

Growth and Development: Animal Performance and Cellular Differentiation

736 Repeated transport influences feed intake, but not feed efficiency in Holstein calves. A. L. Adams*, G. A. Holub, T. H. Friend, A. J. Krenk, S. M. Garey, C. L. Terrill, and M. J. Carter, *Texas A&M University, College Station.*

Previous studies have determined that stress causes decreases in feed intake and efficiency in cattle, but the effect of repeated transport on these parameters has not been well studied. This study determined how repeated transport affected feed intake and growth in calves. Thirty-six 4-mo-old Holstein steer calves were housed in groups of 6 with each group randomly assigned to either transport (T) or control (C) treatments. Each calf was assigned to an individual feed bunk and feed intake was recorded daily. Transported and control calves did not have access to feed during treatment. Calves were transported for 6 h in their group of 6 in a 7.3 m x 2.4 m goose-neck trailer divided into 3 compartments, at an average density of 0.87 m²/calf, every 7 d for 5 consecutive wk. Feed intake was analyzed as a repeated measure in an autoregressive covariance mixed model ANOVA. Average daily gain (ADG) and feed efficiency were analyzed using a diagonal mixed model ANOVA. Control calves had a higher feed intake than transported calves overall (C: 7.29 ± 0.23 kg, T: 6.91 ± 0.23 kg, $P = 0.012$), on the day of treatment (C: 6.78 ± 0.43 kg, T: 6.01 ± 0.43 kg, $P = 0.0074$), and the day after treatment (C: 7.83 ± 0.45 kg, T: 7.08 ± 0.45 kg, $P = 0.015$). On days of treatment, feed intake for transported calves significantly decreased during wk 2, but increased with each successive transport ($P < 0.0001$). Overall, control calves had higher ADG than transported calves (C: 1.53 ± 0.12 kg/day, T: 1.36 ± 0.12 kg/day, $P = 0.024$). The largest difference in ADG occurred during wk 2 (C: 1.69 ± 0.41 kg/day, T: 1.10 ± 0.41 kg/day, $P = 0.031$). Transported calves tended to have decreased feed efficiency during wk 2 and 3, but increased feed efficiency during wk 4 and 5 ($P = 0.072$). These results suggest that calves exposed to repeated transport may decrease feed intake as an initial response to stress, however, overall feed efficiency is not affected and calves may quickly acclimate to repeated transport.

Key words: calf, feed intake, transport

737 Effects of serum protein-based arrival formula and serum protein supplement (Gammulin) on plasma metabolites in transported dairy calves. A. Pineda*, J. K. Drackley¹, J. M. Campbell², and M. A. Ballou³, ¹University of Illinois, Urbana, ²APC Inc., Ankeny, IA, ³Texas Tech University, Lubbock.

Adequate nutrition is important to provide all nutrients for proper health and growth of young calves, especially in the presence of stressors like cold and transport. Serum protein products have been proposed to improve health and diminish effects of stress in dairy calves. Previously we reported that a commercial serum protein supplement (G; Gammulin, APC Inc.) in milk replacer decreased mortality ($P = 0.007$) in transported male calves. The aim of this portion of the study was to assess the effects of a serum protein-based arrival formula (AF) and use of G in milk replacer on plasma indicators of nutritional adequacy and acute-phase response. Nine-3 male Holstein calves were stratified by arrival BW and plasma protein, and then randomly assigned to 1 of 4 treatment groups. Treatments were 1 = control electrolyte, milk replacer with G (n = 24); 2 = control electrolyte, milk replacer without G (n = 25); 3 = AF, milk replacer with G (n = 22); and treatment 4 = AF, milk replacer without G (n = 22). At first feeding, calves were fed either AF or a control electrolyte solution. Six h later, all calves

received either a commercial calf milk replacer (20% CP, and 20% fat; no G supplementation) or the same milk replacer supplemented with G (50 g/d during the first 14 d only). Blood samples were taken at d 0 (before treatments), 2, 7, 14, and wk 4. Calves remained in the experiment until d 56, below the low critical temperature. Data were analyzed using the MIXED procedure of SAS. IgG concentrations at d 0 were not different ($P > 0.17$) among treatments. Cortisol tended to be greater ($P = 0.06$) for calves that received AF. Concentrations of haptoglobin, acid-soluble protein, albumin, and zinc did not differ among treatments. Supplementation with G resulted in greater total protein ($P = 0.04$) and urea N ($P = 0.01$) in plasma at wk 4 (2 wk after G supplementation ended). Despite the marked reduction in mortality of transported cold-stressed male calves fed the serum protein product, indicators of acute-phase response were not affected; however, protein status of calves may have been improved.

