



Differential expression of uncoupling protein gene in feed efficient cattle

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Feed efficiency is one of the important factors for increasing the profitability of beef industry. Hence selection for feed efficiency is important for decreasing the cost of production. Residual feed intake (RFI), defined as the difference between an animal's actual feed intake and its predicted intake has become the preferred measure of feed efficiency for livestock. Uncoupling proteins (UCP) are carrier proteins that release protons during respiration dissipating energy in the form of heat. Expression of these genes plays an important role in regulation of energy balance. Xiao *et al.* (2004) found that UCP1 and UCP3 are downregulated during lactation for saving energy for milk production. Over expression of UCP2 and UCP3 in transgenic mice results in to decreased adiposity but increased hypothalamic NPY concentrations and feed intake (Horvath *et al.* 1997). UCP2 regulates the appetite-promoting effects of ghrelin leading to NPY/AGRP neuron firing and inhibition of neurons that produces satiety hormone POMC in the brain (Andrews *et al.* 2008). Less feed efficient broiler chicken has greater loss of electrons in comparison to high feed efficient animals in mitochondria (Bottje *et al.* 2002). Bottje *et al.* (2006) further reported greater loss of electrons during transport in broiler chickens with low feed efficiency (positive RFI) consequently releasing more energy in the form of heat, produces more reactive oxygen and less ATP. Kolath *et al.* (2006) observed similar result in cattle, suggesting a relationship between mitochondrial respiration and feed efficiency. Therefore present study was undertaken to study the differential expression of UCP2 and UCP3 gene in feed efficient cattle in comparison to low feed efficient animal.

Twenty four Holstein Friesian × Indigenous crossbred calves of 4 to 5 months of age and 71.96±2.22 kg body weight were selected from the institute farm having proven

record of genetic makeup (50:50 HF: Indigenous breed). The animals were housed in semi-open shed with cemented anti-slippery floor and corrugated roof. The animals were dewormed and vaccinated against foot and mouth diseases, black quarter and haemorrhagic septicaemia before the start of the experiment. Deworming was done regularly at 3 month interval. After deworming and vaccination, the calves were maintained for one month for adaptability.

A complete feed mixture was prepared as per NRC (2001) containing 50% wheat straw and 50% concentrate feed. Concentrate feed was prepared by mixing maize grain (20%), wheat bran (21%), deoiled soyabean cake (36%), molasses (20%), mineral mixture (2%) and salt 1%. About 1 kg of seasonal green fodder was offered per day per animal to fulfil the requirement of vitamin A throughout the experimental period. Calves were placed on a receiving diet for 21 days to allow for acclimation to the feeding system. They were provided *ad lib.* feed and water individually for one year. All calves weighed once in a month and residual feed intake was calculated for the entire feeding period. Experimental animals were divided in to two groups, Feed efficient (group 1) and less feed efficient (group 2) on the basis of their RFI. Eight animals were included in group 1 where as six animals were included in group 2 on the basis of their residual feed intake.

Biopsy was done under local anaesthesia (10 ml Xylocaine 2% s/c) to collect longissimus dorsi muscle tissue sample from each animal. Samples were washed with sterile PBS and stored overnight in tms RNA stabilizer (Xcelris, Ahmedabad, India) at 4°C and subsequently at –80°C for long-term storage and further processing. Total RNA was isolated from muscle tissue using TRIzol reagent (Sigma-Aldrich, Germany) and chloroform and subsequently precipitated using isopropanol. The extracted RNA was then treated with the RNase-Free DNase Set (Qiagen) for removal of genomic DNA. A total of 50 mg of each samples were taken for total RNA isolation. The quality of RNA was checked in 1.0% formaldehyde agarose gel. The gel was run at 90 V for 40 min.

First strand of cDNA was synthesized from the individual RNA using RevetAid H Minus First Strand cDNA Synthesis Kit (Fermentas) as per the manufacturer's instructions.

