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The weaning stress response in lambs of different age

Stressová reakce na odstav u jehňat rozdílného věku

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ABSTRACT: Responses to weaning were studied in 50- and 100-days-old lambs. Blood samples were collected before weaning (sampling I) and 15 h after weaning (sampling II). Levels of cortisol and glucose as well as haematocrit were determined in the collected blood samples. Weaning caused a significant increase in cortisol levels in both groups of lambs. More sensitive than 100-days-old lambs were 50-days-old lambs in which an increase in cortisol level from 14.45 to 52.69 nmol/l was found. On the contrary, in 100-days-old lambs the cortisol level increased from 9.13 to 11.57 nmol/l. The level of glucose in 50-days-old lambs (2.79 mmol/l) was significantly higher than the level in 100-days-old lambs (2.39 mmol/l). Weaning stress caused an increase in glucose content in 50-days-old lambs while in 100-days-old lambs it remained on the same level (3.11 and 2.34 mmol/l, respectively). Neither age of lambs nor weaning stress influenced the haematocrit value.

Keywords: lambs; weaning; stress; cortisol; glucose; haematocrit

ABSTRAKT: Reakci na odstav jsme sledovali u jehňat ve věku 50 a 100 dní. Vzorky krve byly odebrány před odstavem (I. odběr) a za 15 hodin po odstavu (II. odběr). V odebraných vzorcích krve jsme zjišťovali hladinu kortizolu, glukózy a hodnotu hematokritu. Odstav způsobil u obou skupin jehňat významné zvýšení hladiny kortizolu. Citlivější než jehňata ve věku 100 dní byla jehňata ve věku 50 dní, u nichž jsme zjistili zvýšení hladiny kortizolu z 14,45 na 52,69 nmol/l. Naproti tomu u jehňat ve věku 100 dní se hladina kortizolu zvýšila z 9,13 na 11,57 nmol/l. Hladina glukózy u jehňat ve věku 50 dní (2,79 mmol/l) byla významně vyšší než u jehňat ve věku 100 dní (2,39 mmol/l). Stres z odstavu přinesl zvýšení obsahu glukózy u jehňat ve věku 50 dní, zatímco u jehňat ve věku 100 dní zůstal na stejné výši (3,11 resp. 2,34 mmol/l). Hodnotu hematokritu neovlivnil ani věk jehňat, ani stress z odstavu.

Klíčová slova: jehňata; odstav; stress; kortizol; glukóza; hematokrit

INTRODUCTION

Generally approved nutritive and taste attributes of the young mutton provoke slaughter of younger and younger lambs. The previous studies showed favourable parameters of slaughter performance and meat quality obtained from sucking lambs slaughtered at the age of both 50 (Tański *et al.*, 1999) and 100 days (Brzostowski *et al.*, 1999). In the case of so young animals, except for routine pre-slaughter handling, weaning and pre-slaughter starvation are also significant stress factors. Stability of the internal environment of the body influencing immunity debilitation, decrease in productivity, and in the case of slaughter animals decrease in meat quality, are the repercussions of those stress factors (Danzer and Mormede, 1983; Apple *et al.*, 1995; Fitko, 1996).

The estimation of stress response magnitude by means of physiological parameters may be very helpful in the evaluation of the load of handling procedures (Danzer and Mormede, 1983; Fenwick and Green, 1986; Broom *et al.*, 1996; Mears and Brown 1997; Sowińska *et al.*, 1998a, b, 1999). In many experiments the levels of the following substances were estimated as stress response parameters: cortisol (Hargreaves and Hutson, 1990; Wrońska *et al.*, 1991; Apple *et al.*, 1995; Broom *et al.*, 1996; Niezgoda *et al.*, 1996), glucose (Wrońska *et al.*, 1991; Apple *et al.*, 1995; Niezgoda *et al.*, 1996;) as well as the haematocrit (Fenwick and Green, 1986; Hargreaves

and Hutson 1990; Knowles *et al.*, 1993; Broom *et al.*, 1996).

Therefore the goal of the present study was to evaluate the magnitude of stress response by means of measurements of cortisol, glucose and haematocrit levels in lambs weaned at 50 and 100 days of age.

MATERIAL AND METHODS

The study was carried out on ten lambs of Pomeranian sheep weaned twice. For the first time the animals were weaned at the age of 50-days, and for the second time at the age of 100-days. Every time at about 5 p.m. the lambs were separated from ewes and placed to pens located in the same sheepfold, their vocal and visual contacts with mothers being maintained. Feed and water were supplied *ad libitum*. The lambs were kept under standard housing and feeding conditions. The next day at about 9 a.m. the lambs returned to the flock.

To evaluate the sensitivity of lambs to stress factors the levels of cortisol and glucose as well as haematocrit were determined in the collected blood samples. Blood samples were collected from the jugular vein just before weaning and 15 h after weaning. Blood collection was made by the experienced staff trying to minimise the influence of stress and to reduce the handling time to 40 seconds. The total number of blood samples was 40 in the group of 10 studied lambs. The full blood was used to estimate haematocrit (a micromethod) and glucose (Biochemtest, Poland) while cortisol was determined in the blood serum by means of a radioimmunological method (Orion Diagnostic, Finland).

The results of the present study were analysed statistically applying the orthogonal two- and one-way variance analysis by means of Fisher's test.

In the experiments, the principles of laboratory care as well as the specific national laws on the protection of animals were followed.

RESULTS AND DISCUSSION

The results of the present experiment are shown in Table 1 and in Figures 1 and 2.

The data presented in Table 1 show that the level of cortisol in the studied lambs (50- and 100-days old lambs taken together) increased from 11.79 to 32.13 nmol/l ($P \leq 0.01$) after 15-hour period of weaning. The standard variance values, in particular, show significant individual variability of the weaning stress response. The age of lambs was also an important factor influencing the level of cortisol (Table 1). The higher concentration of the studied hormone was found in 50-days-old lambs than in 100-days-old animals (33.57 and 10.35 nmol, respectively). Also the standard variance values show significant individual variability of the hormone concentration in the blood of younger lambs.

Data presented in Figure 1 confirm that weaning, found as the psychical factor, was stress inducing for both groups of animals. More sensitive were the younger animals that had 3.64 times higher level of cortisol while 100-days-old lambs had only 1.27 times higher level of the studied hormone.

Sheep are the animals with very well developed social instincts and high sensitivity to the emotional and environmental factors (Stephens, 1980; Moberg, 1987). There are many data dealing with the influence of different stress factors on the level of cortisol in the blood of sheep. Irrespective of the fact that the previous studies were carried out on different breeds of sheep, at different age and sex, and also the time of stress exposition was different, it was found that the stress factors stimulated the pituitary-cortical-adrenal axis to produce cortisol (Fordham *et al.*, 1989; Parrot *et al.*, 1994; Apple *et al.*, 1995; Broom *et al.*, 1996). The magnitude of the response measured by the level of released hormone was influenced not only by the type of stress factor (Hargreaves and Hutson, 1990; Niezgoda *et al.*, 1996; Mears and Brown, 1997) but also by the age of animals (Mellor and Murray, 1989; Kent

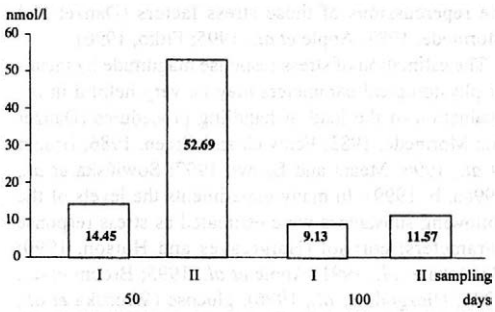

Figure 1. The level of cortisol in 50- and 100-days old lambs ($n = 10$) after blood sampling I and II

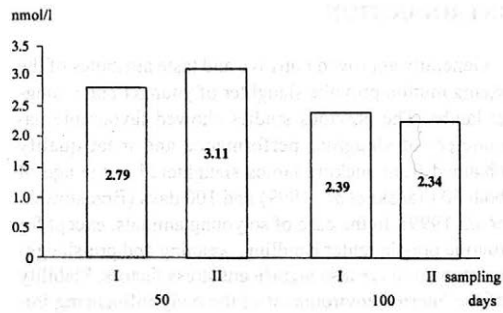

Figure 2. The level of glucose in 50- and 100-days old lambs ($n = 10$) after blood sampling I and II

Table 1. Levels of cortisol, glucose and haematocrit in the blood of lambs in relation to the time of blood sampling and age of lambs (n = 10)

Blood parameters	Age of lambs (days)	Blood sampling	
		I – before weaning	II – after weaning
Cortisol (nmol/l)	50	14.45 ± 5.16	52.69 ± 33.40*
	100	9.13 ± 2.35	11.57 ± 3.56
Glucose (mmol/l)	50	2.79 ± 0.54	3.11 ± 1.08
	100	2.39 ± 0.81	2.34 ± 0.82
Haematocrit (l/l)	50	0.28 ± 0.02	0.30 ± 0.02
	100	0.30 ± 0.03	0.29 ± 0.02

*in rows P = 0.05

and Molony, 1993) and the breed (Mellor and Murray, 1989; Sowińska *et al.*, 1998a, b, 1999). Niezgoda *et al.* (1996) revealed that the isolation of lambs was even a greater stress than blood collection. Mellor and Murray (1989) found different levels of cortisol after physical stress (castration and tail docking) depending on the age and breed of lambs. Kent and Molony (1993) reported that the stress response after castration and tail docking decreased according to the age of lambs. Mears and Brown (1997) found that the isolation with visual and vocal contacts was less stressogenic than the full isolation. Our previous studies (Sowińska *et al.*, 2000a) showed that the weaning response of 50-days-old lambs was stronger than the response after transport to slaughterhouses. However 100-days-old lambs showed the same stress response after both weaning and transport (Sowińska *et al.*, 2000b). Some other data (Sowińska *et al.*, 1998b) indicate that the stress response after pre-slaughter transport, estimated by the level of cortisol, was stronger in 100-days-old lambs than in 50-days-old animals.

Weaning stress caused an increase in glucose level from 2.59 to 2.73 mmol/l but it was not confirmed statistically (Table 1). The data presented in Figure 2 show higher sensitivity of younger lambs to the studied factor. They had 1.11 times higher concentration of glucose while the level of the studied substance in older lambs decreased from 2.39 to 2.34 mmol/l.

The previous studies carried out on 50- and 100-days-old lambs of different genetic groups did not show any significant changes in the glucose level after weaning and pre-slaughter transport (Sowińska *et al.*, 1999, 2000b). Some data document an increase in the glucose level after full isolation, depending also on the duration of this isolation (Pierzchała *et al.*, 1985; Wrońska *et al.*,1991; Apple *et al.*,1995; Niezgoda *et al.*, 1996). During the 6-hour isolation the level of glucose was increasing during the whole period (Apple *et al.*, 1995; Niezgoda *et al.*, 1996). After the 24-hour isolation of Polish Mountain Sheep Pierzchała *et al.* (1985) found that the level of glucose increased from 2.87 to 4.20 mmol/l during the 4th hour of experiment but during the next hours (8th and 14th) it decreased (3.80 and 3.70 mmol/l, respectively). Wrońska *et al.* (1991) reported an increase in glucose level during the 5-hour isolation of Polish Mountain Sheep (from 4.20 to 6.50 mmol/l) and a decrease to 2.80 mmol/l within 4 hours from the end of experiment.

The haematocrit value, 0.28 in 50- and 0.30 in 100-days-old lambs, was not influenced by the age of lambs nor by the handling at weaning.

Changes in the haematocrit value show the response of the sympathetic nervous system to the stress factors. Many experiments suggested that after the initial increase, as the emotional response (Fenwick *et al.*, 1986; Knowles *et al.*, 1993), a decrease in the haematocrit value was recorded after isolation (Parrot *et al.*, 1988; Hargreaves and Hutson, 1990) and transport (Knowles *et al.*,1993; Broom *et al.*, 1996). The previous studies carried out on the 50- and 100-days-old lambs of different genetic groups did not show any significant changes in the haematocrit value after weaning and pre-slaughter transport (Sowińska *et al.*, 1999, 2000b).

Drawing a conclusion from the results of the present study we can state that weaning although vocal and visual contacts with mothers were maintained was a strong stress factor for the lambs. The magnitude of the stress response was related to the age of lambs. More sensitive to weaning in similar environmental conditions were younger animals. This fact was demonstrated by a significant increase in the cortisol level in the blood of 50-days old lambs after weaning.

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The effect of 2-phenoxyethanol, clove oil and Propiscin anaesthetics on perch (*Perca fluviatilis*) in relation to water temperature

Účinek anestetik 2-phenoxyethanol, hřebíčkový olej a Propiscin u okouna říčního (*Perca fluviatilis*) v závislosti na teplotě vody

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ABSTRACT: The goal of a laboratory experiment was to study the onset of the respective phases of anaesthesia and its recovery in perch of 32.2–40.4 g mean body weight using solutions of the following anaesthetics: 2-phenoxyethanol (0.4 ml/l), clove oil (0.033 ml/l) and Propiscin (1.0 ml/l) at 10 min exposure at 4 different water temperatures (12.5; 15.0; 17.5 and 20.0°C). The study was focused on the phases of onset (I, IIa, IIb a III) and recovery of anaesthesia. Terminal phase III of anaesthesia was attained by all specimens under study. The longest time to attain phase III of anaesthesia was registered for clove oil (6.98–7.57 min) under all temperatures studied, compared to Propiscin (4.38–6.06 min) and 2-phenoxyethanol (3.73–6.05 min). Much longer time of anaesthesia recovery was attained using Propiscin: 15.16 min (20°C) to 42.66 min (12.5°C) compared to clove oil (6.06 min at 12.5°C to 9.21 min at 15°C) and 2-phenoxyethanol (3.69 min at 20°C to 7.44 min at 12.5°C), related to water temperature with significance ranging from $P < 0.05$ to $P < 0.005$.

Keywords: laboratory trial; onset of the respective phases; anaesthesia; recovery of anaesthesia

ABSTRAKT: V laboratorních podmínkách byl sledován nástup jednotlivých fází anestezie i jejího odeznívání při použití anestetik 2-phenoxyethanol (0,4 ml/l), hřebíčkový olej (0,033 ml/l) a Propiscin (1,0 ml/l), při expozici 10 min v roztocích anestetik při čtyřech různých teplotách vody (12,5; 15,0; 17,5 a 20,0 °C) u okouna říčního o průměrné kusové hmotnosti 32,2 až 40,4 g. Sledovány byly fáze nástupu (I, IIa, IIb a III) shodně i odeznění anestezie. U všech sledovaných jedinců bylo dosaženo konečné III. fáze anestezie. Nejdelší čas pro dosažení III. fáze anestezie byl zjištěn s hřebíčkovým olejem u všech sledovaných teplot, a to od 6,98–7,57 min, oproti Propiscinu (4,38–6,06 min) a 2-phenoxyethanolu (3,73–6,05 min). Několikanásobně delší čas odeznění byl ale dosažen s anestetikem Propiscin, a to 15,16 min (20 °C) až 42,66 min (12,5 °C) oproti hřebíčkovému oleji od 6,06 (při 12,5 °C) do 9,21 (15 °C) nebo 2-phenoxyethanolu od 3,69 min (při 20 °C) do 7,44 min (12,5 °C) v závislosti na teplotě vody byly statisticky významné rozdíly v rozmezí od $P < 0,05$ do $P < 0,005$.

Klíčová slova: laboratorní podmínky; počátek jednotlivých fází; anestezie; odeznění

INTRODUCTION

Anaesthetics are more and more used in fish culture in order to decrease fish stress and to prevent mechanical injury of fish while handling them, mainly during artificial propagation, marking and tagging the fish, biometric measurements, administration of hormonal preparations, health checks, etc., as well as due to application of regulations for animal protection against cruelty.

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Woynarowich and Horváth (1980) report 2-phenoxyethanol in doses of 0.3–0.4 ml/l to be an appropriate anaesthetic for fish. Kamiński *et al.* (1999) dealt with the effect of water temperature on the survival of cyprinid fry exposed to various concentrations of 2-phenoxyethanol for 15 min. Soto and Burhanuddin (1995) report the clove oil to be a cheap and very effective anaesthetic for fish, easily available in Indonesia. The Propiscin preparation was used by Trzebiatowski *et al.* (1996) as an anaesthetic for wels under three different water temperatures at two different doses and it was recommended as a safe anaesthetic. Hamáčková *et al.* (2000) dealt with assessment of the effect of anaesthetics 2-phenoxyethanol, Propiscin and clove oil on marketable tench under various water temperatures. Kouřil *et al.* (2001) reported on the results of the effects of these anaesthetics on various fish species. Kouřil *et al.* (1998) used 2-phenoxyethanol in concentration of 0.5 ml/l for 2 min prior to artificial propagation of perch.

In our experiments, we used these three anaesthetics (2-phenoxyethanol, clove oil and Propiscin) that were also presented by Svoboda and Kolářová (1999) in a review of anaesthetics used in fish culture. The goal of this study was to compare the effectiveness of these anaesthetics on females and males of perch under four different water temperatures and to assess the onset of the respective phases of anaesthesia and its recovery.

MATERIAL AND METHODS

Experiments were carried out under laboratory conditions in 100 litre aquaria. Anaesthesia was carried out on bisexual population of perch (*Perca fluviatilis* L.) from a pond culture. Ten fishes (5 females and 5 males) were used for every temperature and anaesthetic under study.

The experiment was always carried out synchronously on two specimens registering individual reactions to the anaesthetics used. Anaesthetics tested (commercial pharmaceutical products) were dosed as followed: 2-phenoxyethanol (France) 0.4 ml/l, clove oil (Czech Republic) 0.033 ml/l and Propiscin (Poland) 1.0 ml/l. Concentrations of the preparations were chosen according to general recommendations, appropriately corrected upon results of pilot tests. Experiments were carried out under the following four different water temperatures 12.5; 15, 17.5 and 20°C after previous several days long adaptation of fish to the given temperatures. Water temperatures for the study were chosen according to temperatures at which the perch is handled most frequently under practical conditions. The use of anaesthesia is relevant to such conditions.

An exposure to anaesthesia was chosen in advance to be 10 min, the respective phases of anaesthesia (0, I, IIa, IIb and III) were studied individually on all specimens during this exposure period. Fish were then put into clean aerated water of the same temperature and similarly, the phases of anaesthesia recovery were observed. A description of the respective phases of anaesthesia and of its recovery is given in Table 1. A slightly modified scale given by Kazuń *et al.* (1999) was used.

Mean values and standard deviations of the onset of the respective anaesthesia phases and of its recovery were computed for all temperatures and anaesthetics used. The results were processed statistically by means of *F*-test and two-sample *t*-test.

