

# Implications of supplementation of fermented yeast culture to metritic cross bred Karan Fries cows on plasma parameters and productive performance

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## ABSTRACT

Eighteen number of peripartum Karan Fries (KF) cross bred cows were selected. The animals were monitored post partum for reproductive parameters. Monthly body weight and daily milk yield of all animals were recorded. Wheat straw, maize, concentrate were fed to the lactating animals as per requirements of body weight and maintenance in the ratio 50:50 @ 8kg/animal. Blood samples were collected once a week for the analysis of plasma IGF I, Haptoglobin and Lactoferrin. The KF animals suffering from reproductive problem metritis were grouped separately. The metritic group was supplemented with fermented yeast culture @ 24g/animal/day. Each group had its own control. On supplementation of yeast culture the plasma Haptoglobin level significantly decreased and decreased further during supplementation and antibiotic treatment period ( $P<0.01$ ). The concentration of plasma lactoferrin increased during the period of supplementation but decreased further on rendering antibiotic treatment. The concentration of plasma IGF I increased ( $P<0.01$ ) during the entire period of supplementation. On supplementation even though concentration of inflammatory marker declined it could not cure cows from metritic condition and these animals could not conceive.

**Keywords:** Plasma, IGF 1, Haptoglobin, Lactoferrin, Body weight, Conception, Crossbred cattle.

## INTRODUCTION

The two key components of high reproductive efficiency are: High submission rate of cows for insemination and greater conception rate to each service. Endometritis is a superficial inflammation of the endometrium without systemic or overt signs of illness that often occurs before first ovulation (Sheldon, 2004). The problem arises where pathogenic bacteria multiply due both to the suppressed immune system around parturition and the resultant decrease in migration and the functional capacity of neutrophils to eliminate uterine bacteria. The incidence of endometritis is affected by the method used to diagnose it because of perceived insensitivity and lack of specificity of rectal examination of the uterus and observation of a

genital discharge (Gilbert et al., 2005; Sheldon et al., 2006). The risk factors associated with irregular cycles are dystocia, RFM, abnormal vaginal discharge and metritis (Opsomer et al., 2000). Most of the plasma lactoferrin originates from neutrophils (Lonnerdal and Iyer, 1993). The aim of the present study was to estimate the concentration of plasma Insulin like growth factor I, Haptoglobin and Lactoferrin in post partum KF cows suffering from metritis and correlate with conception rate and see the feasibility of using them as biomarkers. Further effect of supplementation of fermented yeast culture to metritic animals was also evaluated.

## MATERIALS AND METHODS

Freshly calved crossbred Karan Fries cows, included in the study were maintained at general herd of National

Dairy Research Institute, Karnal (Haryana). The experiment was conducted between April 2010 – November 2010. All the animals belonged to second and third parity. A group of cows (6 Nos.) which were observed to be clinically healthy and devoid of reproductive tract problem at 3<sup>rd</sup> – 4<sup>th</sup> week postpartum were considered as healthy animals. Further, these animals, exhibited heat within 60d post partum and they were subjected to A.I. Animals which conceived within three services, and were clinically healthy, were grouped as negative control group. Cows, which developed endometritis within 3-4 week postpartum and could not be cured with routine antibiotic treatment at herd at tenth week postpartum were considered as animals suffering from metritis and were grouped as metritic animals. Out of these, twelve animals were selected and were divided into two groups, one group was supplemented with fermented yeast culture @ 24g/animal/day which was designated as SUP-MET group and the metritic animals, which were not supplemented, were designated as NS-MET group. Purulent discharge from the vaginal opening was observed. Weekly blood samples were collected beginning at four weeks post partum. Weekly, rectal palpation was conducted, to check the ovarian as well as reproductive tract status of the animals.

