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Nutrigenomic effects of different yeast derivatives supplemented in surti buffaloes during early lactation

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Abstract

The present study was conducted on nine postpartum Surti buffalo divided into 3 groups of 3 each categorized as group I (control), group II (supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ 10×10^9 cfu/animal/day and group III (supplemented with selenoyeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm). Whole blood was collected at day 0 of study (beginning), day 45 and day 90 of study (end). Total RNA was isolated, cDNA was synthesized and quantitative gene expression of prolactin, leptin, and GPx1 was studied using real time PCR.

Due to rumen specific yeast supplementation the relative expression of prolactin gene between groups in group II was upregulated more than of control. In group III the downregulation for prolactin between groups was lesser than group I during both 0-45, 45-90 and 0-90 days. Leptin expression was more upregulated in group II (yeast supplemented) than group I (control). The leptin expression in group III between groups was slightly more downregulated during 0-45 and 0-90 day and slightly less upregulated during 45-90 day than in group I. The expression of GPx1 revealed that upregulation was present in all groups and stages postpartum in Surti buffaloes. Between groups it was higher in group II than I. The upregulation of GPx1 between groups was more in group III than in group I during all stages. Thus it was concluded that in Surti buffalo during early lactation supplementation of rumen specific yeast had favourable nutrigenomic effects on prolactin and leptin gene expression whereas selenoyeast supplementation impacted positively the expression of glutathione peroxidase gene.

Keywords: Nutrigenomic effect, yeast derivatives, Surti buffalo, early lactation

Introduction

Animal husbandry plays an important role in the economies like India that is agrarian in practice. One of the major components of Indian agro-livestock sector is milk production. Buffalo (*Bubalus bubalis*) shares significant amount of milk contribution by ruminants to the total milk produced by the country like India. The stage of lactation especially the early part that is immediately after the parturition is stressful. Stress of parturition, postpartum changes and early lactation generally impacts the physiology of buffaloes in a negative manner. Hence routine feeding practices during this period plays a crucial role. Nutrition plays an essential role wherein issues related to feeding cripple the realisation of potential of dairy animal BIRTHAL and Jha (2005) [1]. Study done by Garg *et al.* (2012) [2] is also in agreement with this.

There is increased metabolic demand during these stressful stages of parturition, postpartum and early lactation. At this juncture it is quite beneficial to supplement probiotics that can increase dry matter intake and may meet the increased metabolic demands thus optimizing the nutritional status. Leptin hormone is released from adipocytes and is more in blood of buffaloes that are well fed and have good body condition score. Thus leptin levels are indicative of nutritional status. Pituitary and extra pituitary prolactin mediates stress to some extent apart from its major effect especially involving mammogenesis. Both these hormones apart from indicating stress may also mediate associated physiology.

Selenium dependent enzyme glutathione peroxidase, (GPx) is a major peroxide-removing intracytoplasmic enzyme. Most of the hormones and factors that are present in blood have their regulation by gene expression. Foods have been explored recently to affect the gene expression quantitatively. Commonly probiotic have been supplemented in diet but rumen specific yeast and selenoyeast feeding have gained importance recently. Thus the present study was planned to study the nutrigenomic effects of feeding probiotics (rumen specific yeast and selenoyeast)

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in Surti buffalo on genes of prolactin, leptin and glutathione peroxidase during early lactation.

Materials and Methods

Location of study

The present study was conducted at Department of Veterinary Physiology and Biochemistry, NAU, Navsari, Gujarat following ethical guidelines and was approved by IAEC vide NAU/NVC/IAEC/6/2015 01/08/2015.

Experimental design

9 Surti postpartum buffalo were randomly selected and divided into 3 groups of 3 each categorized as group I (control), group II (supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ 10×10^9 cfu/animal/day and group III (supplemented with selenoyeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm). The buffaloes under study were kept and managed at Livestock Research Station. Feeding and management were adopted. The duration of study was from calving to 90 days postpartum during which supplementation of both yeast derivatives was done in group II and III buffaloes.

