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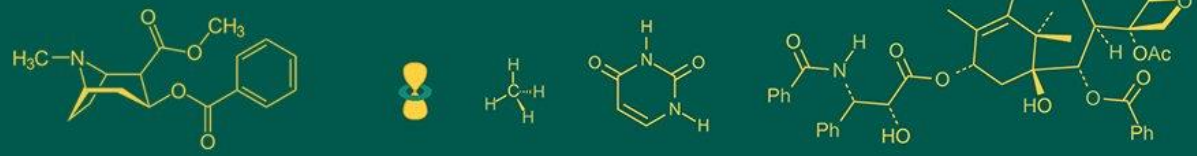

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Mundhe MB
Department of Veterinary Biochemistry, Mumbai Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Dalvi SH
Department of Veterinary Biochemistry, Mumbai Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Bharucha SV
Department of Veterinary Physiology, Mumbai Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Hande ST
Department of Livestock Production & Management, Mumbai Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Gawandi Smita
Scientific Officer 'F', Radiation Medicine Centre, Bhabha Atomic Research Centre, Parel, Mumbai, Maharashtra, India

Corresponding Author:
Dalvi SH
Department of Veterinary Biochemistry, Mumbai Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra, India

Serum thyroid hormones and lipid profile levels and their correlation at different lactation stages of Murrah buffalo

Mundhe MB, Dalvi SH, Bharucha SV, Hande ST and Gawandi Smita

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Abstract
The study was undertaken to estimate the levels of thyroid hormones and lipid profile and their correlation with stages of lactation in Murrah buffalo. Blood samples were collected twice at month interval from 24 healthy lactating Murrah buffaloes during early, mid and late stages of lactation. There was significant increase in the levels of total triiodothyronine (TT_3) during late stage of lactation. However, the levels remained almost same during the early and mid-lactation. total thyroxine (TT_4) levels remained same during early and late stage of lactation, but the levels decreased significantly during mid-lactation stage. There were significant changes in the levels of free T4 during mid lactational stages. All the lipid profile parameters *viz*. Triglycerides, Total Cholesterol, HDL-c, LDL-c and VLDL-c have shown significant increased ($p< 0.005$) during mid-lactation. The higher level of cholesterol and triglycerides with advancement of lactation may be the physiological adjustment to meet the lactation requirement for lactogenesis. Barring HDL-cholesterol thyroxine and free thyroxine hormone in serum was negatively correlated with total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol. However, all the relation were statistically no significant.

Keywords: Thyroid hormones, lipid profile, lactation stage, Murrah buffalo

Introduction
Pregnancy and lactation are physiological periods that result in an increased metabolic demand that, if not satisfied, could provoke a threat to homeostasis (Fiore., (2018) [10]. Periparturient period as well as during the first stage of lactation, increased mammary gland activity results in energy deficiency and increased lipid mobilization from body reserves (Arfuso *et al*., 2016) [1]. Despite the action of homeostatic mechanisms to maintain blood parameters within physiologic levels, changes in metabolites and hormones occur as a result of increased metabolic demands during both pregnancy and lactation (Arfuso *et al*., 2016., Ashmawy NA, 2015) [1, 2]. The peripartum period represents a critical life phase in buffaloes as well (Abdulkareem TA, 2013., Fiore *et al.*, 2017) [9, 3].

The peripartum period and lactation are accompanied by marked changes in some biochemical variables and thyroid hormone values in buffaloes. Moreover, the relationship found between TSH values and milk yield seems to suggest a possible role of the thyroid gland in the maintenance of lactogenesis (Fiore., 2018) [10]. Thyroid gland hormonal activity has an important role in the peripartum period for determining the cell metabolism intensity, the metabolism of lipids and carbohydrates, and the lactation course. (Huszenicza *et al*., 2002) [16].

Thyroid hormones induce HMG-CoA reductase, which is the first step in cholesterol biosynthesis. Triiodothyronine (T_3) upregulates LDL receptors by controlling the LDL receptor gene activation and significantly affects lipid and lipoprotein metabolism. It can influence HDL metabolism by increasing cholesteryl ester transfer protein (CETP) activity. (Rizos, *et al*., 2011) [23]. The hormonal activity of the thyroid gland has an important role in ruminants for the modulation of metabolic variables. (Fiore, *et al*., 2017) [9]. Thyroid hormones are important regulators of mammary gland function. In the absence of thyroid hormones, growth and differentiation of the mammary epithelium are reduced (Vonderhaar and Greco, 1979) [31]. Thyroid hormones and lipid profile changes during the postpartum period are extensively studied in cows but the information is scanty in buffalo.

