejvyšší korelaci s hmotností kýty. Hmotnost těchto pěti částí se ukázala nejpřesnějším ukazatelem podílu masa I. jakosti.

Klíčová slova: býci; křížení; jatečná hodnota; korelace

INTRODUCTION

In order to meet an increasing demand for heavier, very muscular carcasses with bright red, marbled meat, but without excessive intermuscular and subcutaneous fat (Branscheid, 1990; Wajda, 1996), methods for the evaluation of primary parts of the animal body which determine carcass muscularity must be improved. Measurements of the body – fundamental for one of the slaughter value assessment methods on live animals – are weakly correlated with the main traits of the carcass value (Pogorzelska, 1999). Much stronger genetic links with fat content of the carcass can be attributed to characters which are relatively easy to assess at slaughter. These are mainly carcass weight, internal fat weight, half-carcass weight, longissimus dorsal muscle area and weight of primary cuts (Reklewski, 1995). More accurate results of evaluation of the carcass composition are attainable through dissection of complete sides of beef or some cuts. High correlations between contents of meat, fat and bones in the carcass and the percentage of these components in each cut make it possible to determine the carcass composition on the basis of the cuts selected for evaluation (Dobicki, 1996).

Considering the above, the author's aim was to determine a degree of phenotypic correlations between the basic fattening performance traits and post-slaughter parameters in the slaughter value assessment of crossbred bulls from Black × White Landrace cows mated by bulls of some beef breeds, widely used in Poland for commercial crossbreeding.

MATERIAL AND METHODS

The experiment was conducted on 255 young bulls obtained by commercial crossing. The following groups were assessed: Charolaise × Black and White Landrace, Limousine × Black and White Landrace, Simental × Black and White Landrace, and Kortowska synthetic line × Black and White Landrace. To the age of 18 months, half the bulls from each parental group were fattened in the semi-intensive, and the other half – in the intensive feeding system (Table 1). All the bulls aged 18 months were fasted for 24 hours and slaughtered. The slaughter, pre-processing and dissection of the right side of beef to cuts were performed according to the method elaborated by Janicki and Chrząszcz (1962). Five prime cuts were further dissected to: fore ribs, best ribs, rump, round of beef, and shoulder. The weight of each part and the percentage of the muscle, fat and bone tissues were determined in each part (Pogorzelska, 1999).

Simple correlation coefficients were calculated between some fattening performance traits and post-slaughter parameters versus the basic traits of slaughter value (Żuk, 1989).

RESULTS AND DISCUSSION

The phenotypic correlation coefficients between the basic fattening performance traits and some traits of slaughter value of bulls are shown in Table 2.

Body weight at 18 months of age and daily gains of bulls were most strongly correlated with the weight of 5 cuts ($r = 0.80$–0.92) and the weight of meat in dissected cuts ($r = 0.74$–0.84). Negative correlations were found between the consumption of nutrients per 1 kg of body gain and the traits of slaughter value mentioned above. It can be concluded that for both feeding systems energy consumption per 1 kg of body gain showed the highest correlation with the weight of 5 cuts.

Phenotypic correlation coefficients between the basic slaughter value characters and measures of bull carcasses are presented in Table 3. For most of the slaughter value traits the measurements taken on carcasses turned out to be slightly more highly correlated with the basic slaughter value traits than those taken on live animals. In each feeding system, the highest correlation coefficients were recorded for the round length and half-carcass weight ($r = 0.49$ and 0.54), round weight ($r = 0.44$ and 0.47), weight of 5 cuts ($r = 0.47$ and 0.52) and weight of meat in 5 cuts ($r = 0.47$ and 0.49). Nearly all the dimensions of carcasses were either weakly correlated or not correlated at all with the internal fat weight, carcass rump weight, percentage of 5 prime cuts and longissimus dorsal muscle area. These results indicate that carcass dimensions are only weakly correlated with the basic traits of carcass value, and as such they cannot function as selection traits for the evaluation of the slaughter value of animals. The results reported by Dobicki (1996), who also pointed to weak correlation between carcass dimensions and slaughter value traits, seem to confirm this conclusion.

Phenotypic correlation coefficients between the basic carcass traits and some slaughter value traits of bulls are included in Table 4. Their analysis leads to the conclusion that the weight of half-carcasses showed a high correlation with the weight of 5 cuts ($r = 0.95$–0.97) and weight of meat in these cuts ($r = 0.86$–0.92) for either of the feeding systems. Significant, although much lower correlations were obtained for the weight of perirenal fat and weight of fat in 5 cuts. The weight of 5 cuts and their meat content were most strongly correlated with the weight of round ($r = 0.90$–0.93). Also slaughter performance was relatively highly correlated with these two traits, but only for bulls in the semi-intensive feeding system. The longissimus dorsal muscle area is traditionally taken as an indicator of the carcass muscularity. The values of the

Table 1. Body weight and daily gains of bulls

Trait	Feeding system	Genotype CH × BWL		KLS × BWL		SIM × BWL		LIM × BWL		Total	
		$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Body weight at 6 months of age (kg)	P	168.2^{B}	19.29	160.0^{AB}	14.71	159.1^{AB}	16.39	154.8^{A}	7.46	160.5	15.62
	I	170.4^{b}	15.89	163.9^{ab}	14.59	166.3^{ab}	19.71	160.5^{a}	10.65	165.3^{xx}	15.70
Body weight at 18 months of age (kg)	P	421.9^{Bb}	46.02	426.2^{ABa}	27.89	417.0^{ABa}	30.49	444.3^{Aa}	21.73	426.2	34.10
	I	569.7^{B}	43.89	543.0^{A}	29.31	543.6^{A}	45.10	568.7^{B}	26.49	556.2^{xx}	38.90
Daily b.w. gain from 6 to 18 months of age (g)	P	702^{A}	85	739^{A}	71	714^{A}	79	804^{B}	58	740	83
	I	1109^{B}	105	1053^{A}	84	1048^{A}	115	1134^{B}	71	1086^{xx}	101
Net daily gain (g)	P	423^{A}	48	421^{A}	34	409^{A}	39	448^{B}	28	426	40
	I	620^{B}	55	581^{A}	41	575^{A}	58	620^{B}	32	600^{xx}	51

S = semi-intensive feeding

I = intensive feeding

Means for genotype groups marked with different capital letters differ significantly at $P \leq 0.01$, those marked with small letters – at $P \leq 0.05$

Significance of the effect of a feeding system was marked with ^{xx} – at $P \leq 0.01$

Table 2. Phenotypic correlation coefficients between growth rate traits and some slaughter value traits of bulls

Trait	Feeding system	Perirenal fat (kg)	Internal fat (kg)	Right half-carcass without fat and kidney (kg)	Slaughter performance (%)	Round weight	Longissimus dorsal muscle weight (kg)	Longissimus dorsal muscle area (cm^2)	Meat of 5 cuts (kg)	Fat of 5 cuts (kg)	Bones of 5 cuts (kg)	Weight of 5 cuts (kg)
Body weight (6 months, kg)	S	0.28	0.26	0.35	0.21	0.23	–	–	0.28	–	0.44	0.34
	I	0.18	0.20	0.35	–	0.24	–	–	0.26	–	–	0.29
Body weight (18 months, kg)	S	0.42	0.45	0.95	0.45	0.84	0.68	–	0.84	0.59	0.57	0.92
	I	0.53	0.44	0.96	0.30	0.80	0.56	0.18	0.80	0.48	0.64	0.91
Daily gain (6–18 months, kg)	S	0.30	0.35	0.83	0.39	0.78	0.73	0.23	0.75	0.55	0.37	0.80
	I	0.49	0.38	0.89	0.30	0.76	0.57	0.19	0.74	0.44	0.63	0.85
Dry matter consumption per 1 kg	S	–0.29	–0.29	–0.66	–0.33	–0.63	–0.44	–	–0.60	–0.35	–0.48	–0.66
	I	–0.21	–0.24	–0.72	–0.35	–0.70	–0.45	–	–0.77	–	–0.59	–0.77
Consumption of oat feed units per 1 kg	S	–0.23	–0.27	–0.72	–0.39	–0.63	–0.49	–	–0.66	–0.33	–0.41	–0.68
	I	–0.39	–0.35	–0.87	–0.34	–0.79	–0.58	–0.22	–0.80	–0.33	–0.59	–0.86
Total protein consumption per 1 kg	S	–0.29	–0.30	–0.64	–0.26	–0.64	–0.56	–	–0.57	–0.46	–0.35	–0.63
	I	–0.40	–0.34	–0.85	–0.33	–0.75	–0.54	–0.20	–0.76	–0.36	–0.58	–0.84
MJ consumption per 1 kg	S	–0.23	–0.27	–0.72	–0.39	–0.63	–0.49	–	–0.66	–0.33	–0.41	–0.68
	I	–0.39	–0.35	–0.87	–0.34	–0.79	–0.58	–0.22	–0.80	–0.33	–0.59	–0.86

S = semi-intensive feeding

I = intensive feeding

Critical values r in S and I groups at $P \leq 0.05$–0.17, $P \leq 0.01$–0.23

Table 3. Phenotypic correlation coefficients between some slaughter value traits and dimensions of bull carcasses