Table 1. Description of the respective phases of anaesthesia and their recovery in fish (according to Kazuń *et al.*, 1999)

Anaesthesia	phase 0 Quiet behaviour	physiological position, regular respiratory motion, normal locomotor activity, effortless evading obstacles when swimming
	phase I Excitation	physiological position, increased activity, restlessness, fast swimming, not evading the obstacles when swimming, strong withdrawal reflex, irregular respiratory motion, in some species shallow respiratory motion or wide opened opercula
	phase IIa Total superficial anaesthesia	decreased activity, slight tilting on the flank, weakened or no withdrawal reflex, respiratory motions regular, slower and deep
	phase IIb Total complete anaesthesia	flank position, loss of motility, none of the withdrawal reflexes but the acoustic one, respiratory motions regular, deep, retarding
	phase III Respiration block	flank position, respiratory motions blocked or superficial to involuting, no withdrawal reflex, neither the acoustic one
Anaesthesia recovery	phase IIb	flank position, regular respiration, acoustic reflex
	phase IIa	flank position changed to physiological one, uncoordinated motions, regular respiration
	phase I	physiological position, slow swimming initiated, uncoordinated motions, not evading the obstacles when swimming (impinging against them)
	phase 0	physiological position, normal locomotor activity, normal swimming, evading the obstacles when swimming

RESULTS

Randomly sampled fish were used for the experimental groups. Their biometric data are given in Table 2.

Table 2. Total length (TL) in mm, standard length (SL) in mm and individual weight (W) in g of perch in the respective experimental groups

Anaesthetics	Parameter	TL (mm)	SL (mm)	W (g)
2-phenoxyethanol	Mean	149.1	125.5	40.4
	SD	23.0	19.8	21.2
	Min	108.0	92.0	17.0
	Max	222.0	193.0	140.0
Propiscin	Mean	140.2	119.0	32.2
	SD	18.5	16.8	14.2
	Min	117.0	95.0	16.0
	Max	195.0	168.0	74.0
Clove oil	Mean	143.7	122.5	34.5
	SD	15.9	14.7	13.0
	Min	120.0	100.0	17.0
	Max	180.0	165.0	75.0

Under all water temperatures studied, anaesthesia phase IIb was always attained first with Propiscin followed by 2-phenoxyethanol and clove oil. For attaining anaesthesia phase III, this sequence was maintained in the case of 12.5°C temperature only. With all three remaining temperatures studied, this phase was attained first using 2-phenoxyethanol followed by Propiscin and clove oil. It could be stated for all studied cases that with clove oil, both phase IIb and phase III of anaesthesia were attained in the latest time compared with the other anaesthetics.

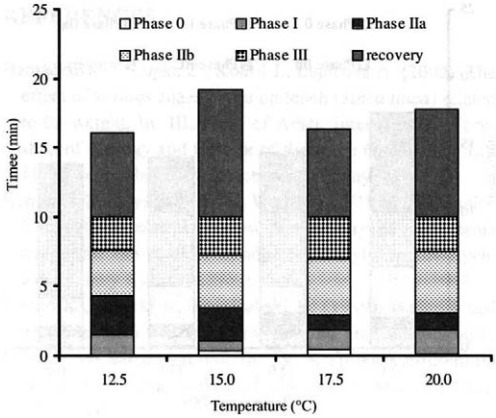

Figure 1. Anaesthesia and recovery time for perch (*Perca fluviatilis*) using clove oil at different water temperatures

On the contrary, when observing the anaesthesia recovery, expressively longer times were registered for the Propiscin anaesthetic under all temperatures studied, compared with other anaesthetics. The duration of anaesthesia recovery with Propiscin ranged from 15.16 ± 3.14 min at 20°C to 42.33 ± 11.89 min at 12.5°C (Figure 1). With 2-phenoxyethanol, the recovery ranged from 3.69 ± 0.79 min at 20°C to 7.44 ± 1.77 min at 12.5°C (Figure 2). It is evident from the values reported that with Propiscin and 2-phenoxyethanol the duration of anaesthesia recovery was longer with falling temperature. This was not valid for clove oil (Figure 3) where the duration of anaesthesia recovery was 6.06 ± 1.08 min at 12.5°C, the longest recovery 9.21 ± 2.17 min was at 15°C while at the other

Table 3. The percentage of fish when attaining the respective phases

Anaesthetics (Concentration)	Temperature (°C)	The onset of anaesthesia (%), $x \pm SD$					Recovery of anaesthesia (%), $x \pm SD$			
		phase					phase			
		0	I	IIa	IIb	III	IIb	IIa	I	0
2-phenoxyethanol (0.4 m/l)	12.5	100	100	80	90	100	100	50	70	100
	15.0	100	100	100	100	100	100	100	100	100
	17.5	100	100	100	100	100	100	100	100	100
	20.0	100	100	87.5	100	100	100	100	100	100
Propiscin (1.0 ml/l)	12.5	100	70	40	80	100	100	90	80	100
	15.0	100	100	90	90	100	100	100	100	100
	17.5	100	70	100	100	100	100	100	100	100
	20.0	100	100	80	100	100	100	100	100	100
Clove oil (0.033 ml/l)	12.5	100	0	90	100	100	100	90	90	100
	15.0	100	40	70	90	100	100	100	80	100
	17.5	100	80	70	100	100	100	100	100	100
	20.0	100	90	90	100	100	100	100	100	100

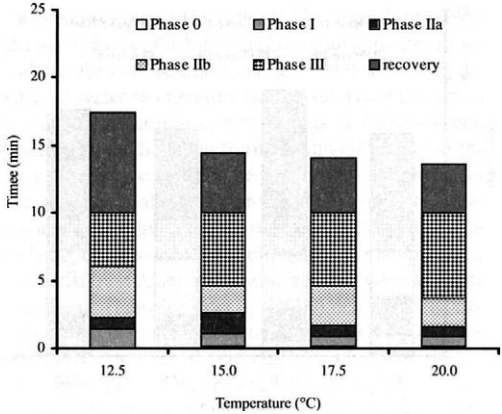

Figure 2. Anaesthesia and recovery time for perch (*Perca fluviatilis*) using 2-phenoxyethanol at different water temperatures

temperatures studied the recovery ranged from 6.38 ± 2.21 (17.5°C) to 7.77 ± 2.30 min (20°C).

No statistically significant differences were found in the time interval for attaining phases IIb and III with clove oil under the temperatures studied. On the contrary, for the recovery of anaesthesia with clove oil significant differences at ($P < 0.005$) were found between temperatures 15°C and 17.5°C; 12.5°C and 15°C.

Using 2-phenoxyethanol, significant differences in attaining phase IIb were found between temperatures 15 and 17.5°C as well as between 15 and 20°C ($P < 0.05$). For attaining phase III of anaesthesia it was between temperatures 12.5 and 20°C ($P < 0.01$). Testing the recovery of anaesthesia, statistically significant differences were found between the temperature 12.5°C and all other temperatures studied, at $P < 0.005$.

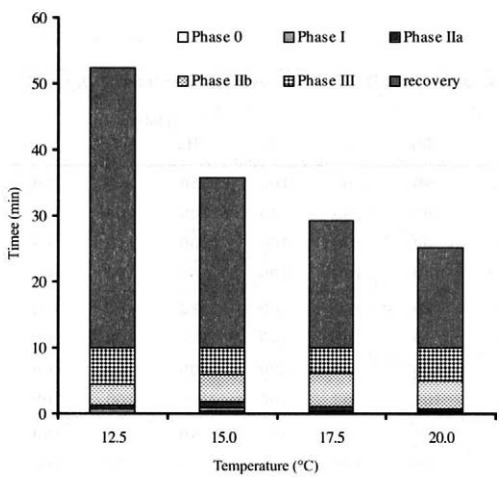

Figure 3. Anaesthesia and recovery time for perch (*Perca fluviatilis*) using Propiscin at different water temperatures

With the Propiscin anaesthetic, statistically significant differences were found in time for attaining phase IIb of anaesthesia between the temperature 20°C and temperatures 12.5 and 15°C ($P < 0.05$). For attaining phase III it was between temperatures 12.5 and 17.5°C ($P < 0.01$). When testing the duration of the time interval from fishing the specimens out of the anaesthetic solution into clean water, statistically significant differences were found at ($P < 0.05$) between the temperature 17.5°C and temperatures 15 and 20°C, at ($P < 0.005$) between the temperature 12.5°C and other temperatures studied, as well as between 15 and 20°C temperatures.

When assessing statistically the attainment of phases IIb and III of anaesthesia and its recovery with the respective anaesthetics it can be stated that at water temperature 12.5°C and assessment of phase IIb, there was a statistically significant difference between 2-phenoxyethanol and Propiscin ($P < 0.01$) or clove oil ($P < 0.05$). At 15°C water temperature, a statistically significant difference was obtained only between Propiscin and clove oil ($P < 0.005$). At 17.5°C water temperature, significant differences were determined at $P < 0.005$ between Propiscin and 2-phenoxyethanol, as well as between Propiscin and clove oil. Differences at the same level ($P < 0.005$) were also found at 20°C water temperature between all anaesthetics tested.

When assessing the duration of the time interval till the recovery of anaesthesia, statistically significant differences ($P < 0.005$) were found between all anaesthetics at 15 and 20°C, and at temperatures of 12.5 and 17.5°C between Propiscin and clove oil or between Propiscin and 2-phenoxyethanol.

The percentage of fish that could be visually detected to progress from one phase to another one is given in Table 3. If phase III was attained by all specimens, it was obvious that all of them passed through the respective phases but they could not be registered visually by changing the fish behaviour. It can be stated that with anaesthesia recovery this case happened at 12.5°C using all anaesthetics studied and at 15°C in the case of clove oil. Checking the onset of anaesthesia the changes could not be registered most frequently using clove oil, followed by Propiscin and 2-phenoxyethanol.

Regardless of the water temperature, the fastest recovery of fish from anaesthesia was with 2-phenoxyethanol (3.69–7.44 min), followed by clove oil (6.06–9.21min) and the longest recovery was found with Propiscin (15.16–42.66 min). The most rapid attainment of phase III of anaesthesia at 15; 17.5 and 20°C was with 2-phenoxyethanol, followed by Propiscin and the slowest one with clove oil. At 12.5°C, Propiscin was the fastest anaesthetics followed by 2-phenoxyethanol and clove oil.

DISCUSSION

Assessing the respective positions compared to the cited review of Kazuń (1999) it can be stated that with perch, phase I of anaesthesia with 2-phenoxyethanol was characterised by a wide opening of the opercula and, appropriately opening the mouth. The females that had not spawned yet did not take the flank position at phase IIb.

In our experiment, phase III of anaesthesia was attained by all specimens under study using all anaesthetics studied, contrary to the results of Kouřil *et al.* (2001) where the last phase of anaesthesia was not attained with 2-phenoxyethanol at the same concentration 0.400 ml/l and water temperature 15°C.

For the reason that anaesthesia was not attained at lower temperatures, we used a higher concentration of 2-phenoxyethanol (0.4 ml/l) in our experiment with perch than recommended (0.2–0.3 ml/l) by Svoboda and Kolářová (1999). Comparing the results obtained in this way with these anaesthetics used for tench at two temperatures nearly identical to those used for perch, it was concluded that the time necessary for attaining the anaesthesia as well as for its recovery was longer for tench (Hamáčková *et al.*, 2000) compared with perch for all anaesthetics studied.

Identically to Munday and Wilson (1997), comparing the recovery of anaesthesia with clove oil and 2-phenoxyethanol, twice longer time was attained in the case of using the clove oil but at higher temperatures (15, 17.5 and 20°C) only. At 12°C water temperature the time was comparable. Using the Propiscin preparation at all temperatures, much longer time for the recovery of anaesthesia was necessary compared with the other anaesthetics used.

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Impact of incorrect weighting of traits in the aggregate genotype of pigs on genetic gain

Vliv neadekvátního vážení znaků v agregovaném genotypu prasat na genetický zisk

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ABSTRACT: Genetic gain for the traits in the aggregate genotype of pig breeds used in a three-way crossing system and total return from one round of selection within an 8-year investment period were calculated for five aggregate genotypes (AG). The AG differed in the relative weighting of the traits included. For AG 1, "true" economic weights were used calculated as the product of marginal economic values and the number of discounted expressions (NDE) for each trait and each selection group in all breeds; in AG 2, the differences in NDE between the selection groups within breeds were neglected; in AG 3 and AG 4 the differences in NDE between dam breeds were omitted using the weights for breed A or B, respectively; marginal economic values were used for all breeds in AG 5. The traits in the aggregate genotypes were average daily gain (ADG), weight of valuable cuts in half-carcass (VC) and number of piglets born alive (NBA). The differences in total return between the alternatives were small (maximally 2.5%). But compared with "true" weighting in AG 1, there were substantial losses (up to 25%) in genetic gain for ADG in dam breed B (using AG 3), for NBA in dam breed A (using AG 4) and for NBA in both dam breeds (AG 5).

Keywords: pig; economic weights; aggregate genotype; genetic gain; gene flow

ABSTRAKT: Byl vypočten genetický zisk znaků v čistokrevných populacích prasat užívaných pro trojplemenné křížení a celkový výnos z jednoho cyklu selekce za osmileté investiční období pro pět souhrnných genotypů (AG). Tyto AG se lišily v relativním vážení znaků. U AG 1 byly použity „správné" ekonomické váhy znaků stanovené jako součin mezní ekonomické hodnoty znaků a počtu odúrokovaných projevů znaků (NDE) pro každou selekční skupinu u všech plemen; u AG 2 byly zanedbány rozdíly v NDE mezi selekčními skupinami uvnitř plemen; u AG 3 a AG 4 byly zanedbány diference v NDE mezi oběma mateřskými plemeny a bylo použito vážení jako u plemene A (AG 3) nebo B (AG 4); u AG 5 byly k vážení použity mezní ekonomické hodnoty znaků pro všechna plemena. Znaky v agregovaném genotypu byly: průměrný denní přírůstek (ADG), hmotnost hlavních masitých částí (VC) a počet živě narozených selat (NBA). Diference v celkovém výnosu selekce mezi alternativami byly malé (maximálně 2,5 %). Došlo však k významnému snížení genetického zisku pro ADG u plemene B (při použití AG 3), pro NBA u plemene A (AG 4) a pro NBA u obou plemen (AG 5) až o 25 % ve srovnání s použitím „správných" ekonomických vah v AG 1.

Klíčová slova: prasata; ekonomické váhy; agregovaný genotyp; genetický zisk; tok genů

INTRODUCTION

Production systems in pigs often consist of different levels such as nucleus, multiplier and commercial herds. Furthermore, purebred animals of several breeds from nucleus herds are used in different crossing systems. Depending on the crossbreeding scheme, the genetic superiority of animals in individual traits is transferred to the commercial level with different frequency and time delay. As shown in several papers (Wilton and Danell, 1981; Wünsch, 1998; Wolfová *et al.*, 2001), the relative economic importance of traits in the aggregate genotype

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differs between breeds according to their position in a specific crossing system. Differences in the relative economic importance of traits can also occur between the selection groups (sires, dams) within a breed (Danell *et al.*, 1976; Dekkers, 1994). The question is if the magnitude of these differences in the relative importance of traits is large enough to be of practical importance for breeding companies.

Genetic gain per time unit obtained for all traits in the aggregate genotype and total return per animal in the population are the main indicators of the efficiency of a selection program. Considering the genetic gain and return from selection of animals in the breeding herds for a special crossing system, the aim of the present investigation was to study the consequences of not taking into account different frequencies and time delays in the transfer of the genetic superiority of a trait through different selection paths.

MATERIAL AND METHODS

Population structure and breeding program

The pig production system in the Czech Republic consists of three tiers – breeding, multiplier and commercial herds. Genetic evaluation is carried out for two dam breeds – Large White (breed A) and Landrace (breed B) – and six sire breeds (breeds C). Young boars and gilts from the breeding herds of breeds A and B are sold to multiplier herds where they produce reciprocal F_1-dams. These are used in commercial herds for the production of terminal hybrids that are mostly three- and four-way crosses. Dams and sires of breed C in breeding herds are kept to produce the terminal sires for crossing.

Wolfová *et al.* (2001) have shown that there is hardly any difference in the impact of three- and four-way crossing systems on the relative weighting of production and reproduction traits in the aggregate genotype for dam breeds. Therefore, only the three-way crossing system was considered in this study. For simplicity, all sire breeds were taken as one breed C. The numbers of sows and boars in all tiers are given in Table 1. Biological and technical parameters used in the calculations (Table 2) were set to values representing the situation in the Czech Republic in 2000 (Pražák *et al.*, 2001). The basic time unit for all calculations is half a year, referred to as "season".

Young boars and gilts reared in breeding herds are field tested for their own performance. The traits measured are average daily gain calculated as weight at the end of test divided by age at the end of test (ADG) and lean meat content at the end of test. Information about fattening and carcass performance is collected on stations with the traits weight of trimmed valuable cuts in half-carcass (VC) and average daily gain on station from 30 to 100 kg live weight. Four groups of full sibs (one gilt and one castrate each) are tested per sire. For dam breeds only, the trait number of piglets born alive (NBA) is available for all parities in the breeding herds.

After the field test, the animals are pre-selected for their conformation and health. On the basis of their total breeding value, the boars suitable for breeding are further divided into two groups – into sires for breeding herds and sires for the lower tiers of the production system. The best 700 dams of breed A and the best 400 dams of breed B from all gilts and sows kept in the breeding herds are selected as sires' dams. The best 70 sires of breed A and 40 sires of breed B from all boars used in the breeding herds are selected as sires' sires (Table 3).

Calculation of genetic gain and return from selection for differently weighted traits in the aggregate genotype

The aggregate genotype for the j-th genetic groups of breed b can be described as follows:

$$T_{jb} = \mathbf{w}'_{jb}\,\mathbf{BV}_{jb}$$

where: $\mathbf{w}'_{jb}$ = the row vector of economic weights with the elements w_{tjb}

$\mathbf{BV}_{jb}$ = the column vector of breeding values for the individual traits with the elements BV_{tjb}

The index t refers to the trait (ADG, VC or NBA).

