Relationships between immunoglobulin and fat-soluble vitamins in colostrum of Japanese Black multiparous cows

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Running title; Ig and fat-soluble vitamins in colostrum

ABSTRACT

Data from 19 Japanese Black multiparous cows were collected to clarify the relationships among Immunoglobulin (Ig) G, IgA, β-carotene, vitamin A and α-tocopherol contents in colostrum of cows in order to evaluate the role of fat-soluble vitamins on colostral IgG and IgA production. Mean colostral IgG was 141 mg/mL, ranging from 65 to 208 mg/mL, whereas mean colostral IgA was 8.7 mg/mL, ranging from 1.0 to 34.6 mg/mL. Colostral IgG was increased with aging in multiparous cows. There were positive correlations between colostral IgG and colostral vitamin A or colostral α-tocopherol in cows, and the higher adjusted R^2 was obtained in the prediction model of colostral IgG from age and colostral vitamin A. Colostral vitamin A was positively correlated with colostral β-carotene or colostral α-tocopherol in cows, but there were no relationships between colostral IgA and colostral IgG or colostral fat-soluble vitamins. These results indicate that fat-soluble vitamin contents in colostrum of cows may be changed in similar patterns and high colostral vitamin A is related with high colostral IgG.

Key Words: colostral IgA, colostral IgG, Japanese Black cows, β-carotene, vitamin A.

INTRODUCTION

Mortality and morbidity of neonates continue to be major problems in calves, and their most common disease is diarrhea, which can cause growth retardation and death of calves. Successful neonatal health depends on many factors related to management and nutrition, but the improvement of immune system is required for preventing diarrhea. Passive immunity is critical to the survival and health of neonates, and colostrum is a source of nutrients and immune components for neonatal calves (Blum 2006).

Immunoglobulin (Ig) antibodies are main immune components in colostrum and the most abundant Ig in bovine colostrum is IgG (Stelwagen *et al.* 2009). Compared with colostral IgG, colostral IgA in cows is very low (Ishikawa *et al* 1992), but IgA is the most abundant Ig isotype in mucosal secretions and provides protection against microbial antigens at mucosal surfaces in guts (Fagarasan & Honjo 2003; Mora & von Andrian 2009). In the previous study (Yasumatsuya *et al.* 2012), feeding whey protein is useful to enhance mucosal IgA induction in calves, because feeding whey protein increased fecal IgA in Japanese Black calves after 14 days of age.

The transfer of passive immunity reduces the incidence and severity of scours in calves, although the disease resistance acquired from colostral Ig is only temporary and scours are common at 5 days to 3 weeks of age in calves (Quigley & Drewry 1998). However, the improvement of colostral IgG and IgA in Japanese Black cows is needed for the appropriate calf health and immune system, because the lower transfer of IgG and IgA from colostrum to neonatal calves was found in Japanese Black calves at 2 days of age (Yasumatsuya *et al.* 2013).

Supplemental vitamin A and β-carotene enhance the immune system in neonates (Chew & Park 2004; Rühl 2007), and vitamin E deficiency is associated with the impairments in the cellular and humoral immunity (Maydani *et al.* 2005; Webb & Villamor 2007). High-quality silages contain large amounts of β-carotene and vitamin E, and β-carotene is converted to vitamin A by enzymes in the intestinal mucosa of cows (Johansson *et al.* 2014). Dietary levels of β-carotene and vitamin A affected not only colostral vitamin contents in cows but also vitamin status in newborn calves (Kume & Toharmat 2001). Supplemental β-carotene drastically increased serum β-carotene in Japanese Black calves (Nishiyama *et al.* 2011a), but β-carotene deficient calves were found to have a higher incidence of diarrhea in the first week of life (Kume & Toharmat 2001). Supplemental β-carotene in maternal mice during pregnancy and lactation increased the numbers of IgA antibody-secreting cells (ASC) in the mammary glands of lactating mice and enhanced IgA transfer from maternal milk to neonatal mice (Nishiyama *et al.* 2011a,b). These results indicate that supplemental fat-soluble vitamins have been expected to improve colostral Ig in cows and health status in neonatal calves. However, it is not clear whether colostral IgG and IgA in cows were affected by dietary fat-soluble vitamins.

