Growth and Development: Early Development and Fetal Programming

635 Evaluation of the NCAPG I442M locus, a major gene for bovine prenatal growth, for effects on postnatal development compared to a disruptive mutation in the myostatin encoding gene GDF8. C. Kuehn*, P. Widmann, R. Pfuhl, and R. Weikard, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.

Recently, we identified the I442M mutation in the bovine non-structural maintenance of chromosomes condensin I complex, subunit G (NCAPG) gene affecting bovine prenatal growth in an F2 resource population. Due to consistent application of embryo transfer during generation of the resource population, the direct genetic background of divergent postnatal growth dissected from putatively persistent maternal allelic effects could be addressed. In addition to the NCAPG I442M locus, also the disrupting mutation Q204X of the growth differentiation factor 8 (GDF8) segregated in our resource population. Thus, the effects of both loci could be evaluated on an identical genetic background. For our study, male calves were fed a milk replacer/hay/concentrate diet until d 121 followed by a semi ad libitum feed ration of concentrates and chaffed hay. Body weight was recorded monthly until 18 mo of age. All P0, F1 and F2 individuals were genotyped for NCAPG I442M and GDF8 Q204X. Association analyses were performed with a single locus and a 2 locus model fitting a fixed effect of the year of birth, an additive genetic SNP effect and an infinitesimal polygenic animal effect. The NCAPG 442M allele that had been associated with increased birth weight, showed a significant effect ($P = 0.0001$) increasing body weight at 18 mo of age. The locus explained 9.3% of the variance in the model. The effect of the loss-of-function allele GDF8 204X on body weight was also significant ($P = 0.006$). The difference between alleles amounted to 25.4 kg (std. err. 6.34 kg) for NCAPG I442M and 30.0 kg (std. err. 12.5 kg) for GDF8 Q204X, respectively. For the 2-locus model, effects of essentially the same magnitude were obtained. In conclusion, both, the NCAPG I442M locus and the GDF8 Q204X locus, exhibit significant, independent effects on postnatal growth.

Key Words: postnatal growth, cattle, NCAPG


Maternal nutrition during mid-late gestation influences adipocyte development in the fetus of various species, however bovine research is limited. The objective of this experiment was to determine the effects of maternal nutrition on the expression of genes in bovine fetal tissues. Genes of interest were selected because each has been demonstrated previously to influence body composition. Twenty-two Angus cross-bred heifers (BW = 527.73 ± 8.3 kg) were assigned randomly to 3 dietary treatments. Maternal dietary treatments were formulated and intake was controlled to provide 150% (HIGH; $n = 7$), 100% (INT; $n = 7$), and 80% (LOW; $n = 8$) of maintenance energy requirements for growing pregnant heifers. Heifers received dietary treatment from d 85 to d 180 of gestation, at which time fetuses were removed via cesarean section and muscle, subcutaneous fat, and liver samples were collected. At trial initiation, dam BW was similar among treatment groups. Final BW was lowest for the LOW dams ($P < 0.05$), however final BW for INT and HIGH were similar. Ribfat thickness increased in the HIGH treatment group compared with LOW and INT dams ($P < 0.05$). Thus, dam growth was influenced by diet during the treatment period, however dietary treatment did not influence fetal weight, crown rump length, or liver weight. Preadipocyte factor-1 showed increased expression in fetal LM ($P < 0.05$) of HIGH fetuses as compared with INT and LOW. Peroxisome proliferator-activated receptor gamma and C/EBPα did not differ as a result of dietary treatment. However, LOW fetuses showed increased C/EBPβ expression as compared with INT ($P < 0.05$). Collectively these results suggest that fetal growth characteristics are not affected by maternal nutritional manipulation during mid-gestation in beef cows. However, differences in expression of fetal genes regulating adipose tissue growth and development could lead to differences in composition of growth and warrants further investigation.

