

Growth and Development: Regulatory Mechanisms in Growth and Development

105 The effect of feeding frequency on circulating thyroid hormones in turkey chicken. A. Towhidi*, A. Yahyabeig, and E. Dirandeh, *University of Tehran, Karaj, Tehran, Iran.*

A strategy that improves growth efficiency is to reduce basal metabolic rate, providing additional energy for growth. Thyroid hormones enhance metabolism and lipolysis. Previous studies have shown that increase of feeding frequency could change blood hormonal profile affecting metabolism and growth in some animals. The objective of this study was to investigate the effect of increased feeding frequency on thyroid hormones status in turkey. Twenty turkey chickens were randomly assigned to 2 groups. In control group, turkey chickens were fed ad libitum, whereas birds in treatment group fed every 4 h and blood samples were collected at 4 intervals. Data were analyzed with Proc Mixed of SAS. Overall mean plasma concentration of triiodothyronine in treated group was higher than control (4.05 ± 0.34 vs. 2.59 ± 0.30 ng/mL, $P = 0.0001$). Mean plasma concentration of thyroxin in treated group was higher than control (47.50 ± 1.6 vs. 24.08 ± 1.7 μ g/dL, $P = 0.0001$). It was concluded that increasing feeding frequency failed to decrease thyroid hormones level. As a result, this kind of feeding management may not be efficient to improve growth performance in turkey chicken.

Key Words: turkey, thyroid hormones, feeding frequency

106 The role of syndecan-4 cytoplasmic domain in turkey skeletal muscle growth and development. Y. Song*¹, D. C. McFarland², and S. G. Velleman¹, ¹*Ohio Agricultural Research and Development Center, The Ohio State University, Wooster;* ²*Department of Animal and Range Sciences, South Dakota State University, Brookings.*

Skeletal muscle formation is a complex process involving the interactions between cells and their extracellular matrix. Syndecan-4 is a cell membrane heparan sulfate proteoglycan that passes signals from the extracellular matrix into the cell. Syndecan-4 core protein is composed of an extracellular domain, a transmembrane domain, and a cytoplasmic domain. The cytoplasmic domain is important in regulating signal transduction into the cell and the formation of focal adhesion complexes which is critical for cell survival. In the current study, the role of the syndecan-4 cytoplasmic domain in muscle cell proliferation, differentiation, fibroblast growth factor 2 (FGF2) responsiveness, and apoptosis was explored. Turkey wild type syndecan-4 (S4) and syndecan-4 without the cytoplasmic domain (S4C) were cloned into the pCMS-EGFP vector and then subcloned into the pcDNA3.1/V5-His TOPO TA expression vector. Wild type syndecan-4, S4C, and the pCMS-EGFP empty vector were transfected into turkey skeletal muscle satellite cells. After transfection, cell proliferation, differentiation, and FGF2 responsiveness were measured. Wild type syndecan-4 and S4C subcloned into the pcDNA3.1/V5-His TOPO TA expression vector were used to test cell apoptosis by a CaspACE assay using CaspACE FITC-VAD-FMK in situ marker which fluorescently labels apoptotic cells. The pcDNA3.1/V5-His TOPO TA expression vector was used because it does not express green fluorescence protein as the pCMS-EGFP vector which will interfere with the CaspACE assay. Results showed that the overexpression of S4C decreased cell proliferation ($P < 0.05$) but did not change cell differentiation, or responsiveness to FGF2 during proliferation and differentiation compared with the cells transfected with S4. The cells transfected with S4C had more apoptotic cells compared with those transfected with S4. These results suggest that syndecan-4 plays a critical role during cell

proliferation, and the syndecan-4 cytoplasmic domain regulates cell proliferation and survival in an FGF2-independent manner.