The objective of this study was conducted to clarify the relationships among IgG, IgA, β-carotene, vitamin A and α-tocopherol contents in colostrum of Japanese Black multiparous cows in order to evaluate the role of fat-soluble vitamins on colostral IgG and IgA production.

MATERIALS AND METHODS

Animals and diets

This research was approved by the guide for the care and use of cows in accordance with “Regulation on Animal Experimentation at Kyoto University” (Animal Research Committee, Kyoto University, revised 2007). Data from 19 Japanese Black multiparous cows kept at Kyoto University Livestock Farm (Kyotanba, Japan) were collected from August 2013 to

February 2014. The parity of cows at parturition was 4.9 ± 2.0 (mean ± SD), ranging from 2 to 9, and body weight of cows at 10 days before the expected calving date was 575 ± 46 kg (mean ± SD), ranging from 478 to 650 kg. The cows were managed in paddocks during the dry period and an individual calving pen from 10 days before the expected calving date to parturition.

The cows were given 2 kg/day of wheat bran and appropriate amounts of Italian ryegrass or Sudangrass round baled silages to meet the TDN requirements of breeding cows (Agriculture, Forestry, and Fisheries Research Council Secretariat 2008), but synthetic vitamins did not offer to the cows. Italian ryegrass silages were produced from the first, second and third cuttings in 2012 and the first cutting in 2013, and Sudangrass silages were produced from the first and second cuttings in 2012. The wheat bran contained 0.07 mg/kg β-carotene and 31.3 mg/kg α-tocopherol, but vitamin contents in silages were not determined.

Sample collection and analyses

Body weights of calves were measured at birth. Samples of colostrum were taken from each udder of the cows by hand approximately within 1 h after parturition, and 100 mL colostrum were stored at -20°C for chemical analyses.

The immunoassay of colostral IgG and IgA was determined as previously described (Nishiyama *et al.* 2011a; Yasumatsuya *et al.* 2013). Colostral IgG and IgA contents were measured using the Bovine IgG and IgA ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, USA) and ELISA Starter Accessory Package (Bethyl Laboratories) according to the manufacturer's instructions, respectively.

Colostral β-carotene, vitamin A and α-tocopherol were determined by high-performance liquid chromatography (HPLC). Briefly, 50-300 µL colostrum were homogenized in 3 mL of ethanol with 1 (vitamin A) or 6 (β-carotene and α-tocopherol) % pyrogallol. The homogenate was mixed with 300 μL of 60% KOH and heated for saponification. After the addition of 3 mL of water, vitamins were extracted with 6 mL of n-hexane and the extract was evaporated to dryness. The residue was dissolved in 150-500 μL of ethanol/chloroform (19:1) solution and 20 μL of the sample was subjected to HPLC equipped with Shimadzu (Kyoto, Japan) LC-10 AT pump and SPD-10A UV-VIS detector set at 480 (β-carotene), 325 (vitamin A), and 292 (α-tocopherol) nm absorbance, respectively. The combinations of mobile phase, column, and column temperature were methanol:tetrahydrofuran:water(94:5:1 v/v)/Vydac 201TP54 (Grace, Columbia, MD, USA)/21-24°C for β-carotene, methanol:water(92:8)/Shim-pack CLC-ODS(M) (Shimadzu)/40°C for vitamin A, and methanol(100)/Shim-pack CLC-ODS(M)/40°C for α-tocopherol, respectively. The flow rate of mobile phase was 0.8 mL/min.

Statistics

Relationships between Ig and fat-soluble vitamins in colostrum or other variables were examined by correlation and regression analyses of Statistical Analysis Systems (SAS 1997). Significance was declared at $P<0.05$.

RESULTS

The dystocia occurred in one cow, but no metabolic disorders were detected in the other cows and the health status of their calves was good at birth. The birth weight of calves was 34.3 ± 3.8 kg (mean ± SD), ranging from 28.0 to 41.0 kg, and the calf birth weight was positively correlated ($r=0.59$; $P<0.01$) with the gestation length of dams.

