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Supplementing pasteurized waste-milk with vitamins A, D, and E improves vitamin status of dairy calves

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Abstract

Holstein calves fed pasteurized waste milk were blocked by sex and randomly assigned to 1 of 3 treatment groups, control with no vitamin supplementation (CON, n = 18) and 2 levels of an oral vitamins A, D, and E supplement: 0.25 mL/d of supplement (0.25ADE, n = 12) or 0.5 mL/d of supplement (0.5ADE, n = 10). The supplemented calves also received a 5 mL subcutaneous injection of vitamins A, D, and E at birth. The oral and injectable supplements contained 50,000 international units (IU) of vitamin A as retinyl-palmitate, 50,000 IU of vitamin D_3, and 500 IU of vitamin E as *RRR*-α-tocopherol per milliliter of product. Concentrations of 25-hydroxyvitamin D and α-tocopherol, but not retinol, in serum of 0.25ADE and 0.5ADE calves were greater than CON calves. We observed that supplemented calves gained less weight compared with CON calves in the first 28 d; however, height and starter grain intakes did not differ among treatments. Incidence of respiratory infection or diarrhea did not differ among groups. In conclusion, injection plus supplementation of pasteurized waste milk with vitamins A, D, and E increased concentrations of 25(OH)D and α-tocopherol in serum but decreased body weight gain, due possibly to over-supplementation of the vitamins.

Key words: vitamins, dairy calves, growth, pasteurized waste-milk

Résumé

Des veaux Holstein nourris avec du lait rejeté pasteurisé ont été bloqués par sexe et alloués aléatoirement à l'un des trois groupes suivants : témoin sans supplémentation vitaminique (TEM, n = 18) et deux niveaux d'un supplément oral de vitamines A, D et E : 0.25 ml/j de supplément (0.25ADE, n = 12) ou 0.5 ml/j de supplément (0.5ADE, n = 10). Les veaux supplémentés avaient aussi reçu 5ml d'une injection sous-cutanée de vitamines A, D et E à la naissance. Les suppléments oraux et injectables contenaient 50 000 unités internationales (UI) de vitamine A sous la forme de palmitate de retinyl, 50 000 UI de vitamine D_3 et 500 UI de vitamine E sous la forme de RRR alpha-tocophérol par ml de produit. La concentration de 25-hydroxyvitamine D et de l'alpha-tocophérol mais pas celle du rétinol dans le sérum des veaux du groupe 0.25ADE et du groupe 0.5ADE était plus élevée que dans le sérum des veaux du groupe témoin. Nous avons observé que les veaux supplémentés gagnaient moins de poids que les veaux témoins lors des premiers 28 jours. Toutefois, la taille et la prise alimentaire de grain de démarrage n'étaient pas différentes entre les groupes. Il n'y avait pas non plus de différence entre les groupes au niveau de l'incidence de problèmes respiratoires et de diarrhée. En conclusion, l'injection puis la supplémentation de vitamines A, D et E dans le lait rejeté pasteurisé a augmenté la concentration sérique de 25(OH)D et de l'alpha-tocophérol mais a réduit le gain de poids possiblement en raison d'un apport trop élevé en vitamines.

Introduction

Proper management and nutrition of neonatal dairy calves has tremendous impacts on health and well-being of dairy calves and overall productivity of dairy herds. A common practice on dairy farms is to utilize non-saleable milk in calf-feeding programs.[25] Pasteurized waste-milk can be an economically efficient way to raise dairy calves; however, recent reports indicate pasteurized waste-milk does not adequately meet requirements of vitamins A, D, and E of dairy calves.[13,16]

The fat-soluble vitamins A, D, and E contribute to growth, development, and immunity of dairy calves.[26] Dietary recommendations for the fat-soluble vitamins A, D, and E, however, are loosely defined and supplementation practices in dairy calf rearing programs are quite variable.[25] The National Research Council recommendations for supplemental vitamins A, D, and E in milk replacer for dairy calves are 4090 IU/lb (9,000 IU/kg) of DM, 273 IU/lb (600 IU/kg) of DM, and 23 IU/lb (50 IU/kg) of DM, respectively.[15] Industry practices for milk replacers often provide vitamins A, D, and E well above the NRC recommendations. Concentrations of vitamins

