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## Research Article

# Comparative Changes in Levels of Circulating Metabolic Biomarkers and Leptin Hormone in High and Low Yielding Breeds of Indian Dairy Cows During Pregnancy and Lactation

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## Abstract

**Background and Objectives:** Changes in body condition and fat depots crucially affects the postpartum negative energy balance and succeeding milk production in dairy cows. Thus, this study was planned to elucidate the comparative changes in circulating levels of biochemical profile and leptin hormone during different physiological stages in high and low yielding breeds of Indian dairy cattle. **Materials and Methods:** Total 6 pregnant Sahiwal and Hariana cows of same age group (approximately 3-5 years) with similar body condition and in their first parity were selected. Blood samples were collected 1 month antepartum (dry) and 1st (early lactation), 2nd (early mid lactation) and 3rd month (late mid lactation) postpartum twice in a month (fortnightly). Plasma was harvested and analyzed for levels of leptin, glucose, urea, triglyceride (TG), cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and non-esterified Fatty Acid (NEFA). **Results:** The significant lower levels of leptin, TG, cholesterol, HDL, LDL and NEFA were observed in high yielding Sahiwal cows compared to low yielding Hariana cows while period of lactation showed significant effect on all the parameters except leptin and NEFA in both the breeds. Interaction of breed and period revealed substantial effect on levels of TG, cholesterol, HDL and LDL. **Conclusion:** The study suggests their possible role in differential production potential of these high and low yielding animals. Moreover, further studies are warranted to explore the real cause of differential production potential of animals.

**Key words:** Adipokine, metabolic biomarkers, pregnancy, lactation, high yielding, low yielding, dairy cows

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Sahiwal is one of the best Indian dairy cattle breed with average milk yield of 2325 kg per lactation while Hariana is a prominent dual purpose breed with average milk yield of 997 kg per lactation<sup>1</sup>. The milk production of animals primarily depends on energy metabolism which is principally affected by feeding behavior, nutrition, physiological condition and genetic makeup of the animals. The leptin hormones is primarily synthesized in White Adipose Tissue (WAT) and serves to regulate feeding behavior, lipid metabolism and energy homeostasis by centrally acting in hypothalamus<sup>2</sup>, thus, said to affect the milk production in animals. Furthermore, during transition from pregnancy to lactation, dairy cows generally encounter Negative Energy Balance (NEB) due to enormous rise in energy and protein demands for growth of foetus and initiation of lactation which is synchronized with fall in Dry Matter Intake (DMI). Consequently the increased energy demands are met from body reserves and fats are mobilized from WAT concomitant decline in leptin levels<sup>3</sup>. Since, NEB and consequent fat mobilization during periparturient period significantly affects milk yield in animals. Furthermore, regular assessment of the circulating metabolic biomarkers in dairy cows and comparing them to normal reference values may facilitate in discovering the metabolic abnormalities in the herd and assist in better management of the metabolic problems at the herd level. Thus, this study was conducted to elucidate the comparative changes in circulating levels of leptin and metabolic biomarkers during different physiological stages in high and low yielding breeds of Indian dairy cattle.

## MATERIALS AND METHODS

**Study area:** In the present study dairy cows were selected from Institutional Livestock Farm Complex, College of Veterinary Science, Mathura, India. Geographically, this area is situated in semi-arid zone of northern India (27° in latitude and 78° longitudes) and 160 m above sea level. The experiment was conducted during August-December, 2015.

**Animals and blood collection:** Total 6 pregnant Sahiwal and Hariana cows each of same age group (approximately 3-5 years) in their first parity with similar Body Condition Score (BCS) were selected for the study and maintained in similar management and nutritional conditions during the experimental period. Blood samples were collected in

heparinized vials 1 month antepartum (dry) and 1st (Early Lactation, EL), 2nd (Early Mid Lactation, EML) and 3rd month (Late Mid Lactation, LML) postpartum twice in a month (fortnightly) and plasma were harvested by centrifugation just after the collection.

**Biochemical analysis:** Samples were subjected to estimation of levels of leptin hormone by bovine specific ELISA kit (Cusabio, GenxBio) and NEFA by modified copper soap extraction method<sup>4</sup>. The biochemical profiling was done by estimating levels of glucose, urea, triglycerides (TG), cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) by using commercial diagnostic kits (Autospan, India Ltd.).

**Statistical analysis:** Data were presented as Mean  $\pm$  SE and p-value 0.05 was considered statistically significant. The statistical differences among the groups were analyzed by two way ANOVA was calculated by using SPSS software.

