J. Dairy Sci. 97:7144–7150
http://dx.doi.org/10.3168/jds.2014-8276

Effects of feeding lutein on production performance, antioxidative status, and milk quality of high-yielding dairy cows

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ABSTRACT

This experiment was conducted to determine the influences of supplementing different levels of an additive containing lutein in the diet of Chinese Holstein lactating cows on production performance, antioxidative plasma metabolites, and milk quality. This study was performed on 60 multiparous Holstein dairy cows in peak lactation. The cows were randomly allocated to 1 of 4 homogeneous treatments, with lutein preparation (extracted from marigolds; effective lutein content was 2%) added at levels of 0, 100, 150, and 200 g/d per head, with the actual available amounts being 0, 2, 3, and 4 g of lutein/d per head, respectively. The experiment lasted for 13 wk, with the first week for adaptation. Milk yield and milk compositions were recorded weekly, and milk concentrations of lutein, dry matter intake, and antioxidative blood index were analyzed in the first, fourth, seventh, and thirteenth week of the study. The results showed that adding lutein in the diet had no effect on dry matter intake compared with the control group; however, it slowed down the trend of decline in milk yield, and had a linear incremental effect on milk yield with increasing concentration of lutein. Dietary lutein tended to quadratically increase the percentage of milk fat, and linearly increased milk lactose concentration, with the highest value when treated at 200 g of lutein preparation/d per head, and decreased somatic cell count, with the lowest values when treated with 150 and 200 g of lutein preparation/d per head. The concentration of lutein in milk linearly increased with the incorporation of the additive, with a value of 0.59, 0.70, 1.20, and 1.50 μg/100 mL when treated with 0, 100, 150, and 200 g/d, respectively. Total plasma antioxidant capacity tended to linearly increase in cows fed lutein preparation, whereas plasma superoxide dismutase and glutathione peroxidase activities did not differ significantly. In conclusion, addition of lutein in the diet could improve the production performance and health status of dairy cows.

Key words: lutein, dairy cow, milk performance, antioxidative status

INTRODUCTION

With the improvement of people's living standards, the demand for high-quality milk has increased. Exogenous nutrients are used to make the most of the potential of lactating cows, while enhancing the ability to resist disease. Recently, interest has increased to improve milk quality by manipulating the composition of the fat-soluble fraction in milk and dairy products by feed-management practices (Martin et al., 2004). In dairy products, along with certain FA, carotenoids and fat-soluble vitamins are recognized as tracer compounds indicating good quality of milk and animal-feeding management (Martin et al., 2005).

Lutein, an antioxidative nutrient, is a natural carotenoid widely found in vegetables, flowers, fruits, and certain algae species (Mangels et al., 1993). It is also a main component of macular pigment in the human retina. The potential benefits reported for lutein supplementation include cancer prevention and enhanced immune function (Chew et al., 1996), inhibition of the autoxidation of cellular lipids (Zhang et al., 1991), protection against oxidant-induced cell damage (Martin et al., 1996), inhibition of the growth of mammary tumors in mice (Chew et al., 1996), increase the antioxidative function of rat to stay -galactose-induced consenescence (Pei et al., 2007), and prevention of age-related macular degeneration (Fullmer and Shao, 2001). Lutein influences the sensory properties of dairy products not only indirectly via antioxidant activity, but also directly because it confers a yellow color that is perceived positively (Prache et al., 2002).

At present, lutein has been commercialized internationally and is used as an additive for poultry feed (Levy, 2001). It has been demonstrated that feeding

Received April 24, 2014.
Accepted July 9, 2014.
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lutein could enhance the production performance of yellow catfish (Wang et al., 2012) and improve the reproductive performance of quail (Cucco et al., 2007). However, information is limited with regard to the effects of lutein on the performance of lactating cows. The objective of this study was to determine the effects of lutein addition on milk performance, serum metabolic variables, health status, and the quality of milk in lactation dairy cows.

