

115 Phenotypic and genetic parameters of muscle fiber number and size. I. Fiedler, C. Rehfeldt*, G. Diel, and K. Ender, *Research Institute for Biology of Farm Animals, Dummerstorf, Germany.*

Numerous studies have reported on relationships between microstructure traits of muscle and criteria of growth, stress-susceptibility, and meat quality in cattle, swine, and poultry. Therefore, it could be efficient in improving animal performance to alter muscle fiber characteristics. To investigate whether the use muscle fiber number and fiber size could be efficient in selection, two experiments, one on 2940 mice and another on 2020 pigs, were conducted to estimate coefficients of heritability and of phenotypic and genetic correlations. The parameters were calculated by using statistic models of full-and-half-pairs-analysis (pig) and of father-son-regression (mouse). The coefficients of genetic variability for fiber number and fiber size were 9.3 and 8.2% in mice and 17.1 and 11.7% in pigs. The coefficients of heritability ranged from low to moderate ($h^2=0.14$ to 0.30). Phenotypic and genetic correlation coefficients revealed negative correlations between fiber size and total fiber number ($r_p = -0.23$ to -0.40 ; $r_g = -0.50$ to -0.80). Simulated selection by data from mice using an optimized index of fiber number and fiber size was able to increase fiber number and fiber size simultaneously against their antagonism. Moreover, muscle mass, body weight, and daily gain were increased. Genetic correlations were also apparent between fiber diameter and criteria of pork quality such as drip loss and colour ($r_g = 0.40$ to 0.78). Phenotypically extreme fiber hypertrophy was related to stress-susceptibility in pigs and to low endurance fitness in mice, whereas high fiber numbers had correspondingly positive effects. An increased grade of pale, soft, exudative meat (PSE) was associated with large fiber diameters and low total fiber numbers. Moreover, pigs with moderate or high marbling values in *longissimus* muscle had fewer muscle fibers than pigs with small marbling values. In conclusion, fiber characteristics of muscle are sufficiently variable and heritable, and they are correlated to animal performance. Consequently, they could be effectively used as criteria of selection in farm animals to affect performance and meat quality.

Key Words: Skeletal muscle, Muscle fiber, Genetic parameter, Pigs, Mice

116 Fetal and maternal responses to feed intake from d 29 to 45 of gestation. R. E. Mussø, D. L. Davis*, R. D. Goodband, M. D. Tokach, and J. L. Nelssen, *Kansas State University, Manhattan.*

Parity-four sows were fed 1.82 kg/d (control, $n = 6$) and 6.36 kg/d (high, $n = 9$) from d 29 to 45 of gestation. Blood samples were taken from sows on d 43 of gestation two hours after morning and evening feeding for analysis of IGF-I concentration. On d 45 of gestation, sows were slaughtered and uteri collected for fetal and placental measurements. High feed intake sows had increased weight gain compared to controls (34.0 vs 4.32 kg, respectively; $P = .0047$). No differences were detected in number of fetuses, mummies, length of unoccupied uterus, implantation length, allantoic fluid volume, placental or fetal weight, and crown-rump length ($P > .10$). Residuals were tested to estimate the variation of fetal and placenta weight, and crown-rump length. No differences were observed between the fetuses from sows fed control or high feed in fetal weight variation (1.94 vs 2.02 g, respectively; $P = .81$), or placental weight variation (18.28 vs 15.58 g, respectively; $P = .21$). However, crown-rump length variation differed (4.77 vs 3.16 mm, respectively; $P = .03$). Sows fed high feed had increased IGF-I concentrations in plasma on d 43 (32.35 vs 77.10 ng/ml, respectively; $P = .006$). Control fetuses demonstrated expected negative relationship between fetal number and fetal weight ($wt = -1.02 \times \text{fetal no} + 32$; $R^2 = .48$), but fetuses from high sows did not show this relationship ($R^2 = .003$). Providing feed in excess of established requirements to gestating sow from d 29 to 45, increased IGF-I concentrations in maternal plasma and decreased crown-rump length variation of the fetus. We postulate the increased maternal IGF-I, or other maternal responses to high feed removed the maternal limit on fetal growth at this stage of gestation.