Mean colostral IgG in cows was 141 mg/mL, ranging from 65 to 208 mg/mL, whereas mean colostral IgA was 8.7 mg/mL, ranging from 1.0 to 34.6 mg/mL (Table 1). Colostral IgG was positively correlated ($P<0.01$) with the age of cows, but colostral IgG was not correlated with the gestation length. There were positive correlations between colostral IgG and colostral vitamin A ($P<0.01$) or colostral α-tocopherol ($P<0.05$) in cows, but there were no relationships between colostral IgG and colostral IgA or colostral β-carotene (Fig. 1). The regression equations of age (X_{Age}), colostral vitamin A (X_{VA}) and colostral α-tocopherol (X_{VE}) on colostral IgG (Y_{IG}) were as follows.

$$Y_{IG} = 0.928(\pm 0.245)^{**}X_{Age} + 73.6(\pm 18.7)^{**}\ (R^2=0.43,\ ^{**}P<0.01)$$

$$Y_{IG} = 0.082(\pm 0.023)^{**}X_{VA} + 101(\pm 12)^{***}\ (R^2=0.40,\ ^{**}P<0.01,\ ^{***}P<0.001)$$

$$Y_{IG} = 0.031(\pm 0.012)^{*}X_{VE} + 111(\pm 13)^{***}\ (R^2=0.24,\ ^{*}P<0.05,\ ^{***}P<0.001)$$

There were no relationships between colostral IgA and fat-soluble vitamins in cows (Fig. 2). Colostral β-carotene was positively correlated with colostral vitamin A ($r=0.69$; $P<0.001$) and colostral α-tocopherol ($r=0.63$; $P<0.01$), and colostral vitamin A was positively correlated ($r=0.78$; $P<0.001$) with colostral α-tocopherol. The regression equations of colostral β-carotene (X_{BC}) on vitamin A (Y_{VA}) and colostral α-tocopherol (Y_{VE}) and the regression equation of colostral vitamin A (X_{VA}) on colostral α-tocopherol (Y_{VE}) were as follows.

$$Y_{VA} = 3.23(\pm 0.82)^{***}X_{BC} + 85(\pm 112)\ (R^2=0.45,\ ^{***}P<0.001)$$

$$Y_{VE} = 6.33(\pm 1.87)^{**}X_{BC} + 168(\pm 256)\ (R^2=0.37,\ ^{**}P<0.01)$$

$$Y_{VE} = 1.68(\pm 0.32)^{***}X_{VA} + 141(\pm 177)\ (R^2=0.59,\ ^{***}P<0.001)$$

There was no relationship between colostral vitamin A and age of cows, but a highly significant relationship was obtained between colostral IgG (Y_{IG}) and age (X_{Age}) and colostral vitamin A (X_{VA}).

$$Y_{IG} = 0.664(\pm 0.232)^{**}X_{Age} + 0.056(\pm 0.021)^{**}X_{VA} + 65.1(\pm 16.3)^{***}\ (R^2=0.58,\ ^{**}P<0.01,\ ^{***}P<0.001)$$

DISCUSSION

Colostrum contains not only nutrients but also biologically active substances that are essential for proper calf nutrition and health (Blum 2006; Stelwagen *et al.* 2009). The importance of adequate consumption of high-quality colostrum for acquisition of optimal nutrition and passive immunity is widely recognised in neonatal calves (Quigley & Drewry 1998; Stelwagen *et al.* 2009). The increased transfer of IgG and IgA from maternal milk to neonates is needed for maintaining normal health in calves, but serum IgG and IgA were not detectable in colostrum-deprived calves at less than 2 days of age (Nonnecke *et al.* 2012). Additionally, colostral IgG contents as well as colostral protein of primiparous cows were lower than those of multiparous cows (Devery-Pocius & Larson 1983; Kume & Tanabe 1993a). The bovine mammary gland plays an active role in regulating Ig concentrations in colostrum, and also immune factors in colostrum play an important role in the host defense of the mammary gland, because the mammary gland is very susceptible to infection (Stelwagen *et al.* 2009). In the present study, colostral IgG was increased with aging in Japanese Black multiparous cows. Thus, the aging in multiparous cows may be a factor altering colostral IgG owing to the protection of the mammary gland from pathogenic organisms.