Key words: calf, acute phase response, serum protein

738 Digestive function and plasma oxidative status of intra-uterine growth retarded fully weaned piglets. J. Michiels*^{1,3}, M. De Vos², J. Missotten³, A. Ovyn³, S. De Smet³, and C. Van Ginneken², ¹Faculty of Biosciences and Landscape Architecture, University College Ghent, Ghent, Belgium, ²Laboratory for Veterinary Anatomy, Embryology and Pathology, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium, ³Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Melle, Belgium.

Intra-uterine growth retarded (IUGR) pigs, relative to normal birth weight (NBW) littermates, have lower birth weights and vitality scores, both of which are associated with a higher incidence of post-natal mortality and a lower growth rate in surviving IUGR pigs. To better understand developmental differences, digestive function and plasma oxidative status of fully weaned IUGR and NBW pigs were studied. Newborns from 13 hyper-prolific sows were weighed. At weaning (26.3 ± 1.2 d after parturition) pairs (n = 21) of IUGR and NBW littermates were selected and fed a commercial starter diet until sampling day (22.3 ± 4.3 d post-weaning). An IUGR piglet was defined as having a birth weight <1 kg and < mean litter birth weight - 1.5 SD. For each piglet 97 variables were determined including anatomy of digestive organs, digesta characteristics, small intestinal morphology, secretory and absorptive capacity, barrier function, brush-border enzyme activities, cytokines and density of growth-factor receptors, serum IGF-I, and plasma oxidative status. Data were analyzed using the paired Student's T-test (continuous variables) or the Wilcoxon rank test (categorical). Few parameters showed significant differences. However, in the IUGR pig, the structural and functional maturation of the digestive tract post-weaning was delayed: lower small intestinal weight to length ratio ($P < 0.001$) due to a thinner Tela submucosa and Tunica muscularis (both $P < 0.001$) and a higher numerical secretory capacity in the distal jejunum. These observations might be a consequence of lower circulating IGF-I concentrations (126 ± 43.7 vs. 152 ± 44.9 ng/mL, $P = 0.034$ for IUGR and NBW, respectively) and a lower density of IGF-I receptors in proximal jejunal tissues ($P < 0.05$). Additionally, the plasma antioxidant capacity was lower for the IUGR pigs (ferric reducing ability, 106 ± 14.4 and 114 ± 9.7 μmol Fe²⁺/L, $P = 0.042$ and glutathione-peroxidase enzyme activity, 0.29 ± 0.054 and 0.33 ± 0.047 U, $P = 0.001$ for IUGR and NBW, respectively). The

described changes in fully weaned IUGR pigs resemble those reported for IUGR neonates.

Key words: intra-uterine growth retardation, gastrointestinal tract, pig

739 Effect of dietary energy manipulation on mares and their foals: Glucose and insulin dynamics. K. N. Winsco^{*1}, J. L. Lucia¹, C. J. Hammer^{2,3}, and J. A. Coverdale¹, ¹Department of Animal Science, Texas A&M University, College Station, ²Department of Animal Sciences, North Dakota State University, Fargo, ³Center for Nutrition and Pregnancy, North Dakota State University, Fargo.