The "correct" economic weight w_{tjb} of trait t in the aggregate genotype for the selected group j in breed b was obtained for breeding herds as

Table 1. Population structure assuming a three-way crossing system with reciprocal crossing in dam breeds A and B

Production level	Breed A		Breed B		Breed C		F_1-cross	Total
	dams	sires	dams	sires	dams	sires	dams	dams
Breeding herds	5 640	430	1 830	230	1 750	140		9 220 (3.3%)
Multiplier herds	20 250	400	6 750	730				27 000 (9.7%)
Commercial herds						1 710	240 800	240 800 (87%)
Total	25 890	830	8 580	960	1 750	1 850	240 800	277 020
	(9.3%)		(3.1%)		(0.6%)		(87%)	(100%)

Table 2. Biological and technical parameters for the Czech pig population in 2000

Parameter (Unit)	Value
Number of offsprings reared per sow and season[1]	
– dam breeds in breeding and multiplier herds	9.8
– sire breeds in breeding herds	8.0
– F_1-sows in commercial herds	9.8
Farrowing interval (seasons)	1.0
Age of animals when their first offspring is born (seasons)	2.0
Productive lifetime of animals in breeding herds (seasons)	
– sires' dams of breeds A and B	2
– sires' sires of breeds A and B	2
– dams' dams of breed A	3
– dams' dams of breed B	4
– dams' sires of breeds A and B	4
– sires' sires and sires' dams of breed C	2.5
– dams' sires and dams' dams of breed C	2.5
Productive lifetime of animals in multiplier herds (seasons)	
– dams of breed A	4
– dams of breed B	5
– sires of breed A	2
– sires of breed B	3.5
Productive lifetime of animals in commercial herds (seasons)	
– F_1 dams	5
– sires of breed C	2.3
Investment period (seasons)	16
Interest rate for return per season	0.03

[1]season = half a year

$$w_{tjb} = NDE_{tjb} a_t$$

where: NDE_{tjb} = the number of discounted expressions (NDE) for trait t in selection group j of breed b

a_t = the marginal economic value of trait t

The marginal economic values are given in Table 4. The selection groups in breeding herds are sires to breed sires, sires to breed dams, dams to breed sires and dams to breed dams, i.e. there are 12 groups altogether (4 groups for each breed).

The variable NDE_{tjb} defines the amount of expressions of the genetic superiority of selected group j in breed b for trait t discounted to its year of birth with interest rate r and investment period S. The equation for the calculation of NDE was given by Nitter *et al.* (1994):

$$NDE_{tjb} = \mathbf{h}'_t \sum_{s=1}^{S} \mathbf{m}_{jbs} (1+r)^{-s}$$

where: $\mathbf{h}'_t$ = row vector whose elements are the realizations of trait t in the individual sex-age classes

$\mathbf{m}_{jbs}$ = column vector whose elements are the gene proportions that the animals of various age-sex classes of breed b carry from selection group j in season s

The NDE_{tjb} were calculated by the gene-flow method using S and r from Table 2.

Five different aggregate genotypes (AG) were considered:

AG 1 – "correct" weights w_{tjb} as shown above; NDEs specific for each path (reference AG)

AG 2 – different economic weights between breeds but not between paths within breeds; NDEs for the path sires to breed sires were used

Table 3. Number of tested and selected animals per season (half a year), number of parents for production of the next generation of sires and selection proportion in the breeding herds

Group of animals	Breed A	Breed B	Breed C
Gilts			
– performance tested	10 900	2 850	1 440
– suitable for breeding[1]	7 200	1 850	710
Young boars			
– performance tested	1 400	850	2 300
– suitable for breeding[1]	325	210	900
– suitable for use in breeding herds[2]	110	60	140
Number of sires' dams	700	400	1 750
Number of sires' sires	70	40	140
Selection proportion in the path (in %)			
– sires to breed sires	10.8	9.5	6.2
– dams to breed sires	4.9	10.8	98.6
– sires to breed dams	33.1	27.4	6.2
– dams to breed dams	26.1	24.7	98.6
Mean generation interval (seasons)	2.88	3.00	2.80

[1]after selection on conformation and health, [2]on the basis of breeding values

Table 4. Genetic parameters and marginal economic values (from Wolf *et al.*, 1999)

Trait (Unit)	Standard deviation		Heritability	Correlation[1]			Marginal values (CZK/unit)
	genetic	phenotypic		ADG	VC	NBA	
ADG (g/day)	25.8	61.9	0.17		–0.08	–0.09	8
VC (kg)	0.83	1.1	0.59	0.06		0.13	270
NBA (piglets)	0.67	1.9	0.13	–0.01	0.03		440

[1]genetic correlation above, phenotypic below the diagonal

ADG = average daily gain in the field test, VC = weight of valuable cuts in the half-carcass, NBA = number of piglets born alive

AG 3 – equal economic weights for both dam breeds; NDEs for the path sires to breed sires of breed A

AG 4 – equal economic weights for both dam breeds; NDEs for the path sires to breed sires of breed B

AG 5 – marginal values a_t not corrected for NDEs for all traits in all breeds and paths

The program ZPLAN (Karras *et al.*, 1993) was applied for the calculation of NDEs, genetic gain and return from selection. In this program, the classical theory of index selection is used.

Equal information sources were used for the indices of all selection groups and breeds. These were the own performance of the selected animal, the performances of sire, dam, sire's sire and sire's dam, dam's sire and dam's dam, halfsibs of the sire and dam, fullsibs of the sire and dam, paternal halfsibs and fullsibs. In dam breeds, the information on both reproduction and production traits (from field and station tests) was included in the index whereas only production traits were included in the sire breed.

The genetic superiority of trait t in breed b and selection group j achieved in one round of selection with selection intensity i_{jb} was calculated as follows:

$$\Delta G_{tjb} = i_{jb}\mathbf{b}'_{jb}\mathbf{G}_{tjb} / \sqrt{\mathbf{b}'_{jb}\mathbf{P}_{jb}\mathbf{b}_{jb}}$$

where: $\mathbf{G}_{tjb}$ = the t-th column of genetic covariance matrix $\mathbf{G}_{jb}$ between the traits in the aggregate genotype and the information sources in the index

$\mathbf{P}_{jb}$ = phenotypic covariance matrix between the information sources of the index

$\mathbf{b}_{jb}$ = the column vector of index weights and the prime stands for the transposition of the vector

All quantities refer to selection group j of breed b. The genetic superiority of the aggregate genotype is then simply calculated by weighting the genetic superiority of the individual traits with the appropriate marginal economic values. The parameters required for the construction of genetic and phenotypic covariance matrices are summarised in Table 4.

The genetic gain per time unit for trait t in breed b (ΔG_{tb}) was obtained analogically to the formula of Rendel and Robertson (1950):

$$\Delta G_{tb} = \left(\sum_j q_{jb}\Delta G_{tjb}\right) / \left(\sum_j q_{jb} l_{jb}\right)$$

where: q_{jb} = the proportion of selection group j of breed b contributed to the next generation

l_{jb} = the generation interval of selection group j of breed b

The present value of overall return (R) from one round of selection was obtained by summing the returns over breeds, traits and selection groups from all tiers (breeding, multiplier and commercial herds) within the investment period (16 seasons = 8 years):

$$R = \sum_b \sum_t \sum_j \Delta G_{tjb} NDE_{tjb} a_t$$

For further details see also Nitter *et al.* (1994).

Table 5. Numbers of discounted expressions for production and reproduction traits in the four selection paths in breeding herds (summarised within the investment period of 16 seasons, discounted to the birth year of selected groups by a discounting rate of 3% and expressed per sow in the whole population)

Selection group	Numbers of discounted expressions					
	dam breed A		dam breed B		sire breed C	
	ADG, VC	NBA	ADG, VC	NBA	ADG, VC	NBA
Sires' sires	0.120	0.238	0.088	0.154	0.273	0.004
Sires' dams	0.120	0.238	0.088	0.154	0.273	0.004
Dams' sires	0.106	0.221	0.072	0.129	0.273	0.007
Dams' dams	0.115	0.238	0.072	0.129	0.273	0.007

For abbreviations of traits see Table 4

Table 6. Relative economic weights in aggregate genotype 1 (true weighting) for selection groups in nucleus herds for all involved breeds

Selection group	Economic weight for NBA expressed as the proportion of economic weight for ADG[1]		
	dam breed A	dam breed B	sire breed C
Sires' sires	109.33	96.43	0.87
Sires' dams	109.33	96.43	0.87
Dams' sires	114.51	98.50	1.42
Dams' dams	113.78	98.50	1.42

Table 7. Relative economic weights in aggregate genotypes 2 to 5 (incorrect weighting) for all involved breeds

Aggregate genotype	Economic weight for NBA expressed as the proportion of economic weight for ADG[1]		
	dam breed A	dam breed B	sire breed C
2	109.33	96.43	0.87
3	109.33	109.33	0.87
4	96.43	96.43	0.87
5	55.00	55.00	55.00

[1]The relation of the economic weights for VC and ADG is 33.76 to 1 for all aggregate genotypes and all breeds

For abbreviations of traits see Table 4

For footnotes see Table 6

RESULTS

The numbers of discounted expressions for the production traits (ADG and VC) and the reproduction trait NBA for the four paths in each breed are summarised in Table 5. In dam breeds, NDE for the reproduction traits is about twice as high as NDE for the production traits; whereas, as expected, it is negligible in the sire breeds. A difference in NDE between the sexes in the paths sires to breed sires and dams to breed sires or sires to breed dams and dams to breed dams occurs only if there is a difference between the productive lifetime of sires and dams.

The economic weights for the traits were obtained by multiplying these NDEs by the marginal values of the traits. For comparing the economic weights of different aggregate genotypes or different paths within an aggregate genotype, not the absolute values of the weights but only their relations are of interest. The economic weights for NBA were therefore expressed as proportions of the economic weights for ADG (Tables 6 and 7). As NDE for the traits VC and ADG was assumed to be equal, the relation of the economic weights for VC and ADG was constant (33.76 to 1) over all aggregate genotypes and all breeds. In AG 1, the reproduction trait NBA had a higher relative economic weight in dam breed A than in dam breed B (Table 6). The relation of weights for breeds A and B differed little between the four paths, which justifies the use of NDE values for the sire to sire path as reference when comparing the various alternatives. Because of the high marginal value of NBA and the low marginal value

Table 8. Genetic gain per season and return from the selection program per sow in the whole population for different aggregate genotypes

Trait (Unit)	Genetic gain per season (in the unit of the given trait) for aggregate genotype					Genetic gain per season (in %, relatively to aggregate genotype 1) for aggregate genotype			
	1	2	3	4	5	2	3	4	5
Breed A									
ADG (g/day)	2.01	2.06	2.06	2.31	3.89	102.5	102.5	114.9	193.5
VC (kg)	0.08	0.08	0.08	0.09	0.10	100.0	100.0	112.5	125.0
NBA (piglet)	0.12	0.12	0.12	0.10	0.09	100.0	100.0	83.3	75.0
Breed B									
ADG (g/day)	2.13	2.15	1.92	2.16	3.63	100.9	90.1	101.4	170.4
VC (kg)	0.09	0.09	0.08	0.09	0.09	100.0	88.9	100.0	100.0
NBA (piglet)	0.10	0.10	0.11	0.10	0.08	100.0	110.0	100.0	80.0
Breed C									
ADG (g/day)	4.57	4.57	4.57	4.57	4.14	100.0	100.0	100.0	90.6
VC (kg)	0.03	0.03	0.03	0.03	0.04	100.0	100.0	100.0	133.3
NBA (piglet)	–0.007	–0.007	–0.007	–0.007	–0.005	100.0	100.0	100.0	71.4
Return/sow[1] (CZK)	661.91	661.89	669.59	646.65	645.81	100.0	101.2	97.7	97.6

[1] Return/sow: total return from one round of selection per one sow in the whole population after an investment period of 16 seasons

For abbreviations of traits see Table 4

of ADG, the relative weighting of NBA and ADG in the sire breed was nearly 1 to 1 despite of the very low value of NDE for the reproduction trait in this breed.

The relative weighting of the traits in aggregate genotypes 2 to 5 is summarised in Table 7. As expected from the results of correct weighting in AG 2 (with NDEs for the sire to sire path only), in AG 3 the relative weight for the trait NBA was overestimated for breed B whereas in AG 4 it was underestimated for breed A. The AG 5 differed substantially from all other alternatives.

For each of the three breeds, genetic gain per season obtained for the individual traits and for the aggregate genotype as well as total return per one sow in the whole population are given in Table 8. The values calculated for aggregate genotypes 2 to 5 were additionally expressed as percentage of the corresponding values for the AG 1 (the genotype with the correct weighting).

As expected, due to the minor differences in NDE between sires and dams within a breed, there were practically no differences between AG 1 and AG 2 for all values. For all five aggregate genotypes, there were also negligible differences in the total return per sow (from –2.4% to + 1.2%). But a lower genetic gain for production traits in dam breed B was observed, if the weighting that was calculated for breed A was also used for breed B. On the other hand, a lower genetic gain for the reproduction trait in dam breed A was observed if the weighting calculated for breed B was also inserted for breed A.

If the position of the breeds in the crossing system was not taken into account (i.e. no weighting with NDE-values in AG 5), there were losses up to 25% in the genetic gain for the reproduction trait NBA in both dam breeds in comparison with AG 1. But these losses were compensated by a higher genetic gain in production traits (especially in growth rate) so that the total return per sow was only slightly reduced. In the sire breed, the genetic gain in ADG and NBA decreased in AG 5 in comparison with genotype 1.

DISCUSSION

The impact of different factors (production system, sex of animals being selected, population and technical parameters that influence the population structure) on the number of discounted expressions of a trait and therefore on the relative economic importance of traits in the aggregate genotype was investigated in several papers (Danell et *al.*, 1976; Wilton and Danell, 1981; Přibyl *et al.*, 1999; Wünsch *et al.*, 1999). Based on these investigations, some authors recommended to consider the differences in the relative expressions of the traits when defining selection objectives and criteria for different breeds used in different crossing systems or for groups of animals used at different production levels (Wünsch *et al.*, 1999; Wolfová *et al.*, 2001). Wilton and Danell (1981) proposed to define separate selection objectives even for both sexes within a line used for crossbreeding in beef cattle.

However, the bias in economic weights due to omitting NDE for the traits should be considered in its effect on the selection response. The loss of efficiency in index selection due to biased estimates of parameters describing the production circumstances (prices, costs, production limitations) that enter into the calculation of the marginal economic values has already been referred by Rönningen (1971), Brascamp (1977), Groen (1990) and Přibyl and Přibylová (2001). A common suggestion of all these studies was that the loss in the overall monetary genetic gain was not probably too serious for moderate deviations (± 50 %) from the true economic weights for one trait at a time. This was confirmed in our study where decreasing the relative economic importance of reproduction versus production traits by 50% (aggregate genotypes 1 to 3 versus AG 5) led to a loss in total revenues of 2.5% only.

As mentioned by Danell (1980), the relative stability of the actual overall return can be explained by mutual compensation between gains in the various traits. This was demonstrated by Brascamp (1977) who concluded that, although the overall return was rather insensitive, the best individuals differed to some extent, thus causing different gains in individual traits. The same outcome was found by Dekkers (1994) when comparing the response for the traits direct and maternal calving ease using single or dual indices for sires mated to primiparous versus multiparous dams. These results were confirmed in our study where the incorrect weighting (omitting the NDE for the individual traits in the given production system) led to losses in genetic gain for the reproduction trait (NBA) in dam breeds up to 25%, but to an increase in the genetic gain of production traits up to 93%.

Giving the same relative weighting of reproduction and production traits for both dam breeds in the three-way crossing system (AG 3 and AG 4), the loss in the genetic gain for the reproduction trait in these breeds was lower than when omitting NDE (AG 5). In this case, the difference in the relative NDE for reproduction and production traits between both dam breeds was lower than if no reciprocal crossing was used (Wolfová *et al.*, 2001). If the dam breeds were strictly distinguished as a grandam and a grandsire breed, a higher loss than that given in Table 8 in the genetic gain for the reproduction traits could be expected for equal weighting of traits in both breeds. These losses would not have to be accepted by producers of F_1-dams in multiplier herds.

CONCLUSIONS

The overall monetary genetic gain and total return from selection seem to be relatively robust to deviations from

the "true economic weighting" of traits in the aggregate genotype. But the genetic gain of an individual trait can be substantially influenced by incorrect economic weights.

Therefore, the weighting factors for the traits in the aggregate genotype of pig breeds used in crossing systems should be obtained by weighting the marginal economic values with the numbers of discounted expressions (NDE) for each trait and breed separately. But the differences between NDEs for the four selection paths within a breed can be omitted and an equal aggregate genotype can be formulated for all the paths.

If only one aggregate genotype for both dam breeds is demanded, the "true" economic weights of the breed used in the grandam position (breed A) can be applied for both dam breeds to avoid a loss in genetic gain for reproduction traits.

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Studies on the relationship between body weight, trunk length and pelt size in common foxes (*Vulpes vulpes*)

Studium závislosti mezi tělesnou hmotností, délkou trupu a velikostí kožek u lišek obecných (*Vulpes vulpes*)

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ABSTRACT: The aim of the present research was to determine the relationship between live body weight, trunk length and pelt size in foxes. The studies were conducted on 134 silver foxes. Measurements of body weight and trunk length were made directly before slaughter. After slaughter pelts were subjected to standard processing. Dried pelts were measured and the values were compared with those concerning the body weight and trunk length of a given animal. Statistical differences between males and females were determined. The multiple regression method was employed to calculate the coefficients of correlation between body weight, trunk length and pelt length. Multiple regression formulas were derived to estimate the pelt size (trait Y) before slaughter on the basis of body weight (trait X_1) and trunk length (trait X_2). Those equations make it easier for breeders to determine the expected size of pelts in a given season before animals are slaughtered and to estimate their market value.

Keywords: common fox; body weight; size; pelt; correlation

ABSTRAKT: Cílem tohoto výzkumu bylo stanovení závislosti mezi živou tělesnou hmotností, délkou trupu a velikostí kožek u lišek. Sledování jsme prováděli na 134 stříbrných liškách. Tělesnou hmotnost a délku trupu jsme měřili těsně před usmrcením zvířete. Kožky pak byly zpracovány standardním způsobem. Usušené kožky jsme změřili a naměřené hodnoty jsme porovnali s hodnotami tělesné hmotnosti a délky trupu daného zvířete. Stanovili jsme statistické rozdíly mezi samci a samicemi. K výpočtu korelačních koeficientů mezi tělesnou hmotností, délkou trupu a délkou kožky jsme použili metodu vícenásobné regrese. Pro odhad velikosti kožky (znak Y) na živém zvířeti na základě jeho tělesné hmotnosti (znak X_1) a délky trupu (znak X_2) byly odvozeny rovnice vícenásobné regrese. Chovatelům tyto rovnice usnadňují stanovit očekávanou velikost kožek v dané sezoně dříve, než jsou zvířata usmrcena, a odhadnout jejich tržní hodnotu.

Klíčová slova: liška obecná; tělesná hmotnost; velikost; kožka; korelace

INTRODUCTION

Fur-bearing animal breeding is aimed at obtaining large pelts of good quality. Size and quality parameters are important as they provide the basis for estimating the commercial value expressed as a sales price. The quality of fur covers depends on numerous factors that may be divided into genetic and environmental ones. The price of pelts depends first of all on their size (length). It is common knowledge that the pelt size is connected with the size and body weight of animals, so those traits are taken into account in the course of selection. The research on the correlation between those traits was conducted on foxes, raccoon dogs and minks (Nurominen and Seppponen, 1996; Piórkowska, 1996, 1998). The results indicate that such relationships exist, but may take different forms in different species or sexes. The correlation between the trunk length and pelt length has been confirmed more frequently than that between the body weight and pelt length. It seems that the fattening of animals after they have reached certain body measurements does not result in obtaining longer pelts. On the contrary, it may lead to some defects (e.g. signs of damage/wear in abdominal parts) and decrease their market value. In order to compare and evaluate pelts produced in different countries and by various manufacturers, the size and shape of blocks for pelt forming have been standardized. The size of pelts depends not only on the trunk length and body

weight of animals, but also on the stretching force and way of forming on the blocks. The length of pelts is also affected by the way of making cuts during the process of fur cover removal.

