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TNF-α level and metabolic status in α-tocopheryl acetate supplemented high body condition periparturient crossbred cows during summer and winter

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ABSTRACT

The study was conducted to investigate the effect of α-tocopheryl acetate supplementation on tumor necrosis factor-alpha (TNF- α) level and metabolic status in periparturient crossbred cows during summer and winter. High body condition periparturient crossbred cows (24) were selected, 12 each during summer and winter. For each season, 6 cows were kept as controls and 6 cows were supplemented with α-tocopherol acetate @ 1000 IU/ day/ cow, supplementation beginning 60 days prior to the expected date of calving. Blood samples were collected at –20, –10, –5, 0, 5, 10 and 20 days in relation to expected date of calving. Glucose value observed higher in winter treatment than other group cows. Non-esterified fatty acid concentration levels increased towards calving and were higher at day 5 postpartum in summer control in comparison to all other groups. Plasma TNF-α levels also increased toward calving but were higher at the day of calving, after that levels decreased towards day 20 postpartum in all the groups. The study indicated that metabolic statuses of high body condition crossbred cows were improved by supplementation of α-tocopherol acetate.

Key words:Cows, Glucose, Nonesterified fatty acid, Tumor necrosis factor-alpha

High yielding dairy cows experience major changes in energy metabolism (Barber *et al.* 1997, Bauman 2000). The shortage in energy input relative to energy output is called negative energy balance (NEB) (Jorritsmaa *et al.* 2003). Adequate body fat reserves can promote milk production and health during NEB, but obese cows have much higher risks of poor conception rates, metabolic problems, and increased susceptibility to infectious diseases (Dechow *et al.* 2004, Castillo *et al.* 2005). Parturition is an inflammatory response and the peripartum period is associated with significant increases in various cytokines and their mRNA expression in various reproductive tissues, maternal circulation, and mammary gland tissues (Chapwanya *et al.* 2009, van Engelen *et al.* 2009, Bruno *et al.* 2010). The enhanced expression of cytokines such as TNF-α and IL6 in obese patients can induce a pro-inflammatory environment and facilitate oxidative damage, leading to initiation and progression of diseases (Furukawa *et al.* 2004). Supplementation of vitamin E can be useful against oxidative stress (Brezezinska *et al.* 1994, Arechiga *et al.* 1998) and reduces effect of summer stress in cows. Periparturient cows undergo mammary growth, synthesis and secretion of carbohydrate, fat and proteins and marked accumulation of colostrum and milk. Colostrum is rich in vitamins A and E, therefore, cows require their increased supply prior to parturition. As the information is not available on effect of α-tocopherol acetate supplementation on TNF- α levels and metabolic status, therefore, the present research was, aimed to investigate the effect of α-tocopherol acetate supplementation on TNF- α levels and metabolic status in periparturient-crossbred cows during summer and winter.

MATERIALS AND METHODS

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the article number 13 of the CPCSEA-rules, laid down by Government of India.

Experiment design: The experiment was carried out in summer (August to September) and winter (January to February) at Institute Farm, which is located at 250 m above mean sea level, latitude 29.43° North and longitude 77.02° east. In each season, equal number (n=12) of adult peripartum crossbred Karanfries (Holstein fresian × Tharparkar) high body condition (BCS > 4.5, 4.85±0.03 BCS) cows (510.04±7.94 kg body weight) free from any anatomical and reproductive disorders and not suffering from any health problems were selected 2 months before calving for study. Six cows were kept as control and 6 were supplemented with

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α-tocopherol acetate @ 1000 IU/ day/ animal during 60 days prior to the expected date of calving. Twelve cows of summer gave birth from August 20 to September 10. The other 12 cows of winter gave birth between January 20 and February 10. All animals were kept in loose housing system. Each paddock had rough brick-cemented flooring and asbestos roofing and sufficient space for free movement of the animals. All animals were fed as per NRC (2001). Animals were fed as per the standard management practices followed at the NDRI. Throughout the experiment animals were given concentrate mixture at 8.30 AM @ 1 kg / cow / day, then 2 kg / cow / day 15 days before calving. After calving, the cows were given concentrate mixture @ 1 kg / 2.5kg of milk producing morning, noon and evening at the time of milking. Green roughage (maize fodder/*jowar*) was fed approximately 35 kg and 4.5 kg of dry fodder during the experimental period.

