



Effect of transition diet fortification on lameness and blood metabolites in pre- and post- partum lame Karan Fries cows

PRAGYA BHADARIA¹, S S LATHWAL², Y S JADOUN³, A P RUHIL⁴, INDU DEVI⁵ and ROHIT GUPTA⁶

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Peri-parturient period in high yielding cows always associated with a series of nutritional, biochemical, hormonal changes that leads to lameness in later lactation. Extra supplementation of vitamins and minerals responsible for hoof health is recommended in some dairy herds during this period. The present study was conducted by fortifying 10, 20 and 30 mg/d of biotin and 2 g/d of zinc sulphate during pre- and post-partum period (2 months before and 2 months after calving) of 40 high yielding lame crossbred KF cows, distributed into control and three treatment groups. The study revealed that, fortification of transition diet with biotin and Zn significantly reduced the lameness score and recovery time in supplemental groups compare to control group. In T-1, the lameness score was reduced by 1.3, T-2 reduction was maximum by 2, T-3 by 1.5, and in control 0.02 with their respective recovery period of hoof lesions were 38.9±1.1 (T-1) 19.12±1.8 (T-2), 25.3±1.4 (T-3) and 56.2±2.7 days. The overall glucose concentration was observed significantly higher in T-2 group as compared to control group, while overall NEFA and BHBA concentrations were significantly higher in control group as compared to other supplemental groups after 120 days of supplementation. Thus, it can be concluded from the study that, lameness in high yielding cows can be reduced by fortification of transition diet with biotin and zinc.

Key words: Blood metabolite, Fortification, Lameness, Transition diet, Zinc

The modern husbandry and management practices have increased the milk production in dairy cows but at the cost of higher incidence of health problems. Lameness has not been considered as transition cow disease, but now it has emerged as one of the most severe problem and welfare concern at organized farms (Enting *et al.* 1997, Zahid *et al.* 2014). Factors have been recognized as contributing to lameness, like physiological, nutritional, managerial, environmental, genetic etc. (Livesey and Fleming 1984, Manson and Leaver 1988). Nutritional management is a key component in the development of lameness in high yielding cows (Randhawa *et al.* 2012), either due to non availability of balanced diet or imbalance of specific nutrients in the diet during this phase. More recently, emphasis is being placed on the metabolic disturbances and mechanical changes of the hoof/claw which occur during the peri-parturient period. Blood measures are frequently used in assessment of disease risk, as they are significantly correlated to nutritional status of the animals (Claypool *et*

al. 1975, Mills *et al.* 1976). Trace minerals that have been identified as important role in normal hoof health include: calcium, zinc, copper, cobalt and manganese (Kilic *et al.* 2007, Karkoodi *et al.* 2012). In vitamins, biotin is essential for the formation and integrity of the keratinized tissues like hoof (Campbell *et al.* 2000, Hedges *et al.* 2001, Randhawa *et al.* 2008b, Al-Qudah and Ismail 2010, Bhaduria *et al.* 2013). Keeping these problems in view, the research was undertaken to assess the effect of zinc-biotin fortification on lameness score and their resultant effects on blood metabolites profile and dry matter intake of high yielding Karan Fries (KF) cows during pre- and post- partum period.

MATERIALS AND METHODS

The present study was conducted on peri-parturient period (2 months before and 2 months after calving) of high yielding lame crossbred Karan Fries (KF) cows maintained at Livestock Research Centre (LRC), NDRI, Karnal. Skilled workers at the veterinary dispensary and animal health section, NDRI, diagnosed the animals for hoof disorders. Only those animals, having hoof disorders and showing lameness, based on locomotion scoring system (Sprecher *et al.* 1997), were selected for the present study. Lame, high yielding KF cows (40) were distributed equally into 3 treatments and a control group. The grouping of the animals

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Present address: ¹Scientist (pragyacari@gmail.com), Central Avian Research Institute, Izatnagar. ²Senior Scientist (lathwal314@gmail.com), ^{5,6}Research Scholar (indulathwal@gmail.com, rohitgpt2009@gmail.com). ³Assistant Professor (yogivet2248@gmail.com), GADVASU, Ludhiana. ⁴Principal Scientist (apruhil@gmail.com), ASRB, New Delhi.

