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Author	Xue-Cheng Jin[1], Dong-Qiao Peng[2], Wang-Yong Jeong[1], Jun-Hee Lee[1], Bo-Mi Kim[1], Danil Kim[3], Yuan H. Brad Kim[4], and Hong-Gu Lee[1]*
Affiliation	[1]Department of Animal Science, Konkuk University, Seoul 05029, Republic of Korea [2]College of Animal Science, Jilin University, Changchun 130062, China [3]Farm Animal Clinical Training and Research Center, Institute of Green-Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea [4]Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA
ORCID (for more information, please visit https://orcid.org)	Xue-Cheng Jin (https://orcid.org/0000-0002-2828-2359) Dong-Qiao Peng (https://orcid.org/0000-0002-0408-0381) Wang-Yong Jeong Jun-Hee Lee (https://orcid.org/0000-0001-6739-6028) Bo-Mi Kim (https://orcid.org/0000-0002-0110-9900) Danil Kim (https://orcid.org/0000-0002-2426-9122) Yuan H. Brad Kim (https://orcid.org/0000-0003-1483-4391) Hong-Gu Lee (https://orcid.org/0000-0002-0679-5663)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Hong-Gu, Lee
Email address – this is where your proofs will be sent	Hglee66@konkuk.ac.kr
Secondary Email address	
Address	Department of Animal Science, Konkuk University, Seoul 05029, Korea
Cell phone number	010-3307-4387
Office phone number	02-450-0523
Fax number	

Abstract

While early nutritional programming critically shapes offspring development, the integrated effects of maternal and neonatal vitamin A (VA) supplementation remain unexplored in beef cattle. In this study, we demonstrate that combining maternal and neonatal VA supplementation exerts pronounced sex-specific effects on growth performance, blood variables, hepatic and skeletal muscle gene expression in Hanwoo calves. Forty-four multiparous Hanwoo cows were assigned to a maternal control (7,000 IU VA/kg DM) or maternal VA group (20,520 IU VA/kg DM) from the third month of gestation until parturition. Calves were further assigned to a neonatal control (17,000 IU/d of VA, until d 60) or VA group (53,000 IU/d of VA, until d 60), forming a 2 × 2 factorial arrangement within each sex. Maternal VA supplementation increased ($p < 0.05$) birth weight in female calves, but not males, an effect that persisted through weaning. It also increased ($p < 0.01$) neonatal red blood cell counts and hemoglobin across sexes. In female calves, neonatal VA supplementation elevated ($p < 0.05$) serum VA concentration at weaning. In the liver, significant maternal × neonatal interactions ($p < 0.05$) occurred for aldehyde dehydrogenase 1 family member A1 (*ALDH1A1*) and retinoic acid receptor alpha (*RARA*) in females, peaking in the dual-supplementation group. In female skeletal muscle, maternal and neonatal VA upregulated ($p < 0.05$) myogenic factor 5 (*MYF5*). Interactions ($p < 0.05$) for myogenic differentiation 1 (*MYOD*) and myogenin (*MYOG*) showed that neonatal VA supplementation upregulated both genes in females from maternal control dams. Maternal VA supplementation increased ($p < 0.05$) zinc finger protein 423 (*ZNF423*) expression, whereas neonatal VA supplementation decreased ($p < 0.05$) peroxisome proliferator-activated receptor gamma (*PPARG*). Male calves showed no significant muscle gene expression effects. Collectively, these findings suggest that maternal VA supply may enhance the transcriptional capacity for hepatic retinoic acid (RA) metabolism, with neonatal supplementation potentially providing additional substrate for this pathway. The associated transcriptional responses in skeletal muscle appear sexually dimorphic, consistent with a preferential effect on myogenic and adipogenic gene programs in female offspring.

Keywords: vitamin A, nutritional programming, sex-specific response, Hanwoo calves, gene expression

Introduction

Maternal nutrition during gestation can permanently alter offspring development, a phenomenon termed fetal or developmental programming [1]. In beef cattle, maternal nutrient restriction or overnutrition during specific gestational periods has been shown to affect birth weight, postnatal growth, and carcass characteristics of the progeny [2, 3]. The balance between skeletal muscle mass and intramuscular fat deposition is a key determinant of beef quality [4]. The cellular foundation for this balance is largely established during fetal and early postnatal development rather than solely during the finishing phase [2]. These programming effects are not confined to the prenatal period; early postnatal nutritional interventions can further modulate the developmental trajectory established in utero, as tissue-specific progenitor cells remain highly plastic during this stage [5]. Among the various nutrients investigated, micronutrients functioning as signaling molecules or transcriptional regulators have attracted considerable interest due to their capacity to directly influence cell fate decisions during development [6, 7]. However, most studies to date have examined a single developmental stage in isolation, and systematic approaches that integrate prenatal and early postnatal interventions remain limited.

The rationale for targeting specific developmental stages lies in the temporal hierarchy of skeletal muscle and adipose tissue ontogeny in cattle. During early to mid-gestation, primary myogenesis establishes the basic framework of muscle fibers, followed by secondary myogenesis, which determines the final fiber number [8]. Concurrently, multipotent mesenchymal stem/stromal cells residing within the muscle undergo lineage commitment toward myogenic, adipogenic, or fibrogenic fates [9]. This competitive process ultimately dictates the ratio of muscle fibers to intramuscular adipocytes in postnatal muscle [10]. Notably, unlike myofiber number, which is essentially fixed at birth, intramuscular preadipocyte hyperplasia initiates around mid-gestation and continues through approximately 11 to 15 months of postnatal age in cattle [11, 12]. This prolonged developmental timeline provides a substantial window during which adipogenic potential can be modulated [13]. Meanwhile, the early postnatal period is also characterized by active satellite cell proliferation and myofiber hypertrophy [14]. These two successive stages—fetal lineage commitment and early postnatal cellular expansion and differentiation—therefore represent the periods of greatest susceptibility to nutritional signals for both muscle and intramuscular fat development. Identifying a nutrient capable of targeting the cellular events within both stages is a logical strategy for maximizing programming efficiency.