Recording of climatic variables and temperature humidity index: Microclimatic data, viz. dry bulb temperature (Cdb), and wet bulb temperature (Cwb) in degree Celsius was recorded at 7.30 AM and 2.30 PM every day from 20 day prepartum to 20 day postpartum on days±20,±10,±5 and 0 with respect to the day of parturition (day 0). The THI was calculated from dry bulb and wet bulb temperatures using the equation (McDowell 1972).

Blood collection and analysis: Blood samples were collected at 7.30 AM, from jugular vein of animals on –20, –10, –5, 0, 5, 10 and 20 days in relation to expected date of calving, in vacutainer tubes containing heparin as an anticoagulant. During the collection, care was taken to pose minimum disturbance to the animal. Blood collected was immediately placed in ice and brought to the laboratory. Plasma was separated by centrifugation at 1 200×g in a refrigerated centrifuge for 20 min and stored at –20°C till analysis of different parameters. Glucose, NEFA and TNF-α were analyzed in plasma. Glucose was estimated in plasma samples by using a commercial kit. NEFA was estimated by copper soap extraction method modified by Shipe *et al*. (1980). Plasma TNF-α was estimated by bovine TNF-α kit. Minimum detection limit was 15.60 pg/ml. Intra- and interassay coefficients of variation were 6.54% and 10.35%, respectively.

Statistical analysis: Data for all measured variables were analyzed as repeated measures using the MIXED procedure of SPSS version 19. The model included the main effects of vitamin E treatment (groups), days around calving and their interactions. The pair-wise comparison of means was carried out using Tukey's Multiple Range Test (Snedecor and Cocharan 1994).

RESULTS AND DISCUSION

The THI varied significantly (P<0.01) between the 2 seasons on all the pre- and post-partum days of study. The mean microclimatic THI during winter was significantly lower (P<0.01) than the corresponding THI in hot–humid season (Fig. 1).

Plasma TNF-α did not differ significantly in groups (Table 1). No significant two -way interactions occurred (Table 1). Plasma TNF-α levels on days –5 (prepartum) and 0 (calving) higher (P<0.05) in comparison to levels on day –20 and –10 prepartum and day 5, 10 and 20 postpartum in SC, whereas, level on day 0 (calving) higher (P<0.05) than its levels on all the days of pre- and post-partum in ST (Table 1). In WC plasma TNF-α levels on day –5 and 0 differ significantly (P<0.05) from each other and to its levels on day –20, –10, 5, 10 and 20. In WT plasma TNF-α levels were followed same patterns as that of ST (Table 1). The correlation between TNF-α and microclimate THI was

Table 1. Effect of α-tocopheryl acetate supplementation on glucose, non esterified fatty acid (NEFA) and tumor necrosis factor-alpha (TNF-α) in control and treatment group cows around parturition during summer and winter

	Groups	Day in relation to calving							SEM	P value		
		–20	–10	–5	0	5	10	20		G	D	G×D
Glucose (mg/dL)	SC	42.06^{aB}	40.80^{aB}	45.36^{aC}	54.54^{aD}	40.90^{aB}	33.16^{aA}	30.97^{aA}	1.47	0.010	0.010	0.226
	ST	46.67^{bC}	50.4^{bD}	56.49^{cE}	63.96^{bF}	48.74^{bCD}	40.00^{bB}	33.03^{abA}	1.94			
	WC	43.45^{aB}	43.53^{aB}	48.76^{bC}	58.56^{cD}	42.75^{aB}	36.66^{cA}	34.26^{bA}	1.99			
	WT	48.95^{bC}	50.97^{bC}	59.57^{cD}	68.06^{dE}	50.41^{bC}	45.01^{dB}	38.62^{cA}	2.59			
NEFA (μM)	SC	250.12^{aA}	335.53^{aB}	410.95^{aC}	455.73^{aD}	5 552.59^{aE}	451.11^{aD}	415.45^{aC}	13.41	0.010	0.010	0.010
	ST	219.77^{abA}	251.22^{bB}	304.86^{bCD}	336.50^{bE}	376.44^{bF}	325.25^{bDE}	296.78^{bC}	14.31			
	WC	235.52^{bcA}	283.79^{cB}	324.49^{bC}	408.84^{cE}	454.61^{cF}	418.14^{cE}	382.65^{cD}	15.51			
	WT	206.23^{cA}	219.07^{dA}	273.23^{cB}	307.78^{dD}	327.12^{dD}	302.90^{dCD}	281.74^{bBC}	14.17			
TNF-α (pg/ml)	SC	21.30^{B}	14.02^{A}	46.33^{C}	55.05^{D}	20.94^{B}	23.89^{B}	22.06^{B}	4.93	0.411	0.010	0.191
	ST	25.52^{B}	25.52^{B}	32.35^{BC}	43.64^{D}	26.70^{B}	34.27^{C}	16.64^{A}	6.67			
	WC	19.40^{A}	22.57^{A}	37.86^{B}	50.05^{C}	23.60^{A}	24.28^{A}	17.20^{A}	4.29			
	WT	22.58^{AB}	21.56^{A}	29.09^{BC}	41.33^{D}	31.45^{C}	20.53^{A}	22.46^{AB}	5.14			