Key Words: turkey, muscle satellite cell, growth and development

107 Comparative phylogenetic analysis of gut microbiota of broilers fed with and without antibiotics. P. Singh*¹, A. Karimi², P. W. Waldroup¹, and Y. M. Kwon¹, ¹*University of Arkansas, Fayetteville,* ²*University of Kurdistan, Sanadaj, Kurdistan, Iran.*

Antibiotics growth promoters (AGP) have been used for growth promotion of chickens in poultry industry since 1950. Recently, concerns have been raised to the use of AGP in livestock due to development of antibiotic resistance in bacteria. The objective of our study is to investigate the effect of AGP in cecal microbiota of broiler chickens. Two groups ($n = 30$) of chickens were fed corn-soybean meal diets with (ANT) and without supplementation of Penicillin (CON) at the concentration of 55 mg/kg. At 18 d of age, ANT group had significantly ($P < 0.05$) higher mean body weight than CON group (668.6 vs. 570.0 g). Cecal samples of 5 randomly selected birds were pooled from each group and used for genomic DNA isolation and PCR amplification of 16S rRNA gene. 454 pyrosequencing of the amplicons resulted in 7,881 and 11,214 sequence reads for CON and ANT groups, respectively. BLAST and phylogenetic analysis using MEGAN-3 indicate that AGP supplementation in ANT group resulted in elevated proportion of phylum Firmicutes from 16.67% to 17.47% and a decreased proportion of phylum Bacteroidetes from 15.71% to 0.14% as compared with CON group. Recent studies conducted in humans, pigs and mice have shown a similar shift in gut microbiota in obese individuals as compared with lean ones, indicating that this shift could be responsible for increase in energy harvest and body weight. The results of this study suggest that the growth promoting effect of AGP supplementation in broilers may be mediated by a similar microbial process. Hence, new and alternative methods for promoting the growth of the birds need to be sought for that may alter gut microflora in a similar pattern.

Key Words: antibiotics growth promoter, 16S rRNA gene, cecal microbiota

108 Impact of feeding raw materials on intestinal viscosity and performance of broilers. F. Nuyens¹, I. Somers¹, S. Van De Craen¹, W. Röser¹, C. Chudaske², and S. Van Dyck*¹, ¹*Kemin AgriFoods Europe, Herentals, Belgium,* ²*Südzucker AG Mannheim/Ochsenfurt, Ochsenfurt, Germany.*

Alternative cereals and cereal by-products can be used to reduce the costs of poultry diets. However, the application of these new raw materials may call for an evaluation of their effect on gut viscosity. This paper evaluates the changes that appear in fiber analysis and in vitro viscosity for diets including 10% of wheat-based distillers dried grains with solubles (DDGS). It is the objective of this paper to assess the impact of feed viscosity changes on broiler performance and intestinal viscosity. Furthermore, the beneficial effect of feed enzymes will be demonstrated. A broiler growth trial was performed with 200 birds divided over 4 treatments (see Table 1) in a randomized block design. On d 21 of the trial, 9 broilers were sacrificed per treatment and viscosity of jejunum and ileum contents was measured (Dusel et al., 1997). The use of feed enzyme in the diet had significant effects ($P < 0.05$) on the intestinal viscosity (Table 1). By use of the feed enzyme in the diets that included

DDGS, the intestinal viscosity was decreased by 40% in the jejunum and 24% in the ileum. The results show that the fiber content in DDGS is much greater than in regular wheat. The DDGS did not induce a greater viscosity than regular wheat. The bioethanol production process might have reduced part of the soluble, long-chain arabinoxylan fibers that are responsible for the viscosity effect. The low viscosity values of both wheat and DDGS after treatment with a feed enzyme indicate the potential to control intestinal viscosity when wheat or DDGS are used in the feed formulation. The treatment with highest intestinal viscosity showed poorest daily gain and feed conversion ratio (FCR), while the group with lowest intestinal viscosity showed the best zootechnical performance (Table 1, $P < 0.05$).

Table 1. Intestinal viscosities and FCR for broilers fed a regular wheat diet and a modified wheat diet with 10% DDGS. The Feed Enzyme was supplemented at a dosage of 500 g/tonne.

Treatment	Jejunum	Ileum	FCR
Control	3.73 ^a	5.28 ^{ab}	1.83 ^{ab}
Control + DDGS	4.67 ^b	6.50 ^b	1.89 ^b
Control + Feed Enzyme	2.60 ^c	4.14 ^a	1.75 ^a
Control + DDGS + Feed Enzyme	2.81 ^c	4.95 ^a	1.85 ^b
SEM	0.048	0.018	0.001
p-value	0.0001	0.0039	0.0006

Means in same column with different superscripts are significantly different ($P < 0.05$).