Vitamin A (VA) is a fat-soluble micronutrient essential for embryonic development and cell differentiation [15]. Its bioactive metabolite, retinoic acid (RA), activates nuclear receptors that regulate both myogenic and adipogenic gene expression [16]. Maternal VA supplementation

during late gestation has been reported to increase offspring birth weight in beef cattle [17]. Similarly, VA supplementation in neonatal calves [18] and lambs [19] promoted skeletal muscle development by upregulating myogenic gene expression. Early postnatal oral VA administration also improved calf growth and modulated intramuscular fat deposition [18, 20]. In our previous *in vitro* study, RA treatment of bovine skeletal muscle-derived cells (BSMCs) promoted myogenic differentiation, enhanced early preadipocyte lineage commitment, and simultaneously suppressed terminal adipogenic differentiation [21]. These findings collectively indicate that VA can influence both muscle and intramuscular fat development across prenatal and postnatal stages. Existing *in vivo* studies, however, have largely focused on a single developmental stage, and the factor of sex has rarely been incorporated into experimental designs. Sex hormones can modulate RA signaling by competing for shared transcriptional co-regulators and altering local retinol metabolism [22, 23]. Whether integrating VA supplementation across both stages produces additive or interactive effects on offspring development remains unknown.

We hypothesized that combining maternal and neonatal VA supplementation would alter hepatic RA metabolism in the offspring and modify the transcriptional profiles of myogenic and adipogenic genes in skeletal muscle, thereby affecting growth performance. Therefore, the objectives of this study were to evaluate the effects of maternal and neonatal VA supplementation on growth performance, blood variables, and hepatic and skeletal muscle gene expression in male and female Hanwoo calves.

Materials and Methods

Maternal management and dietary treatments

All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee of Konkuk University (permit number: KU21206), in compliance with the "Guide for the Care and Use of Laboratory Animals." Forty-eight multiparous Hanwoo cows were stratified by parity (2.5 ± 0.3) and age (49.1 ± 0.6 months at artificial insemination) and randomly assigned to either a maternal control (n = 24; no supplemental VA) or a maternal VA group (n = 24; supplemental VA). Four cows were subsequently excluded due to abortion or twin births, resulting in 44 singleton calves available for the study. Cows were housed in pens (four animals per pen) with *ad libitum* access to water. Artificial insemination was performed by a technician blinded to treatment allocation, using semen from 15 sires randomly distributed across treatments. Pregnancy was confirmed by ultrasonography at three months of gestation. Each cow was offered 3.5 kg/d of commercial concentrate (Jeil Feed Co., Daejeon, Korea) and 10 kg/d of

rice straw (as-fed basis), with no orts observed throughout the trial. The basal diet met the nutrient requirements for gestating beef cows as recommended by the Korean Feeding Standard for Hanwoo (NIAS, 2022) and Nutrient requirements of beef cattle (NASEM, 2016). Beginning at three months of gestation, cows in the maternal VA group received retinyl acetate (5×10^5 IU/g, Hanyou Feed, Wuxi, China) as a top dressing mixed with 15 g/d of corn meal as a carrier. Vitamin A concentrations in the total diet were 7,000 IU/kg DM for the maternal control group and 20,520 IU/kg DM for the maternal VA group. Supplementation continued until parturition (duration: 193.5 ± 1.5 d). Daily health checks were conducted, and dry sawdust bedding was replaced monthly.

Calf management and neonatal treatments

Immediately after birth, calves were separated from their dams, weighed, and individually housed in pens (2.0 × 1.4 × 1.2 m; length × width × height). Within 3 h of birth, all calves were administered commercial bovine dried colostrum (Nursemate, Brookings, SD, USA; reconstituted per manufacturer's instructions) via an esophageal tube, ensuring a minimum IgG intake of 175 g. Calves from each maternal treatment group were then stratified by sex and birth weight (32.1 ± 3.4 kg) and randomly assigned to either the control or VA treatment, producing a 2 × 2 factorial arrangement (maternal treatment–neonatal treatment) within each sex: CON-CON (CC; n = 12, 6 males and 6 females), CON-VA (CV; n = 11, 6 males and 5 females), VA-CON (VC; n = 10, 5 males and 5 females), and VA-VA (VV; n = 11, 5 males and 6 females). From d 1 to 30, calves were fed milk replacer (Nursemate, reconstituted at 160 g/L) using an automated feeder (CalfRail, Foerster-Technik, Engen, Germany) four times daily (0700, 1100, 1700, and 2100 h). The allowance was set at 4 L/d from d 1 to 15 and increased to 6.4 L/d from d 16 to 30. At d 31, calves were relocated to larger individual pens (2.0 × 1.5 × 1.8 m) and offered *ad libitum* access to a mixed ration comprising 80% commercial calf starter (Jeil Feed Co.) and 20% oat hay. Milk replacer was then bottle-fed manually twice daily (0700 and 1700 h), with the allowance linearly reduced from 6.4 to 0 L/d by d 61 to achieve complete weaning [24]. Throughout the pre-weaning period (d 2 to 60), calves in the neonatal VA groups were administered a daily dose of 36,000 IU water-dispersible retinyl acetate dissolved in 20 mL of water (Hanyou Feed, Wuxi, China) via an oral syringe after the morning feeding. Consequently, the total daily VA intakes for the neonatal control and VA groups were 17,000 and 53,000 IU/d, respectively. Water was available *ad libitum* to all calves throughout this period. Dry sawdust bedding was replaced daily. Health status was monitored daily, and calves presenting with diarrhea received supplemental commercial electrolyte solution (Kim's Ade, BEBE COW, Icheon, Republic of Korea) between

milk feedings to prevent dehydration. Daily dry matter intake (DMI) was recorded individually throughout the pre-weaning period. Body weight was recorded before the morning feeding on d 61 to determine average daily gain (ADG) and feed efficiency (ADG/DMI).