Trait	Feeding system	Round		Carcass length (cm)	Sirloin length (cm)	Carcass width (cm)
		length (cm)	girth (cm)			
Internal fat (kg)	S	0.26	–	–	–	–
	I	0.24	–	–	–	
Right half-carcass weight (kg)	S	0.49	0.44	0.29	0.29	0.29
	I	0.54	0.27	0.44	0.35	–
Slaughter performance (%)	S	0.30	0.31	–	–	0.22
	I	–	–	0.32	0.22	–
Carcass fore part weight (kg)	S	0.23	0.22	–	0.19	–
	I	–	0.18	–	–	–
Carcass middle part weight (kg)	S	0.22	0.23	–	0.19	–
	I	–	0.21	–	–	–
Carcass rump part weight (kg)	S	–	–	0.22	–	–
	I	–	–	0.21	–	–
Perirenal fat (kg)	S	0.21	–	0.30	–	–
	I	0.21	–	0.21	0.25	–
Round weight (kg)	S	0.44	0.47	0.27	0.26	0.19
	I	0.47	0.27	0.40	0.26	–
Meat of 5 cuts (kg)	S	0.47	0.45	0.24	0.24	0.27
	I	0.49	0.21	0.40	0.30	
Fat of 5 cuts (kg)	S	0.18	0.27	0.32	0.25	–
	I	0.23	0.24	–	–	–
Bones of 5 cuts (kg)	S	0.28	0.21	0.39	0.25	0.31
	I	0.26	–	0.36	0.39	–
Weight of 5 cuts (kg)	S	0.47	0.46	0.34	0.30	0.26
	I	0.52	0.24	0.45	0.36	–
Percentage of 5 cuts (%)	S	–	–	–	–	–
	I	–	–	–	–	–
Longissimus dorsal muscle weight (kg)	S	0.31	0.34	0.19	0.28	–
	I	0.29	0.19	0.35	0.26	–
Longissimus dorsal muscle length (cm^2)	S	–	0.23	–	–	–
	I	–	–	–	–	–

S = semi-intensive feeding
I = intensive feeding
Critical values in groups S and I at $P \leq 0.05$–0.17, $P \leq 0.01$–0.23

correlation coefficients for this trait and the weight of 5 cuts and weight of meat in these cuts ranged between $r = 0.23$ and $r = 0.28$ in our experiment. The results of the studies conducted to date are ambiguous. Dobicki (1996) and Wajda (1996) reported much higher correlation coefficients.

The highest correlation for both feeding systems was recorded between the weight of half-carcass and weight of round ($r = 0.86$ and 0.90). Slightly lower correlation coefficients were found for the weight of half-carcass and weight of the longissimus dorsal muscle or slaughter performance. Młynek and Litwińczuk (1999) cited much higher coefficients for these correlations. In the present study slaughter performance was most highly correlated with the round weight for semi-intensively fed bulls ($r = 0.62$). A highly significant correlation was also determined between slaughter performance and the longissimus dorsal muscle weight.

The lowest correlations were found between the longissimus dorsal muscle area and the slaughter value traits included in Table 4. The researchers, however, do not seem to agree about the usefulness of the longissimus dorsal muscle area as a measure in the evaluation of slaughter value traits. The discrepancies found in the literature could have resulted from some imprecision of measuring techniques.

Phenotypic correlation coefficients between the weight of the carcass fore part, middle part and rump versus the weight and tissue composition of 5 most valuable cuts of bull carcasses in the semi-intensive and intensive feeding systems are presented in Table 5. The highest correlation coefficients were obtained between the weight of the fore part and the weight of the middle and rump parts versus the weight of the rump parts of the bulls in the intensive system ($r = 0.94$–0.96). Significant, although much lower, correlations were found between the weight

Table 4. Phenotypic correlation coefficients between the basic carcass traits and some slaughter value traits

Trait	Feeding system	Weight (kg)						
		right half-carcass	round	M.l.d.	5 cuts	meat of 5 cuts	fat of 5 cuts	bones of 5 cuts
Right half-carcass weight (kg)	S	–	0.90	0.70	0.97	0.92	0.52	0.61
	I	–	0.86	0.61	0.95	0.86	0.45	0.60
Perirenal fat weight (kg)	S	0.30	0.21	–	0.27	–	0.39	0.21
	I	0.43	0.20	0.17	0.29	–	0.61	0.23
Slaughter performance (%)	S	0.67	0.62	0.39	0.64	0.65	–	0.46
	I	0.46	0.38	0.31	0.42	0.42	–	–
Longissimus dorsal muscle area (cm^2)	S	0.19	0.20	0.38	0.23	0.25	–	–
	I	0.22	0.24	0.34	0.24	0.28	–	–
Round weight (kg)	S	0.90	–	0.74	0.93	0.92	0.41	0.50
	I	0.86	–	0.64	0.92	0.90	0.27	0.53

S = semi-intensive feeding

I = intensive feeding

Critical values in groups S and I at $P \leq 0.05$–0.17, $P \leq 0.01$–0.23

Table 5. Phenotypic correlation coefficients between the weight and tissue composition of 5 prime cuts

Trait	Feeding system	Carcass			Meat of 5 cuts (kg)	Fat of 5 cuts (kg)	Bones of 5 cuts (kg)	Weight of 5 cuts (kg)
		fore part weight (kg)	middle part weight (kg)	rump part weight (kg)				
Carcass fore part weight (kg)	S	–	0.73	0.66	0.43	0.27	–	0.44
	I	–	0.96	0.95	–	0.29	–	0.19
Carcass middle part weight (kg)	S	–	–	0.67	0.47	0.34	–	0.47
	I	–	–	0.94	0.19	0.33	–	0.25
Carcass rump part weight (kg)	S	–	–	–	0.39	–	–	0.32
	I	–	–	–	0.20	0.21	–	0.21
Meat of 5 cuts (kg)	S	–	–	–	–	0.28	0.53	0.95
	I	–	–	–	–	–	0.54	0.95
Fat of 5 cuts (kg)	S	–	–	–	–	–	0.17	0.51
	I	–	–	–	–	–	–	0.33
Bones of 5 cuts (kg)	S	–	–	–	–	–	–	0.64
	I	–	–	–	–	–	–	0.65
Weight of 5 cuts (kg)	S	–	–	–	–	–	–	
	I	–	–	–	–	–	–	–

S = semi-intensive feeding

I = intensive feeding

Critical values in groups S and I at $P \leq 0.05$–0.17, $P \leq 0.01$–0.23

of the fore, middle and rump parts of the carcass versus the weight of meat and fat of 5 cuts and the weight of these cuts. For the bulls in both systems of fattening the weight of 5 cuts turned out to be very highly correlated with the weight of 5 cuts ($r = 0.95$). All these characters can be easily determined at slaughter, which makes them very useful for the evaluation of animal muscularity. The results of Reklewski's study (1995) seem to confirm this. In the present experiment the weight of 5 cuts was also quite highly correlated with the weight of bones in these cuts ($r = 0.64$ and 0.65) and the weight of fat of 5 cuts ($r =$ 0.51 and 0.33).

CONCLUSIONS

Correlation coefficients between the growth rate traits and some slaughter value traits of bulls from commercial crossing were determined. The weight of 5 cuts and the weight of meat of 5 cuts were most highly correlated with the energy consumption per 1 kg body gain of bulls. The weight of 5 cuts and the meat content in 5 cuts were most highly correlated with the weight of round. The weight of 5 cuts turned out to be the best trait of the meat content in 5 prime cuts.

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The evaluation of breeding work in nucleus herds of pigs

Posouzení plemenářské práce ve šlechtitelských chovech prasat

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ABSTRACT: The suitability of breeding procedures in 40 nucleus herds of Large White (White Improved) breed has been evaluated by means of correlation analysis of relations between utility traits and breeding measures. The level of reproductive traits in the herds did not significantly influence the levels of fattening and carcass traits (from the field test) in reared breeding animals. Herd reproduction traits levels are not fully related to the levels of traits of station test. Correlation coefficients between the traits of field test and traits of station test confirm known physiological relations with animals, especially among the traits of carcass value. Surprisingly low are coefficients of correlations between the gains in reared animals and the gains in descendants of breeding animals ($r = -0.08$, resp. $r = 0.15$). Coefficients of correlations between the average daily gain and the traits of leanness ranged from $r = -0.31$ to $r = -0.35$, between average daily gain and backfat thickness it reached $r = 0.26$. Herds with lower feedstuff consumption manifested higher leanness ($r = -0.32$ to $r = -0.42$) and lower backfat thickness ($r = 0.43$; $P \leq 0.01$). Somewhat higher level of relations confirms simultaneously practised selection both for fattening traits and for carcass traits. Correlations between selection intensity in the rearing of young breeding animals and reproductive traits, resp. traits observed in field test were low and non significant. Selection intensity was markedly influenced by the traits of station test, especially by lean meat percentage and ham percentage. From methodology point of view, analysis of correlations is appropriate procedure for evaluation of management practices in nucleus herds.

Keywords: pig; Large White; field test; station test; selection intensity; correlations