Key Words: DDGS, Feed enzyme, intestinal viscosity

109 Ontogenic changes in the activation of translation initiation factors post feeding are not seen in adolescent Thoroughbred mares. A. L. Wagner*, J. C. Gould, R. B. Ennis, and K. L. Urschel, *University of Kentucky, Lexington.*

Following consumption of a protein meal, signaling proteins associated with the mTOR pathway are activated, triggering muscle protein synthesis due to increases in both insulin and amino acid (AA) concentrations. In neonates, the activation of the mTOR pathway decreases with age resulting in lower skeletal muscle protein synthesis. The slower growth seen during the adolescent age has not been studied with regards to mTOR-related signaling in any species. The purpose of this study was to determine the effects of an 18h feed withholding period, either with (postprandial; PP) or without (post-absorptive; PA) the re-feeding of a protein meal ($t = 0$ min), on the activation of translation initiation factors in gluteal muscle of yearling, 2y old, and mature Thoroughbred mares ($n = 18$). Blood samples were taken during the experimental protocol to measure plasma AA via HPLC. A gluteal muscle biopsy was taken after the last blood sample ($t = 90$ min) to measure the phosphorylation (P) of Akt at Ser⁴⁷³ and Thr³⁰⁸, 4EBP1 at Thr^{37/46}, rpS6 at Ser^{235/236;240/244}, and p70 S6 Kinase at Thr³⁸⁹ using Western blotting. For all horses, indispensable plasma AA were ~25- 110% higher in the PP versus PA period ($P < 0.01$). There was a significant increase in Akt P-Ser⁴⁷³ ($P < 0.01$), 4EBP1 P-Thr^{37/46} ($P < 0.01$), rpS6 P-Ser^{235/236} ($P < 0.01$), rpS6 P-Ser^{240/244} ($P < 0.01$), and p70 S6 Kinase P-Thr³⁸⁹ ($P < 0.01$), and a trend for higher AktP-Thr³⁰⁸ ($P = 0.10$) in the PP versus PA state. There was an interaction of age and treatment on the P of 4EBP1 ($P = 0.05$), and a trend for an interaction for the P of p70 S6 Kinase ($P = 0.08$). Age did not have an effect on the P of any protein ($P > 0.05$), although there was a trend for an age effect on AktP-Thr³⁰⁸ ($P = 0.10$). Consumption of a high protein meal, appeared to activate proteins associated with the mTOR pathway in adolescent and mature horses. However, growth of

adolescent horses did not have a greater effect on the P of the proteins in the mTOR pathway compared with mature horses. More research is necessary to determine whether an effect of age is present in the rapidly growing equid neonate.

Key Words: mTOR pathway, growth, protein synthesis

110 Productive performance of pigs vaccinated against gonadotropin releasing factor compared to surgically castrated males and gilts from two different sire lines. J. I. Morales¹, M. P. Serrano*², L. Cámara², C. H. Zúñiga², J. P. López¹, and G. G. Mateos², ¹Copiso S.A., Soria, Spain, ²Universidad Politécnica de Madrid, Madrid, Spain.

A total of 360 pigs was used to study the influence of gender (immunocastrated males, IM; surgically castrated males, CM; intact females, IF) and terminal sire line (Top York; Tempo) on productive performance of pigs slaughtered at 125 kg BW. The female line used was Large White × Landrace in all cases. Improvac (Pfizer, Madrid, Spain) was used for active immunization against GnRH. The IM pigs received a first dose of Improvac at 76 d of age (16 d on trial) and a second dose at 124 d of age (64 d on trial; 7 wk before slaughter). There were 6 treatments (6 replicates of 10 pigs each) arranged factorially (3×2) with 3 genders and 2 terminal sire lines. From 60 to 76 d of age (IM were still entire males), IF and IM had lower ADG than CM (729 and 749 vs. 792 g/d; $P < 0.001$) but no differences were observed for feed intake. From 76 (injection of the first dose of the immune vaccine) to 124 d of age, IM had lower ($P < 0.001$) ADFI and ADG than CM with IF being intermediate. However, from 124 (injection of the second dose of the immune vaccine) to 172 d of age, IM recovered and in fact, they had better ($P < 0.001$) ADG and FCR than IF and CM. For the entire experimental period, IF and IM ate less feed than CM (2.43 and 2.43 vs. 2.59 kg/d; $P < 0.001$) whereas IM and CM had higher ADG than IF (952 and 945 vs. 913 g/d; $P < 0.01$). Also, IM had better FCR than CM with IF being intermediate (2.55 vs. 2.66 vs. 2.74 g/g; $P < 0.001$). From 60 to 172 d of age, crossbreds from Tempo sires had better ADG than crossbreds from Top York sires (952 vs. 922 g/d; $P < 0.001$) but no differences were observed for ADFI or FCR. We conclude that IM have better ADG and are more efficient than CM. Consequently, based on growth performance data, IM are a good alternative to CM for the production of 125 kg BW pigs. Also, the Tempo sire line is a good alternative to the Top York sire line for the production of heavy pigs.

