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Expression of deiodinase 2 (DIO2) and integrin alpha 9 (ITGA9) genes as indicators' of adaptability and their relationship with physio-biochemical parameters in Tharparkar and Karan Fries heifers during different seasons

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ABSTRACT

The aim of the study was to observe the influence of ambient conditions (seasons) on expression pattern of deiodinase 2 (DIO2) and integrin alpha 9 (ITGA9) genes in peripheral blood mononuclear cells (PBMC) and their relationship with physiological and biochemical parameters of Tharparkar and Karan Fries heifers. Healthy heifers of Tharparkar and Karan Fries breed (6 each) were selected and blood samples were collected from these animals at weekly interval. These blood samples were centrifuged to separate the plasma (for biochemical parameters) and buffy coat (for total RNA isolation). The expression of DIO2 gene was down-regulated during hot humid season and up-regulated during winter season in both the breeds. The DIO2 gene showed a positive correlation with thyroid hormones (T3, T4) and negative correlation with physiological responses (RR, PR, RT, ST), cortisol hormone, antioxidant enzymes (SOD, GPx) and non-esterified fatty-acids (NEFA). The expression pattern of ITGA9 gene was opposite to DIO2 gene, i.e. up-regulated during hot humid season and down-regulated during winter season in both the breeds. ITGA9 gene expression showed a positive correlation with cortisol, antioxidant enzymes, NEFA and physiological responses, and negative correlation with thyroid hormones. The fold change in expression of DIO2 and ITGA9 genes was higher in Karan Fries than Tharparkar heifers during hot humid and winter season. The dry matter intake was lower during hot-humid season than autumn and winter season in both the breeds of heifer. The expression pattern of the DIO2 and ITGA9 genes and their relationship with physio-biochemical parameters revealed the essential role in adaptability of cattle as candidate gene. Based on the results of present study, it can be stated that Tharparkar heifers are better adapted to tropical climatic conditions than Karan Fries heifers.

Key words: Cattle, DIO2, ITGA9, Karan Fries, Physio-biochemical, Season, Tharparkar

Animals cope with different climatic conditions through physiological, biochemical and behavioural adjustments. During the process of adjustment, they also acquired the genes that confer the thermo-tolerance at physiological and cellular levels, which make them resilient to the given environments (Hansen 2004, Prayaga and Henshall 2005, Barker 2009). Animals living in hot environment have been seen to compromise the metabolic rate as an adaptive mechanism (Rasooli *et al*. 2004, Pereira *et al*. 2008). One of the important features of the breeds adapted to hot climate is that they can recycle the nutrients more efficiently than temperate breeds (Bayer 2003). Higher ambient temperature and humidity during summer affect the thermal equilibration of the body that can lead to heat stress resulting in reproductive and productive problems (De Rensis and Scaramuzzi 2003). The comfortable ambient temperature for better performance varies from 15° to 25°C for crossbred cattle and 15°–28°C for indigenous cattle under tropical climatic conditions (Singh and Upadhyay 2009). The higher metabolic rate is also one of the main ways to gain heat during cold to maintain a physiological equilibrium. The protein encoded by deiodinase type 2 (DIO2) belongs to the iodothyronine deiodinase family, which catalyzes the conversion of thyroxine (T3) to tri-iodothyronine (T4), plays a crucial role in maintaining the intracellular T3 level in different extra-thyroidal tissues and directly affect their metabolic rate (Darras and Van-Herck 2012). Lower circulating levels of T3 and T4 were reported by Collier *et al*. (1982) and Nardone *et al*. (1997) during elevated temperature in cattle. Cattle not only compromise metabolism during summer, but also upsurge the respiration rate to maximize the heat loss through respiratory vaporization. Integrin alpha 9 (ITGA9) reported to play a significant role in airway smooth muscle by facilitating the free ventilation (minimizing the contraction of smooth muscle) (Chen *et al*. 2012). The deiodinase 2 and integrin alpha 9 presumed to play important role in adaptation of cattle in different climatic conditions. Therefore, the present study was undertaken to observe the seasonal variations of

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deiodinase 2 (DIO2) and integrin alpha 9 (ITGA9) with their relationship with physio-biochemical parameters in Tharparkar and Karan Fries heifers.