ABSTRAKT: Pro posouzení vhodnosti metodických postupů ve šlechtitelském programu populace bílého ušlechtilého plemene, byla využita korelační analýza vztahů mezi základními ukazateli užitkovosti a plemenářskými opatřeními charakterizující jednotlivé šlechtitelské chovy. Z celkového počtu 90 šlechtitelských chovů v roce 1997 bylo do šetření zařazeno 40 chovů, které jednak za poslední roky vykazovaly stabilní úroveň užitkovosti, jednak splňovaly zadanou podmínku, a to, že šlechtitelský chov musí mít minimálně čtyři kanečky klasifikovány a 10 zvířat v testu výkrmnosti a jatečné hodnoty za rok. U vybraných šlechtitelských chovů byly do sledování zahrnuty průměrné hodnoty znaků reprodukce a znaků z polního a staničního testu. U každého šlechtitelského chovu byla stanovena tzv. plemenářská (šlechtitelská) opatření, která do určité míry charakterizují intenzitu selekce při odchovu plemenných zvířat. Korelační vztahy mezi ukazateli reprodukce poukazují na skutečnost, že chovy s vyšším průměrným počtem dochovaných selat ve vrhu budou vykazovat i vyšší průměrnou hmotnost vrhu v 21 dnech jejich věku ($r = 0,64$; $P \leq 0,01$), ale bez výrazného vztahu k délce mezidobí ($r = -0,26$). Z hlediska průkaznosti korelačních koeficientů je možno odvodit, že úroveň reprodukčních vlastností v jednotlivých chovech nemá výrazný vliv na úroveň ukazatelů výkrmnosti a jatečné hodnoty, zjištěnou u odchovávaných plemenných zvířat polním testem. Pouze korelační koeficient mezi počtem dochovaných selat a průměrným denním přírůstkem u kanečků byl statisticky významný ($r = 0,36$; $P \leq 0,05$). Obdobně lze odvodit, že i úroveň znaků reprodukce chovu nesouvisí plně s úrovní znaků ze staničního testu. Korelační koeficienty mezi znaky z polního testu a znaky ze staničního testu potvrzují známé fyziologické vztahy u zvířat, zvláště mezi znaky jatečné hodnoty. Překvapující jsou nízké korelační koeficienty mezi přírůstkem odchovávaných zvířat a přírůstkem u potomstva plemenných prasat ($r = -0,08$, resp. $r = 0,15$). Korelační koeficienty mezi průměrným denním přírůstkem a znaky zmasilosti se pohybovaly od $r = -0,31$ do $r = -0,35$, mezi průměrným denním přírůstkem a výškou sádla byla zjištěna hodnota $r = 0,26$. Chovy s nižší spotřebou krmiva vykazovaly vyšší úroveň ukazatelů zmasilosti ($r = -0,32$ až $r = -0,42$) a nižší výšku sádla ($r = 0,43$; $P \leq 0,01$). Poněkud těsnější závislost uvedených znaků potvrzuje prováděnou simultánní selekci, jak na znaky výkrmnosti, tak na znaky jatečné hodnoty. Korelační koeficienty mezi údaji charakterizujícími intenzitu selekce

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při odchovu plemenných zvířat na jedné straně a znaky reprodukce, resp. znaky z polního testu na straně druhé, byly nízké a statisticky nevýznamné. Výrazněji byla intenzita selekce ovlivněna znaky ze staničního testu. To je patrné zvláště u podílu hlavních masitých částí a podílu kýty z jatečného těla. Z metodického hlediska, je provedená korelační analýza vhodným postupem pro posuzování chování managementu ve šlechtitelských chovech.

Klíčová slova: prase; bílé ušlechtilé plemeno; polní test; staniční test; intenzita selekce; korelace

INTRODUCTION

Achieving high precision in estimation of breeding value of utility traits is a continual process in a breeding work, both in the field of mathematic-statistical procedures (Peškovičová *et al.*, 1999; Wolf and Wolfová, 1999a, b; Wolf *et al.*, 1999a) and in organisational arrangements applied in the nucleus herds. It is valid especially in a large population distributed into the rank of nucleus herds in spite of uniform methods of utility and heritability recording (test of utility traits) practised.

A condition of the use of mathematical models in the breeding practice is not only appropriate standardisation of breeding conditions (treatment, nutrition, feeding and housing technology, health of animals etc.), but breeding measures, as herd size, type of mating (AI or natural mating), intensity of selection in the rearing of young breeding animals, herd replacement, age structure of the sow herds and standardised way of utility traits testing (field and station test). It can be assumed that high variability of utility traits and breeding measures decreases accuracy of breeding value estimation.

Every change of selection program can cause unforeseen effects, and permanent change of genetic parameters (Groeneveld *et al.*, 1998; Wolf *et al.*, 1999b) and economic values of utility traits (Fiedler *et al.*, 1995; Fiedler and Houška, 1999). Main criterion of successful breeding work is the achievement of breeding aim, resp. the reach of genetic and economic gain (Peškovičová *et al.*, 1998; Wolf *et al.*, 1998).

For evaluation of suitability of breeding methods used in the breeding program, an analysis of correlations between main utility traits and breeding measures characterising individual nucleus herds, can be exploited.

Recent paper immediately links the evaluation of nucleus herds on the basis of their economic efficiency (Houška *et al.*, 2001). Analysis of correlations pointed out efficiency of selection methods, especially if the differences in the traits levels among the herds are significant, as shown in above mentioned paper.

MATERIAL AND METHODS

With respect to outlined problems the population of most spread breed in the Czech Republic, i.e. Large White breed has been used. Forty herds of total number of 90 nucleus herds (in 1997) have been used for this evaluation. Only herds with stable level of utility traits and those with minimum of 4 classified young breeding boars within a year (performance test and type, constitution and exterior evaluation) and with minimum of 10 animals tested in performance test were included. The performance levels of the herds have been described in the paper of Houška *et al.* (2001).

In the set of observed herds the average levels of reproductive traits were evaluated:

average litter size at 21 day (LS)
litter weight at 21 day (LW)
farrowing period (FP)

Following groups of traits were also included in observation:

traits of fattening capacity and carcass quality from performance (field) test:

average daily gain from birth to 90 kg in gilts (ADGg)
average daily gain from birth to 100 kg in young boars (ADGb)
backfat thickness in gilts (BFg)
backfat thickness in young boars (BFb)
lean meat percentage in gilts (LMg)
lean meat percentage in young boars (LMb)

traits from progeny (station) test:

average daily gain from 30 to 100 kg (ADG)
consumption of metabolisable energy (ME; MJ/kg of daily gain)
eye muscle area (MLD)
main lean cuts percentage (MLC)
ham percentage (HAM)
average backfat thickness (BF)

breeding measures characterising selection intensity in the rearing of young breeding animals were determined in every nucleus herd:

the share of gilts not included into rearing (PTg, number of weaned female piglets = 100%)
the share of gilts reared, but not permitted into classification (CLg, number of weaned female piglets = 100%)
the share of gilts discarded at classification (SDg, number of gilts permitted to classification = 100%)
the share of young boars not included into rearing (PTb, number of weaned male piglets = 100%)
the share of young boars reared but not permitted into classification (CLb, number of weaned male piglets = 100%)

the share of young breeding boars discarded at classification (SDb, number of young breeding boars permitted to classification = 100%)

Above mentioned data characterising individual herds were evaluated using correlation analysis.

RESULTS AND DISCUSSION

Table 1 presents all calculated correlation coefficients. Correlations among the reproductive traits pointed out the fact, that herds with higher average number of weaned piglets per litter showed higher average litter weight at 21st day ($r = 0.64$; $P \leq 0.01$), but without significant relation to farrowing interval ($r = -0.26$).

Because of significance of correlation coefficients, it can be deduced that level of reproductive traits in the herds does not markedly influence the level of fattening and carcass traits from field test in breeding animals reared. Only relation between the number of weaned piglets and average daily gain in young boars was significant ($r = 0.36$; $P \leq 0.05$).

It can be deduced by analogy, that levels of reproductive traits in the herd are not fully related to the levels of traits from station test, too. It can be assumed that there is no direct relation between selection for reproduction and selection for fattening and carcass traits. Although breeders used to keep sows with above the average level of reproductive traits in the herd, for rearing of young breeding animals they used to choose animals with adequate type, constitution and exterior when weaned, whose parents have high breeding value in the fattening and carcass traits. By analogy brought out by Pavlík (1988), that with selection of young gilts for breeding, an account is taken of their weight. Presumably average and above average animals are classified, with respect of their growth rate in early age.

According to Honko *et al.* (1991), 27.7% of reared young breeding gilts come from the 1st and 2nd parities. It means that large amount of breeding gilts come of sows not yet proven in reproductive traits. Representation of gilts according to the number of weaned piglets in the litter of origin confirms the fact, that the most gilts reared (37.7%) descend from litters of 10 and 11 (on average 10.7) piglets. Above mentioned facts are supported by low correlation coefficients between the parity of gilt's litter of origin and average daily gain ($r = 0.16$), resp. backfat thickness ($r = 0.21$) and between the litter size of gilt's litter of origin and average daily gain ($r = -0.03$), resp. backfat thickness ($r = 0.08$).

The relations among the traits of field test seems to be logical in the fact, that herds with high gains in gilts have high gains in young boars ($r = 0.70$; $P \leq 0.01$). Analogous relation ($r = 0.84$; $P \leq 0.01$) was found with backfat thickness. No such relation however was found among fattening and carcass traits in the field test (from $r = -0.17$ to 0.22). The relations among the carcass traits both in gilts and in young boars are of course high and significant ($r = -0.94$; $P \leq 0.01$, resp. $r = -0.96$; $P \leq 0.01$).

Correlation coefficients between the traits of field and of station test confirm known physiological relations in animals, especially it is obvious in the carcass traits. Surprisingly low correlation coefficients were found between the daily gain in animals reared and daily gain in descendants of breeding animals ($r = -0.08$, resp. $r = 0.15$), while genetic correlation between the average daily gain in field test and daily gain in station test is relatively high ($r = 0.48$; Wolf *et al.*, 1999a).

Pulkrábek *et al.* (1988) pointed out analogous reflexion of physiological relations in animals in relations between the traits of field and of station test, especially traits characterising meatiness and amount of fat.

Correlation coefficients between the daily gain and meatiness ranged from $r = -0.31$ to $r = -0.35$ and between the daily gain and backfat thickness it was $r = 0.26$. Favorable relations were found between average consumption of metabolisable energy per kg of gain and carcass traits. In another words, the herds with lower feed consumption manifested higher meatiness ($r = -0.32$ to $r = -0.42$) and lower backfat thickness ($r = 0.43$; $P \leq 0.01$). Somewhat higher level of relations confirms simultaneous selection practised for fattening traits as well as for carcass traits.

Correlation coefficients between the traits characterising selection intensity in the rearing of young breeding animals and reproductive traits or traits from field test were low and non-significant. Selection intensity was markedly influenced by the traits from station test, especially by main lean cuts percentage and ham percentage. Theoretically, we would expect relations that are more expressive. In another words it can be presumed that herds with high level of utility traits verified in field and station tests will produce more breeding animals, especially young boars. Realised observations however did not prove this assumption. Nevertheless, it can be stated that higher emphasis in the rearing of young breeding animals is put on the traits from station test.

