Status of Circulating Adipokines and Lipid Profile at Different Stages of Lactation in Three Parities of Indian Dairy Cows

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ABSTRACT

Adiponectin and resistin are vital adipokines which influence the energy homeostasis, insulin sensitivity and mobilization of body fat stores. The present investigation was performed to elucidate the influence of the stage of lactation (early, mid and late lactation) and parity (first, second and third) on circulating levels of adipokines and lipid profile in Indian dairy cows. Blood samples were collected from 18 Sahiwal cows, six each in their first, second and third parity in their early (30th day), mid (90th day) and late (200th day) lactation. The Body condition score (BCS), plasma levels of resistin, progesterone, estrogen and high density lipoprotein (HDL) affected with stages of lactation, insulin and Ttriglyerides (TG) were found to be affected by different parities while cholesterol, low density lipoprotein (LDL) and non-esterified fatty acids (NEFA) were found to be affected with both parity and stage of lactation of cows. The plasma levels of adiponectin showed positive association with progesterone, TG, cholesterol, HDL and LDL and negative association with NEFA in first lactation, and showed positive correlation with resistin (RETN) in third lactation in Sahiwal cows. The plasma levels of resistin showed a positive association with adiponectin and NEFA and negative correlation with BCS, progesterone and HDL in second lactation in Sahiwal cows. In conclusion, this investigation is endow with data of plasma levels of adipokines and lipid profile at different stages of lactation in cows of different parities that may be useful for clinical and experimental interpretations. However further studies are needed to elucidate the physiological significance of adipokines in lactogenesis in dairy animals.

Key words: Adiponectin; Resistin; Parity; Stage of Lactation; Cow.

INTRODUCTION

Adipose tissues (AT) synthesize and secrete several distinct biologically active compounds called adipokines or adipocytokines. Among these adiponectin, resistin and leptin are important adipokines which contribute in regulation of energy metabolism by affecting insulin sensitivity in vital organs (1) and centrally mediated feed intake (2). Thus, these play vital roles in regulation of energy homeostasis, immunity, reproduction and cardio-vascular function (3). Since milk production and energy status of animals, changes with their stage of lactation and parity, thus circulating levels of these adipokines and their association with other hormones and lipid profile is expected to be affected with parity and stage of lactation of animals. The study aims to elucidate the status of circulating adipokines and other hormones as well as their lipid profile during different stages of lactation and parity in dairy cows.

MATERIALS AND METHODS

Animals and blood sampling

Eighteen Sahiwal cows, six each in their first, second and third parity were selected from Instructional Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Mathura and blood samples were collected in their early (30th day), mid (90th day) and late (200th day) lactation period. Using these cows the influence of the stage of lactation and parity on circulating levels of adipokines and lipid profile was studied.

The animals were kept under uniform management conditions and provided *ad libitum* clean potable water and they all received the same recommended balanced ration (4) throughout the study period. Blood samples were collected during morning within two hours after milking before feeding of cows. All the procedures were carried out in accordance with the guidelines laid down by the Institutional Ethics Committee and in accordance with local laws and regulations. Body condition score (BCS) of animals were recorded on the day of blood sampling using a 5-point scale (1 = lean, 5 = fat) as per standard method (5). Total and average daily milk production of animals during the fortnight period was also recorded for studying the association of circulating adipokines with milk production of cows.

Estimation of circulating adipokines and other hormones

Blood samples were collected and plasma was harvested by centrifugation and stored at -20°C till analysis. The plasma samples (in duplicate) were analyzed for levels of adipokines *viz* adiponectin (ADIPOQ), resistin (RETN), leptin (LEP) and hormones *viz* insulin (INSU), estradiol (ESTD) and progesterone (PROG) by commercially available bovine specific ELISA kits (Cusabio, GenxBio). Coefficients of variation (CV) for both inter-assay and intra-assay were <10% for all the hormones estimated.

The plasma samples were also analyzed for lipid profile including triglycerides (TG), total cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL) (calculated from levels of TG, cholesterol and LDL), and non-esterified fatty acids (NEFA) using commercially available diagnostic kits (Autospan, Span Diagnostic Kits Ltd., India) as per the standard protocol.

Statistical analysis

The obtained data were presented as mean ± SEM and $p<0.05$ was considered statistically significant. The statistical differences among the means of groups were analyzed by two-way analysis of variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) and correlations between the variables were analyzed by calculating Pearson correlations coefficient using SPSS software for Windows (version 16.0).