MATERIALS AND METHODS

The present study was conducted at the Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute (NDRI), Karnal (Haryana). Karnal is situated at an altitude of 250 m above mean sea level, latitude and longitude position being 29°42′′ N and 79°54′′ E, respectively. The maximum ambient temperature during summer goes > 42°C and minimum temperature during winter come down <2°C with a diurnal variation of 15–20°C.

Selection of animals and sampling: Six each of healthy (1.5–2 year-old) Tharparkar and Karan Fries heifers were selected from the LRC of ICAR-NDRI, Karnal, for the study. All the animals were fed a ration consisting of concentrate mixture and roughages (berseem, maize, sorghum fodder etc). Concentrate mixture consisted of 28% maize, 10% groundnut cake, 13% mustard cake, 15% wheat bran, 11% rice polish, 15% soyabean deoiled, 5% bajra, 2% mineral mixture and 1% salt with approx. 16% CP and 70% TDN. Freshwater was available for drinking round the clock. Feed intake in terms of dry matter intake (DMI) was recorded during the different seasons.

Blood samples were collected in sterile heparinized vacutainer tubes at weekly interval during autumn, hot humid and winter season. Blood samples were immediately centrifuged at 2,500 rpm for 25 min to separate the plasma and buffy coat. The plasma samples were stored at –20°C for biochemical analysis [superoxidase dismutase (SOD), glutathione peroxidase (GPx), non-esterified fatty acids (NEFA), cortisol, tri-iodothyronine (T3) and thyroxine (T4)]. Lymphocytes were also isolated from the buffy coat and processed for total RNA isolation to study the relative mRNA expression of DIO2 and ITGA9 genes. Physiological responses (reparation rate, pulse rate and rectal temperature) were recorded on the day of sampling. The peripheral skin temperature (ST) at different anatomical sites of the experimental animals, viz. forehead, dorsal, ventral and flank regions were recorded using non-contact telethermometer (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, Surrey, UK) by keeping it 2–3 inches away from the surface of the desired site. The environmental variables were recorded during the experimental period (Table 1). The temperature humidity index (THI) was calculated using the following equation of McDowell (1972):

THI = 0.72 (dry bulb temperature °C + wet bulb temperature °C) + 40.6.

Estimation of biochemical parameters: Plasma cortisol, NEFA, SOD and GPx was analyzed using bovine ELISA kit as per the manufacturer's protocol. The sensitivity of bovine SOD and GPx ELISA kit was 2.0 U/ml and the intra-assay coefficient of variation (CV) and inter-assay CV (%) was less than 15%. The optical density was recorded using a TECAN infinite PRO200 ELISA reader (TECAN Asia Pvt. Ltd., Singapore) at 450 nm. Bovine RIA Kit (Beckman Coulter) was used for estimation of T3 and T4. The sensitivity assay of T3 was 0.5 ng/ml and the intra-assay and inter-assay were 6.3% and 7.7%, respectively. The sensitivity assay of T4 was 16.7 ng/ml and the intra-assay and inter-assay CV were 6.2% and 8.6%, respectively.

Table 1. Average environmental parameters recorded during hot humid, autumn and winter seasons

Environmental parameter	Hot humid (August–September)	Autumn (October–November)	Winter (December–January)
Dry bulb temperature (°C)	32±0.5	17±3	8.4±0.1
Wet bulb temperature (°C)	27±0.7	16±3	8.2±0.2
Relative humidity (%)	80±1.2	59±2	82±0.2
Maximum temperature (°C)	33.1±0.4	27.7±2.3	18.2±0.1
Minimum temperature (°C)	23.9±1.2	15±2.6	6.8±0.1
THI	83.1±0.2	65±4.3	52±0.5

Lymphocyte separation: The buffy coat was washed properly with 6 ml of phosphate buffer solution (PBS). The mixture was then suspended in 3 ml of lymphocyte separation media (HISTOPAQUE@1077) and centrifuged again at 2,500 rpm for 25 min. The white ring containing lymphocytes was collected and washed with 3–4 ml of phosphate buffer solution and centrifuged at 1,500 rpm to collect the pellets.