D, and E in whole milk, on the other hand, are well below NRC recommendations[15] and put calves at risk for deficiency. For example, concentrations of 25-hydroxyvitamin D_3 (25(OH) D) and α-tocopherol in serum decreased to less than 10 ng/mL and 1 μg/mL in dairy calves fed pasteurized waste-milk without supplemental vitamins.[13] Those concentrations are respective indicators of subclinical vitamin D and vitamin E deficiencies in cattle.[16,27] Improvements in intestinal development of calves also have been reported with supplemental vitamin A.[22] We hypothesized that supplementing pasteurized waste milk with vitamins A, D, and E at rates similar to that of calf milk replacers would improve fat-soluble vitamin status of dairy calves. Therefore, objectives were to test effects of a commercial source of injectable and liquid supplemental vitamins A, D, and E on concentrations of retinol, 25-hydroxyvitamin D, and α-tocopherol in serum of calves fed pasteurized waste milk.

Materials and Methods

Animals

Holstein calves (19 bulls and 21 heifers) born to cows (primiparous and multiparous) at the University of Florida Dairy Research Unit were used for the experiment. Calves were housed in individual pens in an open-sided barn with shade-cloth curtains and temperature-controlled fans. The pens were aligned in 2 rows and calves from each treatment were randomly located in each row. Calves enrolled in the experiment were born from May 2015 to July 2015. Within 4 h after birth, calves were separated from the dam, transferred to the calf barn, and fed 3.8 L of colostrum that was previously collected from dams at the dairy and stored frozen until feeding. All colostrum had IgG concentrations > 50 g/L as indicated by Brix refractometer measurements. Calves received another 2.85 L of colostrum at 12 h after birth. Rations for lactating cows, which served as source of pasteurized waste-milk utilized in the study, were formulated to provide approximately 140,000 IU vitamin A, 24,000 IU vitamin D, and 600 IU vitamin E.

Diets and Treatments

The experiment was a randomized complete block design. Within 2 d after birth, calves were assigned to 1 of 3 treatment groups: control (**CON**, no supplement), and 2 levels, 0.25mL/d (**0.25ADE**) or 0.5 mL/d (**0.5ADE**), of a liquid dietary supplement of vitamins A, D, and E.[a] The dietary vitamin supplement provided 50,000 IU of vitamin A as retinyl-palmitate, 50,000 IU of vitamin D_3, and 500 IU of vitamin E as *RRR*-α-tocopherol/mL of product and was added to the milk at the morning feeding. Calves were blocked by sex and assigned to treatments in alternating order on the basis of birth date. In total, 9 heifers and 9 bulls were enrolled in the CON group, 6 heifers and 6 bulls were enrolled in the 0.25ADE group, and 6 heifers and 4 bulls were enrolled in the 0.5ADE group. In addition, calves in 0.25ADE and 0.5ADE groups were given a single 5 mL subcutaneous injection of A, D, and E in the neck at birth.[b] The injectable vitamin A, D, and E supplement provided 50,000 IU of vitamin A as retinyl-palmitate, 50,000 IU of vitamin D_3, and 500 IU of vitamin E as *RRR*-α-tocopherol/mL of product, resulting in a total of 250,000 IU vitamin A, 250,000 vitamin D_3, and 2,500 IU vitamin E delivered by subcutaneous injection to 0.25ADE and 0.5ADE calves at birth.

Calves were individually fed 2.85 L of pasteurized waste-milk twice daily at 0600 and 1500 hours. Milk was pasteurized by heating to 145°F (63°C) for 30 minutes. The pasteurized milk was then cooled to 111°F (44°C) before feeding to calves. Additionally, calves were provided ad libitum water and starter pellets.[c] The calf starter pellets were formulated to provide 20% crude protein, 2% fat, 13% crude fiber, 18% ADF, 0.7 to 1.2% Ca, 0.45% P, 0.3 ppm Se, 13,890 IU/kg vitamin A, 3,175 IU/kg vitamin D_3, and 38 IU/kg vitamin E. The amount of offered and refused milk and starter grain were recorded each day. Heifer calves were kept on experimental treatments until 42 d of age. Bull calves were kept on experimental treatments only until 28 d of age because of space limitations.

Sample Collection and Health and Growth Measurements

Growth and health measures were recorded for descriptive analysis of calf performance. Body weights were measured using a full body calf scale, and wither heights were measured using a meter stick. Blood was sampled from the jugular vein every 7 d until 28 d for bulls, and 42 d for heifers (10 mL collection tubes).[d] Blood samples at 0 d were taken after colostrum and before first treatment. Samples were allowed to clot for approximately 2 hours at room temperature. Serum was separated by centrifugation at 1,500 × *g* for 15 minutes at 39°F (4°C) and frozen at -4°F (-20°C) until analyzed.