RESULTS

The circulating levels of adipokines, hormones and lipid profile of Sahiwal cows in different parity and stages of lactation are depicted in Table 1 and Table 2. The BCS of cows revealed significant influence of stage of lactation which showed lower BCS values in early lactation compared to mid and late lactation in all three parities. While the parity of animals and their interaction with stage of lactation (Parity*Lactation) did not reveal significant effect on BCS. In contrast significant influence of parity as well as stage of lactation was observed on average milk yield (AMY) of cows while interaction of parity with stage of lactation (Parity*Lactation) did not reveal significant effect.

The results showed significant influence of stage of lactation; lower levels of PROG, ESTD, CHOL, LDL and LDL concentration in early lactation compared with mid and late lactation. The levels of RETN and NEFA were higher in early lactation compared to mid and late lactation.

Furthermore the circulating levels of insulin and TG were reduced in the third parity compared to first and or second parity while cholesterol and LDL were lower in second compared to first parity. Moreover NEFA levels were higher in second and or third parity compared to first parity.

The results of Pearson's correlation (Table 3 and Table 4) revealed positive association between plasma levels of ADIPOQ with PROG, TG, CHOL, HDL and LDL and negative association with NEFA in first lactation and positive correlation with RETN in second and third lactation. The plasma level of RETN was positively associated with ADIPOQ and NEFA and negative correlation with BCS, PROG and HDL in second lactation.

Table 1: Circulating levels of adipokines and other hormones in different parities and lactation stages of Sahiwal cows.

Parity	First Parity			Second Parity			Third Parity			Pooled SEM	P value		
Lactation	EL	ML	LL	EL	ML	LL	EL	ML	LL		Parity	Lactation	Parity * Lactation
BCS	2.50^{a}	2.87^{b}	2.82^{b}	2.63^{a}	2.97^{b}	2.85^{b}	2.67^{a}	2.97^{b}	2.90^{b}	0.06	0.06	0.01	0.91
ADIPOQ (µg/ml)	11.99^{a}	12.72^{a}	17.08^{a}	16.80^{a}	15.43^{a}	19.19^{a}	16.07^{a}	17.60^{a}	17.11^{a}	2.46	0.21	0.31	0.36
RETN (ηg/ml)	36.04^{a}	38.10^{b}	42.82^{b}	69.01^{a}	38.37^{b}	39.02^{b}	55.93^{a}	45.57^{b}	38.15^{b}	6.97	0.21	0.03	0.08
LEP (ηg/ml)	4.76^{a}	4.55^{a}	3.58^{a}	4.17^{a}	3.74^{a}	4.68^{a}	4.18^{a}	3.60^{a}	4.70^{a}	0.39	0.90	0.38	0.05
INSU (µU/L)	7.18^{ab}	7.46^{ab}	7.35^{ab}	7.49^{a}	7.95^{a}	7.52^{a}	6.74^{b}	7.22^{b}	6.78^{b}	0.37	0.05	0.37	0.99
PROG (ηg/ml)	0.77^{a}	2.06^{b}	2.34^{b}	0.86^{a}	2.25^{b}	2.45^{b}	0.84^{a}	2.20^{b}	2.30^{b}	0.16	0.62	0.01	0.98
ESTD (ρg/ml)	20.08^{a}	25.31^{ab}	31.07^{b}	19.23^{a}	25.08^{ab}	33.50^{b}	20.46^{a}	23.44^{ab}	32.55^{b}	5.11	0.99	0.02	0.99

Means bearing a same superscript a, b, c in a row differ non-significantly (P>0.05)

Table 2: AMY and circulating levels of lipid profile in different parities and lactation stages of Sahiwal cows.

Parity	First Parity			Second Parity			Third Parity			Pooled SEM	P value		
Lactation	EL	ML	LL	EL	ML	LL	EL	ML	LL		Parity	Lactation	Parity * Lactation
TG (mg/dl)	27.12^{a}	25.04^{a}	26.06^{a}	24.77^{a}	30.62^{a}	27.59^{a}	17.00^{b}	22.35^{b}	20.82^{b}	2.98	0.01	0.47	0.67
CHOL (mg/dl)	126.2^{a}	179.8^{b}	163.66^{b}	101.45^{c}	151.10^{d}	143.48^{d}	112.47^{ac}	149.53^{bd}	162.06^{bd}	11.76	0.04	0.00	0.81
HDL (mg/dl)	42.6^{a}	58.15^{b}	60.37^{b}	43.76^{a}	61.94^{b}	62.62^{b}	45.65^{a}	61.67^{b}	63.41^{b}	1.64	0.06	0.00	0.94
LDL (mg/dl)	78.14^{a}	116.71^{b}	98.06^{b}	52.74^{c}	83.04^{d}	75.34^{d}	63.42^{ac}	83.39^{bd}	94.48^{bd}	11.35	0.02	0.01	0.77
NEFA (mM/L)	0.17^{a}	0.08^{b}	0.11^{b}	0.25^{c}	0.15^{d}	0.17^{d}	0.36^{e}	0.24^{f}	0.10^{f}	0.01	0.00	0.00	0.98
AMY (Liters/Day)	4.59^{a}	5.45^{b}	5.36^{a}	4.84^{ac}	7.56^{bd}	5.27^{ac}	5.45^{c}	8.02^{d}	6.32^{c}	0.53	0.01	0.00	0.23