Isolation of total RNA: Total RNA was isolated using RNase kit (Qiagen RNeasy Mini Kit). Briefly, 700 µl RLT buffer was added to the pellet. Cells were lysed by pipetting. 700 µl of 70% ethanol was added to the above mixture and then 700 µl of the mixture was transferred to the spin column and centrifuged at 10,000 rpm for 1 min at room temperature. Buffer RW1 (350 µl) was added and centrifuged at the same speed. DNase-1 (10 µl DNase + 70 µl RDD buffer) was added to the residue, centrifuged as above and incubated for 10–15 min at room temperature. Again 350 µl of RW1 buffer was added and centrifuged at 10,000 rpm for 25 sec followed by 500 µl of RPE buffer and centrifuged for 2 min. Then 20 µl of nuclease-free water was added to the column and the filtrate (RNA) was collected in a new RNase DNase free tube by centrifugation at 10,000 rpm for 1 min. Total RNA concentrations and optical density were measured (40–200 ng/µl) using nanodrop (Biospec-Nano, Shimadzu Corporation). The samples having 1.8 to 2.1 purity ratio (260/280) were selected for the expression study.

Reverse transcription—first strand cDNA synthesis: About 200 ng of total RNA was used for cDNA synthesis using Revert Aid First strand cDNA synthesis kit (Fermentas, USA) by reverse transcription-polymerase chain reaction (RT-PCR) according to the manufacturer's protocol. Briefly, the mixture of RNA, oligo (dt) primer, 5× reaction buffer, Ribolock RNase inhibitor (20 U/µl), 10 mM dNTP mix and revertAid M-MuLVRT (200 U/µl) were added to a 0.2 ml sterile tube and made it to 20 µl by