Daily feed intake of the KF cows was also recorded. The diet of the cows comprised of wheat straw and concentrate in the ratio 1:1. @ 8kg/animal/day. They were supplemented with green matter @ 4 Kg/animal/day. The composition of concentrate is given in Table 1. Daily milk yield was also recorded. At three weeks postpartum, each cow was examined for uterine involution, till the confirmation of involution (Table 2). Results for other reproductive parameters are also given (Table 2). Body weight of all the cows was recorded at monthly intervals. The supplementation of yeast culture to the cows was done in two phases. Till ten weeks, postpartum, only antibiotic treatment was given for metritic group. From the beginning of eleventh week, yeast culture was supplemented along with concentrate, and antibiotic treatment was withdrawn for eight weeks in both the groups and then further from 19<sup>th</sup> to 27<sup>th</sup> week, antibiotic treatment was resumed along with pro-biotic supplementation. Newly calved animals which conceived with in three or equal to three services were grouped as control and healthy animals and further served as negative control group. This type of pattern was followed to evaluate the efficacy of supplementation of fermented yeast culture in reducing, number of animals suffering from metritis and also to study its effect on plasma parameters, associated with reproduction or health of animal. The total study period was classified as presupplementation period when yeast culture was not supplemented to the experimental group of animals, and post supplementation period, during which yeast culture was supplemented, with and without antibiotic treatment.

The concentration of plasma IGF I, Haptoglobin and

Lactoferrin was measured using Enzyme Immunoassay kit (Cusa Biotech Inc., USA). Blood plasma was separated immediately by centrifugation at 1000g for 20 min. The plasma was transferred to plastic tubes and these samples were stored at - 20<sup>o</sup> C for estimation of hormones in plasma. The collection of blood samples initiated at one month postpartum, continued till one month after confirmation of pregnancy. Commercially available kits were used to determine the concentration of IGF-I, Haptoglobin, Lactoferrin in the plasma samples. Parallelism with standard curves and serial dilution of bovine plasma was performed for all assays. No significant difference was observed. The intra and inter assay coefficients of variation were < 10% for all the parameters. Bovine IGF-I was measured in plasma samples using EIA kit (Cusa Biotech. Co. Ltd., USA), The detection range for concentration of standards varied between 3.12 ng/ml to 200ng/ml. Haptoglobin in plasma samples was estimated using EIA kit (Immunology consultants laboratory inc., USA). It is based on immunoperoxidase assay. It is double antibody sandwich ELISA. Concentration of Hp standards ranged between 15.6-1000ng/ml. Lactoferrin in plasma samples was estimated by ELISA method. The concentration of lactoferrin standards ranged between 7.8-500ng/ml. ELISA was performed with the kit procured from Bethyl Laboratories Inc., USA. Enzymatic-colorimetric methods were used to determine plasma concentrations of NEFA (Wako Chemicals, USA)

## RESULTS AND DISCUSSION

It was observed that during pre supplementation period, dry matter intake (DMI) was less in metritic group of animals when compared with control group. But the difference was not significant. The average milk yield/animal/day was significantly greater for metritic group when compared with control group. ( $14.5 \pm 0.9$  vs.  $12.25 \pm 0.25$ ,  $P < 0.01$ , Figure 2). The NS-MET group served as positive control. The Mean  $\pm$  SE of milk yield of the NS group declined from  $14.5 \pm 0.9$  l to  $12.10 \pm 0.15$  l from the 67<sup>th</sup> day of lactation period. Supplementation of *Saccharomyces Cerevisiae* to metritic animals, from tenth week postpartum restricted the decline in milk yield in SUP-MET group, Mean  $\pm$  SE being  $13.8 \pm 0.15$  l and DMI increased significantly from  $11.8 \pm 0.12$  to  $13.2 \pm 0.31$  Kg/animal/day, ( $P < 0.05$ , Figure 1). In dairy cows, it has been reported that supplementation of yeast brought about an improvement of 4% in milk yield, and increased feed intake (Ali Haimoud-Lekhal et al., 1999). It has been observed that *Saccharomyces Cerevisiae* supplementation in animal nutrition increases productive performance (Breul, 1998).