The aim of the present paper was to calculate the coefficients of correlation between body weight, trunk length and pelt length in common foxes. Multiple regression equations were derived to determine the expected size of pelts in a given season before animals are slaughtered, and to estimate their market value on the basis of zoometric measurements carried out for a representative group of farm animals.

MATERIAL AND METHODS

The aim of the research was to determine the relationship between live body weight, trunk length and pelt size in foxes. The studies were conducted at the beginning of December on a commercial fox and mink farm in Olsztyn. 134 silver foxes: 62 males and 72 females constituted the experimental material. Measurements were taken during two working days and concerned the foxes slaughtered on those days, that means the experimental animals were selected at random. It follows that they were representative of the farm and their selection was consistent with production conditions.

Measurements of body weight and trunk length were made directly before slaughter that was carried out at the optimum time, i.e. when fur covers were fully developed. The foxes were measured with a measuring tape, to the nearest 1 cm, from the tip of the nose to the base of the tail. Their body weight was determined to the nearest 10 g. The pelts were marked permanently with numbered metal ear studs and subjected to standard processing. They were formed on openwork blocks designed for the forming and drying of pelts of arctic and common foxes, type P-14. When the process of drying (2 days) was over, the pelts were measured with a measuring tape, to the nearest 1 cm, from the tip of the nose to the base of the tail. The values were compared with those concerning the body weight and trunk length of a given animal. The results were processed statistically, taking into account the sex of the animals. At the first stage, statistical differences between males and females were determined employing an analysis of variance for one-factor non-orthogonal designs (Ruszczyc, 1981). This allowed to find out if coefficients of correlation can be calculated, and regression equations formulated, for the whole population or males and females separately. At the next stage, the multiple regression method was applied to determine the coefficients of correlation between body weight, trunk length and pelt length, separately for males and females. Multiple regression formulas were also derived to estimate the pelt size (trait Y) before slaughter on the basis of body weight (trait X_1) and trunk length (trait X_2).

RESULTS AND DISCUSSION

Table 1 presents the characteristics of live measurements concerning the body weight, trunk length and pelt length in foxes. The material subjected to a statistical analysis (males and females separately) indicates highly statistically significant differences between males and females in all the traits examined. Foxes do not show such clear sexual dimorphism as minks, but statistical differences in the body weight between males and females have been confirmed. The average body weight of males was equal to 7.41 kg; it was somewhat higher than the standard values given by Jarosz (1993). According to this author, males of this species achieve in November the body weight of 7.0 kg. Cholewa (1988) claims that in the case of common foxes the body weight of adult males amounts to 6.0–8.5 kg. In the experimental group of animals, the heaviest male achieved the weight of 10.1 kg while the lightest – 4.90 kg. The average body weight of females in the studied population turned out to be by ca. 0.5 kg lower than that quoted by Jarosz (1993). The heaviest female achieved the weight of 6.80 kg while the lightest – 4.0 kg. According to Cholewa (1988), in late autumn females should weigh from 5.0 to 7.0 kg.

In the case of fox populations, the most common zoometric measurement is the length of the trunk, taken during the appearance evaluation in order to determine the size of a given animal. The average trunk length in the

Table 1. Characteristics of live measurements (body weight and trunk length) and pelt length in common foxes

Specification	Statistical measures	Males	Females
Body weight (kg)	n	62	72
	x	7.41^A	6.07^B
	Range	(4.9–10.1)	(4.0–6.8)
	v	18.29	19.10
Trunk length (cm)	n	62	72
	x	72.19^a	67.00^b
	Range	(59–82)	(59–79)
	v	9.65	10.60
Pelt length (cm)	n	31	36
	x	104.42^A	97.44^B
	Range	(94–118)	(93–115)
	v	8.31	8.07

x = mean
v = coefficient of variation (%)
a, b = $P \leq 0.05$
A, B = $P \leq 0.01$

Table 2. Coefficients of correlation between body weight, trunk length and length of pelts obtained from male and female foxes

Trait	Body weight – X_1	Trunk length – X_2	Pelt length – Y
Male foxes			
Body weight – X_1	1.000	0.907^{xx}	0.762^{xx}
Trunk length – X_2		1.000	0.877^{xx}
Pelt length – Y			1.000
Female foxes			
Body weight – X_1	1.000	0.844^{xx}	0.681^{xx}
Trunk length – X_2		1.000	0.897^{xx}
Pelt length – Y			1.000

$^{x}P \leq 0.05$; $^{xx}P \leq 0.01$

group of males was 72.19 cm (it ranged from 59 to 82 cm), while in that of females – 67.00 (from 59 to 79). According to the Standards of the Appearance Evaluation in Common Foxes, issued in 1998 by the Central Station for Animal Evaluation, the trunk length should exceed 73 cm in males and 68 in females. It follows that the trunk length of common foxes in the examined population oscillated around the maximum values. The average length of pelts amounted to 104.42 cm in males. This allowed to classify them to the commercial category 0 (97.1–106.0 cm). The maximum pelt length was 118 cm – the commercial category 000 (115.1–124 cm) while the minimum – 94 cm, classified to the category 1 (88.1–97.0 cm). As concerns females, the average pelt length was 97.44 cm (the commercial category 0). The maximum pelt length in this group amounted to 115 cm (category 00) while the minimum – to 93 cm (category 1).

An interesting problem is an increase in the length of "raw" pelt in relation to the length of the trunk (Table 1). The results show that pelts of male and female foxes became longer, on average by 32.23 and 30.44 cm, respectively, compared with the average length of their trunks. Expressed as percentage (assuming that the trunk length constitutes 100%), the increase in the pelt length would be equal to 45% in both males and females.

Table 2 presents the coefficients of correlation between live body weight, trunk length and length of pelts obtained from male foxes. All the above coefficients of correlation are characterized by positive values and statistically significant differences. The highest correlation coefficient was determined between the body weight and trunk length – 0.907. The coefficient of correlation between the trunk length and pelt length amounted to 0.877, while that between the body weight and pelt length to 0.762. Those values indicate that both parameters (body weight and trunk length) influence the final length of pelts, which may suggest that the length of "raw" pelts increases with an increment in the trunk length and body weight.

As regards females (Table 2), the coefficients of correlation between individual traits were somewhat different than those recorded in the group of males. Their values were lower for the relationship between the body weight and trunk length (0.844), as well as the body weight and pelt length (0.681), but higher for that between the trunk length and pelt length (0.897). It seems that the body weight of female foxes has a slight effect on the pelt length while there is a significant correlation between the trunk length and pelt length.

In the studies on arctic foxes, conducted by Piórkowska (1996), the coefficient of correlation between the body weight and trunk length was 0.520 while that between the body weight and pelt length was 0.620. As concerns raccoon dogs, the coefficient of correlation between the body weight and pelt length was 0.227, and that between the trunk length and pelt length – 0.449. So they were relatively low, compared with those calculated for foxes. This is probably connected with a different constitution of raccoon dogs. Those animals are rather stocky and capable of depositing fatty tissue in autumn (Piórkowska, 1998).

The correlation coefficients included in Table 2 provided the basis for deriving multiple regression equations (Table 3), in which the value estimated was the length of

Table 3. Regression equations for estimating the pelt length (cm) in foxes on the basis of live measurements: body weight and trunk length

Equation No.	Multiple regression equation	S_y	R
	Common foxes – males		
1	$Y = -1.231\ X_1 + 1.311\ X_2 + 18.870$	4.25	0.881
	Common foxes – females		
2	$Y = -1.798\ X_1 + 1.242\ X_2 + 25.123$	3.39	0.908

Y = pelt length (cm)
X_1 = body weight (kg)
X_2 = trunk length (cm)
Sy = standard error of estimation (cm)
R = coefficient of multiple correlation between Y, X_1 and X_2

dried pelts (variable Y), determined by means of live measurements: body weight (X_1) and trunk length (X_2). The paper presents two equations calculated for males and females separately. The usability of regression equations depends on: the degree of difficulty connected with the measurement of the traits constituting their variables, possibility to repeat measurements and to make them in live animals, effect of the trait measurement on the future commercial value of pelts. The equations discussed in the present paper are easy to apply as they require making two simple measurements only that does not decrease the quality of fur covers in any way. Another advantage of those equations is the possibility to use them in breeding work, especially while selecting animals for herd replacement.

The standard error of estimation in the equations varied from 3.39 cm in the group of females to 4.25 in that of males. The coefficients of multiple correlation (R) between Y, X_1 and X_2 amounted to 0.881 in males and 0.908 in females.

CONCLUSIONS

High values of the correlation coefficients allow to derive multiple regression equations that may be applied to determine the pelt length in foxes on the basis of live measurements of their body weight and trunk length. These equations make it easier for breeders to determine the expected size of pelts in a given season before slaughter, and to estimate their market value.

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Nutrient digestibility and nitrogen retention in arctic foxes fed a diet containing cultures of probiotic bacteria

Stravitelnost živin a retence dusíku u polárních lišek krmených směsí obsahující kultury probiotických bakterií

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ABSTRACT: The studies on the effect of cultures of probiotic bacteria such as *Lactobacillus acidophilus* and *Enterococcus faecium* on nutrient digestibility and nitrogen retention in growing arctic foxes were conducted on 8 females aged ca. 4 months. The animals were placed in individual cages adapted for digestibility-balance analyses and feces/urine collection. A five-day research period was preceded by a preliminary one. Experimental animals were given probiotics in the amount of 1 g per 600 g of feed mixture. The results show that the addition of probiotic bacteria cultures resulted in better utilization of both carbohydrates and gross energy from the ration. A slightly higher level of nitrogen retention was also observed in the experimental group.

Keywords: arctic fox; digestibility; nitrogen retention; probiotic bacteria

ABSTRAKT: Vliv kultur probiotických bakterií, *Lactobacillus acidophilus* a *Enterococcus faecium*, na stravitelnost živin a retenci dusíku jsme sledovali u rostoucích polárních lišek, a to u osmi samic ve věku asi čtyř měsíců. Zvířata jsme umístili do individuálních klecí upravených pro provádění bilančních analýz a pro sběr exkrementů/moči. Pětidennímu pokusnému období předcházelo přípravné období. Pokusná zvířata dostávala probiotika v dávce 1 g na 600 g krmné směsi. Výsledky naznačují, že přídavek kultur probiotických bakterií vedl k lepšímu využití jak uhlohydrátů, tak energie z krmné dávky. U pokusné skupiny jsme rovněž zjistili nepatrně vyšší hladinu retence dusíku.

Klíčová slova: polární liška; stravitelnost; retence dusíku; probiotické bakterie

INTRODUCTION

Probiotics are living microorganisms which have a positive effect on the organism, manifested by an improvement in the microbiological equilibrium of the alimentary tract. This phenomenon is known as probiosis. Some strains of bacteria and yeasts are considered probiotics. The most valuable among them are lactic acid bacteria. The main function of this kind of bacteria is protection of the alimentary tract against its colonization by pathogenic strains. This is especially important in the nutrition of fur-bearing carnivorous animals, due to the kind of feed they receive, because environment acidification is a barrier to the development of putrefactive bacteria. It should also be mentioned that these bacteria produce natural substances – bacteriocins – whose effect is similar to that of antibiotics.

The digestibility of particular nutrients in fur-bearing animals depends on numerous factors. This problem has not been fully investigated yet and still constitutes the subject of scientific research (Szymeczko and Podkówka, 1994; Lorek *et al.*, 1994, 1997; Oleinik, 1994; Gugołek *et al.*, 1997; Barabasz *et al.*, 1999).

The activity of digestive enzymes in particular segments of the alimentary tract depends on the rate of food transportation, diet composition and individual predisposition of animals. A high degree of activity of some enzymes in lower parts of the intestine is characteristic of carnivorous animals (Oleinik, 1994). It seems that also bacterial microflora may affect the level of digestibility in these animals, although its influence is much lower in this case than in ruminants. Some bacteria, e.g. bifidobacteria, have a beneficial effect on the processes of maturation and regeneration of enterocytes in the alimentary tract. They

also stimulate the immune system, preventing infections. Cultures of probiotic bacteria are used in clinical practice to treat patients with lactose intolerance. They provide additional amounts of lactase – an enzyme indispensable for milk sugar digestion.

A positive influence of probiotics on the production results of foxes and minks was already confirmed by many authors (Touson, 1986; Skrede and Ness, 1989; Gugołek *et al.*, 1999).

The aim of the present investigations was to determine the effect of probiotic bacteria cultures on nutrient digestibility and nitrogen retention in growing arctic foxes.

MATERIAL AND METHODS

The experimental factor in the studies on the effect of probiotic bacteria cultures on nutrient digestibility and nitrogen retention in polar foxes was the preparation ALL-LAC (manufacturer – Alltech Biotechnology Center Inc., USA). It was added to rations in the amount of 1g per animal. This preparation contains lyophilized, micro-capsulated strains of probiotic bacteria *Lactobacillus acidophilus* 10^9 cfu and *Enterococcus faecium* 10^9 cfu per gram of probiotic preparation. It is produced in a friable form.

The experiment was carried out in September, on 8 females aged ca. 4 months. They were taken from two litters, placing 2 females selected at random from each litter in each of the groups. The animals were clinically healthy and characterized by similar body weight.

They were put into individual cages adapted for digestibility-balance analyses and feces/urine collection. A five-day research period was preceded by a preliminary one to let the animals adapt to new environmental conditions. The foxes were fed once a day, at the same hour. Each time both groups were given 600 g of feed. The ration was prepared using typical feed components: beef, pluck, slaughter offal (wastes, by-products), hard poultry offal, steamed ground barley, green forage, vegetables and wheat bran; they were supplemented with Polfamix L-N (a vitamin-mineral preparation commonly applied in Poland as diet supplementation in fox and mink feeding). The animals had free access to drinking water. Feed wastes and excreted feces were collected every day and weighed to the nearest 10 g. One half of the feces was preserved in concentrated sulfuric acid. The nitrogen content was determined after taking the average sample for the whole collection. The other half was partly dried, and the content of the other nutrients was determined. Urine was preserved in 20% sulfuric acid, and the volume of the whole collection was determined after the research period. The nutrient content of feed and feces, and the nitrogen content of urine, were determined by the method described by Skulmowski (1974). The balance method, commonly used in this type of studies, was employed to estimate nutrient digestibility and nitrogen retention.

Numerical data, in the form of nutrient digestibility coefficients and results of nitrogen retention, were processed statistically applying the analysis of variance for one-factor orthogonal designs (Ruszczyc, 1981).

RESULTS AND DISCUSSION

Table 1 presents the chemical composition and energy value of the diet applied during digestibility-balance examinations. An addition of the preparation ALL-LAC in the amount of 1g per 600 g of feed did not change the chemical composition of rations. Its dry matter content was equal to 32.52%, and – according to other authors – it was typical of wet feed.

The crude ash content was 4.97%. This component may affect the digestibility of other nutrients (Sławoń, 1987). Its relatively high content was caused by the fact that hard poultry offal, containing large amounts of bones, was used for feed preparation. The organic matter content was at a level of 27.55%, including 12.50% of crude protein, 4.09% of crude fat, 0.74% of crude fiber and 10.22% of N-free extractives.

The gross energy value was 6.033 MJ per kg of feed. This satisfies the needs of growing foxes at the age of 4 months (Sławoń, 1987).

Table 2 presents the coefficients of nutrient and energy digestibility in the case of feed containing the above-

Table 1. Chemical composition of the ration for foxes

Specification*		%
Dry matter	a	32.52
	b	100.00
Crude ash	a	4.97
	b	15.28
Organic matter	a	27.55
	b	84.72
Crude protein	a	12.50
	b	38.44
Crude fat	a	4.09
	b	12.58
Crude fiber	a	0.74
	b	2.27
N-free extractives	a	10.22
	b	31.43
Gross energy (MJ/kg)	a	6.033
	b	18.552

*see Material and Methods for ingredients
a = in fresh matter, b = in dry matter

Table 2. Coefficients of nutrient and energy digestibility (%) in control and experimental foxes

Specification	Statistical variables	Group	
		control – I	experimental* – II
	n	4	4
Dry matter	*x*	74.06	75.26
	v	0.84	0.72
Organic matter	*x*	85.27	87.01
	v	0.74	0.89
Crude protein	*x*	86.25	87.67
	v	0.62	0.54
Crude fat	*x*	92.30	92.59
	v	0.24	0.23
Crude fiber	*x*	18.64	21.23
	v	10.12	8.03
N-free extractives	*x*	56.79 ^{B}	60.97 ^{A}
	v	3.21	4.01
Gross energy	*x*	87.97^{B}	89.04^{A}
	v	0.84	0.76

*The probiotic preparation ALL-LAC was added to the diet of experimental animals

a, b = $P \leq 0.05$

A, B = $P \leq 0.01$

x = mean

v = coefficient of variation (%)

Table 3. Daily nitrogen balance and retention

Specification	Statistical variables	Group	
		control – I	experimental* – II
Nitrogen (g/animal)	*n*	4	4
Intake	*x*	10.31	10.49
	v	0.10	0.07
Excretion:			
in feces	*x*	1.35	1.32
	v	12.51	10.17
in urine	*x*	6.13	5.61
	v	7.18	5.12
Digestion	*x*	8.96	9.17
	v	4.02	3.15
Retention	*x*	2.83	3.56
	v	27.15	20.19
Retention in relation to:			
nitrogen intake (%)	*x*	27.45	33.93
	v	25.18	21.13
nitrogen digestion (%)	*x*	31.58	38.82
	v	29.77	21.01

x = mean

v = coefficient of variation (%)

No statistically significant differences

mentioned preparation. No statistical differences were found in the level of dry matter digestibility between the groups although the value of the digestibility coefficient was higher in the experimental one.

Also the digestibility of organic matter was higher by 1.74% in the experimental group, but this difference was not confirmed statistically. The difference in the organic matter digestibility was affected by the digestibility of particular nutrients. A higher digestibility level was determined in group II for crude protein, crude fat, crude fiber and N-free extractives.

There were no statistically significant differences in the utilization of crude protein between the groups. In group I (control) it was equal to 86.25% while in group II (experimental) to 87.67%. The coefficient of crude protein digestibility was 86–87%, which is considered an average value. A similar level of digestibility (84–85%) was observed by Lorek *et al.* (1997). In the studies conducted by Lorek *et al.* (1994) crude protein digestibility amounted to 93–94%. Szymeczko and Podkówka (1994) reported nitrogen digestibility in foxes at a level of 87 to 95%. Lower protein utilization, compared with the results obtained by Lorek *et al.* (1994), may be connected with the use of hard poultry offal contained in rations. Barabasz *et al.* (1999) found out that in the case of mink nutrition protein digestibility rapidly decreases when large amounts of these wastes are included in the diet.

