

## RELATIONSHIP BETWEEN PLASMA HAPTOGLOBIN, MONOCYTE TOLL LIKE RECEPTOR 4 EXPRESSION WITH GROWTH AND THE EFFECT OF SUPPLEMENTATION OF FERMENTED YEAST CULTURE ON LOW BODY WEIGHT CROSSBRED CALVES

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### ABSTRACT

The relationship between circulating Haptoglobin, Toll like receptor 4 (TLR 4) expression and growth of post weaned low body weight crossbred female calves with supplementation of fermented yeast culture. The expression of TLR 4 receptor and plasma Haptoglobin concentration may act as indicators for post weaning stress and subclinical infection. At nine months of age, Karan Fries calves were grouped in to normal (HBW) and low body weight (LBW) calves fermented yeast culture (*Saccharomyces cerevisiae*) was supplemented @ 300g/ quintal of concentrate mixture for feeding of low body weight. The calves were offered wheat straw and green fodder to meet their nutritional requirements. The concentration of plasma Haptoglobin (Hp) was observed to be negatively correlated with body weight. Expression of TLR 4 in monocytes was down regulated in low body weight group female crossbred KF calves (LBW) when compared with normal growing calves (HBW). Fermented yeast culture supplementation improved the average daily gain, metabolic body weight ( $BW^{0.75}$ ) and expression of TLR 4 and decreased concentration of plasma Haptoglobin in LBW group calves.

**Key words:** Cattle calves, Fermented yeast culture, Growth, Haptoglobin, Stress, Toll like receptor4.

### INTRODUCTION

Management practices, environmental and post-weaning stress may negatively impact growth performance and immune function in calves. Subclinical infections may be indirectly reflected by impaired growth performance (Wright *et al.*, 1999). Haptoglobin (Hp) is a potent acute phase protein which is exhibited at low level in plasma and increases during acute phase reactions and mild infections in cattle and sheep (Nowroozi-Asl, *et al.*, 2008). In healthy animals, serum Hp concentration was observed to be < 0.35 g/L (Horadagoda *et al.*, 1994). Measurement of Hp proved to be a useful tool for evaluation of health parameter in calf herds. In many of these cases, the calves many shown one to only mild, clinical symptoms that could easily be missed in a group of calves in a farm (Ganheim *et*

*al.*, 2007). Toll like receptors are critical component of the innate immune system for detection of pathogen associated molecular patterns (PAMPS) (Kotsougiani *et al.*, 2010). Supplementation of fermented yeast culture (*Saccharomyces cerevisiae*) to calves improved feed intake/live weight gain and decreased effects of transport stress (Fallon and Harte, 1987; Hughes, 1988; Philips and Von Tungela, 1985). Live yeast has been reported to be beneficial in improving live weight gain in calves and lamb. Anandlaxmi *et al.* (2012) reported that supplementation of fermented yeast culture improved growth performance in low body weight group female crossbred calves. The present study was attempted to establish the circulatory levels of plasma Haptoglobin in growing crossbred calves and also the effect of supplementation of fermented yeast

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culture to LBW group on plasma Hp level and TLR 4 expression in monocytes in order to study their relationship with growth in crossbred calves.

### MATERIALS AND METHODS

The experiment was conducted at NDRI, Karnal from Aug 2009 to March 2010. A group of 15 healthy crossbred Karan Fries post-weaned calves were selected and housed individually in sheds. Wheat straw and concentrate were provided in the ratio (50:50) on DM basis and 2kg green fodder was also given daily to meet the vitamin A requirements of calves. The experiment was started when calves were seven months old age. Body weight of the animals was recorded every month. Feed intake (FI) and feed refusal were monitored daily. Weekly blood samples were collected in heparinised tubes; plasma was separated at 2500 rpm for 15 minutes and stored at -20°C till analysis. After 122 days of experimental feeding, calves were grouped based on their body weight. The cutoff point for normal body weight (HBW) was 100 kg. The body weight of the HBW group calves ranged between 110-128 kg and LBW group calves between 68-98 kg (Sharma, *et al.*, 2010). Each group consisted of seven numbers of calves. One calf suffered from severe fever, hence was discarded. The LBW group calves were supplemented with fermented yeast culture of '*Saccharomyces cerevisiae*' (Diamond V, XP) in concentrate mixture after two months post selection of low bodyweight calves till the end of the experimental period of 230 days along with equal quantity of wheat straw and green fodder to meet their nutritional requirements.