Value with different superscript in capital letter in a row and small letter in column differ significantly (P<0.05). SC, Summer control; ST, summer treatment; WC, winter control; WT, winter treatment; G, group; D, day; G×D, group and day interaction.

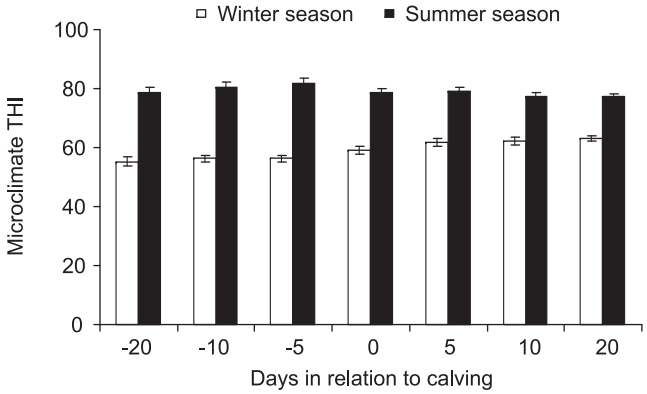

Fig. 1. Microclimate temperature humidity index (THI, mean±SE) during the 2 seasons on different days in relation to calving (day 0=day of parturition).

positive (r=0.39) and significant (P<0.01). We documented a significant increase of plasma TNF-α in the early postpartum period, which confirms the tendency of dairy cows to develop a certain degree of systemic inflammation in the first weeks after calving, which showed agreement with Rontved *et al*. (2005). Adipose tissue secretes pro-inflammatory cytokines such as TNF-α and IL6, which play a major role in the pathophysiology of several inflammatory-based diseases in dairy cattle. Increased plasma TNF-α in high BCS cows may originate from adipocytes and macrophages infiltrating the adipose tissue as described previously in rat models of obesity (Wellen and Hotamisligil 2005). TNF-α levels were higher around transition period indicated higher susceptibility to inflammatory based diseases (mastitis) in dairy cattle (Boyle *et al.* 2006), which supported our study.

Changes of glucose in each 4 groups of cows are reported in Table 1. Overall plasma glucose level differ significantly (P<0.01) in groups. The glucose level on day–5 (prepartum), 0 (calving) was higher (P<0.05) than its level on all the days prepartum and postpartum in all the groups. At day 0 (calving) and 10 (postpartum) all groups differ significantly (P<0.05) from each other. No significant 2-way interactions occurred. In present study, plasma glucose level increased slightly before calving and increase was more at calving and then decreased immediately after calving. Vazquez-Anon *et al.* (1994), Drackley *et al.* (2001) and Dann *et al.* (2005) also reported similar trend of changes in glucose level during periparturient period. Propionate production from the low dry matter intake (DMI) during the early postpartum period is insufficient to synthesize the total amount of glucose needed, therefore, the concentration of glucose decreased following parturition as observed in our studies. Plasma glucose concentration is important energy metabolite and higher level in treatment groups could be attributed to more feed intake resulting in higher levels of glucose (LeBlanc *et al.* 2004). In present study glucose levels are higher in winter as compared to summer which was also reported by Jonsson *et al.* (1997). After calving glucose concentration decreased and NEFA concentration are increased due to lypolysis, which acts as alternative energy source (Leroy *et al.* 2008, Shehab-El-Deen *et al.* 2010). This is supported by the significant negative correlation coefficient between glucose and NEFA concentration found in the present study.

