J. Dairy Sci. 88:3971–3981

Expression of Nuclear Receptor and Target Genes in Liver and Intestine of Neonatal Calves Fed Colostrum and Vitamin A

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ABSTRACT

Nuclear receptors (NR), including retinoic acid and retinoid X receptors (RAR, RXR), pregnane X receptor (PXR), constitutive androstane receptor, and peroxisome proliferator-activated receptor (PPARα) modify the expression of other genes, such as cytochrome p450 enzymes (CYP), sulfotransferases (SULT), and UDP glucuronosyl transferases (UGT). Nuclear receptor expression is influenced by exposure to ligands (e.g., vitamin A). We tested the hypothesis that vitamin A feeding influences the expression of hepatic and intestinal NR and their target genes and that colostrum or formula feeding influence these traits differently. Calves (n = 7/group) were fed colostrum (CO) or a milk-based formula with or without vitamin A (FA, FO, respectively) for 4 d and were euthanized on d 5, followed immediately by tissue collection. Thereafter, RNA was extracted and gene expression quantified by real-time reverse transcription-polymerase chain reaction. Expression relative to housekeeping genes of mRNA was profiled for NR, CYP, SULT, and UGT enzymes. Hepatic mRNA levels of RARβ and CYP26 were higher in FA than FO cows; expression of CYP2E1, CYP2C8, CYP26, and UGT1A1 was higher in CO than FO cows; and expression of CYP2E1, UGT1A1, and p450 reductase was higher in CO than FA. In colon tissue, abundance of RXRα mRNA was lower in FO than CO, and CYP2B6 expression was lower in FO than in CO and FA. In jejunal tissue, there were no significant differences in gene expression among groups. In conclusion, effects of vitamin A feeding were limited, but colostrum feeding had several selective effects on expression of nuclear receptors and target genes.
(**Key words:** nuclear receptor, retinoic acid, cytochrome P450, neonatal calves)

Abbreviation key: CAR = constitutive androstane receptor, **CO** = colostrum-fed group, **CYP** = cytochrome p450, **FA** = formula plus vitamin A-fed group, **FO** = formula-fed group, **LRAT** = lecithin: retinol acyltransferase, **NR** = nuclear receptor, **PPAR** = peroxisome proliferator-activated receptor, **PXR** = pregnane X receptor, **RA** = retinoic acid, **RAR** = retinoic acid receptor, **RXR** = retinoid X receptor, **SULT** = sulfotransferase, **UGT** = UDP-glucoronosyl transferase.

INTRODUCTION

Vitamin A (retinol) is the primary source of retinoids, which include all-*trans* retinoic acid and 9-*cis*-retinoic acid. Retinoids are essential nutrients and critical components of a broad array of important physiological processes, which include growth, bone formation, reproduction, immune function, vision, and maintenance of a healthy epithelium, in part through specific interaction with endocrine systems (Franklin et al., 1998). Retinoid function in the embryo begins soon after conception and continues throughout the life span of all vertebrates (Ross et al., 2000). The effects of retinoids are regulated by specific receptors, the retinoid receptors, which are members of a superfamily of nuclear receptors (**NR**; Evans, 1988). The NR are ligand-activated enhancer proteins that include steroid and thyroid hormone receptors as well as the vitamin D receptor (Riaz-Ul-Haq et al., 1991). The structural organization of the NR molecules is similar, but they show a wide variation in ligand sensitivity (Riaz-Ul-Haq et al., 1991). Generally, NR contain an NH_2-terminal region that has a ligand-independent transcriptional activation function and a core DNA-binding domain. The DNA-binding domain (which contains 2 zinc finger motifs) mediates specific binding to target DNA sequences (ligand response elements), a region that permits protein flexibility to allow for simultaneous receptor dimerization and DNA binding, and a ligand-dependent activation function region (Mangelsdorf and Evans, 1995).

The biological effect of retinoic acid (**RA**) is thought to be mainly modulated by RA receptors (**RAR**) and retinoid "X" receptors (**RXR**; Evans, 1988). All-*trans* retinoic acid is the ligand for RAR isoforms, and 9-cis-RA (which is derived from all-*trans* retinoic acid) is the ligand for RXR isoforms. These receptors each include

Received June 2, 2005.
Accepted July 7, 2005.
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*Part of a thesis of K.A.K. for Dr. med. vet., submitted 2005 to the Faculty of Veterinary Medicine, University of Bern, Switzerland.