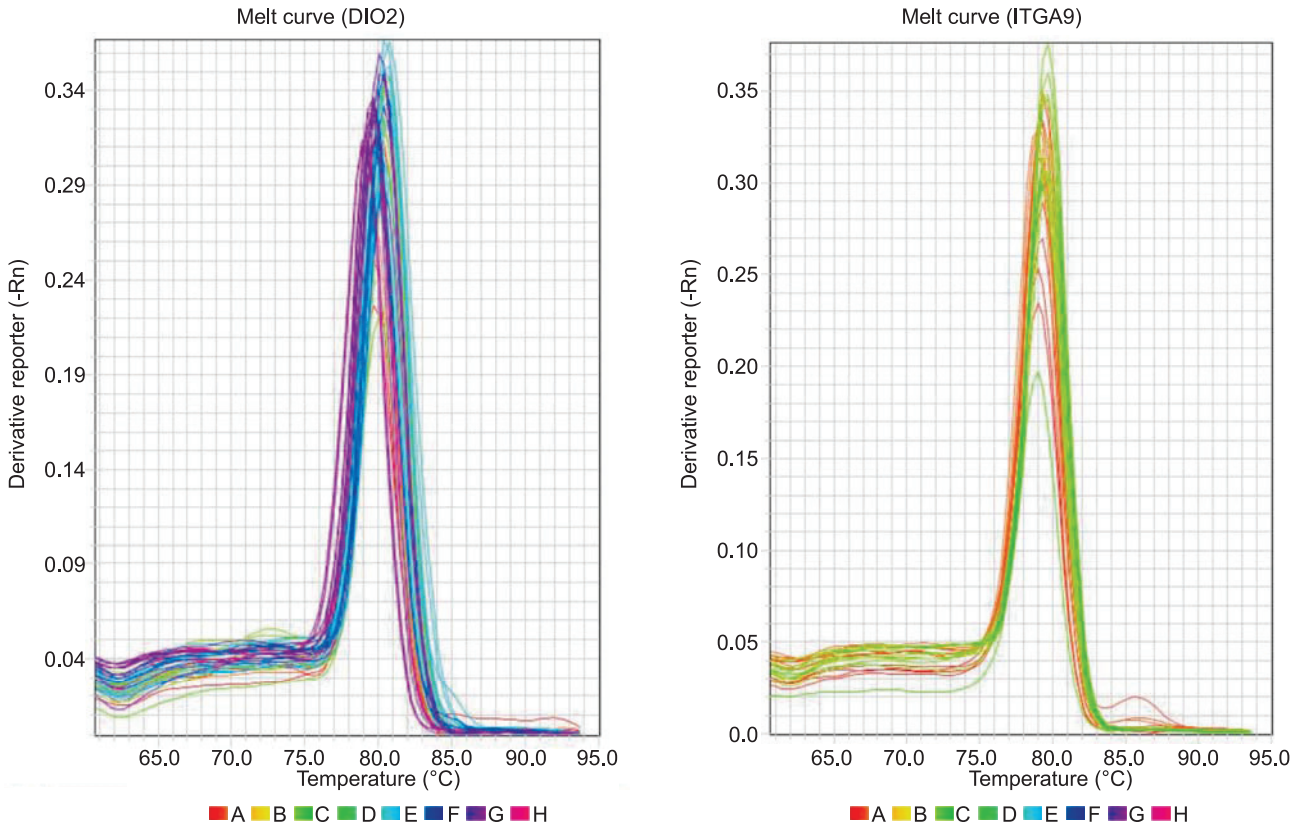

Fig. 1. Dissociation/melting curve of DIO2 and ITGA9 gene.

nuclease-free water. The RT-PCR was carried out at 65°C for 5 min, 42°C for 60 min and 70°C for 5 min in a thermocycler (AB Applied Biosystem). The cDNA product was diluted into 1:1 dilution and stored at –20°C to perform downstream PCR amplification and qPCR.

Semi-quantitative PCR: Semi-quantitative PCR (qPCR, Applied Biosystems® 7500 Real-Time PCR) was carried out to analyze the relative expression of DIO2 and ITGA9 genes. The annealing temperatures for all the primers were evaluated through gradient PCR (Bio-Rad, USA) (Fig. 1), amplification of genes was confirmed by observing the product size (electrophoresis using 2.5% agarose) in a Gel documentation system (Fig. 2). The semi-quantitative PCR reaction was carried out using Maxima SYBR green real-time PCR (qPCR) mAXer mix

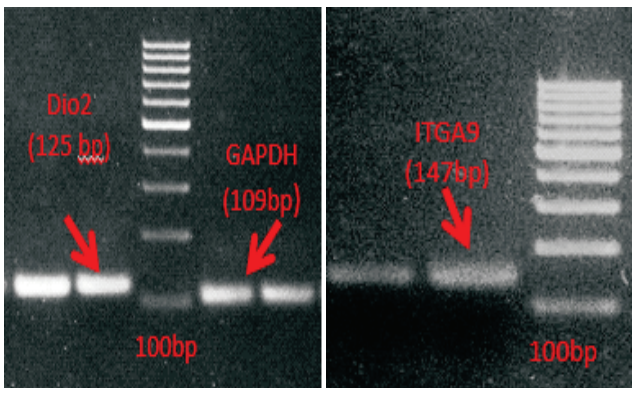

Fig. 2. Size of PCR products under gel-documentation system.

(10 µl) along with forward and reverse primers (1 µl, 10 pmol), nuclease-free sterile water (7 µl) and template (1 µl). Negative controls were run in each PCR assay without template (cDNA). The real-time PCR products of genes were confirmed (2.5% agarose) under gel documentation system. Lack of PCR product on negative control well was assumed to be a complete lack of contamination. The qPCR program consisted of initial heating at 50°C for 2 min followed by 95°C for 10 min, annealing for 60 sec, and amplified for 40 cycles. The final extension at 72°C incubation was continued for a further 10 min.

Real-time primers: The sequence of reference genes (GAPDH) and target genes (DIO2 and ITGA9) were procured from Europhins Genomics India Pvt. Ltd. The sequence of the genes used in the present investigation and the size of the PCR amplified products are given in Table 2.