In the present paper the coefficients of crude fat digestibility were at a similar level, i.e. 92.30% in group I and 92.59% in group II.

The coefficients of crude fiber digestibility were slightly higher in group II (21.23% vs. 18.64% in group I), but the difference was not statistically significant. Fur-bearing carnivorous animals are characterized by low utilization of crude fiber. Until quite recently it was believed that they are not able to use this nutrient. However, according to the latest investigations, the digestibility of crude fiber varies from 25 to 35% (Skrede and Eldegard, 1989; Lorek *et al.*, 1994).

The digestibility of N-free extractives, the main fraction of carbohydrates, was equal to 56.79% in group I and 60.97% in group II. The difference was statistically highly significant. Also in the studies carried out by Gugołek *et al.* (1999), where the experimental factor was a preparation containing probiotic bacteria cultures, an improvement was observed in the digestibility of N-free extractives. This suggests that probiotics have a positive effect on the digestibility of carbohydrates. Maybe they cause quantitative and qualitative changes in bacterial microflora, conducive to carbohydrate decomposition. There is also a hypothesis of changes in the count of bacteria participating in starch assimilation in relation to that of bacterial flora performing other functions. This may lead to better utilization of N-free extractives contained in feed due to activation of cells of intestinal villi (Touson, 1986; Skrede and Ness, 1989).

Animals from the experimental group were characterized by higher utilization of gross energy. The difference between the groups was statistically highly significant – 1.07%. This was caused by higher nutrient digestibility in this group, especially N-free extractives.

Table 3 shows daily nitrogen balance and retention. The daily nitrogen intake was similar in both groups (10.31 g in group I and 10.49 g in group II), which was related to similar chemical composition of both diets.

Average nitrogen excretion in feces was at a level of 1.35g in group I and 1.32 g in group II.

It was found that animals from the control group excreted in urine by 0.52 g of nitrogen more than those from the experimental group.

Nitrogen digestion amounted to 8.96 g and 9.17 g in groups I and II, respectively.

Nitrogen retention was higher in the experimental group (3.56 g) than in the control one (2.83 g) but no statistically significant differences were determined. The level of nitrogen retention may be connected with the coefficient of crude protein digestibility, which was somewhat higher in the experimental group (Table 2).

The percentage ratio between nitrogen retention and intake, and its retention and digestion, was higher in group II. In group I the ratio between nitrogen retention and intake was 27.45% while that between its retention and digestion was 31.58%. In group II it was equal to 33.93% and 38.82%, respectively. In the studies conducted by Lorek *et al.* (1997), where foxes were fed a diet containing a whey-fat concentrate, the results were as follows: 23.96%–27.50% for the retention-to-intake ratio, and 28.14%–32.49% for the retention-to-digestion ratio. In the research carried out by Gugołek *et al.* (1997), in which foxes were given pelleted feed, the ratios were at a level of 30.72% and 43.74% respectively.

CONCLUSIONS

The addition of probiotic bacteria cultures resulted in a higher level of utilization of both carbohydrates and gross energy from the rations.

A slightly higher level of nitrogen retention was observed in the group of foxes fed a diet containing cultures of probiotic bacteria.

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Optimum digestible threonine and sulphur amino acid requirements of high-lean growing pigs

Optimální potřeba stravitelného threoninu a sirných aminokyselin pro rostoucí prasata masného typu

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ABSTRACT: Two balance experiments involving a total of 24 high-lean growing gilts (mean body weight 47.9 ± 0.36 kg) were carried out to estimate the optimum requirements for threonine and sulphur amino acids. In each experiment, six semisynthetic diets based on casein and crystalline amino acids were prepared in which threonine or sulphur amino acid concentrations ranged from deficiency to excess while the other essential amino acids were maintained at levels exceeding the requirement. N retention was used as a response criterion. In both experiments, N retention increased linearly as the dietary concentration of the limiting amino acid increased up to a presumed optimum and then remained relatively unchanged. N digestibility gradually increased with increasing N intake. Using a linear model, the requirements for true ileal digestible threonine and sulphur amino acids were estimated to be 12.1 and 9.5 g/day, respectively. These values were approximately 0.69 and 0.54 relative to ileal digestible lysine, respectively.

Keywords: growing pigs; digestible threonine; digestible sulphur amino acids; optimum requirement

ABSTRAKT: Ve dvou bilančních pokusech na 24 prasničkách masného typu (průměrná hmotnost v bilanci 47,9 ± 0,36 kg) byla studována optimální potřeba stravitelného treoninu a sirných aminokyselin. Pro každý pokus bylo připraveno šest polosyntetických pokusných směsí, v nichž byl použit kazein a krystalické aminokyseliny jako zdroj dusíku. V těchto dietách se obsah treoninu nebo sirných aminokyselin postupně zvyšoval od suboptimálních hodnot až po hodnoty přesahující očekávané optimum, zatímco obsah ostatních esenciálních aminokyselin byl vyrovnán na úroveň přesahující optimum potřeby. Sledovaným ukazatelem byla retence dusíku. V obou pokusech se retence N zvyšovala lineárně s rostoucí koncentrací limitující aminokyseliny ve směsi až k hodnotám odpovídajícím předpokládané optimální potřebě dané aminokyseliny a poté se již dále nezvyšovala. Optimální potřeba skutečně stravitelného treoninu a sirných aminokyselin byla určena pomocí lineárního modelu a činila 12,1 respektive 9,5 g/den. Poměr stravitelného threoninu a sirných aminokyselin ke stravitelnému lyzinu činil 0,69 a 0,54.

Klíčová slova: rostoucí prasata; stravitelný treonin; stravitelné sirné aminokyseliny; optimální potřeba

INTRODUCTION

In practical diets for growing pigs based on cereals and soybean meal, threonine is usually the second-limiting and methionine the third-limiting amino acid (Taylor *et al.*, 1981; Günther and Badewein, 1987; Askbrant *et al.*, 1994). Thus, good knowledge of threonine and sulphur amino acid needs is important for the formulation of well-balanced pig diets. While the optimum requirement of growing pigs for lysine has been studied extensively, the experimental data on the requirements for threonine and sulphur amino acids as well as on their relations to the

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requirement for lysine are scarce and conflicting. The optimum ratio of threonine to lysine suggested by various authors ranges from 0.60 to 0.72 (ARC, 1981; Fuller *et al.*, 1989; Chung and Baker, 1992; Cole and Van Lunen, 1994). Even more uncertainty exists about the relative requirement for sulphur amino acids. The estimates of the ratio of methionine + cystine to lysine vary widely from 0.47–0.50 (ARC, 1981; Knowles *et al.*, 1998) up to 0.70 (Chung et *al.*, 1989; Baker and Chung, 1992). This variability may be due to various factors such as body weight, lean growth potential or the availability of amino acids in experimental diets. Current estimates suggest that the ratio of both threonine and sulphur amino acids to lysine increases with animal weight and the associated increased maintenance requirements (Baker and Chung, 1992). This increase is most dramatic during the 50- to 115-kg growth period, when feed intake and maintenance requirements continue to increase but the proportional rate of lean gain declines. Since the availability of amino acids measured as ileal digestibility varies widely among feedstuffs (Lenis *et al.*, 1990), the calculations based on ileal digestible amino acids may improve the precision of diet formulation. To date, there is only a limited number of studies focused on the requirements for digestible amino acids. Therefore the objective of the experiments described here was to estimate the optimum requirements of growing pigs for true ileal digestible threonine and sulphur amino acids.

Table 1. Composition of experimental diets and calculated nutrient content (g/kg) – Experiment 1

Ingredient	Diet					
	TH 1	TH 2	TH 3	TH 4	TH 5	TH 6
Casein	55.00	85.00	110.00	140.00	170.00	200.00
Cellulose	55.00	55.00	55.00	55.00	55.00	55.00
Polyethylene	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	250.40	250.10	250.20	250.40	250.40	250.30
Soybean oil	50.00	50.00	50.00	50.00	50.00	50.00
Mineral mix[1]	61.50	61.50	61.50	61.50	61.50	61.50
Premix[2]	5.00	5.00	5.00	5.00	5.00	5.00
Wheat starch	501.60	471.20	441.60	407.20	374.10	341.60
Chromic oxide	4.00	4.00	4.00	4.00	4.00	4.00
L-lysine HCl	2.24	2.76	4.74	6.24	7.49	8.61
L-threonine	0.93	1.30	1.87	2.24	2.61	2.98
DL-methionine	0.99	1.06	1.28	1.69	2.02	2.32
L-tryptophan	0.37	0.42	0.54	0.71	0.85	0.97
L-arginine	0.50	0.55	1.08	1.44	1.72	1.96
L-valine	0.45	0.35	0.54	0.79	0.92	0.99
L-leucine	0.53	0.38	0.66	0.98	1.13	1.19
L-isoleucine	0.43	0.39	0.57	0.81	0.95	1.04
L-histidine	0.27	0.40	0.54	0.74	0.88	0.98
L-cystine	0.42	0.53	0.67	0.86	1.04	1.21
Phenylalanine + tyrosine	0.42	0.06	0.19	0.40	0.42	0.34
Total	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00
Nutrient content						
CP	53.15	78.93	104.46	133.75	161.94	189.58
Lysine	5.65	8.17	11.50	14.80	17.90	20.90
Threonine	3.00	4.50	6.00	7.50	9.00	10.50
Methionine + cystine	3.05	4.14	5.24	6.74	8.16	9.52
Tryptophan	1.00	1.40	1.80	2.32	2.80	3.27

[1]Amounts per kg of diet: $Ca_3(PO_4)_2$ 40 g; salt 4 g; $NaHCO_3$ 7.5 g; MgO 3 g; K_2CO_3 7.5 g

[2]Amounts per kg of diet: retinol 1.2 mg; cholecalciferol 25 mg; α-tocopherol 10 mg; d-pantothenic acid 10 mg; niacin 20 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamin 30 μg; choline 500 mg; Fe 92 mg; Zn 103 mg; Mn 40 mg; Cu 9 mg; Co 0.5 mg; Se 0.16 mg

MATERIAL AND METHODS

Animals and procedures

A total of twenty-four high-lean growing gilts, the progeny of Hampshire boars and Large White × Landrace sows, with the initial body weight of 44.7 kg (SE = 0.34), were used in two experiments of twelve animals each. The pigs were divided into six pairs of similar body weight. During three successive experimental periods, each pair received three diets in such a way that none of the animals had the same diet twice. Thus each diet was given to six pigs. The pigs were placed into metabolic cages 7 days before the start of the experiment for adaptation. For the first 5 days, they were fed a commercial diet A2 *ad libitum* while experimental diets were offered on days 6 and 7. The last day of the adaptation period, the pigs were fitted with sterile bladder catheters, draining into carboys containing 50 ml 5M HCl.

Each experimental period consisted of a 3-day preliminary period followed by two successive 48 h collection periods during which urine was collected quantitatively. At the end of every collection period aliquots of urine were taken and immediately analyzed for total N. Faeces were collected for 5 days by random sampling, starting on the last day of the preliminary period. They were stored at –20°C, freeze-dried and finely ground for subsequent analysis of N and Cr_2O_3.

Table 2. Composition of experimental diets and calculated nutrient content (g/kg) – Experiment 2

Ingredient	Diet					
	SA 1	SA 2	SA 3	SA 4	SA 5	SA 6
Casein	55.00	85.00	110.00	115.00	135.00	160.00
Cellulose	55.00	55.00	55.00	55.00	55.00	55.00
Polyethylene	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	250.00	250.00	250.00	250.00	250.00	250.00
Soybean oil	50.00	50.00	50.00	50.00	50.00	50.00
Mineral mix[1]	61.50	61.50	61.50	61.50	61.50	61.50
Premix[2]	5.00	5.00	5.00	5.00	5.00	5.00
Wheat starch	501.90	471.60	448.60	431.80	406.40	377.00
Chromic oxide	4.00	4.00	4.00	4.00	4.00	4.00
L-lysine HCl	2.20	2.80	4.60	4.40	5.60	6.60
L-threonine	1.74	2.05	2.95	3.24	4.03	4.79
DL-methionine	0.38	0.22	0.55	0.87	1.14	1.36
L-tryptophan	0.37	0.42	0.65	0.74	0.93	1.11
L-arginine	0.50	0.55	1.07	1.87	2.36	2.80
L-valine	0.45	0.35	1.12	1.57	2.08	2.48
L-leucine	0.53	0.38	1.51	1.85	2.52	3.01
L-isoleucine	0.43	0.39	1.03	0.59	0.87	1.04
L-histidine	0.27	0.40	0.80	1.70	2.14	2.54
L-cystine	0.27	0.32	0.45	0.58	0.71	0.84
Phenylalanine + tyrosine	0.42	0.06	1.18	0.29	0.70	0.84
Total	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00	1 000.00
Nutrient content						
CP	53.27	78.83	99.20	114.38	136.55	161.96
Lysine	5.65	8.17	10.68	11.56	13.92	16.52
Threonine	3.80	5.24	6.70	7.55	9.09	10.78
Methionine + cystine	2.30	3.10	4.00	4.90	5.90	7.00
Tryptophan	1.00	1.40	1.80	2.06	2.48	2.94

[1] Amounts per kg of diet: $Ca_3(PO_4)_2$ 40 g; salt 4 g; $NaHCO_3$ 7.5 g; MgO 3 g; K_2CO_3 7.5 g

[2] Amounts per kg of diet: retinol 1.2 mg; cholecalciferol 25 mg; α-tocopherol 10 mg; d-panthothenic acid 10 mg; niacin 20 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamin 30 μg; choline 500 mg; Fe 92 mg; Zn 103 mg; Mn 40 mg; Cu 9 mg; Co 0.5 mg; Se 0.16 mg

Diets and feeding

For each experiment, six semisynthetic diets containing casein and crystalline amino acids as sources of N were prepared in which threonine (Experiment 1) or sulphur amino acids (Experiment 2) ranged from severe deficiency to levels exceeding the expected optimum requirement. All other essential amino acids were given in a 30% excess relative to the level of the limiting amino acid to avoid their possible deficiency. Casein and amino acids were added to the diets at the expense of wheat starch. The composition of diets and their calculated nutrient contents are shown in Tables 1 and 2. To ensure the accuracy of amino acid additions, the diets were prepared without amino acids which were weighed for each animal and each feeding separately and mixed to the food allowance by hand. Pigs were fed twice a day, at 7:30 and 15:00 h in two equal parts at a daily rate of 80 g/kg$^{0.75}$. Water was provided according to a total feed : water ratio of 1 : 2.5, both before and after each feeding.

Chemical analyses

Amino acid composition of casein was analysed by ion exchange chromatography (Llames and Fontaine, 1994), chromic oxide content of diets and faeces by atomic absorption spectroscopy (Petry and Rapp, 1970/71) and total N of urine, freeze-dried faeces and diets by macro-Kjeldahl methodology (AOAC, 1984).

Statistical analysis

The Statgraphics 7.0 package was used to compute the analysis of variance and to assess the differences between means by Tukey's test. Where applicable, the results were expressed as means with pooled standard errors. The optimum requirements for threonine and sulphur amino acids were estimated by a linear regression model relating N retention to the intake of the respective amino acid (Robbins *et al.*, 1979). The optimum requirement was estimated as the amino acid intake equivalent to the breakpoint in the response (Heger *et al.*, 1987).

The experiments were conducted in accordance with the standards of the Ethics Committee for Animal Experiments of the Research Institute of Animal Nutrition.

RESULTS

Experiment 1

The results of Experiment 1 are summarized in Table 3. Addition of different levels of dietary threonine to the basal diet resulted in a significant ($P < 0.05$) linear increase in N retention up to maximum (24.5 g/day) found in group TH 4 which corresponded to the daily dietary threonine intake of 12.4 g/day. Further increase in daily threonine intake did not influence N balance markedly. Urinary N output gradually increased with increasing dietary threonine. N output in faeces was affected only slightly. N digestibility significantly increased ($P < 0.05$) with increasing dietary threonine. Based on data obtained in the experiment, regression equations describing the relation between N balance and digestible threonine intake were calculated. These equations together with calculated breakpoints describing the optimum requirement are given in Table 5. A linear model fitted to the experimental data is shown in Figure 1. The calculated optimum digestible threonine requirement was 12.1 g/day.

Table 3. The effect of increasing dietary threonine on nitrogen balance – Experiment 1

		Experimental group						
		TH 1	TH 2	TH 3	TH 4	TH 5	TH 6	Pooled SE
Threonine intake	(g/d)	5.09	7.69	10.10	12.40	14.90	17.24	
Live weight	(kg)	49.1	49.7	50.4	48.4	47.3	47.3	0.46
N intake	(g/d)	15.0^{a}	21.3^{b}	28.2^{c}	35.4^{d}	42.9^{e}	49.8^{f}	0.14
Faecal N	(g/d)	1.6^{a}	1.9^{ac}	2.1^{ab}	2.1^{ab}	2.3^{bc}	2.6^{b}	0.06
Urinary N	(g/d)	1.4^{a}	2.5^{a}	5.1^{b}	8.9^{c}	17.3^{d}	22.4^{e}	0.23
N digestibility	(%)	89.3^{a}	91.1^{ac}	92.7^{cd}	94.2^{bd}	94.7^{b}	94.8^{b}	0.17
N retention	(g/d)	12.0^{a}	17.0^{b}	21.0^{c}	24.5^{d}	23.4^{cd}	24.8^{d}	0.28

^{a,b,c,d,e,f}within rows, the means followed by the same superscript are not significantly different $P < 0.05$

Table 4. The effect of increasing dietary sulphur amino acids on nitrogen balance – Experiment 2

		Experimental group						
		SA 1	SA 2	SA 3	SA 4	SA 5	SA 6	Pooled SE
Sulphur AA intake	(g/d)	3.89	5.18	6.70	8.03	9.63	11.23	
Live weight	(kg)	45.7	46.9	47.3	48.4	47.3	47.3	0.55
N intake	(g/d)	15.1^{a}	20.8^{b}	26.8^{c}	29.9^{d}	35.6^{e}	41.5^{f}	0.15
Faecal N	(g/d)	2.0^{ac}	1.8^{a}	2.0^{ac}	2.3^{bc}	2.2^{ac}	2.1^{ac}	0.04
Urinary N	(g/d)	2.1^{a}	2.5^{a}	6.3^{b}	7.3^{bc}	9.3^{c}	15.5^{d}	0.21
N digestibility	(%)	86.7^{a}	91.1^{b}	92.7^{cd}	92.2^{bd}	93.8^{ce}	94.8^{e}	0.11
N retention	(g/d)	11.0^{a}	16.5^{b}	18.5^{bc}	20.3^{c}	24.1^{d}	23.8^{d}	0.23

^{a,b,c,d,e,f}within rows, the means followed by the same superscript are not significantly different $P < 0.05$

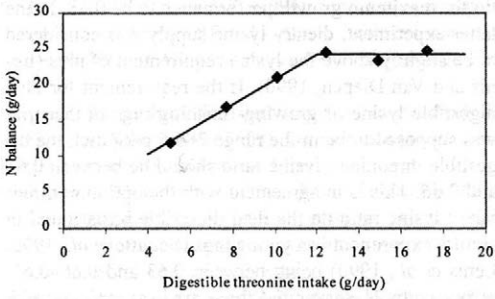

Figure 1. Relation of nitrogen balance to digestible threonine intake

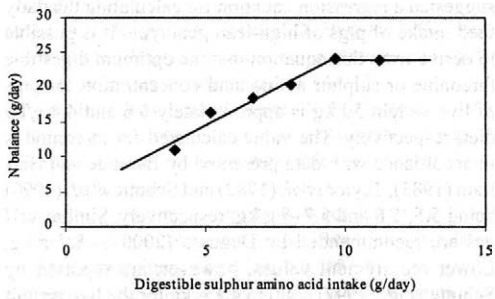

Figure 2. Relation of nitrogen balance to increasing digestible sulphur amino acid intake

Experiment 2

The effect of different levels of sulphur amino acids on N retention of pigs is presented in Table 4. Nitrogen retention increased linearly as the intake of dietary sulphur amino acids increased up to 9.63 g/day (group SA 5) and then remained relatively unchanged. Maximum N retention was found to be 24.1 g/day. Similarly like in Exp. 1, there was an increase in urinary N output and N digestibility as the sulphur amino acid concentration increased. Regression equations with calculated breakpoints are presented in Table 5. The breakpoint of the response was equivalent to the intake of 9.5 g/day dietary sulphur amino acids (see Table 5). Pairs of regression lines showing the relation between the N balance and the intake of digestible sulphur amino acids are given in Figure 2.