It is logical, that in the herds with higher number of breeding gilts and young breeding boars reared, higher share of animals is classified ($r = 0.89$; $P \leq 0.01$, resp. $r = 0.66$; $P \leq 0.01$). The share of produced (sold) gilts (of the number of classified gilts) shows the same tendency ($r = 0.44$; $P \leq 0.01$), too.

This relation, however was not found in the rearing of breeding boars ($r = 0.17$). On the other side a negative relation was found ($r = -0.48$; $P \leq 0.01$) between the share of young boars not included into rearing and the share of sold boars (of the number of classified boars). This fact indicates that the more breeding animals does the breeder produce, the less animals he would sell. Further it is indicated that herds producing more breeding gilts do not

Table 1. Correlation coefficients in observed traits

Traits	LW21	FP	ADGg	ADGb	BFg	BFb	LMg	LMb	ADG	ME	MLD	MLC	HAM	BF	PTg (%)	CLg (%)	PTb (%)	CLb (%)	SDg (%)	SDb (%)
LS21	0.64^{++}	–0.11	0.22	0.36^{+}	–0.26	–0.29	0.03	0.09	0.04	–0.29	0.08	–0.07	–0.04	0.09	0.16	0.10	0.06	–0.06	–0.11	–0.21
LW21		–0.26	0.09	0.18	–0.17	–0.17	0.28	0.22	0.11	–0.30	0.23	0.11	0.12	0.03	0.09	–0.04	–0.08	–0.01	–0.26	0.05
FP			0.03	0.20	0.15	–0.02	0.13	0.17	0.01	0.14	–0.12	–0.05	–0.19	0.06	0.08	0.03	0.17	–0.11	–0.03	–0.27
ADGg				0.70^{++}	0.04	–0.09	0.10	–0.04	–0.08	0.01	–0.05	0.03	0.21	–0.09	0.19	0.14	0.14	0.02	–0.01	–0.13
ADGb					–0.07	–0.17	0.14	–0.08	0.15	0.11	–0.08	–0.11	–0.01	0.04	0.03	–0.05	0.04	0.01	0.01	0.06
BFg						0.84^{++}	–0.95^{++}	–0.83^{++}	–0.12	0.25	–0.47^{++}	–0.29	–0.33^{+}	0.43^{++}	0.07	0.17	–0.08	–0.11	0.17	–0.10
BFb							–0.78^{++}	–0.97^{++}	0.17	0.33^{+}	–0.58^{++}	–0.40^{+}	–0.43^{++}	0.55^{++}	0.01	0.11	0.02	–0.10	0.21	–0.17
LMg								0.82^{++}	–0.08	0.07	0.12	–0.03	0.08	0.06	0.01	0.03	0.08	–0.09	0.09	–0.30
LMb									–0.01	0.19	0.05	–0.16	–0.09	0.21	0.02	0.07	0.02	–0.19	0.11	–0.44^{++}
ADG										–0.12	–0.31	–0.35^{+}	–0.31	0.26	–0.28	–0.30	0.04	0.16	–0.01	0.18
ME											–0.42^{++}	–0.34^{+}	–0.32^{+}	0.43^{++}	–0.03	–0.07	–0.31	–0.29	0.18	0.14
MLD												0.66^{++}	0.63^{++}	–0.62^{++}	0.17	0.09	0.04	–0.06	–0.15	–0.10
MLC													0.86^{++}	–0.70^{++}	0.26	0.13	0.23	0.08	–0.32^{+}	–0.31
HAM														–0.74^{++}	0.34^{+}	0.17	0.10	–0.13	–0.31	–0.34^{+}
BF															–0.24	–0.07	–0.01	0.09	0.32^{+}	–0.18
PTg(%)																0.89^{++}	–0.02	–0.04	0.01	–0.06
CLg(%)																	–0.06	–0.02	0.44^{++}	0.02
PTb(%)																		0.66^{++}	–0.12	–0.48^{++}
CLb(%)																			–0.01	0.17
SDg(%)																				0.16

$^{+}P < 0.05$; $^{++}P < 0.01$

produce more breeding boars, too ($r = -0.02$). It follows from this fact, that in some herds emphasis are on production of gilts, while others emphasise production of boars.

It follows from above mentioned facts that production of breeding animals exceeds the needs, and rigorous selection of animals for rearing and milder selection at classification is not being applied. Contingent antagonism between the reproductive and utility traits, as mentioned by Tvrdoň *et al.* (1999), has not been expressed in negative correlation coefficients.

We suppose that the correlation analysis is an appropriate method for evaluation of management practices in nucleus herds. This method can increase precision of methodology in the realisation of breeding programs.

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Genetic trends of some performance traits in Whiteheaded Mutton sheep

Genetické trendy některých znaků výkrmnosti u bělohlavých masných ovcí

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ABSTRACT: The DFREML algorithm was used to estimate direct and maternal heritabilities as well as respective genetic and environmental trends for some fattening performance traits of the first Polish mutton-type sheep under long-term selection. The obtained direct heritability estimates were low and ranged from 0.0042 (for daily weight gain 1–28 days of age) to 0.1734 (for the birth weight). The maternal heritability estimates were also low. Additionally, maternal permanent environmental variance estimates were also negligible. Both direct and maternal genetic trends turned out to be positive for the majority of analysed traits, especially since the sixth year of selection.

Keywords: mutton sheep; genetic trends; variance components; maternal effects; selection

ABSTRAKT: Pro odhad přímé a mateřské dědivosti i příslušných genetických trendů a trendů prostředí některých znaků výkrmnosti u prvních polských ovcí masného typu v dlouhodobé selekci jsme použili algoritmus DFREML. Hodnoty odhadů přímé dědivosti byly nízké a pohybovaly se od 0,0042 (pro denní přírůstek mezi 1. a 28. dnem věku) do 0,1734 (pro hmotnost při narození). Odhady mateřské dědivosti vykazovaly rovněž nízké hodnoty. Kromě toho odhady rozptylu mateřského trvalého prostředí byly také zanedbatelné. Přímé i mateřské genetické trendy se ukázaly jako pozitivní u většiny analyzovaných znaků, zejména od 6. roku selekce.

Klíčová slova: masné ovce; genetické trendy; složky rozptylu; mateřské efekty; selekce

INTRODUCTION

The main breeding objective in the majority of European sheep populations is to obtain genetic improvement especially in growth traits. Therefore, monitoring of genetic and environmental trends for these traits is suitable for the determination of breeding strategies.

The first Polish population of mutton sheep (called the Whiteheaded Mutton sheep), originated as a result of crossbreeding sheep of domestic breeds: the Polish Merino and the Wielkopolska sheep; the East Friesian sheep and mutton sheep breeds, such as Texel, Ile de France and Berrichone du Cher (Gut *et al.*, 1986). After the crossbreeding was completed, during the second stage of breeding the sheep were mated and selected according to the simplified index of individual fattening performance (Rzepecki *et al.*, 1988).

It should be noted that growth and conformation traits in sheep are known to be influenced, apart from direct genetic and environmental effects, also by indirect maternal genetic effects (Bradford, 1972; Hanrahan, 1976). Hence, these effects were included into our considerations.

The aim of this study is to estimate direct and maternal heritabilities and the ratio of maternal environmental variance to the total variance as well as respective genetic trends for some fattening performance traits of the Whiteheaded Mutton sheep line under long-term selection. Environmental trends for analysed traits were also evaluated.

MATERIAL AND METHODS

Material

Growth, body conformation and fleshiness tests of lambs coming from the first and following matings within consecutive generations of the synthetic line (after crossbreeding had been completed) were analysed. A total of full records of 1889 unselected lambs born between January and March in the years 1985 to 1995 in one flock at the Experimental Station of Złotniki (Poland) were available.

Lambs were weighted on the second day after birth, at the age of 28 days (± 3 days) and at the age of 70, 100 (after weaning) and 152 days (± 7 days). At the age of 152 days the body conformation and fleshiness of lambs were evaluated using a 5-trait score system (with the total number of maximum 25 points). The point score assessment of conformation throughout the testing period was performed by the same selector.

Lambs were selected at the age of 5–6 months on the basis of the phenotypic index (I_m) of individual performance (Rzepecki *et al.*, 1988):

$$I_m = \frac{x_1 - \bar{x}_1}{s_1} * 22.2 + \frac{x_2 - \bar{x}_2}{s_2} * 11.1$$

where: x_1 – daily weight gain of an individual at the age of 28–152 days (till 1987 at the age of 28–100 days)

x_2 – point score for conformation and fleshiness of an individual at the age of 5 months

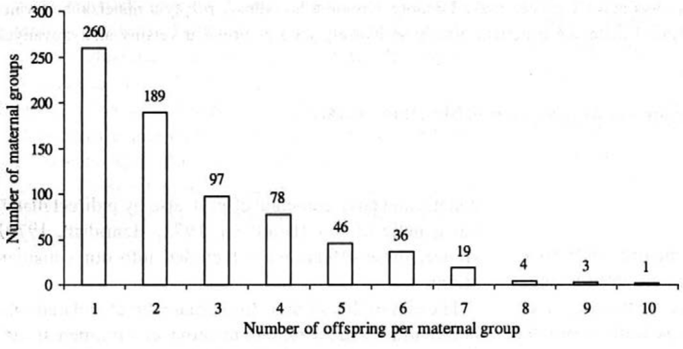
$\bar{x}_1$, $\bar{x}_2$, s_1, s_2 – averages and standard deviations for these traits, respectively

In order to improve genetic gain, in the years 1988–1990 and 1993–1995, progeny testing of stud rams of the White-headed Mutton sheep was performed. Each year, ten preselected rams, at the age of 18 months, were ranked on the basis of daily weight gains and feed consumption per 1 kg weight gain of their ram-lamb progeny and all plus-variant rams were incorporated into the flock of stud-rams.