Key Words: adipose tissue, beef cattle, fetal programming


Enhancing adipogenesis in muscle increases intramuscular adipocytes, while increasing fibrogenesis would affect meat tenderness. The objective was to evaluate the effects of maternal obesity on the intramuscular fat and collagen content of offspring muscle. Multiparous ewes (Rambouillet/Columbia cross) were fed a control diet (100% energy requirement, Con, $n = 8$) or an obesogenic diet (150% energy requirement, OB, $n = 9$) from 2-mo before pregnancy to parturition. Then, offspring lambs were fed commercial feeds to 22 mo old. The Longissimus dorsi (LD) muscle (2g) was biopsied at the left 12th rib for histochemical examination, mRNA and protein expression analyses and collagen content assessment. Mean ± standard errors of means are reported. No difference was observed in maternal body weight (68.3 ± 2.9 kg vs. 71.6 ± 3.2 in Con and OB) or body condition score (4.9 ± 0.4 in Con and 5.0 ± 0.3 in OB) before dietary treatments. Following 2 mo treatment (before mating), both maternal body weight (73.1 ± 4.0 vs. 108.8 ± 3.1 in Con and OB, $P < 0.05$) and maternal body condition score (4.9 ± 0.3 vs. 8.6 ± 0.2, $P < 0.05$) were higher on OB compared with Con. More intramuscular adipocytes were observed in OB offspring muscle compared with Con muscle; the mRNA expression of peroxisome proliferator-activated receptor (PPAR) γ, an adipocyte marker, was 33.6 ± 15.6% higher ($P < 0.05$) in OB, and the protein content was 51.1 ± 5.1% greater ($P < 0.05$), consistent with the 32.1 ± 9.8% higher intramuscular triglyceride content in OB compared with Con muscle ($P < 0.05$). The mRNA and protein contents of fatty acid transport protein 1 (FATP1) were increased in OB group by 61.8 ± 24.8% and 40.8 ± 9.3% ($P < 0.05$) respectively. We also detected 39.4 ± 8.8% higher mRNA expression for fatty acid translocase (FAT/CD36) ($P < 0.05$). In addition, 50.6 ± 15.3% higher collagen content was detected in OB compared with Con muscle; the mRNA expression of fibroblast growth factor (FGF)-2 and C/EBPβ expression as compared with INT ($P < 0.05$) were different. Thus, differences in expression of fetal genes regulating adipose tissue growth and development could lead to differences in composition of growth and might affect the quality of resulting meat.

Key Words: maternal obesity, collagen, muscle

638 Enhanced transforming growth factor β (TGF-β) signaling and fibrogenesis in ovine fetal skeletal muscle of obese dams at late gestation. Y. Huang*, X. Yan, M. J. Zhu1, R. J. McCormick1, S. P. Ford1, P. W. Nathanielsz2, and M. Du1, 1Department of Animal Science, University of Wyoming, Laramie, 2University of Texas Health Science Center, San Antonio.

Recently, we identified the I442M mutation in the bovine non-structural matrix growth and development could lead to differences in composition of growth and warrants further investigation.
Maternal obesity is increasing at an alarming rate. The objective was to evaluate the effect of maternal obesity on fibrogenesis in fetal skeletal muscle at late gestation when ovine fetal muscle matures. Non-pregnant ewes were assigned to a control diet (Con, fed 100% of NRC nutrient recommendations, n = 6) or an obesogenic diet (OB) fed 150% of NRC recommendations, n = 6) from 60 d before conception, and fetal semitendinous (ST) muscle was sampled at 135 d of gestation (term 148 d). The total concentration and the area of collagen in the cross-sections of muscle increased by 27.0 ± 6.0% (P < 0.05) and 105.1 ± 5.9% (P = 0.05) in OB compared with Con group. The expression of TGF-β was 177.3 ± 47.6% higher (P < 0.05) in OB fetal muscle. The concentration of phospho-p38 was 74.7 ± 23.6% higher (P < 0.05) in OB group. An increase of 327.9 ± 168.0% (P < 0.05) and 189.0 ± 82.1% (P < 0.05) respectively for the mRNA expression of Smad7 and fibronectin was observed in OB compared with Con samples. In addition, enzymes involved in collagen synthesis, including lysyl oxidase, lysyl hydroxylase 2b and prolyl 4-hydroxylase α1 were increased by 350.2% ± 90.0% (P < 0.05), 236. Five ± 25.2% (P < 0.05) and 82.0 ± 36.2% (P < 0.05) respectively in OB muscle. In conclusion, maternal obesity enhanced fibrogenesis in fetal muscle at late gestation which was associated with upregulation of TGF-β/p38 signaling pathway. Because muscle fibrosis is a hallmark of aging, enhanced fibrogenesis at such an early stage of development is expected to negatively affect the properties of offspring muscle.