Key Words: immunocastration and gender, sire line, pig performance

111 Effects of nutrient balance and implant status on IGF-1 and PUN concentrations of feedlot calves. T. Lee*, L. K. Mamedova, S. Guillosoy, B. J. Bradford, C. D. Reinhardt, and D. U. Thomson, *Kansas State University, Manhattan.*

To simulate nutritional conditions in highly stressed feedlot calves with low nutrient intake, 16 predominantly English-breed calves (BW = 293.3 ± 5.41 kg) were used in a 2×2 factorial experiment to evaluate the main and interactive effects of poor vs. adequate nutrient intake and implant status on growth and PUN and serum IGF-1 concentrations. All calves were individually fed a common, pelleted, complete diet (15.3% CP; 1.44 Mcal NE_m/kg DM). After a 28-d period of adaptation to the Calan gates and the diet, 4 calves each were randomly assigned to receive either (1) implant + 2× maintenance energy intake; (2) implant + 1× maintenance energy intake; (3) no implant + 2× maintenance energy intake; or (4) no implant + 1× maintenance energy intake. Dietary NE_m content was estimated using dietary ingredient concentrations and NRC (1984) nutrient values. Animal NE_m requirement was determined based on d0 BW and NRC (1984) requirement tables; 1× maintenance calves

were fed to meet their NE_m requirement; 2× maintenance calves were fed twice their NE_m requirement; intake was subsequently adjusted based on d14 BW. Implanted calves were implanted (Revalor-XS; 40 mg estradiol-17β + 200 mg trenbolone acetate) on d0; all calves were weighed and processed through the chute on d0 and fed for 28 d. Blood samples were drawn on d0, d14, and d28 for analysis of serum IGF-1 (analyzed using ELISA) and plasma urea nitrogen (colorimetric assay). Data were analyzed as a repeated measures design using the MIXED procedure of SAS (v. 9.1). Diet affected ($P < 0.05$) ADG (1.65 vs. 0.04 kg/d for 2× vs. 1×), and d28 PUN (6.21 vs. 5.35 mM/L for 2× vs. 1×) but there was no effect of implant or any interaction between diet and implant on weight gain, PUN, or IGF-1 concentrations. These data suggest that nutrient restriction does not alter IGF-1 response or subsequent cellular nutrient uptake response due to implants.

Key Words: diet, feedlot, implant

112 Growth hormone and insulin-like growth factor I have different effects on bovine myoblasts and myotubes in culture. X. Ge* and H. Jiang, *Virginia Polytechnic Institute and State University, Blacksburg.*

Growth hormone (GH) and insulin-like growth factor I (IGF-I) are 2 major regulators of skeletal muscle growth in animals. The objective of this work was to compare the effects of GH and IGF-I on proliferation and fusion of primary bovine myoblasts, and their effects on protein synthesis and degradation in cultured bovine myotubes. Myoblasts were isolated from extensor carpi radialis of adult cattle by Pronase digestion and were allowed to proliferate or induced to form myotubes in culture. Recombinant bovine GH at the concentrations of 10 ng/mL and 100 ng/mL did not affect myoblast proliferation and fusion. However, both concentrations of GH increased protein accumulation by 15% compared with no-GH control ($P < 0.01$) without changing protein degradation. Although IGF-I at concentrations of 50 ng/mL and 500 ng/mL had no effect on myoblast fusion, IGF-I at concentration of 500 ng/mL increased myoblast proliferation by 20%, compared with no-IGF-I control ($P < 0.05$). Both concentrations of IGF-I increased protein accumulation in bovine myotubes by more than 75% ($P < 0.01$) and decreased protein degradation by 30% ($P < 0.05$), compared with no-IGF-I control. These data indicate that GH and IGF-I have largely different effects on proliferation and fusion of bovine myoblasts and on protein synthesis and degradation in bovine myotubes. These results suggest that GH and IGF-I might stimulate skeletal muscle growth in cattle through different mechanisms.