MATERIALS AND METHODS

Animal, Diets, and Experimental Design

Sixty multiparous Chinese Holstein dairy cows in peak lactation period were blocked based on DIM (39 ± 10 d; mean ± SD), milk production (36.3 ± 1.3 kg/d; mean ± SD), average weight (605 ± 52 kg; mean ± SD), and parity (2.9 ± 1.1; mean ± SD), and were randomly assigned to 1 of 4 dietary treatments within block: 0 (control), 100, 150, or 200 g of lutein preparation/d per head. The lutein preparation, AbsoLUTEIN (dark yellow, short-strip particles), was provided by Kemin Industries (Zhuhai, China). It was composed of 15% oleoresin extracted from marigolds; silica and rice bran were used as carriers. The content of lutein was 2% in this product. Thus, the actual amount of lutein fed was 0, 2, 3, and 4 g of lutein/d per head, respectively. Cows were housed in individual tie-stalls with access to water and feed ad libitum, and were milked at 0700, 1400, and 2000 h daily. The use of the animals was approved by the Animal Care Committee, Zhejiang University (Hangzhou, China).

The ingredients of the experimental diet and nutrient content of the feed are presented in Table 1. Feed was offered 3 times per day. All ingredients were mixed in a TMR, offered to each cow daily, and the lutein preparation was top-dressed on the TMR based on diet assignment. All cows had free access to water throughout the entire experiment.

Sampling, Measurement, and Analyses

Feed offered was recorded daily and was adjusted to yield 5 to 10% orts. Feed intake was calculated based on the feed offered and orts. Adjustments to the TMR were made weekly based on DM content of dietary components. During the sampling period, proportional amounts of each feed offered were collected, pooled according to treatment, and sampled for chemical analysis. All samples were dried in a forced-air oven at 60°C for 48 h, and placed in sealed containers until analysis. Samples were ground to pass a 2-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA) and then through a 1-mm screen in a Cyclotec mill (Tecator 1093; Tecator AB, Höganäs, Sweden). Feed samples were analyzed for DM, total nitrogen (AOAC International, 2000; method 984.3), NDF (AOAC International, 2000; method 973.18), ADF (Van Soest et al., 1991), dietary lutein content (Cardinault et al., 2006), and ether extract, Ca, P, and ash (AOAC International, 2000).

Milk yield was recorded daily and milk samples were collected midweek from each milking using Waikato Milking System meters (Waikato Milking Systems NZ Ltd., Hamilton, New Zealand). Milk samples were collected from each milking, proportional to yield (4:3:3 ratio, composite). A 50-mL subsample was treated with Broad Spectrum Microtabs II milk preservative (D & F Control Systems Inc., Norwood, MA) and stored at 4°C for later determination of fat, protein, lactose, and TS content by infrared spectrophotometry (MilkoScan; Foss Electric A/S, Hillerød, Denmark; Laporte and Paquin, 1999), and SCC by automatic counter (Fossomatic 5000; Foss Electric A/S). The concentration of MUN was determined using the diacetyl monoxime-binding assay described by Wang et al. (2010). Another

Table 1. Ingredients and composition (% of DM, unless otherwise specified) of basal diet used in the experiment

Item	Content
Ingredient	
Ground corn	22.9
Soybean meal	9.9
Barley	3.9
Wheat bran	2.4
DDGS[1]	3.3
Cottonseed meal	4.8
Corn silage	11.9
Alfalfa hay	16.1
Grass hay	9.6
Leaf mustard	1.6
Beet pulp	8.8
Premix[2]	4.9
Composition[3]	
DM, %	44.8
CP	15.2
EE	3.85
NDF	36.9
ADF	22.1
Ca	0.62
P	0.31
Ash	8.17
Lutein, mg/kg	15.1
NE_L,[4] Mcal/kg	1.65

[1]Dried distillers grains with solubles.

[2]Provided per kilogram of premix: 80,000 to 145,000 mg of vitamin A, 20,000 to 39,000 mg of vitamin D, ≥700 IU of vitamin E, 180 to 345 mg of Cu, 190 to 330 mg of Fe, 950 to 1,800 mg of Zn, 350 to 650 mg of Mn, ≥7% Ca, ≥1.3% P, ≥1% CP, ≥15% ether extract (EE), ≤6% crude fiber, and ≤12% moisture.

[3]Calculated from the analyzed value of the dietary ingredients.

[4]Calculated value [based on Ministry of Agriculture of P.R. China (2004)].

subsample was treated without preservatives and stored at −20°C to be used for determining the concentration of lutein in milk by HPLC according to Emenhiser et al. (1996) and Dachtler et al. (1998).