**Key Words:** collagen, TGF, muscle

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**639 Up-regulation of nutrient transporters in the placenta of nutrient-restricted pregnant ewes.** Y. Ma*,1, M. J. Zhu,1 P. W. Nathanielsz2, and S. P. Ford1,1 Center for the Study of Fetal Programming. Univ. of Wyoming, Laramie, 2Center for Pregnancy and Newborn Research, Univ. of Texas Health Sciences Center, San Antonio.

In sheep, maternal:fetal exchange occurs in placentomes, comprised of uterine caruncular and placental cotyledonary (COT) tissues. Glucose transporters (GLUT) and fatty acids transporters (FATP) in COT deliver glucose and long chain fatty acids (LCFA) to the fetal compartment. We have reported that fetuses from ewes fed to 50% NRC recommendations from 28 to 78 d of gestation (dGA; nutrient restricted, NR) weighed ~30% less than fetuses gestated by control ewes (C, 100% of NRC) at 78dGA. In contrast, NR fetuses exhibited a marked increase of LCFA storage in their lung, liver and muscle. When NR ewes were re-alimented to a C diet from 78dGA, their fetuses exhibited weights similar to C fetuses by 135dGA. COT tissue collected on 78 and 135dGA was used to investigate GLUT and FATP systems via Realtime PCR and Western blot. Ewes assigned to C (n = 5) and NR (n = 6) groups were necropsied on 78dGA, while 6 C and 7 NR-realimented ewes were fed the C diet from 78 to 135dGA before necropsy. At 78dGA, COT GLUT1 mRNA and protein levels were greater (P < 0.05) in NR than C ewes. Similarly, COT FATP4 mRNA and protein levels were greater (P < 0.05 and P = 0.06, respectively), and CD36 mRNA and Protein levels tended to be greater (P = 0.06) in NR versus C ewes. At135dGA, COT FATP4 mRNA and protein levels tended to remain elevated (P = 0.06 and P = 0.09, respectively) in NR-realimented versus C ewes. CD36 and GLUT3 protein levels also tended to remain elevated (P = 0.08 and P < 0.05, respectively) in NR-realimented versus C ewes on 135dGA. The increased COT GLUT and FATP expression in NR versus C ewes at 78dGA is consistent with increased placental efforts to increase maternal nutrient transfer to the fetus. The continued elevation in COT GLUT3, FATP4 and CD36 expression after realimentation of NR ewes, would facilitate delivery of the increased blood levels of maternal nutrient to the fetus, accelerating its growth, and possibly causing metabolic problems in postnatal life.

**Key Words:** maternal nutrient restriction, placental nutrient transport, sheep

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**640 Effect of grouping calves post-weaning according to pre-grouping feed intake on animal performance.** C. M. Matuk1, M. Chahine1, A. Bach1, B. Ozer1, M. E. de Haro Martí1, J. B. Glaze Jr1, T. Fife1, and M. Nelson1,1 University of Idaho, Twin Falls, 2IRTA, Cadiles de Montbui, Spain, 3ICREA, Barcelona, Spain, 4University of Idaho, Gooding.