Due to the increase in the size of the flock, by over 50% in the years 1985–1991, the possibility to reduce the generation interval was limited and ewes were utilised on average for more than 5 years, the rams for approximately 3 years. For the same reason, the obtained selection differentials were not too large and e.g. for the selection of ewes in the years 1988–1991 they ranged from 54 to 84% of the maximum differential (Gut, 1994). The numbers of offspring per base dam as well as the sizes of dam groups are shown in Figure 1. Brief statistical description of the data set is summarised in Table 1.

Table 1. Description of the data sets (number of observations = 1 889)

Trait	Average	Standard deviation
Birth weight (kg)	4.59	0.977
Weight at 100 days (kg)	29.70	5.449
Weight gain 1–28 (g)	286.79	64.374
Weight gain 28–70 (g)	247.52	67.187
Weight gain 28–152 (g)	212.08	50.461
Weight gain 70–152 (g)	193.94	58.468
Point score (25 pts.)	20.84	2.175



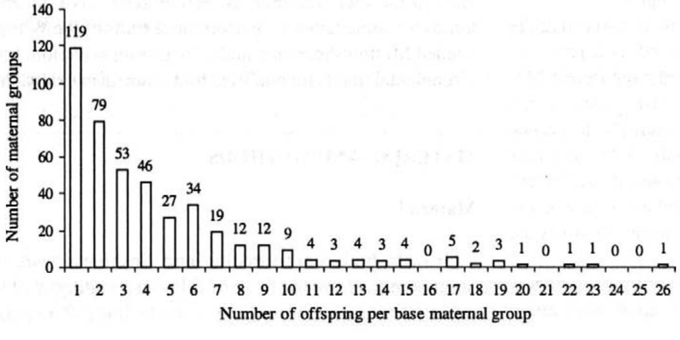


Figure 1. Empirical distribution of size of maternal and base maternal groups

Methods

A single trait animal model is used to estimate the direct and maternal heritability within strains:

$$y = X_1b_1 + X_2b_2 + X_3b_3 + X_4b_4 + Z_1a + Z_2m + Z_3c + e$$

where: y – the vector of observations

b_1 – the vector of fixed effects of birth years

b_2 – the vector of fixed sex effects

b_3 – the vector of fixed effects of litter type (single, multiple)

b_4 – the vector of the age of ewe at lambing included as linear covariable

a – the vector of random direct additive genetic effects

m – the vector of random maternal additive genetic effects

c – the vector of random maternal permanent environmental effects

e – the vector of random errors

$X_1, X_2, X_3, Z_1, Z_2, Z_3$ – these incidence matrices relating the effects to y

X_4 – the incidence matrix containing the regression coefficient for the age of ewe at lambing

First and second moments for the model were assumed to be:

$$E\begin{bmatrix} a \\ m \\ c \\ e \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \text{ and } D\begin{bmatrix} a \\ m \\ c \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & A\sigma_{am} & 0 & 0 \\ A\sigma_{am} & A\sigma_m^2 & 0 & 0 \\ 0 & 0 & I\sigma_c^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

where: A – the *qxq* additive relationship matrix

σ_a^2 – the direct additive genetic variance

σ_m^2 – the maternal additive genetic variance

σ_m – the covariance between direct and maternal additive effects

σ_c^2 – the maternal permanent environmental variance

σ_e^2 – the error variance

Hence, $y \sim N(X_1b_1 + X_2b_2 + X_3b_3 + X_4b_4, V)$

where:

$$V = Z_1AZ_1'\sigma_a^2 + Z_2AZ_2'\sigma_m^2 + (Z_2AZ_1' + Z_1AZ_2')\sigma_{am} + I\sigma_c^2 + I\sigma_e^2$$

The following parameters were estimated:
- direct heritability ($h_a^2 = \sigma_a^2/\sigma_p^2$), where σ_p^2 is the phenotypic variance
- maternal heritability ($h_m^2 = \sigma_m^2/\sigma_p^2$)
- covariance between direct and maternal effects as a proportion to phenotypic variance ($d_{am} = \sigma_{am}/\sigma_p^2$)
- total heritability ($h_T^2 = (\sigma_a^2 + 0.5\sigma_m^2 + 1.5\sigma_{am})/\sigma_p^2$)
- maternal permanent environmental variance as a proportion to total variance ($c^2 = \sigma_c^2/\sigma_p^2$)

The derivative-free restricted maximum likelihood (DFREML) algorithm (Graser *et al.*, 1987; Meyer, 1989) under a simplex procedure is employed. The value of 10^{-8} was used as the convergence criterion for all analysis.

Direct and maternal additive genetic trends were evaluated by taking averages of the predicted direct and maternal effects for individuals born in a given year. Environmental trends were estimated from solutions for the best linear unbiased estimates (BLUE) of year effects. The computations were performed using DFREML software package of Meyer (1993).

RESULTS AND DISCUSSION

Achieving selection response in case of meatiness traits in the Whiteheaded Mutton sheep depends not only on the applied method of selection, but also on other factors affecting the genetic improvement efficiency of a population. Thus, the rate of the obtained genetic gain is influenced, among others, by the length of utility periods for ewes and rams (the increasing size of the generation interval), the selection differential and the heritabilities of traits under selection.

These obtained heritability estimates are shown in Table 2. Additionally, the respective genetic and environmental variance estimates are listed in Table 3. Estimated direct heritabilities for growth traits as well as the point scores for conformation and fleshiness were low, and ranged from 0.0042 (for daily gain 1–28 days) to 0.1734 (for the birth weight), whereas relatively high standard deviations of some heritability estimates were found. The obtained maternal heritability estimates were also low, but they were higher for lamb growth traits at a younger age (daily weight gain 1–28 and 28–70 days) compared to the daily weight gains at an older age (70–152 days). It corresponds to results reported by others authors (Al-Shorepy and Notter, 1996; Maria *et al.*, 1993) where weaning traits of progeny were influenced in a more pronounced way by the dam. Small maternal genetic variance estimates (ranging from 0.02 to 0.06) with the use of various genetic models are also reported by Hagger (1998). In consequence, the total heritability estimates of the studied traits were also low: from 0.0519 for weight gain in the period 1–28 days to 0.1919 for the weaning weight (100 days of age). It should be noted that low estimates of $\hat{h}_a^2$, $\hat{h}_m^2$ and $\hat{h}_T^2$ for lamb growth traits were also reported by other authors. For instance, Wolf *et al.* (1981) – for lambs of mutton breeds and hybrids – showed heritability within the range from 0.02 (for weight at the age of 4 weeks) to 0.05 (for weight at 8 weeks). Croston *et al.* (1988) for Suffolk ram lambs found heritabilities from 0.02 (weight at 4 weeks) to 0.20 (at the age of 124 days). Basically, similar results were reported by Maria *et al.* (1993) for Romanov sheep lambs, where the estimated direct heritabilities ranged from 0.04 (birth weight) to 0.26 (weight gains up to 40 days), where-

Table 2. Heritability estimates and standard deviations (in parentheses) of traits studied

Trait	$\hat{h}^2_a$	$\hat{h}^2_m$	$\hat{d}_{am}$	$\hat{h}^2_T$	$\hat{c}^2$
Birth weight	0.1734 (0.5420)	0.0207 (0.0187)	0.0062 (0.0111)	0.1707 (0.0548)	0.0012 (0.0048)
Weight at 100 days	0.1636 (0.0492)	0.0183 (0.0176)	0.0057 (0.0102)	0.1919 (0.0570)	0.0010 (0.0041)
Weight gain 1–28	0.0042 (0.0084)	0.0523 (0.0298)	0.0144 (0.0156)	0.0519 (0.0297)	0.0017 (0.0054)
Weight gain 28–70	0.0911 (0.0393)	0.0458 (0.0278)	0.0156 (0.0163)	0.1375 (0.0483)	0.0000
Weight gain 28–152	0.0927 (0.0396)	0.0521 (0.0297)	–0.0081 (0.0117)	0.1066 (0.0425)	0.0000
Weight gain 70–152	0.1392 (0.0646)	0.0171 (0.0735)	–0.0488 (0.0622)	0.0746 (0.0355)	0.0527 (0.0299)
Point score 25 pts.	0.1285 (0.0467)	0.0476 (0.0284)	–0.0134 (0.0151)	0.1322 (0.0473)	0.0008 (0.0037)

Note on symbols:

$\hat{h}^2_a$ – direct heritability estimate

$\hat{h}^2_m$ – maternal heritability estimate

$\hat{d}_{am}$ – estimate of covariance between direct and maternal genetic effects as proportion to phenotypic variance

$\hat{h}^2_T$ – total heritability estimate

$\hat{c}^2$ – maternal permanent environmental variance as a proportion to phenotypic variance

Table 3. Estimated genetic and environmental variance components for trait studied

Trait	σ^2_a	σ^2_m	σ_{am}	σ^2_c	σ^2_e
Birth weight	0.1069	0.0113	0.0038	0.0006	0.4939
Weight at 100 days	3.6019	0.38027	0.1293	0.0216	16.6329
Weight gain 1–28	16.8596	209.1808	57.4664	6.8537	3711.6514
Weight gain 28–70	330.0841	165.7627	56.6645	0.0001	3069.0255
Weight gain 28–152	154.0904	86.5866	–13.4353	0.00002	1434.4021
Weight gain 70–152	316.0582	38.8152	–110.7344	119.5842	1906.9292
Point score 25 pts.	0.5292	0.1959	–0.0551	0.0033	3.4443

Note on symbols:

σ^2_a – direct additive genetic variance estimate

σ^2_m – maternal additive genetic variance estimate

σ_{am} – estimate of covariance between direct and maternal effects

σ^2_c – maternal permanent environmental variance estimate

σ^2_e – residual variance estimate

as maternal heritabilities were from 0.01 (weight at 90 days) to 0.22 (birth weight). For the Spanish Merino, Jurado *et al.* (1994) observed heritability ranging from 0.03 (weight gain at 10–30 days) to 0.15 (weight at 90 days). Olsen *et al* (1995) reported $\hat{h}^2_a = 0.13$ and $\hat{h}^2_m = 0.11$ for weaning weight of the Norwegian breed lambs. Al-Shorepy and Notter (1996) for synthetic line lambs at 65 days observed $\hat{h}^2_a = 0.04$, $\hat{h}^2_m = 0.10$ and $\hat{h}^2_T = 0.10$, whereas at the age of 120 days the figures were 0.18, 0.05 and 0.18, respectively.