Plasma Hp was estimated by enzyme immunoassay (EIA) using bovine EIA Kit (Immunology Consultants Limited Co., USA). An aliquot of plasma was processed for collection of leukocytes. RNA was extracted using RNA extraction Kit (Qiagen Co. USA). The QIAmp RNA Blood Mini Kit was used for isolation of RNA from plasma with a fast spin-column procedure. Purity of RNA was checked by taking ratio of absorbances at 260 and 280 nm. cDNA was synthesized from RNA using primers.  $\beta$  Actin was kept as house keeping gene. NCBI accession no. for  $\beta$  Actin gene is AY141970 (FP- GCCCTGAGGCTCTCTTCCA; RP- AAGTGTACGTCGACATCCG); For TLR 4 gene

NCBI accession no: AF310952 (FP- GTGTTCAGAGCCTTTAGATATGA; RP - CATCAGTGTGTCGGTGGTCACT).

RT reaction was performed using Oligo-dT primer and MMLuV reverse transcriptase enzyme (Chromous Co. Bengaluru, INDIA). First strand cDNA was used for PCR and real time PCR was performed using Qiagen SYBR PCR Kit (Genetix Biotech Asia Pvt. Ltd., New Delhi). Quantification of mRNA for TLR 4 gene of monocytes was performed. Relative quantification was carried out for TLR 4 gene against house keeping gene  $\beta$  Actin and expression of TLR 4 was quantified for LBW group and HBW group monocyte samples. In triplicates. The expression of TLR 4 gene was normalized with respect to  $\beta$  Actin gene. Status of the TLR 4 expression was studied immediately 60d post initiation of experiment and at 60 and 110 d post supplementation of fermented yeast culture respectively. Plasma samples for individual calf were pooled for monitored time periods (6 samples/ time period/ group) for both the groups respectively.

### Statistical analysis

The graphs for data were drawn by using statistical software 'PRISM' version 3.02, GraphPad Software, Inc., 1999. The data was analyzed using Two way ANOVA method with bodyweight and month as factors, using software package SYSTAT VERSION 6.0.1, (1996), SPSS INC. For correlation studies, Pearson correlation coefficient method was used.

### RESULTS AND DISCUSSION

Calves belonging to HBW group exhibited higher body weight ( $P < 0.001$ ) than LBW group (Fig-1). The significant difference between the body weights of two groups of calves (HBW vs. LBW) could not be reduced even at 60 days post supplementation. The rate of ADG increased from pre to post supplementation period within respective groups, although no significant difference was observed between the groups during post supplementation period. The percentage of animals exhibiting ADG  $> 700$ g/day was more for LBW group compared to HBW (Fig. 2). There was no significant difference in Dry matter intake (DMI), DMI/day, body weight gain (BWG), BWG/day and percentage of feed conversion efficiency (FE) between the groups either in the pre supplementation

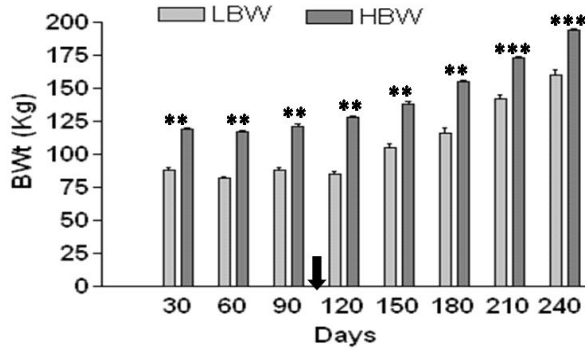


FIG. 1. Body weight of KF calves at different days after weaning. Supplementation of *Saccharomyces cerevisiae* to LBW group; \*\*\*P< 0.01 between groups, \*\* P< 0.001 between groups;

period (110 days) or post supplementation period (120 days). But a significant difference (P< 0.01) was observed within a group from pre to post supplementation period. The metabolic body weight (MBW) and average BW for HBW group was significantly higher (P< 0.01) in both pre and post supplementation periods respectively (Fig- 3a, b).

The quantitative gene expression studies for TLR 4 of monocytes of HBW group revealed that the TLR4 expression was 12 times greater at 60 days (pre supplementation) when compared with expression level at 150 and 210 d periods (Fig-4a), whereas in LBW group, the expression was significant only at 210d post supplementation period, (Fig-4b). When expression level of TLR 4 of LBW group at 60d and 150d was compared with expression exhibited around 60d by HBW group, it was