The absorption of colostral Ig by neonatal calves is considered adequate when serum IgG concentrations exceed 10 mg/mL, because the mortality rates of calves with serum IgG < 10 mg/mL were over twice than those of calves with higher IgG contents (Quigley & Drewry 1998). On the other hand, passive immune protection of the newborn gastrointestinal tract is dependent on an active process of IgA ASC accumulation in the lactating mammary glands of the mother, and IgA antibodies produced from IgA ASC in the mammary glands are secreted into milk (Morteau *et al.* 2008; Nishiyama *et al.* 2011b; Wang *et al.* 2013). Mean colostral IgG and IgA contents in Japanese Black cows in the present study agreed with those of the other report (Ishikawa *et al.* 1992), but colostral IgG and IgA varied widely and colostral IgG was not correlated with colostral IgA. In the previous study (Yasumatsuya *et al.* 2013), there was no relationship between serum IgG and fecal IgA in Japanese Black calves at 2 days of age. These results suggest that the improving methods on IgG and IgA production in colostrum are different in cows, although the adequate supply of IgG and IgA from colostrum is essential to gain sufficient passive immunity in neonatal calves.

The composition of colostrum varied with a number of factors, including individuality, breed, parity, prepartum diet and occurrence of mastitis, and the production and composition of colostrum varied with the uptake of nutrients by the mammary gland, which was influenced by mammary blood flow and utilization of nutrients in the mammary gland (Foley & Otterby 1978). In basic dairy cow diets, provitamin A (β-carotene) and vitamin E are mainly found in pasture and in grass and legume silages, but the contents were highly variable and synthetic vitamins are often supplemented in the diets (Johansson *et al.* 2014). As a result, colostral β-carotene in 46 Holstein cows was 169 ± 85 μg/dL (mean ± SD), ranging from 17.8 to 342.9 μg/dL, and colostral vitamin A was 122 ± 77 μg/dL (mean ± SD), ranging from 32.9 to 450.0 μg/dL (Kume & Toharmat 2001). Additionally, colostral β-carotene and vitamin A in Holstein multiparous cows were not affected by the parity, but colostral vitamin A in primiparous cows was high level (Kume & Tamabe 1993b). The wide range of colostral β-carotene and vitamin A in dairy cows may be due to the variable dietary levels of β-carotene and vitamin A and their uptake by the mammary gland (Kume & Tanabe 1993b; Kume & Toharmat 2001). In the present study, β-carotene, vitamin A and α-tocopherol contents in colostrum of Japanese Black multiparous cows varied widely, but highly positive correlations were obtained among β-carotene, vitamin A and α-tocopherol contents in colostrum. Thus, β-carotene, vitamin A and α-tocopherol contents in colostrum of cows may be changed in similar patterns owing to the quality of silages and their uptake by the mammary gland.

Several effects of carotenoids are thought to be mediated by their metabolism to vitamin A and subsequent mediation of retinoic acid (RA) receptor and retinoid X receptor response pathways (Rühl 2007). β-carotene supplementation is effective to enhance mucosal IgA induction in the jejunum or ileum in weanling mice, and these effects may be mainly due to the RA-mediated immune response (Nishida *et al.* 2014). In the present study, high colostral vitamin A was related with high colostral IgG in Japanese Black cows, and the higher adjusted R^2 was obtained in the prediction model of colostral IgG from age and colostral vitamin A. Additionally, colostral α-tocopherol was positively correlated with colostral IgG, but colostral fat-soluble vitamins were not related with colostral IgA. These results indicate that feeding high-quality silages in pregnant cows has been expected to improve not only colostral fat-soluble vitamins but also colostral IgG contents. However, it is not clear whether supplemental vitamin A or β-carotene increases colostral IgG contents in cows owing to the RA-mediated immune response. Further study is needed to clarify the effects of fat-soluble vitamins on the passive immunity in calves, because a higher incidence of diarrhea occurred in the β-carotene deficient calves at 6 days of age (Kume & Toharmat 2001).

ACKNOWLEDGMENT

The present study was supported in part by a grant from Ito Kinenzaidan (Tokyo, Japan).

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Figure legends:

Figure 1 Relationships between colostral IgG and colostral IgA, vitamin A, α-tocopherol or age in Japanese Black multiparous cows (n=19). IgA, Immunoglobulin A; IgG, Immunoglobulin G.