Calf health was assessed daily using the calf health scoring system previously described.[14] Briefly, feces were scored on scale of 0 to 3 in which 0 = normal, 1 = pasty, 2 = loose, and 3 = watery. Nasal and lung (respiratory) health were scored on a scale of 0 to 3 in which 0 = normal, 1 = slight discharge and induced cough, 2 = noticeable buildup of discharge and occasional cough, and 3 = heavy discharge and repeated coughing.

Vitamin Measurements

Concentrations of retinol and α-tocopherol in serum samples were measured by HPLC. Serum (200 μL) was added to 200 μL ethanol containing 1 μg/mL retinyl-acetate[e] as an internal standard. Samples were vortexed for 10 sec, then 1.5 mL hexane was added to samples and vortexed for 10 min. The lipid-soluble fraction was separated by centrifugation for 15 min at 1,000 × *g*. The hexane layer was collected and dried with vacuum centrifuge. The extracts were reconstituted in 200 μL ethanol and filtered through a 0.2 μm filter prior to HPLC. The samples were analyzed using an Agilent 1290

Infinity II system with quaternary pump and 10 mm flow cell diode array detector.[f] A Poroshell 120 EC-C18 2.1 mm × 50 mm column with particle size of 1.9 μm[g] was used for separation of analytes. Run times of 6 min at 113°F (45°C) were initiated with mobile phase of 50% HPLC-grade water and 50% ACN:MeOH mix (75% acetonitrile and 25% methanol). The mobile phase was changed to 25% water and 75% ACN:MeOH at 1 min run time and 100% ACN:MeOH at 2.5 min by continuous gradient. The mobile phase remained at 100% ACN:MeOH until 5.5 min before switching back to 50% water and 50% ACN:MeOH. Retinol and retinyl-acetate were measured at 325 nm wavelength light and α-tocopherol was measured at 292 nm wavelength. Retention times for retinol, retinyl-acetate, and α-tocopherol were 2.2, 2.6, and 3.4 min, respectively. Standards for retinol[h] and α-tocopherol[i] were prepared at 50, 100, 500, and 1,000 ng/mL and 0.5, 1.0, 5.0, and 10 μg/mL, respectively, in ethanol. Solvents for sample extractions and HPLC mobile phases were HPLC-grade.[j]

Concentrations of 25(OH)D in serum of all calves were measured using an ELISA for 25(OH)D[k] as previously described.[18] The ELISA was performed according to manufacturer instructions except that a custom standard prepared in bovine serum was used in place of the standard provided with the kit. Inter and intra-assay CVs for the ELISA were 1.5% and 5.4%, respectively.

Statistical Analysis

Residuals were tested for normal distribution and analyzed using Glimmix procedure of SAS.[l] For repeated measures data, models included fixed effects of sex, treatment, time, and interaction between treatment and time. Calf nested within treatment was included as a random effect. Day 0 measurements of retinol, 25-hydroxyvitamin D, and α-tocopherol were used as covariates in respective models. Because data were only collected to 28 d for bull calves, only data collected up to 28 d were used in analyses when data from both sexes were combined. Contrasts of CON vs 0.25ADE and 0.5ADE treatments were performed to determine the effect of supplementation compared with no supplementation. Significance was declared at $P<0.05$ and tendencies were declared at $0.1 > P < 0.05$.

Results

Serum Vitamin Concentrations

Mean concentrations of retinol in serum were between 120 and 240 ng/mL and were not affected by treatment, but did increase with time ($P=0.005$; Table 1). In contrast, concentrations of 25(OH)D and α-tocopherol were increased ($P<0.01$) in 0.25ADE and 0.5ADE calves compared with CON (Table 1). Concentrations of 25(OH)D in CON calves remained

Table 1. Concentrations of vitamins in serum of calves.

