EFFECT OF THI ON HORMONE PROFILE, BLOOD METABOLITES, MILK COMPOSITION AND BCS IN LACTATING MURRAH BUFFALOES

T H E S I S

Submitted

in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

VETERINARY PHYSIOLOGY

BY

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Enrolment No: V/12/0282

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(INDIA)

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"Effect of THI on hormone profile, blood metabolites, milk composition and BCS in lactating Murrah buffaloes"** or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

Date: **25.09.2019**

Place: Mumbai

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DECLARATION OF ADVISORY COMMITTEE

Ms. BHARUCHA SIMIN VISPI has satisfactorily prosecuted her course of research for a period of not less than two semesters (for Ph.D.) and that the thesis entitled, **"Effect of THI on hormone profile, blood metabolites, milk composition and BCS in lactating Murrah buffaloes"** submitted by her is the result of research work is sufficient to warrant its presentation to the examination in the subject of **Veterinary Physiology** for the award of **Doctor of Philosophy (Ph.D.)** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by her for a degree of any other University.

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Date: **27th September 2019**

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CERTIFICATE

This is to certify that the thesis entitled, **"Effect of THI on hormone profile, blood metabolites, milk composition and BCS in lactating Murrah buffaloes"** submitted by **Ms. BHARUCHA SIMIN VISPI** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **Doctor of Philosophy (Ph.D.)** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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(Name)

PROF. DR. A. S. RANADE

All things bright and beautiful,
All creatures great and small,
All things wise and wonderful,
The Lord God made them all.

TO THOSE WHO INSPIRED IT, BUT SHALL NOT READ IT

If having a soul means being able to feel love and loyalty and gratitude, then animals are better off than a lot of humans

James Herriot

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%	:	Percentage, Per cent
@	:	At the rate of
<	:	Less than
>	:	More than
Δ	:	Increment
ΔA/minute	:	Absorbance per minute
µmol/L	:	Micromole (10^{-6} moles)
µl	:	Microliter (10^{-6} liters)
µg/ ampoule	:	Microgram per ampoule
µU/ml	:	Micro units per millilitre
1, 25-$(OH)_2$ D	:	1,25 – Dihydroxyvitamin D
A	:	Absorbance
A.M.	:	Ante meridian
A/G ratio, A: G	:	Albumin to Globulin ratio
AA	:	Amino acid
Abs	:	Absorbance
AI	:	Artificial insemination
Alb	:	Albumin
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
Avg.	:	Average
BCS	:	Body condition score
bGH	:	Bovine growth hormone
BSA	:	Bovine serum albumin
BUN	:	Blood urea nitrogen
BW	:	Body weight
Ca	:	Calcium
CD	:	Critical difference
Cl	:	Chloride
Conc.	:	Concentration
D	:	Day
DIM	:	Days in milk

DMI	:	Dry matter intake
DMY	:	Daily milk yield
ELISA	:	Enzyme linked immunosorbent assay
et al. (et alii / alia,)	:	And others
F%	:	Fat percentage
g/dl	:	Grams per decilitre
g/kg, g kg^{-1}	:	Grams per kilogram
g/L	:	Grams per litre
GH	:	Growth hormone
GIT	:	Gastrointestinal tract
Glu	:	Glucose
HF	:	Holstein Friesian
HPA	:	Hypothalamic-pituitary-adrenal axis
i.e. (*id est*)	:	In other words
I^{125}	:	$Iodine^{125}$
IGF1	:	Insulin-like growth factor 1
IU/L	:	International units per litre
Kg	:	Kilogram
Kg/day, kg/d	:	Kilogram per day
kU/l	:	Kilo units per litre
M	:	Molar
Max	:	Maximum
mEq/l	:	Milliequivalent per litre
Mg	:	Magnesium
mg/dl	:	Milligrams per decilitre
Mg^{++}	:	Magnesium ion
Min	:	Minimum
ML, Ml, mL, ml	:	Millilitre (10^{-3} litre)
mmol/L	:	Millimoles (10^{-3} moles) per litre
mol/L	:	Moles per litre
MY	:	Milk yield
NABL	:	National Accreditation Board for Testing and Calibration Laboratories
NEB	:	Negative energy balance

ng/dl	:	Nanograms per decilitre
ng/ml	:	Nanograms per millilitre
NHPP	:	National Hormone and Peptide Program
nm	:	Wavelength
nmol/L	:	Nanomoles per litre
NRC	:	National Research Council
P	:	Phosphorous
P%	:	Protein percentage
P.M.	:	Post meridian
PBS	:	Phosphate buffered saline
PEG	:	Polyethylene glycol
pmol/L	:	Picomoles per litre
RB	:	reagent blank
RIA	:	Radioimmunoassay
RPM	:	Revolutions per minute
SCC / SCS	:	Somatic cell count / somatic cell score
SE	:	Standard error
SGOT	:	Serum glutamic-oxaloacetic transaminase
SGPT	:	Serum glutamate-pyruvate transaminase
SNF	:	Solid not fat
Sr. No.	:	Serial number
Std.	:	Standard
T_3	:	3,5,3'-tri-iodothyronine
T_4	:	Tetraiodothyronine / thyroxin
T_a	:	Ambient temperature
THI	:	Temperature humidity index
TNZ	:	Thermoneutral zone
TP	:	Total protein
U	:	Urea
U/l	:	Unit per litre
UV	:	Ultraviolet
viz. (*videre licet*)	:	Namely / that is to say / which is

Introduction

INTRODUCTION

India is by and large an agrarian country with nearly 60% of its population netting their livelihood from agronomic undertakings in one form or another. Worldwide, milk has been appreciated as a beneficial staple of pastoral diets and it is a highly nutritious produce (Frelich *et al.,* 2012). Our country is home to a prodigious buffalo germplasm biodiversity. The India buffalo population is 105.3 million (NDDB, 2012) and these animals are the mainstay of the milk production system in India. The buffalo, an economically imperative multipurpose livestock principally for the baseline income clusters, plays an enriching role by contributing in many cultural and social aspects in India (Boro *et al.,* 2018). Buffaloes also have a key role in the Indian economy as nearly 56% of the total national milk production is supplied by the buffalo. India, with 32 million tons, is world's topmost buffalo milk producer accounting for 64% of the world's total of 49 million tons (FAO, 2008).

The home tract of the world famous Murrah buffaloes, renowned for high milk production capability is Haryana, but graded Murrah buffaloes are found throughout the country owing to their nonpareil milk production potential coupled with acclimatization to wide ecological conditions and feed conversion proficiency (Kumar *et al.*, 2017). In Mumbai City alone, there are more than 200,000 buffaloes inside the main city and probably another 100,000 in suburban areas like Thane (Thomas, 2004).

The Indian and specifically Maharashtra state dairy and animal husbandry sector has remained a marginalized and under-developed sector of agriculture for numerous years, but now is deemed as a priority area due to augmented internal mobility of investors with greater interest. Conception of a separate ministry of livestock and dairy development is a recognition of the relevance of this sector in this age and time. Further, milk yield is the single most important fiscal trait determining economic returns from the dairy animals. The demand for value addition in dairy products by the populace as well as industries has resulted in an

emerging trend of budding dairy entrepreneurs who are greatly concerned with the quality and quantity of the different milk components produced by their livestock.

Milk encompasses most of the essential nutrients needed for growth and development of the offspring as well as man. Buffalo milk when compared with cow's milk embodies superior concentrations of fats, lactose, proteins and incorporates about 1.5 times higher calcium and magnesium quantities but is poorer in chloride (Khedkar *et al.,* 2016). A study by Sheehan and Phipatanakul (2009) showcased an important advantage of buffalo milk tolerance in people having allergies with cow milk, thus adding to its benefits. Another benefit of buffalo milk consumption is that indigenous Indian dairy buffaloes have only A2 allele and hence are a source for safe A2 milk (Mishra and Joshi, 2009). Going a step further, the somatic cell counts (SCC) in buffalo is always lower when compared to cows (Singh and Ludri, 2001 and De *et al.,* 2011). However, like cows, disparity in the daily milk SCC has also been reflected in Murrah buffaloes.

The buffalo milk components are superior, casein micelles are larger and this positively influences the concoction of traditional milk products. Essentially the milk water content regulates its fat and protein concentrations and the rate of this water secretion depends upon lactose synthesis, which consecutively determines the osmolarity of the milk produced. Lactose levels in succession are reliant on the glucose concentration variations, somatic cell count, and energy harnessed for physiological processes (Miglior *et al.,* 2006). Piccione *et al.,* (2009) established that in the course of lactation the mammary gland secretory cells utilise 80% of the blood-circulating metabolites for milk synthesis, subject to the infiltration spend of the milk compounds' precursors. The blood total protein and albumin, are prerequisites for the production of proteins by the dam for milk synthesis and immunoglobulin production.

An imperative yardstick of a ruminant's energy status is its glucose concentration which is expended by the mammary gland for the amalgamation of milk lactose (Surya Prakash *et al.,* 2018a). It has been ascertained that in cows yielding 20 liters of milk a day, glucose above one kilogram is coveted by the mammary glands (Stamatović *et al.,* 1983) and this delivery and its mammary gland

uptake regulates the milk synthesis proficiency. Ensuing the inception of lactation, it is thought that insulin-independent glucose uptake in the body except the mammary gland regresses and insulin resistance in the whole body progresses (Komatsu *et al.,* 2005). Ingvartsen *et al.,* (2003), Ingvartsen (2006) and LeBlanc (2010) have identified plasma glucose as the foremost metabolite that correlates to the intensity of physiological imbalance.

The quantification of blood metabolites are a handy guide for the animals' nutritional status and potential performance (García *et al.,* 2017). The fundamental analytes for assessing the blood profile are total proteins which furnish knowledge about impairments to organs like the kidney and liver as well as are a portrayal of its nutritional health (Stojević *et al.,* 2005); albumin that connotes hepatic insufficiency by dwindling in concentration (Whitaker, 2000) and globulin that escalations in response to an inflammatory progression (Kaneko *et al.,* 2008). The blood urea nitrogen (BUN), a strategic marker of the animal's energy intake is used as an elicitation of the synchronization between fermentable carbohydrates and rumen degradable protein (Van Saun, 2010). The renal functions, principally embodied by urea and creatinine concentrations are significantly affected during the different physiological phases.

Electrolytes, have multifaceted functions in animal's body like incorporating plasma osmolarity conservation, acid-base equilibrium, nerve impulse propagation and are co-factors for various enzymes, thus playing a decisive role in sustaining metabolic homeostasis (Jacob *et al.,* 2011). The status of macro minerals like calcium (Ca), phosphorus (P) and magnesium (Mg), due to their significance in the metabolic reactions expeditiousness and their role in the transmembrane transport systems (Houillier, 2014) along with chloride (Cl) the most abundant anion in extracellular fluid (Soetan *et al.,* 2010) are of critical relevance because of their role in metabolic challenges like ketosis and milk fever wherein they play a crucial role. Alterations in the biochemical and electrolyte profile occur in the body around parturition and during peak lactation. High producing dairy livestock are vulnerable due to the elevated turnover of fluids, minerals and organic matter in the body during the transition periods thereby, disturbing the homeostasis which in due

course leads to anomalous clinical situations as reported by Blood and Radostits (1989).

As per Samanc *et al.,* (2011) most of these minerals are regrettably, tightly synchronized in the body by an array of homeostatic processes and the macro mineral blood concentrations are not reflective of the dietary status when the homeostatic system is functioning properly. Nevertheless the blood concentrations of phosphorus and magnesium are reasonably sensitive to dietary intake. Assessment of calcium concentrations around the time of calving is a useful indicator of how well the calcium regulatory system is functioning. Excluding the two weeks prior to and following calving, as a result of the intact regulatory system the blood calcium levels are not a very sensitive diagnostic measure (Goff, 2004). Consequently macro mineral blood concentrations need to be judiciously deciphered in the light of whether or not the homeostatic system is in proper operation.

The enzymes SGPT / ALT (serum glutamate-pyruvate transaminase / alanine aminotransferase) and SGOT / AST (serum glutamic-oxaloacetic transaminase / aspartate aminotransferase) are vital catabolic enzymes which play a critical role in liver function of animals. In high producing dairy cows during the period of postpartum and negative energy balance, there is excessive mobilization of body fat due to the body's augmented physical consumption that ensues into liver injury and the amendment of liver cell permeability which initiates variable degrees of increased enzymatic activity (Bjerre-Harpoth *et al.,* 2012). Blood plasma and serum SGPT and SGOT activities are reported to be useful for postpartum dairy cows (Stojevic *et al.,* 2005 and Samanc *et al.,* 2011).

Physiological imbalance is elucidated as the digression of physiological parameters from the normal and consequently, amplified risk of developing production maladies (clinical or subclinical) and reduced production. Diminishing the degree of physiological imbalance in individual animals causes reduction in the risk of disease and, thereby, enhances production performance (Ingvartsen, 2006 and Moyes *et al.,* 2013). The serum biochemical reference values are used to establish normality and to diagnose disease and physiological alteration. Textbook

reference intervals generated by European or American veterinary laboratories are normally founded on animals living under good husbandry conditions in temperate climates, and the reference sample groups may fluctuate from Indian normal values. Potential variances may be ascribed to genetic factors, the quality and quantity of nutrition, presence or absence of water, electrolyte losses in sweat, internal parasites and climatic conditions and this makes it tricky to depend on reference intervals from other countries to interpret results for animals living in India or elsewhere in the tropics.

Lactation is high profile production phase and animals have to adjust their biology to cater to the milk production. The maintenance of lactation is regulated by an interplay of hormones like growth hormone (GH), insulin, triiodothyronine (T_3) and tetraiodothyronine / thyroxin (T_4) which influence the blood metabolites circulatory levels and the milk yield and composition in dairy livestock (Knight, 1993 and Knight and Flint, 1995). It is noteworthy that while hormones play a lead role in the physiological process of milk secretion, the apparent seasonal intervention of cortisol (stress hormone) in milk secretion is obvious during lactation. Accompanying the above mentioned hormones, integrated in this cascade, hormones like IGF – 1 and leptin play prominent roles in the different aspects of lactation.

The involvement of growth hormone in galactopoiesis and lactogenesis has been authenticated for more than 70 years, initiating with the Russian scientists' use of crude pituitary extract to augment milk production. It has now been concluded that growth hormone, and not prolactin, is the primary regulator of lactation in the cow (Sinowatz *et al.,* 2000), whereas prolactin and not growth hormone, performs this role in the laboratory species (Tucker, 2000). In bovines, a positive correlation is perceived between serum growth hormone concentration and milk yield (Sartin *et al.,* 1988) and GH exerts a long lasting homeorhetic control that synchronizes the utilization of absorbed nutrients (Bauman and Vernon, 1993). Growth hormone imparts an exceptional range of biological effects on growth and lactation. One well- corroborated biological action of GH is stimulation of insulin-like growth factor 1 (IGF1) production (Argetsinger and Carter-Su, 1996) and it is

now postulated that many of the effects of GH are mediated by IGF1. One of the important effects of GH and IGF1 in ruminants is on mammary gland development and lactation. GH does not alter mammary glucose transporter capability, but changes the intracellular glucose metabolism favoring lactose synthesis (Nielsen *et al.,* 2001).

The thyroid hormones uphold homeostasis of energy and protein metabolism, thermo-regulation, growth and productivity parameters (Huszenicza *et al.,* 2002). T_3 and T_4 concentrations are deemed to be indicators of homeorhetic adaptation to negative energy balance (NEB) until energy balance is achieved (Pethes *et al.,* 1985, Reist *et al.,* 2002, Djoković *et al.,* 2007, Remppis *et al.,* 2011).

Insulin, a fundamental metabolic hormone, can influence the cow's homeorhesis during its varied physiological states (from dry to lactation periods and back). The tissue sensitivity to insulin is of significant relevance in lactating animals (Sternbauer and Luthman, 2002). Both the responsiveness of insulin to glucose and the tissue responsiveness to insulin can amend during the various physiological states of dairy cows. Tucker (2000) stated that insulin is irrefutably encompassed in partitioning of nutrients to the mammary gland during lactation. Insulin secretion and action deviates with the decline in milk production subsequent to exposure to environmental stress (Sartin *et al.,* 1985). The relationship between increased growth hormone (GH) and decreased insulin during early lactation suggests a role of metabolic hormones in promoting mobilization of adipose tissue stores to fulfil energy needs (Randel, 1990).

Leptin, a valuable biomolecule, can be employed as a marker for distinguishing high performing individuals leading to better adaptability and productivity (Jamre *et al.,* 2016). Leptin is a proteic hormone produced in the adipose tissue which inhibits the feed intake (Liefers *et al.,* 2002) and down regulates the deposition of the adipose tissue (Morrison *et al.,* 2001). Houseknecht *et al.,* 1997 concluded that leptin has a sizable influence in orchestrating the whole body energy metabolism and could therefore be classified as a "metabolism modifier". Buchanan *et al.,* (2003) indicated that the leptin TT genotype is associated with increased milk and protein yield, without changing the milk fat and

has influence on the cow, sheep and goat milk quality. Not much work has been published concerning this aspect at buffalo milk. Leptin, because of its role in the regulation of feed intake and energy disposition, could also partake in the co-ordination of metabolism during the transition from pregnancy to lactation (Block *et al.,* 2001) and it could be employed as a biomolecule for enriching productivity in farm animals. Taking into consequence one of the main characteristics of the buffalo's milk is its high fat and protein content, this hormone has been included in the study. Houseknecht *et al.,* (2000) proposed that GH mitigates the ability of insulin and cortisol to stimulate leptin synthesis in bovine white adipose tissue.