Statistical analysis: Statistical analysis of the data was performed using the SPSS 16.0 software by three-factor analysis of variance for season, breed and season × breed interaction. ANOVA was followed by post-hoc Fisher's LSD test for pairwise comparisons where appropriate. The relative expression of DIO2 and ITGA9 gene was calculated by comparing the expression level of reference gene GAPDH as per Livak and Scmittgen (2001). Graphs were plotted using GraphPad Prism software.

Ethical permission: The experiment was approved by the Institutional Animal Ethics Committee (IAEC) established as per the article number 13 of the Committee

Table 2. Real-time primers for targeted and housekeeping genes

Primer name	Sequence	Tm (°C)	Product size	References
DIO2F	ATGTCACAAGG CCAACACAA	58.8	125	(Howard *et al*. 2014)
DIO2R	AGCCATACGCA GGAGAAGAA			
ITGA9F	TGGGAATCCT CATCTTCCTG	61	147	(Howard *et al*. 2014)
ITGA9R	GGGTCACTGG TTTTCTGGA			
GAPDHF	CCAACGTGTCTG TTGTGGATCTGA	59	109	(Choudhary *et al*. 2016)
GAPDHR	GAGCTTGACAAAG TGGTCGTTGAG			

for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

RESULTS AND DISCUSSION

Physiological responses: The physiological responses, i.e. respiration rate (RR), pulse rate (PR) and skin temperature (ST) were higher (P<0.05) during hot humid season (THI, 83.1±0.2) and lower (P<0.05) during winter (THI, 52.0±0.5) compared to autumn (THI, 65.0±4.3) in Tharparkar and Karan Fries heifers (Table 3). The magnitude of increase in these physiological parameters was higher in Karan Fries heifers than Tharparkar (Table 3). Our results were in accordance with those of Singh and Upadhyay (2009) who reported higher respiration rate and rectal temperature in Karan Fries (Tharparkar × Holstein Friesian) than Sahiwal cattle during heat stress. The deviation in physiological responses is important to cope up under adverse climatic conditions. Stressors of some systems are detectable as modifications of rectal temperature, respiration or heart rates, which are the valid index of social stress/discomfort in large animals (Guyton and Hall 2006). The higher magnitude of physiological responses in Karan Fries than Tharparkar heifers indicates the sensitivity of this breed to heat stress (Table 3). Gaughan *et al.* (2000) also reported a lower respiration rate under heat stress as an indicator of lesser discomfort in cattle. Under control climatic conditions at 44°C in climatic chamber, the RR, RT, PR and ST were recorded higher in Karan Fries than Tharparkar cattle (Bhan *et al.* 2012, 2013). THI and ambient temperature showed positive correlation with physiological responses (Singh and Upadhyay 2009).

Antioxidant enzymes: The higher (P<0.05) levels of antioxidant enzymes (SOD, GPx) was found during hot humid season compared to winter and autumn (Table 3). The levels of SOD increased from 26.59±1.64 (winter) to 76.74±2.87 U/ml (hot humid) in Tharparkar; and similar trend of increase, i.e. 39.84±0.80 to 69.58±3.30 U/ml was observed in Karan Fries heifers (Table 3). The basal levels of SOD during autumn and winter were higher (P<0.05) in Karan Fries than Tharparkar heifers (Table 3). The levels

Table 3. Physiological responses, antioxidant enzymes, thyroid hormones and dry matter intake during different seasons