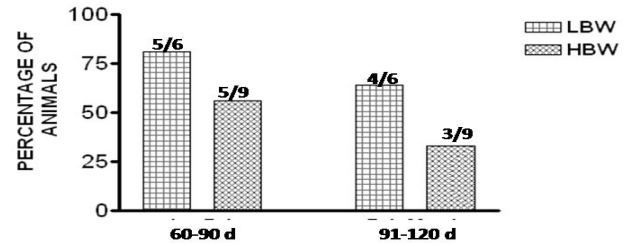


FIG. 2. Percent of animals gaining > 700g/day after supplementation LBW> HBW group

observed that expression was down regulated (Fig-4c). It was detectable only at 110d post-supplementation period associate with decrease in level of plasma Hp and significant increase in body weight gain and ADG during the same tenure of post-supplementation period (Fig. 4c). Continuous exposure to endotoxin or certain peptides can cause down regulation of TLR4 receptors but not in healthy individuals (Foster *et al.*, 2007). TLR4 signaling presents itself as a pharmacological target and may allow therapeutic modulation of leukocyte survival by direct and indirect mechanisms at sites of inflammation (Wu *et al.*, 2008). If there is traumatic/stress conditions persisting, it leads to non-responsiveness of immune cells and down regulation of TLR 4 (Vergees *et al.*, 2009). In LBW group animals expression was not significant at early stage perhaps due to suffering from post weaning and environmental stress.

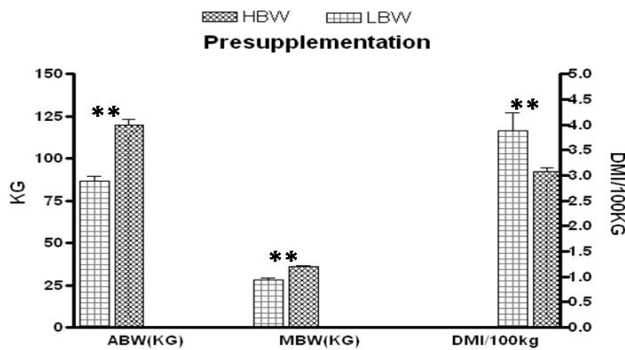


FIG. 3A. Presupplementation- Comparison of different body weight and feed parameters between HBW and LBW group (110 days)

\*\*P< 0.001

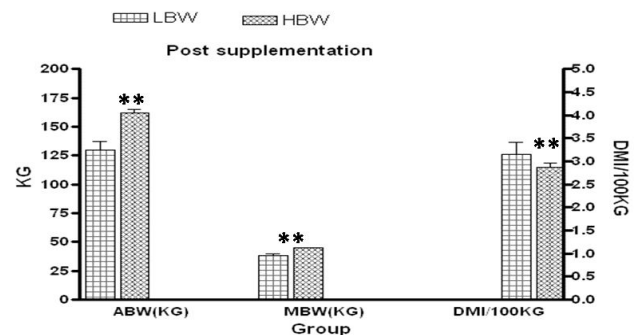


FIG. 3B. Post supplementation- Comparison of different body weight and feed parameter between HBW and LBW group (120 days) \*\*P< 0.01

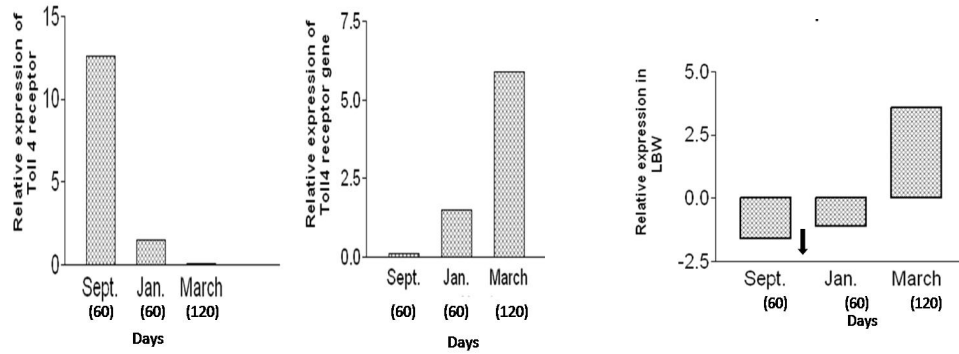


FIG. 4 A, B, C. Relative expression of Toll 4 like receptor expression in HBW and LBW groups respectively. Supplementation of fermented yeast culture.