Means bearing a same superscript a, b, c in a row differ non-significantly (P>0.05)

DISCUSSION

The results revealed lower BCS values in early lactation compared to mid and late lactation in all three parities. Similarly, lowest BCS was reported around 30th day post-partum (6) and (7), observed loss of body condition 3 weeks post-partum in both multiparous (MP) and primiparous (PP) cows.

In present study parity and stage of lactation did not show significant change in circulating levels of ADIPOQ in Sahiwal cows. However higher levels of serum ADIPOQ concentrations (1.4-fold) was reported in MP cows than in PP cows (8). The present results also revealed significantly higher levels of RETN in early lactation compared with mid and late lactation in second and third parity. Similar higher plasma RETN concentration was also demonstrated one week postpartum compare to six weeks and 5 months postpartum during first and second lactation, (9). This finding corroborates with the findings of present study. These changes may be due to the fact that blood hormones and

Table 3: Correlation of circulating ADIPOQ hormone with levels of other plasma hormones in different parities and lactation stages of Sahiwal cows.

Lactation	Stage	BCS	RETN	LEP	INSU	PROG	ESTD	TG	CHOL	HDL	LDL	NEFA
I	EL	-0.21	-0.51	0.53	-0.15	0.53	0.62	0.48	-0.21	-0.76	-0.12	-0.42
	ML	-0.51	0.32	0.54	-0.68	-0.23	-0.14	0.51	0.46	-0.08	0.61	0.26
	LL	-0.07	0.47	-0.16	-0.37	-0.68	0.40	-0.10	-0.27	0.14	-0.26	-0.80
	Overall	0.28	0.12	-0.09	-0.23	0.71**	0.31	0.60**	0.62**	0.72**	0.53*	-0.70**
II	EL	-0.55	0.88*	-0.34	0.15	0.75	-0.61	-0.23	0.16	-0.30	0.23	-0.82*
	ML	0.18	0.76	0.64	-0.05	-0.70	0.80	0.28	0.81	0.47	0.80	0.15
	LL	0.86*	0.57	-0.75	0.82*	-0.56	0.59	-0.58	0.84*	0.24	0.84*	0.14
	Overall	0.10	0.53*	-0.02	0.18	-0.13	0.45	-0.25	0.49	0.08	0.57	-0.05
III	EL	0.24	0.69	0.32	0.08	-0.01	-0.74	-0.03	0.71	0.49	0.69	0.24
	ML	0.12	0.64	0.33	0.09	0.07	0.34	-0.06	-0.52	0.02	-0.52	-0.55
	LL	-0.06	0.55	0.51	-0.62	0.03	-0.08	0.02	-0.94**	-0.07	-0.96**	-0.71
	Overall	0.16	0.55*	0.32	-0.10	0.13	-0.06	0.04	-0.15	0.16	-0.21	-0.18

Correlation coefficient bearing asterisk (*) exhibiting significant correlation (P<0.05) and double asterisk (**) exhibit highly significant correlation (P<0.01)

Table 4: Correlation of circulating levels of RETN hormone with levels of other hormones in different parities and lactation stages in Sahiwal cows.