		Treatment*				*P* value†	
Metabolite	**Day**	**CON**	**0.25ADE**	**0.5ADE**	**SEM**	**Trt**	**CON vs ADE**
Retinol, ng/mL	0	134	121	119			
	7	144	154	124	23	0.92	0.94
	14	166	196	189			
	21	193	169	174			
	28	208	240	184			
	Overall	178	190	168	11		
25(OH)D, ng/mL‡	0	7.3	12.3	14.4			
	7	3.4[a]	73.1[b]	81.3[b]	10.3	<0.001	<0.001
	14	9.0[a]	69.3[b]	80.2[b]			
	21	4.8[a]	71.8[b]	142[c]			
	28	10.8[a]	76.4[b]	99.8[b]			
	Overall	7.1[a]	72.6[b]	100.8[b]	8.8		
α-Tocopherol, mg/mL	0	2.5	3.0	3.0			
	7	1.4	1.8	1.9	0.7	0.02	0.006
	14	2.6	4.4	4.5			
	21	2.7	3.3	4.5			
	28	3.0	4.6	5.3			
	Overall	2.4[a]	3.6[ab]	4.0[b]	0.4		

* Treatments: CON = no vitamin supplement; 0.25ADE = mL/d dietary vitamin supplement; 0.5ADE = 0.5 mL/d dietary vitamin supplement. Treatments were added to pasteurized waste milk once per day. Values represent LSM estimates.

† Significance for effects of treatment (Trt), contrast of CON vs 0.25ADE and 0.5ADE (CON vs ADE) and linear contrast. Samples from 0 d were collected after first colostrum and before start of treatments and were used as covariates in the model.

‡ Interaction between treatment and day was significant, $P=0.002$.

[abc]Means with different letters within row are different, $P<0.05$.

below 10 ng/mL of serum until 28 d, whereas mean concentrations of 25(OH)D in 0.25ADE and 0.5ADE calves were 72.6 and 100.8 ng/mL of serum, respectively, during the 28-d period (Table 1). Concentrations of α-tocopherol in serum remained below 3 μg/mL in CON calves but were 3.6 and 4.0 μg/mL in 0.25ADE and 0.5ADE calves, respectively, during the 28-d period (Table 1). Overall, supplemented calves had greater concentrations of 25(OH)D and α-tocopherol in serum compared with CON calves (0.25ADE and 0.5ADE vs CON, $P<0.01$), and a linear response ($P<0.01$) was observed for 25(OH)D and α-tocopherol in serum as a result of increasing supplementation from 0.25 mL to 0.5 mL of oral supplement per day compared with CON. Serum 25(OH)D of supplemented heifers was greater than supplemented bulls, but not different between control heifers and bulls ($P<0.001$; Figure 1). Mean concentrations of retinol and α-tocopherol in serum did not differ between heifers and bulls (Figure 1).

Growth and health outcomes were analyzed for descriptive purposes, but were not the main objective of the experimental design. Vitamin supplementation decreased ($P=0.008$) weight gain in the first 28 d in that 0.25ADE and 0.5ADE calves weighed 6 lb (3 kg) less than CON calves at 28 d (Table 2). Body weights of heifers at 42 d were 137.3 ± 4.9, 135.6 ± 3.5, and 137.6 ± 3.7 lb (62.3 ± 2.2, 61.5 ± 1.6, and 62.4 ± 1.7 kg) for CON, 0.25ADE and 0.5ADE calves, respectively, and did not differ among treatments. Wither heights and starter grain intakes of calves did not differ among treatments in the first 28 d (Table 2).

Incidence of scours in the first 28 d was 39% (7/18) for CON calves and 41% (9/22) for supplemented calves. Incidence of respiratory illness was 28% (5/18) for CON calves and 23% (5/22) for supplemented calves. Severity of scours and respiratory illness did not differ between treatments (Table 3).

Discussion

The importance of vitamins A, D, and E in calf growth and development are appreciated as indicated by common use of vitamin supplements.[25] Early studies on requirements of vitamins A, D, and E for dairy calves demonstrated the critical importance of each vitamin for growth and development.[2,4,10] However, guidelines for optimal vitamin supplementation practices for calves are unclear. Here, we show that calves fed pasteurized waste-milk benefit from supplemental vitamins D and E in regards to measures of vitamin concentrations in serum. We also observed that combined injectable and oral supplementation of vitamins A, D, and E potentially has negative consequences for growth in neonatal dairy calves, due possibly to over-supplementation. The results of our experiment have relevance for raising dairy calves as pasteurized waste-milk is commonly fed, injectable vitamins are commonly administered with little guidance, and calf milk replacers provide vitamins A, D, and E at rates similar to those provided in this experiment.