Key Words: GH, IGF-I, muscle

113 Trenbolone regulates myogenic differentiation via inducing androgen receptors and β-catenin interaction in muscle derived stem cells of cattle. J. X. Zhao*, J. Hu, M. J. Zhu, W. J. Means, and M. Du, *Department of Animal Science, University of Wyoming, Laramie.*

Anabolic steroid hormones have been widely used in the beef cattle industry for more than 50 years. Trenbolone is a synthetic analog of anabolic steroid hormone which can promote both skeletal muscle and bone growth; however the underlying mechanisms remain obscure. Because canonical Wnt/β-catenin signaling is known to promote myogenesis, we hypothesized that trenbolone regulates myogenesis through promoting the interaction of androgen receptor with β-catenin, increasing the transcription of β-catenin targeted genes. Muscle derived stem cells were prepared from male fetal skeletal muscle of cattle at mid-gestation, and treated with or without trenbolone (10 nM) in a myogenic medium consisting of DMEM plus 2% horse serum.

Results showed that trenbolone treatment increases the protein contents of MyoD (0.72 ± 0.18 vs. 1.45 ± 0.19 arbitrary units, $P < 0.05$), myosin heavy chain (0.22 ± 0.03 vs. 0.38 ± 0.02 arbitrary units, $P < 0.05$), and androgen receptor (0.92 ± 0.04 vs. 1.18 ± 0.03 arbitrary units, $P < 0.05$) compared with the control group. The myogenic effect of trenbolone was blocked by cyproterone acetate, a specific inhibitor of androgen receptor, showing that the myogenic effect of trenbolone is mediated by androgen receptor. Immunoprecipitation showed that androgen receptor and β-catenin formed a complex; addition of trenbolone increased interaction between androgen receptor and β-catenin. Both cytoplasmic and nuclear β-catenin levels were increased following trenbolone treatment. The enhanced translocation of β-catenin to the nuclei following trenbolone treatment was correlated with higher β-catenin/T-cell transcription factor 1 (TCF) mediated transcriptional activity compared with that of control group (11.7 ± 1.6 vs. 6.9 ± 0.9 relative luciferase activity, $P < 0.05$). In conclusion, these data provide evidence that trenbolone promotes the interaction between androgen receptor and β-catenin, which promotes the expression of β-catenin targeted genes and myogenesis in the muscle derived stem cells of cattle.

Key Words: catenin, beef, androgen

114 Increasing days on the finishing diet equalizes carcass grade distributions of zilpaterol-HCl fed heifers. B. C. Bernhard*, R. S. Swingle², T. E. Lawrence³, W. T. Nichols⁴, D. A. Yates⁴, J. P. Hutcheson⁴, M. N. Streeter⁴, J. C. Brooks¹, M. F. Miller¹, B. J. Johnson¹, and R. J. Rathmann¹, ¹Texas Tech University, Lubbock, ²Cactus Research Ltd., Amarillo, Texas, ³West Texas A&M University, Canyon, ⁴Intervet Schering Plough Animal Health, DeSoto, Kansas.