Metritis is an important postpartum disease due to its negative effect on the reproductive performance of dairy cows (Gilbert et al., 2005, LeBlanc et al. 2002). Huzzey et

**Table 1.** Percentage of different componants in concentrate mixture

Components of Concentrate	Per 100 kg
maize	30 parts,
deoiled mustard cake	25 parts
soybean extraction	10 parts
wheat bran	10 parts
deoiled rice bran	15 parts
molasses	7 parts
mineral mixture	2 parts
common salt	1 part

**Table 2.** Different parameters recorded with respect to reproduction

Causes of metritis	Retained placenta, Abortion, Dystocia
Days required for uterine involution	30-40d
Estrus cycles	15-30d (irregular)
Heat first observed post partum	<60d

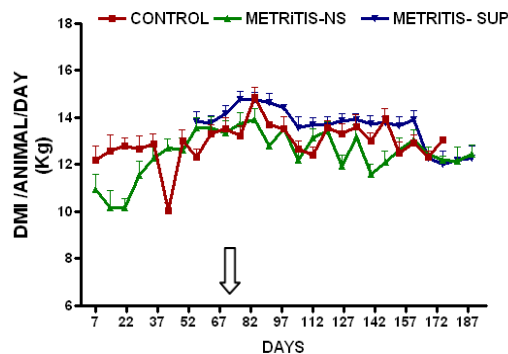


Fig-1 Initiation of supplementation,  
 Presupplementation –  
 CONTROL vs. METRITIC group,  
 Post supplementation-  
 Control, METRITIS-SUP vs. METRITIS-NS ( $P<0.05$ )

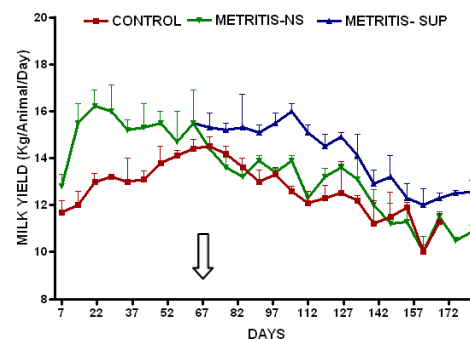


Fig-2 Initiation of supplementation  
 Presupplementation-  
 CONTROL vs. METRITIS ( $P<0.01$ )  
 Postsupplementation  
 METRITIS-SUP vs. CONTROL, METRITIS-NS ( $P<0.01$ )

al. (2007) reported that the cows that developed metritis ate less than the healthy animals in the early postpartum period and yielded less milk during the first 28 weeks of lactation. In the present study also it was observed that, cows that developed chronic metritis exhibited decline in milk yield when compared with early period of lactation ( $P<0.01$ ). Bartlett et al. (1986) reported that functions like reproductive efficiency and milk production were affected depending on degree of metritic functions. At an early stage, plasma haptoglobin is a physiological inflammation indicator (Jahoor et al., 1996). It was observed that the Mean  $\pm$  SE of plasma Hp concentration in metritic group

was observed to be  $670.40 \pm 4.71$  ng /ml whereas in healthy group it was  $278.37 \pm 4.20$  ng /ml. Plasma Hp is a protein that is connected with inflammation (Humblet et al., 2004). The concentration of plasma Hp estimated in the present study in both metritic and healthy group is less when compared with the reports of Humblet et al. (2004), Nazifi et al., (2008). The differences observed in the concentration of plasma Hp may be due to the usage of different EIA kit and different method used for estimation of Hp. At the end of supplementation period i.e. 27 weeks postpartum, the plasma Hp level declined ( $418.23 \pm 3.38$  ng /ml.) in SUP-MET group, whereas in

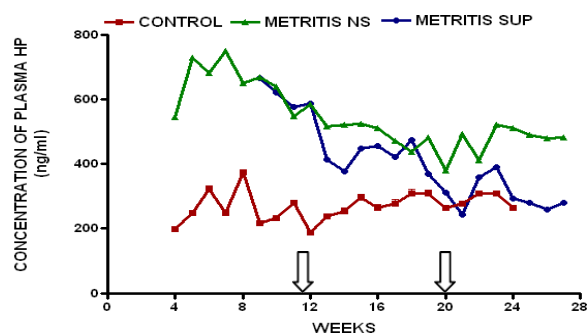


Fig- 3 ↓ Supplementation initiated at tenth week postpartum,  
Pre supplementation period - -  
CONTROL vs. METRITIC group ( $P < 0.001$ ),  
↓ Post supplementation period -  
METRITIS-SUP vs. METRITIS-NS ( $P < 0.05$ )

