Effects of β-Carotene on Immunoglobulin A Concentrations in Feces, Serum, and Milk of Pregnant Sows (Postprint)

Authors: Zhang Xiaoyin, Ji Yubin, Li Yanqiang, Wu Min, Ma Sihui, Zheng Xin

Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary β-carotene supplementation during late gestation on β-carotene content in feces and immunoglobulin A (IgA) concentrations in feces, serum, and milk of pregnant sows. Thirty pregnant sows with similar body condition and parity were randomly divided into 3 groups with 10 sows per group and raised for 60 d. The control group was fed a basal diet, while the half-month group and one-month group received the basal diet supplemented with 200 mg/kg β-carotene at half a month and one month before the expected farrowing date, respectively. β-carotene content in feces was determined by high-performance liquid chromatography (HPLC), and IgA concentrations in feces, serum, and milk were measured by enzyme-linked immunosorbent assay (ELISA). The results showed that β-carotene content in feces of the half-month and one-month groups was higher than that of the control group, with most differences being significant ($P < 0.05$); at one week before farrowing, IgA concentrations in feces of the half-month and one-month groups were mostly higher than those of the control group, but at one week after farrowing, IgA concentrations in feces of the half-month and one-month groups were mostly lower than those of the control group; IgA concentrations in serum and milk of the half-month and one-month groups were higher than those of the control group, with no significant differences ($P > 0.05$) except for serum IgA concentration in the one-month group. In conclusion, dietary β-carotene supplementation can increase β-carotene content in feces of pregnant sows and elevate IgA concentrations in serum and milk, thereby enhancing the immune function of pregnant sows.

Full Text

Effects of β-Carotene on Immunoglobulin A Concentration in Feces, Serum, and Milk of Late-Pregnancy Sows

ZHANG Xiaoyin, JI Yubin*, LI Yanqiang, WU Min, MA Sihui, ZHENG Xin
(College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China)

Abstract

This experiment was conducted to investigate the effects of dietary β-carotene supplementation on fecal β-carotene content and immunoglobulin A (IgA) concentrations in feces, serum, and milk of late-pregnancy sows. Thirty sows with similar body condition and parity were randomly divided into three groups (n=10 per group) and fed for 60 days. The control group received a basal diet, while the half-month and one-month groups received the basal diet supplemented with 200 mg/kg β-carotene starting at half a month and one month before the expected delivery date, respectively. Fecal β-carotene content was determined by high-performance liquid chromatography (HPLC), and IgA concentrations in feces, serum, and milk were measured by enzyme-linked immunosorbent assay (ELISA). The results showed that fecal β-carotene content in both the half-month and one-month groups was significantly higher than in the control group ($P<0.05$) for most sampling days. One week before delivery, fecal IgA concentrations in the treatment groups were mostly higher than in the control group; however, one week after delivery, they were mostly lower. Serum and milk IgA concentrations in the treatment groups were higher than in the control group, though most differences were not significant ($P>0.05$), except for serum IgA in the one-month group. These findings indicate that dietary β-carotene supplementation can increase fecal β-carotene content and elevate IgA concentrations in serum and milk, thereby enhancing the immune function of late-pregnancy sows.

Keywords: β-carotene; late-pregnancy sows; HPLC; immunoglobulin A

Introduction

Swine production is a pillar industry of China' s animal husbandry sector, yet it is plagued by widespread reproductive problems, including low farrowing rates and piglet survival rates. Moreover, the intensification of farming practices has led to increased disease prevalence, particularly severe infectious diseases that pose significant threats to the industry. Kume et al. [**?**] demonstrated that β-carotene promotes animal reproduction, immunity, and health. As a type of carotenoid, β-carotene is a fat-soluble compound widely found in plants and serves as the most biologically active provitamin A, listed as a food and feed

additive in 157 countries. Supplementing β-carotene in the diets of pregnant and lactating sows can prevent metritis and reduce piglet scours, thereby improving piglet survival rates and weaning litter weights [**?**]. Previous studies have shown that β-carotene can modulate immunity and reduce disease incidence [**?**]. This experiment investigated the effects of dietary β-carotene supplementation on fecal β-carotene content and IgA concentrations in feces, serum, and milk of late-pregnancy sows, providing further theoretical basis for understanding β-carotene absorption, metabolism, and immune function in pregnant sows.