was based on “quasirandom distribution” of cows. As a result, the animals were evenly distributed between the groups with regard to parity, stage of lactation, average milk yield per day based on weekly milk yield in the start of study. Supplemented cows were identified with paint marking on their backs and brand number given to them at the time of birth. Following diets were offered to the experimental animals up to 60 days before their predicted calving date and 60 days post-partum. Control diet (NRC 2001) along with 2 g of ZnSO_4 and 10 mg of biotin was offered to treatment group-1 (T-1), control diet with 2 g of ZnSO_4 and 20 mg of biotin was offered to treatment group-2 (T-2) and control diet with 2 g of ZnSO_4 and 30 mg of biotin was offered to treatment group-3 (T-3) once a day at the time of morning in the form of ‘Laddoos’ of jaggery. Locomotion scoring were carried out on a 5-point scale (0 to 5: normal to severely lame) described by locomotion scoring guide (Sprecher *et al.* 1997) at 60 days pre-partum and 60 days post-partum. Blood samples were collected through jugular venipuncture using blood collection tubes with heparin as anticoagulant early in the morning (7.00 to 8.00 AM) before feeding. Samples were collected 60d, 15d pre-partum, on the day of calving 15d and 60d post-partum. Immediately after collection, the blood samples were centrifuged at 3,000 rpm for 15 to 20 min, plasma were separated and stored in cryo vials of 2 ml capacity at -20°C until analysis. The blood biochemical parameters viz., glucose (by end point O-Toluidine method), copper soap extraction method modified by Shipe *et al.* (1980) was adopted for the determination of plasma non-esterified fatty acids (NEFA) and the β -hydroxyl butyrate (BHBA) was estimated using f-HB assay kit in the experimental animals. The data obtained were analysed by using sigma plot version 11.0 by using appropriate statistical methods.

RESULTS AND DISCUSSION

Effect of biotin and zinc fortification on lameness score during peri-parturient period in KF cows: The results of mean and SE values for lameness scores of control and biotin supplemented KF cows are presented in Table 1. The results showed that biotin and zinc fortification significantly reduced ($P<0.05$) the lameness score in supplemental groups, while the lameness score after 120 days of experiment was significantly ($P<0.01$) high in control group. In treatment group 1 (10 mg/d) the lameness score was reduced by 1.3 (41.94%), in treatment group 3 (30 mg/d) by 1.5 (45.45%) and in treatment group 2 (20 mg/d) reduction was maximum i.e. 2 (51.28%) and minimum in control group by 0.02 (6%). The findings (Table 1) also revealed that the recovery period of hoof lesions under biotin supplemented (T-1, T-2, and T-3) and control groups averaged 38.9 ± 1.1 19.12 ± 1.8 25.3 ± 1.4 and 56.2 ± 2.7 days respectively. This suggested that recovery period is significantly slow in control group and early recovery was observed in cows supplemented with 20 mg/d biotin followed by 30 mg/d and 10 mg/d and 2 gm ZnSO_4 . As there was no further benefit on lameness score and recovery

period with 30 mg/d biotin supplementation over 20 mg/d, so it may be assumed as optimum dose for supplementation of dietary biotin during peri-parturient period.

These findings are in agreement with the findings of Hagemeister and Steinberg (1996), Hochsteer (1998), Midla *et al.* (1998), Bergsten (1995), Fitzgerald *et al.* (2000), Campbell *et al.* (2000), Hedges *et al.* (2001) and Lean and Rabiee (2011), who found that biotin supplementation @ 20 mg/d increased hoof health and early recovery in lame cows. Inclusion of 20 mg/day of biotin in the ration of dairy cattle was found beneficial to hoof health; however, frequently supplementation has to be long term before any effects are seen (Green *et al.* 2000, Midla *et al.* 1998). However, Yang *et al.* (2009) found that dietary biotin supplementation could improve the performance and milk quality of Holstein cows at optimal level of 30 mg/d. On the contrary, Geyer and Schulze (1994) found no difference in hoof health in equines following biotin supplementation. There is evidence that biotin supplementation improves both the cellular and intercellular structure of horn (Sarasin 1994, Budras *et al.* 1996, Hochstetter 1998, Fitzgerald *et al.* 2000, Hedges *et al.* 2001) and biotin-mediated resistance of the epidermis, both of the soft skin and the keratinized epidermis of claw horn (Bergsten *et al.* 2003). Koster *et al.* (2002) reported that ultrastructure and composition of intercellular cementing substance is sensitive to biotin status. Improvements of horn strength and quality caused by biotin supplementation may be, in part, due to its effects on horn lipid metabolism.