The indispensable adrenal steroid hormone, cortisol, enacts the starring role in gluconeogenesis as well as carbohydrate and lipid metabolism regulation. This is of critical significance in high-yielding dairy cows during elevated metabolic load periods such as late pregnancy and lactation (Bertoni *et al.,* 2005). These cows have to confront immense metabolic challenges throughout the gestation-lactation cycles and the onset of lactation intensifies the energy deficit by routing the glucose to the mammary gland for lactose syntheses resulting in depressed blood glucose levels (Bell and Bauman, 1997). During this crucial timeframe of lactation the probability of manifestation of production diseases surges (LeBlanc, 2010). The activation of the hypothalamic-pituitary-adrenocortical axis (HPA) is paramount to the cows' physiological endocrine response to stress. In dairy cows pituitary and adrenocortical activity are linked to milk yield, energy balance and plasma concentrations of glucose and may possibly be arbitrated by the cows' individual adaptation process to the negative energy balance (Beerda *et al.,* 2004). Cortisol serves as an endpoint in the investigation of HPA-activity and consequently, its investigation is judged to be a beneficial tool to quantify stress response (Mostl and Palme, 2002).

Body condition score (BCS) first defined by Murray (1919), improved to be the process of subjectively and visually evaluating the flesh cover for assessment of the amount of metabolizable energy stored (Russel, 1984) is now extensively applied as a management tool in animal production systems. It has been effective in monitoring the energy intake of cows and buffaloes (Jeffrey and James, 1989)

and is a ready reckoner for ascertaining the animal's energy balance by gaging its outer appearance thus getting a perception of its body fat reserves. It gives a direct appraisal of the animal's body state and can be effortlessly amalgamated in effective assessment (Gransworthy, 1988). BCS systems have been instituted by various researchers in different species using a 0 to 5 scale, *viz*. Jefferies (1961) in ewes, Lowman *et al.,* (1976) in beef cattle, Edmonson *et al.,* (1989) in HF cows, Sarjan-Rao *et al.,* (2002) in Indian crossbred dairy cows and Anitha *et al.,* (2011) in Murrah buffaloes. In the present scenario, BCS is used for precise determination of energy reserves as energy balance during the entire lactation period (Coffey *et al.,* 2003). As per Roche *et al.,* (2009), the BCS in which a cow calves, her highest BCS, and the amount of BCS she exhausts post calving are all coupled with milk production, reproduction and health. Thus, BCS may also be a valid indicator of animal welfare, but further research is required to determine the effect of BCS and BCS change on how a cow "feels."

Livestock performance is influenced by different elements due to complex interactions between the individual animal and the different environmental factors (Lambertz *et al*., 2013). The levels of the various metabolic indices in the blood may diverge in response to assorted dietary, physiological and environmental stimuli (Bova *et al.,* 2014). One of the greatest challenges confronted by domestic animals around the world is thermal stress, which, in the tropics, is the chief limiting factor in livestock production. It has a decidedly unfavorable consequence on the animal bioenergetics, performance and health. The most crucial influencers of heat stress are temperature and humidity (Bohmanova *et al.,* 2005). There are many techniques for assessing the thermal load, and one of the most efficacious is gauging the temperature humidity index (THI) that combines dry bulb and wet bulb temperatures along with relative humidity to quantify heat stress (Thom, 1959).

Behera *et al.,* (2018) identified the THI model developed by NRC (1971) as the most suitable temperature humidity index (THI model) to study the impact of thermal stress. In terms of THI, the values of THI >72 is considered as stressful and THI >78 is considered traumatic for dairy cows and buffaloes (Ganaie *et al*., 2013). The potential effect of climate change on cattle has been linked to its pecuniary

sustainability since increase in ambient temperature during summer is associated with the cutback of voluntary intake of food, eliciting diminutions in body weight and milk production in dairy cattle.

Buffaloes can become stressed upon exposed to elevated temperatures and direct sunlight, which consequently diminishes their productivity. Buffaloes are predisposed to extreme climatic conditions due to their barely present sweat glands, dark colour and scant hair cover as compared to cattle. These attributes compromise the buffaloes' ability to endure heat stress and so vigilance is required to maintain production under hostile conditions (Vo and Wang, 2007). The water buffalo has only one tenth the number of sweat glands per unit area of skin as compared to zebu cattle and must rely on wallowing or wetting of the skin during heat conditions to reduce heat load. Air temperatures between 13 – 18 °C, relative humidity around 55 – 65% and wind velocity of 5 – 8 km/h are the optimum environmental conditions for buffaloes as alluded by Payne (1990).

In hot-humid climates, the buffalo endeavors to acclimatize by physiological alterations like cutting down on feed intake and heat production. This is a detrimental state of affairs due to the sacrifice of a portion of the animal's productivity towards its maintenance and consequentially resulting in decrease in performance (Santhosh Kumar *et al.,* 2018). The thyroid and adrenal glands play imperative roles in animal adaptation (Silva *et al.,* 2014). The concentration of thyroid hormones and the production of metabolic heat decrease (Morais *et al.,* 2008), whereas the blood concentration of cortisol increases (Marai and Haeeb, 2010) under heat stress. Milk hormones depend primarily on the unremitting supply of hormones to the mammary gland. This hormone transport may be disturbed due to exposure of the animals to high ambient temperature. Johnson *et al.,* (1988) reported that most of pituitary, thyroid and adrenal glands hormones in both plasma and milk were affected by stage of lactation under thermoneutral and short term heat exposure.

Evaluation of the blood and milk profiles to assess the animal health and milk yield has been underlined by multiple authors and diverse discrepancies have been observed in both blood and milk yield results. This is because the overall effects on

the animal are multifaceted, so varied physiological outputs must be studied in order to understand in what way these impact animal health and productivity. Further, livestock production and welfare is influenced by interactions between the individual animal and the environment. Since the milk constituents are secreted and synthesized in different metabolic pathways and essentially transported by blood, it is contemplated that studies on blood hormonal profile would furnish valuable scientific information in relation to lactation. Although studies have assessed the effect of environment on milk production and its composition on plasma hormones and plasma metabolites in buffaloes, there is very scanty literature on the relationship of the THI, BCS, milk composition and plasma hormones and metabolites in buffaloes kept open (not bred by AI nor allowed natural service) throughout lactation.

Thus, given the limited amount of information concerning the relationships and interrelation among and between these factors with regard to female buffaloes in this region, the present study has been conceived with the below mentioned objectives:

1. To study the hormone profile *(T_3, T_4, IGF1, Insulin, GH, Leptin and Cortisol)* and blood metabolites *(glucose, total proteins, albumin, calcium, phosphorus, chloride and magnesium)* during lactation in buffaloes.

2. To quantify the milk yield and determine the milk *composition (fat, protein, lactose and SNF)* in buffaloes during lactation.

3. To study body condition score (BCS) during lactation in buffaloes.

4. To correlate relationship between milk yield and composition, hormone profile, BCS, blood metabolites with THI.

Review of Literature

REVIEW OF LITERATURE

Buffaloes command an exalted status in the Indian dairy sector as they hold the greatest promise to resolve food security by generating a fluid cash income from selling milk, its varied products, meat, live buffalo sale and by provision quality protein, energy and other obligatory nutrients to solve malnutrition (Balain, 1999). Several region-specific, time-honoured milk preparations and products owe their characteristic physiognomies to buffalo milk. The physiological status of the lactating buffalo causes several metabolic adaptations. During such transitional or production stages, there is intense pressure on the dam for coping with the excess demand, often at the steep cost of sacrificing its reserves to meet the inflated nutrimental demands. Subsequently the dry period is the body reserves' restoration and rebuilding period provided appropriate feed is offered. These metabolic adaptations principally, are linked to several hormones that trigger major-minor up scaling and downgrading of the available resources and its stores. Homeostasis is the choreographed control of body tissues imperative to support its physiological state (Bauman and Currie, 1980). The buffalo's physiological adjustment to lactation is unlike other ruminants as it is supported by low incidences of metabolic disorders (Bertoni *et al.,* 1994). Metabolic profiling for dairy cattle pioneered in the early seventies (Payne and Laws, 1978) illuminates the status of the metabolic pathways of an individual animal or a herd. Each of the parameters has an important role in assessing the physiological status of the animal to enable speedy rectification of any irregularities. The endocrine system shoulders the essential responsibility in the adaptations for lactogenesis and galactopoeisis and so the understanding of a few prominent players is imperative. The available literature on blood metabolites, hormone profile, milk yield and its composition and body condition score (BCS) during lactation, relevant to the present study has been reviewed in brief:

Herbein et al., (1985) measured concentrations of glucose and growth hormone in blood plasma relative to days in milk, milk production, type of housing, and

season. Blood samples were obtained from lactating Holstein cows on three consecutive days in July, October, January and April. They reported that while glucose increased with increasing days in milk, growth hormone decreased with increasing days in milk. Above average milk production throughout lactation was associated with lower glucose. The relationship was not significant between growth hormone concentration and milk production. Cows on summer pasture with limited grain supplement had higher growth hormone and lower glucose than cows eating *ad libitum* in barns or feedlot. They concluded that all the cows had higher glucose and lower growth hormone in July than in cooler months.

Jindal and Ludri (1990) conducted studies on six lactating cows and six lactating buffaloes in second and third lactation. At fortnightly intervals, jugular blood samples were drawn at morning, noon, evening and night hours. The authors found that plasma growth hormone concentrations were highest during morning and thereafter decreased. In both species, there was a definite trend in the change of growth hormone concentrations during the day. In general growth hormone concentrations decreased as the stage of lactation advanced. The overall average values of plasma growth hormone in cows and buffaloes were 2.95 and 2.48 ng/ml which were not statistically different. With the advancing lactation, the decline in milk yields in both the species was positively correlated with the growth hormone concentrations.

Chaudhry (1992) studied the effect of factors such as buffalo status, season of calving and parity on lactation length and total lactation yield in 391 Nili-Ravi buffaloes. The average lactation length observed by them was 301.73 ± 1.87 (mean ± SE) days with a range of 181 to 505 days. The effect of parity on both traits under study was significant ($p < 0.01$). The milk yield was maximum (2150.38 ± 58.79 kg) in the seventh lactation and minimum (1818.31 ± 60.04 kg) in the sixth lactation.

Jindal and Ludri (1993) investigated six lactating crossbred cows and six Murrah buffaloes, maintained under similar conditions of feeding and management

for body composition by the antipyrine dilution technique. Measurements were made at the start of the experiment when the animals had completed about 50 days in lactation and thereafter at monthly intervals up to 90 days of the experimental period. The correlation coefficient between body composition parameters and various hormones (growth hormone, insulin, T_3 and T_4) were generally low and non-significant. They concluded that body composition studies using body water was not sufficiently sensitive to predict changes in body composition of lactating cows and buffaloes and/or the changes in body composition during lactation were not very drastic.

Jindal and Ludri (1994) explored the relationship between certain hormones and metabolites and between hormones and milk yield during different stages of lactation in six lactating Karan Swiss cows and six Murrah buffaloes. Highly negative relationship was ascertained between growth hormone with T_3 in cows and marginally negative in buffaloes. In both the species the relationship between T_3 and milk yield was negative and between T_4 and milk yield was positive. However, it was significant only in cows and not in buffaloes.

Habeeb et al., (1996) researched the effect of lactation number and ambient temperature on T_3 and cortisol levels in milk and blood and milk composition of lactating water buffaloes. The experiment was carried out on during two periods and included 72 animals. The first was carried out on 36 animals in February where the average ambient temperature was 17.5 °C, while the second was conducted on another 36 animals in July where the average ambient temperature was 37.1 °C. In both periods, the animals were classified according to lactating number into 6 equal groups from the 1^{st} to 6^{th} lactation number. The data indicated that milk yield and T_3 in milk and in blood and milk fat, protein and lactose were significantly lower in July than in February, whereas the opposite was true with cortisol levels.

Sharma et al., (2000) compared the gross composition of the milk from buffalo and cross-bred cows and found that the total proteins, lactose and SNF (solids-not-

fat) contents were comparatively higher in buffalo than in cow's milk. They concluded that the SNF contents remained almost unchanged throughout the lactation period. The protein contents of both cow and buffalo milk increased towards the end of lactation.

Block et al., (2001) measured plasma concentration of leptin from 35 days before to 56 days after parturition. They reported that the plasma leptin concentration was reduced by 50% after parturition and remained depressed during lactation despite a gradual improvement in energy balance. To determine whether negative energy balance caused this reduction in circulating leptin, cows were either milked or not milked after parturition. Absence of milk removal eliminated the energy deficit of early lactation, and doubled the plasma concentration of leptin. The plasma concentration of leptin was positively correlated with plasma concentrations of glucose, and negatively correlated with plasma concentrations of growth hormone.

Bouraoui et al., (2002) conducted two experiments using lactating Friesian-Holstein cows to measure the effects of heat stress, using temperature-humidity index (THI), on milk production and milk composition under the Mediterranean climate. These trials were carried out in two periods differing in average THI values (68 ± 3.75 vs. 78 ± 3.23 for the spring and summer periods, respectively). Daily THI was negatively correlated to milk yield (r = –0.76), when the THI value increased from 68 to 78, milk production decreased by 21%. Milk yield decreased by 0.41 kg per cow per day for each point increase in the THI values above 69. Milk fat (3.24 vs. 3.58%) and milk protein (2.88 vs. 2.96%) were lower for the summer group. THI was positively correlated to cortisol (0.31), and negatively with free thyroxin (–0.43). The average concentration of cortisol increased from 21.75 to 23.5 nmol/L ($P > 0.05$), while that of free thyroxin decreased from 15.5 to 14.5 pmol/L, ($P > 0.05$). Summer heat stress reduced milk yield and altered milk composition and affected the physiological functions of confined lactating Holstein cows.

Singh and Ludri (2002) selected eighteen crossbred goats to determine the changes in hormones, blood metabolites and yield and composition of milk during lactation. The blood and milk samples were collected from each goat at fortnightly interval for a period of 150 days. They observed that plasma concentration of GH was high during early lactation when the goats acquired peak milk yield, but during the remainder of lactation its concentration varied. The ambient temperatures did not influence plasma concentration of GH, insulin, T_3 and T_4 during the lactation cycle. The fat content of milk varied significantly ($p<0.01$) but protein and lactose content of milk remained unchanged during different stages of lactation. Growth hormone was positively correlated with insulin ($p<0.05$) during lactation.

Roy et al., (2003) carried out an analysis to investigate the effect of test day milk yield, test day evening milk yield, parity, stage of lactation and body weight on milk protein concentration. A total of 319 milk samples was collected from buffaloes over four month's period and subjected to protein analysis. They deduced that milk protein did not vary significantly with the test day milk yield as well as test day evening milk yield. Parity and stage of lactation did not have any significant effect on milk protein concentration.

Yoon et al., (2004) conducted a study to assess the effect of milk production, parity, stage of lactation, season and individual milk components of 3,219 Holstein dairy cows in Korean dairy farms. Milk yield negatively correlated with fat and protein contents and somatic cell counts (SCC) in milk ($p<0.01$). They also found that when the somatic cell increased, milk yield reduced and cows in spring and winter produced more milk over 1.43 and 0.93 kg/day, respectively, than cows in summer ($p<0.01$). Milk yield and SCC related positively to the lactation period.

Hayashi et al., (2005) surveyed ten small-scale farms to identify the feeding traits, milk productivity and nutritional status of lactating buffalo and cattle in Terai, Nepal. Body condition score (BCS), milk yield (MY) and plasma metabolites were obtained in the pasture-sufficient, pasture-decreasing and fodder shortage periods

which were August, November and March, respectively. Milk yield of 305-day lactation was estimated by the milk yield of seven consecutive days a month. They reasoned that the buffalo milk yield was significantly higher in the pasture sufficient period than in the other periods (7.7 litres/day vs. 6.5 litres/day, on an average, $p<0.01$). The buffalo decreased MY from the pasture-sufficient period to the pasture-decreasing period but maintained BCS even in the pasture-decreasing period. They ascertained that concentrations of total protein, globulin and BUN in buffalo were significantly higher in the pasture-sufficient period. They also noticed that the average BUN concentration was higher in buffalo than in cattle (13.4 mg/dl vs. 6.0 mg/dl, $p<0.01$).

Ahn et al., (2006) explored the effects of stage of lactation on daily milk yield (DMY), somatic cell score (SCS) and blood glucose in Holsteins (n = 200). The average lactational means and standard deviations of DMY, SCS and blood glucose in the experimental herd were 23.35±7.75 kg, 3.81±2.00 and 44.91±13.12 mg/dl, respectively. SCS was lowest in mid-lactation. The highest blood glucose was observed during mid-lactation.

