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Plasma IGF-I and lactoferrin as biomarkers of post-weaning stress and the effect of feeding probiotic to low body weight calves for the improvement of growth performance in crossbred KF calves

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ABSTRACT

Management practices as well as environmental and pathological stresses have an impact on growth performances and health status of calves. Lactoferrin is a glycoprotein and is a prominent component of host-defense against infection. Insulin like growth factor I (IGF-I) has been established as a biochemical indicator for different production traits. The present study was under taken to evaluate the role of IGF-I and lactoferrin as biomarkers for growth performances and health status in female crossbred KF calves and the effect of probiotic *(Saccharomyces cerevisiae)* supplementation on growth performance in post-weaned calves. Plasma concentration of these factors were correlated with growth performances in terms of body weight gain, dry matter intake, feed conversion efficiency, metabolic body weight gain in post-weaned calves. Calves exhibiting normal body weight (HBW) and the ones exhibiting lesser body weight (LBW) with respect to age ($P<0.01$) were divided into 2 groups. The body weight of LBW group was significantly less ($P<0.001$) during the pre-supplementation period (122 days). At 60d post-supplementation period, the significant difference between the body weight decreased ($P<0.01$). The circulatory level of IGF-I was significantly high ($P<0.01$) in HBW group when compared with LBW. During the post-supplementation period, the concentration of lactoferrin was not significantly different in both the groups and had decreased to the basal level (500–420 vs.150–250ng/ml) in comparison to pre-supplementation period, suggesting normal immune status and reduced stress/ inflammation. Supplementation of probiotic improved growth performance to a lesser extent in LBW group. IGF-I can act as a marker for growth performance in crossbred calves.

Key words: Body weight, Calves, IGF-I, Lactoferrin, Post-weaned, Stress

Studies on post-weaning stress in crossbred (KF) calves has not been studied in terms of circulatory levels of IGF-I with respect to body weight and feed intake. IGF-I is strongly associated with cell proliferation, anti apoptosis and transmits proliferation signals in hematopoietic cell lineages. It also acts as a biological marker for growth in neonatal calves (Graham *et al.* 2010). Lactoferrin is a multi-functional member of the transferring family of non-haeme iron binding glycoprotein. This protein is an abundant component of the granules of the neutrophils and is the first line of host-defense against infection and inflammation. Lactoferrin (LF) binds to various cells of immune system that lead to activation of the same (Legrand *et al.* 2005, Gonzαlez-Chαvez *et al.* 2009).

The objective of the present study was to find out the relationship between the different plasma parameters and the body weight of the calves during the course of the study; and also to identify these parameters as biomarkers for body weight gain and health status of post weaned calves. This would further allow us to screen the calves with less growth performance at an early post-weaning stage, in relation to plasma biomarkers and they can be supplemented with probiotics in the diet to improve growth and health status.

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MATERIALS AND METHODS

Crossbred calves (15), weighing 28±1.5 kg, born between December 2008 and January 2009 were chosen for this study. Each calf was housed separately in individual pens and offered a weighed quantity of ration consisted of wheat straw: concentrate in the ratio 1: 1 along with 2 kg of green fodder/ animal/day. Feed intake (FI) was recorded every day by weighing back the refusal. Samples of wheat straw: concentrate, green fodder and refusal were analyzed for dry matter every fortnight to estimate the DM intake. Jugular blood samples were collected in the morning, before

Table 1. Composition of concentrate mixture

Ingredient	Percentage (%)
Maize	33
Groundnut cake	21
Mustard cake	12
Wheat bran	20
Deoiled rice	11
Mineral mixture	2
Common salt	1

providing feed. The blood samples were centrifuged. The plasma was separated out by centrifugation at 2000 rpm for 15 min from the blood samples and stored at –20°C for analysis of IGF-I and lactoferrin by EIA kits.

After 4 months of post weaning age, the calves were divided based on their body weights into high (HBW, 110–130 kg) and low body weight (LBW, 68–98 kg) groups. The calves of LBW group in addition to normal feed were supplemented with probiotic (yeast culture) @ 300g/quintal of concentrate mixture. The composition of the concentrate mixture is given in Table 1. The duration for pre- and post-supplementation period of probiotic was 4 months (120 days) respectively. The body weight, feed intake, plasma IGF-I and lactoferrin were monitored for 8 months post-weaning. Experiments were performed from August 2009 to March 2010.