The effect of grouping calves post-weaning according to pre-grouping feed intake on animal performance was evaluated using 752 replacement Holstein calves raised on a large operation in southern Idaho. In 4 different periods, individual feed intake was recorded 4 times a week during the last 3 wk that calves were individually hutch (56 d of age). Calves were classified as ‘high eaters’ (highest feeding level quartile) and ‘low eaters’ (lowest feeding level quartile). When leaving the individual hutches in each period, calves formed 6 groups: 20 animals randomly chosen without considering their level of feed intake (Control), 20 calves within the highest quartile of feed intake during the 3 wk prior leaving the hutch (HH), 20 within the lowest quartile (LL), 5 calves from the lowest and 15 from highest feeding level (LHH), 15 calves from the lowest and 5 from highest feeding level (LLH), and 10 calves from the highest and 10 from lowest feeding level (HL). Thus, out of 752 initially-tracked heifers, 480 heifers were chosen to form the 20 groups (6 groups per period) that were studied. Pen feed intake was recorded during the first 4 wk after grouping. After grouping, calves received a TMR composed of 95% starter and 5% alfalfa. Final weight was recorded at the end of the 12 wk of study. Pen was the experimental unit. Data were analyzed using a mixed-effects model with repeated measures accounting for the random effect of period and pen and the fixed effects of treatment, intake level class, time of measurements, and their 2-way interaction. Average DMI after grouping was greatest (P < 0.05) in HH (2.24 kg/d) and HHL (2.15 kg/d) followed by HL (2.07 kg/d), Control (2.06 kg/d), LLH (1.92 kg/d and LL (1.77 kg/d). Similarly, ADG was greatest (P < 0.05) in HH (694 g/d) and HHL (658 g/d) than in HL (584 g/d), LLH (571 g/d), Control (546 g/d), and LL (531 g/d). The coefficient of variation of final BW (at 84 d of age) was lowest (P < 0.05) for HH (9.3%) and LL (11.7%), followed by Control (12.9%), LLH (15.8%), HH (13.5%), and HL (17%). Grouping calves according to pre-weaning intake improves overall animal performance and diminishes variation.

**Key Words:** calves, heifers, intake

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**641 Evaluation of serum protein-based arrival formula and serum protein (Gammulin) on growth, morbidity, and mortality of stressed dairy calves.** A. Pineda*,1 J. K. Drackley1, and J. M. Campbell2, 1University of Illinois, Urbana, 2APC, Inc., Ankeny, IA.

Appropriate nutrition is a crucial factor to decrease morbidity and mortality of pre-weaning dairy calves. Several nutritional additives are available that may help to achieve this goal; however, their effectiveness is uncertain. The objective of this study was to evaluate a serum protein-based arrival formula (AF) and use of a commercial serum protein supplement (G, Gammulin, APC Inc.) in milk replacer for stressed (transport, cold) male calves on performance, morbidity, and mortality. Ninety-three male Holstein calves were stratified by arrival BW and plasma protein, and then randomly assigned to 1 of 4 treatment groups: Treatment A = AF, milk replacer without G (n = 22); Treatment B =
control electrolyte, milk replacer without G (n = 25); Treatment C = AF, milk replacer with G (n = 22); and Treatment D = control electrolyte, milk replacer with G (n = 24). At arrival, calves were fed either AF or a control electrolyte solution. At the next feeding, all calves received either a commercial calf milk 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). Feed offered and refused was recorded daily. Calf health was assessed by daily assignment of scour and respiratory scores. Body weight, withers height, body length, heart girth, hip height, and hip width were measured weekly. Calves remained in the experiment until d 56. Data were analyzed using the MIXED procedure of SAS (v. 9.2). Results indicated that, during the first 2 wk of dietary treatment, calves fed AF had significantly greater heart girth (P = 0.05) and body length (P = 0.03), while G supplementation resulted in greater BW (P = 0.05). In addition, a significant (P = 0.03) interaction of G × week was observed for ADG. Mortality was greater (P = 0.007) for calves that did not receive G. Addition of a serum protein product improved early growth and decreased mortality in transported male calves.