Blood samples (10 mL) were collected from the coccygeal vein by syringe at approximately 3 h after feeding on the first day of the first, third, sixth, and twelfth week, immediately placed into a 10-mL tube with anticoagulation, and then centrifuged at 3,000 × *g* for 10 min at 4°C to collect plasma. The plasma was stored at −20°C away from light until the quantification of NEFA (McCutcheon and Bauman, 1986), glucose (McCutcheon and Bauman, 1986), malondialdehyde (Zhang et al., 2006), glutathione peroxidase (**GSH-Px**; Zhang et al., 2006), superoxide dismutase (**SOD**; Zhang et al., 2006), and total antioxidant capacity (**TAOC**; Tang et al., 2009). These indicators were analyzed by automatic biochemical analyzer.

Calculations and Statistical Analysis

The intake of NE_L was calculated as the sum of the net energy content of individual feeds and their proportion in the ration multiplied by DMI. Statistical analysis was conducted using PROC MIXED of SAS (SAS Institute, 2000) with the covariance type autoregressive 1 [AR(1)]. A randomized block design with repeated measurements was used, with week, treatment, treatment × week, and block as the main effects, and cow within treatment as a random effect. The effect of week was included as a repeated measure. Results are reported as least squares means. Linear and quadratic effects of treatment were tested for milk yield, milk composition, and plasma variables using orthogonal polynomial contrasts. Transfer efficiency of lutein to milk was calculated individually for each cow, using the ratio between the daily intake of lutein and that excreted in milk. Regression analysis of average milk lutein concentration on lutein intake was also carried out for individual cows. The REG procedure of SAS (SAS Institute, 2000) was applied to determine the milk lutein concentration relative to lutein consumption. A *P*-value of ≤0.05 was used to define statistical significance, and values of $0.05 < P < 0.10$ were considered a trend.

RESULTS

Lactation Performance

The effect on production performance of the addition of lutein is shown in Table 2. No significant effect on DMI was detected. During the experimental period, milk yield declined over time in all groups; however, that of the control was the lowest compared with those of the experimental groups (Figure 1). Milk yield was the highest in the group fed 200 g of lutein preparation/d per head ($P < 0.05$), whereas milk yield tended to be higher in 100 and 150 g of lutein preparation/d per head compared with that of the control. Average daily milk yield increased by 2.8, 1.5, and 0.7 kg/d per head, respectively, for treatments of 200, 100, and 150 g of lutein preparation/d per head. Milk yield linearly increased in cows receiving lutein preparation compared with that for the control. Addition of lutein had a quadratic effect on milk fat concentration ($P = 0.02$; Table 2). Contents of milk protein and TS were not different among the 4 treatments ($P > 0.05$). All the cows fed lutein had higher lactose contents than that of the control ($P < 0.001$). Also, a trend of decreased SCC was observed for all cows fed lutein ($P = 0.06$), with a 33.7% decrease in 150 and 200 g of lutein preparation/d per head compared with that of the control.

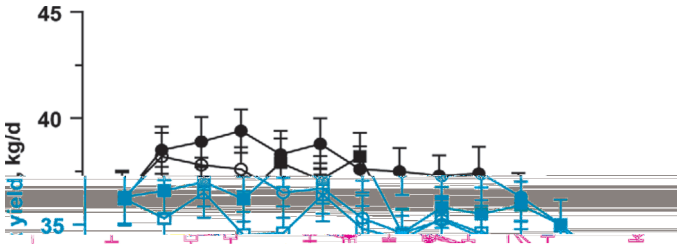
Milk Lutein and Transfer Efficiency

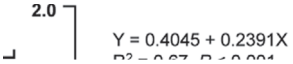
As shown in Table 3, the milk lutein concentration was 0.59 μg/100 mL for the control group and increased with the addition of lutein. Milk lutein concentration

Table 2. Effects of dietary addition of lutein preparation[1] on DMI, milk yield, and milk composition in dairy cows

	Lutein preparation, g/d per head					*P*-value	
Item	0	100	150	200	SEM	Linear	Quadratic
DMI, kg/d	21.1	21.1	21.2	20.6	0.35	0.37	0.42
Milk yield, kg/d	34.6	36.1	35.3	37.4	0.70	0.02	0.72
Milk composition, %							
Fat	3.74	3.83	3.89	3.75	0.05	0.57	0.02
Protein	3.09	3.02	3.11	3.06	0.03	0.99	0.83
Lactose	4.94	4.97	4.99	5.04	0.02	<0.01	0.61
TS	13.3	13.2	13.5	13.2	0.10	0.58	0.19
Urea nitrogen	13.4	13.2	13.7	14.4	0.17	<0.01	0.03
SCC, ×1,000/mL	175	154	116	116	24.4	0.06	0.67

[1]AbsoLUTEIN (Kemin Industries, Zhuhai, China).