The graphs were drawn by using statistical software 'PRISM' version 3.02, Graph Pad Software, Inc., 1999. The data were analyzed using 2–way ANOVA method with bodyweight and days as factors, using software package SYSTAT VERSION 6.0.1, (1996), SPSS INC. and the following equation model was used in this analysis. For correlation studies, pearson correlation coefficient method was used.

RESULTS AND DISCUSSION

At the time of weaning, the body weight of the animals indicated ADG @ 400g/d for HBW and 300g/d for LBW group calves, but during 120 days post weaning the ADG was merely 0.030+0.01 for HBW vs. –0.02+0.01 g/day for LBW group calves reflecting static growth during 120 d post weaning (Fig 1a). The retarded growth may be due to stressful effects of and post-effect weaning, seasonal and post-effect of hot humid (July-September) climate.

The change in body weight and feed parameters observed within the group, from pre- to post-supplementation period was significant (P<0.001) for both the groups, respectively (Fig 1a, 1b). Difference observed from pre to post was more significant for LBW than for HBW group calves. But there was no significant difference for these parameters between the groups. This may be due to supplementation of probiotic to LBW group and the favourable environment (December-March) available for growth of animals. However, MBW

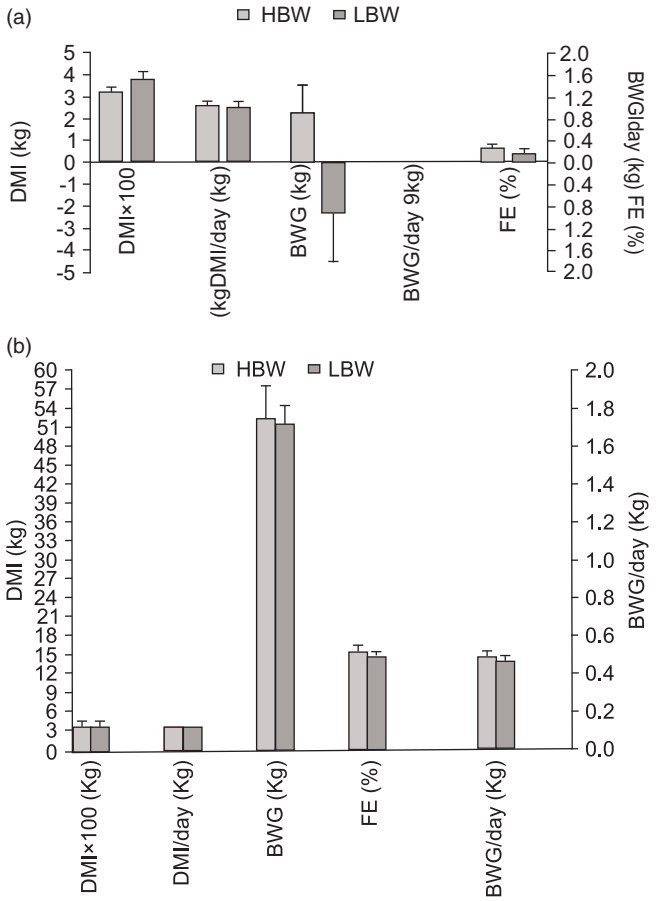

Fig 1a–b. **1a.** Comparison between HBW and LBW group during pre-supplementation period (122 days). **1b.** Comparison between HBW and LBW group during post-supplementation period (110 days).

BWG, body weight gain; DMI, dry matter intake; FE, feed conversion efficiency (in per cent).

and average body weight at pre- (120 days) and post-supplementation (110 days) was significantly different (P<0.01) between the 2 groups (Fig 2a, 2b). It was observed that by 90d post weaning, plasma IGF-I level was not significantly different between the groups reflecting static growth of animals during this period. In HBW group, the plasma IGF-I increased significantly from 90d of post-weaning till the 240d period of study (P<0.01), without supplementation of probiotic, whereas in LBW group the significant increase in IGF-I level (P<0.01) was observed only at 30d post supplementation. Prior to supplementation, there was no significant increase in plasma IGF-I concentration when compared with respect to post supplementation period (Fig. 4). The significant increase in body weight and plasma IGF-I concentration within LBW group was observed within the same time period of post weaning (Fig. 4). It is known that plasma IGF-I level is a potential physiological indicator, since it is not secreted in a pulsatile manner and associated with gain in body weight and growth rate (Davis *et al.* 1996). Plasma insulin-like