1.1 Experimental Design

Thirty healthy Junmu No. 1 pregnant sows with identical parity, similar expected delivery dates, and uniform body condition were selected from the Jilin University Original Breed Pig Farm and randomly divided into three groups (n=10 per group) for a 60-day feeding trial. The control group received a basal diet, while the half-month and one-month groups received the basal diet supplemented with 200 mg/kg β-carotene starting at half a month and one month before the expected delivery date, respectively.

Received date: 2015-08-12
Funding: International Science and Technology Cooperation Project (20130413037GH)
Author introduction: ZHANG Xiaoyin (1991-), female, from Shangqiu, Henan, master' s student, engaged in cellular immunity and animal breeding research. E-mail: 951770634@qq.com
*Equal contribution author
**Corresponding author: ZHENG Xin, professor, doctoral supervisor, E-mail: zhengxinjilin@126.com

1.2 Experimental Materials

β-carotene (10%) was purchased from Wuhan Xingchen Biological Technology Co., Ltd. (food-grade, suitable for feed additives). The high-performance liquid chromatography (HPLC) system (1260 Infinity quaternary liquid chromatography) was purchased from Agilent Technologies. Porcine IgA ELISA kits were purchased from Shanghai Langdun Biotechnology Co., Ltd.

1.3 Feeding Management and Sample Collection

All sows were housed in individual gestation crates under identical environmental conditions and managed by the same caretaker throughout the trial. Basal diets were formulated according to NRC (2012) standards for gestating and lactating sows, with composition and nutrient levels shown in Table 1 . Feed intake was limited to 2.5 kg per sow per day, divided into three meals, with free access to water. Seven days before the expected delivery date, sows were transferred to farrowing crates for careful monitoring. Routine farm vaccinations followed the Jilin University Original Breed Pig Farm immunization program, with proper

management and disease prevention measures implemented during the feeding period.

Fecal samples were collected daily from 7 days before delivery to 7 days postpartum; no samples were collected on the day of delivery due to minimal defecation. All fecal samples were immediately frozen at -20°C. Blood samples (5 mL) were collected via ear vein on days 7 prepartum, 1 day postpartum, and 7 days postpartum, allowed to clot for 30 minutes, then centrifuged at 3,000 rpm for 10 minutes at 4°C. Serum was harvested and stored at -80°C. Milk samples (5 mL) were collected on the day of delivery and stored at -80°C.

1.4.1 Determination of Fecal β-Carotene Content

Fecal samples were ground and homogenized, and 1.00 g was accurately weighed and extracted repeatedly with petroleum ether and acetone (8:2, v/v). β-carotene content was determined by HPLC (4.6 mm × 250 mm, 5 m) and quantified using a standard curve.

1.4.2 Determination of IgA Concentration

Fecal samples were diluted with phosphate-buffered saline (PBS) and centrifuged at 3,000 rpm for 10 minutes; the supernatant was collected for analysis. Serum and milk samples were analyzed by indirect ELISA according to the kit instructions. Optical density (OD) values were measured at 450 nm using a fully automated microplate reader, and IgA concentrations were determined from standard curves.

1.5 Statistical Analysis

Data were initially processed using Excel 2007 and analyzed statistically using SPSS 19.0 software. Differences among groups were tested using Duncan' s multiple comparison test. Results are expressed as mean ± standard deviation, with $P<0.05$ considered statistically significant.

2.1.1 Standard Curve Construction by HPLC

β-carotene standard solutions were filtered through a 0.45 m organic membrane before HPLC analysis. The standard curve was constructed by plotting peak area (y-axis) against standard solution concentration (x-axis). Representative HPLC chromatograms are shown in Figures 1-3 [FIGURE:1-3], and the standard curve is presented in Figure 4 [Figure 4: see original paper].

2.1.2 Effects of β-Carotene Supplementation on Fecal β-Carotene Content in Late-Pregnancy Sows

Fecal samples were collected from 7 days prepartum to 7 days postpartum, with sampling days arranged as shown in Table 2 ; day 8 (delivery day) was excluded due to minimal defecation. As shown in Table 2, fecal β-carotene content in

the control group remained nearly unchanged throughout the sampling period. In the half-month group, β-carotene content was significantly higher than in the control group ($P<0.05$) on most days except days 10 and 11. Similarly, in the one-month group, β-carotene content was significantly higher than in the control group ($P<0.05$) on most days except days 9, 12, and 13. Notably, β-carotene content on day 1 (pre-supplementation) and day 9 (1 day postpartum) was markedly higher than on other sampling days in the treatment groups.