Five ml of whole blood was collected from each animal from jugular vein into vacutainers containing K_3EDTA . Total RNA

was isolated from whole blood for relative quantification of gene expression of prolactin, leptin, GPX₁ using GAPDH as housekeeping gene. Trizol reagent TRI Reagent BD (Sigma-Aldrich, USA) was used to isolate total RNA from whole blood as per protocol. After isolation the RNA pellet was reconstituted with nuclease free water. Determination of concentration and purity of RNA was done by Nanodrop spectrophotometric (Thermo Scientific ND 2000C) and samples having 260/280 ratio ≥ 1.7 were selected for reverse transcription and relative gene expression study. Integrity of RNA was assessed by agarose gel electrophoresis. First strand cDNA was synthesized. RT-PCR was done using QuantiTect Reverse Transcription Kit (Qiagen, India) following manufacturers protocol. The qRT PCR for Prolactin, Leptin and GPx1 gene was performed against housekeeping gene GAPDH which also acted as internal control using Applied Biosystems 7500 software v 2.0.5. For relative expression quantification, QuantiFast SYBR Green PCR Kit (qiagen) was used. The primers specific for bovine were synthesized (Eurofins Genomics, India). Published primer sequences for prolactin and leptin gene and designed primer sequences for GPx1 and GAPDH gene were used for primer synthesis. The specificity of primers was checked by NCBI blast program (<http://www.ncbi.nlm.nih.gov/BLAST/>). Following primers were used for qPCR:

Table 1: Primer pair sequences, amplicon length and accession number/reference of genes under study

Target Gene	Sequence of nucleotide (5'-3')	Fragment size (bp)	EMBL/Reference
Prolactin Forward	CGAGTCCTTATGAGCTTGATTCTT	156	Mitra (1994) [3]
Prolactin Reverse	GCCTTCCAGAAGTCGTTTGTTC		NM_001290885.1
Leptin Forward	GGCTCCACCCTCTCCTGAGT	123	Dhanoa <i>et al.</i> (2016) [4]
Leptin Reverse	CCCGGAGGTTCTCCAGGTCA		NM_173928
GPx1 Forward	ACGAGGAGATCCTGAATTGC	91	JQ031269.1
GPx1 Reverse	CCATTCACCTCGCACTTTTC		
GAPDH Forward	TCATTGACCTTCACTACATGGTC	109	HQ434960.1
GAPDH Reverse	GCCTTCCATTGATGACGAG		

Relative expression quantification of a target gene to a reference gene was done according to Livak and Schmittgen (2001) [5] and Pfaffl (2001) [6]. Housekeeping gene i.e. GAPDH was used as reference gene for normalization of target gene for relative quantification. The ΔCq values were calculated as $\Delta Cq = Cq \text{ target gene transcript} - Cq \text{ reference gene transcript}$ Calculations for relative quantification (RQ) was done by using following formula Fold increase/decrease in target = $2^{-\Delta\Delta Cq}$ (Livak and Schmittgen, 2001) [5]

Statistical analysis

The collected data were compiled, tabulated and analyzed by using SAS 9.3 software. Statistical analysis was carried using repeated measure two-way ANOVA with PROC GLM procedure.

Results

The fold increase or decrease in gene transcripts of prolactin, leptin, and GPx1 in Surti buffalo postpartum were calculated and are presented in table 2.

Table 2: Fold increase/decrease of gene transcripts in postpartum Surti buffaloes

Prolactin			
	0-45 day	45-90 day	0-90 day
Group I	0.80±0.03	0.78±0.13	0.63±0.12
Group II	0.87±0.03	0.80±0.14	0.70±0.14
Group III	0.82±0.08	0.81±0.08	0.67±0.11
Overall	0.83±0.03	0.80±0.06	0.67±0.06
Leptin			
	0-45 day	45-90 day	0-90 day
Group I	0.76±0.06	1.06±0.02	0.81±0.07
Group II	0.83±0.04	1.03±0.02	0.85±0.03
Group III	0.75±0.08	1.05±0.01	0.79±0.08
Overall	0.78±0.03	1.05±0.01	0.82±0.04
GPx1			
	0-45 day	45-90 day	0-90 day
Group I	1.56±0.39	1.02±0.06	1.59±0.38
Group II	1.64±0.11	1.08±0.02	1.77±0.14
Group III	1.82±0.39	1.12±0.04	2.01±0.39