Lactation	Stage	BCS	RETN	LEP	INSU	PROG	ESTD	TG	CHOL	HDL	LDL	NEFA
I	EL	-0.21	-0.51	-0.84*	-0.40	0.11	-0.52	-0.86*	-0.13	-0.01	-0.09	0.90*
	ML	-0.17	0.32	0.92**	0.17	-0.13	0.41	0.20	-0.50	-0.78	-0.32	0.09
	LL	-0.04	0.47	0.53	-0.87*	-0.28	0.32	0.05	-0.54	-0.11	-0.50	-0.65
	Overall	0.13	0.12	-0.33	-0.32	0.02	0.02	-0.27	-0.14	-0.07	-0.15	0.12
II	EL	-0.67	0.88*	-0.61	0.07	0.83*	-0.63	0.16	0.43	-0.12	0.43	-0.57
	ML	0.28	0.76	0.40	-0.52	-0.61	0.91*	0.41	0.43	-0.10	0.46	-0.09
	LL	0.52	0.57	-0.60	0.62	-0.39	0.42	-0.65	0.36	-0.35	0.42	-0.27
	Overall	-0.49*	0.53*	-0.19	-0.12	-0.65**	-0.01	-0.15	-0.17	-0.66**	0.01	0.49*
III	EL	-0.38	0.69	0.83*	0.67	0.62	-0.57	0.59	0.34	0.32	0.31	0.55
	ML	-0.04	0.64	0.17	-0.12	-0.14	0.67	-0.11	0.07	0.16	0.06	-0.47
	LL	-0.17	0.55	-0.04	-0.59	-0.32	0.29	-0.63	-0.56	-0.14	-0.53	-0.59
	Overall	-0.33	0.55*	0.36	0.08	-0.34	-0.01	-0.27	-0.21	-0.29	-0.16	0.34

Correlation coefficient bearing asterisk (*) exhibiting significant correlation (P<0.05) and double asterisk (**) exhibit highly significant correlation (P<0.01)

metabolites are affected by parity because primiparous animals are still growing thus nutrients are directed both to maternal growth as well as milk production, thus levels are affected with parity (10).

The plasma levels of LEP in early, mid and late lactation in different parities did not reveal significant changes in present study. Analogous non-significant variations during lactation were also documented by authors (11, 12, 13, 14, 15, 16). The results of present study also revealed non-significant influence of parity of cows on plasma levels of LEP, similar (17) in PP and MP cows. However significant variation in serum LEP after seven month of lactation was recorded in Holstein cows (18).

In the present study the influence of lactation stages and their interaction with parity did not show significant influence on INSU levels. Likewise, no change in levels of INSU from early to late lactation was also reported in cows by various authors (13, 18, 19, 20, 21). However higher levels of INSU was also reported in mid lactation compared to early and late lactation (22, 23). In the present study the influence of parity on levels of INSU concentration showed significant difference between second and third parity in cows. However no change in INSU levels in different parities have been observed in dairy cows (18).

The results revealed significant effect of stage of lactation on plasma levels of PROG which showed higher levels of PROG in mid and late lactation compared to early lactation in Sahiwal cows, while parity and their interaction with stage of lactation revealed non-significant effect on levels of PROG. Similarly, a rise in levels of serum PROG was also documented in mid lactation after 45 days of lactation (24). Likewise significantly higher values at day 45 and/or 60 in buffaloes was also observed and it was suggested that this rise may be due to presence of luteinized follicles and/or corpora lutea preceded by silent ovulations from day 45 onward postpartum (25). However no effect of lactation was reported on plasma levels of PROG in cattle (19, 26). Besides, positive correlation between PROG concentration and parity with significant rise in PROG levels after 50 days postpartum was also documented in MP cows compared to PP cows (27). Similarly, Petersson *et al.* (28) found that cows in first parity had a higher incidence of atypical PROG profiles and subsequently a longer interval from calving to conception, than cows of higher parity. A possible explanation for this is that metabolic profiles might differ between young cows and older because young cows are still growing but generally produce less milk (29). Additionally, a possible alternative speculation may take into account a different metabolism of PROG between MP and PP cattle.

The results revealed significant influence of stage of lactation on plasma ESTD levels which revealed significant high levels of ESTD in late lactation compared to early lactation. Similar rise in ESTD levels were also reported in mid and late lactation compared to early lactation (19, 26), corroborated the findings. Additionally rise in levels of serum ESTD documented in mid lactation compared to early lactation in MP cows (27), simulated the findings of present study. The present study also revealed non-significant effect of parity and their interaction with stage of lactation on plasma ESTD concentration. However rise in levels of ESTD was demonstrated in PP cows than MP cows (27). Moreover lower ESTD concentrations also documented in PP cows than in MP cows (30). Tomomi *et al.* (31) documented that most PP cows have the first pattern and most MP have the second pattern of follicular development during the post-partum period, suggesting that the longer interval from calving to the first ovulation in PP cows than in MP cows is due to repetition follicular wave of non-ovulatory follicles. A preovulatory rise in ESTD concentration was observed during the process of growth of the first dominant follicle in MP cows, whereas a low level of ESTD concentration continued in PP cows regardless of development of the first dominant follicle (27).