To investigate the effect of dietary DE manipulation of mares in the last third of pregnancy on mare and foal glucose and insulin dynamics, 30 Quarter Horse mares (538 to 695 kg BW and 4 to 19 yr) were blocked by expected foaling date. All mares were allowed ad libitum access to bermudagrass (*Cynodon dactylon*) hay and randomly assigned within block to dietary treatment: forage (F), or forage + grain (FG; grain fed at 0.75% BW as-fed). Treatments began 110 d before expected foaling date and terminated at parturition. A modified frequent sampling i.v. glucose tolerance test (FSIGT) was performed on mares 20 d before expected foaling date and on foals at 80 and 160 d of age. Jugular catheters were placed 1 h before FSIGT, and horses were allowed ad libitum access to bermudagrass hay and water throughout. A baseline plasma sample was harvested, then glucose bolus of 0.3 g/kg BW administered. An insulin bolus of 30 mU/kg BW was given 20 min after the glucose bolus. Blood samples were harvested at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min into tubes containing sodium heparin, immediately placed on ice, and centrifuged within 20 min. Glucose concentrations were analyzed using a colorimetric assay and insulin concentrations determined using a commercial RIA kit. All data were analyzed using the PROC MIXED procedures of SAS. Mare glucose area under the curve (AUC) was greater for FG compared with F ($P < 0.01$). Mare insulin AUC and peak insulin were also greater for FG compared with F ($P \leq 0.05$). Foal glucose AUC and peak glucose were not influenced by maternal treatment ($P \geq 0.82$), but both increased with age ($P \leq 0.05$). Foal insulin AUC and peak insulin were influenced by maternal treatment with foals from FG mares having greater peak insulin compared with foals from F mares ($P \leq 0.10$ and $P \leq 0.05$, respectively). An influence of age was observed on foal insulin AUC ($P \leq 0.05$), with all foals decreasing from d 80 to d 160. In summary, dietary treatments affected both mare and foal glucose and insulin dynamics.

Key words: glucose, insulin, maternal nutrition

740 Expression of key transcription factors during differentiation of equine bone marrow mesenchymal stem cells into osteoblast cells. E. R. Ackell^{*1}, A. Sanchez², C. Mora¹, S. A. Zinn¹, T. A. Hoagland¹, and K. E. Govoni¹, ¹Department of Animal Science, University of Connecticut, Storrs, ²Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA.

A novel method to improve fracture healing in horses is the use of equine bone marrow mesenchymal stem cells (eBMSC). Additional research is needed to identify optimal culture conditions and determine the mechanisms involved in regulating eBMSC differentiation into osteoblasts. We hypothesize that eBMSC have greater proliferation potential in allogenic serum and expression of key transcription factors is required for optimal differentiation. The eBMSC were iso-

lated from the sternum of 3 Morgan horses (3.4 ± 0.3 yr of age). Cells were treated with fetal bovine serum (FBS) or horse serum (HS) and cell proliferation was determined by alamarBlue assay and bromodeoxyuridine (BrdU) incorporation. To induce osteoblast differentiation, cells were incubated with L-ascorbic acid-2-phosphate and glycerol-2-phosphate in the presence or absence of human bone morphogenetic protein 2 (hBMP-2), dexamethasone (DEX), or both. Alkaline phosphatase (ALP) activity, an indicator of osteoblast differentiation, was determined by ELISA in these cultures. Total RNA was isolated from cells at d 0, 3, 6, 9, 12, 15, and 18 of culture to determine expression of RUNX2 and Tbx3 using real-time RT-PCR. Data were analyzed by ANOVA using SAS and expressed as mean ± SE. Relative to control (CON) (serum-free media), FBS and HS increased cell number (133 ± 5 and 116 ± 5%, respectively; $P < 0.001$) and BrdU incorporation (173 ± 16 and 172 ± 16%, respectively; $P < 0.001$). Treatment with DEX (1,638 ± 38%; $P < 0.001$), but not hBMP-2 ($P > 0.05$) increased ALP activity compared with CON. Osteocalcin expression increased 260-fold by d 18 of culture ($P < 0.001$) demonstrating differentiation into osteoblasts. By d 6 of culture RUNX2 expression increased 3-fold ($P < 0.001$). Tbx3, a gene involved in regulating osteoblast function, increased 1.8-fold at d 3 ($P < 0.01$), however expression was 4-fold less at d 18 ($P < 0.01$). In summary, FBS induced greater proliferation of eBMSC, DEX is essential for differentiation into osteoblast cells, and inhibition of Tbx3 may be required for optimal differentiation of eBMSC.