Chemical composition analysis

Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF). DM was determined by oven-drying ground samples at 100 °C overnight (AOAC, 1990; method 930.15). Total nitrogen was quantified by the Kjeldahl method, and CP was derived by multiplying nitrogen content by 6.25 (AOAC, 1990; method 984.13). EE was measured by solvent extraction following AOAC (1990; method 920.39). Ash concentration was obtained by combustion at 550 °C overnight according to AOAC (1990; method 942.05). Calcium (Ca) and phosphorus (P) were determined by inductively coupled plasma spectroscopy (AOAC, 1990; method 985.01). The NDF and ADF fractions were analyzed using the methodology of Van Soest et al. [26]. Nutritional composition of the experimental diets is presented in Table 1.

Blood collection and preparation

Blood samples were collected from the jugular vein of calves before the morning feeding (0700 h) at 2 and 61 days of age. Each sample was aliquoted into two types of evacuated blood tubes: a K2 EDTA tube (BD Vacutainer, Franklin Lakes, NJ, USA) for whole blood and an SST tube (BD Vacutainer) for serum. Following collection, serum was separated by centrifugation at 2,700 × g for 15 min at 4 °C and then stored at −80 °C for subsequent analysis of VA concentration and metabolic profiles. Throughout this process, serum samples were handled under light-protected conditions to prevent VA degradation. The whole blood was utilized for the complete blood count (CBC) test.

Complete blood count (CBC)

The CBC test included four parameters, measured using a hematology analyzer (Microsemi LC-660; HORIBA, Kyoto, Japan). These parameters were: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), and platelets (PLT).

Metabolic profile test (MPT)

The MPT included five items, determined using an automated clinical chemistry analyzer (FUJI DRI-CHEM 7000i; Fujifilm, Tokyo, Japan). These items comprised serum levels of glucose (GLU), creatinine, total cholesterol (TCHO), and aspartate transaminase (AST).

Serum VA analysis

Serum VA concentration was analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC), as previously described by Jin et al. [27]. Briefly, serum samples were deproteinized using ethanol, followed by extraction with hexane. The extracts were then evaporated under nitrogen gas and reconstituted in 95% methanol, which served as the mobile phase. Separation of VA was performed using a stainless-steel Nova-Pak C18 column (4-μm reversed-phase column, 150 mm × 3.9 mm ID, Waters, Dublin, Ireland) in an Agilent 1100 Series HPLC Value System (Agilent Technologies, Waldbronn, Germany), with UV detection at 325 nm. A standard curve was established using retinol (>99%, Sigma-Aldrich, Saint Louis, MO, USA) as an external standard with a twofold serial dilution. Retinyl acetate (>99%, Sigma-Aldrich) was used as an internal standard to correct the results, ensuring a recovery rate of over 80% in all samples. All procedures were performed under red light to prevent the degradation of VA, and butylated hydroxytoluene (BHT, Sigma-Aldrich) was used as a stabilizer for sample preparation. Each sample was analyzed in duplicate.

Longissimus lumborum muscle and liver biopsy

Longissimus lumborum muscle and liver samples were collected from each calf at 65 days of age through biopsy procedures carried out by a veterinarian. The area around the biopsy site was shaved and locally anesthetized with 2% lidocaine (Jeil Pharmaceutical, Yongin, Gyeonggi, Republic of Korea). Approximately 2 g of muscle sample was collected from the right lumbar region between the 3rd and 4th lumbar vertebrae (L3-L4) using blunt-ended scissors. The sample was transferred into a 5 mL diethyl pyrocarbonate (DEPC)-treated microtube for mRNA quantification. Approximately 0.4 g of liver sample was obtained through the right 9th and 10th intercostal spaces using an Achieve coaxial biopsy system (14 G, 15 cm, CareFusion, San Diego, CA, USA), and then transferred into a 2 mL DEPC-treated microtube. Once the samples were placed in the microtubes, they were immediately flash-frozen in liquid nitrogen and transported to the laboratory on dry ice. For subsequent analysis, the frozen samples were stored at −80 °C.

Total RNA isolation and cDNA synthesis

Total RNA was extracted from 0.1 g each of muscle and liver samples, with the muscle tissue being ground to a fine powder in liquid nitrogen. The prepared samples were then homogenized in TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) using an IKA T10 basic ULTRA-TURRAX disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) equipped with a dispersing tool S10N-5G (IKA Werke). The extraction was then performed following the manufacturer's protocol. Briefly, phase separation was achieved by adding chloroform, and the aqueous phase containing RNA was collected. RNA was precipitated with isopropanol, washed with 75% ethanol, air-dried, and reconstituted in DEPC-treated water. The concentration and purity of the RNA were measured on a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The 260/280 ratio of the RNA samples used in this study was in the range of 1.8–2.0. RNA integrity was measured using an RNA Nano 6000 assay kit (Agilent Technologies) on an Agilent Bioanalyzer 2100 system (Agilent Technologies). The RNA integrity number (RIN) of the samples used in this study was 8.9 ± 0.5. cDNA was synthesized from 1 μg of RNA using a cDNA synthesis kit (AccuPower CycleScript RT PreMix, Bioneer, Seoul, Republic of Korea) on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA).