Yokus and Cakir (2006) reported the seasonal and physiological variations of chloride, calcium, phosphorus, urea, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, globulin, and total protein concentrations in cattle. Two groups of mated (n = 14) and non-mated (n = 10) healthy cows were selected for the study. Serum samples were collected at each of four periods: (1) early pregnancy (May), (2) mid-pregnancy (August), (3) late pregnancy (October), and (4) lactation (February). They showed that physiological variations resulted in changes in the calcium and total protein concentrations. Phosphorus varied only with seasonal but not physiological changes, whereas ALP concentrations changed with physiological and seasonal conditions. Neither the seasonal nor the physiologic variations affected chlorine, calcium, urea, albumin and globulin values in both groups in all periods. They further concluded that the measured total protein might not have reflected its true value because of dehydration

during the hot season. These observations suggested that seasonal and physiologic variations need to be taken into consideration for the correct interpretation of serum chemistry and elements status in cattle.

Zvorc et al., (2006) described the changes in serum concentrations of some micronutrients during pregnancy and lactation of sows. They reasoned that the serum phosphorus concentration decreased towards the end of lactation, while glucose concentration increased during lactation.

Carcangiu et al., (2007) evaluated the state of some haematochemical parameters in dry and lactating Sarda sheep breed. Twenty lactating sheep (Group A) and twenty dry sheep (Group B) were selected for this study; all the sheep were pluriparous and non-pregnant. Blood samples were collected from each animal of Group A on days 7^{th} and 30^{th} of the lactation period and the results were determined as glucose (68.8±8.6 and 60.1±8.2), total proteins (6.8±1.2 and 6.7±1.4), urea (24.6±10.4 and 26.5±12.6), Ca (9.3±1.2 and 9.5±0.9), P (4.8±0.8 and 4.9±1.1) and Mg (2.0±0.2 and 2.0±0.3). Further, in Group A the plasma glucose concentration was higher on day 7^{th} ($P<0.01$) than that determined on day 30^{th}.

Gentry (2007) conducted a series of experiments to evaluate the potential role of leptin in bovines. In his second experiment he found that mean leptin concentrations were not correlated with female age or bodyweight but were positively correlated with body condition scores of beef cattle. Plasma leptin concentrations were lower for lactating cows (1.0 ng/ml) compared with non-lactating cows (2.1 ng/ml) and that the female age did not affect circulating leptin concentrations.

Jabbar et al., (2007) conducted a study on 21 Nili-Ravi lactating buffaloes with similar milk production and stage of lactation. The duration of study was 5 months. The milk production was 6.81 ± 1.80 liter. They disclosed that there were non-significant differences between the fat and SNF percent as well that among the haematological and biochemical parameters among the different groups.

Mishra et al., (2007) selected 22 post-partum Murrah buffaloes of first or second lactation to determine the length of post-partum period for onset of cyclicity by growth hormone (GH). They found that in high yielders, a significant ($P<0.01$) positive correlation between GH and milk yield. GH did not show any significant correlation with plasma glucose concentrations. Concentration of glucose was unaffected by milk yield and was non-significant ($P>0.05$).

Upadhyay et al., (2007) studied the sensitivity of lactating Murrah buffaloes to sudden temperature (T_{max}, T_{min}) change and THI analysed from milk production and climatic records (1994 – 2004) of Karnal. A sudden change *(rise or fall)* in the maximum / minimum temperature during summer and winter was observed to affect milk production. The decline in minimum temperature (>3 °C) during winter and increase (>4°C) during summer than normal were observed to negatively impact milk production upto 30% on the next or subsequent days after extreme event. The return to normal milk production depended on severity and time period of thermal stress/ event occurrence. The R^2 was very low for cool period observed during February – April / Sept – November and actual effect on milk production was minimum. This indicated that low THI had a relatively small effect on milk production performance. The lactation period of animals was shortened during extreme summer when THI was more than 80 and reproductive functions were also adversely affected. They concluded that milk production was likely to be affected due to the warming effects.

Jilek et al., (2008) confirmed the relationship among milk yield in subsequent lactation, reproductive efficiency and BCS development in Czech Fleckvieh dairy cows. The BCS and milk yield were measured once a month and the cows were divided into groups according to their BCS before and after calving. Cows with lower BCS in the 1st month after calving showed an increase in both milk yield and fat and protein. No significant relationship was found between the BCS level before calving and subsequent milk yield. The group of cows with the highest BCS level before calving retained a high BCS level in the first five months of lactation. Adequately, the group of cows with the lowest BCS in the first month of lactation had the lowest BCS

in the next four months. The group of cows with BCS > 3.5 in the 1st month after calving had the most favourable reproduction indicators, also when the milk yield level was taken into account.

Mishra et al., (2008) selected 22 post-partum Murrah buffaloes of first or second lactation from NDRI and reported a significant ($P<0.01$) positive correlation between GH and milk yield in high yielders, whereas no significant difference ($P>0.05$) was seen in the plasma GH profiles in between the high and low yielders.

Nawaz et al., (2008) fed four early lactating Nili-Ravi buffaloes four fat source varied diets and found that milk protein, lactose and solids-not-fat did not differ significantly ($p>0.05$) in control versus those fed fat from various sources, whereas feeding different sources of supplemental fat increased the daily solids-not-fat yield.

Mushtaq (2009) undertook a study to evaluate the role of body condition score (BCS) as an indicator of milk yield and composition in Nili-Ravi buffaloes under subtropical conditions. A total of 36 buffaloes within 1st week of parturition were selected from a private peri-urban dairy farm. Milk yield (kg/d) and BCS (scale 1 – 5) were recorded weekly and milk samples (n = 1008) were collected for analysis of fat, protein and lactose contents. The study continued for 7 months. BCS significantly affected milk yield and fat and protein contents. Lactose was least affected with changes in BCS during lactation. Highest yield was recorded with moderate BCS in buffaloes. BCS correlated positively with milk fat and protein and negatively with milk yield. Milk yield decreased while BCS increased with advancing lactation.

Aggarwal and Singh (2010) carried out an experiment on twelve lactating Murrah buffaloes during early lactation (50-70 days). Six buffaloes were kept under water showers (Group 1) while another group of buffaloes were allowed to wallow in a water pond (Group 2) from 11.00 A.M. to 4.00 P.M. daily for a period of 30 days. Blood samples were collected from buffaloes of both the groups at 3 day intervals and analysed for plasma T_3, T_4, cortisol and insulin hormones. The THI was 80.3 and 83.6 during the hot-dry and hot-humid seasons, respectively. During the hot-dry

season, average plasma T_4 and insulin levels were significantly ($P<0.01$) higher in Group 2 as compared to Group 1. Plasma T_3 levels did not vary significantly in Groups 1 or 2. Plasma cortisol concentration in Group 1 was higher ($P<0.01$) in comparison to Group 2 buffaloes (4.80 vs. 2.60 ng/ml). During the hot-humid season, average T_3, T_4 and insulin concentrations were significantly higher ($P<0.01$) in Groups 2 buffaloes than in Group 1 buffaloes. The overall average value of cortisol was higher in Group 1 when compared to Group 2 buffaloes.

Cincović et al., (2010) performed an experiment including 90 cows divided in batches of 30 cows so that the first third of lactation occurred during summer in 30 cows (G1), the second third of lactation occurred during summer in 30 cows (G2), and in the last 30 cows the last third of lactation was in summer period (G3). The value of THI was between 72 and 82, which indicates the existence of the moderate intensity of heat stress. Heat stress did not damage the milk yield, milk fat and protein percentage on the level of the whole lactation, regardless of the lactation period in which the cows were exposed to stress. There was no correlation between THI and milk yield and quality at the level of the whole lactation. Increased value of THI showed non-significant effect on yield and quality of milk in the first third of lactation. In the middle and at the end of lactation THI was in a significant negative correlation with the yield and quality of milk. Their study showed a significantly lower heat-induced milk yield, milk fat and protein percent in the middle and at the end of lactation.

Hadiya et al., (2010) observed the effect of supplementation of minerals + proteins-vitamins and enzymes on fortnightly plasma profile of macro-micro minerals in 20 freshly calved healthy triple crossbred (HF x J x K) cows, randomly divided into four groups of five animals each, from the day of calving to up to 105 days postpartum. The animals of Group-I served as control. They found that the plasma calcium, inorganic phosphorus and magnesium concentrations in the control group were 9.76±0.46, 4.62±0.13 and 3.52±0.16 mg %, respectively. Calcium level was

significantly higher in enzyme treatment as compared to the control group, while phosphorus showed inverse trend.

Mushtaq et al., (2010) investigated the effect of body condition and pregnancy on milk yield and composition in F1 crossbred cows (Holstein- Friesian X local cows). Fifty-eight cows (60 days postpartum) were selected and the study continued up to 9 months postpartum. They found that non pregnant animals had lower milk yield compared to pregnant ones. BCS affected fat and protein contents as well as milk yield. Milk yield was higher with moderate BCS while fat and protein with higher BCS. BCS correlated negatively with milk yield while positively with fat and protein contents, thus resolving that moderate BCS supported higher milk yield.

Qureshi et al., (2010) examined milk fatty acid variations with body condition in Nili-Ravi buffaloes within 60 days after parturition up to 6 months. The body condition score (BCS), milk yield and composition were recorded once a week. The mean milk yield and fat content were 9.28 kg/d and 5.36%, respectively. The correlation analysis showed that milk yield was negatively affected by BCS and milk fat positively affected, though non-significantly.

Anitha et al., (2011) developed a new body condition score (BCS) system for Murrah buffaloes. The skeletal check points were identified based on the anatomical features and carcass fat reserves and a new BCS chart with a 1 – 5 scale having 0.5 increments examining eight skeletal check points was established.

Antunovic et al., (2011) determined changes in concentrations of biochemical parameters, and metabolic hormones in the blood of Tsigai ewes in the first third of lactation. The study included 10 ewes Tsigai breed monitored during three periods of lactation: 20, 40 and 60 days of lactation. A significant decrease in calcium concentration was recorded in sheep blood at the 40^{th} day of lactation and later an increase at the 60^{th} day of lactation. The opposite trend was determined for concentrations of P-inorganic. They observed a significant increase in the concentrations of glucose and total protein in the first third of lactation. On the 40^{th}

day of lactation there was a significant decrease in the AST activity in contrast to ewes at 20th day of lactation. Concentrations of T_3 and T_4 hormones were slightly increasing in the first third of lactation, but the differences were not significant, whereas the blood insulin concentrations significantly increased in the first third of lactation.

De et al., (2011) in order to observe the effect of different physiological stages on the amount of milk somatic cells secreted by Murrah buffaloes udders, collected milk from 64 Murrah buffaloes, which were then divided into various groups according to their stage of lactation, parity, body condition score (BCS), season and milking practices. There were non-significant changes in milk somatic cell counts (SCC) in early, mid and late lactation. Milk SCC increased non-significantly from the 1st to 4th parity. Milk SCC were significantly higher ($P<0.01$) in day-1 colostrum samples and then decreased when colostrum transformed into milk. No relationship was found between milk SCC and body weight and body condition score. Milk SCC was significantly higher ($P<0.01$) in the summer season *vis-a-vis* winter season. Their results indicated that milk SCC is greater in buffaloes of higher parity and during the months of summer and recommended proper care of these animals to maintain their milk quality.

Gantner et al., (2011) determined the microclimatic conditions in stables in three different climactic regions of Croatia and evaluated the effect of temperature-humidity index (THI) values on the daily production of dairy cattle. Absence of heat stress during autumn and winter season was a characteristic of all three regions. Highly significant ($P<0.01$) decrease of daily milk yield as well as of daily fat and protein content due to enhanced THI was observed in all cows regardless the parity class and in all three climatic regions.

Hussein et al., (2011) investigated the plasma leptin and T_3 in dairy cows and its relationship with blood plasma components and mammary gland functions. They found that the plasma leptin concentration started to decrease one month pre-partum

and reached 3.29 ng/dl; at parturition and continued in low level during postpartum period reached nadir levels at 60 days postpartum (2.71 ng/dl). Plasma leptin concentration correlated negatively with milk solid not-fat yield ($r=-0.26$; $P<0.0985$) and milk protein yield ($r=-0.28$; $P<0.0695$).

Vidu et al., (2011) highlighted the influence of season on the quantity and chemical composition of buffalo milk. In autumn-winter season, the fat content of milk was high, increasing continuously until the end of lactation. Protein content decreased during the winter months and began to rise with the onset of spring. The amount of milk showed a peak in the first month of lactation-winter season, after which there was a steady decline.

Bampidis et al., (2012) used forty lactating Greek buffalo cows in an experiment to determine effects of parity on productivity and milk composition. During the experiment, which commenced on week 6 postpartum and lasted 24 weeks, buffalo cows were allocated, relative to parity, into treatments GBP1 (21 buffalo cows with parity 1, 2, and 3) and GBP2 (19 buffalo cows with parity 4, 5, and 6). During the experiment, there were differences ($P<0.001$) between GBP1 and GBP2 treatments in average milk yield (4.1 vs. 5.3 kg/day), fat yield (0.33 vs. 0.41 kg/day), protein yield (0.19 vs. 0.24 kg/day) and lactose yield (0.21 vs. 0.27 kg/day). In contrast, milk fat (80.8 g/kg), protein (45.9 g/kg), lactose (51.2 g/kg) and somatic cell counts (82.9 ×1000/ml) were not affected ($P>0.05$) by parity.

Farouk (2012) used eighteen lactating buffaloes to study the effect of season on milk production performance and some blood biochemical indicators. The study included two seasons, summer and winter. Results indicated that, during summer season THI values were higher than those of the thermoneutral zone (TNZ), while the winter THI was within the TNZ of lactating buffaloes. Milk production was significantly lower under heat stress in summer than that in winter when ambient temperature (Ta) was around the TNZ. Daily milk yield average (DMY) and total milk yield (TMY) in addition to milk protein % were significantly higher in winter

than those in summer season. Whereas, milk lactose and fat % did not differ between winters and summer seasons. Alanine aminotransferase (ALT) was significantly higher in winter than in summer season, while aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin and globulin showed no significant differences. Albumin/globulin ratio (A/G ratio) was within normal values and did not differ between seasons. A significant positive correlation was found between plasma ALT and each of DMY and TMY, and between plasma ALP levels and each of lactose and SNF %. He concluded that during winter season, buffaloes produced more milk and milk components than summer season. Plasma ALT activity could be used to predict TMY and average of DMY, meanwhile plasma ALP activity could be used to predict milk SNF contents.

Gurmessa and Melaku (2012) evaluated the effect of stage of lactation, on yield and major components of milk in lactating crossbred Holstein Friesian cows. The result showed that lactation stage significantly ($P<0.05$) affected the milk yield. The highest yield was recorded in mid stage and lowest in late stage of lactation. The yield was higher in non-pregnant than pregnant cows. The fat content of the milk was significantly higher ($P<0.05$) in early and late than mid stage of lactation. The solid not fat (SNF) and protein contents of the milk were not significantly affected by stage of lactation.

Han et al., (2012) analysed the physicochemical contents of buffalo milk during a one – year cycle. They found that the average contents of fat, lactose and protein in milk ranged from 6.57 – 7.97 %, 4.49 – 4.73 % and 4.59 – 5.37 % respectively.

Herbut and Angrecka (2012) evaluated changes of temperature and relative air humidity inside a free-stall barn affecting the welfare of cows of three groups during a hot summer. The effects of selected microclimate parameters of the barn were assessed based on the THI (temperature-humidity index) in relation to milk

production. The research revealed that the animals suffered from thermal stress which resulted in decreased milk production in particular groups.

Jozwik et al., (2012) evaluated the effect of milk yield and stage of lactation on the activity of liver enzymes in blood of milking cows. The experiment was carried out on Polish Holstein-Friesian Black and White dairy cows with two different milk yield levels: M – medium (about 7000 kg per lactation) and H – high (about 10 000 kg per lactation). The AST and ALT activities in the blood serum were lower in M group than in H group, however within M and H groups there were no differences in both amino-transferases activity between the 60th and the 200th day of lactation.

Monteiro et al., (2012) analyzed the influence of lactation and dry period on the glucose metabolism of buffaloes using one hundred forty seven blood samples. They concluded that it was difficult to appraise the nature of influence of the mean blood glucose contents despite the significant statistical difference between groups.

Mushtaq et al., (2012) investigated BCS as a regulator of milk yield (MY) and composition (MC) in dairy animals. They observed that higher BCS was maintained by Azakheli (AZ), crossbreds (XB) and Holstein Friesian (HF), medium by NR and lowest by Achai (AC) Sahiwal (SW), Jersey (JC), cattle and Beetal (BT) goats. Highest yield was recorded in buffaloes with moderate BCS. BCS correlated positively with fat and protein and negatively with lactose contents. MY decreased while BCS increased with advancing lactation and MY and BCS correlated inversely.

Piccione et al., (2012) carried out a study on five clinically healthy pregnant and lactating HFs, in good nutritional condition. Blood samples were collected two days before the expected parturition (late gestation), during the post-partum, in early lactation, during the 2nd, 5th and 15th weeks after parturition, at the end of lactation and at the dry period. Serum wad analysed for total proteins, albumin, calcium, phosphorus and magnesium. A significant effect of the physiological phase was observed on total proteins, calcium and phosphorus.

Sarubbi et al., (2012) estimated the genetic parameters for daily milk yield, milk fat and milk protein contents and found that the average, milk production during lactation was 9.21 ± 2.79 kg/d with 8.73% of fat and 4.98% of protein.