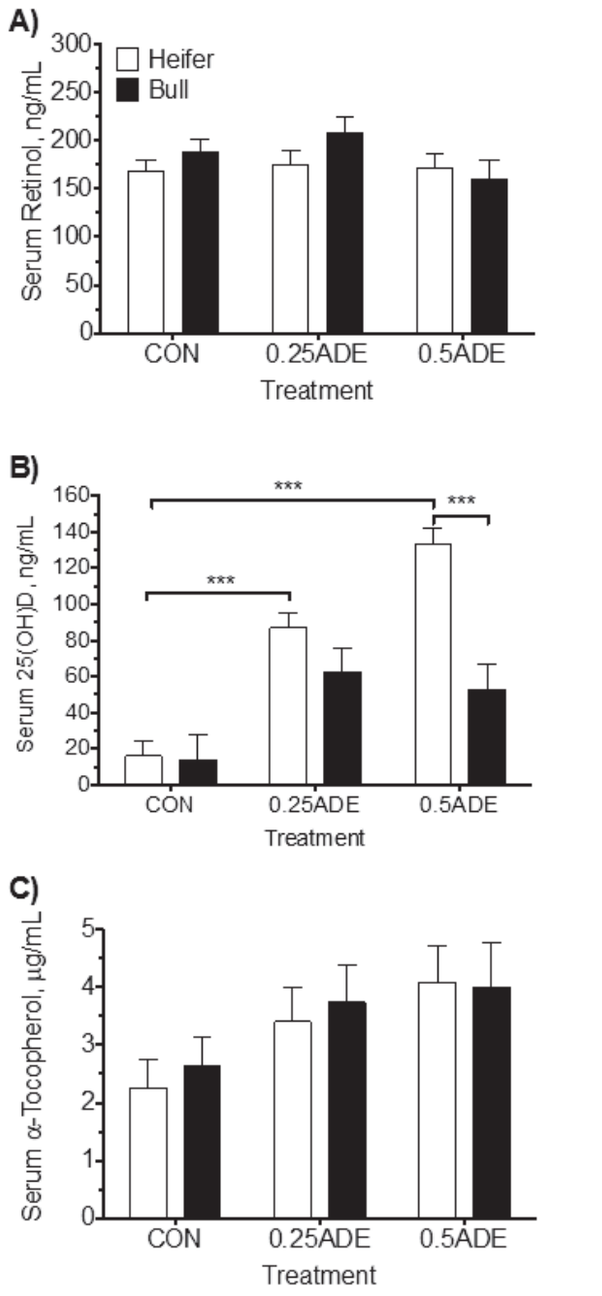

Figure 1. Effects of vitamin A, D, and E supplementation on serum retinol (A), 25-hydroxyvitamin D (25(OH)D) (B), and α-tocopherol (C) concentrations in serum of heifers (white bars) and bulls (black bars). Forty Holstein calves were fed pasteurized waste milk with no supplement (CON, n = 18), 0.25 mL/d (0.25ADE, n = 12) or 0.5 mL/d (0.5ADE, n = 10) dietary vitamin supplement. Data represent treatment LSM ± SE of each respective metabolite in serum sampled from 7 to 28 d of age. Probabilities for effects of treatment, contrasts of CON vs supplemented and linear contrasts are presented in Table 1. Effects of sex and interactions between treatment were not significant for retinol and a-tocopherol, $P>0.1$. Interaction between treatment and sex on 25(OH)D was significant, $P<0.01$. The Tukey-Kramer adjustment was applied to account for multiple means comparison.
***Means are different, $P<0.001$.

Table 2. Effects of supplemental vitamins on growth and starter intake.*

		Treatment × Day LSM				*P* value†	
Measure	**Day**	**CON**	**0.25ADE**	**0.5ADE**	**SEM**	**Trt**	**CON vs ADE**
Bodyweight, lb (kg)	0	89.3 (40.2)	87.6 (39.4)	90.9 (40.9)			
	7	100.2 (45.1)	99.3 (44.7)	94.0 (42.3)	2.9 (1.3)	0.02	0.008
	14	106.4 (47.9)	102.0 (45.9)	98.4 (44.3)			
	21	115.1 (51.8)	109.8 (49.4)	104.7 (47.1)			
	28	125.6 (56.5)	119.6 (53.8)	118.4 (53.3)			
ADG, lb/d (kg/d)	0-28	1.24 (0.56)	1.09 (0.49)	0.84 (0.38)	0.13 (0.06)	0.07	0.05
Height, in (cm)	0	28.7 (73)	28.0 (71)	29.1 (74)			
	7	29.9 (76)	30.3 (77)	29.9 (76)	0.5 (1)	0.48	0.58
	14	31.1 (79)	31.5 (80)	31.1 (79)			
	21	31.9 (81)	31.9 (81)	31.9 (81)			
	28	32.3 (82)	33.1 (84)	32.3 (82)			
Starter intake,‡	0-7	0.29 (0.13)	0.22 (0.10)	0.4 (0.18)	0.49 (0.22)	0.33	0.48
lb/week	7-14	1.15 (0.52)	0.51 (0.23)	1.13 (0.51)			
(kg/week)	14-21	3.24 (1.46)	2.47 (1.11)	3.09 (1.39)			
	21-28	4.13 (1.86)	3.18 (1.43)	4.47 (2.01)			