Table 5. Pairs of regression equations relating N retention (y, g/d) to amino acid intake (x, g/d) in pigs receiving different levels of threonine or sulphur amino acids and the calculated breakpoints

Amino acid	Regression equation	R^2	SE	Breakpoint
Threonine	$y_1 = 3.39 + 1.73x$	0.98	0.95	12.05
	$y_2 = 24.23$			
Sulphur AA	$y_1 = 4.00 + 2.11x$	0.94	1.22	9.47
	$y_2 = 23.99$			

DISCUSSION

In these experiments, basal diets were supplemented by amino acids in order to be sure that no other amino acids than the amino acids studied were limiting.

Maximum values of N retention obtained in both experiments ranged from 24 to 25 g/day, which corresponded to daily protein deposition of 150 to 156 g. This shows a high genetic capacity of pigs to deposit body protein. These results are comparable with those achieved in other studies with the high-lean genotypes in the same weight category. The results showed that the ileal digestible threonine and sulphur amino acid level required to support maximum N retention of pigs of 50 kg live weight was 12.1 and 9.5 g/day, respectively. The finding for threonine is higher than the values recommended by NRC

(1998) being 9.7 g/day for pigs of the live weight range 20 – 50 kg and 11 g/day for pigs from 50 to 80 kg live weight (true digestible basis). The value found for digestible sulphur amino acids is close to NRC recommendation (1998) for pigs of 50–80 kg live weight, being 10 g/kg.

Literature data on the daily ileal digestible threonine or sulphur amino acid requirement of growing pigs are scarce and the requirement of pigs is often expressed as a dietary concentration of individual amino acids. Therefore it is necessary to know the daily feed intake of pigs of particular live weight. However, voluntary feed intake varies considerably from day to day and among individual genotypes. Thus, the estimation of the requirement based on daily basis is more reliable than that based on the concentration of amino acids in the diet. The recommendation of nutrition commission (Šimeček *et al.*, 2000) suggested a regression equation for calculating the daily feed intake of pigs of high-lean genotype. It is possible to derive from this equation that the optimum digestible threonine or sulphur amino acid concentration for pigs of live weight 50 kg is approximately 5.6 and 4.4 g/kg diet, respectively. The value calculated for threonine is in accordance with data presented by Berende and Bertram (1983), Taylor *et al.* (1982) and Schutte *et al.* (1990) being 5.5, 5.6 and 5.7–6 g/kg, respectively. Similar values are recommended by Degussa (2000) – 5.7 g/kg. Lower requirement values, however, are reported by Schutte *et al.* (1997) – 4.0 to 4.1 g/kg for the live weight from 50 to 95 kg. The latter authors reported that these estimates were mainly based on the results for food conversion efficiency rather than on those for weight gain. To achieve maximum weight gain, the requirement was found to be somewhat higher. However, amino acid requirements based on N balance are generally higher than the requirements based on growth performance and carcass characteristics (Baker, 1986). Lenis *et al.* (1990) and Lenis and Van Diepen (1990) also derived the requirement for ileal digestible threonine of boars and gilts together in the live weight range 35–105 kg as amounting to 4.2 g/kg. It is important to note that in the latter reports the average daily feed intake of pigs was considerably higher than that calculated in our study. On the other hand, Saldana *et al.* (1994) estimated that the dietary digestible threonine content required to maximize the growth rate of finisher pigs (initially 58 kg) was 2.8 g/kg, which is substantially lower than 5.6 g/kg suggested in our study.

The requirement for ileal digestible sulphur amino acids found in the present experiment (4.4 g/kg) is close to the results reported by Lenis *et al.* (1990) and Taylor *et al.* (1983): 4.2 and 4.4-4.8 g/kg, respectively. A considerably higher concentration of digestible sulphur amino acids is recommended by Degussa (2000) – 5.3 g/kg.

The requirement for threonine and other essential amino acids is often expressed relative to the dietary level of lysine. To determine the ratios of individual amino acids to lysine, it is important that animals are fed diets containing no excess lysine. Feeding excess lysine would result in a lower ratio than the ratio determined if the lysine level fed was in agreement with the requirement (Knowles *et al.*, 1998). In one of our previous experiments studying the optimum lysine requirement carried out on gilts of the same genotype and weight category (Heger *et al.*, 1997) the requirement for true ileal digestible lysine was estimated to be 17.6 g/day. Based on this data, the ratio of digestible threonine and sulphur amino acid to lysine was calculated to be 0.69 and 0.54, respectively.

The ratio calculated for threonine is close to the values recommended by NRC (1998) and Degussa (2000) – 0.65. On the other hand, Lenis and Van Diepen (1990) found the optimum ileal digestible threonine : lysine ratio for maximum growth performance to be 0.58. In the latter experiment, dietary lysine supply was considered to be slightly above the lysine requirement of pigs (Lenis and Van Diepen, 1990). If the requirement for ileal digestible lysine of growing-finishing pigs in that trial was supposed to be in the range 7–6.5 g/kg diet, the digestible threonine : lysine ratio should be between 0.61 and 0.66. This is in agreement with the optimum threonine : lysine ratio on the ileal digestible basis found in similar experiments on young pigs (Schutte *et al.*, 1990, Lenis *et al.*, 1990) being between 0.63 and 0.66–0.63, respectively. The data cited above are in accordance with our estimates but they are higher than the ileal digestible threonine : lysine ratio of approximately 0.58 found by Schutte *et al.* (1997) who, however, based this ratio on data for food conversion efficiency, and 0.59 on the total basis estimated by Taylor *et al.* (1982).

In the literature, a wide range of optimum digestible sulphur amino acids : lysine ratios is reported for diets of growing pigs. The estimate found in the present study (0.54) is in accordance with the ratio 0.58 (on the faecal digestible basis) reported by Lenis *et al.* (1990) and with the NRC recommendation (0.57) but it is higher than the other values reported by Loughmiller et al. (1998), Taylor *et al.* (1983) and Knowles *et al.* (1998). On the other hand, our estimate is considerably lower than the ratio 0.63 reported by Wang and Fuller (1989) or 0.62 recommended by Degussa (2000).

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The influence of commercial types of bulls on fatty acid composition in beef

Vliv užitkového typu býků na obsah mastných kyselin v mase

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ABSTRACT: The objective of the present paper was to study the level of fatty acids in the intramuscular fat of crosses of the domestic Czech Pied cattle with beef breeds of cattle. The level of unsaturated fatty acids (mono-, di-, tri- to hexaenic) was significantly higher in the progeny of Piemontese (Pi) and Aberdeen Angus (Aa) bulls. In terms of PUFA the worst results were discovered in the muscles of Hereford (He) and Blonde d'Aquitaine (Ba) cattle. As concerns the nutrition, the most important group was the group with 3 to 6 double bonds (C18 : 3, C20 : 4, C20 : 3, C20: 5, C20 : 6). The range of the level of linolenic acid (C18 : 3) was significant, i.e. between 0.18 and 0.64%, with the highest value in the group of Pi bulls. Among the fatty acids the level of C20 : 4 was 0.29% in the Hereford (He) and Belgian Blue (Bm) groups, and lower than 0.67% in the Pi group. The levels of C20 : 5 and C20 : 6 were relatively low (on the level of the tenths of per cent) and the differences between the groups of bulls were insignificant.

Keyword: crossing; beef bulls; beef; intramuscular fat; saturated and unsaturated fatty acids

ABSTRAKT: V práci je studován profil mastných kyselin ve vnitrosvalovém tuku u kříženců domácí červenostrakaté populace skotu s masnými plemeny. Významně vyšší obsah nenasycených mastných kyselin (mono-, di-, tri- až hexaenových) byl zaznamenán u potomstva po býcích piemontského (Pi) a aberdeen anguského (Aa) plemene. Při hodnocení polyenových mastných kyselin byla nejhůře hodnocena svalovina jatečných býků herefordského (He) a plavého akvitánského (Ba) plemene. Z nutričního hlediska má nejvyšší význam skupina se třemi až šesti dvojnými vazbami (C18 : 3, C20 : 4, C20 : 3, C20 : 5, C20 : 6). Signifikantní rozpětí obsahu kyseliny linolenové (C18 : 3) se pohybovalo v rozsahu 0,18 až 0,64 %, s nejvyšší hodnotou ve skupině býků Pi. Kyselina C20 : 4 byla zastoupena v profilu mastných kyselin v rozsahu 0,29 % u skupin He a belgického modrého (Bm) do 0,67 % u skupiny Pi. Poměrně nízké hodnoty obsahu (na úrovni desetin procenta), s nesignifikantními diferencemi mezi skupinami býků, jsme stanovili i u kyselin C20 : 5 a C20 : 6.

Klíčová slova: křížení; býci masných plemen; hovězí maso; intramuskulární tuk; nasycené a nenasycené mastné kyseliny

INTRODUCTION

Meat is one of the basic human foodstuffs and its consumption is the topic of frequent discussions and considerations in the pursuit to provide nutrition conforming to the physiological requirements of each individual. In this respect an adequate portion of meat with intramuscular fat is a component of good human nutrition. Fat is a necessary ingredient of food and no other component can substitute it. Particularly the energy value and content of essential fatty acids are basic components of human nutrition. In terms of weight they represent approximately 19% of the nutrients and according to the recommendations of FAO their energy share in the daily consumption should be 20–30% in the future (at present it is more than 35%). The basic components of fats are fatty acids, which can have a positive effect on the consumer's metabolism provided that they are taken up in certain concentrations. Therefore the proportion of fat lower than 20% would not provide the recommended doses of some important nutritional factors and would unfavourably affect the consumer's health (Strapák, 1998).

The important properties of meat, i.e. the important quality characteristics, are based on the chemical com-

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position of meat. Due to high heterogeneity, the content of chemical substances of the respective cuts that are analysed always shows a higher variability. It is characteristic of animal fat that it contains a high amount of saturated fatty acids that are considered as negative in view of nutrition. Saturated and monounsaturated fatty acids are not quite necessary in food because the body itself is able to produce them from glycides, alcohol and proteins. However, the body is not capable of producing some polyunsaturated fatty acids (so called essential fatty acids) and has to draw them from foodstuffs. Acids of the *n*-3 series are of great importance, particularly eicosapentaenic and eicosahexaenic acids, which are important antisclerotic factors in human nutrition and are extremely scarce in our nutrition. Only fish oil, the fat of mammals and of other edible animals contain higher amounts of these acids, but the share of this meat in our "overland" diet is wholly insufficient (Kopecký, 1995).

There are great differences in the composition of fatty acids from one organism, which are based on the body tissue from which the fat was isolated. A number of authors studied the changes in the structure of fatty acids as a consequence of changes in the nutrition of cattle and when feeding a modified feed ration (Palanská *et al.*, 1993; Knight and Death, 1997; Nürberg *et al.*, 1998; Horník *et al.*, 1998; Kirchneim *et al.*, 1998 and others). The number of analyses of differences between the levels of fatty acids in muscles and fat of various commercial types of cattle was lower. Šubrt *et al.* (1991) analysed the spectrum of fatty acids in the muscles and fat of crosses of the Czech Pied cattle with dairy breeds fattened to a weight from 686 to 702 kg. In association with the declining share of the Czech Pied cattle in the crossings they found a significant trend towards a reduced proportion of the C14 : 0, C16 : 1 and C17 : 1 acids in the intramuscular fat. Mojto *et al.* (1996) compared the proportion of fatty acids in the meat of Slovakian Pied, Slovakian Pinzgau and Black Pied Lowland bulls. In their studies of the effect of the father, length of fattening period and feeding strategy, Rule *et al.* (1997) reported a higher proportion of some saturated fatty acids in steers – crosses of the Charolais, Angus, Hereford, Red Angus and Tarentaise breeds. In meat samples from crosses of the Black Pied cattle with Italian breeds (Piemontese, Marchigiana and Chianina) Weglarz *et al.* (1999) observed important differences in the content of intramuscular fat and of most of the mono and polyunsaturated fatty acids. Mojto *et al.* (1998) reported significant differences in the proportion of oleic acid and some other less frequent fatty acids in the fat of Holstein and Belgian Blue crosses.

MATERIAL AND METHODS

For intentional crossing of mothers of the Czech Pied cattle we used special beef breed bulls; Aberdeen Angus (Aa), Blonde d'Aquitaine (Ba), Belgian Blue (Bm), Charolais (Ch), Limousine (Li), Hereford (He) and Piemontese (Pi). Czech Pied bulls were used as a control group (C). Altogether 224 meat samples were analysed. Table 1 gives the number of analyses carried out in each group. The bulls were fattened under the same systems of management and they were slaughtered at the same age after fattening was terminated (497 to 501 days). The slaughter weight ranged between 526 and 597 kg. Sirloin samples were taken from the 9th to 10th thoracic vertebrae deprived of superficial fascias and intermuscular fat and chemical analyses were carried out. After fat extraction in diethyl ether, the fat extracted from the muscles was esterified and after transfer to methyl esters it was analysed on a CHROM 5 gas chromatograph with flame ionising detector (PID). The identification of the analysed fatty acids was based on the elution times and compared with elution times of the standards of methyl esters of fatty acids. The CI-105 integrator was used for quantitative evaluations of the chromatographic analyses, resulting in the percentage content of fatty acids. In the intramuscular fat we identified 21 fatty acids with at least 12 carbon atoms. The differences in the data between the groups of animals were tested using Kruskal-Wallis test in the Unistat 5.0 statistical programme. Indications of fatty acids based on the type are given in the following survey:

Saturated fatty acids		Unsaturated fatty acids					
$C_n : 0$		$C_n : 1$		$C_n : 2$		$C_n \geq 3$	
12 : 0	lauric acid	14 : 1	myristoleic acid	12 : 2	lauroleic acid	18 : 3	linolenic acid
14 : 0	myristic acid	16 : 1	palmitoleic acid	14 : 2	myristeoleic acid	20 : 3	eicosatrienic acid
16 : 0	palmitic acid	18 : 1	oleic acid	16 : 2	palmitoleic acid	20 : 4	arachidonic acid
18 : 0	stearic acid	20 : 1	gadoleic acid	18 : 2	linoleic acid	20 : 5	eicosapentaenic acid (EPA)
20 : 0	arachic acid			20 : 2	eicosadienic acid	20 : 6	eicosahexaenic acid
						22 : 4	docosatetraenic acid
						22 : 5	dokosapentaenic acid
						22 : 6	dokosahexaenic acid (DHA)

RESULTS AND DISCUSSION

The percentage fat content in meat was within a relatively wide range, i.e. between 0.98% (Pi) and 2.70% (He) (Table 1). The differences between the groups of animals were statistically significant, i.e. $P > 0.05$ – standard letters and $P > 0.01$ – bold type. Significant differences were recorded between Pi, Li, Bm, Ba, Ch and He, Aa, C.

Mojto *et al.* (1998) found an insignificantly higher level of intramuscular fat (i.e. 1.10%) in the Holstein × Blue Belgian crosses. Šubrt and Schmidt (1994) discovered a smaller proportion of intramuscular fat when the level of bulls' nutrition was lower.