Darwesh et al., (2013) used nineteen black does in a study to investigate the impact of lactation stage on body condition, blood metabolism and milk composition. Body condition score (BCS) was assessed together with blood and milk samples, which were collected at week 6 (early), 14 (mid) and 22 (late) of lactation. The overall mean of BCS was 2.35±0.08 and serum total protein and glucose, averaged, respectively 5.80±0.10 g/dl, 120.87±5.34 mg/dl. The milk fat, protein, lactose and solid non-fat percentages averaged 3.24±0.15, 4.49±0.10, 4.44±0.01 and 9.71±0.11, respectively. BCS after kidding decreased significantly at week 4 and then increased significantly during mid and late part of lactation. Lactation stage also significantly affected all milk constituents and glucose.

Djoković et al., (2013a) evaluated the metabolic status of 15 late pregnant and 15 early lactation dairy cows to measure glucose, total protein (TP), albumin, total bilirubin, urea and the activity of aspartate transaminase (AST). Significantly lower glycaemia was found in early lactation cows, AST activities above 100 U/L was detected in 2 early lactation. Glucose levels below 2.5 mmol/L were found in 10 (66.6%) of the early lactation cows, whereas these cows had lower blood serum albumin and urea and higher concentrations of TP and AST.

Djoković et al., (2013b) chose fifteen early-lactation cows and fifteen mid-lactation cows to measure glucose (Glu), total protein (TP), albumin (ALB), urea (U) and the activity of aspartate transaminase (AST). Early lactation cows showed significantly lower glycaemic levels when compared to mid-lactation cows. AST activities above 100 IU/l were detected in two early-lactation and none of the mid-lactation cows. Glucose levels were below 2.5 mmol/l in 10 (66.6%) early-lactation and 5 (33.3%) mid-lactation cows. Early-lactation cows showed lower blood serum

concentrations of ALB, TP and U and higher concentrations of AST, as compared to mid-lactation cows.

Ducháček et al., (2013) evaluated the body condition score (BCS) in the first 4 weeks of lactation of 50 Czech Fleckvieh cows. Average BCS values ranged from 4.14 at calving to 3.6 points in the 4th week of lactation. They detected particularly high BCS values (average 4.14 points) at the beginning of lactation and observed a considerable BCS change from 4.14 to 3.6 points during the entire first month of lactation. Basic statistics of milk composition (fat %, protein % and daily milk yield) and BCS were quite homogeneous.

Hafez (2013) conducted an experiment on two buffalo farms located at Giza (F1) and Qena (F2) governorates to assess leptin profiles as affected by Temperature Humidity Index (THI). The results demonstrated that leptin concentration was greater ($P<0.05$) in F1 (4.15±0.41 ng/ml) than F2 (1.86±0.41 ng/ml). There was a negative ($P<0.05$) correlation between leptin and THI.

Kapa and Alapati (2013) developed a new body condition score (BCS) system for Murrah buffaloes. The skeletal check points were identified based on the anatomical features and carcass fat reserves. A new BCS chart in a 1 – 5 scale using 0.5 increments examining eight skeletal check points was developed. The ultrasonography assessment of the precision of BCS system in 10 buffaloes for each point of the 1 – 5 scale indicated that BCS adequately reflected in the actual fat reserves. The milk production traits like total milk yield upto 18 weeks of lactation (1658.67 kg), 305 day predicted lactation yield (3187.3 kg), peak milk yield (16.5 kg), milk protein and solids not fat were also higher in BCS at calving of 3.5 – 3.99 followed by the BCSc groups of 4.0 – 4.49, 3.0 – 3.49 and 2.5 – 2.99.

Krsmanović et al., (2013) evaluated the blood levels of glucose, total proteins, albumin, urea, total bilirubin in peripartal and peak lactating dairy cows and found them to be within the physiological ranges. The cows in puerperium showed declined ($p <0.05$) concentrations of blood albumin at 29.54 ± 3.89 g/l as compared to the

albumin concentration in cows in peak lactation which was 35.43 ± 3.84 g/l. The lowest blood urea values were detected in cows in early lactation at 4.44 ± 1.60 mmol/l which were significantly lower ($p < 0.05$) when compared to urea nitrogen in the cows during lactation at 5.50 ± 1.30 mmol/l. The highest serum AST activity was found in early lactation cows and was 64.41 ± 18.08 IU/L and was significantly higher ($p < 0.01$) when compared to the AST values during lactation maximum at 39. 47 ± 17.36 IU/L. The cows that were in the maximum established lactation had the highest amount of glucose in the blood (3:10 ± 0:46 mmol/l).

Mir et al., (2013) studied certain biochemical parameters of lactating Murrah buffaloes supplemented with Tinospora cordifolia. Overall plasma GH concentration was 51.80 ± 1.5 and glucose concentration was 14.00 ± 0.5 in the control group.

Mouffok et al., (2013) investigated the correlation between body condition score (BCS), blood biochemical metabolites, milk yield (MY) and quality (Mfat) in Montbéliarde cattle (31 cows) reared in 5 farms of Algerian semi-arid area. The BCS was measured in dry and peak of lactation (6 weeks after calving). They found that the cows' produced 20±4 kg of milk with 3.17±0.72 % of fat at sixth week of lactation. The results showed a significant decrease in postpartum BCS. However, the level of milk production decreased significantly with high glucose.

Pawar et al., (2013) used temperature humidity index (THI) to assess the effect of temperature and relative humidity on performance in animals. In summer, the THI, ranged from 74 – 89 and in winter months THI ranged from 49 – 70. The results showed a significant effect of heat stress on daily milk yield and milk composition. There was decrease in milk yield of 0.028kg per buffalo per day. Heat stress reduced milk yield by 18.2% and significantly reduced milk fat content from 8.3% during the winter to 7.19% during the summer. Milk protein percentage significantly decreased as a result of summer heat stress (3.08 vs.2.9 %, respectively for the winter and summer). SNF decreases from 9.08 to 9.05 %, heat stress reduced SNF % as the THI value went from ≤74 to ≥ 83 in summer.

Tariq et al., (2013) explored a workable and reliable method of predicting BW by using body measurements and body condition scoring (BCS) for implementation of management recommendations for the Nili-Ravi buffalo in small and medium scale commercial dairy production systems in Pakistan. They found a close correlation between the body weight of Nili-Ravi buffaloes and the morphometric variables including heart girth, body length and BCS.

AkhandPratap et al., (2014) evaluated the effect of lactation on yield and components (fat, protein, solid not fat and lactose) of milk in HF cows. The result showed that lactation stage significantly affected the milk yield. The highest yield was recorded in mid stage and lowest in late stage of lactation. The fat content of the milk was significantly higher ($P<0.05$) in early and late than mid stage of lactation. The solid not fat and protein contents of the milk were not significantly affected by stage of lactation and parity.

Kohli et al., (2014) assessed the effect of heat stress on milk production, using thermal humidity index (THI) in 20 high (cross bred cattle) and 20 low milk producing (LMP) cows (native cows). They found that while the low yielding cows did not show any significant change in their milk yield when the THI was above 72 from month June to October during stress condition the high yielders showed a significant decrease in milk yield when THI was above 80 (severe stress zone) in the month of June to October and milk production decreased from an average of 18±1.4 to 10.9±0.92 L. whereas in November – December when THI declined and the cows were in the comfort zone the milk yield did not show any significant rise. T_3 and T_4 levels were lower in summer heat stress condition for a high yielding cattle. They concluded that summer heat stress significantly decreased milk yield in high milk producing (HMP) crossbred cows. As THI rose from comfort zone to stress zone milk yield decreased by 30 – 40% and this loss in milk production was irreversible and management strategies should be used for the HMP crossbred cattle to minimize the heat stress.

Mir et al., (2014) depicted the effect of supplementation of feed with Tinospora cordifolia on milk production and composition, and relative changes in SCC, glucose and somatotropin profile in lactating Murrah buffaloes during summer season. It was found that the average milk yield (kg/day) in the control group was 7.16 ± 0.10, while the differences in milk constituents (protein %, fat %, SNF%, lactose %) between the groups were non-significant. The average somatic cell count (x 10^6/ml) in the milk in the control group was 1.52 ± 0.07, average plasma glucose concentration was 46.64 ± 1.96 and average level of somatotropin (ng/ml) was 9.06 ± 0.79.

Petrovska and Jonkus (2014) analysed the body condition score (BCS) relationship with milk productivity from 49 different breed and lactation dairy cows. They found that the BCS (evaluated using the 5 points system) of all cows decreased from 2.8 ± 0.05 to 2.5 ± 0.04 points in research period. Milk yield increased from 35.6 ± 0.79 kg at around day 14 of lactation to 40.9 ± 1.12 kg around day 47 of lactation. Milk yield decreased around day 80 of lactation. Fat content was the lowest 35.5 ± 0.09 g kg-1 around day 47 of lactation, whereas the protein content differed significantly between 14 and 47 of lactation. Somatic cell changes were not significant. BCS decreased of older lactation cows, but milk yield increased at the same time. Body condition score significantly affected live weight in BCS groups of<2.5 points, 2.75 to 3.0 points. Milk productivity and quality traits were not affected by the body condition score ($p<0.05$).

Silva et al., (2014) assessed the hormonal responses of 20 female buffaloes raised under the sun (SS group) or in the shade (CS group) in Brazil. Blood sample collections to quantitatively determine levels of triiodothyronine (T_3), and thyroxine (T_4) were performed every 14 days, at 13.00 h. Different seasons of the year were also assessed: rainy (January-April), transition (May-July), and less rainy (August-December). The highest $T_3$and T_4 levels were recorded only during the rainy season. T_3 and T_4 were negatively correlated with dry-bulb temperature and globe temperature and humidity index and positively correlated with relative humidity.

Singh et al., (2014a) studied the effect of hot- dry, hot- humid and winter season on plasma hormones and physiological responses in lactating crossbred cows. Blood samples, milk samples, physiological responses and climatic variables were collected daily for a period of seven days in each season. During the hot-humid season there was lowering of plasma T_4 and glucose levels without affecting plasma insulin and T_3 levels. Plasma T_4 was more in winter season in comparison to hot dry and hot humid season. Milk fat, protein, lactose and SNF content varied between seasons and were more in winter than the summer.

Singh et al., (2014b) studied the effect of feeding prill fat on milk production and hormonal changes during mid-lactation (150 days) in crossbred cows. The observations on body condition score (BCS) were recorded and milk composition, plasma metabolites and glucose were measured in control (CON) and experimental prill fat group (PFG) cows. Milk yield, growth hormone, triiodothyronine and thyroxine were significantly lower in CON group. However leptin levels were not affected. Milk fat, protein and lactose were similar in both the groups. Furthermore, plasma glucose varied non-significantly between the groups.

Ashalatha et al., (2015) studied the lactation curve characteristics in relation to BCS in 40 buffaloes and showed that the milk production increased from calving until two months of lactation, reaching peak production and then gradually showed a decline for all the BCS groups of the test herd. The total milk yield upto 18 weeks of lactation was higher for the BCS group of 3.5 – 3.99. For every one unit increase in BCS, an increase of 432.01 kg in the 18 weeks lactation yield was observed, but as the BCS exceeded 3.99, a decrease in milk yield was noticed. The predicted lactation yield was higher for the BCS group of 3.5 – 3.99. The peak milk yield was higher for the BCS group 3.5 – 3.99. For every one unit increase in BCS, an increase of 3.64 kg of peak yield was noticed. Also, the peak yield and persistency index showed a decrease as BCS exceeded 3.99. As the BCS increased from 6 – 8 weeks after calving to 16 – 18 weeks after calving the milk components i.e., fat, protein and SNF showed an increasing trend.

Ashmawy (2015a) determined the influence of physiological status on blood metabolic profile, enzymes and some hormones concentration in the blood of Egyptian buffalo. Investigations were carried out on 12 lactating buffalo cow from 10^{th} day of lactation during winter. The study indicated that there was drop in the calcium and phosphorus levels during early stage of lactation and an opposite trend was recorded for chloride levels.

Ashmawy (2015b) studied the variations in the levels of some hormones in blood plasma of Egyptian buffaloes. Plasma IGF-1 concentrations increased during the first week of lactation and then fell slightly. The plasma leptin concentrations in the dams showed evident decrease from (2.38±0.93 ng/ml) at calving, to (1.87±0.32 ng/ml) at four weeks of lactation.

Atasever and Stádník (2015) determined the effective factors on the variation of daily milk yield (DMY), fat (F%) and protein percentage (P%) in Holstein cows. A total of 278 primiparous cows were examined by four parameters in four calving seasons (CS), three years and six test days (TD) post-calving. While fat values were affected by CS ($P<0.05$), no significant difference was found among DMY and P% by CS. Both year and TD caused significant differences ($P<0.05$) among DMY, F% and P% and correlation of DMY with fat and protein was determined as negative ($P<0.01$; $r= -0.363$ and $r= -0.335$, respectively).

Chaudhary et al., (2015) evaluated the impact of hot dry, hot humid and comfortable season on biochemical parameters in ten lactating Surti buffaloes. They found that with increase in the temperature-humidity index (THI), there was a significant rise in the biochemical parameters such as alanine aminotransferase (ALT), creatinine and blood urea nitrogen and significant decline in glucose and triiodothyronine (T_3).

Cinar et al., (2015) investigated the effect of somatic cell count (SCC) on milk yield and milk composition in 30 first and 49 second lactation Holstein dairy cows. They observed that SCC had a high significant effect on milk yield, milk protein,

milk lactose, total solids and milk urea-N, however, the effect of SCC on milk fat was not significant. Their study indicated that high SCC negatively affects not only milk yield but also milk composition and quality.

Djoković et al., (2015a) investigated the metabolic and endocrine status in Simmental dairy cows during peripartum period and mid lactation for relationships between growth hormone (GH), triiodothyronine (T_3), thyroxine (T_4), glucose, total protein (TP) and albumin. They found that the early lactation cows had higher serum concentrations of GH, TP and lower blood serum concentrations of T_3, glucose, albumin and urea as compared to mid lactation cows.

Djoković et al., (2015b) evaluated the endocrine and metabolic changes in Simmental dairy cows during the transition period and mid lactation. Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T_3) and thyroxine (T_4) by ELISA methods and glucose, total protein (TP), albumin and urea by different colorimetric techniques. Early lactation cows were found to have higher blood serum concentrations of GH and lower blood serum concentrations of insulin, T_3, T_4, glucose, albumin and urea compared to late pregnant and mid lactation cows.

Mithuna et al., (2015) conducted a farm study on 60 buffaloes to elucidate the effect of pre-partum supplementation on the body condition score (BCS) of the dam and milk production among local buffaloes reared under a mixed farming system in Bidar district, Karnataka. Supplementation was done for 90 days till parturition using concentrate feed of known composition. Results revealed that prepartum supplementation had a significant ($P \leq 0.01$) effect on BCS of dam before and at the end of the 3rdmonth of lactation. Milk yield was significantly ($P \leq 0.01$) higher in dams supplemented (4.45±0.20, 4.62±0.20, 4.90±0.20) compared to the non-supplemented groups (3.50±0.20). However, no significant difference was observed in the milk constituents like milk fat, solid not fat and total solids.

Patbandha et al., (2015) studied the effect of season and lactation stage on milk components of Jaffrabadi buffaloes. Overall milk fat, protein, lactose and solid not fat (SNF) were 8.31±0.37, 4.31±0.06, 5.66±0.09 and10.93±0.18, respectively. There was significantly higher protein percent during rainy and lower during winter (4.37±0.05 and 4.20±0.05%, respectively), but milk lactose percent was significantly higher during winter (5.46±0.07%) and lower during rainy (5.79±0.07%). Stage of lactation had also significant effect on milk components; milk fat and protein increased significantly with the advancement of lactation stage; whereas, milk lactose decreased significantly. Milk fat during early, mid and late lactation was 7.65±0.10, 8.36±0.10 and 8.92±0.11%; protein was 4.25±0.04, 4.24±0.05 and 4.44±0.05%, respectively. However, milk lactose percent was 5.83±0.06, 5.65±0.07 and 5.51±0.07%, respectively during early, mid and late lactation. The results of their investigations indicated that season and stage of lactation affect certain milk components in Jaffrabadi buffaloes and could be minimized by better farm management practices.

Singh et al., (2015) assessed the effect of body condition score (BCS) on milk yield and composition of 50 HF cross cows and 50 Murrah buffaloes. The animals were divided into three groups on the basis of BCS at calving and kept in observation for 120 days. The BCS values in Group III animals were significantly higher than those in Group I animals. The daily milk fat was higher in groups with high BCS than in groups with lower BCS; however, SCS was lower in the milk of Group III than other groups. They proposed that milk fat could be positively correlated with BCS at calving.

Al Reyad et al., (2016) observed the effect of heat stress on milk yield and milk compositions of Holstein Friesian crossbred (HF) dairy cows. The temperature humidity index (THI) of July, August, September and October were 84.95, 81.99, 81.40 and 79.57, respectively. The highest THI was found in July which indicated higher heat stress during this month. A significant difference ($p<0.05$) in milk yield of cows was found among different months from July to October. The highest milk yield (6.10±0.50 l/h/d) was found in October among observed months. The

compositions of milk such as solids-not-fat (SNF), fat, protein and lactose also differed significantly ($p<0.01$). The highest values (%) of SNF, fat, protein, lactose and ash content of milk were found in October as 8.80, 3.83, 3.69 and 4.39 respectively and lowest values (%) were in July as 8.50, 3.71, 3.50 and 4.30 respectively due to the high THI value.