2.2.1 Effects of β-Carotene Supplementation on Fecal IgA Concentration in Late-Pregnancy Sows

As shown in Table 3 , during the prepartum period (days 1-7), fecal IgA concentrations in the half-month group were higher than in the control group, with significant differences on days 5 and 7 ($P<0.05$). Similarly, the one-month group showed higher fecal IgA concentrations than the control group on days 1, 3, 5, 6, and 7 prepartum. Thus, during the week before delivery, both treatment groups exhibited mostly higher fecal IgA concentrations than the control group. In contrast, during the postpartum period, fecal IgA concentrations in the half-month group were lower than in the control group on all days, with significant differences except on day 14 ($P<0.05$). The one-month group also showed lower fecal IgA concentrations than the control group on most postpartum days, except days 9 and 14. Therefore, during the week after delivery, both treatment groups displayed mostly lower fecal IgA concentrations than the control group.

2.2.2 Effects of β-Carotene Supplementation on Serum IgA Concentration in Late-Pregnancy Sows

As shown in Table 4 , serum IgA concentrations in both the half-month and one-month groups were higher than in the control group, indicating that dietary β-carotene supplementation can increase serum IgA levels, which tended to rise with prolonged supplementation. However, an anomaly was observed on day 9 (1 day postpartum), where serum IgA concentration in the half-month group was higher than in the one-month group ($P>0.05$). This corresponded with the abnormal fecal IgA data on day 9 shown in Table 3, where the half-month group had significantly lower fecal IgA concentration than the one-month group ($P<0.05$).

2.2.3 Effects of β-Carotene Supplementation on Milk IgA Concentration in Late-Pregnancy Sows

As shown in Figure 5 [Figure 5: see original paper], colostrum collected on day 1 postpartum exhibited a trend of increasing IgA concentration with β-carotene supplementation, though the differences were not statistically significant ($P>0.05$).

IgA effectively neutralizes bacterial toxins and plays a crucial protective role in intestinal and respiratory mucosa [**?**]. Primarily produced by mucosa-associated

lymphoid tissue, IgA prevents microbial adhesion to mucosal surfaces and inhibits penetration by commensal microorganisms [**?**]. Additionally, through binding to viral receptors on cell surfaces, IgA can disrupt viral structure and reduce viral infection [**?**]. Furthermore, mammary tissue during lactation contains abundant IgA-producing cells, and mothers can transfer secretory IgA to infants via colostrum, representing an important form of natural passive immunity. Therefore, IgA concentration can serve as an indicator of immune status [**?**].

Numerous studies have reported the antioxidant and immune-enhancing effects of β-carotene. Research in poultry, cats, and dogs has shown that β-carotene can increase superoxide dismutase (SOD) activity and enhance various immune defense functions [**?**]. MA Sihui et al. [**?**] demonstrated that β-carotene can increase serum IgA concentration in immunosuppressed mice. However, studies on the effects of β-carotene on immune function in large livestock such as pigs and cattle remain limited. HE Wenjuan et al. [**?**] found that dietary β-carotene supplementation during the periparturient period significantly increased lymphocyte transformation rates in dairy cows, with plasma β-carotene and vitamin A levels rising as supplementation increased. Brief et al. [**?**] observed that daily injection of 32.6 mg β-carotene in gilts increased plasma β-carotene content and immunoglobulin concentrations.