The content of higher fatty acids in the meat characterises the quality of fat in the meat. The fatty acids defined in the present study were divided into saturated and unsaturated acids. Table 2 gives a survey of the percentage content of saturated fatty acids in the respective groups of bulls. The percentage level of lauric acid (C12 : 0) was low and the variability was medium to high. The

Table 1. The content of intramuscular fat in the *musculus longissimus* thoracis of bulls based on the breed of fathers (%)

Statistical parameters	1 – Aa	2 – He	3 – Li	4 – Pi	5 – Bm	6 – Ba	7 – Ch	8 – C
	$n = 21$	$n = 9$	$n = 34$	$n = 16$	$n = 11$	$n = 30$	$n = 92$	$n = 11$
$\bar{x}$	1.89	2.70	1.18	0.98	1.01	1.19	1.39	1.93
$s_{\bar{x}}$	0.629	1.265	0.697	0.271	0.500	0.711	0.840	1.078
V %	33.3	46.8	59.3	27.7	49.6	59.7	60.4	55.8
$P < 0.05$								
$P < 0.01$	**3, 4, 5, 6, 7**	**3, 4, 5, 6, 7**	**1, 2, 8**	**1, 2, 8**	**1, 2, 8**	**1, 2, 8**	**1, 2, 8**	**3, 4, 5, 6, 7, 8**

Table 2. Saturated fatty acids (C_n : 0) in the meat of commercial cattle (%)

Fatty acids	Statistical parameters	Group no. – commercial type							
		1 – Aa	2 – He	3 – Li	4 – Pi	5 – Ch	6 – Ba	7 – Bm	8 – C
C12 : 0	$\bar{x}$	0.07	0.10	0.10	0.11	0.06	0.09	0.08	0.10
	$s_{\bar{x}}$	0.029	0.020	0.033	0.024	0.022	0.027	0.046	0.021
	V %	42.51	20.82	32.01	21.92	36.96	31.14	54.22	21.17
	$P < 0.05$							**2, 3, 4,**	
	$P < 0.01$	**2, 3, 4, 6, 8**	**1, 7**	**1, 5, 6, 7**	**1, 5, 6, 7**	**3, 4, 7, 8**	1, 3, 4, 7	**5, 6, 8**	**1, 5, 7**
C14 : 0	$\bar{x}$	2.47	2.84	2.34	2.09	2.35	2.32	2.58	2.22
	$s_{\bar{x}}$	0.325	0.408	0.409	0.242	0.502	0.346	0.412	0.367
	V %	13.15	14.35	17.45	11.59	21.35	14.88	16.00	16.54
	$P < 0.05$								
	$P < 0.01$	2, 4	**1, 3, 4, 6, 7, 8**	**2, 4, 5**	**1, 2, 3, 5, 6, 7**	**3, 4, 6, 8**	**2, 4, 5**	2, 4	**2, 5**
C16 : 0	$\bar{x}$	23.57	25.73	22.99	21.27	22.91	22.91	24.09	22.74
	$s_{\bar{x}}$	1.626	2.477	2.036	1.370	1.732	1.991	1.935	1.576
	V %	6.90	9.63	8.85	6.44	7.56	8.69	8.03	6.93
	$P < 0.05$				**1, 2, 3, 5,**				
	$P < 0.01$	2, 4	**1, 3, 4, 6, 7, 8**	**2, 4, 5**	**6, 7, 8**	**3, 4, 6, 8**	**2, 4, 5**	2, 4	2, 4, 5
C18 : 0	$\bar{x}$	21.44	21.82	23.34	23.75	22.73	24.02	22.43	23.23
	$s_{\bar{x}}$	2.398	1.888	2.827	2.761	3.583	2.986	2.783	3.115
	V %	11.18	8.65	12.12	11.63	15.76	12.43	12.41	13.41
	$P < 0.05$								
	$P < 0.01$	**3, 4, 6**	6	**1**	1	6	**1, 2, 5**	–	–
C20 : 0	$\bar{x}$	0.33	0.21	0.39	0.25	0.58	0.530	0.41	0.23
	$s_{\bar{x}}$	0.167	0.027	0.377	0.044	0.167	0.368	0.274	0.035
	V %	50.74	12.89	96.97	17.61	29.09	69.88	68.23	14.71
	$P < 0.05$					**2, 4,**	**1, 2, 3,**	**1, 2, 3,**	
	$P < 0.01$	2, 6, 7	**1, 3, 5, 6, 7**	2, 6, 7	5, 6, 7	6, 7, **8**	**4, 5, 8**	**4, 5, 8**	**5, 6, 7**

Table 3. Monoenic fatty acids (C_{nj} : 1) in the meat of commercial cattle (%)

Fatty acids	Statistical parameters	Group no. – commercial type							
		1 – Aa	2 – He	3 – Li	4 – Pi	5 – Ch	6 – Ba	7 – Bm	8 – C
C14 : 1	$\bar{x}$	0.86	0.64	0.66	0.44	1.04	0.85	0.92	0.44
	$s_{\bar{x}}$	0.285	0.138	0.293	0.047	0.190	0.322	0.314	0.083
	V %	33.20	21.42	44.32	10.63	18.28	37.80	34.21	18.82
	$P < 0.05$			**1, 4, 5,**	**1, 2, 3,**			**2, 3, 4,**	**1, 2, 3,**
	$P < 0.01$	**3, 4, 8**	**4, 5, 7, 8**	**6, 7, 8**	**5, 6, 7**	**2, 3, 4, 8**	**3, 4, 7, 8**	**6, 8**	**5, 6, 7**
C16 : 1	$\bar{x}$	4.07	2.91	3.21	2.66	4.75	4.01	4.18	2.69
	$s_{\bar{x}}$	1.375	0.389	0.973	0.484	0.724	1.201	1.117	0.556
	V %	33.71	13.35	30.29	18.16	15.22	29.93	26.72	20.64
	$P < 0.05$						**2, 3, 4,**	**2, 3, 4,**	
	$P < 0.01$	**2, 3, 4, 8**	**1, 5, 6, 7**	**1, 5, 6, 7**	**1, 5, 6, 7**	**2, 3, 4, 8**	**7, 8**	**6, 8**	**1, 5, 6, 7**
C18 : 1	$\bar{x}$	41.13	40.31	39.84	41.49	38.26	37.85	39.04	41.96
	$s_{\bar{x}}$	2.382	3.128	3.538	2.401	2.310	2.656	3.162	3.164
	V %	5.79	7.76	8.88	5.79	6.04	7.02	8.10	7.54
	$P < 0.05$						**1, 2, 3,**		
	$P < 0.01$	**5, 6, 7**	**6**	**6**	**5, 6, 7**	**1, 4, 6, 8**	**4, 5, 8**	**1, 4, 8**	**5, 6, 7**
C20 : 1	$\bar{x}$	0.46	0.53	0.54	0.46	0.43	0.54	0.49	0.43
	$s_{\bar{x}}$	0.097	0.300	0.404	0.038	0.106	0.421	0.231	0.039
	V %	21.16	56.66	75.09	8.33	24.65	77.71	46.40	8.98
	$P < 0.05$								
	$P < 0.01$	–	–	–	–	–	–	–	–

group average of the content of myristic acid (C14 : 0) ranged between 2.09% in bulls of Pi fathers and 2.84% in crosses with He. The most frequently occurring saturated fatty acids in the animal fat were palmitic and stearic acid. The highest content of palmitic acid (C16 : 0) was found in the progeny of bulls from He fathers, i.e. 25.73%, and the variability was lower (6.4–9.6%). Mojto *et al.* (1998) discovered higher levels of palmitic acid. The highest significant proportion of stearic acid (C18 : 0) was reported in Ba crosses and the lowest in Aa crosses (i.e. 24.02 and 21.44%, respectively). Based on the analyses it was evident that the ratio of palmitic to stearic acid ranged between 1.00 and 1.18. According to Tsuneishi *et al.* (1989) the ratio of C16 : 0/C18 : 0 in the *musculus longissimus thoracis* was 1.65 to 2.25 and considerably differed from our final values. Rule *et al.* (1997) also reported higher proportions of myristic and palmitic acid (2.8–6.6% and 25.2–30.0%, respectively) and a lower proportion of stearic acid (12.1–18.1%). Kirchneim *et al.* (1998) stated that a higher amount of unsaturated fatty acids in the feed ration reduced the proportion of palmitic and stearic acids in the meat. The proportion of arachic acid (C20 : 0) was relatively low, between 0.57% (Ch) and 0.21% (He).

Higher numbers were discovered in the group of unsaturated fatty acids, which was divided on the basis of double bonds into unsaturated fatty acids with one double bond – monoenic (C_n : 1), with two double bonds – dienic (C_n : 2) and with three or more double bonds – tetra- to hexaenic ($C_n \geq 3$).

The difference in the proportion of myristoleic acid (C14 : 1) was significant and ranged between 0.44% (Pi, C) and 1.04% (Ch) (Table 3). The level of palmitoleic acid (C16:1), i.e. 4.75%, was highest in the group Ch. As for the unsaturated fatty acids, the highest content was that of oleic acid in all commercial types. The highest percentage (41.96%) was reported in bulls from fathers of breed C. The variability of this fatty acid was low and ranged between 5.79% and 8.88%. Lawrie (1976) found higher values for oleic acid, i.e. ca. 52%. Šubrt *et al.* (1991) found the level of oleic acid in the meat and in kidney fat to be 34.93 and 37.08%, respectively, and Mojto *et al.* (1998) reported 41.49%.

The nutritive value of fat is largely dependent on the content of PUFAs, frequently indicated as vitamin F. It was found on the basis of the latest information that these fatty acids have anticancerous effects and that they reduce the incidence of cancerous diseases of the thoracic cavity, stomach and skin. As for the unsaturated fatty acids with two double bonds (dienic) in the meat of commercial types, the proportion of lauroleic acid (C12 : 2) was lowest (Table 4). It was studied only exceptionally

Table 4. Dienic fatty acids (C_n : 2) in the meat of commercial cattle (%)

Fatty acids	Statistical parameters	Group no. – commercial type							
		1 – Aa	2 – He	3 – Li	4 – Pi	5 – Ch	6 – Ba	7 – Bm	8 – C
C12 : 2	*n*	11	–	9	–	58	21	10	–
	$\bar{x}$	0.03	n.a.	0.05	n.a.	0.04	0.04	0.04	n.a.
	$s_{\bar{x}}$	0.008	–	0.020	–	0.014	0.015	0.012	–
	V %	26.91	–	38.77	–	35.60	35.12	29.68	–
	$P < 0.05$								
	$P < 0.01$	**3, 5, 6**	–	**1**	–	1	1	–	–
14 : 2	$\bar{x}$	0.139	0.124	0.19	0.11	0.19	0.16	0.18	0.10
	$s_{\bar{x}}$	0.061	0.068	0.119	0.033	0.048	0.052	0.236	0.036
	V %	43.88	54.82	63.58	29.34	25.79	33.21	130.17	34.17
	$P < 0.05$								**1, 3,**
	$P < 0.01$	**7, 8**	**7**	**4, 8**	**3, 5, 6, 7**	**4, 8**	**4, 8**	**2, 1, 4, 8**	**5, 6, 7**
C16 : 2	$\bar{x}$	0.57	0.57	0.55	0.57	0.56	0.51	0.52	0.58
	$s_{\bar{x}}$	0.092	0.068	0.075	0.069	0.141	0.088	0.101	0.072
	V %	16.23	11.83	13.51	12.10	25.05	17.47	19.35	12.25
	$P < 0.05$						**1, 2, 3,**		
	$P < 0.01$	6	6	5, 6	5, 6	3, 4, 8	**4, 7, 8**	6	5, 6
C18 : 2	$\bar{x}$	3.45	2.76	3.75	4.36	4.67	4.31	3.42	3.49
	$s_{\bar{x}}$	0.812	0.754	1.380	0.826	0.976	1.941	1.032	0.986
	V %	23.56	27.28	36.80	18.95	20.90	45.05	30.13	28.22
	$P < 0.05$				**1, 2,**			**1, 2,**	
	$P < 0.01$	**4, 6, 7**	**3, 4, 5, 6, 7**	**2, 4, 7**	3, 5, 8	**2, 4, 6, 7**	1, 2, 5	3, 5, 8	4, 7
C20 : 2	$\bar{x}$	0.26	0.09	0.37	0.20	0.26	0.46	0.44	0.14
	$s_{\bar{x}}$	0.260	0.046	0.642	0.086	0.136	0.623	0.556	0.065
	V %	100.18	51.08	171.49	43.47	52.09	136.19	125.61	46.99
	$P < 0.05$		**1, 3, 4,**						
	$P < 0.01$	2, 5	**5, 6, 7**	2, 5	**2**	**1, 2, 3, 8**	2, 8	**2**	5, 6

n.a. = not analysed

in some animals (Table 4 shows the numbers of animals in which lauroleic acid was studied). The average values in the groups ranged between 0.03 and 0.05%. The proportion of acids C14 : 2 and C20 : 2 was below 0.5%. The acid C16 : 2 slightly exceeded this figure in the studied groups of bulls. As for the dienic fatty acids, the highest significant proportion was that of linoleic acid (C18 : 2), which ranged between 2.76% (He) and 4.67% (Ch).

From the nutritional point of view, the most important group is the group with 3 to 6 double bonds (Table 5). The most important acids are linolenic acid (C18 : 3), arachic acid (C20 : 4) and other so called eicosa acids with 20 C (C20 : 3, C20 : 5, C20 : 6). The proportion of polyenic acids in the total amount of fatty acids of the intramuscular fat of cattle is relatively low. The level of linolenic acid (C18 : 3) was significant and ranged between 0.18 and 0.64%; the value was highest in the group of Pi bulls. The level of eicosatrienic acid (C20 : 3) ranged between 0.09% in the Aa, He, Ch and Bm crosses and 0.17% in the group of Pi crosses. Arachic acid (C20 : 4) was also classified as an essential fatty acid with one common name – vitamin F. Within the profile of fatty acids the proportion was highest in the Pi group (0.67%) and lowest (0.29%) in the He and Bm groups. This value is in accordance with data on this fatty acid reported by Lawrie (1976). The levels of eicosapentaenic (C20 : 5) and eicosahexaenic (C20 : 6) acids were relatively low (tenths of per cent) and the differences between the groups were insignificant. The highest levels were discovered in the intramuscular fat of bulls of Ba fathers. The content of the C22 : 4 acid was also low. The limit of one per cent content of the acid (0.1%) was exceeded in the Pi group only. In the bulls of Pi, Li and C fathers the content of the C22 : 5 acid was significantly highest. Attention has recently been focused also on DHA (C22 : 6) due to health reasons; its content in beef is very low and ranges between 0.10 and 0.20%. The total levels of saturated and unsaturated fatty acids and their correlations

Table 5. Trienic to hexaenic fatty acids ($C_n = 3$) in the meat of commercial cattle (%)

Fatty acids	Statistical parameters	Group no. – commercial type							
		1 – Aa	2 – He	3 – Li	4 – Pi	5 – Ch	6 – Ba	7 – Bm	8 – C
C18:3	$\bar{x}$	0.36	0.53	0.51	0.64	0.18	0.32	0.32	0.56
	$s_{\bar{x}}$	0.279	0.090	0.244	0.086	0.069	0.261	0.242	0.149
	V %	77.97	16.81	48.18	13.41	39.37	81.66	75.74	26.74
	$P < 0.05$			1, 4,	1, 3,	2, 3,	2, 3,	2, 3,	1, 5,
	$P < 0.01$	3, 4, 8	5, 6, 7	5, 6, 7	5, 6, 7	4, 8	4, 8	4, 8	6, 7
C20 : 3	$\bar{x}$	0.09	0.09	0.14	0.17	0.09	0.10	0.09	0.14
	$s_{\bar{x}}$	0.044	0.041	0.084	0.069	0.033	0.067	0.060	0.059
	V %	50.46	43.03	61.66	41.31	37.69	67.76	67.65	40.92
	$P < 0.05$			1, 4,	1, 2, 3,				1, 5,
	$P < 0.01$	3, 4, 8	4	5, 6	5, 6, 7	3, 4, 8	3, 4, 8	4, 8	6 ,7
C20 :4	$\bar{x}$	0.30	0.29	0.45	0.67	0.41	0.43	0.29	0.42
	$s_{\bar{x}}$	0.182	0.200	0.359	0.310	0.272	0.406	0.225	0.279
	$V_{(\%)}$	60.76	70.11	79.78	46.45	66.38	94.62	78.28	66.31
	$P < 0.05$				1, 2, 3,	3, 4,			
	$P < 0.01$	4	4	4, 5	5, 6, 7, 8	6, 8	4, 5	4	4, 5
C20 : 5	$\bar{x}$	0.10	0.09	0.13	0.10	0.14	0.14	0.13	0.08
	$s_{\bar{x}}$	0.060	0.022	0.076	0.020	0.096	0.071	0.081	0.024
	$V_{(\%)}$	62.79	24.97	59.91	19.31	66.98	49.63	63.57	28.96
	$P < 0.05$								
	$P < 0.01$	3, 5, 6	6	1		1	1, 2, 8		6
C20 : 6	$\bar{x}$	0.04	0.06	0.05	0.05	0.05	0.06	0.05	0.05
	$s_{\bar{x}}$	0.016	0.033	0.018	0.012	0.016	0.082	0.027	0.016
	V %	41.21	59.76	37.17	21.78	32.30	136.29	50.76	37.18
	$P < 0.05$								
	$P < 0.01$	3, 4, 5		1	1	1			
C22 : 4	$\bar{x}$	0.05	0.04	0.09	0.11	0.08	0.08	0.06	0.06
	$s_{\bar{x}}$	0.026	0.027	0.044	0.050	0.034	0.046	0.030	0.030
	V %	48.21	67.89	50.29	44.00	42.60	55.79	46.59	47.62
	$P < 0.05$		3, 4,	2, 1,	2, 1,	2, 3,	1, 2,		
	$P < 0.01$	3, 4, 6, 7	5, 6, 7	5, 8	5, 6, 8	4, 6	4, 5	1, 2	3, 4
C22 : 5	$\bar{x}$	0.14	0.18	0.20	0.30	0.13	0.15	0.13	0.19
	$s_{\bar{x}}$	0.089	0.076	0.106	0.115	0.075	0.091	0.075	0.098
	V %	63.22	42.86	53.71	38.47	59.15	62.43	58.42	50.87
	$P < 0.05$			1, 4,	1, 2, 3,				
	$P < 0.01$	3,4	4	5, 6, 7	5, 6, 7, 8	3, 4, 8	3, 4	3, 4	4, 5
C22 : 6	$\bar{x}$	0.11	0.10	0.15	0.20	0.09	0.11	0.11	0.13
	$s_{\bar{x}}$	0.045	0.035	0.076	0.078	0.012	0.044	0.045	0.055
	V %	44.44	36.63	49.49	39.07	13.13	39.45	41.58	43.76
	$P < 0.05$			1, 2, 5,	1, 2,				
	$P < 0.01$	3, 4	3, 4	6, 7, 8	5, 6, 7	3, 4	3, 4	3, 4	4

are important indicators of the nutritive value of fat. In view of healthy nutrition it is desirable to increase the proportion of linoleic, linolenic, arachic and other polyenic acids because their uptake is very important in human nutrition in terms of health. Rumsey *et al.* (1972), Šubrt *et al.* (1991) and Mojto *et al.* (1995) reported that the changes in the proportions of saturated and unsaturated fatty acids were largely affected by the changes in

the amount of stearic, oleic and linoleic acid. Yoshimura and Namikawa (1985) also detected considerable differences in the content of fatty acids in the intramuscular fat of carcasses.

CONCLUSION

The objective of the study was to investigate if specialised breeds of cattle used for commercial crossing could influence the proportion of fatty acids in the intramuscular fat. Based on the requirements for "healthy nutrition" and good quality beef, unsaturated fatty acids are considered to be better. Higher contents of (mono- and poly) unsaturated fatty acids were reported in the progeny of Piemontese and Aberdeen Angus bulls. As for PUFA, the least favourable results were determined in the muscles of Hereford and Aquitaine Blond slaughter bulls. The results have shown that it is well-founded to consider the choice of the commercial type of fattened cattle when evaluating the effect of the factors on the desirable structure of fatty acids.

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Analysis of genetic linkage in ovine α_{S1}- and β-casein (*CSN1S1* and *CSN2*) loci

Analýza genetické vazby v lokusech α_{S1}- a β-kazeinu (*CSN1S1* a *CSN2*) u ovcí

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ABSTRACT: Using the restriction fragment length polymorphisms technique the genetic linkage of ovine α_{S1}- and β-Cn (*CSN1S1* and *CSN2*) genes was analysed. The segregation of gametes was studied in families of double heterozygous males belonging to the Churra and Manchega breeds. The lod-score value (*Z*) showed genetic linkage between the α_{S1}- and β-Cn genes.