Bhat et al., (2016) undertook an investigation to study some blood biochemical parameters during different stages in different lactations in Toggenberg goats. These included control group, consisting of dry goats and group I, II, III and IV containing goats in 1st, 2nd, 3rd and 4th lactation period. The total protein concentration showed an increasing trend from early to late lactation stages and increasing levels of albumin in group I, II and IV and globulin in group II and III were seen. Lowest total protein concentration was observed in third lactation (group III). Albumin and globulin ratio was higher in early lactation in group III (third lactation); whereas, in group II and IV, highest ratio was observed during mid-lactation. Glucose concentration showed a definite increasing trend from early to late lactation stages in different lactations. Significantly lower ($P<0.05$) glucose levels were found in early as well as mid lactations as compared to late stage in all the four groups. Increasing trend of urea concentration was observed from early to late stage of lactation in all the lactating groups. The total protein, globulin, glucose and urea levels were found higher in lactating goats as compared to dry animals.

Das et al., (2016) investigated the biochemical profile of 18 Mehshani buffaloes categorized into three groups based on the length of their lactation: early stage, mid stage and late stage. The glucose level was recorded to be the lowest in the early stage of lactation; whereas, the protein and creatinine concentrations were slightly higher in this stage. No significant alteration in the concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was noticed amongst the three groups of buffaloes.

Pande et al., (2016) monitored 12 high yielding buffaloes in their transition period to assess the alterations in haemato-metabolic status and body condition. Glucose and BUN concentrations peaked at calving, the levels at 3-week postpartum were higher as compared to the prepartum values.

Patel et al., (2016) reported the reference values for different blood metabolites, minerals and some clinically important enzymes as well as their variation in serum concentration at different age, sex, physiological stages *viz*. pregnancy, dry and lactation of Banni buffalo (*Bubalus bubalis*). They observed that the concentrations of glucose, total protein, creatinine, BUN, inorganic phosphorus, ALP and ALT showed significant variation with lactation unlike the other biochemical analytes. The levels of albumin, A/G ratio and creatinine were apparently lower in lactating buffaloes than dry buffaloes. Conversely, BUN, cholesterol and uric acid were higher in lactating buffaloes than dry buffaloes.

Anarjan et al., (2017) investigated the relationships and correlations among some blood metabolites associated with energy and protein balance in lactating Holstein dairy cows. Blood and milk samples were collected from fifty-six lactating Holstein dairy cows based on their reproductive status *(in 35–45 d post AI)* and blood urea and glucose were measured by enzymatic colorimetric method. Cows at 56 to 63 day post AI were divided in to two groups of Pregnant (PG; n=25) and Non–pregnant (NPG; n=31) by touché rectal pregnancy diagnosis. In NPG group, there were significant correlations among milk yield and some energy balance related metabolites (glucose) concentrations and correlations among milk yield and protein balance related blood urea was significant in both NPG and PG groups. Furthermore, there were significant differences between means of monthly milk yield between two groups. In conclusion, although evidence exists for adverse effects of elevated circulating urea on fertility, pregnant cows were able to adapt to elevated circulating urea over several days.

Behera et al., (2017) tried to identify the most suitable temperature humidity index (THI) model among seven reported THI models for analyzing the impact of thermal stress on monthly test day fat % (MTDF%), monthly test day SNF% (MTSNF%), monthly test day fat yield (MTDFY) and monthly test day SNF yield (MTDSNFY) of Murrah. Regression analysis was performed for identifying the best THI to assess the impact of heat stress on milk constituent traits under study and a negative association was found between the milk constituent traits and monthly average THI values. The THI model [THI = (0.55 × Tdb + 0.2 ×Tdp) × 1.8 + 32 + 17.5] developed by NRC (1971) was identified as the most suitable THI model to assess the impact of heat stress on milk composition traits of Murrah indicating maximum decline in MTDF% (-0.005), MTDFY (-0.68 g), MTDSNF% (b=-0.0008) and MTDSNFY (-2.25 g) per unit rise in THI.

Dhillod et al., (2017) correlated the milk yield of Murrah buffaloes with certain body parts measurements. Their study revealed that buffaloes had positive significant correlation between 305 days milk yield (MY) and body weight and the buffaloes had an average 2604.8 ± 39.5 kg MY.

Faith et al., (2017) analysed some blood biochemical parameters (urea, albumin, globulin and creatinine) as well as, calcium (Ca) and magnesium (Mg) by spectrophotometer. A total of 20 ewes were divided into two groups of 10 pregnant (P) and 10 lactating (10) ewes ranging from 2 to 4 years of age. The pregnant ewes had statistically higher concentration of albumin in the blood compared to the lactating ewes due to decreased in albumin over the lactation which could be explained by a rapid extraction of immunoglobulin from the plasma during the last few months of pregnancy when colostrum's is being formed in the mammary gland. Calcium concentration of both pregnant and lactating ewes were not significant. Magnesium (Mg^{++}) concentration for both pregnant and lactating ewes were not significant but there was an increase in the concentration of pregnant ewes while a gradual decrease was observed in lactating ewes though the concentration remained within the normal range. The creatinine concentrations were significant and the

highest concentration was recorded at lactating ewes. Globulin concentrations were not significant for both pregnant and lactating ewes, although there was superior increase in concentration in lactating ewe compared to pregnant ewes.

Lasheen et al., (2017) evaluated the effect of nutrition and temperature humidity index (THI) on dairy cattle milk production and composition. Milk production and composition were negatively affected by increasing of THI. As the THI increased from 63 to 76, the animal performance decreased in milk production and composition.

Nagre and Kuralkar (2017) carried out a study to assess the levels of glucose, insulin and leptin during late gestation and early lactation in Murrah buffaloes. They found that all the variables were significantly affected by transition period. The levels of insulin, glucose and leptin was significantly higher 15 days before parturition, followed by 15 days after parturition and lowest on the day of parturition.

Nasr and El – Tarabany (2017) explored the impact of temperature-humidity index (THI) on somatic cell count (SCC), milk production and composition on daily milk test records (33600) of Holstein cows under subtropical Egyptian conditions with different levels of THI. Their results revealed that daily milk yield (MY) and composition (fat%, protein %, and the lactose %) were higher in low THI when compared with high THI. SCC significantly increased 36% from low to high. At high THI level, SCC was negatively correlated with total MY, protein %, fat% and lactose %. They concluded that dairy cows performance was better in most of the investigated parameters at low THI than those in high THI, thus, indicating a detrimental effect of THI on both welfare and economic return.

Behera et al., (2018) identified the most suitable temperature humidity index (THI model) among seven reported THI models as heat stress indicator on daily milk yield (DMY) of Murrah buffaloes at subtropical climatic conditions of Karnal, India. The overall least-squares mean for daily milk yield was 7.55±0.002 kg and the

average daily THI was calculated using each of the seven models under study. Regression analysis was performed to conclude the most suitable THI model for assessing the effect of heat stress on DMY and a negative association was found between DMY (kg) and THI. THI model developed by NRC (1971) was identified as the most suitable THI model to study the impact of thermal stress.

Chitra et al., (2018) investigated the effect of genetic and non-genetic factors of first lactation 305 days milk yield (FLMY305), fat yield (LFY), fat percentage (FAT%) and solid not fat percentage (SNF%) in Murrah buffaloes. Very high and positive genetic and phenotypic correlations of FLMY305 with milk constituents' yield traits inferred that selection based on FLMY305 would result in correlated response in milk constituents yield traits and therefore need not to be considered separately for their improvement.

Das et al., (2018) executed a study in 18 clinically healthy lactating Mehshani buffaloes. They observed that although the level of different minerals and electrolytes varied numerically during the three lactation stages, only Ca was found to be significantly lower in early stage unlike the Cl, which was significantly higher in early lactation stage.

Kekana et al., (2018) studied the effects of high thermal stress on serum protein metabolites, milk production of transition dairy cows in semi-arid areas in South Africa. Summer thermal-humidity index (THI) of the areas were THI-1 (72–83: extreme caution) and THI-2 (75–87: danger). Milk yield was recorded daily and samples collected for milk fat, protein, lactose and urea nitrogen analysis. Heifers in THI-2 had lower total serum proteins, albumin and blood urea nitrogen than THI-1. Post-calving, cows in THI-1 had higher ($P < 0.05$) TP (73.4 *vs* 67.9 g/l) and BUN (4.61 *vs* 3.77 mmol/l) at 21 days in milk (DIM), and lower creatinine at 21 and 75 DIM than THI-2 group. Milk yield, fat and protein in THI-2 were all lower ($P < 0.05$) than THI-1 21 DIM. The results confirm that heat stress affects utilisation of nutrients in transition dairy cows.

Kiran and Dey (2018) examined the effect of composite feed additive on fluctuations and correlation of milk lactose concentration the lactating buffaloes. A total of 18 Murrah lactating buffaloes (*Bubalus bubalis*) (avg. milk yield 10.83± 1.56 kg) and (avg. live weight, 507.24 ± 44.18 kg; parity, 2-5) at early stage (30 days) of lactation were selected. In the initial stages of lactation milk lactose concentrations was 4.51±0.23 in the control group. The concentration of milk lactose in weekly milk samples throughout the experiment remained variable among the buffaloes.

Kumar et al., (2018) assessed the haemato-biochemical profile of healthy Murrah buffaloes (n=30) in different phases of lactation viz. (I) early lactation (II) mid lactation (III) dry animals. Haemato-biochemical alterations showed decreased glucose levels in group-III, lowest levels in group-I and highest levels in group-II. Significant decrease in serum calcium was recorded in animals during early lactation. Significantly higher levels of blood urea levels were recorded in group-I and II, than the other groups and serum creatinine levels were significantly increased in group III. Higher activity of serum enzymes were recorded during mid-lactation.

Sales et al., (2018) studied the buffalo milk composition used in manufacturing Mozzarella cheese. The mean ± standard deviation values for physico-chemical characteristics of raw buffalo milk estimated by them were 6.40 ± 0.17 fat, 3.80 ± 0.16 total protein, 5.11 ± 0.07 lactose and 10.15 ± 0.21 solid-not-fat.

Silva et al., (2018) investigated the relationship among somatic cell scores (SCS) and certain traits viz. milk yield, fat, protein, lactose and no-fat-solids contents in milk in Holstein dairy cows in a semi-arid climate. The results showed positive correlations among SCS and fat, protein and solids-non-fat contents, while the SCS and lactose content and milk yield were negatively correlated. The highest milk yield (34.43 kg / cow / day) was obtained for the lowest SCS (0; 0 to 24 cells x 1000/mL).The milk yield and lactose decreased while protein and fat contents increased when SCS increased mostly above score five (400 to 799 cells x 1000/mL).

It was observed that the increase in SCS influenced negatively milk yield and composition in Holstein cows in the semi-arid climate in Brazil.

Surya Prakash et al., (2018a) compared the biochemical profile and its correlation with milk production in Gir, Kankrej and crossbred cattle. The animals were divided in to three groups *viz.* 0 – 90 days of lactation (Group I), 91 – 180 days of lactation (Group II) and 181 till dry stage (Group III). The values of glucose, were higher in Gir, as compared to Kankrej and crossbreds, while cholesterol, total proteins and albumin, were higher in crossbreds as compared to two other breeds. Stages of lactation had significant effect on all biochemical parameters except albumin. Non-significant differences were observed between the milking phases in all the stages and the breeds.

Surya Prakash et al., (2018b) compared the activity of important endocrine parameters and enzymes and its correlation with milk production in six Gir, Kankrej and Crossbred cattle. The animals were divided in to three groups' *viz.* 0 – 90 days of lactation (Group I), 91 – 180 days of lactation (group II) and 181 till dry stage (Group III). The levels of T_3 andT_4 increased with advancement of lactation and they were negatively correlated with milk yield.

Tamami et al., (2018) investigated the effect of the temperature humidity index (THI) and days in milk (DIM) on milk production traits and somatic cell score (SCS) of dairy cows raised in north area of Iran. Greatest milk yields were recorded in THI $\leq$60 ($P<0.05$). The highest decrease in milk yield in connection with THI values were recorded in the early lactation (0 to 100 DIM). SCS was positively associated with the THI and increased more in early period of lactation.

Wildridge et al., (2018) assessed the effect of temperature-humidity index (THI) on milk yield. Daily measures of average milk yield per cow during December to February (Australian summer) were assessed for associations with maximum, minimum, and average THI. Average daily milk yield per cow was negatively associated with an increasing maximum, minimum, and average THI (-0.11, -0.08,

and -0.15 kg/THI unit increase, respectively) on the collection day and up to three days prior. Their results suggested that high THI was negatively associated with milk yield.

Yadav et al., (2018) chose twelve advance pregnant Murrah buffaloes of identical parity and similar previous lactation yield and randomly assigned to two groups of six animals each; CON as control with basal diet and SBO as soybean oil supplementation @ 200 ml/animal/day upto 90 days post-partum. The BCS during entire study were statistically similar in both the groups. The fortnightly average daily milk yield was 7.90±0.06 and the analysis of milk constituents *viz*. milk SNF, protein, lactose and fat (%) in the control group were 9.84±0.09, 3.82±0.03, 5.18±0.05 and 6.89±0.01, respectively.

Zhou et al., (2018) compared the milk protein, fat and lactose profiles of Murrah buffalo, Nili-Ravi buffalo and crossbreed buffalo. Milk protein and total solids contents of Murrah buffalo were higher than those of Nili-Ravi buffalo. The average milk protein, fat and lactose contents of all buffalo samples were 4.76, 7.31 and 5.19 /100g of milk, respectively.

Çinar et al., (2019) investigated the physio – chemical properties of Anatolian water buffalo milk, and from six different provinces in Turkey. The fat amount in water buffalo milk samples was in the range of 5.97% to 9.19% and the mean fat was 6.96%, lactose was in the range of 4.38% to 5.44%, and the mean lactose was 4.97%, SNF was in the range of 9.96 to 8.12 and the mean SNF was 9.14% and the milk proteins were in the range of 3.77% to 3.12% with a mean protein was 3.48%.

Gianesella et al., (2019) assessed the changes in the concentration of serum protein fractions and milk in clinically healthy in Italian Mediterranean buffaloes during early lactation. Blood for serum total proteins, albumin, globulins and albumin/globulin ratio (A/G) values and milk for fat, protein and lactose percentages along with milk yield were collected from 30 buffaloes at 7, 30 and 50 days after

calving. Milk yield and fat % changed significantly throughout the monitoring period ($P < 0.005$).

Golla et al., (2019) assessed negative energy balance (NEB) indicators in Murrah buffaloes by estimating serum biochemical and endocrine parameters (growth hormone [GH], insulin-like growth factor1 [IGF1], insulin, and leptin). They deduced that the NEB condition was probably restricted to the first month of early lactation and simultaneous higher free fatty acids and lower leptin levels could act as direct plausible metabolic indicators of NEB in buffaloes.

Luke et al., (2019) scrutinized the use of midinfrared (MIR) spectroscopy on milk for predicting serum metabolite concentrations. Serum and milk samples were collected from 773 early-lactation Australian HF cows (between July and October 2017). The serum albumin, globulin, urea, calcium and magnesium concentrations were measured and milk samples were analyzed by MIR spectroscopy. The predictions for calcium, magnesium, albumin, and globulin concentrations were poor. They concluded that MIR spectroscopy of milk showed promise for predicting the serum urea concentration, however, more data would be needed to improve prediction accuracies.

Savaliya et al., (2019) carried out an experiment to understand the effect of fogger cooling on Temperature-Humidity Index (THI), milk yield and milk composition in Jaffrabadi buffaloes during summer season at Cattle Breeding Farm, Gujarat (India) for a period of 9 weeks (from April to June, 2017). Thirty lactating Jaffrabadi buffaloes were divided in two groups of 15 each. Buffaloes of Group I (control) were kept under loose housing without any cooling system, while buffaloes of Group II (experiment) were kept under loose housing with fogger cooling system operated from 11.00 a.m. to 4.00 p.m. Significantly ($P<0.05$) lower THI value was observed in Group II buffalo shed. Milk yield and fat percent increased significantly ($P<0.05$) in Jaffrabadi buffaloes of group II. They therefore resolved that the fogger cooling system was beneficial in terms of body comfort of the animals by reducing

heat stress as well as increasing milk yield and fat percent in Jaffrabadi buffaloes during summer season.

Wang et al., (2019) explored the physicochemical composition of milk produced by Chinese local buffalos which were crossed with Murrah and Nili-Ravi over a 210-day period. They found that the protein, fat, ash and total solids contents of milk from the hybrids decreased and as the lactation progressed, the lactose content also increased, but this change was not significant after the 15^{th} day postpartum.

Materials & Methods

3. MATERIAL AND METHOD

The study was conducted on 15 apparently healthy lactating Murrah buffaloes in their 2nd – 4th lactation maintained at a farm in Bhiwandi, Thane District, Mumbai, Maharashtra. Majority of the samples processing and analysis was carried out in the Department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai. The enzyme-linked immunosorbent assay was done in the Department of Veterinary Public Health, Mumbai Veterinary College, Parel, Mumbai and the radioimmunoassay was facilitated by the Laboratory Nuclear Medicine Section (LNMS), Tata Memorial Centre, Mumbai.