Quantitative real-time PCR (qPCR)

The qPCR reactions were performed using a SYBR green kit (AccuPower 2X GreenStar qPCR MasterMix, Bioneer) on a CFX Connect Real-Time PCR Detection System (Bio-Rad) with a final primer concentration of 100 nM. Specific primers spanning exon-exon junctions were designed within coding sequences (CDS) using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to avoid amplification of potential genomic DNA in the samples. Primers were synthesized by Bioneer via the AccuOligo system. All primers were validated using no-template controls and melt curve analysis to confirm both amplification specificity and the absence of primer-dimer formation. The amplification efficiencies were calculated from standard curves generated using 10-fold serial dilutions and were above 95%. The primer sequences and annealing temperatures are provided in Table S1. Gene expression data were normalized to the geometric mean of three reference genes: eukaryotic translation initiation factor 3 subunit K (*EIF3K*), ribosomal protein lateral stalk subunit P0 (*RPLP0*), and ribosomal protein S9 (*RPS9*) [28]. The resulting ΔCT values were subjected to statistical analyses, whereas relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method [29] and expressed as fold changes for presentation.

Statistical analysis

All data were analyzed using the GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Birth weight and neonatal blood variables (hematological parameters, serum metabolites, and VA concentration) were analyzed with maternal treatment, sex, and their interaction as fixed effects. Because a significant maternal treatment × sex interaction was detected for birth weight, all subsequent analyses were stratified by sex. For each sex, growth performance, weaning blood variables, and post-weaning hepatic and skeletal muscle mRNA expression were analyzed with maternal treatment, neonatal treatment, and their interaction as fixed effects. Residual normality was verified via the Shapiro–Wilk test, and Levene's test was used to confirm homogeneity of variances. Post hoc pairwise comparisons were conducted using the Tukey–Kramer test. Statistical significance was declared at $p < 0.05$, and a tendency was considered at $0.05 \leq p < 0.10$.

Results

Birth weight and neonatal blood variables

At birth, a significant main effect of sex ($p < 0.05$) and a maternal treatment × sex interaction ($p < 0.05$) were observed for birth weight (**Table 2**). Specifically, regardless of the maternal treatment, male calves had higher birth weights than female calves. Furthermore, female calves born to cows in the maternal control group exhibited significantly lower ($p < 0.05$) birth weights compared with all other groups. Regarding hematological parameters, calves born to cows supplemented with VA during gestation exhibited significantly greater ($p < 0.05$) RBC and HGB compared with those in the maternal control group. However, WBC and PLT did not differ significantly ($p > 0.05$) among the groups. For serum metabolites, a significant maternal treatment × sex interaction ($p < 0.05$) was detected for creatinine concentration. Female calves in the maternal VA group had significantly higher ($p < 0.05$) creatinine levels than female calves in the maternal control group. Additionally, maternal VA supplementation resulted in significantly higher ($p < 0.05$) serum VA concentrations in newborn calves, regardless of sex. Finally, GLU, TCHO, and AST did not differ significantly ($p > 0.05$) between groups.

Growth performance from birth to weaning

Regarding pre-weaning growth performance, maternal and neonatal VA supplementation did not significantly affect ($p > 0.05$) birth weight, weaning weight, ADG, DMI, or feed efficiency in male calves (**Table 3**). For female calves, those born to cows supplemented with VA during gestation had significantly greater ($p < 0.05$) birth weights and weaning weights than those in the

maternal control group. However, ADG, DMI, and feed efficiency did not differ significantly ($p > 0.05$) among the treatments.

Hematological parameters at weaning

At weaning, maternal and neonatal VA supplementation did not significantly affect ($p > 0.05$) hematological parameters, including WBC, RBC, HGB, and PLT, in either male or female calves (**Table 4**).

Serum metabolites and VA concentration at weaning

Regarding serum metabolites at weaning, no significant differences ($p > 0.05$) were observed in the concentrations of GLU, creatinine, TCHO, or AST among the treatments for both sexes (**Table 5**). Furthermore, serum VA concentration in male calves was not significantly ($p > 0.05$) affected by the treatments. However, in female calves, neonatal VA supplementation resulted in significantly higher ($p < 0.05$) serum VA concentrations compared with the neonatal control group.

Hepatic mRNA expression of VA-related genes

The relative mRNA expression of VA metabolism-related genes in the liver post-weaning, neonatal VA supplementation tended to increase ($p = 0.058$) the expression of aldehyde dehydrogenase 1 family member A1 (*ALDH1A1*) in male calves, while other genes were not significantly affected ($p > 0.05$) (**Table 6**). In female calves, however, both maternal and neonatal VA supplementation significantly increased ($p < 0.01$) *ALDH1A1* expression. Furthermore, significant maternal × neonatal treatment interactions ($p < 0.05$) were observed for both *ALDH1A1* and retinoic acid receptor alpha (*RARA*). Specifically, female calves in the VA-VA group exhibited significantly higher expression levels of both *ALDH1A1* and *RARA* compared with all other groups. Additionally, maternal VA supplementation significantly increased ($p < 0.05$) the expression of retinol binding protein 4 (*RBP4*). The expression of alcohol dehydrogenase 1C (*ADH1C*) did not differ significantly ($p > 0.05$) among the treatments.

Skeletal muscle mRNA expression of myogenic genes

For the relative mRNA expression of myogenic and adipogenic genes in the *Longissimus lumborum* muscle, treatments did not significantly affect ($p > 0.05$) the expression of any evaluated genes in male calves (**Table 7**). In female calves, significant maternal treatment × neonatal treatment interactions ($p < 0.05$) were observed for the myogenic genes myogenic

differentiation 1 (*MYOD*) and myogenin (*MYOG*). Specifically, female calves in the CON-VA group had significantly higher ($p < 0.05$) expression of both *MYOD* and *MYOG* than those in the CON-CON group. Additionally, both maternal and neonatal VA supplementation significantly increased ($p < 0.05$) the expression of myogenic factor 5 (*MYF5*). Furthermore, the expression of the adipogenic gene zinc finger protein 423 (*ZNF423*) in female calves was significantly increased ($p < 0.05$) by maternal VA supplementation, whereas neonatal VA supplementation significantly decreased ($p < 0.05$) the expression of peroxisome proliferator activated receptor gamma (*PPARG*). Finally, the expression of paired box 7 (*PAX7*), myogenic factor 6 (*MYF6*), preadipocyte factor-1 (*PREF1*), CCAAT/enhancer-binding protein beta (*CEBPB*), and fatty acid binding protein 4 (*FABP4*) did not differ significantly ($p > 0.05$).