45
40
35
yield, kg/d


2.0
Y = 0.4045 + 0.2391X

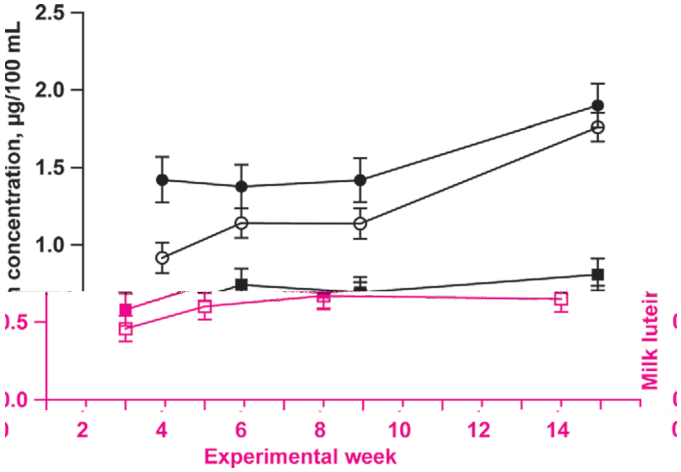

2.5
2.0
1.5
1.0
concentration, µg/100 mL
2
4
6
8
10
12
14
Experimental week
Milk luteir

Table 3. Effects of dietary addition of lutein preparation[1] on milk lutein concentration and transfer efficiency of lutein in dairy cows

	Lutein preparation, g/d per head					*P*-value	
Item	0	100	150	200	SEM	Linear	Quadratic
Milk lutein, μg/100 mL	0.59	0.70	1.20	1.50	0.08	<0.01	0.32
Milk lutein excretion,[2] mg/d	0.20	0.25	0.41	0.54	0.01	<0.01	0.39
Transfer efficiency,[3] ‰	0.00	0.025	0.071	0.087	0.00	<0.01	0.45

[1]AbsoLUTEIN (Kemin Industries, Zhuhai, China).

[2]Calculated from milk lutein concentration multiplied by milk yield.

[3]The ratio between milk lutein excretion per day and the daily intake of lutein.

in the growth of animals, production performance, and has antioxidant properties. In the study of Wang et al. (2012), supplementation of 24.2 to 1,700 mg of xanthophylls/kg significantly improved growth performance of *Pelteobagrus fulvidraco*. Little literature is available about the effects of lutein on dairy cow production performance. In the present study, no effect on DMI of supplementing lutein was detected; however, milk yield was significantly increased (P = 0.02). The feed conversion (kg of milk/kg of DMI) was 1.64, 1.71, 1.67, and 1.82 for the control, and 100, 150, and 200 g of lutein preparation groups, respectively, suggesting that the enhanced milk yield in cows fed lutein is likely associated with increased feed conversion. In addition, all the cows fed a lutein preparation had higher milk fat and lactose contents than those of the control. Some studies have reported that diets containing β-carotene can also increase milk yield and increase milk fat concentration (Aréchiga et al., 1998; Nalecz-Tarwacka et al., 2003).

Higher milk production may be associated with a decrease in SCC, and a linear relationship between daily milk yield and $\log_2$-transformed SCC was reported by Hagnestam-Nielsen et al. (2009). A maximum of −0.346 to −0.66 kg of milk yield per unit of SCS was observed by Miller et al. (2004). Results of another study suggested strong and negative effects of SCS on milk yield (approximately −0.9 to −1.8 kg per unit of SCS; Wu et al., 2007). Boland et al. (2013) reported that the change in test-day milk yield was −0.82 kg/d with increasing SCC from <101 to <201 × 1,000/mL. Similarly, in the current study, milk yield increased 0.7 kg/d with a decrease in SCC from 175 to 116 × 1,000/mL. The number of somatic cells increases in response to pathogenic bacteria such as *Staphylococcus aureus*, a cause of mastitis. Cows are in negative energy balance during the peak lactation. Consequently, cows are more susceptible to udder infection in early lactation, and a more severe depression of milk yield would be expected to occur (Suriyasathaporn et al., 2000). In the present study, a trend existed to reduce the SCC for all cows fed lutein. Thus, feeding lutein could reduce the incidence of mastitis, which is consistent with the increase in the concentration of plasma TAOC (P = 0.07).