Key words: bone marrow mesenchymal stem cell, osteoblast, transcription factor

741 Inter-relationship of BW with linear body measurements in Hissardale sheep at different stages of the life cycle. M. Abdullah^{*}, U. Younas, J. A. Bhatti, T. N. Pasha, M. Nasir, and M. A. Jabbar, *University of Veterinary & Animal Sciences, Lahore, Punjab, Pakistan.*

Determining animal live BW, linear body measurements, and their inter-relationship and correlation is imperative for determining genetic potential, breed standards, and improved breeding programs for greater meat production. Hissardale is the only fine wool sheep breed maintained in Pakistan and developed by crossing exotic Merino with a local carpet wool sheep Bikaneri. Monthly BW data from 314 Hissardale sheep and linear body measurements including heart girth (HG), height at wither (HAW), body length (BL), ear length (EL), ear width (EW), neck length (NL), neck width (NW), tail length (TL), and tail width (TW) were analyzed after random selection at the Livestock Experiment Station, Jahangirabad, Khanewal, Pakistan to determine the appropriate model for estimating BW at different ages of life cycle including pre-weaned and post-weaned sheep. Animals were categorized according to their age (≤ 6 , 7 to 12, 13 to 18, 19 to 24, and > 24 mo). The BW (mean ± SD) of animals was found to be 10.87 ± 1.82, 16.40 ± 1.40, 21.04 ± 1.44, 25.57 ± 2.94 and 47.10 ± 4.41 kg in all age groups (≤ 6 , 7 to 12, 13 to 18, 19 to 24, and > 24 mo, respectively). Body weight was found to be significantly ($P < 0.001$) and positively correlated with HAW for all 5 age groups (0.79, 0.85, 0.67, 0.73, and 0.54, respectively), BL (0.69, 0.83, 0.53, 0.82, and 0.46, respectively) and HG (0.58, 0.85, 0.70, 0.76, and 0.42, respectively). However, the relationship between BW and other linear body measurement like EL, EW, NL, NW, TL, and TW were found significant ($P < 0.05$) but comparatively less correlated except for neck width in 0 to 6- and 7 to 12-mo age groups; neck length, neck width, tail length, tail width in 13 to 18- and 19 to 24-mo age groups. The recorded body measurements had a strong positive correlation with BW, indicating that they can be

used to estimate BW in Hissardale sheep of varying ages under field conditions.

Key words: Hissardale sheep, live body weight, linear body measurements

742 Gene expression of Red Angus sired steers and heifers evaluated for residual feed intake. C. M. Welch^{*1}, G. K. Murdoch¹, C. S. Schneider¹, K. C. Chapalamadugu¹, K. J. Thornton¹, J. K. Ahola², J. B. Hall¹, and R. A. Hill¹, ¹University of Idaho, Moscow, ²Colorado State University, Fort Collins.

To improve our mechanistic understanding of variation in residual feed intake (RFI), gene expression studies have been suggested. In the present study, 42 progeny (25 steers, 17 heifers) of Red Angus sires divergent for maintenance energy (ME) EPD were RFI-tested during an 84-d post-weaning period. Subsequently, biopsy samples were collected from the biceps femoris muscle and mRNA expression of key genes in various regulatory pathways was evaluated through quantitative real-time PCR. A 2-sample t-test at a 2-tailed $P < 0.05$ was used to identify differentially expressed genes in the 2 extreme RFI quartile groups. Fatty acid synthase (FASN) was expressed in greater abundance in inefficient (high RFI) compared with efficient (low RFI) animals ($P = 0.03$). Gene expression of C/EBP α ($P = 0.06$) and PPAR γ ($P = 0.13$) also tended toward greater abundance in inefficient animals. When these data were analyzed by sex, inefficient heifers showed a similar expression pattern for C/EBP α ($P = 0.003$) and PPAR γ ($P = 0.08$), while FASN tended to be up-regulated in inefficient steers ($P = 0.11$). These patterns of gene expression in skeletal muscle suggest that lipogenesis was up-regulated in inefficient heifers, while fatty acid synthesis was up-regulated in inefficient steers. In addition, IGFBP5 ($P = 0.06$) tended to be up-regulated in inefficient animals although there was no difference ($P > 0.05$) in expression of the type 1 IGF receptor, IGFBP2, IGFBP3 or growth hormone receptor (GHR). When analyzed by sex, inefficient heifers tended to show higher expression of IGFBP2 ($P = 0.12$), IGFBP3 ($P = 0.15$), IGFBP5 ($P = 0.08$), and GHR ($P = 0.13$), thus muscle anabolic processes may be altered. No differences in expression of these genes were detected in steers. Thus, differential gene expression provides a tool to suggest metabolic pathways that may be involved in RFI variation of beef cattle.