Plasma NEFA levels began to increase from last 20 days of pregnancy and reached maximum at the day 5 postpartum in relation to calving, which was maximum (P<0.05) in summer control than all other groups (Table 1). NEFA levels were higher (P<0.05) on days 0 (calving) and 5 postpartum in comparison to NEFA levels on days –20, –10, and –5 prepartum and 20 postpartum in SC whereas, same patterns observed in ST cows. In WC same patterns of NEFA levels was observed as that of summer whereas, NEFA levels on day 5 (postpartum) was higher (P<0.05) than days –20, –10 and -5 prepartum and day –20 postpartum in WT. At day –10 (prepartum), 0 (calving), 5 and 10 (postpartum) all groups differ significantly (P<0.05) from each other. Group and days interaction was significant (Table 1). Significant positive correlation (r=0.42, P<0.01) was found between NEFA and microclimate THI. Plasma concentrations of NEFA are used as a measure of energy balance. Fat is mobilized from adipose in the form of NEFA when dietary energy intake is insufficient to meet animal metabolic needs. High BCS cows tend to lose more body weight and body condition during the transition period and dramatic elevations in NEFA around the time of calving reflect this condition of negative energy balance (Coffey *et al.* 2002, Adewuyi *et al.* 2005, Boyle *et al.* 2006), as observed in our results. Bernabucci *et al.* (2005) suggested that the energy status of over-conditioned cows was related to oxidant status during the periparturient period. It was reported that cows with elevated plasma NEFA levels also had higher plasma levels of reactive oxygen metabolites, but lower levels of antioxidants (Bernabucci *et al.* 2005). Concentration of NEFA was lower in vitamin E treatment groups in comparison to control groups in both the seasons (Bouwstera *et al.* 2008). NEFA levels were observed higher in control in comparison to treatment groups in both the seasons, but levels were maximum in summer indicating that effect of α-tocopherol acetate supplementation was more beneficial in dry period during summer and winter, but more in summer. Greater decline of BCS were found related to plasma NEFA concentration (Rukkwamsuk *et al.* 1998) and, possibly, to incidence of metabolic disorders (Buckley *et al.* 2003).

In conclusion, our results indicated that supplementation of α-tocopherol acetate reduces metabolic stress during transition period. Plasma TNF-α level around transition period were lower in winter than summer, which indicated higher susceptibility to inflammatory based diseases in dairy cattle during summer. Therefore, supplementation of some antioxidant like α-tocopherol acetate was more beneficial in summer as comparison to winter.

REFERENCES

Adewuyi A A, Gruyse E and van Eerdenburg F J. 2005. Non-esterified fatty acids (NEFA) in dairy cattle: A review. *Veterinary Quarterely* **27**: 117–26.

Arechiga C F, Vazques-Flores S, Ortiz O, Hernandes-Ceron J, Porras A, McDowell L R and Hansen P J. 1998. Effect of injection of beta-carotene or vitamin E and selenium on fertility of lactating dairy cows. *Theriogenology* **50**: 65–76.

Barber M C, Clegg R A, Travers M T and Vernon RG. 1997. Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta* **1347**: 101–26.

Bauman D E. 2000. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. Ruminant physiology, digestion, metabolism, growth and reproduction. *Proceedings of the 9th International Symposium on Ruminant Physiology*. pp 311–28. (Ed.) Cronje P B. CABI Publishing, New York

Bell A W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* **73**: 2804–19.

Bernabucci U, Ronchi B, Lacetera N and Nardone A. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science* **88**: 2017–26.

Bouwstera R J, Goselink R M A, Dobbelaar P, Nielen M, Newbold J R and Werven T. 2008. The relationship between oxidative damage and vitamin E concentration in blood, milk and liver tissue from vitamin E supplemented and non supplemented periparturient heifers. *Journal of Dairy Science* **91**: 977–87.

Boyle N O, Corl C M, Gandy J C and Sordillo L M. 2006. Relationship of body condition score and oxidant stress to tumor necrosis factor expression in dairy cattle. *Veterinary Immunology and Immunopathology* **113**: 297–304.

Brezezinska S E, Miller J K, Quigley J D, Moor J R and Madsen F C. 1994. Antioxidant status of dairy cow supplemented prepartum with vitamin E and selenium. *Journal of Dairy Science* **77**: 3087–95.

Bruno D. R, Rossitto P V, Bruno R G, Blanchard MT, Sitt T, Yeargan B V, Smith W L, Cullor J S and Stott J L. 2010. Differential levels of mRNA transcripts encoding immunologic mediators in mammary gland secretions from dairy cows with subclinical environmental *Streptococci* infections. *Veterinary Immunology and Immunopathology* **138**: 15–24.