As an antioxidant, lutein has strong antioxidant capacity and can eliminate active oxygen free radical activity and prevent damage to normal cells, thereby protecting the body against metabolic damages (Zhang et al., 1991; Chew et al., 1996; Martin et al., 1996). In the current study, lutein additive tended to increase plasma SOD concentration and TAOC, and numerically increase plasma GSH-Px concentration (P = 0.20), which can inhibit the activity of the reactive oxygen species (**ROS**), and prevent ROS damage to normal cells (Chew and Park, 2004). Experiments show that ROS can react with DNA, protein, and lipid to weaken their physiological functions and thus cause cancer,

Table 4. Effects of dietary addition of lutein preparation[1] on plasma metabolic variables in dairy cows

	Lutein preparation, g/d per head					*P*-value	
Item[2]	0	100	150	200	SEM	Linear	Quadratic
GLU, mmol/L	3.35	3.27	3.18	3.22	0.06	0.07	0.37
NEFA, μmol/L	113.9	134.9	111.5	130.3	7.49	0.45	0.88
MDA, nmol/mL	2.72	2.90	3.22	3.20	0.17	0.13	0.56
SOD, U/mL	65.0	66.8	68.6	68.8	1.40	0.10	0.53
GSH-Px, U/mL	75.1	84.1	86.2	84.9	5.30	0.20	0.34
TAOC, U/mL	1.67	1.89	1.71	1.94	0.09	0.07	0.98

[1]AbsoLUTEIN (Kemin Industries, Zhuhai, China).

[2]GLU = glucose; MDA = malondialdehyde; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; TAOC = total antioxidant capacity.

hardening of the arteries, and age-related macular degeneration (Mares-Perlman et al., 2002). Our results were consistent with previous studies (Woodall et al., 1996; Tian et al., 2010), showing that supplementation of lutein could effectively increase plasma SOD and GSH-Px concentrations and TAOC in chicks. Lutein can inactivate singlet oxygen through physical or chemical quenching effects, thus protecting the body from harm and strengthening the body's immune ability.

In the current study, lutein added in the diet raised the milk lutein concentration to 1.5 μg/100 mL. However, a gap exists between the milk lutein concentration in our study and that found by Calderón et al. (2007; 1.5 vs. 2.4 μg/100 mL, respectively). The concentration of milk carotenoids depends on the dietary supply (Nozière et al., 2006). Therefore, the higher milk lutein concentration found by Calderón et al. (2007) may be associated with more fresh forage resources used, whereas the ingredients of the diet in the present study were mostly dried feed or silage, which are low in carotenoids (Nozière et al., 2006). The relationship between milk lutein concentration (Y) and lutein intake (X) was calculated using the concentrations from wk 1 to 13 of the feeding period, and was expressed as follows:

$$Y\ (\mu g/100\ mL) = 0.4045 + 0.2391X\ (g/d\ per\ head)$$
$$(R^2 = 0.67;\ P < 0.01).$$

A correlation between milk lutein concentration and lutein intake was observed. The regression implies that milk lutein concentration increases linearly as lutein intake increases. It can be calculated from the above regression that with a daily lutein intake of <0.49 g/d per head, milk lutein concentration would be below the detectable limit (<0.005 mg/kg). The regression also implies that when cows consume diets that do not contain any detectable levels of lutein, the milk can also be expected to contain no detectable lutein. We speculate that when a high level of lutein is fed and the time fed is long enough, relatively large amounts of lutein could be absorbed into the blood, which would lead to a higher level of lutein in the milk. More research is needed to confirm this hypothesis. Some other factors, such as tolerance dose and transfer efficiency, may also affect the transfer efficiency of lutein from feed to milk.