3.1. Selection of experimental animals

The farm owner's conscent was procured and animal selection completed before the commencement of the study and only those buffaloes who were at the farm for more than three months were included. Buffaloes having a BCS score of minimum 2.5 and about to parturate were included in the study. Twenty apparently healthy lactating Murrah buffaloes in their 2nd to 4th parity, maintained at a farm in Bhiwandi, Thane District, Mumbai were selected. These buffaloes were aged between 5 – 7 years and their average milk yield was 8 – 16 liters per day.

Out of the twenty animals selected, fifteen completed 210 days of lactation and were included in the study. The animals were neither artificially inseminated nor allowed to mate and were therefore were open throughout the duration of the study.

3.2. Nutrition and management of the experimental animals

The buffaloes were maintained under uniform and standard conditions of feeding and management. The animals were housed in animal shed with asbestos cement roof, under natural daylight and temperature conditions. They were given a maintenance ration of 15 kg of para grass and 2.5 kg of concentrate mixture and *ad libitum* clean drinking water, twice daily at the time of milking. Paddy straw or locally procucred grass when available was given *ad libitum*. The buffaloes were milked by hand milking by expert milkers twice daily.

Deworming was done regularly. Routine vaccinations were carried before the onset of the monsoons against hemorrhagic septicemia, black quarter and brucellosis as per the schedule prescribed by the farm veterinarian. The foot and mouth disease vaccination was repeated six monthly in all buffaloes present on the farm and periodic brucellosis check-ups were a farm mandate.

3.3. Temperature Humidity Index and Body Condition Score Evaluation

The Temperature Humidity Index (THI) was measured using the National Research Council formula THI = (Tdb + Twb) × 0.72 + 40.6 (NRC, 1971). An NABL laboratory accredited Wet and Dry Thermometer was placed at a suitable location on the farm and the readings on the day of collection were documented.

Fortnightly body condition score (BCS) of the experimental animals was recorded using the scoring system of 1 to 5 point scale using 0.50 increments (Anitha *et al.,* 2011).

3.4. Sample collection and processing: Milk

During the afternoon milking, just before blood collection, milk samples were collected from the milk weighing bucket after complete milking and through mixing. About 10 – 20 ml of milk was collected aseptically into clean and dry plastic bottles and these vials were immediately placed into the chilled ice box to carry out subsequent physiochemical analysis in the college laboratory.

3.5. Sample Analysis: Milk

The milk samples were collected on the 7^{th} and 15^{th} day of parturition and thereafter at fortnightly intervals (days 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th}, 105^{th}, 120^{th}, 135^{th}, 150^{th}, 165^{th}, 180^{th}, 195^{th} and 210^{th} of lactation) till 210 days of lactation (drying off) from the same buffaloes throughout their lactation period. The milk yield (litres) was recorded on each sampling day, pooling the morning and evening collection. Milk composition of each buffalo was determined using the automatic milk analyzer for the percentages of fat, protein, lactose and solids not fat (SNF) on the day of collection itself. The milk analyser "Milkotronic" marketed by "New Dairy Engg & Trading Co. (P) Ltd." (www.milkotronic.com, Made in Bulgaria) was used for this analysis.

3.6. Sample collection and processing: Blood

Blood samples for the study were collected aseptically, just after milking, on 7^{th} and 15^{th} day of parturition and thereafter at fortnightly

intervals (days 30th, 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th, 180th, 195th and 210th of lactation) till 210 days of lactation (drying off) from the same buffaloes throughout their lactation period by jugular vein puncture into serum clot activator tubes to separate out the serum.

The serum was separated by centrifugation at 2000 rpm, made into duplicates and stored collectionwise. Samples for proteins and enzymes were processed within 24 hours in the Department of Veterinary Physiology, Mumbai Veterinary College, Parel, Mumbai. The remaining serum was stored at –20 ºC for hormone and electrolyte analysis.

3.7. Sample Analysis: Serum metabolites and enzymes

The analysis of serum total proteins, albumin, urea, creatinine, glucose, glutamate-pyruvate transaminase / alanine aminotransferase (SGPT / ALT), glutamic-oxaloacetic transaminase / aspartate aminotransferase (SGOT / AST), calcium, phosphorous, magnesium and chloride was done on the Prietest Touch Autoanalyser (Robonik, India).

3.7.01. Estimation of total protein

Method:

Spectrophotometric method according to Biuret method, End point

Principle:

Proteins together with copper ions form a violet blue colour complex in alkaline solution. The absorbance of the colour is directly proportional to the concentration.

Proteins + Cu^{2+} ⟶ Coloured complex

Reagents: Components and concentrations:

Sodium Hydroxide: 0.1 N

Potassium Sodium Tartarate: 16 mmol / l

Copper Sulphate: 6 mmol / l

Preservative and Stabilizer

Standard: 6.0 g/dl

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Reaction Slope	:	Increasing
Wavelength 1	:	546 nm
Temperature	:	37 °C
Zero Setting	:	Reagent Blank
Standard Concentration	:	6
Units	:	g/dl
Sample Volume	:	20 µl
Reagent Volume	:	1000 µl
Incubation Time	:	10 minutes
Range Linearity	:	15

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	---	---
Standard	---	20 µl	---
Sample / Control	---	---	20 µl

Mix and read absorbance (A) after 10 minutes of incubation but within 60 minutes

Calculation: With standard or calibrator.

$$\text{Conc in sample (g/dl)} = \frac{\text{conc of std}}{\text{A std} - \text{A RB}} \times \text{A sample} - \text{A RB}$$

where, conc = concentration, RB = reagent blank and std = standard

3.7.02. Estimation of albumin

Method:

Spectrophotometric method using Bromocresol Green (BCG)

Principle:

Serum albumin in the presence of bromocresol green at a slightly acid pH produces a colour change of the indicator from yellow-green to green-blue.

$$\text{Albumin + BCG} \xrightarrow{\text{pH = 4.20}} \text{Albumin – BCG complex}$$

Reagents: Components and concentrations:

Concentration are those in final Test reaction

Succinate Buffer: 75 mmol / l

Bromocresol Green: 0.15 mmol / l

Preservatives and Stabilizer

Standard: 4.0 g/dl

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Reaction Slope	:	Increasing

Wavelength 1	:	630 nm
Temperature	:	37 °C
Zero Setting	:	Reagent Blank
Standard Concentration	:	4
Units	:	g/dl
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Incubation Time	:	05 minutes
Range Linearity	:	6

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	---	---
Standard	---	10 µl	---
Sample / Control	---	---	10 µl

Mix and read absorbance (A) after 05 minutes of incubation but within 60 minutes

Calculation: With standard or calibrator.

$$\text{Conc in sample (g/dl)} = \frac{\text{conc of std}}{\text{A std} - \text{A RB}} \times \text{A sample} - \text{A RB}$$

where, conc = concentration, RB = reagent blank and std = standard

3.7.03. Estimation of globulin

Globulin (g/dl) = Total Proteins – Albumin

3.7.04. Estimation of Albumin to Globulin (A: G) ratio

A : G = Albumin / Globulin

3.7.05. Estimation of urea

Method:

Enzymatic – UV, Kinetic

Principle:

Enzymatic determination according to the following reactions

$$\text{Urea} + 2H_2O \xrightarrow{\text{urease}} 2NH^+ + Co_3^{2-}$$

$$NH_4 + \text{Tris Base} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glu} + \text{NAD} + H_2O$$

Reagents:

R1:	**R2:**
Tris base: 120 mmol / l	NADH: 0.25 mmol / l
2 – Oxo glutarate: 7 mmol	Preservatives & Stabilizer
Urease: > 6 KU / l	
Glutamate dehydrogenase: > 1 KU / l	

Standard: 40 mg/dl (6.66 mmol / L)

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	Fixed Time
Reaction Slope	:	Decreasing
Wavelength 1	:	340 nm
Temperature	:	37 °C
Zero Setting	:	Distilled Water
Lag Time	:	30 Seconds
Read Time	:	60 Seconds
Standard Concentration	:	40
Units	:	mg/dl

Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Range Linearity	:	300
Initial OD	:	> 1.0
Max Delta / Min	:	0.5

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Standard	Sample / Control
Reagent R1	800 µl	800 µl
Reagent R1	200 µl	200 µl

Mix and incubate at 37 °C for 2 minutes then add

	Standard	Sample / Control
Standard	10 µl	---
Sample / Control	---	10 µl

Calculation: With standard or calibrator.

$$\text{Conc in sample (mg/dl)} = \frac{\text{conc of std}}{\Delta \text{ A std}} \times \Delta \text{ A of sample}$$

where,
conc = concentration and std = standard

Conversion factor:

Urea (mg / dl) x 0.1665 = Urea mmol / l

Urea (mg / dl) / 2.14 = BUN mg / dl

3.7.06. Estimation of Creatinine

Method:

Kinetic test without deproteinization according to Jaffe method.

Principle:

Creatinine forms a coloured orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the creatinine in the sample.

Creatinine + Alkaline Picrate ⟶ Creatinine picrate complex

Reagents: Components and Concentration:

R1:

Picric Acid: 9 mmol / l

R2:

Sodium Hydroxide: 0.4 mol / l

Preservatives and Stabilizer

Standard: 2 mg/dl (177 µmol / L)

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	Fixed Time
Reaction Slope	:	Increasing
Wavelength 1	:	510 nm
Temperature	:	37 °C
Zero Setting	:	Distilled Water
Lag Time	:	30 Seconds
Read Time	:	90 Seconds
Standard Concentration	:	2
Units	:	mg/dl
Sample Volume	:	100 µl
Reagent Volume	:	1000 µl

Range Linearity	:	20
Initial OD	:	> 0.400
Max Delta / Min	:	0.60

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Standard	Sample / Control
Working Reagent	1000 µl	1000 µl
Standard	100 µl	---
Sample / Control	---	100 µl

Mix and read the variation of absorbance between 30 seconds and 90 seconds (ΔA).

Calculation: With standard or calibrator.

$$\text{Conc in sample (mg/dl)} = \frac{\text{conc of std}}{\Delta \text{ A std}} \times \Delta \text{ A of sample}$$

where, conc = concentration and std = standard

3.7.07. Estimation of SGPT / ALT (serum glutamate-pyruvate transaminase / alanine aminotransferase)

Method:

IFCC method without pyridoxal phosphate, Kinetic, UV.

Principle:

Kinetic determination of the GPT activity:

$$\text{L – Alanine} + \alpha\text{ – Ketoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L – Glutamate}$$

$$\text{Pyruvate} + H^+ + \text{NADH} \xrightarrow{\text{LDH}} \text{L – Lactate} + NAD^+$$

Reagents: Components and Concentrations

R1:

Tris-Buffer: 100 mmol / l

L-Alanine: 500 mmol / l

Lactate Dehydrogenase: >1200 U / L

R2:

α – Ketoglutarate: 150 mmol/l

NADH: 0.18 mmol / l

Reagent Preparation: The reagents are ready to use.

Automated Parameters:

Reaction	:	Kinetic
Reaction Slope	:	Decreasing
Wavelength 1	:	340 nm
Temperature	:	37 °C
Zero Setting	:	Distilled Water
Factor	:	1746
Units	:	IU/L
Sample Volume	:	100 µl
Reagent Volume	:	1000 µl
Lag Time	:	60 Seconds
Read Time	:	180 Seconds
Reference Range	:	0 to 40
Range Linearity	:	400
Initial OD	:	> 1.0
Max Delta / Min	:	0.229

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Sample / Control
Reagent R1	800 µl
Reagent R1	200 µl

Mix and incubate at 37 °C for 2 minutes then add

	Sample / Control
Sample / Control	100 µl

Mix and after a 60 seconds incubation at 37 °C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

Calculation: Activity of sample (U/L) = (ΔA / min) x 1746

3.7.08. Estimation of SGOT / AST (serum glutamic-oxaloacetic transaminase / aspartate aminotransferase)

Method:

IFCC method without pyridoxal phosphate, Kinetic, UV.

Principle:

Kinetic determination of the GOT activity:

$$\text{L – Alanine} + \alpha\text{ – Ketoglutarate} \xrightarrow{\text{GOT}} \text{Oxaloacetate} + \text{L – Glutamate}$$

$$\text{Oxaloacetate} + H^+ + \text{NADH} \xrightarrow{\text{MDH}} \text{L – Malate} + NAD^+$$

Reagents: Components and Concentrations

R1:

Tris: 80 mmol / l

L - Aspartate: 240 mmol / l

Lactate Dehydrogenase: >600 U / L

Malate dehydrogenase: > 600 U / L

R2:

α – Ketoglutarate: 150 mmol/l

NADH: 0.18 mmol / l

Preservatives and Stabilizer

Reagent Preparation: The reagents are ready to use.

Automated Parameters:

Reaction	:	Kinetic
Reaction Slope	:	Decreasing
Wavelength 1	:	340 nm
Temperature	:	37 °C
Zero Setting	:	Distilled Water
Factor	:	1746
Units	:	IU/L
Sample Volume	:	100 µl
Reagent Volume	:	1000 µl
Lag Time	:	60 Seconds
Read Time	:	180 Seconds
Reference Range	:	0 to 38
Range Linearity	:	400
Initial OD	:	> 1.0

Max Delta / Min : 0.229

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Sample / Control
Reagent R1	800 µl
Reagent R1	200 µl

Mix and incubate at 37 °C for 2 minutes then add

	Sample / Control
Sample / Control	100 µl

Mix and after a 60 seconds incubation at 37 °C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

Calculation: Activity of sample (U/L) = (ΔA / min) x 1746

3.7.09. Estimation of glucose

Method:

Enzymatic (GOD / POD), Spectrophotometric method according to Biuret method, End point

Principle:

Glucose oxidase (GOD) converts the sample Glucose into gluconate. The hydrogenperoxide (H_2O_2) produced in the reaction is degraded by peroxidase (POD) and gives a coloured product phenol and 4-Aminoantipyrine which is measurable using trinder indicator reaction at 505

nm. The increase in absorbance correlates with the glucose concentration of the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4\ H_2O$$

Reagents: Components and concentrations:

Phosphate Buffer: 100 mmol / l

Glucose Oxidase: >8 U/ml

Peroxidase: >0.6 U/l

4 – Amino Antipyrine: 0.28 mmol/l

Preservative and Stabilizer

Standard: 100 mg/dl (5.55 mmol/L)

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Reaction Slope	:	Increasing
Wavelength 1	:	510 nm
Temperature	:	37 °C
Zero Setting	:	Reagent Blank
Standard Concentration	:	100
Units	:	mg/dl
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Incubation Time	:	10 minutes
Range Linearity	:	500

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into

the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	---	---
Standard	---	10 µl	---
Sample / Control	---	---	10 µl

Mix and read absorbance (A) after 10 minutes of incubation at 37 °C but within 60 minutes.

Calculation: With standard or calibrator.

$$\text{Conc in sample (mg/dl)} = \frac{\text{conc of std}}{\text{A std} - \text{A RB}} \times \text{A sample} - \text{A RB}$$

where,
conc = concentration, RB = reagent blank and std = standard

Conversion factor:

Glucose (mg / dl) x 0.0555 = Glucose mmol / L

3.7. 10. Estimation of calcium

Method:

Spectrophotometric method using Arsenazo III, End Point

Principle:

Calcium with Arsenazo III [2, 7 – (bis (2 – arsonophenyfazo)) – 1, 8 – dihydroxynaphtaiene – 3, 6 – disulphoric acid], at neutral pH yields a blue coloured complex, whose intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by addition of 8-Hydroxyquinoline-5-sulfonic acid.

Reagents: Components and concentrations:

Imidazole Buffer: 100 mmol / l

8 – Hydroxy Quinoline: 5 mmol / l

Arsenazo III: 120 µmol / l

Peroxidase: >0.6 U/l

Preservative and Stabilizer

Standard: 10 mg/dl (2.50 mmol/L)

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Wavelength 1	:	630 or 623 nm
Temperature	:	37 °C
Zero Setting	:	Reagent Blank
Standard Concentration	:	10
Units	:	mg/dl
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Incubation Time	:	02 minutes
Range Linearity	:	15

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given overleaf:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	---	---
Standard	---	10 µl	---
Sample / Control	---	---	10 µl

Mix and read absorbance (A) after 02 minutes of incubation.

Calculation: With standard or calibrator.

$$\text{Conc in sample (mg/dl)} = \frac{\text{conc of std}}{\text{A std} - \text{A RB}} \times \text{A sample} - \text{A RB}$$

where,

conc = concentration, RB = reagent blank and std = standard

Conversion factor:

Calcium (mg / dl) x 0.2495 = Calcium mmol / L

3.7. 11. Estimation of phosphorous

Method:

Spectrophotometric method, End Point, UV

Principle:

Inorganic phosphorous reacts in acid environment with molybdic acid to form an unreduced phosphomolybdic acid complex, which absorbs light at 340 nm. The absorbance is directly proportional to the phosphorous concentration in the sample.

Reagents: Components and concentrations:

Ammonium molybdate: 0.4 mmol / l

Sulphuric acid: 210 mmol / l

Preservative and Stabilizer

Standard: 5 mg/dl (1.62 mmol/L)

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Wavelength 1	:	340 nm
Temperature	:	37 °C
Zero Setting	:	Reagent Blank
Standard Concentration	:	5
Units	:	mg/dl
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Incubation Time	:	5 minutes
Range Linearity	:	15

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	---	---
Standard	---	10 µl	---
Sample / Control	---	---	10 µl

Mix and read absorbance (A) after 05 minutes of incubation.