Figure 2 Relationships between colostral β-carotene and colostral vitamin A or α-tocopherol and relationships between colostral vitamin A and colostral α-tocopherol or IgA in Japanese Black multiparous cows (n=19). IgA, Immunoglobulin A.

Table 1 Correlation between IgG, IgA and fat-soluble vitamins in colostrum of Japanese Black multiparous cows (n=19).

	Mean	SD	Min.	Max.	r	
					IgG	IgA
Age, months	72.7	23.5	34.0	118.3	0.68**	0.25
Gestation length, days	287.7	4.6	277.0	296.0	0.32	0.37
Colostrum						
β-carotene, μg/dL	125.7	56.0	40.0	221.0	0.35	-0.07
Vitamin A, μg/dL	490.5	259.4	69.0	1096.0	0.66**	-0.01
α-tocopherol, μg/dL	963.2	554.3	117.0	2606.0	0.53*	0.17
IgA, mg/mL	8.7	9.7	1.0	34.6	0.32	-
IgG, mg/mL	140.9	32.2	65.0	208.0	-	−

**P<0.01, *P<0.05.

IgA, Immunoglobulin A; IgG, Immunoglobulin G.

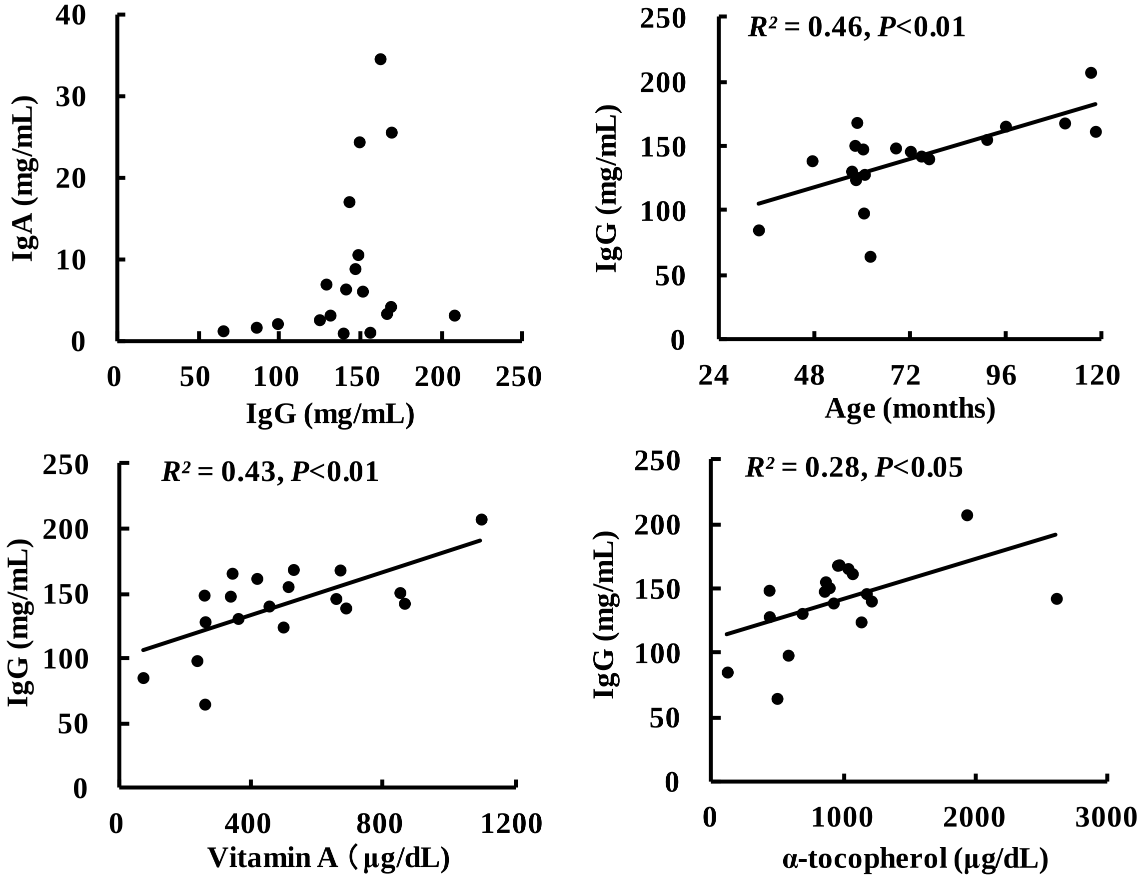
IgA (mg/mL)
IgG (mg/mL)
R² = 0.46, P<0.01
IgG (mg/mL)
Age (months)
R² = 0.43, P<0.01
IgG (mg/mL)
Vitamin A（μg/dL)
R² = 0.28, P<0.05
IgG (mg/mL)
α-tocopherol (μg/dL)