Overall	1.67±0.17	1.08±0.03	1.79±0.17
Group I (Control-no dietary supplement); Group II (diet supplemented with rumen specific yeast <i>Saccharomyces cerevisiae</i> CNCM I-1077@10x10 ⁹ cfu/animal/day and Group III (diet supplemented with selenoyeast-inactivated yeast <i>Saccharomyces cerevisiae</i> containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm) (n=3)			
Mean bearing different superscripts in upper case letters differ significantly between groups and in lower case letters differ significantly within groups; Mean bearing superscripts with ‘*’ differ significantly at P≤0.01 and without ‘*’ at P≤0.05			

Prolactin downregulation was observed in all the groups and at all stages. Between groups, downregulation during 0-45 days was minimum in group II (yeast supplemented) (0.87±0.03) and maximum in group I (control) (0.80±0.03), for the duration of 45-90 days maximum in group I (0.87±0.06) and minimum in group III (0.81±0.08) and during 0-90 days between groups the downregulation was maximum in group I (0.63±0.12) and minimum in group II (0.70±0.14). Within group the extent of downregulation increased in all the groups from 0-45 days to 45-90 days of study.

The expression of leptin was downregulated during 0-45 and 0-90 days and upregulated during 45-90 days postpartum. Between the groups, during 0-45 days maximum downregulation in group I (control) (0.75±0.08) and minimum in group II (yeast supplemented) (0.83±0.04), during 45-90 upregulation was maximum in group I (1.06±0.02) and minimum in group II (1.03±0.02), during 0-90 days the downregulation was maximum in group III (0.79±0.08) (selenoyeast supplemented) and minimum in group II (0.85±0.03). Within groups the expression of leptin increased from 0-45 to 45-90 day in all the groups.

The expression of GPx1 was upregulated in all the groups during all time periods of postpartum. For 0-45 day, 45-90 day and 0-90 day it was maximum in group III (1.67±0.17, 1.08±0.03 and 1.79±0.17) (selenoyeast supplemented) and minimum in group I (1.56±0.39, 1.02±0.06 and 1.59±0.38) (control) between the groups during all stages postpartum. Within group all groups showed a decrease in upregulation from 0-45 to 45-90 day postpartum.

Discussion

The relative expression of prolactin gene between groups in group II (yeast supplemented) was upregulated more than in group I (control) during all the stages of postpartum studied. Within groups the expression of prolactin decreased in both group I and II.

The stimulus for prolactin increase includes feeding (Johansson *et al.*, 1999) [7], suckling (McNeilly, 1983) [8] and reducing levels of progesterone during approaching parturition (Ashmawy, 2015) [9]. A probable reason for increased expression of prolactin could be improved feeding in group II (yeast supplemented) which may have led to higher prolactin gene expression owing to stimulus from feeding. Prolactin stimulates mammary development and promotes the formation and action of the corpus luteum in the female reproductive cycle in mammals (Sharifi *et al.*, 2010; Le Provost *et al.*, 1994) [10, 11]. Allelic variation in the structural or regulatory sequences of PRL and variations in genes upstream and downstream to PRL in lactation pathway is of interest because of its possible direct or indirect effect on milk production (Ghasemi *et al.*, 2009; Alipanah *et al.*, 2007; He *et al.*, 2006; Brym *et al.*, 2004) [12, 13-15]. Verma and Agsatian (2013) [16] have compared expression profile major milk producing genes of different breeds of cattle and found that Holstein Friesian exhibits highest expression of prolactin gene, and is known for its high milk yield, and an average Fat% though the SNF% is low. Thus a higher expression would ensure improved production performance in dairy animal.