Discussion

This study aimed to investigate the effects of combined vitamin A (VA) supplementation during gestation and the pre-weaning period on early growth performance, blood parameters, and mRNA expression related to hepatic metabolism and skeletal muscle development in Hanwoo cattle. Based on our prior findings regarding late gestation [17] and the neonatal calf period [18], the VA supplementation level for cows in the current study was set at three times that of the control group, which corresponds to approximately seven times the level recommended by NASEM (2016) for gestating beef cows (2,800 IU/kg DM). Similarly, the VA intake for neonatal calves was three times that of the control, equating to roughly five times the adequate intake (approximately 10,000 IU/d) suggested by NASEM (2021) for milk replacer-fed calves. Hematological and serum biochemical parameters of calves measured at both birth and post-weaning remained within normal reference ranges, confirming the *in vivo* safety of the high-dose supplementation regimen employed in this study.

Previous studies have reported that maternal VA supplementation can influence offspring growth and development. In goats, VA supplementation during late gestation increased offspring birth weight [32]. Similarly, our previous study found that late-gestational VA supplementation increased birth weight in Hanwoo calves [17]. However, that study lacked a balanced sex ratio, which limited the assessment of sex-specific effects. In the present study, we found that the effect of maternal VA supplementation on calf birth weight was sex-dependent. Specifically, maternal VA selectively increased the birth weight of female calves to a level comparable to that of males, without affecting male calves. Interestingly, the interaction pattern for serum creatinine at birth mirrored this finding, with elevated levels observed only in female calves from VA-supplemented dams. Serum creatinine primarily originates from the non-enzymatic degradation of

phosphocreatine in skeletal muscle and can partially reflect the muscle mass of animals [33, 34]. Therefore, this result may provide indirect biochemical support for enhanced fetal skeletal muscle accretion in female calves. However, neonatal serum creatinine may also be influenced by residual placental clearance [35], and this interpretation should be treated with caution. Additionally, maternal VA supplementation significantly increased RBC counts and HGB levels in neonates regardless of sex. This is consistent with findings of Moosavian et al. [36], suggesting that improved maternal VA status may enhance neonatal oxygen-carrying capacity by promoting fetal erythropoiesis [37]. Notably, the birth weight advantage in female calves from VA-supplemented dams persisted through the weaning period. However, no significant differences in ADG or feed efficiency were detected among treatment groups. This indicates that the weaning-stage weight advantage likely originated from a higher initial birth weight rather than accelerated postnatal growth. Similarly, direct VA supplementation to neonatal calves had no significant effect on growth performance in either sex. This may be partly explained by the relatively short supplementation period under the early weaning system (2 months of age) employed in this study. Furthermore, potential weaning-associated stress could have attenuated the growth-promoting effects of VA supplementation during this period [38].

The effective delivery of maternal VA to the offspring is a prerequisite for evaluating the efficacy of gestational nutritional interventions. In this study, maternal VA supplementation significantly increased serum VA (retinol) concentrations in neonatal calves regardless of sex, confirming effective placental transfer of maternal VA and a consequent improvement in fetal circulating VA status. This finding is consistent with previous reports in beef cattle [17] and goats [32]. However, by weaning, the serum VA response pattern diverged between sexes. While no significant differences in serum VA concentrations were observed among treatment groups in male calves, neonatal VA supplementation significantly elevated serum VA levels at weaning in female calves. This discrepancy suggests that inherent physiological differences may exist between sexes in VA absorption, retention, or peripheral tissue clearance.

The liver is the central organ for VA metabolism, responsible for oxidizing circulating retinol into biologically active retinoic acid (RA) and regulating its distribution to peripheral tissues [39]. Consistent with the serum VA response patterns, the expression of hepatic VA metabolism-related genes also exhibited pronounced sex specificity. Male calves showed lower sensitivity to VA supplementation, with only a tendency for upregulated *ALDH1A1* expression in response to neonatal VA supplementation. In female calves, the elevated serum VA concentration at weaning was driven by neonatal VA supplementation. However, the mRNA expression of *RBP4*, which is responsible for transporting hepatic retinol into the circulation [40], was significantly upregulated

only by maternal VA supplementation. This discordance between transcriptional levels and circulating metabolite profiles suggests that maternal VA may have exerted a lasting metabolic programming effect on the fetal liver [41], potentially altering its capacity to mobilize and export VA during the pre-weaning period. Furthermore, ALDH1A1, the rate-limiting enzyme catalyzing the irreversible oxidation of retinal to RA [42], and RARA, the nuclear receptor mediating RA-dependent transcriptional regulation [43], both exhibited significant maternal × postnatal interactions in female calves, with the highest expression observed in the dual-supplementation group (VV). This result is consistent with the possibility that maternal VA intervention primed the hepatic RA metabolic network at the transcriptional level, with combined maternal and neonatal supplementation associated with the highest expression of these key components. The relatively large within-group variation observed for *ALDH1A1* expression in the VV group likely reflects individual differences in baseline hepatic VA reserves and in the responsiveness of the hepatic RA metabolic network to dual VA stimulation. In contrast, no significant differences in *ADH1C* expression were detected among treatment groups, possibly because this enzyme primarily participates in the reversible oxidation of retinol to retinal rather than the rate-limiting step of RA synthesis [44].