Parameter	Season	Tharparkar	Karan Fries
Rectal temperature (ºC)	Hot humid	38.66 ± 0.09^{ax}	39.01 ± 0.06^{ax}
	Autumn	38.41 ± 0.06^{axy}	38.68 ± 0.03^{ay}
	Winter	37.87 ± 0.02^{az}	38.28 ± 0.03^{bz}
Respiration rate (/min)	Hot humid	24.33 ± 0.50^{ax}	26.58 ± 0.98^{bx}
	Autumn	21.63 ± 0.90^{ay}	22.88 ± 0.25^{by}
	Winter	19.08 ± 0.20^{az}	21.33 ± 0.32^{bxz}
Pulse rate (/min)	Hot humid	73.21 ± 0.77^{ax}	78.83 ± 0.42^{bx}
	Autumn	63.92 ± 0.78^{ay}	66.42 ± 0.85^{by}
	Winter	70.04 ± 0.50^{az}	69.62 ± 0.21^{bz}
Skin temperature (ºC)	Hot humid	34.71 ± 0.16^{ax}	35.48 ± 0.12^{ax}
	Autumn	30.88 ± 0.30^{ay}	31.80 ± 0.20^{ay}
	Winter	23.83 ± 1.3^{az}	26.92 ± 0.51^{bz}
Plasma non-estrified fatty acids (µmol/l)	Hot humid	5.16 ± 0.08^{ax}	5.73 ± 0.24^{ax}
	Autumn	1.50 ± 0.08^{ay}	1.73 ± 0.05^{ay}
	Winter	1.20 ± 0.10^{ay}	1.29 ± 0.08^{ay}
Plasma superoxide dismutase (U/ml)	Hot humid	76.74 ± 2.87^{ax}	69.58 ± 3.30^{ax}
	Autumn	43.18 ± 2.43^{ay}	50.26 ± 2.55^{by}
	Winter	26.59 ± 1.64^{az}	39.84 ± 0.80^{bz}
Plasma glutathione peroxidase (U/l)	Hot humid	25.91 ± 0.70^{ax}	24.60 ± 0.49^{ax}
	Autumn	22.97 ± 0.40^{ay}	21.83 ± 0.56^{ay}
	Winter	20.88 ± 0.11^{ay}	19.77 ± 0.45^{ay}
Tri-iodothyronine (nmol/l)	Hot humid	1.14 ± 0.03^{ax}	1.40 ± 0.06^{bx}
	Autumn	1.59 ± 0.04^{ay}	1.90 ± 0.03^{by}
	Winter	2.30 ± 0.05^{az}	2.80 ± 0.12^{az}
Thyroxine (nmol/l)	Hot humid	46.43 ± 0.70^{ax}	56.29 ± 0.32^{bx}
	Autumn	55.45 ± 0.60^{ay}	61.65 ± 0.57^{by}
	Winter	59.84 ± 0.50^{az}	70.46 ± 0.90^{bz}
Dry matter intake (kg)	Hot humid	4.1 ± 0.04^{ax}	5.4 ± 0.03^{bx}
	Autumn	4.7 ± 0.08^{ay}	6.1 ± 0.01^{by}
	Winter	5.3 ± 0.01^{az}	6.7 ± 0.04^{bz}

The values are means and SE of six observations on six animals. Different superscripts between the columns (x, y, z) and rows (a, b) for individual parameters differs significantly (P<0.05).

of SOD differed significantly (P<0.05) during autumn and winter season in Tharparkar and Karan Fries heifers. Bhan *et al*. (2013) and Singh *et al.* (2014) also reported the almost similar higher levels of antioxidant enzymes (glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase) during summer than winter season and in crossbred than zebu cattle. The antioxidant enzymes (SOD, GPx) showed negative correlation with metabolic hormones (T3 and T4) and positive correlation with cortisol, NEFA and physiological responses.

Metabolic hormones and energy metabolites: The lower levels of thyroid hormones, i.e. T3 and T4 was found in Tharparkar compared to Karan Fries heifers during autumn, hot humid and winter season (Table 3). The average values of T3 and T4 were 2.30±0.05 nmol/l and 59.84±0.50 nmol/L during winter season and the values decreased to 1.14±0.03 nmol/l and 46.43±0.70 nmol/l respectively during hot humid season in Tharparkar heifers (Table 3). Similar patterns of decrease in thyroid hormones were observed in Karan Fries heifers, but the magnitude of decrease was much higher in Karan Fries heifers even though the levels

of these hormones remained higher during all the seasons (Table 3). The higher levels of these metabolic hormones, particularly during hot humid season indicated higher metabolic rate in Karan Fries heifers and making this breed more susceptible to high temperature compared to Tharparkar heifers. Vaidya *et al*. (2015) also reported higher levels of metabolic hormones in Karan Fries cattle during summer season.