* Treatments: CON = no vitamin supplement; 0.25ADE = 0.25 mL/d dietary vitamin supplement; 0.5ADE = 0.5 mL/d dietary vitamin supplement. Treatments were added to pasteurized waste milk once per day.

† Significance for effects of treatment (Trt), contrast of CON vs 0.25ADE and 0.5ADE (CON vs ADE) and linear contrast. Day 0 measurements of bodyweights and heights were used as covariates in respective models. Sex was excluded from the models because it was not significant when bodyweights and heights at 0 d were used as covariates.

‡ Starter intake reported on an as-fed basis for the weekly intake.

Table 3. Effects of supplemental vitamins on health of calves.

		Treatment*				
Measure	**Days**	**CON**	**ADE**	**SEM**	**Trt**	**Trt × time**
Fecal score†						
	0-7	4.4	5.9	0.9	0.36	0.70
	7-14	2.8	2.6			
	14-21	0.2	0.9			
	21-28	0.0	0.3			
	Total‡	7.4	9.7	1.6	0.33	
Respiratory						
score†	0-7	0.6	0.6	0.4	0.55	0.87
	7-14	1.1	1.0			
	14-21	1.0	0.6			
	21-28	1.7	1.1			
	Total‡	4.2	3.2	1.1	0.52	

* Treatments: CON = control, no vitamin supplement, n = 18); ADE = 0.25 mL/d and 0.5 mL/d supplemented calves. Data from 0.25ADE and 0.5ADE calves were combined for analysis of health data because of limited sample size.

† Calves were observed daily for illness and assigned a severity score of 0 (no sickness) to 3 (severe) as described by McGuirk et al, *Vet Clin North Am Food Anim Pract* 2008; 24:139-153. Values represent LSM estimate of cumulative scores for 7 d periods.

‡ Cumulative score for 0 to 28 d of experiment.

Minimum requirements of vitamins A, D, and E for normal growth and development of dairy calves have been established for several decades. The minimum vitamin A requirement for calves was reported to be 13.1 mg/lb (29 µg/kg) of BW (44 IU/lb or 97 IU/kg BW) on the basis of cerebral spinal fluid pressure measurements.[5] The minimum vitamin D intake required to prevent rickets in dairy calves was estimated to be 3 IU/lb (6.6 IU/kg) BW of vitamin D.[2] Determination of the minimum vitamin E requirement of calves is somewhat more elusive as availability of selenium and pro-oxidants influence the use of vitamin E, but a review of vitamin E deficiency experiments report that a minimum of somewhere between (0.23 to 0.68 IU vitamin E/lb (0.5 to 1.5 IU/kg) BW is required to prevent muscular dystrophy.[3] Although whole milk is not a prime source of vitamins A, D, and E, it provides enough of each vitamin to prevent clinical

deficiencies provided the lactating herd consumes adequate fat-soluble vitamins before and after calving. Pasteurization of milk does not decrease vitamins A, D, and E significantly;[11] so, if cows are adequately supplemented, as they were in the present study, then milk from those cows is not expected to result in classical symptoms of vitamin deficiencies.