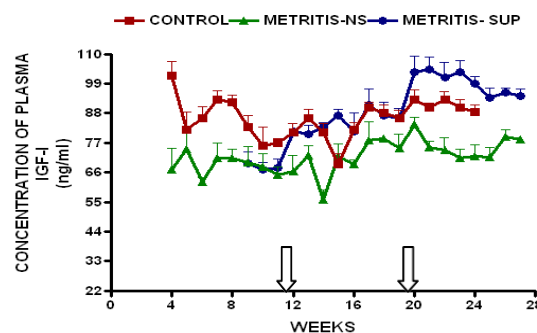


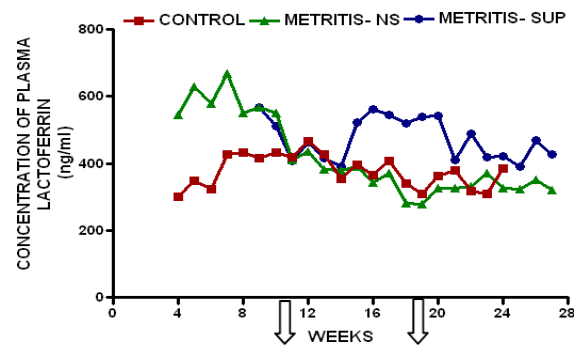
Fig- 4 Presupplementation  
CONTROL vs. METRITIC group ( $P < 0.01$ ),  
↓ Post supplementation  
CONTROL, METRITIS - SUP vs. METRITIS - NS ( $P < 0.01$ )  
↓ SUP+ Antibiotic ( $19^{\text{th}}$  - $27^{\text{th}}$  wk.)

In the NS-MET group, the concentration was  $510.11 \pm 3.56$  which was significantly more ( $P < 0.05$ ). Further greater decrease ( $P < 0.01$ ) in plasma Hp concentration in SUP-MET. group was observed during the later phases of supplementation along with antibiotic treatment, the results indicate that supplementation alone can not decrease the inflammatory conditions. In subclinical infection conditions, the concentration of circulatory Hp can act as an indicator (Jain et al., 2011). The decrease in concentration of plasma Hp in SUP-MET group, but more than negative control group indicates persistence of inflammation due to infection (Figure 3).

Persistent infection beyond 21d postpartum is more likely to reduce the chemotactic and phagocytic activity of neutrophils of endometrium which might have caused the chronic metritic conditions to prevail in the cows (Klucinski et al., 1990). Although in the early lactation period the plasma NEFA concentration was more in metritic group than in healthy animals, it was not significantly different ( $< 350$   $\mu\text{M/L}$ , not given), indicating that, animals suffering from metritis did not experience negative energy balance, and the same trend was observed during post supplementation period also when compared between the three groups, namely negative control, NS-MET and SUP-MET group respectively.

The twelve animals, which turned out to be metritic, six of them experienced retained placenta, two of them abortion and other two dystocia. It is possible that some infection/ inflammation, present in the system persisted for a longer duration and non specific inflammation when induced in the endometrium, intracellular killing by neutrophils is reduced (Meglia et al., 2005). The plasma IGF1 concentration during pre-supplementation period, in the control group was  $91 \pm 3.48$  ng/ml and in group which developed metritis was  $69.40 \pm 1.45$  ng/ml which was significantly different ( $P < 0.01$ ). During post