The plasma NEFA levels of Tharparkar heifers were 1.50±0.08 µmol/l during autumn, which was increased by 3.66 µmol/l during hot humid and decreased by 0.30 µmol/l during winter. Similar trend of increase in NEFA during hot humid (4.0 µmol/l) and decrease during winter (0.44 µmol/l) from the autumn (1.73±0.05 µmol/l) was observed in Karan Fries heifers (Table 3). The data clearly indicated more deviation in NEFA from autumn to other seasons in Karan Fries than Tharparkar heifers. The higher levels of NEFA during hot humid season indicated mobilization of fat due to lower feed intake by Tharparkar and Karan Fries heifers (Table 3). The dramatic increase in energy requirements needed for faster growth of heifers often accompanied by a decrease in voluntary dry matter intake that causes a negative energy balance particularly during heat stress. Energy requirements that cannot meet by the diet must then rely on tissue energy reserves. Therefore, negative energy balance during heat stress causes mobilization of fat from tissue stores and the release of non-esterified fatty acids (NEFA) into the blood stream. The plasma NEFA concentration is more closely related to animal energy status and depot fat mobilization as a consequence of a negative energy balance (Block *et al.* 2001). Vaidya *et al.* (2015, 2017) also reported the similar pattern of NEFA in Karan Fries and Sahiwal cattle during different seasons and levels were higher during summer and just after parturition, which correspond to the higher NEFA levels.

Dry matter intake: The dry matter intake (DMI) was higher, i.e. 6.7±0.04 kg during winter compared to autumn (6.1±0.01 kg) and hot humid season (5.4±0.03 kg) in Karan Fries heifers. Similar pattern of DMI was observed in Tharparkar cattle, i.e. higher during winter and lower during hot humid season. Correspondingly, higher NEFA levels were observed during hot humid season in both the breeds of heifers (Table 3). These results were in accordance with those of Singh *et al*. (2017) who reported lower feed intake in heat stress Karan Fries heifers than other group of animals maintained under fan cum mist system during summer stress. Further, results of study showed higher ($P<0.05$) reduction in DMI (1.3 kg) of Karan Fries heifers than Tharparkar (1.2 kg), indicating higher stress levels in KF than Tharparkar and therefore, these animals reduced their feed intake to cut the metabolic heat production (Table 3). DMI showed positive correlation with metabolic hormones and THI in both the breeds of cattle.

Expression profile of deiodinase 2 (DIO2) gene: The expression profile of DIO2 gene was lower ($P<0.05$) in both the breeds during hot humid season. However, it increased ($P<0.05$) during winter and more pronounced in Karan Fries heifers than Tharparkar (Fig. 3). In addition to environmental conditions, several other factors such as hormones, growth factors, adrenergic agents and nutritional conditions influence deiodinase activity as reported by Bianco *et al.* (2002). Results of the present study showed higher expression of DIO2 gene and simultaneously higher concentration of metabolic hormones in Karan Fries compared to Tharparkar heifers, indicating higher metabolic rate in Karan Fries than Tharparkar heifers. Several researchers (Crantz *et al.* 1982, Bianco and Silva 1987) showed the critical role of DIO2 gene in maintaining intracellular T3 level in specialized tissues, such as the anterior pituitary, central nervous system and brown adipose tissues. DIO2 is localized in the endoplasmic reticulum and its action is directed almost exclusively at the efficient conversion of the prohormone T4 to the active T3 by 5'-deiodination. It is negatively regulated by thyroid hormone both pre- and post transcriptionally (Burmeister *et al.* 1997). DIO2 gene showed a positive correlation with T3 and negative correlation with cortisol, RT, ST, RR, SOD, GPx and NEFA in Tharparkar and Karan Fries heifers.