## RESULTS

The comparative levels of leptin and other biochemical indices (Mean  $\pm$  SE) during periparturient period in Sahiwal and Hariana cows have been depicted in Table 1. The results of 2-way ANOVA revealed significant effect of breed on plasma levels of leptin, cholesterol, HDL, LDL and NEFA while period of lactation showed significant effect on all the parameters except leptin and NEFA. Furthermore, interaction of breed and period revealed substantial effect on levels of TG, cholesterol, HDL and LDL.

The results also revealed lower levels of leptin, TG, cholesterol, HDL, LDL and NEFA in high yielding Sahiwal cows compared to low yielding Hariana cows. However, trends observed in all these biochemical indices from pregnancy to mid lactation period were similar in both the breeds.

The plasma levels of leptin and NEFA remained un-altered from pregnancy to mid lactation in both the breeds. The levels of plasma glucose, HDL and LDL showed non-significant change from pregnancy to early lactation, thereafter, significantly changes in mid lactation. The plasma levels of urea showed significant increase during lactation compared to levels at pregnancy. From pregnancy to early lactation, the plasma levels of TG and cholesterol showed sudden decrease and thereafter levels of these indices increased significantly to early mid lactation and then remained un-altered.

Table 1: Circulating levels of leptin and biochemical markers in high and low yielding breeds of Indian dairy cows during different physiological stages

Stage	Breed	Leptin	Glucose	Urea	TG	Cholesterol	HDL	LDL	NEFA
Dry	Sahiwal	4.52 <sup>a</sup>	52.53 <sup>a</sup>	21.38 <sup>a</sup>	40.27 <sup>a</sup>	111.47 <sup>a</sup>	36.52 <sup>a</sup>	66.90 <sup>a</sup>	0.18 <sup>a</sup>
	Haryana	7.26 <sup>b</sup>	51.16 <sup>a</sup>	19.04 <sup>a</sup>	30.65 <sup>b</sup>	132.23 <sup>b</sup>	41.63 <sup>b</sup>	84.48 <sup>b</sup>	1.46 <sup>b</sup>
	Total	5.89	51.84	20.21	35.46	121.85	39.08	75.69	0.82
EL	Sahiwal	4.54 <sup>a</sup>	51.17 <sup>a</sup>	33.80 <sup>b</sup>	23.53 <sup>c</sup>	110.26 <sup>c</sup>	38.60 <sup>a</sup>	66.96 <sup>a</sup>	0.20 <sup>a</sup>
	Haryana	7.54 <sup>b</sup>	51.99 <sup>a</sup>	37.22 <sup>b</sup>	33.04 <sup>d</sup>	191.61 <sup>d</sup>	47.85 <sup>b</sup>	137.15 <sup>c</sup>	1.58 <sup>b</sup>
	Total	6.04	51.58	35.51	28.29	150.93	43.22	120.05	0.82
EML	Sahiwal	4.69 <sup>a</sup>	67.74 <sup>b</sup>	40.83 <sup>b</sup>	24.07 <sup>c</sup>	160.77 <sup>e</sup>	57.51 <sup>c</sup>	98.45 <sup>d</sup>	0.16 <sup>a</sup>
	Haryana	7.99 <sup>b</sup>	68.27 <sup>b</sup>	41.05 <sup>b</sup>	34.08 <sup>d</sup>	223.60 <sup>f</sup>	50.24 <sup>d</sup>	166.54 <sup>e</sup>	1.01 <sup>b</sup>
	Total	6.34	68.01	40.94	29.08	192.18	53.87	132.49	0.59
LML	Sahiwal	4.91 <sup>a</sup>	57.26 <sup>c</sup>	35.92 <sup>b</sup>	26.43 <sup>ac</sup>	162.46 <sup>e</sup>	52.12 <sup>c</sup>	105.05 <sup>f</sup>	0.10 <sup>a</sup>
	Haryana	7.85 <sup>b</sup>	65.13 <sup>d</sup>	37.29 <sup>b</sup>	37.07 <sup>bd</sup>	230.56 <sup>f</sup>	49.55 <sup>cd</sup>	173.59 <sup>e</sup>	1.54 <sup>b</sup>
	Total	6.38	61.20	36.60	31.75	196.51	50.84	139.32	0.82
Total	Sahiwal	4.66	57.17	32.98	28.58	136.24	46.19	84.34	0.16
	Haryana	7.66	59.14	33.65	33.71	194.50	47.32	140.44	1.40
	Total	6.61	58.16	33.32	31.14	165.37	46.75	112.39	0.78
Pooled SEM		0.34	2.49	2.27	2.25	8.53	2.16	8.15	0.17
Breed		0.00	0.05	0.68	0.00	0.00	0.03	0.00	0.00
Period		0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.33
Breed *period		0.88	0.27	0.64	0.00	0.00	0.00	0.00	0.32