Calculation: With standard or calibrator.

$$\text{Conc in sample (mg/dl)} = \frac{\text{conc of std}}{\text{A std} - \text{A RB}} \times \text{A sample} - \text{A RB}$$

where, conc = concentration, RB = reagent blank and std = standard

Conversion factor:

Phosphorous (mg / dl) x 0.323 = Phosphorous mmol / L

3.7. 12. Estimation of chloride

Method:

Spectrophotometric method, End Point, UV

Principle:

Chloride ions in an acidic environment in presence of ferric nitrate form a coloured complex with mercuric thiocynate. Intensity of the developed colour is proportional to the sample chloride ion concentration.

Reagents:

R1 Chloride reagent: 0.4 mmol / l

Standard: 100 mEq/L

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point (Increasing)
Wavelength 1	:	500 nm
Light path	:	1 cm
Reaction Temperature	:	Room temperature
Blank / Zero Setting	:	Reagent
Standard Concentration	:	100
Units	:	mEq/L
Sample Volume	:	0 µl
Reagent Volume	:	10 µl
Incubation Time	:	01 minute
Range Linearity	:	120

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into

the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Standard	---	10 µl	---
Sample / Control	---	---	10 µl

Mix and read absorbance (A) after 01 minute of incubation time.

Calculation: With standard or calibrator.

$$\text{Concentration in sample (mEq/L)} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

3.7. 13. Estimation of magnesium

Method:

Spectrophotometric method, End Point, UV

Principle:

At alkaline pH magnesium reacts with xylidyl blue and produces a chelating red colored compound. The red increasing or the blue decreasing colors are proportional to magnesium concentration.

Reagents: Components and concentrations:

R1: Xylidyl Blue Reagent

Standard: 2.5 mg/dl

Reagent Preparation: The reagent and standard are ready to use.

Automated Parameters:

Reaction	:	End point
Wavelength 1	:	520 nm
Light path	:	1 cm
Reaction Temperature	:	Room temperature

Blank / Zero Setting	:	Reagent
Standard Concentration	:	2.5
Units	:	mg/dl
Sample Volume	:	10 µl
Reagent Volume	:	1 ml
Incubation Time	:	05 minutes
Range Linearity	:	5

Test procedure:

The kit contents and samples were brought to room temperature prior to use. The test tubes were labelled as sample and control. Then into the duly labelled test tubes was pipetted the reagent followed by either the sample or control as per the table given below:

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Standard	---	10 µl	---
Sample	---	---	10 µl

Mix and read absorbance (A) after 05 minutes of incubation at room temperature.

Calculation: With standard or calibrator.

$$\text{Concentration in sample (mg/dl)} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard}$$

3.8. Sample Hormone Analysis: ELISA (Serum IGF1 and Leptin)

The serum insulin like growth factor 1 (IGF1) and leptin concentrations were estimated using ELISA (Enzyme Linked Immunosorbent Assay) kits. Serum IGF1 was estimated using KINESISDx Bovine IGF1 ELISA kit, Cat No: K04 – 0046 and serum leptin using

KINESISDx Bovine Leptin ELISA kit, Cat No: K04 – 0174, 1179, W 29th St., Apt. 9, Los Angeles, CA 90007, USA (www.kinesisdx.com). The concentrations of IGF-1 and leptin are expressed as nanorgam per milliliter (ng/ml).

3.8.01. Estimation of IGF1

Method:

ELISA

Principle:

The kit uses a double – antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of bovine insulin like growth factor 1 (IGF1) in samples. IGF1 is added to wells pre-coated with monoclonal IGF1 antibody. After incubation, added IGF1 secondary antibodies labeled with biotin followed by Streptavidin-HRP to form immune complex. Unbound immune complex is removed by washing step. Then addition of Chromogenic Solution A and B, develops blue colour, and stop solution is added to stop the reaction. The concentration of bovine IGF1 is directly proportional to the colour developed.

Materials:

1 Microtiter Coated Plate (96 wells)
2 Bovine IGF1 Biotin Conjugated Detection Antibody
3 Standard, 1920 ng/ml
4 Streptavidin: HRP Conjugate
5 Wash Buffer (30X)
6 Standard Diluent
7 Substrate A
8 Substrate B
9 Stop Solution

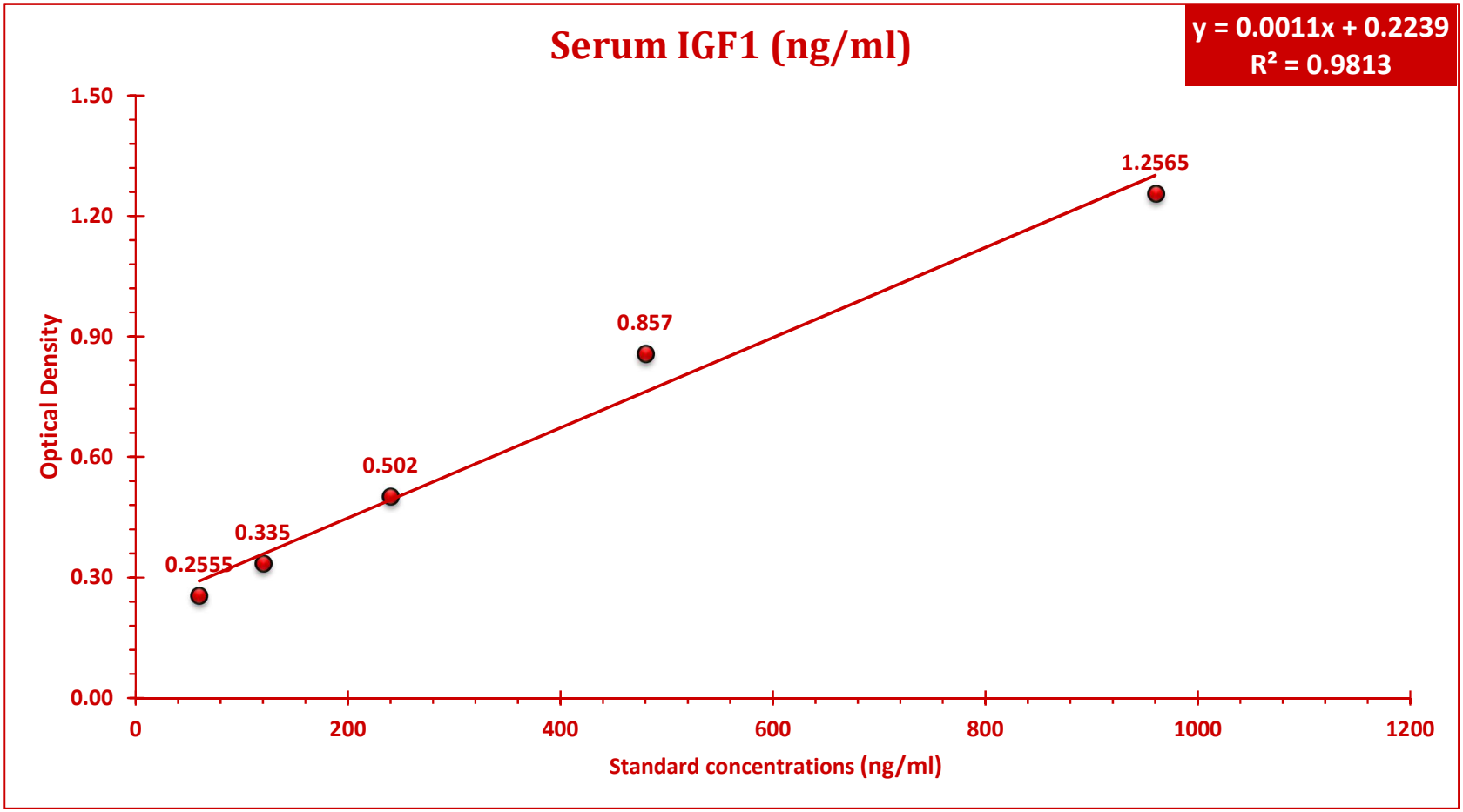

Figure 01. Standard curve for the determination of bovine serum insulin like growth factor 1

Sample: Serum

Reagents:

Reagents were diluted immediately prior to use. To make 1x Wash Solution, 10 ml of 30X Wash Buffer was add to 290 ml of deionized water.

Assay Procedure:

(i) All reagents were brought to room temperature prior to use.

(ii) A standard curve was formulated using the mean of the optical densities (absorbance) got for each assay as the kits were the same.

(iii) Standards Dilution: the standards were prepared as per the table given below using the provided standard concentration and standard diluent.

960 ng/ml	Standard No. 05	120 µl Original Standard (1920 ng/ml) + 120µl Standard diluent
480 ng/ml	Standard No. 04	120 µl Standard No. 05 + 120 µl Standard diluent
240 ng/ml	Standard No. 03	120 µl Standard No. 04 + 120 µl Standard diluent
120 ng/ml	Standard No. 02	120 µl Standard No. 03 + 120 µl Standard diluent
60 ng/ml	Standard No. 01	120 µl Standard No. 02 + 120 µl Standard diluent

(iv) The number of strips required for the assay were remove.

(v) 50 µl of Standards and 40 µl Samples was pipetted out into the respective wells as mentioned in the above list.

(vi) The blank well did not contain the sample, biotin conjugate and streptavidin-HRP.

(vii) 10 µl of biotin conjugate was pipetted into each sample well.

(viii) 50 µl of streptavidin-HRP conjugate was pipetted into each sample and standards well.

(ix) The plates were covered and incubated for 1 hour at 37 °C in the incubator.

(x) Then, they were aspirated and washed 4 times with 1X wash buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper.

(xi) Any liquid from the bottom as well as outside of the microtiter wells

was wiped off as any residue could interfere in the reading. All the washes were performed in the similar fashion.

(xii) Then 50 µl Substrate A followed by 50 µl Substrate B was pipetted into each well including the blank and gently mixed.

(xiii) The plate was again incubated for 10 minutes at 37 ºC in the dark.

(xiv) 50 µl of stop solution was pipetted into which the wells changed colour from blue to yellow.

(xv) Absorbance was read at 450 nm within 15 minutes of adding the stop solution blanking on the zero standards.

Calculation of Results:

The mean optical density was calculated of each standard duplicate. A standard curve was plotted with the the standards concentration on the X-axis and mean optical densities on the Y-axis, which is presented in Figure 01 (previous page). A 4 – parameter curve ELISA software was used to calculate the mean optical density of each unknown. The values of the unknowns were read directly off the standard curve.

3.8.02. Estimation of Leptin

Method:

ELISA

Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Bovine Leptin in samples Leptin is added to wells pre-coated with monoclonal Leptin antibody. After incubation, added Leptin secondary antibodies labeled with biotin followed by Streptavidin-HRP to form immune complex. Unbound immune complex is removed by washing step. Then addition of Chromogenic Solution A and B, develops blue colour, and Stop solution is

added to stop the reaction. The concentration of Bovine Leptin is directly proportional to the colour developed.

Materials:

1 Microtiter Coated Plate (96 wells)

2 Bovine Leptin Biotin Conjugated Detection Antibody

3 Standard, 32 ng/ml

4 Streptavidin: HRP Conjugate

5 Wash Buffer (30X)

6 Standard Diluent

7 Substrate A

8 Substrate B

9 Stop Solution

Sample: Serum

Reagents:

Reagents were diluted immediately prior to use. To make 1x Wash Solution, 10 ml of 30 X Wash Buffer was add to 290 ml of deionized water.

Assay Procedure:

(i) All reagents were brought to room temperature prior to use.

(ii) A standard curve was formulated using the mean of the optical densities (absorbance) got for each assay as the kits were the same.

(iii) Standards Dilution: the standards were prepared as per the table given overleaf using the provided standard concentration & standard diluent.

(iv) The number of strips required for the assay were remove.

(v) 50 µl of Standards and 40 µl Samples was pipetted out into the respective wells as mentioned in the list overleaf.

16 ng/ml	Standard No. 05	120 µl Original Standard (32 ng/ml) + 120µl Standard diluent
8 ng/ml	Standard No. 04	120 µl Standard No. 05 + 120 µl Standard diluent
4 ng/ml	Standard No. 03	120 µl Standard No. 04 + 120 µl Standard diluent
2 ng/ml	Standard No. 02	120 µl Standard No. 03 + 120 µl Standard diluent
1 ng/ml	Standard No. 01	120 µl Standard No. 02 + 120 µl Standard diluent

(vi) The blank well did not contain the sample, biotin conjugate and streptavidin-HRP.

(vii) 10 µl of biotin conjugate was pipetted into each sample well.

(viii) 50 µl of streptavidin-HRP conjugate was pipetted into each sample and standards well.

(ix) The plates were covered and incubated for 1 hour at 37 °C in the incubator.

(x) Then, they were aspirated and washed 4 times with 1X wash buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper.

(xi) Any liquid from the bottom as well as outside of the microtiter wells was wiped off as any residue could interfere in the reading. All the washes were performed in the similar fashion.

(xii) Then 50 µl Substrate A followed by 50 µl Substrate B was pipetted into each well including the blank and gently mixed.

(xiii) The plate was again incubated for 10 minutes at 37 oC in the dark.

(xiv) 50 µl of stop solution was pipetted into which the wells changed colour from blue to yellow.

(xv) Absorbance was read at 450 nm within 15 minutes of adding the stop solution blanking on the zero standards.

Calculation of Results:

The mean optical density was calculated of each standard duplicate. A standard curve was plotted with the the standards concentration on the X-axis and mean optical densities on the Y-axis, which is presented in Figure

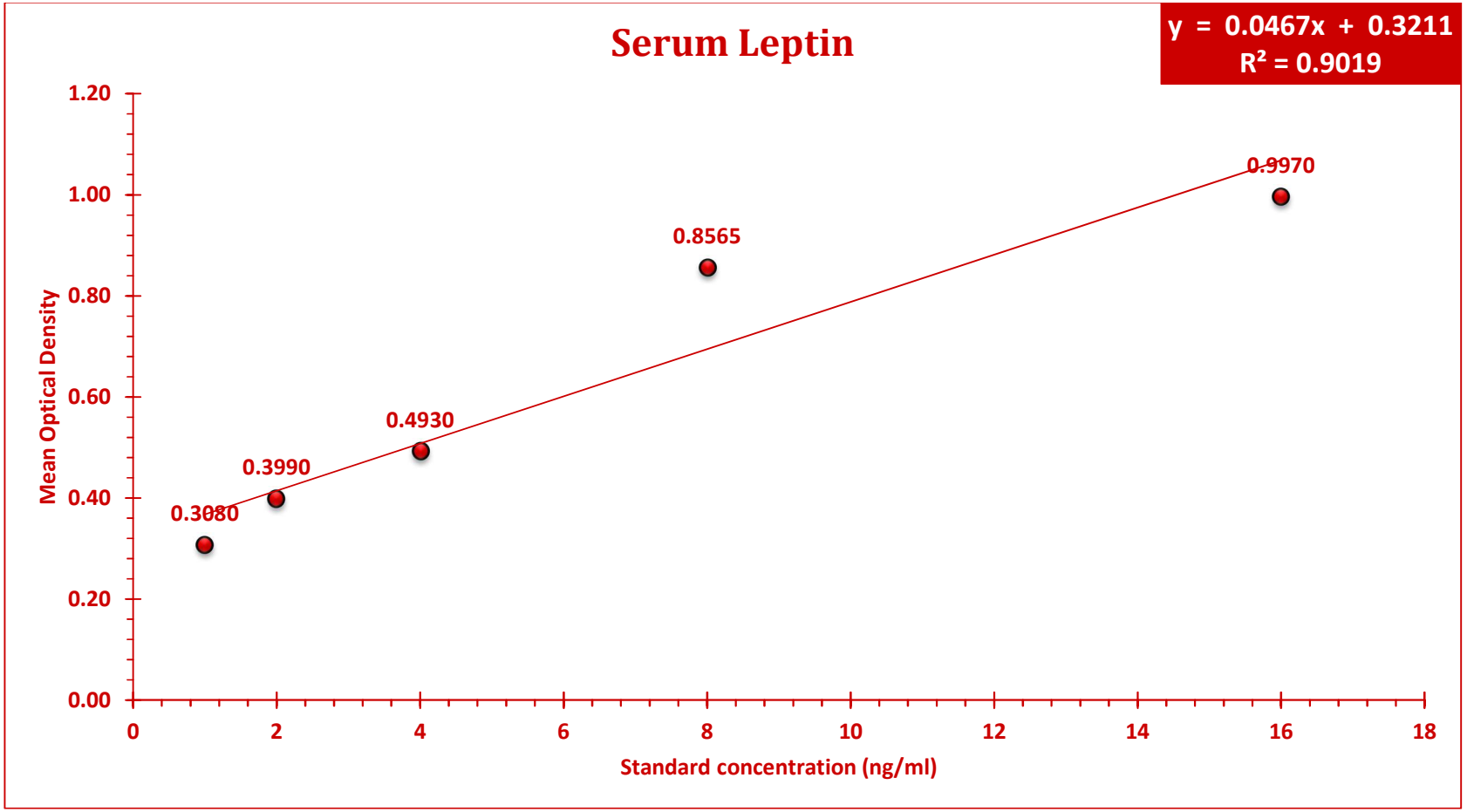

Figure 02. Standard curve for the determination of bovine serum leptin

02 (previous page). A 4 – parameter curve ELISA software was used to calculate the mean optical density of each unknown. The values of the unknowns were read directly off the standard curve.