Key Words: Feed intake, Fetal growth, IGF-I

117 Isolation of porcine myoblasts by flow cytometry. J. R. Blanton, Jr.*, A. L. Grant, D. C. McFarland, J. P. Robinson, and C. A. Bidwell, *Purdue University, West Lafayette, IN.*

The capability to identify and isolate myoblasts readily would allow a broad range of muscle biology research in domestic animals. Cells isolated by flow cytometry retain most of their proliferative capacity, which is a characteristic that allows genetic manipulation and propagation of cells. The objective of this study was to determine parameters for isolating porcine myoblasts from mixed primary cell preparations. As a first step in characterizing cellular parameters for porcine myoblasts, adult myoblasts were clonally selected from *semimembranosus* muscle of 2 to 4 wk old pigs. Individual cells were collected at two separate times by a robotic cell manipulator and transferred to 96-well plates. Three hundred of 481 isolated cells survived collection. At this time, 42 cell lines have been confirmed to be myogenic by cell fusion and reverse transcriptase-PCR analysis of muscle specific mRNA. Non-myoblast clones are referred to as fibroblasts, however their specific cell types have not been characterized. A Coulter EPICS Elite flow cytometer with a 488 nm argon laser was used to characterize clonal myoblasts, clonal fibroblasts, and primary mixed cell preparations. Cell size and nuclear/cytoplasmic ratio were determined by forward scatter (FS) and ninety degree light scatter (90LS), respectively. There were no differences in FS and 90LS between second passage clonal myoblasts and clonal fibroblasts. However, mixed primary cultures that were proliferated on a single plate for 2, 3, 4, 5, 15 or 28 d had two distinct populations based on FS. The two populations were sorted (d 2, 4, 5, 28) and tested for cell fusion. The population of larger cells either detached or remained mononucleated, whereas the smaller cells readily fused to form multinucleated myotubes. Size differences between myoblasts and fibroblasts were lost after a single passage of mixed primary cultures. Therefore, porcine myoblasts can be isolated from mixed primary cultures prior to the first passage based on size alone.

Key Words: Pigs, Muscle Cell, Culture

118 Direct injection of DNA into skeletal muscle of pigs for delivery of recombinant protein. S. K. Jacobi*, D. E. Gerrard, C. A. Bidwell, and A. L. Grant, *Purdue University, West Lafayette, IN.*

One approach for genetically engineering skeletal muscle includes direct injection of DNA constructs into skeletal muscle. The objective of this study was to determine if intramuscular injection of DNA in porcine muscle is effective for obtaining production of recombinant protein. A commercial luciferase reporter plasmid, pGL3C (Promega Biotech Inc.), containing the firefly luciferase cDNA under control of the SV40 promoter and enhancer was used for injections. Preliminary studies were conducted using muscle cell cultures to demonstrate construct functionality. Four pigs (2 wk old) were used for determining effective doses of DNA and extent of DNA and (or) luciferase migration following DNA injection. DNA was suspended in saline containing 1% India ink. Each pig received four 250- μ l injections in the *longissimus* muscles for delivery of 50, 100, 200, and 500 μ g DNA/site. Seven days following injections, pigs were euthanized and injection sites were located by presence of India ink. Muscle samples (3 mm \times 3 mm \times 1 cm) were collected from each injection site and at 3 mm intervals anterior and posterior to the injection site. Luciferase activity was determined by chemiluminescence and samples from uninjected muscles served as negative controls to determine background activity. Amount of luciferase activity was dependent on sample site ($P < .05$). Greatest activities were detected at injection sites and activities decreased with distance from the injection sites. Luciferase activity was not detected in the most distal samples (>9 mm) from the injection sites. Greater doses of DNA appeared to increase the migration of luciferase to more distal sites, even though injection volume was constant. Luciferase activity was greater with 100 μ g and 500 μ g than with 50 μ g ($P < .05$) or 200 μ g ($P < .07$) DNA injections. Variation in luciferase activity may also reflect sampling accuracy. We have demonstrated that direct DNA injection can be used to deliver recombinant protein to porcine skeletal muscle.

Key Words: Pigs, Muscle, DNA