CONCLUSIONS

Addition of lutein increased milk yield, milk lactose content, and milk fat content. The optimum level of lutein preparation (AbsoLUTEIN) was 150 to 200 g/d per head, which was associated with significantly higher concentration of lutein in milk. Feeding lutein can enhance the antioxidant capacity of cows, and prevent diseases. It is concluded that dietary supplementation of lutein is beneficial for milk yield and milk quality in lactating cows.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Beijing, China; No. 31101736), Public Technology Application Research of Zhejiang Province (China; 2013C32069), and the national Spark Program project (2013GA7000023). We are also grateful to the technical staffs of the Institute of Diary Science at Zhejiang University (Lin'an, China) and Kemin Industries (Zhuhai) Co. Ltd. (Zhuhai, China) for their help in this work. Also, staff members of Qiao-Si dairy farm (Hangzhou, China) are appreciated for their care for the cows used in this study.

REFERENCES

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.

Aréchiga, C. F., C. R. Staples, L. R. McDowell, and P. J. Hansen. 1998. Effects of timed insemination and supplemental β-carotene on reproduction and milk yield of dairy cows under heat stress. J. Dairy Sci. 81:390–402.

Biard, C., P. F. Surai, and A. P. Møller. 2006. Carotenoid availability in diet and phenotype of blue and great tit nestlings. J. Exp. Biol. 209:1004–1015.

Blount, J. D., P. F. Surai, D. C. Houston, and A. P. Møller. 2002. Patterns of yolk enrichment with dietary carotenoids in gulls: The roles of pigment acquisition and utilization. Funct. Ecol. 16:445–453.

Boland, F., L. O'Grady, and S. J. More. 2013. Investigating a dilution effect between somatic cell count and milk yield and estimating milk production losses in Irish dairy cattle. J. Dairy Sci. 96:1477–1484.

Calderón, F., B. Chauveau-Duriot, P. Pradel, B. Martin, B. Graulet, M. Doreau, and P. Nozière. 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. J. Dairy Sci. 90:5651–5664.

Cardinault, N., M. Doreau, C. Poncet, and P. Nozière. 2006. Digestion and absorption of carotenoids in sheep fed fresh red clover. Anim. Sci. 82:49–55.

Chew, B. P., and J. S. Park. 2004. Carotenoid action on the immune response. J. Nutr. 134:257S–261S.

Chew, B. P., M. Wong, and T. S. Wong. 1996. Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. Anticancer Res. 16:3689–3694.

Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2007. Effects of β-carotene on adult immune condition and antibacterial activity in the eggs of the Grey Partridge, *Perdix perdix*. Comp Biochem. Physiol. A Mol. Integr. Physiol. 147:1038–1046.

Dachtler, M., K. Kohler, and K. Albert. 1998. Reversed-phase high-performance liquid chromatographic identification of lutein and zeaxanthin stereoisomers in bovine retina using a C_{30} bonded phase. J. Chromatogr. B Biomed. Sci. Appl. 720:211–216.

Emenhiser, C., N. Simunovic, L. C. Sander, and S. J. Schwartz. 1996. Separation of geometrical carotenoid isomers in biological extracts using a polymeric C_{30} column in reversed-phased liquid chromatography. J. Agric. Food Chem. 44:3887–3893.

Fullmer, L., and A. Shao. 2001. The role of lutein in eye health and nutrition. Cereal Foods World 46:408–413.