In this study, fecal β-carotene content and serum and milk IgA concentrations were higher in both treatment groups compared to the control group, indicating that dietary β-carotene supplementation can enhance the immune function of late-pregnancy sows. During the postpartum period (days 10-13), fecal IgA concentration in the control group increased markedly, possibly due to the release of various reproductive, regulatory, and stress hormones in lactating sows. However, this phenomenon was not observed in the treatment groups. Notably, while fecal IgA concentrations in the treatment groups were higher than the control group during the prepartum week, they were lower postpartum, and milk IgA concentrations in the treatment groups were consistently higher than in the control group. This suggests that β-carotene supplementation may enhance the transfer of IgA from sows to newborn piglets, thereby boosting neonatal immunity. Nishiyama et al. [**?**, **?**] at Kyoto University reported that in mice born to β-carotene-supplemented dams, IgA concentration in stomach contents was significantly elevated, and serum and fecal IgA concentrations in the offspring remained elevated throughout subsequent development, indicating that maternal β-carotene supplementation during pregnancy and lactation enhances IgA transfer from mother to neonate. Although piglet samples were not collected in this study, future experiments will verify this hypothesis.

In conclusion, dietary supplementation with appropriate levels of β-carotene in late-pregnancy sows can increase fecal β-carotene content, elevate serum and milk IgA concentrations, and enhance sow immune function.

References

[1] KUME S, TOHARMAT T. Effect of colostral β-carotene and vitamin A on vitamin and health status of newborn calves[J]. Livestock Production Science, 2001, 68(1): 61-65.
[2] Cai Xiaozhan, He Yinfeng. Research progress on β-carotene[J]. Journal of Agricultural Products Processing, 2005(8): 27-30.
[3] Effects of vitamins on reproductive function in pigs[EB/OL]. (2009-06-16). http://www.sdny.com.cn/xwxx_{xiangye}.asp?id=89069&cid=&cnam=.
[4] Li Cairong, Jin Yuting, Hua Chunzhen. Dynamic observation of immunoglobulin and complement content in early breast milk[J]. Zhejiang Preventive Medicine, 2015, 27(3): 308-309, 312.
[5] MACPHERSON A J, UHR T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria[J]. Science, 2004, 303(5664): 1662-1665.
[6] GAN Y J, CHODOSH J, MORGAN A, et al. Epithelial cell polarization is a determinant in the infectious outcome of immunoglobulin A-mediated entry by Epstein-Barr virus[J]. Journal of Virology, 1997, 71(1): 519-526.
[7] YANG Y, YUAN Y J, TAO Y H, et al. Effects of vitamin A deficiency on mucosal immunity and response to intestinal infection in rats[J]. Nutrition, 2011, 27(2): 227-232.
[8] JOHNSON E J, QIN J, KRINSKY N I, et al. β-carotene isomers in human serum, breast milk and buccal mucosa cells after continuous oral doses of all-trans and 9-cis β-carotene[J]. The Journal of Nutrition, 1997, 127(10): 1993-1999.
[9] CANFIELD L M, GIULIANO A R, NEILSON E M, et al. Kinetics of the response of milk and serum β-carotene daily β-carotene supplementation healthy, lactating women[J]. American Journal of Clinical Nutrition, 1998, 67(2): 276-283.
[10] Han Ruili, Li Tongshu, Li Jianqun, et al. Effects of β-carotene and VE on fatty acid composition and oxidative stability of chicken meat fed fish oil diets[J]. Journal of Northwest A&F University: Natural Science Edition, 2006, 34(11): 47-50.
[11] Ma Sihui, Yang Huan, Wu Tiancheng, et al. Effects of β-carotene on immune indices in immunosuppressed mice[J]. Chinese Journal of Veterinary Medicine, 2014, 48(7): 10-14.
[12] He Wenjuan, Meng Qingxiang, Bian Sibei. Effects of periparturient feeding of β-carotene on immune performance in dairy cows[J]. Chinese Journal of Animal Science, 2007, 43(3): 32-35.
[13] BRIEF S, CHEW B P. Effects of vitamin A and β-carotene on reproductive performance in gilts[J]. Journal of Animal Science, 1985, 60(4): 998-1004.
[14] NISHIYAMA Y, YASUMATSUYA K, KASAI K, et al. Effects of supplemental β-carotene with whey on IgA transfer from maternal milk and mucosal IgA induction in neonatal mice and calves[J]. Livestock Science, 2011, 137(1/3): 95-100.
[15] NISHIYAMA Y, SUGIMOTO M, IKEDA S, et al. Supplemental β-carotene

increases IgA secreting cells in mammary gland and IgA transfer from milk to neonatal mice[J]. British Journal of Nutrition, 2011, 105(1): 24-30.

Note: Figure translations are in progress. See original paper for figures.