3 known isoforms (RAR α, β, γ and RXR α, β, γ), which are encoded by different genes (Benbrook et al., 1988; Napoli, 1999). The RAR and RXR proteins interact to form homodimers or heterodimers. In addition, other NR such as the constitutive androstane receptor (**CAR**), pregnane X receptor (**PXR**), peroxisome proliferator-activated receptor (**PPAR**), and several others, may also form functional dimers with RXR. Under physiological conditions, RAR and (or) RXR primarily function as heterodimers with other NR. These heterodimers are capable of binding to RA response elements within the promoters of responsive genes and activate or repress gene transcription in the presence of retinoids and thereby can regulate a myriad of biological processes (Ross, 2003). These responsive target genes are primarily enzymes needed for metabolism, transport, conjugation, and catabolism of exogenous compounds (xenobiotics) as well as endogenous products of metabolism (endobiotics), such as bile acids. Such genes include cytochrome P450 monoxygenases (**CYP**), sulfotransferases (**SULT**), and UDP-glucuronosyl transferases (**UGT**).

At the time of birth, calves have very low liver reserves of vitamin A, which is reflected by the low concentration of vitamin A in blood (Blum et al., 1997; Swanson et al., 2000; Zanker et al., 2000). To prevent vitamin A deficiency, calves need to ingest high amounts of vitamin A immediately after birth, which is supplied by colostrum. Colostrum usually contains large amounts of β-carotene, the precursor of retinol (Blum et al., 1997; Zanker et al., 2000). The conversion of β-carotene to vitamin A occurs in the mucosal cells of the small intestine, but this process in neonatal calves is limited (Nonnecke et al., 2000). Because it is clear that NR play a vital role in the expression of many important enzymes, it is likely that feeding of vitamin A would have major effects on metabolism, health status, and growth performance of young calves. Published studies on NR and NR target genes are very limited in cattle under practical feeding conditions. To the best of our knowledge no studies of this type have been performed in neonatal calves.

The objective of this study was to examine the effects of feeding vitamin A on mRNA expression of genes for NR and metabolically important NR target genes in liver and the gastrointestinal tract of neonatal calves.

MATERIALS AND METHODS

Animals, Experimental Procedures, and Evaluation of Health Status

Twenty-one male calves were selected from a group of 35 calves from a larger study of vitamin A and lactoferrin effects (Muri et al., 2005; Schottstedt et al., 2005). The calves were born between February and May 2003 at the Experimental Station in Posieux, Switzerland, and at neighboring farms. After parturition they were immediately separated from cows. Three groups of 7 calves each were formed. They were fed either a formula deficient in vitamin A (group **FO**), a formula supplemented with vitamin A (group **FA**), or colostrum (group **CO**). Group FO consisted of 1 Red Holstein calf and 6 Simmental × Red Holstein calves; group FA consisted of 3 Holstein-Friesian and 4 Simmental × Red Holstein calves; and group CO consisted of 3 Holstein-Friesian and 4 Simmental × Red Holstein calves. In group FA, 351, 402, 490, and 490 μmol of vitamin A/kg of DM were added to the formula that was fed on d 1, 2, 3, and 4, respectively. The colostrum fed to calves of group CO contained 49, 61, and 76 μmol of vitamin A/kg of DM in milkings 1, 2, and 5, respectively, and was collected from cows at the Experimental Station (Posieux, Switzerland). For this purpose, cows were milked twice daily and colostrums of milkings 1, 3, and 5 after parturition were stored separately in plastic bottles at –20°C. Formulas or colostrum were warmed to 40°C immediately before feeding. Formulas were produced by UFA AG (Sursee, Switzerland) and consisted of calcium caseinate (Emmi Milch AG, Lucerne, Switzerland), lactalbumin (Emmi Milch AG), and a vitamin and mineral premix that supplied the indicated amount of vitamin A (Provimi S.A., Cossonay-Gare, Switzerland). Formula powder was dissolved in water and fat was added during the mixing procedure (49.7% saturated fatty acids, 39.0% unsaturated fatty acids, 6.7% polyunsaturated fatty acids, 2.1% *trans* fatty acids, 2.5% water; Nutriswiss AG, Lyss, Switzerland). The added fat contained no measurable amounts of vitamin A. Lecithin was added as emulsifier (Emulsifier LO-1; UFA AG) as 3% of the total fat. Formulas for meals on d 1, 2, 3, and 4 were formulated to contain comparable amounts of nutrients as colostrums fed on d 1 (milking 1), d 2 (milking 3), d 3, and d 4 (milking 5). Compositions of colostrums and formulas, and feed intake data are shown in Tables 1 and 2, respectively. Pooled mixtures were stored in plastic bottles at –20°C until used. Total fed amounts of formula and colostrum were 6% BW on d 1, 8% BW on d 2, and 10% BW on d 3 and 4.

Before the first meal, each calf received a subcutaneous injection of 2 g of a bovine colostral immunoglobulin preparation (Gammaserin, Gräub AG, Bern, Switzerland) to provide protection against infections. Additionally, calves were fed chicken-egg-derived immunoglobulins that contained high antibody titers against rotavirus and pathogenic *Escherichia coli* type K99 (Globigen 88; Lohmann Animal Health, Cuxhaven, Germany). Amounts per meal fed were 5, 4, 3, and 2 g on d 1, 2, 3, and 4, respectively. Calves were given a subcutaneous