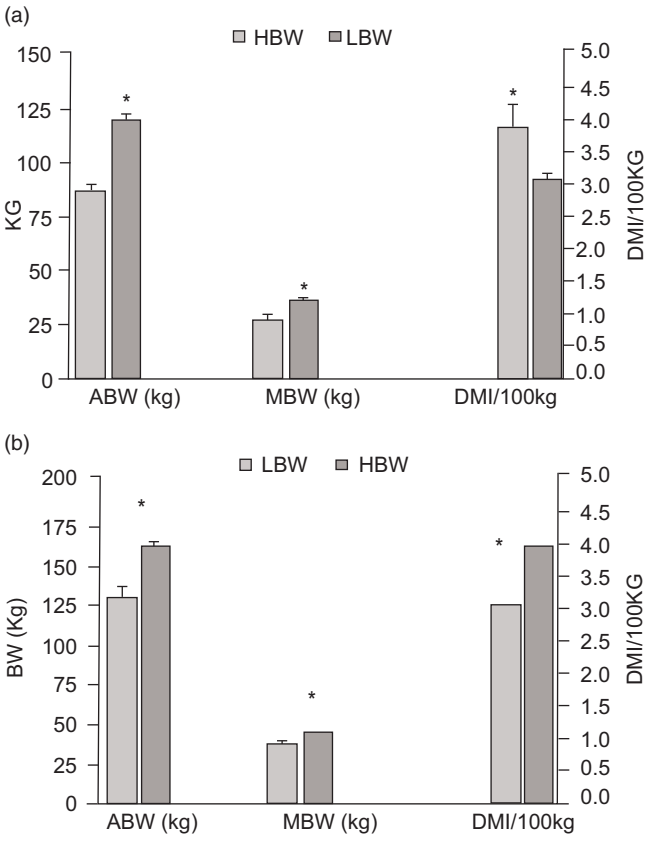

Fig 2a–b. **2a.** Comparison between HBW and LBW group during pre-supplementation period (122 days). **2b.** Comparison between HBW and LBW group during post-supplementation period (110 days).

ABW, Average body weight; MBW,metabolic body weight; DMI, dry matter intake; BW, body weight. *, P<0.01.

growth factor-1 (IGF-I) has been implicated in pre- and post-natal growth, lactation, reproduction, and immune function, and is produced primarily in the liver, although many other tissues produce smaller quantities (Lund *et al.* 1986).

With increase in age of animals, the plasma level of IGF-I also increased (Morel *et al.* 1991). The plasma IGF-I concentration proved to be a physiological indicator trait that can be used for a selection program for post-weaned calves. The same was reported by Johnston *et al*. (2001) and Davis and Simmen (2010). Supplementation of probiotic may result in better growth performance of female calves. In the present study, since the supplementation of probiotic was initiated for LBW group at 120d post weaning, the growth performance in terms of ABW and MBW would not have resulted in improving the growth performance in par with respect to HBW group calves.

ADG >700g/d was observed in 5/6 calves at 60d post supplementation in LBW group, whereas 5/9 animals in HBW group could gain ADG at the same rate. In the subsequent days at 120d post weaning, 4/6 animals (with supplementation in LBW) and 3/9 animals in HBW group

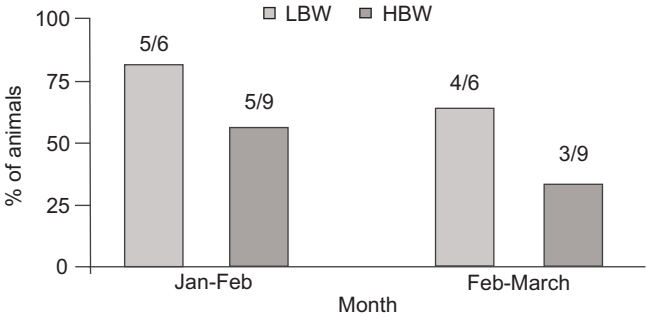

Fig 3. Per cent of animals gaining >700g/day for HBW and LBW group.