In group III (selenoyeast supplemented) the downregulation for prolactin between groups was lesser than group I during both 0-45, 45-90 and 0-90 days. Within group also the downregulation increased in both groups. Extrahypothalamic prolactin secreted from immune cells which postulates its role also in immune regulation (Harvey *et al.*, 2012) [17]. So group III which was supplemented by selenoyeast may have robust immunity from selenium dependent immune defences thus sparing the prolactin mediated immune regulation and consequently lowering its levels.

Prolactin (PRL) is produced by macrophages and T and B-lymphocytes. However, in peripheral blood-mononuclear cells (PBMC), PRL production is mainly associated with the T-lymphocyte (Ben-Jonathan *et al.*, 1996 and Montgomery, 2001) [18, 19]. Jarczack *et al.* (2014) [20] have demonstrated expression of selected immune system genes in the milk somatic cells of dairy goats when they were supplemented with yeast. Between groups the expression of leptin was upregulated only during 45-90 days and the downregulation in group II was lesser than group I during 0-45 and 0-90 days. Within group the downregulation at 0-45 day drastically changed in to upregulation at 45-90 day postpartum in both the groups.

Leptin regulates food intake, energy partitioning and adipose tissue deposition during both short- and long-term changes in nutritional state (Ingvarsen and Boisclair, 2001) [21]. In dairy cows, plasma leptin concentrations are high before calving, proportionally to body condition score (BCS); they then decrease at calving and then remain low even when energy status improves (Ingvarsen and Boisclair, 2001; Wathes *et al.*, 2012) [21, 22]. Thus, even though marginally but leptin expression was more upregulated in group II (yeast supplemented) than group I (control). The leptin expression in group III between groups was slightly more downregulated during 0-45 and 0-90 day and slightly less upregulated during 45-90 day than in group I. Within group the downregulation at 0-45 day drastically changed in to upregulation at 45-90 day postpartum in both the groups. The expression of leptin in group III was almost similar to control and did not show any major variation.

The expression of GPx1 revealed that upregulation was present in all groups and stages postpartum in Surti buffaloes. Between groups it has higher in group II than I. Yue *et al.* (2009) [23] have also found a selenomethionine dose dependent increase in GPx values in Taihang Black Goats which also has been reflected in present study. Oral supplementation of cysteine and glycine leading to higher levels of glutathione has also been proved in various studies (Nguyen *et al.*, 2013) [24] thus proving that glycine and cysteine apart from glutamate are precursor to glutathione synthesis. This result was in coherence with the gene expression results of glutathione peroxidase in the present study. The upregulation of GPx1 between groups was more in group III than in group I during all stages. Within group the expression decreased from 0-45 to 45-90 days postpartum in both the groups. Most antioxidant functions of Se can be attributed to the glutathione peroxidases (GPxs) and thioredoxin reductases (TRxRs), where selenocysteine residues are located in the active site required for catalytic activity. In dairy cattle, however, the

most widely studied selenoprotein is cytosolic GPx1. Previous studies suggest that the antioxidant functions of GPx1 are the primary reason why Se improves bovine innate immune responses (Brzezinska-Slebodzinska *et al.* 1994 and Spears, *et al.* 2008) [25, 26]. Se, through the actions of GPx1 can protect phagocytic cells from oxidative damage potentially occurring during respiratory burst. Leakage of ROS from the phagosome or failure to reduce ROS to less reactive metabolites could cause bystander damage to neutrophils and result in a reduction of bactericidal functions (Larsen, 1993) [27]. These studies also indicate the similarity of results of present study with these that supplementation of seleno yeast can be a reason for increased upregulation of GPx1 gene in group III at all stages. Yuan *et al.* (2012) [28] also showed that selenium enriched food when supplemented increases expression of glutathione peroxidase gene. In the present study the increase was not significant probably because of lesser number of samples.

Conclusion

It was concluded that in Surti buffaloes during early lactation supplementation of rumen specific yeast had favourable nutrigenomic effects on prolactin and leptin gene expression whereas seleno yeast supplementation positively impacted the expression of glutathione peroxidase gene.

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