In contrast, results obtained by Pięta (1993) for Polish Lowland sheep lambs were higher than those presented above (heritability estimates ranged from 0.50 for birth weight to 0.28 for weight at 100 days). However, the results cited above were estimated on the basis of the sire and dam model ignoring existing relationships between parents. At the same time, it should be stressed that relatively high heritability estimates (from an animal model) were presented by Shrestha *et al.* (1996) for a synthetic paternal line (0.25 for birth weight and 0.35 for weight at 91 days).

Relatively low ratio estimates of covariance were found between direct and maternal genetic effects in the phenotypic variance. Certain trends could be observed despite low estimates of and relatively high standard errors. In case of body weight and its changes in the first period of life (up to 70 days), the obtained $\hat{d}_{am}$ values were positive, whereas for body weight gains up to 152 day, the $\hat{d}_{am}$ estimators were negative, which indicates an antagonism between direct and maternal genetic effects. In such cases, simultaneous improvement for maternal ability and growth capacity would be difficult. On the other hand, small both direct and maternal genetic variances lead to a large correlation between the effects (Maria *et al.*, 1993).

All estimates $\hat{c}^2$ instead of weight gain 70–152 were smaller than their standard deviations. Hence, it seems that maternal environmental effects can be omitted in the linear model describing variability of these traits in the analysed population. It corresponds to our earlier studies (Gut *et al.*, unpublished data) for a model without maternal environmental effects. The heritability estimates obtained on the basis of the current and previously applied models are very similar.

Direct genetic trends of the majority of these analysed traits of lambs of the Whiteheaded Mutton sheep turned out to be positive (Figures 2–4), except for the birth weight and daily weight gains in the first month of the lambs' lives (1–28 days). The considerable positive genetic trends were registered only since the sixth year of selection. This could be the positive influence of the applied breeding program, including progeny testing of rams as well as the reduction of the flock size in the years 1992–1995 as a consequence of economic changes in Poland after the year 1989. The positive maternal genetic trend was found mainly since the sixth year of selection with respect to body weight at the age of 100 days, daily weight gains from 28 to 70 days and from 28 to 152 days.

Varied environmental trends were observed for the investigated traits of lambs (Figure 5). It indicates changes in environmental conditions over the years. Genetic gain as a result of the applied strategy of selection (aiming at the improvement of lambs' growth rate) is relatively low. It has also been confirmed by the results reported in liter-

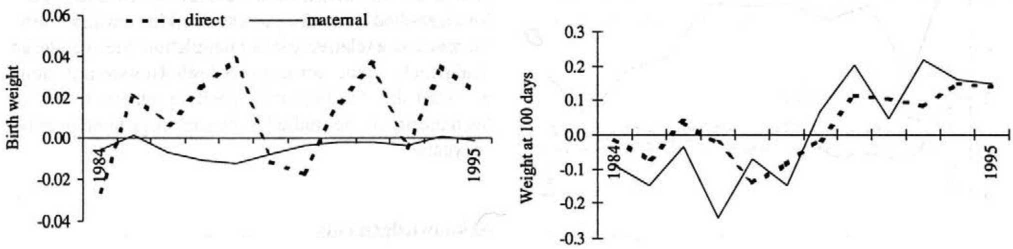


Figure 2. Direct and maternal genetic trends for lamb weight (kg)

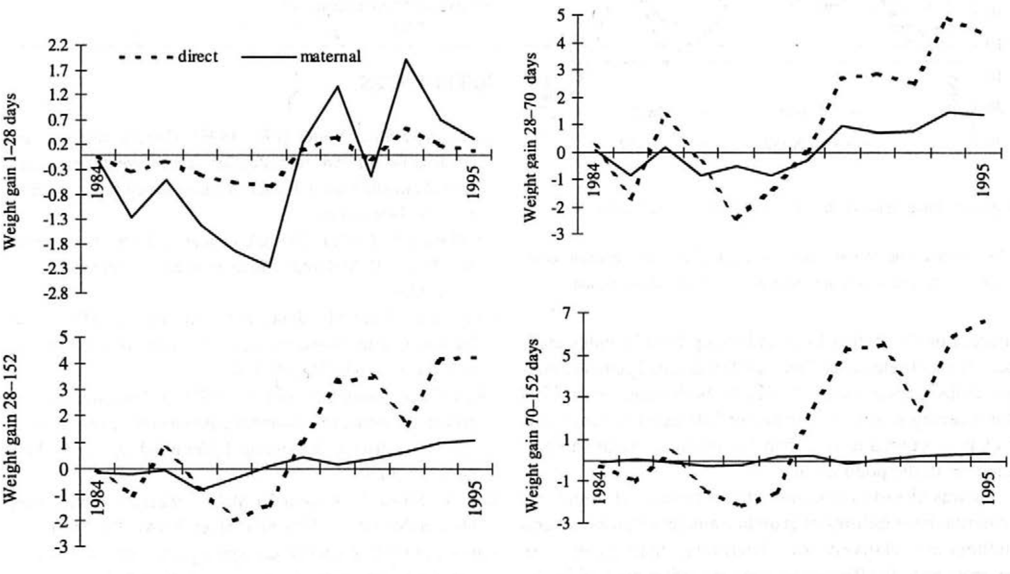


Figure 3. Direct and maternal genetic trends for daily weight gain in lambs (g)

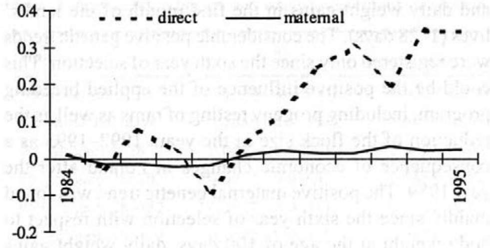


Figure 4. Direct and maternal genetic trends for body conformation score of lambs (pts.)

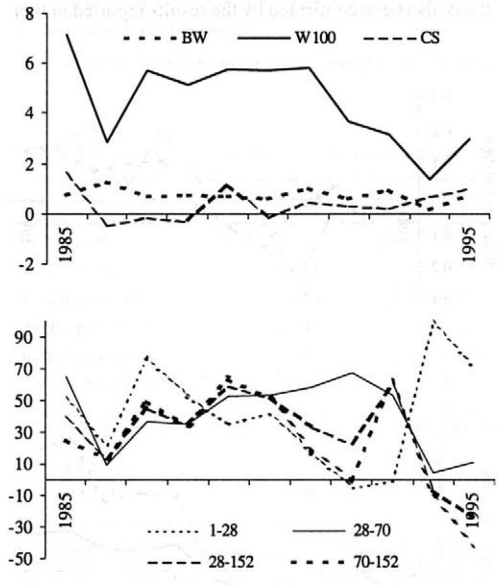


Figure 5. Environmental trends for growth traits of lambs

BW – birth weight, W100 – 100 day weight; CS – conformation score; 1–28. ..., 70–152 – daily weight gains in the successive period

ature. For the Polish Lowland sheep breed (under eighteen years of selection), Pięta (1993) obtained genetic trends for lambs' growth traits as 0.0128 for birth weight and 0.1704 for weaning weight (at the age of 100 days) per year. The trends amounted to less than 1% of the average value of the trait in the population.

As was already mentioned, the estimates of direct and maternal heritabilities of growth traits reported by several authors are relatively low. Therefore, changes in these average genetic effects over time are rather negligible. Jurado *et al.* (1994) estimated the genetic trends for growth traits in the population of the Spanish Merino at the range from 0.003 (weight at 30 days) to 0.184 (weight gain at 30–90 days). As a result of a breeding programme implemented in Norway (Olesen *et al.*, 1995), the genetic trend for the weaning weight in case of different breeds ranged from 0.0003 to 0.0010. Shrestha *et al.* (1996) reported a genetic trend for birth weight, body weights at the age of 21, 70 and 91 days in lambs coming from a synthetic paternal line at 0.014, 0.045, 0.104 and 0.141, respectively. The yearly selection response in terms of the body weight at the age of 91 days was approximately 1.5% of the average trait value for the population.

CONCLUSION

Generally, the obtained direct and maternal heritability estimates were low (not exceeding 0.18). The maternal permanent environmental variance estimates are close to zero for all studied traits. As was expected for low heritability estimates in a relatively small population, the average annual genetic values are not very high. However, it should be noted that the important positive genetic trends for the majority of the studied traits were registered over last six years.

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Meat production and quality of Holstein bulls fattened to 405–480 kg of live weight

Masná užitkovost a jakost masa holštýnských býčků vykrmovaných do živé hmotnosti 405 až 480 kg

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ABSTRACT: The aim of this study was to evaluate parameters of beef production and quality of meat and their mutual relationships in Holstein bulls. The experimental group consisted of 50 young bulls. They were slaughtered at 405–480 kg of live weight. Mean values of observed parameters were as follows: live weight before slaughter 431.6 kg, age at slaughter 412 days, daily gain 1.094 kg, dressing percentage 51.4%, net daily gain 0.544 kg, weight and proportion of kidney fat 5.03 kg (2.28%), fat trim 3.64 kg (3.30%), dry matter content in muscle 24.9%, fat in muscle 1.86%, collagen in muscle 1.15%, pH_{24} 5.54, drip loss 1.08% and remission (525 nm) 7.73%. Feedlot performance parameters affected carcass value to various degree; the total weight gain affected as many as 6 of 7 carcass value parameters (maximum) and the total duration of fattening affected only 1 of 7 parameters (minimum). As for meat quality parameters, the collagen content in muscle was positively affected by the age at slaughter and the fat content in muscle and remission were affected by the total weight gain.