supplementation period the Mean  $\pm$  SE concentration of plasma IGF1 in non supplemented group was  $72.10 \pm 1.67$  ng/ml which was not significantly different from the pre-supplementation period, but was significantly less than that of the supplemented group ( $87.87 \pm 2.55$  ng/ml,  $P < 0.01$ ). The Mean  $\pm$  SE plasma IGF1 level of supplemented group increased and was not significantly different when compared with negative control (Figure 4). It is known that IGF1 directly acts on the ovary for increasing sensitivity to LH and FSH as well as on the uterus (Webb et al., 2004; Bilby et al., 2006). Uterine infection decreases reproductive efficiency (Gilbert, 2012). Hence decrease in IGF1 and persistent uterine infection might have resulted in nil conception rates in metritic group. In humans, it has been reported that, IGF1 promotes granulocyte function (Kooijman et al., 2002) and stimulates cytokine secretion by peripheral blood mononuclear cells. IGF1 is known to enhance degranulations of neutrophils (Bjerknes and Aarskog, 1995). Continuous reduced concentration of plasma IGF1, might have also caused decrease in the plasma level of lactoferrin in non-supplemented animals during mid and later stages of lactation during the course of the study. All elements of IGF1 system are present in the immune system and regulate immune specific stimuli (Kooijman et al., 2004). It has been reported by Oetzel et al. (2007) that cows fed with probiotic during first 85 days in milk received less antibiotic treatment. The term probiotic, is defined as a live microbial supplement that may beneficially affect the host animal upon ingestion by improving its intestinal microbial balance (Fuller, 1989). Most commercial yeast products contain species of *Saccharomyces* and *Aspergillus*. Chaucheyras et al. (1995) reported that *S. cerevisiae* had the ability to provide growth factors such as organic acids and vitamins, thereby improving the numbers of beneficial



**Fig- 5** Supplementation initiated at tenth week postpartum, CONTROL vs. METRITIC group ( $P < 0.01$ ),  
 ↓ Post supplementation period – METRITIS-SUP vs. METRITIS-NS ( $P < 0.05$ )  
 ↓ SUP+ Antibiotic (19<sup>th</sup> -27<sup>th</sup> wk.)

rumen microflora, inhibiting growth of pathogens and stimulating immune function. After the yeast culture is administered, function is taken up by epithelial cells, which are engulfed by macrophages and APC stimulating immune response (Cakiroglu et al., 2010; Parantham, 2011).

During pre-supplementation period,  $MEAN \pm SE$  concentration of plasma lactoferrin was above basal level when compared with that of control group ( $366.37 \pm 2$  vs.  $594.40 \pm 1.71$  ng / ml). During post supplementation period, it was observed that in NS –MET group the concentration declined from pre supplementation period ( $594.40 \pm 1.71$  to  $365.90 \pm 2.67$  ng/ml), which was not significantly different from negative control group ( $380.64 \pm 2.55$ ). In SUP-MET group, supplementation caused significant increase ( $P < 0.01$ ) but further on rendering antibiotic, the concentration of plasma lactoferrin decreased, where as no significant change was observed in NS-MET group (Figure 5). It is known that plasma lactoferrin is involved in immune response and anti-inflammatory properties. The greater concentration of plasma lactoferrin in the presupplementation period, may be due to initial response to infection, but when the infection persisted beyond 15 weeks postpartum, the NS-MET group could not maintain greater concentration of lactoferrin or decreased and maintained normal physiological concentration of plasma lactoferrin. Lactoferrin receptors have been identified on monocytes and neutrophils (Maneva et al., 1994; Birgens et al., 1984). On supplementation of yeast culture to metritic group, higher concentration of plasma lactoferrin and decreased concentration of plasma Hp indicate that supplementation of yeast culture, even though did not cure metritic condition might have reduced inflammatory condition or degree of metritis, which was not studied in detail. In confirmation with reports of Kalmus et al.

(2009), supplementation of yeast culture further restricted the decrease in milk yield. It suggests that supplementation of yeast culture will prove beneficial for improving milk production and health of KF cows. On exhibition of heat by the animals, they were artificially inseminated, but always proved to be negative for conception. It can be concluded that in the early lactation period, the decrease in DMI, higher milk yield around parturition might have led to development of metritic condition in KF cows. Further the reduced concentration of plasma IGF1 could be restored to physiological level only on supplementation of yeast culture. Maintenance of plasma level of lactoferrin and decrease in Haptoglobin concentration on supplementation suggests the beneficiary role of *Saccharomyces Cerevisiae* in maintenance of milk production and plasma parameters in crossbred Karan Fries Cows. From our study on supplementation of fermented yeast culture for longer duration, it was observed that feeding animals at the mentioned particular dose could not cure the animals from metritic condition, but could maintain DMI and milk production. There are many other factors like, minerals and vitamins which also govern the health status of cows.

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