Buckley F, O'Sullivan K, Mee J F, Evans R D and Dillon P. 2003. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calved Holstein–Friesians. *Journal of Dairy Science* **86**: 2308–19.

Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V and Benedito J L. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *Veterinary Journal* **169**: 286–92.

Chapwanya A, Meade K G, Doherty M L, Callanan J J, Mee J F and O'Farrelly C. 2009. Histopathological and molecular evaluation of Holstein–Friesian cows postpartum: toward an improved understanding of uterine innate immunity. *Theriogenology* **71**: 1396–1407.

Coffey M P, Simm G and Brotherstone S. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. *Journal of Dairy Science* **85**: 2669–78.

Dann H M, Morin D E, Bollero G A, Murphy M R and Drackley J K. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. *Journal of Dairy Science* **88**: 3249–64.

Dechow C D, Rogers G W, Sander-Nielsen U, Klei L, Lawlor T J, Clay J S, Freeman A E, Abdel-Azim G, Kuck A and Schnell S. 2004. Correlations among body condition scores from various sources, dairy form, and cow health from the United States and Denmark. *Journal of Dairy Science* **87**: 3526–33.

Drackley J K, Overton T R and Douglas G N. 2001. Adaptations of glucose and longchain fatty acid metabolism in liver of dairy cows during the periparturient period. *Journal of Dairy Science* **84**: 100–12.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M and Shimomura I. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation* **114**: 1752–61.

Gearhart M A, Curtis C R, Erb H N, Smith R D, Sniffen C J, Chase L E and Cooper M D. 1990. Relationship of changes in condition score to cow health in Holsteins. *Journal of Dairy Science* **73**: 3132–40.

Jonsson N, McGowan M, McGuigan K, Davison T, Hussain A, Kafi M and Matschoss A. 1997. Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. *Animal Reproduction Science* **47**: 315–26.

Jorritsma R, Wensinga T, Kruipb T A, Vos P L and Noordhuizena J P. 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Veterinary Research* **34**: 11–26.

Kim I H and Suh G H. 2003. Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. *Theriogenology* **60**: 1445–56.

LeBlanc S J, Herdt T H, Seymour W M, Duffield T F and Leslie K E. 2004. Peripartum serum vitam**in** E, ret**in**ol, and ß-carotene **in** dairy cattle and their associations with disease. *Journal of Dairy Science* **87**: 609–19.

Leroy J L M R, Vanholder T, Van Knegsel A T M, Garcia-Ispierto I and Bols P E J. 2008. Nutrient prioritization in dairy cows early postpartum. mismatch between metabolism and fertility? *Reproduction in Domestic Animals* **43**(2): 96–103.

McDowell R E. 1972. *Improvement of Livestock Production in Warm Climates.* W. H. Freeman and Company, San Francisco.

NRC. 2001. *Nutrient Requirements of Dairy Cattle.* 7th revised edn. Washington D C. National Academy Press.

Rontved C M, Andersen J B, Dernfalk J and Ingvartsen K L. 2005. Effects of diet energy density and milking frequency in early lactation on tumor necrosis factor-alpha responsiveness in dairy cows. *Vet Immunol Immunopathol* **104**: 171–81.

Rukkwamsuk T, Wensing T and Geelen M J H. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. *Journal of Dairy Science* **81**: 2904–11.

Shehab-El-Deen M A M M, Fadel M S, Soom A V, Sherif Y S, Maes D, Leroy M R and Jo L. 2010. Circadian rhythm of metabolic changes associated with summer heat stress in high-producing dairy cattle. *Tropical Animal Health and Production*

42: 1119–25.

Shipe W F, Senyk G F and Fountain K B. 1980. Modified copper soap solvent extraction method for measuring free fatty acids in milk. *Journal of Dairy Science* **63**: 193–98.

Snedecor G W and Cochran W G. 1994. *Stastical Methods*. 8th edn. Iowa State University Press, Iowa.

van Engelen E, Breeveld-Dwarkasing V N, Taverne M A, Everts M E, van der Weijden G C and Rutten V P. 2009. MMP–2 expression precedes the final ripening process of the bovine cervix. *Molecular Reproduction and Development* **75**: 1669–77.

Vazquez-Anon M, Bertics S, Luck M, Grummer R R and Penheiro J. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. *Journal of Dairy Science* **77**: 1521–28.

Wellen K E and Hotamisligil G S. 2005. Inflammation, stress, and diabetes. *Journal of Clinical Investigation* **115**: 1111–19.