Gómez, R., A. Alonso, and M. Martín. 1978. Carotenoid absorption in chicken intestine . Rev. Esp. Fisiol. 34:257–259.
Hagnestam-Nielsen, C., U. Emanuelson, B. Berglund, and E. Strandberg. 2009. Relationship between somatic cell count and milk yield in different stages of lactation. J. Dairy Sci. 92:3124–3133.
Laporte, M. F., and P. Paquin. 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. J. Agric. Food Chem. 47:2600–2605.
Leng, X., Y. Shi, X. Li, H. Xu, and Z. Yang. 2010. Effect of dietary xanthophyll level on body colour of goldfish (*Carassius auratus*). Journal of Zhejiang University 36:168–174.
Levy, L. W., inventor. 2001. Trans-xanthophyll ester concentrates of enhanced purity and methods of making same. Inexa Industria Extractora C.A., assignee. US Patent No. 6,191,293.
Mangels, A. R., J. M. Holden, G. R. Beecher, M. R. Forman, and E. Lanza. 1993. Carotenoid content of fruits and vegetables: an evaluation of analytic data. J. Am. Diet. Assoc. 93:284–296.
Mares-Perlman, J. A., A. E. Millen, T. L. Ficek, and S. E. Hankinson. 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. J. Nutr. 132:518S–524S.
Martin, B., A. Cornu, N. Kondjoyan, A. Ferlay, I. Verdier-Metz, P. Pradel, E. Rock, Y. Chilliard, J. B. Coulon, and J. L. Berdagué. 2005. Milk indicators for recognizing the types of forages eaten by dairy cows. Pages 127–136 in Indicators of Milk and Beef Quality. EAAP Publ. no. 112. J. F. Hocquette and S. Gigli, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
Martin, B., V. Fedele, A. Ferlay, P. Grolier, E. Rock, D. Gruffat, and Y. Chilliard. 2004. Effects of grass-based diets on the content of micronutrients and fatty acids in bovine and caprine dairy products. Grassl. Sci. Eur. 9:876–886.
Martin, K. R., M. L. Failla, and J. C. Smith Jr. 1996. β-Carotene and lutein protect HepG2 human liver cells against oxidant-induced damage. J. Nutr. 126:2098–2106.
McCutcheon, S. N., and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44–51.
Miller, R. H., H. D. Norman, G. R. Wiggans, and J. R. Wright. 2004. Relationship of test-day somatic cell score with test-day and lactation milk yields. J. Dairy Sci. 87:2299–2306.
Ministry of Agriculture of P.R. China. 2004. Feeding Standard of Dairy Cattle. China Standard NY/T 34. China Nong Ye Hang Ye Biao Zhun/Tuijian-34. China Agricultural Publisher, Beijing, China.
Nalecz-Tarwacka, T., A. Karaszewska, and K. Zdziarski. 2003. The influence of carrot addition to cow's ration on the level of vitamins and fatty acids in cow milk. Pol. J. Food Nutr. Sci. 12:53–56.
Nozière, P., B. Graulet, A. Lucas, B. Martin, P. Grolier, and M. Doreau. 2006. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Technol. 131:418–450.
Pei, L. P., B. D. Hui, and F. H. Dong. 2007. Effects of lutein on antioxidative action in aged rat. Journal of the Central University for Nationalities (Natural Sciences Edition) 16:197–201.
Prache, S., A. Priolo, H. Tournadre, R. Jailler, H. Dubroeucq, D. Micol, and B. Martin. 2002. Traceability of grass-feeding by quantifying the signature of carotenoid pigments in herbivores meat, milk and cheese. Grassl. Sci. Eur. 7:592–593.
SAS Institute. 2000. SAS User's Guide: Statistics. Version 8.01. SAS Institute Inc., Cary, NC.
Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, and Y. H. Schukken. 2000. Hyperketonemia and the impairment of udder defense: A review. Vet. Res. 31:397–412.
Tang, Z., Y. Yin, Y. Zhang, R. Huang, Z. Sun, T. Li, W. Chu, X. Kong, L. Li, M. Geng, and Q. Tu. 2009. Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. Br. J. Nutr. 101:998–1005.
Tian, H. S., Q. D. Li, L. Li, X. Y. Zhao, and H. P. Zhang. 2010. Effects of different supplementation ways of lutein on antioxidant capacity in chicks. Chin. J. Anim. Nutr. 22:685–695.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.
Wang, C., H. Y. Liu, Y. M. Wang, Z. Q. Yang, J. X. Liu, Y. M. Wu, T. Yan, and H. W. Ye. 2010. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. J. Dairy Sci. 93:3661–3670.
Wang, L., M. Xue, J. Wang, X. Wu, Y. Zheng, and C. Cao. 2012. Effect of natural xanthophylls on growth performance and body pigmentation of yellow catfish (*Pelteobagrus fulvidraco*). J. Fisheries China 36:1102–1110.
Woodall, A. A., G. Britton, and M. J. Jackson. 1996. Dietary supplementation with carotenoids: Effects on α-tocopherol levels and susceptibility of tissues to oxidative stress. Br. J. Nutr. 76:307–317.
Wu, X.-L., B. Heringstad, Y.-M. Chang, G. de los Campos, and D. Gianola. 2007. Inferring relationship between somatic cell score and milk yield using simultaneous and recursive models. J. Dairy Sci. 90:3508–3521.
Zhang, L.-X., R. V. Cooney, and J. S. Bertram. 1991. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: Relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–2114.
Zhang, X. Y., Y. L. Tan, L. Y. Cao, G. Y. Wu, Q. Xu, Y. Shen, and D. F. Zhou. 2006. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. Schizophr. Res. 81:291–300.