Key words: IGF, C/EBP α , PPAR γ

743 Effects of timing of an initial implant on performance of feedlot heifers. M. R. McDaniel^{*1}, W. C. Murdock¹, K. M. Taylor¹, N. P. Miller¹, B. H. Carter¹, F. Castillo¹, N. A. Elam³, D. U. Thomson², and C. A. Loest¹, ¹New Mexico State University, Las Cruces, ²Kansas State University, Manhattan, ³Nutrition Services Associates, Hereford, TX.

Calves affected by disease repartition nutrients to support immune function, which could interact with anabolic implant modulation of growth. This study evaluated effects of timing of an initial implant on performance of 408 Angus-cross heifers (200 ± 0.8 kg BW). Heifers were assigned to 24 pens and 3 treatments in a completely randomized design. Treatments were: 1) no implant (CON); 2) an implant (Revalor-H; 140 mg trenbolone acetate and 14 mg estradiol; Intervet/Schering-Plough Animal Health) at initial processing (IMP0); and 3) an implant (Revalor-H) 21 d after initial processing (IMP21). Heifers were fed once daily a 68% concentrate diet from d 0 to 21, a 75% concentrate diet from d 22 to 63, and an 82% concentrate diet from d

64 to 126. Statistical analysis used the mixed procedure of SAS and differences of least squares means. From d 0 to 21, DMI of heifers was not affected ($P = 0.34$), and ADG was greater ($P < 0.05$) for IMP0 (1.03 kg/d) than CON (0.86 kg/d) and lesser ($P < 0.05$) for IMP21 (0.65 kg/d) than CON. Also, G:F was greater ($P < 0.05$) for IMP0 (0.299) than CON (0.247) and lesser ($P < 0.05$) for IMP21 (0.196) than CON from d 0 to 21. From d 0 to 42, DMI of heifers was not different among treatments ($P = 0.31$), ADG was greater ($P < 0.05$) for IMP0 (1.23 kg/d) than CON (1.04 kg/d) and intermediate for IMP21 (1.15 kg/d), and G:F was greater ($P < 0.05$) for IMP0 (0.245) and IMP21 (0.238) than for CON (0.211). From d 0 to 63, DMI was not different among treatments ($P = 0.38$), ADG was greater ($P < 0.05$) for IMP0 (1.39 kg/d) and IMP21 (1.36 kg/d) than for CON (1.21 kg/d), and G:F was greater ($P < 0.05$) for IMP0 (0.238) and IMP21 (0.236) than for CON (0.212). From d 0 to 126, DMI was greater ($P < 0.05$) for IMP0 (7.01 kg/d) and IMP21 (7.00 kg/d) than for CON (6.75 kg/d), ADG was greater ($P < 0.05$) for IMP0 (1.31 kg/d) and IMP21 (1.33 kg/d) than for CON (1.24 kg/d), and G:F was not different ($P = 0.28$) among treatments. In summary, heifers that received IMP0 and IMP21 had similar performance by d 42, which implies that delaying the initial implant for 21 d does not affect overall feedlot performance. Authors acknowledge Texas Cattle Feeders Association funding.

Key words: implant, performance, heifer

744 Effect of feeding 25-hydroxycholecalciferol on porcine fetal myoblast proliferation and differentiation. E. A. Hines¹, J. D. Coffey¹, M. A. Vaughn¹, C. W. Starkey¹, T. K. Chung², and J. D. Starkey^{*1}, ¹Texas Tech University, Lubbock, ²DSM Nutritional Products Asia Pacific Pte. Ltd., Singapore.