Similarly, zinc supplementation resulted in a markedly lower incidence of hoof lesions and lameness than non-zinc supplemented group. The complete resolution of white line haemorrhages and reduction in number of white line fissures in a study by Randhawa *et al.* (2012) suggested that the zinc supplementation influenced tissue strength and possibly healing rate. Also, supplementing zinc tended to reduce the incidence and severity of claw disorders. Zinc is also found implicated as an important trace element in keratin synthesis. Moore *et al.* (1989) and Stern *et al.* (1998) reported that organic forms of zinc provided greater improvements in macroscopic horn quality and strength compared to control animals or those supplemented with inorganic zinc oxide. The animals receiving zinc tended towards better clinical scores for microscopic horn quality and traction resistance. The prevalence of lameness was higher in control group than ZnSO_4 supplemented treatment (Nobijaria *et al.* 2012). On the contrary, there was no effect of zinc supplementation on locomotion score in Jersey cows during the 2 week before and after parturition. Hoof hardness improved over the duration of the study but there were no differences between treatments (Nobijaria *et al.* 2012). Thus, it can be concluded that supplementation of biotin and Zn improves the quality of claw horn, which encourages the replacement of defective horn, improves healing, and makes it less likely for sole lesions to develop a lower locomotion score during peri-parturient period in KF cows.

Table 1. Effect of biotin and zinc fortification on lameness score and recovery period during peri-parturient period in lame KF cows

Lameness score	Control (n=10)	T-1 (n=10)	T-2 (n=10)	T-3 (n=10)
Before	3.2±0.326 ^a	3.1±0.233 ^a	3.9±0.233 ^a	3.3±0.3 ^a
After	3.0±0.421 ^a	1.8±0.249 ^b	1.9±0.233 ^b	1.8±0.2 ^b
Reduction rate	0.06	0.41	0.51	0.45
Recovery period (days)	56.2±2.7 ^a	38.9±1.1 ^b	19.12±1.8 ^b	25.3±1.4 ^b

Plasma glucose concentration: The effect of biotin and zinc fortification on plasma glucose concentration during peri-parturient period is presented in the Table 2. The overall glucose values were 47.84±1.31, 49.17±1.25, 50.66±1.02 and 49.25±0.99 mg/dl in control, T-1, T-2 and T-3, respectively. The overall glucose concentration was observed significantly ($P<0.05$) higher in T-2 group as compared to control group. The results showed that the effect of fortification on plasma glucose concentration was observed to be nonsignificant on different groups. There was significant variations in the blood glucose level ($P<0.001$) showing sharp increase from 15d pre-partum till the day of calving where it was maximum followed by sudden decrease on 15d post-partum, the level however came to slight above on 60d post-partum.

The pre-partum plasma glucose level increase in response to the increased demand for initiations of milk synthesis. Bertics *et al.* (1992) reported that glucose supply calculated from digestible energy intake exceeded estimated demand before parturition. It may be associated with increased glycogen synthesis in liver and muscle. After calving, demand for glucose synthesis increases significantly and cows adapt to this requirement by gluconeogenesis from propionates, amino acids, lactate and glycerol. Aiello *et al.* (1984) and Drackley *et al.* (2001) found that rates of gluconeogenesis from propionate in liver slices were three-fold greater at 30 days of lactation as compared to 90 and 180 days of lactation. The transient fall in glucose levels in the first weeks of lactation could be the result of high demands for lactose synthesis and of insufficient gluconeogenesis (Baird 1982, Doepel *et al.* 2002), mainly because energy losses could not be fully compensated by energy intakes. The concentration of glucose is regulated by various hormones such as insulin, cortisol, glucagon, somatotropin and adrenaline and consequently blood glucose levels depends on the nutritive values of the diets, on social or environmental stress conditions as well on physiological phases (Borghese 2005).