Keywords: Holstein bulls; meat production; carcass quality; meat quality

ABSTRAKT: Byl proveden pokus na 50 býčcích holštýnského plemene chovaných v ČR s cílem kvantifikovat základní ukazatele masné užitkovosti a jakosti masa včetně jejich vzájemného vztahu. Býčci byli poráženi v živé hmotnosti od 405 do 480 kg. U celého souboru byly zjištěny následující průměrné hodnoty: živá hmotnost a věk před porážkou 431,6 kg a 412 dnů, denní přírůstek živé hmotnosti 1,094 kg, jatečná výtěžnost 51,4 %, denní nettopřírůstek 0,544 kg, hmotnost a podíl ledvinového loje 5,03 kg a 2,28 %, hmotnost a podíl lojového ořezu 3,64 kg a 3,30 %, obsah sušiny a tuku ve svalovině 24,9 % a 1,86 %, obsah kolagenních bílkovin ve svalovině 1,15 %, pH_{24} 5,54, ztráta masné šťávy samovolným odkapem 1,08 % a remise (při 525 nm) 7,73 %. Ze sledovaných parametrů výkrmnosti ovlivnil nejvíce ukazatelů jatečné hodnoty (6 ze 7) celkový přírůstek živé hmotnosti a nejméně (1 ze 7) celková doba výkrmu. Z ukazatelů jakosti masa byl obsah kolagenu ve svalovině pozitivně ovlivněn věkem při porážce, obsah tuku ve svalovině a remise celkovým přírůstkem živé hmotnosti.

Klíčová slova: holštýnští býčci; masná užitkovost; jatečná hodnota; jakost masa

INTRODUCTION

The cattle population in the Czech Republic has been through considerable changes during last decade. Apart from a significant decrease in number of cattle (nearly 50%), changes in breed structure of the cattle population have taken place. A part of the population of dual-purpose Czech Pied cattle has been gradually replaced by dairy Holstein cattle. Out of the total 501 705 cows monitored in milk recording there were as many as 229 389 cows of Holstein breed (before called Black and White cattle) or its crosses (ČSCH, 2000).

In spite of a generally known negative relationship between milk and meat production in cattle, dairy cattle, and young bulls in particular; can be used for beef production as was proved in a number of studies. Franc *et al.* (1993)

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looked into meat production of young bulls of Black and White cattle. Ten purebred bulls were slaughtered at 516.9 kg live weight (LW); dressing percentage was 58.85% and kidney fat weight was 10.91 kg (2.11% of LW before slaughter). Ptáček and Suchánek (1985) presented results of fattening of 13 Black and White bulls slaughtered at 471 kg LW (510 days of age). The average daily gain in a period from 6 months of age till slaughter was 920 g, net daily gain 475 g, dressing percentage 54.98% and weight of kidney fat 8.76 kg. Comparison with other breeds under observation showed that intensive fattening of Black and White bulls should not be prolonged above 500 kg LW at slaughter.

Danielson and Pehrson (1998) described beef production in 12 bulls (5 Red and White Swedish and 7 Swedish Friesian). The bulls were fattened from 305 kg LW to 514 kg LW while the average daily gain was 1.315 kg. The carcass weight was 263.6 kg which resulted in dressing percentage 52.0%. Kidney and kidney fat weighed 6.8 kg (2.9% of the carcass weight), the weight of fat trim was 11.4 kg.

Meat production of Black and White (Holstein) bulls was also evaluated by Chládek *et al.* (1997). The live weight at slaughter ranged between 430 and 530 kg with the average 436 kg, carcass weight 227 kg, daily gain 1 012 g and net daily gain 536 g.

Teslík *et al.* (1995) described meat production and quality in 16 Black and White bulls. The average slaughter weight was 516.68 kg at the age of 475.93 days with daily gain 1.296 kg and net gain 0.548 kg (calculated from the beginning of fattening period – 223.62 kg LW on average). Dressing percentage was 56.0%, weight of kidney fat 7.58 kg and separable fat 2.25 kg (1.59%). Some of the physical characteristics were observed: pH_{24} 5.69, remission (685 nm) 30.55% and components of sirloin – fat 4.61%, dry matter 26.42% and nitrogen compounds 3.32%.

Teslík *et al.* (1996) presented following physical and chemical characteristics of meat of Black and White bulls: pH_{24} 5.555, remission (522 nm) 8.54% and (685 nm) 32.010%, fat content in sirloin 12.517%, and components of MLD – dry matter 28.638%, fat 6.014% and protein 19.681%. Feedlot performance characteristics of the observed group of bulls were: live weight at slaughter 578.5 kg, age at slaughter 598.4 days, average daily gain during fattening 1.045 kg, kidney fat weight 12.98 kg (2.25%) and separable fat 2.93 kg (1.817%).

Mojto *et al.* (1998) evaluated nutritional and physical-technological quality of meat of 12 Holstein bulls. The average live weight at the age of 500 days was 501 kg. As for meat quality, 100 g of meat contained 74.58 g of water, 21.41 g of total protein, 0.37 g of ligament protein and 2.94% of fat, remission (540 nm) 9.96% and pH_{48} 5.69.

Ingr *et al.* (1996) investigated colour of meat of Black and White bulls. They evaluated 4 groups of young bulls; carcass weight ranged from 188.7 kg to 224.1 kg and remission values (525 nm) ranged between 5.4% and 7.8%. The colour of meat was evaluated using four different methods (sensual evaluation, myoglobin content and remission at two different wave lengths – 525 nm and 685 nm) and the highest correlation (0.692) was found between sensual evaluation and remission (525 nm).

MATERIAL AND METHODS

The aim of this study was to evaluate main parameters of meat quality and quantity and their mutual relationship in young Holstein bulls fattened to 405–480 kg LW. The observation was carried out in a group of 50 young Holstein bulls housed in loose boxes with sawdust bedding.

Diet consisted of ad lib crushed barley and wheat, limited amount of protein concentrate (which contained required quantity of vitamins and minerals) and limited amount of alfalfa hey. The daily ration was calculated to secure 1.3 kg of daily gain, as recommended by Sommer *et al.* (1994). Young bulls were slaughtered at 405–480 kg of LW.

The following parameters were evaluated on the day of slaughter: live weight and age, total gain, duration of fattening, daily gain, net gain, carcass weight, dressing percentage, weight and proportion of kidney fat. Additional characteristics were measured (only in the right half of the carcass) 24 hours after slaughter: weight and proportion of fat trim, percentage of dry matter, fat and collagen in MLLT muscle (sample between 8th and 9th pectoral vertebrae), remission of cut (525 nm) and drip loss. Measured values were analysed for statistical parameters: mean ($\bar{x}$), standard deviation (s), coefficient of variation (V) and correlation (r). For statistic analyses was used GLM procedure of SAS v. 6.03.

RESULTS

Parameters of feedlot performance of Holstein bulls under observation are presented in Table 1. The mean live weight before slaughter was 431.6 kg at the age of 412 days. The overall period of fattening lasted 287 days with the total gain 308.7 kg, which gave the daily gain 1.094 kg. The coefficient of variation was highest in duration of fattening (14.83%) and lowest in weight before slaughter (4.40%).

Meat production parameters are presented in Table 2. The mean carcass weight was 221.7 kg, which gave 51.4% dressing percentage and net weight gain 0.544 kg. The

Table 1. Some parameters of feedlot performance of Holstein bulls (n = 50)

Beef production parameters	$\bar{x}$	s	V
Live weight at slaughter (kg)	431.6	19.18	4.4
Age at slaughter (day)	412	42.85	10.4
Total weight gain (kg)	308.7	27.86	9.03
Duration of fattening (day)	287	42.54	14.83
Daily weight gain (kg)	1.094	0.1613	14.75

Table 2. Some carcass traits of Holstein bulls (n = 50)

Beef production parameters	$\bar{x}$	s	V
Carcass weight (kg)	221.7	9.36	4.22
Dressing percentage (%)	51.4	1.92	3.7
Net daily gain (kg)	0.544	0.0667	12.25
Weight of kidney fat (kg)	5.03	1.834	36.49
Proportion of kidney fat (%)	2.28	0.827	36.35
Weight of fat trim[1)] (kg)	3.64	1.515	41.63
Proportion of fat trim[1)] (%)	3.3	1.347	40.83

[1)]From the right half of carcass

Table 3. Some meat quality parameters of Holstein bulls (n = 50)

Meat quality parameters	$\bar{x}$	s	V
Dry matter content in muscle (mg/100 g)	24.9	0.73	2.93
Fat content in muscle (%)	1.86	0.569	30.56
Collagen content in muscle (%)	1.15	0.412	35.73
Drip loss (48 h p.m.) (%)	1.08	0.492	45.45
pH_{24}	5.54	0.332	5.98
Remission on cut (24 h p.m.) (%)	7.73	2.711	35.05

Table 4. Coefficients of correlation between parameters of feedlot performance and carcass traits (n = 50)

Carcass traits	Parameters of feedlot performance				
	live weight at slaughter	age at slaughter	total weight gain	duration of fattening	daily weight gain
Carcass weight	0.624**	–0.08	0.274	–0.115	0.317*
Dressing percentage	–0.478**	–0.278	–0.510**	–0.2	–0.067
Net daily gain	0.056	–0.940**	–0.117	–0.772**	0.776**
Weight of kidney fat	0.177	–0.292*	0.394*	–0.053	0.314*
Proportion of kidney fat	0.104	–0.268	0.361*	–0.024	0.262
Weight of fat trim[1)]	–0.131	0.085	–0.494**	–0.11	–0.211
Proportion of fat trim[1)]	–0.176	0.082	–0.520**	–0.117	–0.223

[1)]From the right half of carcass
* $P < 0.05$
** $P < 0.01$

Table 5. Coefficients of correlation between feedlot performance parameters and meat quality parameters (n = 50)

Meat quality parameters	Parameters of feedlot performance				
	live weight at slaughter	age at slaughter	total weight gain	duration of fattening	daily weight gain
Dry matter content in muscle	0.176	–0.061	0.245	0.035	0.136
Fat content in muscle	0.275	0.176	0.418**	0.243	–0.046
Collagen content in muscle	0.196	0.321*	0.223	0.254	–0.11
Drip loss (48 h p.m.)	–0.028	–0.164	0.14	0.03	0.095
pH_{24}	0.041	0.167	–0.025	0.215	–0.217
Remission on cut (24 h p.m.)	0.28	0.105	0.410**	0.092	0.11

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

weight of kidney fat was 5.03 kg (2.28%) and fat trim from the right half of the carcass 3.64 kg (3.3%). The highest variability was found in fat trim (41.63%) and lowest in dressing percentage (3.70%).