Little is known regarding the effects of maternal supplementation of different vitamin D sources on fetal muscle development. To determine the effects of feeding the circulating metabolite of vitamin D, 25-hydroxycholecalciferol (25OHD3, Hy•D, DSM Nutritional Products), on fetal myoblast proliferation and differentiation, 38 PIC C22 gilts in 4 replicates were randomly assigned to 1 of 2 corn-soybean meal-based diets. The control diet (CTL) was formulated to contain 2,500 IU D3/kg diet, while the experimental diet (25OHD3) contained 500 IU D3/kg diet + 50 μ g 25OHD3/kg diet. Gilts were fed 2.7 kg of their assigned diet once daily beginning 42 d before breeding. Gilts were artificially inseminated with PIC 337-G semen 12 and 24 h after exhibiting estrus. At gestational d 90 (± 1), pregnant gilts were harvested (CTL n = 12; 25OHD3 n = 13), fetuses were extracted, and the right semitendinosus muscles of all fetuses from each litter were pooled and used for myoblast isolation. Myoblasts were cultured and proliferation index was measured at 24, 48, 72, and 96 h post plating using bromodeoxyuridine labeling and fluorescence microscopy. Myoblast differentiation capacity was determined by enumerating nuclei fused into myotubes 12 d post plating. Data were analyzed using the GLIMMIX procedure of SAS. The percentage of proliferating myogenic cells observed at 48 h was not different ($P > 0.05$) among treatments. However, at 24 ($P = 0.08$), 72 ($P = 0.07$), and 96 ($P < 0.05$) h post plating the percentage of proliferating myogenic cells was greater in the 25OHD3 cultures compared with CTL. Additionally, the differentiation capacity of myoblasts (percentage of nuclei fused into myotubes after 12 d in culture) was not different among treatments ($P > 0.05$). These results indicate that the proliferation phase of myoblasts derived from fetuses from 25OHD3 gilts may be extended. Extension of the proliferative phase of myoblasts in fetal muscle at gestational d 90, when muscle fiber hyperplasia is complete, could result in greater

capacity for muscle fiber hypertrophy and ultimate meat yield in the offspring of gilts fed 25OHD3.

Key words: myoblast, cell proliferation, vitamin D

745 Early postnatal myofiber increase in pig muscle results from myofiber elongation and tertiary myofiber formation. J. Bérard*^{1,3}, D. Loesel¹, A. Tuchscherer², C. Rehfeldt¹, and C. Kalbe¹, ¹*Leibniz Institute for Farm Animal Biology (FBN), Research Unit Muscle Biology and Growth, Dummerstorf, Germany,* ²*Leibniz Institute for Farm Animal Biology (FBN), Research Unit Genetics and Biometry, Dummerstorf, Germany,* ³*Institut Agricole Régional, Aosta, Italy.*

Myogenesis in pigs is commonly considered a biphasic phenomenon with the formation of primary and secondary fibers. Hyperplasia is accomplished around 90 d of gestation. However, some studies suggest a substantial increase in the total fiber number (TFN) from birth to 4 to 5 wk of age, but the origin is uncertain. Few studies indicate that early after birth tertiary myofibers of very small diameter expressing embryonic/fetal isoforms of myosin heavy chains (MyHC) appear. On the other hand, maturation and elongation of existing myotubes may contribute to muscle growth after birth. The aim of this study was to establish in which way TFN increases after birth and whether this increase is imputable to tertiary myofibers and(or) fiber elongation. The

semitendinosus muscle of 128 German Landrace piglets was examined by histological and (immuno-)histochemical techniques at d 1 (n = 63), 7 (n = 12), 21 (n = 12), and 28 (n = 41) of age. Birth weight groups (BWG) were established on the basis of the 25 and 75% quartiles of birth weight frequency distribution of all piglets; low (L-BW \leq 1.16 kg), medium (M-BW $>$ 1.16 to 1.52 kg), and heavy (H-BW \geq 1.52 kg). Data were analyzed by ANOVA using PROC MIXED of SAS including the fixed effects age, BWG and age x BWG. Least squares means were pair-wise tested by Tukey Kramer or Dunnett test. Eosin-stained muscle cross sections allowed determining muscle cross-sectional area and TFN. TFN was increased at d 7, 21, and 28 of age compared with d 1 of age ($P < 0.01$). From d 1 to 28 of age TFN increased from 463×10^3 to 825×10^3 on average and amounted 85% in L-BW, 91% in M-BW, and 61% in H-BW piglets ($P < 0.001$). Microscopy of longitudinal and consecutive (every 110 μ m) transversal sections revealed that at d 7 of age very small fibers expressing the embryonic MyHC isoform were apparent all over the muscle. In addition, intra-fascicular endings of normal-sized fibers expressed embryonic MyHC. These results suggest that the TFN is not fixed at birth and its postnatal increase may be related both to the elongation of existing muscle fibers and to the genesis of tertiary myofibers.

Key words: skeletal muscle, myogenesis, total fiber number