Key Words: calves, serum protein, Gammulin


To determine the effects of maternal exercise of gestating gilts on fetal piglet development and growth, Yorkshire gilts (n = 8), bred to a common boar, were placed in individual gestation stalls at d 30 of gestation. Treatments were assigned and initiated at d 40 of gestation. Exercise gilts (EX) were housed in individual gestation stalls but were individually exercised 3 times per week for 30 min until d 105 of gestation. Control gilts (CON) remained in gestation stalls for the duration of gestation. All farrowings were attended. Within 12 h of completion of farrowing, the lightest (LWT) and heaviest (HWT) male and female from each litter, excluding piglets < 800 g, were selected for necropsy. Adrenal glands, brain, digesta, heart, kidneys, intestines, liver, lung, pancreas, spleen, stomach, gonads, semimembranosus (SM) and semitendinosus (ST) were dissected and weighed. Organ weights (g) and organ weight/live BW (g/g) were analyzed by PROC MIXED. Live weight of necropsied piglets was greater in offspring from EX gilts (P = 0.03; 1282.06 and 1698.70 ± 125.40 g). Piglets from EX gilts had larger adrenal glands (g) (P < 0.01), kidneys (g) (P < 0.01), liver (g) (P < 0.01), and stomach (g) (P < 0.01) than CON gilts. Piglets from EX gilts had a tendency (P < 0.10) to have more digesta (g), heavier hearts (g), intestines (g), spleen (g), and SM (g/g). The brain and pancreas were the only organs not affected by treatment. Treatment by weight interaction occurred in liver (g/g) (P < 0.05), ovaries (g) (P < 0.02), and uterus (g/g) (P < 0.01). Light weight EX offspring had larger liver (g/g) (P < 0.001) compared with LWT CON offspring but were not different compared with HWT CON and EX treatments. LWT CON livers were also smaller than HWT EX (P = 0.01) but not different than HWT CON livers. Ovaries (g) were lightest in gilts from LWT CON gilts compared with all other treatment groups (P < 0.05). Light weight CON offspring had heavier uteri (g/g) compared with all other treatment groups (P < 0.05). Maternal activity during mid to late gestation influenced the developmental composition of the neonate.

Key Words: neonatal offspring, organ weights, pigs


The objective of the present study was to determine the effects of in ovo feeding of exogenous nutrients on the glycogen reserves, protein status and early growth of Pekin ducks. To this end, based on randomized completely block design, 750 fertile eggs were divided into following 5 groups of 150 eggs: 1) Uninjected; 2) 0.35% sodium chloride (NaCl); 3) 2.5% sucrose + 3% maltose (CHO); 4) 0.22% arginine (Arg); and 5) 2.5% sucrose + 3% maltose + 0.22% arginine (CHO + Arg). At 23 d of incubation, 1.2 mL of each solution was injected into amniotic fluid of each group using a 22-gauge needle. Ten eggs/ducklings per treatment were sampled at 25 d of incubation, hatch, 3 and 7 d of age to determine liver and muscle glycogen, glucose-6-phosphatase (G6P) activity and different protein expression including S6K1 (S6 kinase1), phosphorylated S6K1 and phosphorylated adenosine monophosphate-activated protein kinase (AMPK) using iodine reduction test, colorimetric oxidase test and Western blot, respectively. Maximal hatchability was found in the group (P < 0.05) fed with CHO + Arg (94%) followed in order by Arg (90%), CHO (89%), uninjected control (85%) and NaCl (80%). All the ovo fed ducklings improved BW at hatch, 3, 7, 14, 21, 28 and 35 d of age related to uninjected (P < 0.05). Arg and CHO + Arg had significantly enhanced the liver glycogen by 188% and 249%, respectively at hatch (P < 0.01) compared with that of uninjected group.
CHOs and CHO+Arg significantly increased muscle glycogen level (P < 0.01) by 22% and 42%, respectively at 25 d of incubation over the uninjected group. CHO and Arg had significantly decreased (P < 0.01) G6P by 41% and 30%, respectively at 25 d of incubation, whereas NaCl and CHO+Arg increased G6P by 30% and 20%, respectively at hatch in comparison with the uninjected group (P < 0.01). At 25 d of incubation, hatch, 3 and 7d posthatch, greater values of S6K1 and S6 phosphorylation were observed in duck embryos and neonates fed with Arg and CHO+Arg. The activation of AMPK was also detected in the group fed with Arg and CHO+Arg. The present results indicated that in ovo feeding CHO and Arg may improve glycogen storage and muscle protein deposition in ducks

Key Words: in ovo feeding, energy, protein, metabolism, growth, ducks

645 Effect of induced moisture loss on embryonic development of pekin ducks. C. Noonan* and M. S. Lilburn, Ohio State University/OARDC, Wooster.