British × Continental heifers (n = 3,382; 307 kg) were serially slaughtered to determine if increasing days on the finishing diet (DOF) mitigates negative consequences of zilpaterol-HCl (ZH) on quality grade and tenderness. A 2 × 3 factorial arrangement of treatments in a completely randomized block design (36 pens; 6 pens/treatment) was used. Zilpaterol-HCl (8.33 mg/kg DM) was fed 0 and 20–22 d before slaughter plus a 3–5 d withdrawal to heifers spending 127, 148, and 167 DOF. Feedlot and carcass performance data was collected in addition to Warner-Bratzler shear force (WBSF) of strip loin steaks aged for 7, 14, and 21 d. No ZH × DOF interactions were detected ($P > 0.05$). Feeding ZH increased ADG, G:F, carcass ADG, carcass G:F, carcass ADG:live ADG, HCW, dressing percent, LM area and WBSF at 7, 14, and 21 d; decreased 12th-rib fat, YG ($P < 0.01$) and KPH ($P = 0.05$); and tended to decrease marbling score ($P = 0.10$). Feeding ZH decreased empty body fat percentage (EBF) and increased 28% EBF adjusted final BW ($P < 0.01$). Analysis of interactive means indicated that the ZH × 148 DOF group had a similar percentage of USDA Prime, Premium Choice, Low Choice and YG 1, 2, 3, 4, and 5 carcasses ($P > 0.10$); an increased percentage of total Choice ($P = 0.02$); and decreased percentage of Select ($P = 0.03$) and Standard ($P = 0.05$) compared with the Control × 127 DOF group. The ZH × 148 DOF group was tougher than the Control × 127 DOF group, but more tender than the ZH × 127 DOF group ($P < 0.01$) at 21 d. As a result of ZH shifting body composition, an additional number of DOF equalizes carcass grade distributions, but ZH mediated advantages in feedlot and carcass weight gain are sustained.

Key Words: zilpaterol-HCl, beef heifers, serial slaughter

115 Mitochondrial complex I protein is correlated to residual feed intake in beef cattle. M. H. Ramos* and M. S. Kerley, *University of Missouri, Columbia.*

Crossbred beef steers (310kg, n = 48) were fed over a 100 d period where daily feed intake and weight gain were measured. Diet fed to steers consisted of corn (62.5%), soyhulls (15%), distillers grain (10%), glycerol (10%) and mineral/vitamin supplements (2.5%). Dry matter intake and body weight was measured daily using Growsafe feed intake system. Intake was regressed on average metabolic body weight (MBW) and average daily gain (ADG) and resultant coefficients used to calculate predicted feed intake. Difference between actual and predicted intake was residual feed intake (RFI). Efficient animals (- RFI) had lower actual intake than predicted while inefficient animals (+ RFI) had higher actual intake than predicted. Blood was collected via jugular vein puncture and mitochondria isolated from lymphocytes. Mitochondria were processed to measure complex I quantity (CI), complex I enzyme activity (CIE) and pyruvate dehydrogenase quantity (PDH, as a mitochondria marker). Efficient animals had higher ($P = 0.006$) CI compared with average and inefficient animals (68.4, 39.2 and 28.5, respectively). Correlation between RFI and CI was -0.37 ($P = 0.03$) We concluded that efficient animals had higher CI. These results agree with previous research showing that efficiency is correlated to differences among animals in mitochondrial protein concentration.

Key Words: RFI, efficiency, mitochondria

116 Bone tissue-specific over-expression of growth differentiation factor 11 propeptide transgene causes homeotic transformation of the seventh cervical vertebra into a thoracic vertebra in mice. Z.

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Skeletal formation is highly dependent upon sequential switching on and off specific gene activities that control cellular events. Growth differentiation factor 11 (GDF11), also known as bone morphogenetic protein 11 (BMP11), is one of the significant genes that control skeletal formation and development. Complete deficiency of GDF11 function by gene targeting caused abnormal patterning of the anterior/posterior axial skeleton. However, all the GDF11-deficient mice died at birth because of serious kidney defect. To obtain live animals with disrupted GDF11 function, we developed a novel strategy to block the function of GDF11 through its propeptide. Using the intracytoplasmic sperm injection (ICSI) technique in combination with piggyBac transposon-mediated gene transfer, we produced viable transgenic mice overexpressing GDF11 propeptide under the control of a bone tissue-specific promoter, 2.3kb alpha1 type 1 collagen promoter. The transgenic mice exhibited skeletal abnormalities that appeared to represent homeotic transformation of the seventh cervical vertebra into a thoracic vertebra. The GDF11 propeptide transgene was detected as early as at 12.5 dpc in embryonic skeleton. Over 80% of the transgenic mice from a line expressing high level of transgene showed ectopic ribs on the seventh cervical vertebra. The transgene caused expression shifts of *Hoxa-4* and *5* genes from their normal prevertebra locations in embryos. These results strongly suggest that the GDF11 function in the transgenic mice is suppressed in bone tissue. The transgenic mice with overexpressed GDF11 propeptide are useful animal models for investigating the role of GDF11 in skeletal formation and bone metabolism during late embryonic and postnatal growth.

Key Words: GDF11 propeptide, cervical rib, Hox gene