Given the sex-specific activation patterns of the hepatic RA metabolic network, we further examined the expression profiles of myogenic genes in skeletal muscle. In female calves, the expression of the early myogenic determinant *MYF5* [45] was significantly upregulated by both maternal and neonatal VA. Maternal VA supplementation in this study commenced at the third month of gestation, which falls within the active phase of secondary myogenesis in bovine fetuses, reported to extend from approximately 60 to 180 days post-conception [46]. Therefore, the upregulation of *MYF5* may reflect an expanded pool of myogenic progenitor cells established during fetal development [47]. This partly explains the significantly higher birth weight observed in female calves from VA-supplemented dams. Additionally, *MYOD* and *MYOG*, both involved in myogenic differentiation [48], exhibited significant maternal × neonatal interactions. Specifically, neonatal VA supplementation alone (CV group) significantly increased the expression of both genes compared to the un-supplemented control (CC group). One possible explanation is that skeletal muscle tissue not previously exposed to high maternal VA (CV group) displayed a more pronounced transcriptional response to neonatal VA supplementation. In contrast, skeletal muscle that had been exposed to elevated maternal VA (VV group) may have undergone adaptive regulation, thereby attenuating its responsiveness during the pre-weaning period. Unlike the prenatal period for myofiber formation, intramuscular adipogenesis is a continuous process that extends well into the postnatal phase [11]. In this study, maternal VA

supplementation significantly upregulated *ZNF423*, a mediator of early adipogenic lineage commitment in intramuscular preadipocyte progenitors [49]. This suggests that gestational supplementation may have promoted the commitment and recruitment of adipogenic progenitor cells. Conversely, neonatal VA supplementation downregulated the expression of *PPARG*, a core transcription factor for terminal adipocyte differentiation [50], potentially reflecting a modest delay in the differentiation program. The combination of maternal-stage upregulation of adipogenic potential and neonatal-stage suppression of terminal differentiation suggests, at the transcriptional level, that VA supplementation may have the potential to expand the intramuscular preadipocyte pool in offspring.

In the present study, male calves showed no significant response of the examined genes to VA supplementation in post-weaning biopsies, although the modest per-sex sample size precludes detection of smaller effects. The limited transcriptional responsiveness in males may attenuate the potential for downstream phenotypic differences. One possible explanation for this sex-specific responsiveness lies in the crosstalk between sex hormones and RA signaling pathways [22]. It has been reported that the androgen receptor can antagonize the transcriptional activity of RAR through mechanisms such as competition for co-activators [23]. Accordingly, the continuous secretion of endogenous androgens by male calves during the pre-weaning period may have systemically suppressed VA-induced transcriptional signaling. Conversely, RA metabolism in skeletal muscle has been shown to exhibit marked sexual dimorphism. A recent study using an Rdh10 haplodeficient mouse model revealed that estrogen can specifically induce the expression of alternative retinol dehydrogenases, thereby maintaining local RA homeostasis in skeletal muscle when the primary biosynthetic pathway is compromised [51]. In the absence of androgen-mediated antagonism, female calves may possess greater metabolic flexibility through similar estrogen-regulated networks, enabling a more efficient conversion of exogenous VA into RA signaling and the amplification of its transcriptional effects. A notable limitation, however, is that long-term productive outcomes were not assessed in female cattle. Future research should incorporate extended follow-ups of female cattle to determine whether the hepatic and skeletal muscle transcriptional changes observed at weaning can ultimately translate into functional phenotypic outcomes. A further consideration concerns the timing of tissue sampling. Although biopsies were performed four days after weaning, the genes examined in this study are not established direct targets of the stress response, and substantial residual effects of weaning-associated stress on these measurements are unlikely.

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Tables and Figures

Table 1. Nutritional composition of the experimental diets

	Dam	Calf	
Item[1] (% DM)	**Basal diet**	**Milk replacer**	**Mixed ration**
DM, % as-fed	85.84	95.52	90.25
CP	10.68	24.22	21.31
NDF	50.30	-	25.77
ADF	34.28	-	12.44
EE	3.03	17.24	2.87
Ash	9.58	6.87	6.93
NFC[2]	26.41	51.68	43.12
Ca	0.54	0.85	0.78
P	0.24	0.68	0.55

[1]DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fiber carbohydrate; Ca, calcium; P, phosphorus.
[2]Non-fiber carbohydrates (%) = 100 − (CP + EE + NDF + Ash) [52]

Table 2. Effects of maternal VA supplementation on birth weight and blood variables of newborn calves

	Maternal control		Maternal VA			*p*-value		
Item[1]	**Male**	**Female**	**Male**	**Female**	**SEM**	**VA**	**Sex**	**VA × Sex**
Birth weight, kg	33.4[a]	28.9[b]	32.9[a]	32.7[a]	0.54	0.108	0.019	0.033
Hematological parameters (Reference values[2])								
WBC, × 10^3/μL (4.6–16.4)	8.7	8.4	8.8	9.0	0.31	0.578	0.667	0.357
RBC, × 10^6/μL (6.8–14.6)	9.9	9.7	10.5	10.9	0.21	0.007	0.675	0.837
HGB, g/dL (6.5–13.5)	9.8	9.9	11.1	11.9	0.29	0.005	0.483	0.726
PLT, × 10^3/μL (166.0–918.0)	433.0	381.6	421.4	412.0	13.91	0.419	0.122	0.169
Serum metabolites								
GLU, mg/dL (75.30–193.30)	87.60	84.00	76.70	91.10	4.03	0.452	0.326	0.984
Creatinine, mg/dL (0.93–1.65)	1.12[ab]	1.09[b]	1.13[ab]	1.34[a]	0.06	0.393	0.638	0.042
TCHO, mg/dL (20.80–67.50)	35.90	42.18	42.20	36.80	2.22	0.920	0.923	0.204
AST, U/L (49.6–133.4)	45.50	52.90	45.50	51.70	2.09	0.887	0.115	0.884
Serum VA, IU/dL	20.59	23.58	29.39	28.26	1.34	0.011	0.715	0.422

[1]WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; PLT, platelets; GLU, glucose; TCHO, total cholesterol; AST, aspartate transaminase; VA, vitamin A.