A legitimate concern, however, is whether supplies of vitamins A, D, and E in whole milk and pasteurized waste-milk are sufficient to support the various functional roles of each in immunity and tissue development of calves. Recent reports have documented that supplies of vitamins A, D, and E will influence key immune responses of calves.[9,17,20] Accordingly, the supplies of vitamins D and E in pasteurized waste-milk may be insufficient for optimal performance of calves considering the low concentrations of 25(OH)D and α-tocopherol in serum observed for CON calves in the present experiment. The CON calves had serum 25(OH)D concentrations below 10 ng/mL on average compared with 72.6 and 100.8 in 0.25ADE and 0.5ADE calves. In comparison, beef calves on summer pasture have serum 25(OH)D concentrations between 40 to 60 ng/mL.[18] Serum concentrations of 25(OH)D below 5 ng/mL generally are considered vitamin D deficient, and concentrations below 30 ng/mL are argued to be insufficient for proper immunity.[8,9] For vitamin E, concentrations of α-tocopherol near 1 μg/mL of serum are common for neonatal dairy calves; however, concentrations above 3 μg/mL are associated with improved neutrophil function and health of adult cows.[27] Concentrations of α-tocopherol in serum of CON calves here were 2.4 μg/mL on average, compared with 3.6 and 4.0 μg/mL for 0.25ADE and 0.5 ADE calves. Similarly, Krueger et al reported that calves fed pasteurized waste-milk without supplemental vitamin E had serum α-tocopherol concentrations near 2 μg/mL, whereas, calves supplemented with 500 IU/d vitamin E as *RRR*-α-tocopherol increased to near 8 μg/mL by 35 d of age.[13] As far as vitamin A, serum retinol is not the best measure of vitamin A status, but the lack of difference in serum retinol concentrations between supplemented and CON calves suggests amounts of vitamin A in milk, or liver stores of vitamin A, were adequate to maintain serum retinol of calves.[7] Although stark clinical deficiencies of vitamins D and E are not expected from feeding pasteurized waste-milk, low concentrations of 25(OH)D and α-tocopherol in serum of calves fed pasteurized waste-milk without supplemental vitamins should be of concern.

A striking observation of this study that deserves attention for veterinarians and manufacturers of milk additives and milk replacers is that supplementation of vitamins A, D, and E by diet and injection had negative consequences on initial growth rates of calves. The supplementation strategy used in this study mimicked common practices in the industry in that vitamins A, D, and E were administered by subcutaneous injection and daily dietary supplementation. The 2011 Heifer Raiser report indicated that 52% of producers administer vitamins A, D, and E via injection or feed additive.[25] A review of formulations for 9 different milk replacers or milk enhancers indicated they provide calves 40,000 to 60,000 IU of vitamin A, 10,000 to 12,000 IU of vitamin D, and 100 to 200 IU of vitamin E per day. Therefore, the negative effects of supplementation on weight gain in this experiment deserve consideration because of the relevance to industry practices.

Several studies have examined effects of supplemental rates of vitamins A and E, either alone or in combination, on growth and health of calves. Swanson et al reported that supplemental vitamin A at 8,300 and 19,600 IU/lb (18,300 and 44,000 IU/kg) of milk replacer solids did not negatively affect weight gain compared with 4,080 IU/lb (9,000 IU/kg) of solids.[23] Reddy et al found that 125 IU and 250 IU of vitamin E/d improved weight gains of calves compared with no vitamin E supplementation.[21] Krueger et al reported that calves fed to achieve 1.1 lb (0.5 kg)/d of BW gain had slight improvement in BW gains when supplemented with 500,000 IU of vitamin A, 50,000 IU of vitamin D, and 1,500 IU of vitamin E via 1 subcutaneous injection at birth and 500 IU of vitamin E via daily dietary supplementation compared with no supplementation.[13] So, prior reports indicate the amounts of dietary vitamin A or vitamin E used in this study are not detrimental when administered alone. Supplemental vitamin A, however, has negative consequences on vitamin E uptake.[1] The combination of vitamins A, D, and E administered by diet and injection has not been rigorously tested, although it is commonly practiced, and may indeed be detrimental to health and growth of calves if given in excess.

Excess supply of vitamins A and D from combined supplementation by diet and injection, in particular, may explain the decreased growth and digestive health of supplemented calves in this experiment. Researchers have reported that treatment of calves with 2×10^6 IU of vitamin A and 5×10^5 IU of vitamin D via injection resulted in premature growth plate closure.[28] The premature growth plate closure results in a condition known as 'hyena disease' in calves injected with high amounts of vitamin A. Takaki et al reported that vitamin D injection alone did not result in abnormalities, but vitamin A injection combined with vitamin D seemed to exacerbate the deformities.[24] Thus, the injection of vitamins A and D (250,000 IU each) in the present experiment may have contributed to the lesser gains of supplemented calves. Supplemental vitamin A (30,000 IU/d) also increased severity of scours in neonatal calves.[6]

The amount of vitamin D_3 supplied in this experiment also increased serum 25(OH)D concentrations beyond the normal range of 40 to 60 ng/mL observed for calves kept outdoors.[16,18] The 25(OH)D concentrations here were below concentrations associated with toxicity in cattle (200 ng/mL), but negative interactions between vitamins A and D on gut health, liver metabolism, and bone development from supplementation by diet and injection, as in this study, are conceivable and need to be explored further.