Keywords: sheep; casein; RFLP; linkage

ABSTRAKT: Provedli jsme analýzu genetické vazby mezi geny α_{S1}- a β-Cn (CSN1S1 a CSN2) za použití metody RFLP (metoda polymorfismů délek restrikčních fragmentů). Štěpení gamet jsme sledovali u rodin otců s dvojitou heterozygotností, kteří náleželi k plemeni Churra a Manchega. Hodnota lod-score (*Z*) naznačila genetickou vazbu mezi geny α_{S1}- a β-Cn.

Klíčová slova: ovce; kazein; RFLP; vazba

Studies on sheep casein polymorphisms are very scarce. In particular, those concerning the analysis of linkage between ovine casein genes involve only studies carried out by Di Gregorio *et al.* (1991) and Leveziel *et al.* (1991). These studies point out that, like in the case of other ruminants, ovine casein genes constitute a linkage group. Ovine caseins were assigned to chromosome 6 (De Gortari *et al.*, 1998).

This study aims to establish, using DNA polymorphisms, the possible linkage between the α_{S1}- and β-Cn (*CSN1S1* and *CSN2*) genes in Spanish dairy sheep breeds.

The α_{S1}- and β-caseins from randomly chosen complete families, belonging to the Churra and Manchega breeds, were analysed using the restriction fragment length polymorphisms (RFLP) technique. Six males, 14 ewes and 29 offsprings were studied.

The DNA samples were obtained from leukocytes according to the technique of Goossens and Kan (1991), and each DNA sample was digested with the following endonucleases: *Eco*RI, *Hind*III, and *Taq*I. C184 and C468 plasmids containing cDNAs from α_{S1} and 5'β bovine caseins, respectively, were used as probes (Stewart *et al.*, 1984; 1987). Electrophoresis, DNA transfer to nylon membrane, prehybridization, hybridization and autoradiography were carried out according to Di Gregorio *et al.* (1991) and Ordás *et al.* (1997).

The lod-score method, developed by Morton (1955), was used in family data analysis to detect genetic linkage between ovine caseins. *Z* values: $3 \geq +3$ and ≤ -2 were taken as indicators of the presence or absence of linkage, respectively.

The α_{S1}-Cn locus showed fragments of 3.6 and 4.7 kb with *Eco*RI and fragments of 5.0, 8.0 and 10.0 kb with TaqI. The locus β-Cn presented fragments of 1.2 and 1.6 kb with *Hind*III. The size and number of fragments shown by each locus and endonuclease are identical to those described by Di Gregorio *et al.* (1991) in other European ovine breeds, that means they are not specific to the Churra or Manchega breeds. However, the identified fragments differ from those detected by Leveziel *et al.* (1991). They found bands of 4.0, 8.4 and 12.0 kb with *Taq*I in α_{S1}-Cn and fragments of 1.4, 1.7, 4.4 and 7.0 kb with *Hind*III in β-Cn. This discrepancy is attributed to the fact that the cDNA casein probes used in both studies are different.

The genetic linkage of the two casein genes was established by segregation analysis of gametes in families of double heterozygous rams. Table 1 shows the analysed

Table 1. Analysis of the linkage between the α_{S1}- and β-Cn genes

Relation α_{S1}-Cn versus β-Cn	Ram No	Most probable phase for sire	Number of informative offsprings	Segregation parental	Segregation recombinant	Lod-score value (Z) for $\theta = 0$
α_{S1}-Cn^{TaqI} : β-$Cn^{HindIII}$	1CH	8.0 – 1.6/10.0 –1.2[a]	6	6	0	1.50[b]
α_{S1}-Cn^{EcoRI}: β-$Cn^{HindIII}$	1CH	4.7 – 1.6/3.6 –1.2[a]	8	8	0	2.11
α_{S1}-Cn^{TaqI} : β-$Cn^{HindIII}$	2CH	8.0 – 1.6/10.0 –1.2[a]	4	4	0	0.90
	1M	8.0 – 1.6/10.0 –1.2[a]	2	2	0	0.30
	2M	8.0 – 1.6/10.0 –1.2[a]	2	2	0	0.30
						3.61[c]

[a] *Cis* (coupling)

[b] the ram 1CH is analysed twice for the α_{S1}-Cn, but only one Z value is included in the cumulative lod-score

[c] cumulative lod-score for the pair of loci considered

relations (the endonucleases used to detect RFLPs are indicated as exponents), the males analysed in each family, the informative offspring, the segregation and lod-score values (Z) for $\theta = 0$ (limiting values of Z when θ tends to 0.00). The results obtained in the segregation of the different families can be considered representative of the Churra and Manchega breeds. In all cases the analysis of linkage leads to the determination of parental types and the absence of recombinants in progeny. The cumulative lod-score value (Z) is higher than 3. In conclusion, the results suggest the existence of genetic linkage between the α_{S1}- and β-Cn genes in the Churra and Manchega breeds. This confirms what was stated by Di Gregorio *et al.* (1991) and Leveziel *et al.* (1991) in other European ovine breeds.

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Association of polymorphism in the *IGF2* gene with litter size in Black Pied Přeštice pigs

Asociace polymorfismu v genu *IGF2* s velikostí vrhu u přeštického černostrakatého prasete

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ABSTRACT: Insulin-like growth factor 2 (*IGF2*) is a member of the insulin-relaxin growth factor family that shows a significant effect on reproductive traits in pigs. Single nucleotide polymorphism, caused by G → A substitution, was described in the *IGF2* gene. PCR based test and RFLP were used for the detection of mutant allele. The PCR product (~ 1.6 kb) was digested by restriction enzyme NciI to 0.9 kb fragment (allele *A*, in which the restriction locus is absent) or 0.8 kb fragment (allele *B*) and few undetectable fragments. We determined associations between the polymorphism in *IGF2* and the total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets. The investigated population consisted of 90 Black Pied Přeštice (BPP) sows. The association between *IGF2* genotypes and TNB, NBA and NW was evaluated by means of a general linear model (GLM). We did not find any significant effect of polymorphism in *IGF2* gene on TNB, NBA and NW in the 1st litters. In the 2nd–7th litters there was a highly significant ($P \leq 0.001$) effect of polymorphism of *IGF2* gene on TNB. The association with NBA ($P \leq 0.05$) was also found. Significant differences in TNB were observed between the sows of genotypes *AA* and *AB* ($P \leq 0.001$), *AA* and *BB* ($P \leq 0.05$), *AB* and *BB* ($P \leq 0.05$) and in NBA between the sows of genotypes *AA* and *AB* ($P \leq 0.05$). In the 1st –7th litters the polymorphism of *IGF2* gene was shown to exert a highly significant effect on TNB ($P \leq 0.001$) and a significant effect on NBA ($P \leq 0.05$). There are significant differences in TNB between the sows of genotypes *AA* and *AB* ($P \leq 0.001$), *AA* and *BB* ($P \leq 0.01$), *AB* and *BB* ($P \leq 0.05$). Significant differences in NBA were determined between the sows of genotypes *AA* and *AB* ($P \leq 0.01$), *AA* and *BB* ($P \leq 0.05$). The effect of the polymorphism of *IGF*2 gene on TNB and NBA in the 2nd–7th and the 1st–7th litters was detected. The sows of *AB* and *BB* genotypes showed significant differences in TNB and NBA in contrast to the sows of *AA* genotypes.

Keywords: insulin-like growth factor; *IGF2*; PCR-RFLP; reproduction; pig

ABSTRAKT: Insulinu podobný růstový faktor 2 (*IGF2*) je jedním z genů významně podmiňujících reprodukční vlastnosti prasat. Je členem rozsáhlé rodiny genů růstových faktorů a ovlivňuje růst a diferenciaci buněk, embryonální vývoj a průběh gravidity. Sledovali jsme vliv polymorfismu genu *IGF2* na počet všech, živě narozených a odchovaných selat v souboru 90 prasnic přeštického černostrakatého prasete. Uvedené ukazatele byly hodnoceny na 1., 2. až 7. a 1. až 7. vrzích. Detekci genotypů genu *IGF2* jsme provedli metodou PCR-RFLP. Výsledkem štěpení PCR-produktu (o délce 1,6 kb) je fragment dlouhý 0,9 kb (zaniklé restrikční místo – alela *A*) nebo fragment dlouhý 0,8 kb (alela *B*) a několik krátkých fragmentů, které jsou obtížně detekovatelné. Statistické vyhodnocení vlivu polymorfismu genu *IGF2* na velikost vrhu jsme provedli obecným lineárním modelem (GLM). Nezjistili jsme žádný vliv polymorfismu genu *IGF2* na sledované znaky na 1. vrzích. Na 2. až 7. vrzích byl vyhodnocen velmi vysoce průkazný ($P \leq 0{,}001$) vliv polymorfismu genu *IGF2* na počet všech narozených selat a průkazný vliv ($P \leq 0{,}05$) na počet živě narozených selat. Byly vyhodnoceny rozdíly v počtu všech narozených selat mezi prasnicemi s genotypy *AA* a *AB* ($P \leq 0{,}001$), *AA* a *BB* ($P \leq 0{,}05$) a *AB* a *BB* ($P \leq 0{,}05$). Průkazný rozdíl byl také v počtu živě narozených selat u prasnic s genotypy *AA* a *AB* ($P \leq 0{,}05$). Při souhrnném hodnocení sledovaných znaků na 1. až 7. vrzích jsme zjistili velmi vysoce významný ($P \leq 0{,}001$) vliv polymorfismu genu *IGF2* na počet všech narozených selat a významný vliv

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($P \leq 0,05$) na počet živě narozených selat. V počtu všech narozených selat byly shledány rozdíly mezi prasnicemi s genotypy *AA* a *AB* ($P \leq 0,001$), *AA* a *BB* ($P \leq 0,01$) a *AB* a *BB* ($P \leq 0,05$) a v počtu živě narozených selat mezi prasnicemi s genotypy *AA* a *AB* ($P \leq 0,01$) a *AA* a *BB* ($P \leq 0,05$). V naší práci byl zjištěn průkazný vliv polymorfismu genu *IGF2* na počet všech a živě narozených selat na 2. až 7. a 1. až 7. vrzích. Zároveň byl zjištěn průkazně vyšší počet všech a živě narozených selat u prasnic s genotypem *AB* nebo *BB* oproti prasnicím s genotypem *AA*.

Klíčová slova: insulinu podobný růstový faktor; *IGF2*; PCR-RFLP; reprodukce; prase

INTRODUCTION

Reproductive traits in farm animals are conditionally polygenic with low heritability estimates. However, there are some genes that determine the important part of variability in the investigated traits. Molecular genetics contributes to the knowledge of factors influencing animal reproduction by mapping the genes and studying their effects. The knowledge of the mode of inheritance of identified QTL is important for agricultural applications. The numbers of known loci and genes in pigs are about 2 200 and 640, respectively, but imprinting has been reported only for four QTLs (De Koning *et al.*, 2000) and one gene (Nezer *et al.*, 1999; Jeon *et al.*, 1999).

One of the genes with a significant effect on reproduction in pigs is the insulin-like growth factor 2 (*IGF2/IGFII*). The gene *IGF2* is a member of the insulin-relaxin growth factor family and influences the growth and differentiation of cells, embryonic development and progression of pregnancy (Ko *et al.*, 1994). Along with the other members of the IGF-system it stimulates the epithelial cell proliferation via the type II receptor (Badinga *et al.*, 1999).

The gene *IGF2* in interaction with FSH (follicle-stimulating hormone) or estradiol increases progesterone synthesis (Garmey *et al.*, 1993).

Lamberson *et al.* (1996) examined the relation between the concentration of IGF1 and IGF2 in the blood serum of pigs in the age range of 9–21 weeks and their growth and reproduction. The IGF1 and IGF2 concentration was associated with higher backfat thickness and with fewer days to 100 kg. Nevertheless IGF2 did not show any effect on growth nor on litter size nor age at puberty. Owens et al. (1999) determined the concentration of plasma IGF1, IGF2 and IGFBP3 in relation to postnatal growth traits in pigs. Plasma IGF2 was positively associated with backfat thickness.

The *IGF2* gene was mapped to porcine chromosome 2 (SSC2p) by Nezer *et al.* (1999). The polymorphism of *IGF2* gene was described by Knoll *et al.* (2000). This gene is linked with another candidate gene *MYOD*1, the polymorphism of which was described by Knoll *et al.* (1997). The *IGF2* gene as a candidate gene for muscularity and fat deposition was reported by Nezer *et al.* (1999). In the study of the F_2 population from Meishan boars and sows from the commercial Dutch pig lines, de Koning *et al.* (2000) found no evidence for QTL affecting the muscle thickness on SSC2.

The aim of this study was to determine the effect of polymorphism in *IGF2* gene on the total number of born (TNB), number of born alive (NBA) and number of weaned piglets (NW) in Black Pied Přeštice (BPP) sows.

MATERIAL AND METHODS

The investigated sample population consisted of 90 sows BPP from pedigree breeding. DNA was isolated from the leukocytes of blood samples and amplified by means of PCR.

To establish the genotypes of *IGF2* gene we used the PCR-RFLP procedure as described by Knoll *et al.* (2000). *IGF2* gene is characterised by single nucleotide polymorphism (G → A substitution) which causes disappearance of restriction locus. The absence of restriction locus is used for the detection of *IGF2* genotypes, when a fragment of 0.9 kb and some few undetectable fragments are produced by the digestion of the PCR-products (~ 1.6 kb) by endonuclease *Nci*I (allele *A*). If the restriction locus is present, the fragment 0.8 kb (and several short fragments) is produced (allele *B*). The obtained fragments are separated in 2% agarose gel.

The association between *IGF2* genotypes and TNB, NBA and NW were evaluated by means of a general linear model-GLM (SAS, 2000), which contained the following fixed effects: genes *OPN*, *ESR1*, *ESR2*, *MYF4*, *IGF2*; litter parity; herd; year of sow birth. Because of the low significance of the 1st litters we examined the effect of polymorphism in *IGF2* gene separately for the 1st, 2nd–7th and 1st–7th litters.

RESULTS AND DISSCUSION

In the 1st litters no effect of the polymorphism of *IGF2* gene on TNB, NBA and NW was detected.

In the 2nd–7th litters (Table 1) the polymorphism of *IGF2* gene influenced highly significantly TNB ($P \leq 0.001$) and NBA ($P \leq 0.05$). A significant difference ($P \leq 0.001$) in TNB was determined between the sows

Table 1. Associations between the total number of born, number of born alive and number of weaned piglets and genotypes of *IGF2* gene in the 2nd–7th litters in Black Pied Přeštice sows (least-squares means, LSM ± standard error, S_E)

IGF2 genotypes	*n*	NL	TNB	NBA	NW
AA	12	70	$10.77^{Aa} \pm 0.57$	$10.77^{a} \pm 0.50$	9.78 ± 0.44
AB	36	184	$12.23^{Ab} \pm 0.47$	$11.68^{a} \pm 0.41$	10.29 ± 0.36
BB	42	204	$11.65^{ba} \pm 0.45$	11.31 ± 0.40	10.23 ± 0.35

Note:

n = number of sows, NL = number of investigated litters, TNB = total number of born piglets, NBA = number of piglets born alive, NW = number of weaned piglets

Values with the same exponents in columns show significant differences: $^{a,b}P \leq 0.05$; $^{A}P \leq 0.001$

Table 2. Associations between the total number of born, number of born alive and number of weaned piglets and genotypes of *IGF2* gene in the 1st–7th litters in Black Pied Přeštice sows (least-squares means, LSM ± standard error, S_E)

IGF2 genotypes	*n*	NL	TNB	NBA	NW
AA	12	82	$10.49^{AB} \pm 0.49$	$10.42^{Ab} \pm 0.44$	9.71 ± 0.39
AB	36	220	$10.98^{Aa} \pm 0.40$	$11.38^{A} \pm 0.35$	10.23 ± 0.31
BB	42	246	$11.48^{Ba} \pm 0.38$	$11.11^{b} \pm 0.34$	10.19 ± 0.30

Note: see Table 1

Values with the same exponents in columns show significant differences: $^{a,b}P \leq 0.05$, $^{A,B}P \leq 0.01$, $^{A}P \leq 0.001$

of *AA* and *AB* genotypes. There was a significant ($P \leq 0.05$) difference in TNB between the sows of *AA* and *BB* genotypes and also between those of *AB* and *BB* genotypes. The heterozygotes *AB* showed a more significant ($P \leq 0.05$) NBA than homozygotes *AA*.

No association between the polymorphism of *IGF2* gene and NW in the 2nd–7th litters was observed nor were any significant differences in this trait found between the particular *IGF2* genotypes.

In a joint analysis of the reproductive traits in the 1st–7th litters (Table 2), the effect of the polymorphism of *IGF2* gene on TNB ($P \leq 0.001$) and NBA ($P \leq 0.05$) was similar to that of the 2nd–7th litters.

Highly significant differences in TNB were recorded between the sows of *AA* genotypes and *AB* genotypes ($P \leq 0.001$) and significant differences ($P \leq 0.01$) between the sows of *BB* genotypes and *AA* genotypes. TNB from heterozygous *AB* sows was also significantly higher ($P \leq 0.05$) than that from homozygous *BB* sows. The sows of *AB* genotypes had significantly ($P \leq 0.01$) more live piglets than the sows of *AA* genotypes. A significant ($P \leq 0.05$) difference in NBA was also observed between the sows of *AA* and *BB* genotypes.

Neither the effect of polymorphism in *IGF2* gene on NW nor the differences in NW between the sows of the particular *IGF2* genotypes were demonstrated in the 1st–7th litters.

The obtained results prove the association of polymorphism in *IGF2* gene with the reproductive traits in pigs. The polymorphism of *IGF2* gene had a significant effect on TNB and NBA in the 1st–7th and the 2nd–7th litters. In the investigated sample population the sows with genotypes *AB* or *BB* of *IGF2* gene achieved higher TNB and NBA. The relation between the polymorphism of *IGF2* gene and NW was not proved nor were there any differences observed in this trait between the sows of the particular genotypes of *IGF2* gene.

In this study the analysis of association was carried out by the model supposing Mendelian expression of the *IGF2* alleles. After the significant differences were identified, we did not analysed either paternal or maternal imprinting. We do not consider the fact (Nezer *et al.*, 1999; Jeon *et al.*, 1999) that only paternal *IGF2* allele is expressed as a disagreement with our finding of the association with the number of piglets if we assumed Mendelian expression. A possible explanation is that this may be due to specific imprinting for certain tissues and developmental stages (Morison and Reeve, 1998). The analysis of the effect of both sexes on daily weight gain in twelve groups of full sibs (boars and sows) in two developmental stages indicates the possibility of expression of maternal imprinting in the early and Mendelian expression in the late growth period (Dvořák, 2001, unpublished). The *IGF2* gene may be imprinted in tissues participating in meat production while we suppose Mendelian expression in tissues playing a role in fertility.

For a more conclusive proof of the effect on litter size it would be necessary to examine a larger sample popu-

lation of different breeds and hybrid combinations used in breeding programs.

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