The results TG levels showed significant lower values in third parity compared to first and second parity, however, the stage of lactation did not reveal significant effect on TG levels in cows. Nonetheless, no effect of parity on plasma levels of TG was documented by some authors (32, 33, 34). Similarly no effect of lactation stages on circulating levels of TG was also documented by authors (22, 34, 36), replicated the present findings. However Eman *et al.* (42) reported higher values of plasma TG in mid and late lactation than early lactation. The results of present study also revealed lower levels of plasma CHOL in early lactation compared to mid and late lactation while levels were significantly higher in the first and third parity compared to second parity in cows. Analogous higher values of CHOL in mid and late lactation compared to early lactation was also reported in dairy cows (22, 23, 35, 36, 37). Nevertheless Rossato *et al.* (34) observed non-significant effect of parity on plasma CHOL in cows and buffaloes. Furthermore plasma NEFA concentrations revealed a significant influence of parity and stage of lactation in Sahiwal cows which showed higher NEFA concentration in early lactation compared to mid and late lactation. Similar effect of stage of lactation on plasma NEFA was also reported in dairy cows (22). The increase in levels of NEFA was also observed with increase in parity in Sahiwal cows. Similarly the higher NEFA concentration in multiparous compared to primiparous cows was also documented by some authors in dairy cows (8, 18), corroborating with the findings of present study. However the non-significant effect of parity on NEFA concentration was also documented in dairy cows (33).

Possibly, after parturition, characteristic changes in energy demand may lead to hormonal changes which regulate the lipid mobilization for utilizing these reserves for initiation and maintenance of lactation (38).The decline in TG levels in third parity indicates that TGs are used by mammary glands as precursors of milk fat (39, 40). Furthermore the higher level of cholesterol observed with advancement of lactation may be a physiological adjustment to meet the lactation requirements. This rise in cholesterol levels in mid and late lactation reflects an increase in the uptake of lipids in the liver, suggesting that the increase in cholesterol levels is associated with higher tissue mobilization, increase of food intake, greater synthesis of steroid hormones and lipoproteins, which are physiological processes of the lactation (41, 42). Additionally this increasing trend of plasma cholesterol during advancement of lactation may be coupled with the commencement of ovarian activity and establishment of postpartum ovarian cyclicity. Since plasma total cholesterol, acts as a fatty acid carrier in the form of cholesterol ester for milk synthesis, as a result, there is a gradual increase in plasma cholesterol level with advancing lactation. During early lactation, cattle undergo several hormonal changes (particularly a decrease in insulin concentration and an increase in somatotropin) and a reduction in DMI. Moreover high somatotropin concentrations and the low insulin levels stimulate a marked mobilization from adipose tissues, resulting in a higher plasma NEFA concentration (43). This enhanced NEFA concentrations, are useful for the animals to maximize milk synthesis with lower glucose consumption. Moreover additional NEFA are taken up by the liver (44) and completely oxidized to carbon dioxide to provide energy for the liver, partially oxidised to produce ketone bodies such as β-hydroxy butyric acid (BHBA) that are released into the blood and serve as fuels for other tissues, or reconverted to storage TG (45). Recently it has been shown that NEFA activates the AMPKs signaling pathway to increase lipid oxidation and decreases lipid synthesis in bovine hepatocytes, which in turn, could generates more ATP to relieve the negative energy balance in transition dairy cows (46).

The decrease in serum NEFA levels in mid and late lactation probably reflect high NEFA extraction by the mammary gland for milk fat synthesis and the improvement in DMI, hence in energy balance (47).

In conclusion, this investigation privides data of plasma levels of adipokines and lipid profile at different stages of lactation in cows of different parities that may be useful for clinical and experimental interpretations. Since this study revealed significant role of adipokines in nutrient partitioning towards the mammary gland for the milk production and also showed significant association between adipokines and lipid profile in dairy cows. Thus the results of this study will facilitate in understanding the physiological significance of the adipokines in lactation of cows that will aid in untying the molecular mechanism of adipokines involved in regulation of lactation in dairy animals.

However, further studies are required to explore their influence in another breeds and population of cows or in another species to elucidate the mechanisms by which these adipokines influences the lactogenesis in dairy animals.

CONFLICT OF INTEREST

None of the authors have any conflict of interest (financial remuneration, personal relationships and competing interests) to declare.

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