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Table 1. Primer sequences of UCP-2, UCP-3 and β -actin gene

Gene	Primer sequence	Amplicon length (bp)
β -actin	F: 5' GGAATTCGAGCAGGAGATGG3' R: 5' GGATTCCATGCCCAAGG3'	151
UCP2	F: 5' AGCCAACGGATGTGGTGAAG3' R: 5' TCACCAGCTCAGCACAGTTG3'	183
UCP3	F: 5' AGTACAAGGGCATCGTGGAC3' R: 5' GGAATTCTGCCCAACATCAC3'	198

Primers for amplification of UCP-2, UCP-3, ANT 3 and β -Actin were synthesized on the basis of homologous sequence available publicly at NCBI using Biolytic Dr. Oligo 96/192 synthesizer (Table 1).

qPCR reactions were setup for differential expression of UCP-2 and UCP-3 in both experimental groups. β -Actin gene was used as internal control for real time experiment. All the reaction is performed in triplicate using SYBR Green I Master (2 $\times$), in Light Cycler 480 Real time Thermocycler. Reactions were carried out in triplicate using a 96-well plate format and prepared in 20 μ l, with 100 ng cDNA, 10 μ l P SYBR green master mix, and 10 pM forward and reverse primer mix. Reaction samples were incubated at 95°C for 5 min, initially for activation of Taq polymerase. It was followed by 45 cycles of denaturation at 95°C for 10 sec, annealing at 55°C for 15 sec and extension at 72°C for 15 sec then one cycle was run at 95°C for 5 sec, 65°C for 1 min and 97°C for 1 min for melting curve analysis. Gene expression results were calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001)) with β actin used to normalize the data. Fold changes in gene expression between factors were determined as described (Livak and Schmittgen 2001).

Feeding trial was conducted to identify animals with high and low feed efficiency. Calves with negative residual feed intake are included in feed efficient group where as calves with positive residual feed intake is included in less feed efficient group. Differential expression analysis of studied genes in both the group revealed significant difference ($P \leq 0.05$) in the gene expression for the UCP2 gene in the calves having higher feed efficiency than the calves having lower feed efficiency where as UCP3 gene has non significant effect on residual feed intake (Table 2).

UCP 2 gene is upregulated (44.3%) in the less feed efficient group in comparison to feed efficient group. UCP 3 gene was slightly downregulated (5.8%) but it was statistically nonsignificant.

Table 2. Real time PCR result in term of ΔC_t and fold change

Gene name	m ΔC_t less feed efficient group (Group 1)	m ΔC_t feed efficient group (Group 2)	$\Delta\Delta C_t$ value	Fold change in group 1 with respect to group 2
UCP2	6.70 $\pm$ 0.79 ^a	7.23 $\pm$ 0.39 ^b	-0.529	1.443
UCP 3	7.44 $\pm$ 0.59	7.36 $\pm$ 0.71	0.086	0.942

In case of differential expression of UCP2, result disagree with findings of Kolath *et al.* (2006) and Fonseca *et al.* (2015) who found nonsignificant effect of UCP2 expression in *longissimus dorsi* muscle of feed efficient and non feed efficient group of Angus and Nellore cattle, respectively. However this result is in agreement with Ojano-Dirain *et al.* (2007) who found significant higher expression of avUCP gene in less feed efficient group of broiler chicken. This upregulation of UCP2 gene in less feed efficient group suggests that UCP2 gene may affect the feed efficiency through increased energy dissipation via mitochondrial oxidation (Dridi *et al.* 2004).

In case of differential expression of UCP3 this result is in agreement with Kolath *et al.* (2006) and Fonseca *et al.* (2015) who also found nonsignificant effect of UCP3 expression in *longissimus dorsi* muscle of feed efficient and non feed efficient group of Angus and Nellore cattle, respectively. However this result is in disagreement with Kelly *et al.* (2011) who found significant decreased expression of UCP3 feed efficient group of Limousin $\times$ Friesian heifers.

Various workers have found positive correlation between growth rate and milk production traits (Cooke *et al.* 2013, Van De Stroet *et al.* 2016). Profitability of dairy industry is highly dependent on feed conversion ratio. Hence, positive correlation between UCP2 and feed conversion ratio can be used in improving growth and production performances in cattle.

SUMMARY

Uncoupling proteins (UCP) are carrier proteins that release protons during respiration dissipating energy in the form of heat. Expression of these genes plays an important role in feed efficiency through regulation of energy balance. Therefore present study was undertaken to study the differential expression of UCP2 and UCP3 gene in feed efficient cattle in comparison to low feed efficient animal. HFX Sahiwal male calves of same age group were selected randomly and divided in to two groups (feed efficient and less feed efficient) on the basis of residual feed intake (RFI) for the experiment. Differential expression analysis of UCP2 and UCP3 revealed significant upregulation of UCP2 gene in calves having less feed efficiency where as UCP3 gene was having non significant effect on residual feed intake.

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