Figure 1

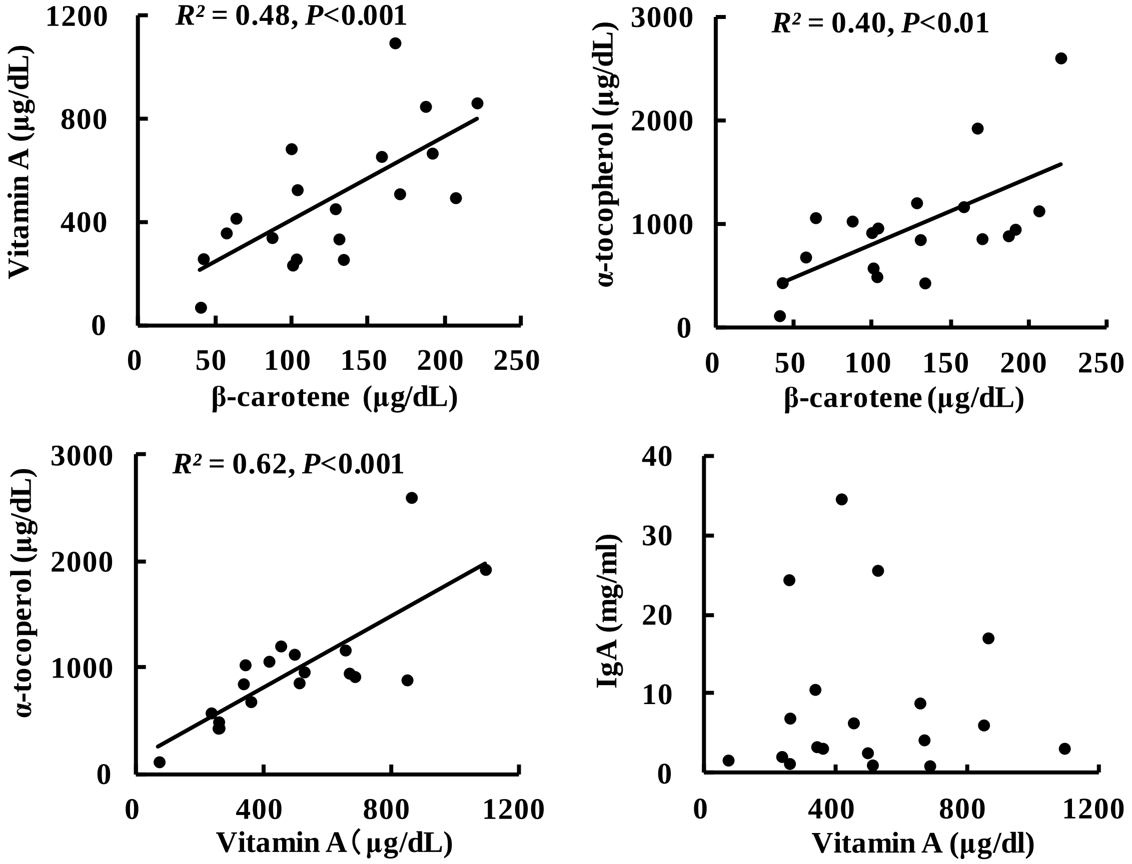
R² = 0.48, P<0.001
Vitamin A (μg/dL)
β-carotene (μg/dL)
R² = 0.40, P<0.01
α-tocopherol (μg/dL)
β-carotene (μg/dL)
R² = 0.62, P<0.001
α-tocoperol (μg/dL)
Vitamin A（μg/dL)
IgA (mg/ml)
Vitamin A (μg/dl)

Figure 2