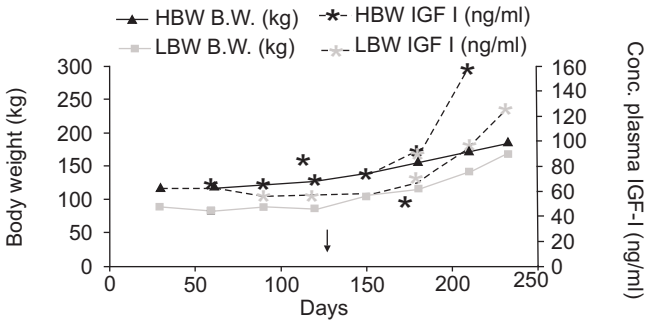

Fig 4. Relationship between body weight (kg) and plasma IGF-I concentration in HBW and LBW group. –, supplementation of probiotic. *,P<0.01 (within a group).

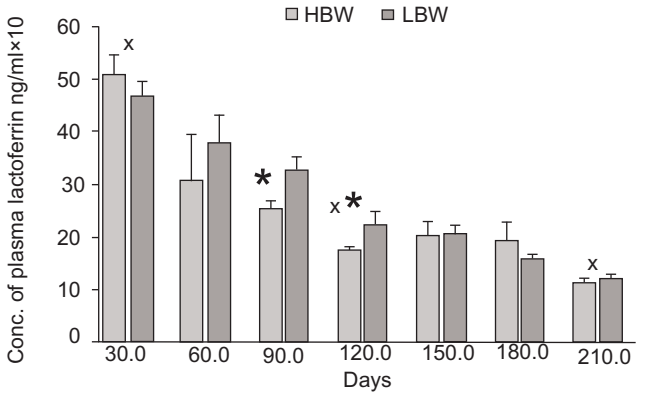

Fig 5. Plasma-lactoferin concentration in HBW and LBW group. *, P<0.05 (between groups); x, P<0.001(within a group).

(without supplementation) gained >700g BW. Comparatively more animals of LBW group exhibited greater ADG in the post-supplementation period (Fig 3). It is known that yeast fermentation product can improve growth performance and benefit-stressed calves (Windschill *et al.* 1991). The yeast culture provided as a supplement in the present study might have improved the growth performance of LBW group calves. When correlation studies were performed between body weight and plasma IGF-I concentration, a strong positive correlation was observed between the 2 parameters for HBW (r^2 = 0.84497) and LBW (r^2 = 0.69042) groups (Table 2).

Table 2. Correlation studies between parameters (Group LBW/HBW).

Group LBW				Group HBW			
	Body weight	IGF-I	Lactoferrin		Body weight	IGF-I	Lactoferrin
Body weight	1.0000			Body weight	1.0000		
IGF-I	0.69042	1.0000		IGF-I	0.84497	1.0000	
Lactoferrin	–0.48432	–0.32330	1.0000	Lactoferrin	–0.62918	–0.59714	1.0000

The plasma level of lactoferrin ranged between 500–250ng/ml and 470–321 ng/ml in LBW vs HBW group respectively during post-weaning period. The plasma level of lactoferrin decreased significantly at 90d post weaning period in comparison to HBW group, indicating lower activity of neutrophils in LBW group to the prevailing stressful conditions. It is known that activity of neutrophils is impaired due to post-weaning stress (Lynch *et al.* 2010). The significant increase in plasma concentration of lactoferrin >200 ng/ml at 120d post-weaning period indicated lower health status/ stressful/ inflammatory conditions in both the groups at 60–90d post-weaning period (Fig. 5). The pearson correlation studies indicated negative relationship between body weight and plasma concentration of lactoferrin (Table 2). The circulatory concentration of lactoferrin was at physiological level during the post-supplementation period and the decrease in concentration of plasma lactoferrin in LBW group during pre-supplementation became similar to that of physiological concentration (<200 ng/ml) in the post-supplementation period. The changes in plasma lactoferrin level indicated that initially at early post weaning period stressful or inflammatory conditions might have prevailed for the calves in both the groups. The lesser concentration of lactoferrin in LBW group calves indicated poor health status of calves which normalized to the plasma level of HBW group on supplementation of probiotic (Fig. 5).

Post-weaning period and seasonal changes during July-October months are stressful to calf. Probiotic supplementation to calves exhibiting low body weight improved growth performance and plasma IGF-I concentration, even though the mentioned plasma parameters in LBW group could not attain the same level as observed in normal growing animals. Thus, supplementation of probiotic to calves exhibiting decreased growth performance at an earlier stage may improve growth performance, which will be further useful for production traits.

IGF-I acts as a physiological marker for body weight gain and also health status which can be used for screening the cross-bred female calves and probiotic supplement may be provided to improve the growth performance and health status.

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