[2]The reference values for hematological parameters and neonatal serum metabolites were obtained from Kim et al. [53] and Yu et al. [54], respectively.

[a,b]Values with different letters are significantly different ($p < 0.05$).

Table 3. Effects of maternal and neonatal VA supplementation on the pre-weaning growth performance of calves

	Treatment					*p*-value		
Item[1]	**CC**	**CV**	**VC**	**VV**	**SEM**	**Mat**	**Neo**	**Mat × Neo**
Male calves								
Birth weight, kg	33.2	33.7	32.7	33.1	0.70	0.735	0.763	0.967
Weaning weight, kg	59.8	61.5	59.8	56.1	1.17	0.278	0.659	0.274
ADG, g/d	444.1	462.4	452.2	382.3	15.44	0.260	0.415	0.171
DMI, g/d	801.3	880.2	790.6	760.8	20.87	0.579	0.335	0.648
Feed efficiency, g gain/g of DM	0.6	0.6	0.6	0.5	0.01	0.849	0.815	0.754
Female calves								
Birth weight, kg	30.6	27.9	31.4	32.3	0.73	0.036	0.447	0.153
Weaning weight, kg	56.1	53.1	58.1	63.4	1.45	0.045	0.924	0.194
ADG, g/d	425.0	420.2	445.0	518.7	15.09	0.205	0.568	0.476
DMI, g/d	776.1	769.5	767.2	820.2	23.13	0.382	0.619	0.525
Feed efficiency, g gain/g of DM	0.5	0.6	0.6	0.6	0.01	0.957	0.827	0.891

[1]ADG, average daily gain; DMI, dry matter intake; CC, CON-CON; CV, CON-VA; VC, VA-CON; VV, VA-VA; Mat, maternal treatment; Neo, neonatal treatment.

Table 4. Effects of maternal and neonatal VA supplementation on hematological parameters of calves at weaning

	Treatment					*p*-value		
Item[1] (Reference values[2])	**CC**	**CV**	**VC**	**VV**	**SEM**	**Mat**	**Neo**	**Mat × Neo**
Male calves								
WBC, × 10^3/μL (4.6–16.4)	9.1	9.3	8.9	9.9	0.51	0.320	0.464	0.227
RBC, × 10^6/μL (6.8–14.6)	13.6	13.8	14.1	13.5	0.28	0.643	0.126	0.512
HGB, g/dL (6.5–13.5)	13.5	12.9	12.8	13.1	0.35	0.279	0.696	0.807
PLT, × 10^3/μL (166–918)	780.5	705.5	690.9	733.7	28.69	0.578	0.554	0.388
Female calves								
WBC, × 10^3/μL (4.6–16.4)	10.5	8.9	8.9	10.2	0.68	0.901	0.915	0.358
RBC, × 10^6/μL (6.8–14.6)	13.7	14.7	13.7	13.9	0.29	0.625	0.507	0.629
HGB, g/dL (6.5–13.5)	12.6	13.2	12.7	12.4	0.37	0.687	0.840	0.581
PLT, × 10^3/μL (166–918)	718.0	699.7	773.3	743.2	35.46	0.581	0.791	0.312

[1]WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; PLT, platelets; CC, CON-CON; CV, CON-VA; VC, VA-CON; VV, VA-VA; Mat, maternal treatment; Neo, neonatal treatment.

[2]The reference values for each item were obtained from [53].

Table 5. Effects of maternal and neonatal VA supplementation on serum metabolites and VA concentration of calves at weaning

	Treatment					*p*-value		
Item[1] (Reference values[2])	**CC**	**CV**	**VC**	**VV**	**SEM**	**Mat**	**Neo**	**Mat × Neo**
Male calves								
GLU, mg/dL (50.44–124.31)	64.00	61.50	70.40	69.75	3.73	0.376	0.847	0.910
Creatinine, mg/dL (0.57–1.23)	0.87	0.85	0.99	0.96	0.03	0.117	0.441	0.845
TCHO, mg/dL (56.30–158.40)	67.50	68.83	69.80	69.50	5.40	0.903	0.966	0.947
AST, U/L (36.19–105.81)	102.33	83.00	88.75	98.33	8.38	0.547	0.775	0.190
VA, IU/dL	69.70	69.60	77.13	74.08	1.89	0.153	0.694	0.713
Female calves								
GLU, mg/dL (50.44–124.31)	73.20	68.40	53.67	72.00	4.13	0.372	0.447	0.202
Creatinine, mg/dL (0.57–1.23)	0.86	0.92	0.84	0.94	0.03	0.533	0.182	0.357
TCHO, mg/dL (56.30–158.40)	84.25	73.00	68.00	84.00	6.05	0.272	0.281	0.250
AST, U/L (36.19–105.81)	81.00	82.75	70.20	86.40	6.32	0.796	0.520	0.603
VA, IU/dL	56.44	85.74	57.14	88.93	5.94	0.857	0.010	0.908

[1]GLU, glucose; TCHO, total cholesterol; AST, aspartate transaminase; VA, vitamin A; CC, CON-CON; CV, CON-VA; VC, VA-CON; VV, VA-VA; Mat, maternal treatment; Neo, neonatal treatment.

[2]The reference values for GLU and creatinine were obtained from Roadknight et al. [55], and those for TCHO and AST from Kazana et al. [56].