Plasma NEFA concentration: The effect of biotin and zinc fortification on plasma NEFA concentration during peri-parturient period is presented in Table 3. The overall NEFA concentration was significantly ($P<0.05$) higher in control group as compared to other supplemental groups, while T-1 and T-3 group holds the intermediate position.

Table 2. Effect of biotin and zinc fortification on plasma glucose concentration (mg/dl) during peri-parturient period in lame KF cows

Periods/days relative to calving	Groups			
	Control (n=10)	Treatment 1 (n=10)	Treatment 2 (n=10)	Treatment 3 (n=10)
-60	36.76 ±1.06 ^{aA}	39.46 ±0.94 ^{abA}	42.25 ±1.21 ^{bA}	41.74 ±1.54 ^{abA}
-15	47.93 ±1.97 ^{aBC}	48.21 ±1.18 ^{aBC}	51.27 ±0.92 ^{aB}	48.17 ±1.127 ^{aB}
0	58.96 ±1.40 ^{aD}	59.59 ±2.59 ^{aD}	60.94 ±0.98 ^{aC}	58.69 ±1.11 ^{aD}
15	42.41 ±1.68 ^{aAB}	45.27 ±2.45 ^{aAB}	45.72 ±0.97 ^{aA}	44.58 ±0.82 ^{aAB}
60	53.17 ±1.72 ^{aCD}	53.34 ±1.04 ^{aCD}	53.14 ±1.030 ^{aB}	53.05 ±1.00 ^{aC}
Overall	47.84 ±1.30 ^a	49.17 ±1.24 ^{ab}	50.66 ±1.02 ^b	49.25 ±0.99 ^{ab}

a, b-significance difference within row and A, B, C, D-within column.

The results showed that the effect of supplementation on NEFA was nonsignificant on -60 pre-partum, -15 days pre-partum while in 15 and 60 days post partum the control group differ significantly to T-2 group. The NEFA concentration increased significantly ($P<0.01$) from 15d pre-partum, reached a peak level till day of calving up to 15d post-partum. After this, NEFA concentration decreased significantly ($P<0.01$) up to 60d post-partum.

Variation and range of plasma NEFA was in agreement as reported by Grum *et al.* (1996), Drackley, (2001), Kanna, (2007), Chandra, (2009) and Aswal, (2009) for transition dairy cows. However, Grummer (1993) reported that plasma NEFA concentration increased approximately 2 fold between day 17 and day 2 pre-partum and increased 2 fold again to peak by calving. Concentration of NEFA typically increased after parturition and peaked at week's post-partum. Near parturition feed intake is reduced and after parturition the demand for energy is progressively increased by the initiation of lactation. The negative energy balance attributable to the reduced feed intake and the initiation of lactation is compensated by the mobilization of NEFA from adipose tissues as reported by Bell (1980) and Baird (1982). An increase in concentration of plasma NEFA results from the mobilization of lipids, which increases gradually in the pre-partum transitions period but rapidly in the lasts 3 days of gestation. A portion of this increase in NEFA is under hormonal control, while another portion is the result of an energy deficit (Bertics *et al.* 1992, Grummer 1993 and Dyk *et al.* 1995). Increased lipolysis triggered by hormonal changes, catecholamine release increases activity of sympathetic nervous around calving results in large increase in NEFA concentration at parturition (Grum *et al.* 1996). Thus optimum nutritional management during transition period can reduces the blood NEFA concentration by decreasing the adipose tissue TG (triglycerides)

Table 3. Effect of biotin and zinc fortification on plasma NEFA concentration ($\mu\text{mol/l}$) during peri-parturient period in lame KF cows