Moisture loss in commercial Pekin duck eggs during incubation is often variable and accelerated moisture loss may contribute to excessive hatchling dehydration and adversely effect later developing systems such as the intestine. Two experiments were conducted to determine the effect of induced moisture loss on embryonic development. In both experiments, commercial duck eggs were individually weighed at set. At 12 d, all eggs were reweighed and randomly allocated to one of four treatments consisting of 0, 1, 2, or 3 holes (Experiment 1; n = 40 per treatment) or 0, 1 or 2 holes (Experiment 2; n = 120 per treatment). The holes (<1 mm) were drilled above the air sac. All eggs were reweighed at approximately 2-d intervals. All data was analyzed by ANOVA using the PROC Mixed program (SAS Inc.) and least squares means were separated using LSD. In Experiment 1, there were no differences in initial egg wt or moisture loss (4.67%; P > 0.05) at 12 d. On D 14 and all sample days thereafter, there were incremental increases in moisture loss with each additional hole (P < 0.01). At D 19, the range was 7.35% (0-hole) to 20.86% (3-hole) and the experiment was terminated. In Experiment 2, the range was from 5.32% (0-hole) to 7.34% (2-hole) on D 14 (P < 0.01) and 8.03% (0-hole) to 15.15% (2-hole) on D 19. On Day 20, a sample of embryos from each treatment (n = 25) was broken out for embryo weight determination. Wet embryo weight was heavier (P < 0.01) in the 1-hole treatment (36.7 g) but no effects on intestinal measures (villus height; crypt depth). In conclusion, major differences in moisture loss during incubation profiles may have more importance on bone development than the genetic background, and the epigenetic effects of parental feed restriction were only observed at hatch.

Key Words: bone development, incubation, epigenetics


The effect of injecting graded levels of selenium (Se) as selenomethionine (SeMet) or sodium selenite (Na2SeO3) into the yolk of incubating eggs on embryo viability and liver Se levels was studied. Fertile eggs were obtained from white shell laying hens (Hy-Line W-36) that were fed a low Se corn-soybean meal diet. On Day 10 of incubation, eggs were candled to ensure embryo viability. The shell surface was disinfected with alcohol and a small hole was drilled over the air cell. The yolk of each of 30 eggs per treatment was then injected with 0.1 mL of a phosphate buffered saline solution providing 0, 2.5, 5, 10 or 20 μg Se as either SeMet or Na2SeO3. In a control group of eggs holes were drilled in the shell, but no injection was administered. The holes were sealed with glue and eggs were returned to the incubator. On Day 20 of incubation, eggs were candled to determine viability. Viable embryos were then killed to obtain tissue samples. Liver samples were analyzed for Se using fluorometric analysis following digestion in nitric and perchloric acids. Embryo viability values for the non-injected eggs and eggs injected with buffer without Se were 100% and 94%, respectively. Viability values for eggs injected with 2.5, 5, 10 and 20 μg Se as SeMet were 97, 94, 90 and 83%, respectively, while the respective values for Na2SeO3 were 87, 94, 74 and 87%, respectively. Injecting graded doses of Se resulted in linear increases (P < 0.001) in liver Se. However, the regression coefficient for Na2SeO3 was greater than that for SeMet (0.059 vs. 0.014). The results indicate that in ovo injection of Se as SeMet or Na2SeO3 at levels up to 20 μg does not have a detrimental effect on embryo viability. The effects of the SeMet and Na2SeO3 on liver Se concentrations suggests that the compounds are metabolized differently by the chick embryo.

Key Words: in ovo injection, selenium, embryo viability