Expression profile of integrin alpha 9 (ITGA9) gene:

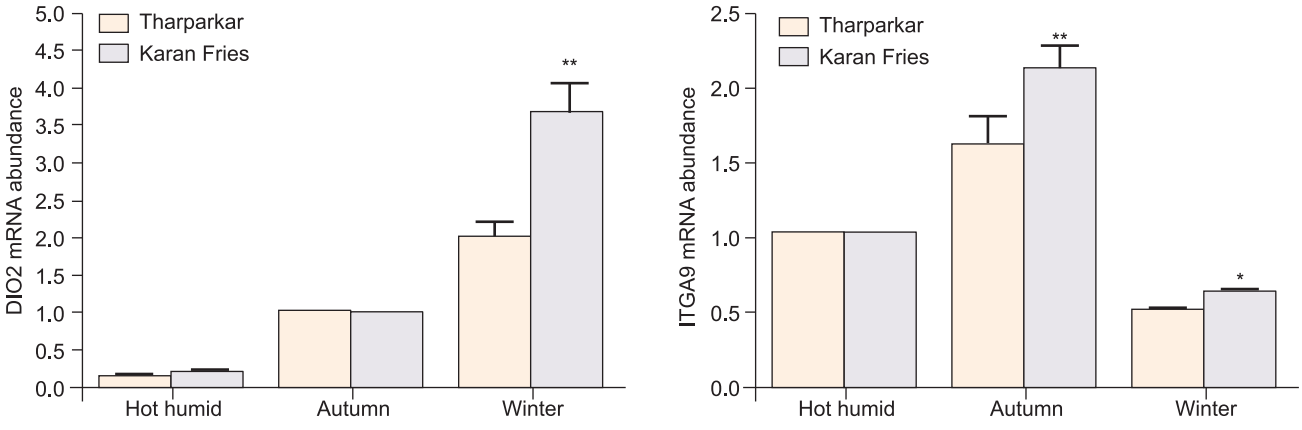

Fig. 3. Relative expression of DIO2 and ITGA9 genes in Tharparkar and Karan Fries heifers during different season.

The expression of integrin alpha 9 (ITGA9) gene was up-regulated ($P<0.05$) during the hot humid season and down-regulated during winter compared to autumn (comfort ambient temperature). The expression levels were more pronounced in Karan Fries than Tharparkar heifers particularly during hot humid season (Fig. 3). ITGA9 showed to play a crucial role in airway smooth muscle contraction, lacking this gene in mice resulted in death due to respiratory failure (Huang *et al*. 2000). The higher expression of ITGA9 in Karan Fries related to higher respiration rate in this breed compared to zebu cattle reason being lower sweating rate in Karan Fries heifers. Chen *et al.* (2012) demonstrated that ITGA9 gene localizes in the respiratory smooth muscles and it prevents exaggerated airway smooth muscle contraction, by which it facilitates the free ventilation in mice. ITGA9 serve as a brake on airway smooth muscle contraction. The higher expression of this gene during the hot humid season might allow the animals for higher vaporization through the respiratory tract. The release of heat from respiratory system is one of the finest ways when the ambient temperature reached to skin temperature. Therefore, ITGA9 can also be taken as a candidate gene for respiration (Howard *et al*. 2014). ITGA9 gene showed negative correlation with thyroid hormones. This might be due to the thermogenesis or minimizing the heat loss through respiratory system to maintain the body temperature during the winter. The higher concentration of cortisol was also reported by various authors and it enhances the protein metabolism to fulfill the requirement of the body (Francisco *et al*. 1992). The body lipid mobilization also occurred during hot humid season indicated by higher concentration of non-esterified fatty acids (NEFA) in the plasma of Tharparkar and Karan Fries heifers (Table 3). The expression of ITGA9 gene showed positive correlation with cortisol, RT, ST, RR, SOD, GPx and NEFA and negative correlation with T3 and T4 in Karan Fries and Tharparkar heifers.

The variation in expression pattern of DIO2 and ITGA9 genes during different seasons and their relationship with physio-biochemical parameters in the present study revealed the important role of these genes in adaptability of cattle under different climatic conditions. Based on our results, it can be stated that Tharparkar heifers are better adapted to tropical climatic conditions than Karan Fries heifers.

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