Periods/days relative to calving	Groups			
	Control (n=10)	Treatment 1 (n=10)	Treatment 2 (n=10)	Treatment 3 (n=10)
-60	107.01 $\pm 9.32^{\text{aA}}$	105.84 $\pm 6.83^{\text{aA}}$	128.30 $\pm 32.29^{\text{aA}}$	105.44 $\pm 10.67^{\text{aA}}$
-15	144.82 $\pm 12.98^{\text{aAC}}$	129.73 $\pm 8.68^{\text{aB}}$	112.92 $\pm 12.48^{\text{aB}}$	141.76 $\pm 14.10^{\text{aB}}$
0	288.43 $\pm 16.48^{\text{aB}}$	279.15 $\pm 12.58^{\text{abC}}$	216.20 $\pm 18.20^{\text{bC}}$	276.48 $\pm 13.27^{\text{abC}}$
15	304.61 $\pm 19.72^{\text{aB}}$	268.23 $\pm 21.88^{\text{abC}}$	232.40 $\pm 19.83^{\text{bC}}$	250.62 $\pm 36.56^{\text{abC}}$
60	193.21 $\pm 9.25^{\text{aC}}$	144.27 $\pm 14.79^{\text{abB}}$	110.59 $\pm 13.94^{\text{bB}}$	124.81 $\pm 22.32^{\text{bB}}$
Overall	207.61 $\pm 12.65^{\text{a}}$	185.44 $\pm 12.05^{\text{ab}}$	160.08 $\pm 11.62^{\text{b}}$	179.82 $\pm 13.59^{\text{ab}}$

a, b-significance difference within row and A, B, C, D-within column.

mobilization.

Plasma BHBA concentration: The effect of biotin and zinc fortification on plasma BHBA concentration during peri-parturient period is presented in the Table 4. The overall BHBA concentration was significantly ($P < 0.05$) highest in control group whereas lowest in T-2 group. The results showed that the effect was significant on day of calving and day 15th post-partum. In T-2 group, the cows had significantly lower level of BHBA concentration compared to control, T-1 and T-3 on day of calving and 15th day post-partum. In addition, nonsignificantly lower BHBA concentration was observed in T-3 group compared to T-1.

The BHBA values followed the same pattern that plasma NEFA followed up to 60d of calving. Concentration of

Table 4. Effect of biotin and zinc fortification on plasma BHBA concentration ($\mu\text{mol/l}$) during peri-parturient period in lame KF cows

Periods/days relative to calving	Groups			
	Control (n=10)	Treatment 1 (n=10)	Treatment 2 (n=10)	Treatment 3 (n=10)
-60	657.18 $\pm 17.29^{\text{aA}}$	645.69 $\pm 16.58^{\text{aA}}$	662.55 $\pm 18.88^{\text{aA}}$	640.71 $\pm 18.82^{\text{aA}}$
-15	734.50 $\pm 20.76^{\text{aB}}$	747.23 $\pm 17.04^{\text{aB}}$	694.84 $\pm 14.19^{\text{aB}}$	694.01 $\pm 23.66^{\text{aA}}$
0	842.03 $\pm 22.81^{\text{aC}}$	838.97 $\pm 17.32^{\text{aC}}$	762.93 $\pm 22.22^{\text{bBC}}$	801.32 $\pm 20.61^{\text{abB}}$
15	913.65 $\pm 16.86^{\text{aC}}$	917.75 $\pm 15.06^{\text{aD}}$	827.69 $\pm 18.29^{\text{bC}}$	850.77 $\pm 19.38^{\text{abB}}$
60	748.98 $\pm 12.73^{\text{aB}}$	727.22 $\pm 27.09^{\text{aB}}$	718.70 $\pm 24.95^{\text{aB}}$	681.64 $\pm 19.43^{\text{aA}}$
Overall	779.27 $\pm 14.99^{\text{a}}$	775.37 $\pm 15.73^{\text{a}}$	733.35 $\pm 11.87^{\text{b}}$	733.69 $\pm 14.29^{\text{b}}$

a, b-significance difference within row and A, B, C, D-within column.

plasma BHBA increased after parturition peaked on 15d post-partum and decreased after that and reached at lower concentrations. The higher plasma biotin concentration revealed incomplete oxidation of NEFA in the tri-carboxylic acid cycle during NEB (Grummer 1993, Doepel *et al.* 2002). Like NEFA, the increase in plasma BHBA during early post-partum decreases the immune function of dairy cows and increases the susceptibility to lameness. Thus BHBA can be used as a diagnostic tool for metabolic disturbance in early lactation. These concentrations were lower than what was reported by Grum *et al.* (1996). The BHBA plasma values increased slightly for the remaining 6 week of lactation in both biotin-supplemented and control cows, suggesting that alimentary ketogenesis promoted with increased intake.