Materials and Method

Experimental animals & Collection of samples

The study was conducted on 24 healthy lactating buffaloes from Kokani Dairy Farm, located at Wadalagaon, Dist. Nashik, Maharashtra, India. Blood samples were collected by jugular venipuncture from each buffalo during morning hours between 8:00 am to 10:00 am. About 10 ml of a blood sample from these animals was collected during the Early (15-45 days), Mid (100-130days), and Late (200-230days) stages of lactation in a clot activator gel tube. A total of 6 samples were collected from each buffalo during the early, mid, and late lactation period at monthly interval and clear serum was separated and used for analysis. The average maximum, minimum atmospheric temperature and humidity during the experimental period were 33.13±0.32 ºC, 22.72±0.30 ºC and 61.31±2.15% respectively.

Estimation of thyroid hormones and lipid profile

To measure the circulating levels of total triiodothyronine (T_3), thyroxine (T_4) and fT_4 in the collected serum samples radioimmunoassay was conducted by implying ready to use kits supplied by Board of Radioisotope Technology (BRIT), Bhaba Atomic Research Centre. The radioimmunoassay was carried out at Radiation Medicine Centre (RMC), Parel, Mumbai - 400 012. The lipid profile *viz*. total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol, estimated using standard biochemical kits and spectrophotometry.

Statistical Analysis

Analysis of variance of the data of the concentration of triiodothyronine, thyroxine, free thyroxine and lipid profile were done using 'R' software and according to Snedecor and Cochran (1994) [25] using one way ANOVA.

Results & Discussion

Thyroid Hormones

Mean ± SE serum thyroid hormones profiles during different stages of lactation in Murrah buffaloes is presented in Table1. The levels of TT_3 remained almost same during early and mid-stage of lactation and the levels increased significantly ($P < 0.002$) during late stage of lactation. Similar trend and range were found in the study of Tiirats (1997) [27] in dairy cows, Tajik *et al*. (2010) [26] in water buffalo, Fiore *et al.* (2017) [9] in buffalo, Dalvi *et al*. (2013) [28] in Pandharpuri buffalo, Vala *et al*. (2020) [29] in lactating Jafarabadi buffalo and Cincovic *et al*. (2015) [7] in dairy cows. However, Kolbeisy and shetaewi (1992) found the higher levels of T_3 values in water buffalo as compare to our study during early stage of lactation. Blood levels of thyroid hormones in peripartum cows decreased, particularly in early lactation, when body reserves are mobilized for high milk production (Bonczek *et al.*, 1988; Tiirats, 1997; Huszenicza *et al.*, 2002) [6, 27, 16]. Ronge *et al.* (1988) [24] has also reported lower circulating levels of T_3 during early lactation and suggest their role in the partitioning of nutrients.

There was no significant variation in the levels of T_4 during early and late lactation but the levels decreased significantly ($P < 0.005$) in mid lactation stage. The mean and range of levels of thyroxine during different stages of lactation agreed with the study of Galdhar *et al.* (2021) in buffalo, Cincovic *et al*. (2015) [7] in cows, and Fiore *et al.* (2017) [9] in postpartum dairy cows. Also, Hassan *et al.*, (1984) in buffaloes and Pichaicharnarong *et al.* (1982) [20] observed that, as lactation stage advances reduction in plasma T_4 in Swamp buffalo. The lower levels of T_4 during mid stage of lactation may be because of peak of milk production. It is supported by the observations of Vanjonack and Johnson (1976) [30] that, the thyroid hormones are excreted by the mammary gland and with high milk production lose greater amounts of these hormones through the udder thus resulting in lowered plasma concentration. Also, may be due to considerable mammary drain of iodine during lactation (Premachandra and Turner, 1961; Ayoub, 1966) [22, 4].

Nixon *et al*. (1988) [19] recorded levels of fT_4 during three stages of lactation in four seasons in serum of Holstein cows which was higher than the findings of present study. Only the free fractions of serum thyroid hormones are available for utilization by peripheral tissues (Bantle *et al*., 1980) [5] and only the free fractions control the rates of secretion of thyroid releasing hormone (TRH) and thyroid-stimulating hormone (Faglia *et al*., 1979) [11]. In our study the levels of fT_4 also fall significantly ($P < 0.005$) during the mid stage of the lactation.