During pre-supplementation period, the plasma concentration of Hp of LBW group was significantly higher ( $P < 0.001$ ) compared to HBW group calves (Fig-5). The plasma Hp concentration for both the groups exhibited higher than the normal basal level of circulatory Hp (200 ng/ml) during the pre-supplementation period of 60-90d post-weaning period. Plasma Haptoglobin was within in the adult bovine reference values, 0.2-0.3 g/l. (Petersen *et al.*, 2004; Nazifi *et al.*, 2008). APP may be useful for determining the spread of the disease in the herd by providing information about the prevalence of ongoing clinical and subclinical infections indicated by the high serum concentration of selected APP (Petersen *et al.*, 2002) and by serving as a prognostic tool. Further the plasma Hp concentration decreased significantly ( $P < 0.01$ ) during the 120days of the experiment period in both the groups, (Fig-5) the concentration still being higher in LBW group. Supplementation of fermented yeast culture to LBW animals for 60 days further reduced the concentration of Hp significantly ( $P < 0.01$ ) in comparison to HBW group (Fig-5). Thus, the supplementation of

fermented yeast culture to low body weight animals could reduce the plasma concentration of Hp in calves. During inflammatory reaction, they are more likely to draw energy and nutrients leading to lower body weight gain and stimulating formation of acute phase proteins (APP) (Arthington., 2007). In ruminants, the circulatory level of haptoglobin is negligible in normal animals, but increases during inflammation and its severity. It is known that activated inflammatory reaction is acting as a nutrient sink, resulting in reduced body weight gain in stressed calves (Arthington *et al.*, 2005). The animals gaining less weight were found to have greater plasma Hp level than those gaining more weight. Hp seemed to be a promising marker of health status by reflecting a lower spectrum of sub-clinical diseases (Eurell *et al.*, 1992). Haptoglobin has been used as a marker more recently, whose plasma concentration increases the first day after onset of inflammation, and remains elevated as long as the inflammation is present. It has been suggested

TABLE 1: Correlation between Haptoglobin and body weight (BW).

GROUP LBW			
B WT	B WT	HAPTOGLOBIN	Haptoglobin is negatively correlated with body weight
HAPTOGLOBIN	1.00000	1.00000	
* P< 0.01	-0.40695*		
GROUP HBW			
B WT	B WT	HAPTOGLOBIN	Haptoglobin is negatively correlated with body weight
HAPTOGLOBIN	1.00000	1.00000	
	-0.05018		

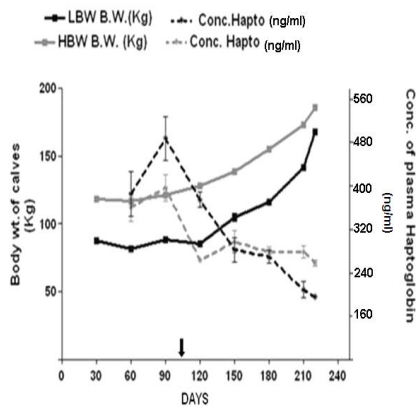


FIG.5. Negative correlation between body weight and plasma Haptoglobin during presupplementation period. Supplementation of fermented yeast culture \* ( $P < 0.001$ ).

that the APR can be used for assessment of cattle health (Ganheim *et al.*, 2003). Initially, the plasma Hp level in both HBW and LBW groups were significantly higher than the normal basal level, which indicated stress and mild infected /inflammation in both the groups, but later continued to be higher in LBW group, which decreased on supplementation.

Pearson's correlation studies revealed that Hp concentration was negatively correlated with BW of animals (Table-1). The negative correlation was more significant in case of LBW than HBW group animals. Since, the metabolic weight of the LBW group was significantly low ( $P < 0.01$ ) in comparison to HBW group and higher plasma Hp level and weak expression of TLR 4 indicated lower health status of the LBW group animals.

Fermented yeast culture has been recently used for controlling and maintaining intestinal bacteria. Yeast has been reported to be beneficial in improving live weight gain in calves and lambs (Hughes, 1988; Wallace and Newbold, 1993). It has been observed that administration of *Saccharomyces cerevisiae* to calves improved feed intake/kg live weight gain and decreased the effects of transportation stress (Philips and Von Tungela, 1985). Beef cows and calves fed a poor quality pasture improved weight gain from 0.57 kg/day to 0.80 kg/day on feeding yeast culture along with the diet (Wiedmeier, 1989). The study suggested that fermented yeast culture can be fed to low body weight growing cross bred calves for better health and body weight gains. There is no published report on the reference value of serum Hp in post-weaned crossbred calves and its association with body weight and the plasma level during phase of less body weight gain. It was also to assess if supplementation of Fermented yeast culture can ameliorate these effects and improve average daily BW gain in calves. Supplementation of fermented yeast culture in the present study is a source of vitamins, amino acids and other nutrients besides viable yeast cells which boosts the growth and immune status of growing calves and reduces stress markers / sub clinical infection markers in growing calves.

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