Effect of biotin and zinc fortification on dry matter intake in KF cows during peri-parturient period: The fortnightly dry matter intake (DMI) in control and treatment groups is presented in Table 5. No specific trend of increase in DMI was observed in different treatment groups. During fourth period i.e. just before calving, dry matter intake was minimum due to foetus growth and other hormonal changes associated with parturition.

Present findings are in agreement with Margerison *et al.* (2002), Reynolds *et al.* (2003) and Ganjkanlou *et al.* (2007). KF cows fed supplemental biotin consumed similar amounts of DM daily as control cows when expressed as kilograms per day and as a percentage of BW during both the pre-partum and post-partum periods (Singh *et al.* 2009). On the contrary to above findings Zimmerly and Weiss (2001) reported that DMI was 0.7 kg/d higher for cows

Table 5. Effect of biotin and zinc fortification on DMI during peri-parturient period in lame KF cows

Periods/days relative to calving	Groups			
	Control (n=10)	Treatment 1 (n=10)	Treatment 2 (n=10)	Treatment 3 (n=10)
1	12.29 $\pm 0.27^{\text{aBC}}$	12.26 $\pm 0.22^{\text{aCDE}}$	13.29 $\pm 0.63^{\text{abBC}}$	13.45 $\pm 0.25^{\text{bD}}$
2	11.45 $\pm 0.21^{\text{aB}}$	11.38 $\pm 0.18^{\text{aAB}}$	11.68 $\pm 0.44^{\text{aAB}}$	11.72 $\pm 0.24^{\text{aB}}$
3	11.61 $\pm 0.19^{\text{aB}}$	11.46 $\pm 0.17^{\text{aABC}}$	11.63 $\pm 0.39^{\text{aAB}}$	11.57 $\pm 0.18^{\text{aB}}$
4	09.85 $\pm 0.35^{\text{aA}}$	10.65 $\pm 0.16^{\text{aA}}$	10.28 $\pm 0.34^{\text{aA}}$	09.73 $\pm 0.20^{\text{aA}}$
5	11.46 $\pm 0.34^{\text{abB}}$	11.32 $\pm 0.24^{\text{aAB}}$	12.45 $\pm 0.50^{\text{bBC}}$	11.17 $\pm 0.24^{\text{aB}}$
6	12.11 $\pm 0.27^{\text{aBC}}$	12.05 $\pm 0.17^{\text{aBCD}}$	13.67 $\pm 0.66^{\text{bBC}}$	12.52 $\pm 0.26^{\text{aBC}}$
7	12.78 $\pm 0.18^{\text{aC}}$	12.53 $\pm 0.15^{\text{aDE}}$	13.35 $\pm 0.39^{\text{aBC}}$	12.74 $\pm 0.09^{\text{aCD}}$
8	13.08 $\pm 0.19^{\text{aC}}$	12.88 $\pm 0.15^{\text{aE}}$	13.92 $\pm 0.44^{\text{aC}}$	13.07 $\pm 0.12^{\text{aD}}$
AVR	11.83 $\pm 0.25^{\text{a}}$	11.82 $\pm 0.18^{\text{a}}$	12.53 $\pm 0.48^{\text{b}}$	11.99 $\pm 0.19^{\text{a}}$

a, b-significance difference within row and A, B, C, D-within column.

between 46 and 160 DIM that were supplemented with 20 mg/d of dietary biotin when compared with the DMI of control cows. Midla *et al.* (1998), Bergsten *et al.* (2003) and Majee *et al.* (2003) reported an increased DMI due to biotin supplementation possibly due to improved hoof health, which leads to increase feeding time than resting. The increase in DMI by 1.7 kg/d higher for cows supplemented with 20 mg/d dietary biotin compared to control cows. But, there was no further benefit on DMI with 40 mg/d biotin supplementation over 20 mg/d (Majee *et al.* 2003).

It was concluded that biotin and zinc fortification during the last 60d pre-partum and 60d post-partum significantly reduced the lameness score and favors early recovery in KF cows. Fortification also elevates the concentrations of glucose in plasma and lowered NEFA and BHBA in lame KF cows. Based on the finding of the study, we can recommend a specific transition diet fortified with biotin (20mg/d) and ZnSO₄ (2g/d) for high yielding KF cows as a preventive measure for lameness.

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