Means bearing a same superscript in a column differ non-significantly ( $p \geq 0.05$ ), EL: Early lactation, EML: Early mid lactation, LML: Late mid lactation, TG: Triglyceride, HDL: High Density lipoprotein, LDL: Low Density lipoprotein, NEFA: Non-esterified fatty acid

## DISCUSSION

The trend of leptin concentration in periparturient cows observed in present study was somewhat similar with the trend reported earlier in cows<sup>5-7</sup> simulate the findings. In addition non-significant variation during lactation period was also reported by Eryavuz *et al.*<sup>8</sup>. However, significant variations in leptin level during periparturient period were also reported in cows and sheep<sup>9-11</sup>. The elevated levels of leptin during pregnancy may be due to high energy intake during pre-partum period which is an essential requirement for growth of foetus and for initiation of lactation. This rise in plasma leptin levels may facilitate the increased intake of food.

The plasma TG levels from pre-partum level to early lactation declined in Sahiwal cows while rose in Haryana cows which remained constant during lactation period. Similar gradual decrease from pregnancy to nadir levels at calving was also reported in Sahiwal, crossbred and Estonian Holstein periparturient cows, respectively<sup>9,12,13</sup>. Moreover, non-significant changes in cholesterol were also reported in lactating cows and buffaloes, respectively<sup>14,15</sup>, corroborated the findings of present study. The TG levels reduced at parturition may be due to the possible utilization of plasma TG as precursors of milk fat by mammary tissue<sup>16</sup> and by the liver in lactating cows<sup>17</sup>.

The plasma cholesterol concentration showed significant lower levels in Sahiwal compared to Haryana breed. In addition, cholesterol level remained higher during dry period which subsequently decreased in early lactation and then drastically increased during mid lactation. The comparable

trend of plasma cholesterol was also reported in periparturient cows<sup>13,18</sup>, simulates the findings of this study. The fall in levels of total cholesterol during early lactation may be due to the increased demand for energy for initiation of lactation causing NEB in animals.

The level of cholesterol rose with progression of lactation reflects rise in the uptake of lipids in the liver, which may be associated with increased tissue mobilization, feed intake, greater synthesis of steroid hormones and lipoproteins, which are physiological processes of the postpartum<sup>19</sup>. Similar to the present findings, the comparable change in plasma levels of HDL and LDL in dry and lactation period were also reported by Mohebbi-Fani *et al.*<sup>20</sup>, Singh *et al.*<sup>21</sup> and Yousuf *et al.*<sup>22</sup>. However, non-significant changes in HDL and LDL concentration were also documented in pregnant and lactating cows<sup>9,14</sup>.

It is reported that the TG production rate in the liver is enhanced at calving and maximum lipid infiltration of the liver takes place 1-2 weeks after calving indicated inclination of the dairy cows to fatty liver after calving<sup>23</sup>. Furthermore, high energy-low protein ration before calving increases LDL and Very Low Density Lipid (VLDL) during early lactation and results into less mobilization of body fats and less accumulation of fats in the liver.

The results showed non-significant changes in NEFA levels from dry period to mid lactation in both breeds of cows. Analogous trend of NEFA observed in this study is somewhat in agreement with the reports of some other authors, substantiate the findings of present study<sup>13,14,18,24</sup>.

Just after parturition demands for glucose significantly enhanced for milk lactose synthesis for initiation of milk production, but concurrently feed intake remains insufficient to meet out the energy demand<sup>25</sup>. Thus, Negative Energy Balance (NEB) sets in just after calving. This NEB may induce fat mobilization from adipose tissues and releases NEFA, which are the principal energy source for the cow in conditions of NEB<sup>26</sup>. The levels of NEFA in blood reflect degree of adipose tissue mobilization<sup>27</sup>. In present study, NEFA did not show significant variation from pregnancy to lactation indicated that the mobilization of fat subsided and the energy level of cows maintained during lactation.

### CONCLUSION

It can be concluded that high yielding Sahiwal cows maintains the lower levels of leptin, TG, cholesterol, LDL and NEFA compared to low yielding Harijana cows during study period. The indicators of the energy profile such as; glucose, cholesterol, TG, HDL and LDL changed from pregnancy to lactation, suggesting their significant physiological role in the energy metabolism of dairy cows during this crucial period.

### SIGNIFICANCE STATEMENT

This study ascertains the possible role of adipokine leptin and other metabolic indices in differential production of high and low yielding dairy cows which can be beneficial for studying the complex lactation physiology of animals. This study will help the researcher to identify biomarker for assessing the production potential of dairy cows that many researchers were not able to explore. It will also assist in discovering the metabolic abnormalities in the herd and support in better management of the metabolic problems at the herd level.

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