Table 1: Mean ± SE Serum Thyroid hormones levels during different stages of lactation in Murrah buffaloes

Parameter	Stage of Lactation		
	Early	Mid	Late
TT3 (ng/dl)	92.57^{a} ±4.88	92.35^{a} ±4.39	113.77^{b} ±4.73
TT4(µg/dl)	2.36^{a} ±0.14	1.83^{b} ±0.12	2.24^{a} ±0.10
fT4 (ng/dl	0.86^{a} ±0.04	0.73^{b} ±0.01	0.84^{a} ±0.02

Means with different superscripts differ significantly

Lipid Profile

As regards to the lipid profile, the Mean ± SE serum levels of triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C during different stages of lactation in Murrah buffaloes is presented in Table 2. All the lipid profile parameters *viz*. Triglycerides, Total Cholesterol, HDL-c, LDL-c and VLDL-c have shown significant increased ($P < 0.005$) during mid-lactation and then levels fall during late lactation.

The levels of total cholesterol and triglycerides increased significantly during the mid and late stage of lactation. Similar trend was observed in study of Tripathi *et al.* (2010) [28], Hagawane *et al.* (2009) [14], Polat *et al.* (2002) [21] in lactating and Grasso *et al*. (2004) [12] in water buffalo. The higher level of cholesterol and triglycerides with advancement of lactation may be the physiological adjustment to meet the lactation requirement (Lone *et al.* 2003) [18] for lactogenesis. The lipoprotein profile *viz*. HDL-c, LDL-c and VLDL-c has shown similar trend of that of triglyceride and total cholesterol. There levels significantly increased during mid lactation and remained higher levels during late lactation than that of early lactation.

Table 2: Mean ± SE Serum lipid profile levels (mg/dl) during different stages of lactation in Murrah buffaloes

Parameter	Stage of Lactation		
	Early	Mid	Late
Triglycerides	15.94^{a} ±0.65	22.46^{b} ±0.79	21.58^{b} ±0.58
Total Cholesterol	67.23^{a} ±2.71	93.54^{b} ±2.96	77.91^{c} ±2.79
HDL-C	36.06^{a} ±1.33	47.85^{b} ±1.51	41.75^{c} ±1.4
LDL-C	27.99^{a} ±1.46	41.42^{b} ±1.45	31.84^{c} ±1.44
VLDL-C	3.18^{a} ±0.13	4.50^{b} ±0.16	4.31^{b} ±0.12

Means with different superscripts differ significantly

Correlation of thyroid hormones and lipid profile

The triiodothyronine hormone in serum was positively correlated with triglyceride (r=0.18), VLDL cholesterol (r= 0.17 and negatively with total cholesterol (r= -0.069), HDL cholesterol (r= -0.039), LDL cholesterol (r= -0.1). However, all the relation were statistically nonsignificant. The thyroxine and free thyroxine hormone in serum was negatively correlated with total cholesterol (r= -0.025), triglyceride (r= -0.17), LDL cholesterol (r= -0.064), VLDL cholesterol (r= -0.16) while positively with HDL-cholesterol. However, all the relation were statistically nonsignificant.

Table 3: The inter correlation matrix of thyroid hormones and lipid profile during different stages of lactation in Murrah buffaloes

Parameters	Total Cholesterol	TG	HDL	LDL	VLDL	fT4	T4	T3
Total Cholesterol	--							
TG	0.28*	--						
HDL-C	0.99***	0.26*	--					
LDL-C	0.97***	0.25*	0.95***	--				
VLDL-C	0.3*	1.0***	0.28*	0.26*	--			
fT4	-0.16	-0.11	-0.14	-0.19	-0.11	--		
T4	-0.025	-0.17	0.0096	-0.064	-0.16	0.1	--	
T3	-0.069	0.18	-0.039	-0.1	0.17	0.25*	0.11	--

Conclusion

There was significant increase in the levels of total triiodothyronine (TT_3) during late stage of lactation. However, the levels remained almost same during the early and mid-lactation. The levels of total thyroxine (TT_4) remained same during early and late lactation but the values decreased significantly in mid lactation stage. Free thyroxine (fT_4) has followed the similar trend of TT_4. There are no reports of the levels of serum fT_4, in lactation buffaloes and it is reported first time in the present study.

The triiodothyronine hormone in serum was positively correlated with triglyceride, VLDL cholesterol and negatively with total cholesterol, HDL cholesterol, LDL cholesterol however, all the relation were statistically nonsignificant. Barring HDL-cholesterol thyroxine and free thyroxine hormone in serum was negatively correlated with total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol. However, all the relation were statistically nonsignificant.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors.

The animal study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of college.

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