Table 6. Effects of maternal and neonatal VA supplementation on the relative mRNA expression of VA metabolism-related genes in the liver of calves post-weaning

	Treatment				*p*-value		
Item[1]	**CC**	**CV**	**VC**	**VV**	**Mat**	**Neo**	**Mat × Neo**
Male calves							
ADH1C	1.00 ± 0.12	1.12 ± 0.16	1.20 ± 0.13	0.88 ± 0.09	0.825	0.873	0.576
ALDH1A1	1.00 ± 0.19	1.46 ± 0.30	1.55 ± 0.39	2.56 ± 1.61	0.299	0.058	0.283
RBP4	1.00 ± 0.12	1.00 ± 0.11	0.99 ± 0.14	1.13 ± 0.31	0.897	0.829	0.855
RARA	1.00 ± 0.09	0.89 ± 0.07	1.08 ± 0.19	0.92 ± 0.26	0.914	0.907	0.966
Female calves							
ADH1C	1.00 ± 0.14	0.94 ± 0.04	0.99 ± 0.05	0.92 ± 0.08	0.901	0.892	0.956
ALDH1A1	1.00 ± 0.31^{b}	1.53 ± 0.29^{b}	1.83 ± 1.00^{b}	11.56 ± 5.57^{a}	0.008	0.007	0.029
RBP4	1.00 ± 0.07	1.24 ± 0.12	1.68 ± 0.25	2.12 ± 0.39	0.019	0.121	0.428
RARA	1.00 ± 0.01^{b}	1.01 ± 0.08^{b}	1.18 ± 0.12^{b}	1.67 ± 0.38^{a}	0.044	0.315	0.036

[1]*ADH1C*, alcohol dehydrogenase 1C; *ALDH1A1*, aldehyde dehydrogenase 1 family member A1; *RBP4*, retinol binding protein 4; *RARA*, retinoic acid receptor alpha; CC, CON-CON; CV, CON-VA; VC, VA-CON; VV, VA-VA; Mat, maternal treatment; Neo, neonatal treatment.

Values are expressed as fold change ± SEM relative to the CC group.

[a,b]Values with different letters are significantly different ($p < 0.05$).

Table 7. Effects of maternal and neonatal VA supplementation on the relative mRNA expression of myogenic and adipogenic genes in the *Longissimus lumborum* muscle of calves post-weaning

	Treatment				*p*-value		
Item[1]	**CC**	**CV**	**VC**	**VV**	**Mat**	**Neo**	**Mat × Neo**
Male calves							
Myogenesis							
PAX7	1.00 ± 0.05	0.89 ± 0.08	0.86 ± 0.16	0.83 ± 0.08	0.928	0.937	0.571
MYF5	1.00 ± 0.13	0.76 ± 0.17	0.69 ± 0.14	0.84 ± 0.19	0.713	0.691	0.662
MYOD	1.00 ± 0.39	1.34 ± 0.14	1.77 ± 0.30	1.14 ± 0.24	0.889	0.810	0.419
MYOG	1.00 ± 0.30	1.35 ± 0.06	1.46 ± 0.24	1.38 ± 0.15	0.805	0.799	0.808
MYF6	1.00 ± 0.12	1.20 ± 0.16	1.10 ± 0.28	1.48 ± 0.52	0.714	0.524	0.485
Adipogenesis							
PREF1	1.00 ± 0.11	1.10 ± 0.27	0.86 ± 0.20	0.77 ± 0.20	0.505	0.684	0.407
ZNF423	1.00 ± 0.14	0.80 ± 0.09	0.91 ± 0.13	1.11 ± 0.22	0.680	0.837	0.778
CEBPB	1.00 ± 0.13	0.85 ± 0.06	0.90 ± 0.28	0.66 ± 0.13	0.913	0.323	0.846
PPARG	1.00 ± 0.17	0.87 ± 0.09	0.87 ± 0.11	0.92 ± 0.06	0.985	0.904	0.895
FABP4	1.00 ± 0.34	1.23 ± 0.71	1.09 ± 0.58	0.88 ± 0.46	0.869	0.957	0.834
Female calves							
Myogenesis							
PAX7	1.00 ± 0.16	1.53 ± 0.08	1.65 ± 0.29	1.48 ± 0.13	0.542	0.820	0.711
MYF5	1.00 ± 0.26	2.05 ± 0.18	1.98 ± 0.40	2.47 ± 0.36	0.033	0.019	0.296
MYOD	1.00 ± 0.50^{b}	4.74 ± 0.87^{a}	2.96 ± 0.57^{ab}	2.91 ± 0.16^{ab}	0.616	0.040	0.014
MYOG	1.00 ± 0.39^{b}	3.25 ± 0.47^{a}	2.41 ± 0.31^{ab}	2.17 ± 0.22^{ab}	0.455	0.038	0.027
MYF6	1.00 ± 0.24	1.04 ± 0.32	0.88 ± 0.28	1.65 ± 0.59	0.119	0.304	0.157
Adipogenesis							
PREF1	1.00 ± 0.30	1.06 ± 0.27	1.23 ± 0.19	0.71 ± 0.11	0.816	0.836	0.404
ZNF423	1.00 ± 0.25	1.49 ± 0.13	1.62 ± 0.05	1.86 ± 0.21	0.047	0.178	0.235
CEBPB	1.00 ± 0.22	1.38 ± 0.24	1.02 ± 0.22	1.07 ± 0.42	0.460	0.299	0.763
PPARG	1.00 ± 0.09	0.62 ± 0.07	0.74 ± 0.12	0.67 ± 0.07	0.728	0.034	0.564
FABP4	1.00 ± 0.34	1.06 ± 0.23	1.08 ± 0.34	1.68 ± 0.70	0.667	0.818	0.870

[1]*PAX7*, paired box 7; *MYF5*, myogenic factor 5; *MYOD*, myogenic differentiation 1; *MYOG*, myogenin; *MYF6*, myogenic factor 6; *PREF1*, preadipocyte factor-1; *ZNF423*, zinc finger protein 423; *CEBPB*, CCAAT/enhancer-binding protein beta; *PPARG*, peroxisome proliferator activated receptor gamma; *FABP4*, fatty acid binding protein 4; CC, CON-CON; CV, CON-VA; VC, VA-CON; VV, VA-VA; Mat, maternal treatment; Neo, neonatal treatment.

Values are expressed as fold change ± SEM relative to the CC group.

[a,b]Values with different letters are significantly different ($p < 0.05$).