Meat quality parameters are shown in Table 3. The content of dry matter in muscle was 24.9%, proportion of fat in muscle 1.86% and collagen 1.15%. Drip loss was 1.08%, pH_{24} 5.54 and remission 7.73%. The highest variability (44.45%) was found in drip loss and lowest (2.93%) in dry matter content in muscle.

Coefficients of correlation between parameters of feedlot performance and carcass traits are presented in Table 4. Highly significant correlations were found between live weight at slaughter and carcass weight ($r = 0.624$) and dressing percentage ($r = -0.478$). Highly significant correlation was also found between net daily gain ($r = -0.940$) and age at slaughter. Total gain was highly significantly correlated with dressing percentage ($r = -0.510$), weight and proportion of fat trim ($r = -0.494$ and $r = -0.520$, resp.) and significantly correlated with weight and proportion of kidney fat ($r = 0.394$ and $r = 0.361$ resp.). Duration of fattening period was highly correlated with net daily gain ($r = -0.772$). Daily gain was highly significantly correlated to net daily gain ($r = 0.776$) and significantly correlated to carcass weight ($r = 0.317$) and kidney fat weight ($r = 0.314$).

Coefficients of correlation between meat quality parameters and feedlot performance parameters are presented in Table 5. A highly significant correlation was found between total gain and fat percentage in muscle ($r = 0.418$) and remission of cut ($r = 0.410$). A significant correlation was found between age before slaughter and collagen content in muscle ($r = 0.321$).

DISCUSSION

Bulls under observation were slaughtered at lower live weight and age in comparison to results of other studies. Daily gain corresponds with results of Teslík *et al.* (1996) and Chládek *et al.* (1997) and it was lower than values presented by Danielson and Pehrson (1998) and Teslík *et al.* (1995) in spite of the fact that we applied an intensive system of fattening. Lower daily gain was probably caused by lower weight (122.9 kg) and age (125 days) at the beginning of the fattening period compare to higher values (305 kg; 249.56 days and 223.62 kg, resp.) presented by authors.

Kidney fat weight was lower than that presented by other authors. Comparable values were presented by Teslík *et al.* (1996) and Danielson and Pehrson (1998). On the contrary, proportion of kidney fat was very close to the results of other studies. The weight and proportion of fat trim was lower than that found by Teslík *et al.* (1995, 1996). Danielson and Pehrson (1998) found the weight of fat trim three times higher however; the method of its measurement was not described. Dressing percentage was low compare to other authors, only values found by Chládek *et al.* (1997) and Danielson and Pehrson (1998) were similar.

However, in our study the dressing percentage was calculated from the live weight before slaughter reduced by the weight of stomach content (up to 8%) and thus calculated dressing percentage is about 5% lower. This could explain the difference between our results and those of Ptáček and Suchánek (1985) and partly those of Franc *et al.* (1993) but in the last study the weight at slaughter was higher, which could have affected dressing percentage too.

Meat quality parameters (dry matter, remission at 522 nm or 540 nm and collagen) correspond with results of Teslík *et al.* (1995, 1996) a Mojto *et al.* (1998). The percentage of fat was considerably lower which could be explained by lower live weight and age at slaughter of our animals.

As for parameters of feedlot performance and carcass quality, expected high positive correlation was found between daily gain and net daily gain and between live weight at slaughter and carcass weight. Higher total gain resulted in higher proportion and weight of kidney fat while higher daily gain affected only weight of kidney fat. This shows a negative effect of total gain and live weight at slaughter on dressing percentage. Total gain positively affected weight and proportion of kidney fat however, negative relationship was found between total gain and fat trim.

Net daily gain was negatively affected by duration of fattening and age at slaughter. No relationship was found between net daily gain and live weight at slaughter or total gain. Thus, age seems to have more important effect on net daily gain than live weight has. A positive relationship was found between total gain and fat content in muscle and remission on cut. Content of collagen in muscle significantly increased with age of animals.

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Leptin in colostrum and blood serum: is there any relation to milk production and weight gain of piglets in Large White sows?

Leptin v mlezivu a krevním séru: existuje vztah k produkci mléka u prasnic plemene bílé ušlechtilé a k přírůstkům jejich selat?

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ABSTRACT: Leptin concentrations in colostrum and blood serum of Large White sows (*n* = 19, 22 resp.) after the first or the fourth parturition were detected by ELISA assay. The average (± SEM) leptin concentrations in colostrum and blood serum were 8.0 ± 1.7 ng/ml and 4.1 ± 1.6 ng/ml, respectively. Leptin concentrations in both media were highly variable, however, the values correlated with each other ($r = 0.4$; $P < 0.05$). The correlations of leptin concentration with the parameters of milk production and litter gain were statistically insignificant.

Keywords: leptin; sows; milk production; weight gain of piglets

ABSTRAKT: Metodou ELISA byly určeny koncentrace leptinu v mlezivu a krevním séru prasnic plemene bílé ušlechtilé (*n* = 19; resp. 22) po prvním a čtvrtém porodu. V mlezivu byla zjištěna průměrná koncentrace leptinu (± SEM) 8,0 ± 1,7 ng/ml, v krevním séru byl průměr koncentrací 4,1 ± 1,6 ng/ml. Koncentrace leptinu v obou médiích byly individuálně značně rozdílné avšak ve statisticky významném pozitivním vztahu ($r = 0,4$; $P < 0,05$). Korelace koncentrací leptinu s parametry produkce mléka a přírůstku vrhu nebyly statisticky průkazné.

Klíčová slova: leptin; prasnice; produkce mléka; přírůstek selat

The product of the obesity gene leptin is included in the regulation of food intake and whole-body energy balance in animals and humans. Leptin is secreted mainly by the adipocytes and is considered a link between the energy metabolism and reproductive functions (Houseknecht *et al.*, 1997). Leptin gene expression in mice decreased to 30–50% during lactation and was restored to the original level after weaning (Aoki *et al.*, 1999). The same authors demonstrated leptin expression in mammary gland of mice. These facts suggest the importance of leptin in mammo- and lactogenesis. Although there are no data about milk leptin absorption, its transport into the neonatal circulation is possible, based on leptin receptors expression in small intestine of neonates (Houseknecht *et al.*, 1997). The aim of this study was to find a possible relation among the leptin concentrations in colostrum and blood serum and milk production rate in sows and gain of piglets. The colostrum samples were collected on the second day after parturition from all teats of Large White sows after the first or the fourth delivery. Blood samples were taken in the same time. Leptin concentrations were detected *in vitro* by assay ELISA for quantitative measurements of human leptin (BioVendor].

The average (± SEM) leptin concentration in colostrum was 8.0 ± 1.7 ng/ml ($n = 19$), ranging from 0.9 to 29.0 ng/ml. The average concentration of leptin in blood serum was insignificantly lower 4.1 ± 1.6 ng/ml ($n = 22$), ranging from 0 to 36.3 ng/ml. Leptin concentrations differed markedly in both the media (coefficient of variance of the leptin concentration in colostrum was 91.5%, in blood serum 187.8%), and the values were in a significant positive relation ($r = 0.4$; $P < 0.05$). The groups of sows (by the order of delivery) did not have statistically significantly differ-

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Table 1. Correlation coefficients between leptin concentration in both media and parameters of milk production and piglets gain

Correlation between leptin concentration			
in colostrum and		in blood serum and	
Leptin concentration in blood serum	$r = 0.40^*$	Leptin concentration in colostrum	$r = 0.40^*$
Litter weight at the parturition	$r = 0.32$	Litter weight at the parturition	$r = 0.18$
Litter weight at 21 days of life	$r = 0.03$	Litter weight at 21 days of life	$r = 0.22$
Milk production	$r = -0.13$	Milk production	$r = -0.26$
Parturition age of sows	$r = 0.21$	Parturition age of sows	$r = 0.14$

$^*P < 0.05$

ent values of leptin concentrations. Previous studies (Čanderle *et al.*, 2000) also showed wide range of serum leptin concentrations in different categories of pigs (0.5–32 ng/ml) and on the day of parturition leptin concentrations of colostrum in sows (6.3 ± 1.2 ng/ml) corresponded with our results. There were no significant correlations with the milk production and litter gain (Table 1). Our results did not support the assumptions about possible role of leptin in the regulation of neonatal growth or milk production but our experiment was performed with very small number of animals, so further studies are needed to confirm our results.

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If any abbreviation is used in the paper, it is necessary to mention its full form for the first time it is used, abbreviations should not be used in the title or in the summary of the paper.

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Only original **methods** should be described, in other cases cite the method used and any modifications. This section should also contain a description of experimental material.

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Detailed instructions to authors are published in No. 2 of this volume.

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