Until more data is available on optimal vitamin nutrition of calves, a moderate approach to vitamin supplementation of calves seems justified. Swanson et al[23] estimated

that Holstein bull calves required 5,000 IU/lb (11,000 IU/kg) of DM of vitamin A added to milk replacer as retinyl-acetate to sustain liver vitamin A concentrations. Pasteurized waste-milk should provide sufficient vitamin A to sustain liver vitamin A concentrations of calves, but an argument can be made to include 5,000 to 10,000 IU of supplemental vitamin A to compensate for potential vitamin A deficiencies of milk. For vitamin D, Nelson et al[16] estimated that serum 25(OH)D concentrations of Holstein bull calves increased approximately 6 to 7 ng/mL from a baseline of 15 ng/mL for every 1,000 IU of supplemental vitamin D_3 to be added to milk replacer. In that case, supplementation of pasteurized waste-milk with approximately 5,000 to 6,000 IU of vitamin D_3/d is estimated to achieve serum 25(OH)D concentrations of 40 to 60 ng/mL. As for vitamin E, Reddy et al[21] reported that 125 IU and 250 IU/d of supplemental vitamin E in the diets of Holstein heifer calves during the first 6 months of age improved weight gain compared to controls (0 IU/d). Feeding 500 IU/d of vitamin E did not provide further benefit. On the basis of data from Krueger et al[13] and Reddy et al[21] and the present experiment, we propose supplementing pasteurized waste-milk with 125 IU of vitamin E. We anticipate a moderate approach to supplementation of calves with vitamins A, D, and E, as proposed here, should provide tissues with sufficient supplies of each vitamin for optimal function, but not result in negative consequences from excess supplementation. Further experiments are needed to determine if our proposed rates of supplementation are optimal for health and growth of dairy calves.

Notable limitations to the present experiment are the lack of data on milk components and relatively small size of treatment groups. In the absence of data on fat, protein, and vitamin concentrations of waste-milk fed in the experiment, we are limited to making assumptions of nutrient intakes for the calves. Day-to-day and farm-to-farm variations in waste-milk composition must be acknowledged and limit the degree to which outcomes in the present experiment can be applied in other settings. In regards to the size of treatment groups, dairy calf nutrition experiments require approximately 20 calves per treatment group to detect reasonable differences in growth,[12] so the number of bulls and heifers in each group was not sufficient to detect interactions between sex and treatment for growth, health, and intake outcomes. On the other hand, the experiment benefited from including bulls and heifers as we observed differences between sexes for the concentration of 25(OH)D in serum. Similar to data here, beef heifer calves had greater serum 25(OH)D concentrations than beef bull calves.[18] Potential differences in responsiveness to vitamin supplementation of dairy calves have relevance for nutritional recommendations, as several previous vitamin supplementation trials used only dairy bull calves.[5,13,22,23] Design and interpretation of future experiments seeking to identify optimal vitamin nutrition of calves should consider the differences between sexes in responses to supplemental vitamins.

Conclusion

Supplementation of pasteurized waste milk with vitamins A, D, and E is effective for increasing fat-soluble vitamin serum concentrations in calves. Data from the present experiment indicate potential negative consequences of high rates of combined supplemental vitamins A, D, and E on growth of neonatal calves. For the time being, a moderate approach to supplementation of vitamins A, D, and E is recommended. On the basis of this experiment and others, we propose supplementing pasteurized waste milk with 6,000 IU of vitamin A, 6,000 IU of vitamin D_3, and 125 IU of vitamin E per day in the first weeks of life for dairy calves. Further research utilizing larger groups of dairy calves is warranted to further validate these recommendations.

Endnotes

[a] Stuart Products, Bedford, TX
[b] VITAL E-Newborn, Stuart Products, Bedford, TX
[c] AMPLI-Calf Starter 20P R50 Medicated, Purina Animal Nutrition, Gray Summit, MO
[d] BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ
[e] Sigma-Aldrich, St. Louis, MO
[f] Agilent Technologies, Inc., Santa Clara, CA
[g] Agilent Technologies, Inc., Santa Clara, CA
[h] Snap-N-Spike Retinol, V-011, Sigma Aldrich, St. Louis, MO
[i] Snap-N-Spike alpha-tocopherol, V-020, Sigma Aldrich, St. Louis, MO
[j] Fisher Chemical, Lenexa, KS
[k] Human VD3 ELISA, Eagle Biosciences, Nashua, NH
[l] SAS/STAT, SAS Institute Inc., Cary, NC

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