3.9. Sample Analysis: RIA (GH, T3, T4, Cortisol and Insulin)

The radioimmunoassay estimations of serum growth hormone concentration was done by using modified adsorption coating technique (Kumarasamy, 2001), those of triiodothyronine (T_3), thyroxine (T_4) and cortisol by using RIA kits supplied by Immunotech, France and insulin by using RIA kits supplied by BRIT, BARC, Mumbai. The tracer I^{125} was used to estimate the binding competition between free and isotope tagged hormones to the limited antibody sites and quantification was done using a calibration curve.

3.9.01. Estimation of growth hormone (bGH)

Method:

Radioimmunoassay (RIA)

Principle:

Radioimmunoassay involves the separation of a protein (from a mixture) using the specificity of antibody - antigen binding and quantitation using radioactivity.

Immuno-reactants for GH assay

The bovine growth hormone antigen and bovine growth hormone antiserum were procured from Dr. A.F. Parlow, Scientific Director, National Hormone & Peptide Program (NHPP).

(i) Bovine growth hormone antigen highly purified, for iodination and standard USDA-bGH, approximately 114 µg / ampoule, lyophilized.

(ii) Bovine growth hormone antiserum (monkey), NIDDK-anti-bGH 1.0 ml, 1:50 dilution lyophilized, in 2% normal monkey serum in phosphosaline buffer (PBS).

Dilution of bGH antibody

Bovine growth hormone antibody (antiserum) was acquired from National Hormonal and Pituitary Programme. The vial had, in a lyophilized form, 1 ml of anti-bovine GH antibody (1:50 diluted in 2% standard monkey serum) which was then reconstituted with 1 ml distilled water. Working dilutions of 1:25000, 1:30000, 1:40000 and 1:50000 stocks were prepared from this 1:50 diluted antibody stock, in 0.02 M phosphate buffer (pH 8.5).

bGH antibody coating of tubes

(i) Heat activation of the polystyrene (star) tubes was done using an ovan (microwave) for 15 seconds.

(ii) 300 µl of 1:40000 diluted anti-bGH antibody was then added to the appropriately labelled activated tubes.

(iii) The tubes were kept at room temperature overnight, to enable the antibodies to coat the tubes.

(iv) The following day, the excess antibody from the tubes was regained by incubation, aspirated and then stored for future coating.

(v) The labelled activated tubes were blocked using 500µl of 2% BSA solution for 3 – 4 hours (room temperature).

(vi) After blocking, they were aspirated and washed thrice with 1 ml PBS (0.02 M).

(vii) These washed tubes were allowed to air dry and upon drying stored in self – sealing bags with desiccant sachets.

bGH iodination

(i) The G25 sephadex column was soaked in distilled water overnight.

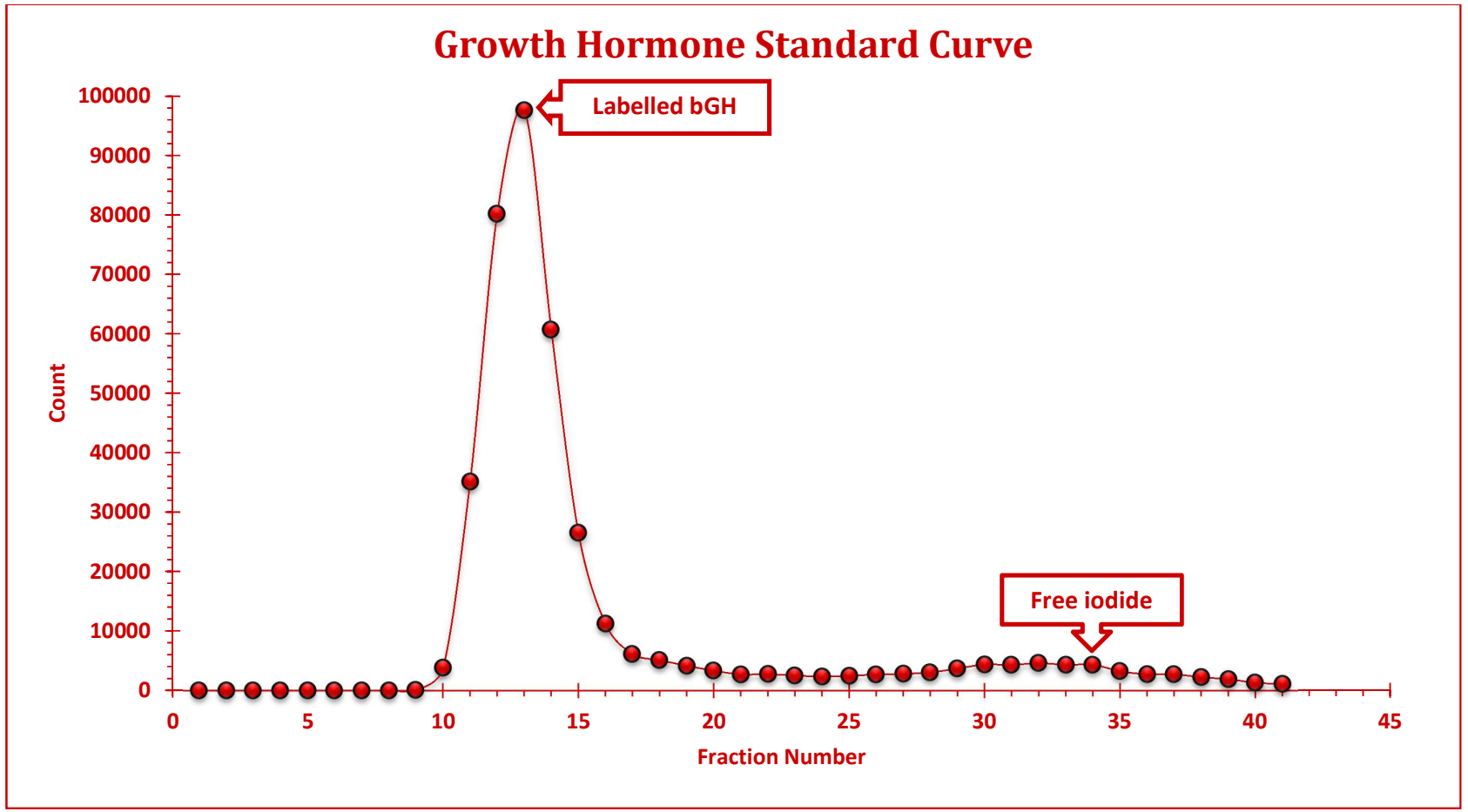

Figure 03. Bovine growth hormone iodination

(ii) This slurry was used for packing 10 cm columns in disposable syringes. The column was equilibrated in PBS with 2% BSA.

(iii) 1 mg of iodination grade bGH was dissolved in 1 ml of 0.01 M sterilized bicarbonate buffer (pH 8.6). This stock was aliquoted and frozen at -20 °C.

(iv) Iodogen coated tubes were used for the iodination reaction. 50 µl of 0.5M PBS (pH 7.4) was pipetted into the iodogen coated tubes and 5 µg of bGH was suspended in it. 500 µCi ^{125}I was added and the reaction was carried out for 3 minutes.

(v) The whole reaction mixture was passed through Sephadex G25 column. 1% BSA – PBS solution was used as elution buffer. 40 fractions of 500 µl were collected. The radioactivity was determined using a gamma counter and iodination graph is presented in Figure 03.

(vi) Fractions 12, 13, 14 showed the maximum count and were used for tracer preparation. 2 ml of 3% BSA-PBS solution was added to each of the peak tubes as stabilizer and these peak tubes were aliquoted and stored.

(vii) The working tracer was prepared in 3% BSA-PBS solution by taking 200 µl of iodinated tracer from tube no. 12. The count in the working tracer solution was adjusted to about one lakh counts per minutes.

bGH Assay

(i) The standard curve and concentration of serum GH were determined by adsorption coating tubes technique (Kumarasamy, 2001). The anti-bGH raised in monkey was coated to polystyrene (PS) tubes as per the method developed in Radiation Medicine Centre, BARC, Mumbai.

(ii) The GH antibody coated tubes were arranged serially. In Tube No. 01 and 02, 100 µl of labeled hormone was added (approximately 1,00,000 cpm Total count tubes).

(iii) In Tube No. 03 and 04, 200 µl HFS and 100 µl labeled hormone was added (‘0’ standard tubes).

(iv) Tube No. 5 onwards standards and samples were added in duplicate as stated in the table overleaf:

Tube No.	Standard hormone (GH) with different concentrations	Labeled (I^{125}) (GH) hormone
1, 2 (Total Count)	---	100 µl
3, 4 (Zero Binding)	200 µl HFS	100 µl
5, 6	200 µl (0.65 ng/ml std)	100 µl
7, 8	200 µl (1.25 ng/ml std)	100 µl
9, 10	200 µl (2.5 ng/ml std)	100 µl
11, 12	200 µl (5.0 ng/ml std)	100 µl
13, 14	200 µl (10.0 ng/ml std)	100 µl
15, 16	200 µl (20.0 ng/ml std)	100 µl
17, 18 onwards (Samples)	200 µl	100 µl

(v) These tubes were then incubate at room temperature for 24 hours.

(vi) After incubation, they were washed with 2 ml wash solution and aspirated.

(vii) Using a gamma counter for one minute each tube was calculated.

(viii) The serum bGH concentration was calculated using a logit log graph for corresponding tube.

(ix) Serum GH concentration was expressed in ng/ml and the assay sensitivity was 0.65 ng/ml.

(x) The inter – assay and intra – assay coefficients of variation were 11.5 % and 8.5 %, respectively.

3.9.02 Radioimmunoassay of triiodothyronine

Radioimmunoassay kits procured from Immunotech, France were used to determine the serum triiodothyronine (T_3) concentration. The samples were analysed in duplicates. After pipetting the serum samples (25 µl) in the RIA antibody coated tubes 25 µl of the standards ranging from 0 – 12 nmol/l was added and this was followed by the addition of 200 µl of I^{125} triiodothyronine tracer in all the tubes. The tubes were gently vortex mixed

and then placed in a horizontal shaker at 280 rpm at 25 °C for an hour. Then the supernatant was decanted and tubes were wiped clean without disturbing the pellet. The radioactivity was measured using a gamma counter for a minute and the results were expressed in ng/ml.

3.9.03 Radioimmunoassay of thyroxine

Radioimmunoassay kits procured from Immunotech, France were used to determine the serum thyroxine (T_4) concentration. The samples were analysed in duplicates. After pipetting the serum samples (20 µl) in the RIA antibody coated tubes, 20 µl of the standards ranging from 0 – 420 nmol/l was added and this was followed by the addition of 500 µl of I^{125} thyroxine tracer in all the tubes. The tubes were gently vortex mixed and then placed in a horizontal shaker at 280 rpm at 25 °C for an hour. Then the supernatant was decanted and tubes were wiped clean without disturbing the pellet. The radioactivity was measured in gamma counter for a minute and the results were expressed in ng/ml.

3.9.03 Radioimmunoassay of insulin

Radioimmunoassay kits procured from Board of Radiation and Isotope Technology, BARC, Mumbai were used to determine the serum insulin concentration. Multiple RIA tubes were prepared using different dilutions of the standards (between 7.5 µU/ml to 200 µU/ml) with 200 µU/ml assay buffer. The samples were analysed in duplicates. Insulin free serum (100 µl) was added in the blank and standard tubes. This was followed by pipetting 100 µl insulin antiserum in all the tubes except the total count and NSB tubes. The contents were mixed carefully and incubated overnight in a refrigerator (4 °C). The next day 100 µl of ^{125}I insulin tracer

was added in each tube and mixed using the vortex mixer. After allowing the tubes to rest at room temperature for three hours, 100 µl of the second antibody (antiguinea pig IgG) was added to all the tubes except the total counts tube. 1000 µl of polyethylene glycol (PEG) was then added. The tubes were mixed well and allowerd to rest at room temperature for 20 minutes after which they were centrifuged using a refrigerated centrifuge at 4 °C at 1500 rpm for 20 minutes. Decanting of the supernatant was followed by drying the pellets. Gamma counter counting of the radioactivity of the bound fraction was carried out and results were expressed in µU/ml.

3.9.04 Radioimmunoassay cortisol

Radioimmunoassay kits procured from Immunotech, France were used to determine the serum cortisol concentration.

Principle

The radioimmunoassay of cortisol is a competition assay. Samples, control and calibrators are incubated in monoclonal antibody coated tubes with ^{125}I-labelled cortisol tracer. After incubation, the liquid contents of the tube are aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve.

RIA procedure for cortisol

(i) Antibody coated tubes for cortisol calibrators, control and samples were labeled and two tubes for total counts.

(ii) 50 µl calibrators control and samples were pipetted into the appropriate tubes. Total count tubes remain empty at this stage.

(iii) To all tubes 500 µl of cortisol tracer including the total count tubes was added.

(iv) All tubes were mixed briefly on a vortex mixer.

(v) Tubes were incubated for an hour at 18 – 25 °C with shaking at 400 rpm.

(vi) The tubes were decanted except the total count tubes against adsorbent paper.

(vii) Each tube was counted using a gamma counter for a minute.

3.10. Statistical analysis

Analysis of variance of the data was done according to Snedecor and Cochran (1998) using complete randomised design. Differences in means were tested using critical difference (CD) test.

Results & Discussion

4. RESULTS AND DISCUSSION

The present investigation was designed to investigate the effect of temperature humidity index (THI) on blood metabolites, hormone profile, milk composition and body condition score (BCS) in lactating Murrah buffaloes. The lactation period lasted for 210 days. The buffalo remained healthy and were kept open until the end of the lactation. The observations of the different parameters are represented in tabular and graphical form for better comprehension.

4.1 Milk Yield

The mean milk yield on day 7^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th}, 90^{th}, 105^{th}, 120^{th}, 135^{th}, 150^{th}, 165^{th}, 180^{th}, 195^{th} and 210^{th} of lactation in lactating Murrah buffaloes is presented in Table 1.01, its analysis of variance in Table 1.02 and its graph is depicted in Figure 04.

The average milk yield (litres) on day 07 of lactation was 7.92 ± 0.54 litres, which augmented numerically, but non-significantly upto Day 45. Significant ($P < 0.05$) rise in the milk yield was observed after Day 45 *i.e.* from Day 60 to Day 75 which showed the highest milk yield of 11.09 ± 0.52 litres. Further, it declined significantly ($P < 0.05$) to 8.88 ± 0.36 litres on Day 90, remained almost similar till Day 150 of lactation, and from thereon decreased non-significantly on Day 165. Later there was a highly significant ($P < 0.01$) fall in the milk yield from Day 180 upto the Day 210 which was the end of lactation recording the lowest milk yield of 4.55 ± 0.36 litres. The average milk yield of the lactation was 8.24 ± 0.48 litres.

Lactation length is an important trait influencing milk yield in buffaloes and in Murrah buffaloes it ranges from 245 days to 355.39 days as per Singh and Barwal, 2010 and Afzal *et al.,* (2007) who have chronicled a significant positive correlation between lactation length and milk yield.

The total lactational yield of the buffaloes in our experiment was approximately 1734 litres and the lactation length was 210 days and this is within the average range (1200 to 2100 kg per lactation) proposed by Ibrahim *et al.,* 2012 for a buffalo having a lactation length of 210 to 280 days.

Higher yield than that in the present study has been reported by Afzal *et al.,* (2007) who recorded 1831.6 ± 530.9 liters per lactation for an average lactation length of 273.3 ± 52.8 days in Nili-Ravi buffaloes. Other authors who cited higher yields per lactation in Nili-Ravi buffaloes are Chaudhry (1992) who reported a total lactational milk yield of 2031.08 kg, Cady *et al.,* (1983) of 1811 kg, Khan and Chaudhry (2000) of 1984 kg and Malhado *et al.,* (2007) of 1,863.50. Higher yields in Murrah buffaloes were quoted by Shabade *et al.,* (1993) at 1,892.21 kg, Aspilcueta-Borquis *et al.,* (2010) at 1,813.5 and Jakhar *et al.,* (2016) at 2182.82 kg. Better feeding and longer lactation could be probable incentive for this alteration.

Ours results are in tandem with those reported by Dhar and Deshpande (1995) at 1704.36 kg in Indian Murrah buffaloes. Conversely, the results obtained in this examination were higher than those surmised by Tonhati *et al.,* (2000a) of 1,259.47 kg, Tonhati *et al.,* (2000b) of 1,496.00 kg and Ramos *et al.,* (2006) of 1,650 kg respectively.

AkhandPratap *et al.,* (2014) concluded that the highest yield was recorded in mid stage and lowest in late stage of lactation. In the current investigation, the peak was reached on day 60, maintained on Day 75 and then dropped. This peak milk yield is lesser than that reported of Mech *et al.,* (2008) in buffaloes wherein the milk yield augmented up to 90 days and remain high for a while and then declines in late stage of lactation.

As postulated by Roche (2003) and AkhandPratap *et al.,* (2014) in cows and Khan *et al.,* (2011) in dairy buffaloes, pregnancy has a significantly negatively effect on milk yield. Further, Syed *et al.,* (1996) reported a decline in the milk yield from approximately 90 days in pregnant cows. This is in agreement with our investigation, wherein, as the buffaloes were kept open the milk yield was more or less constant from Day 90 upto Day 165.

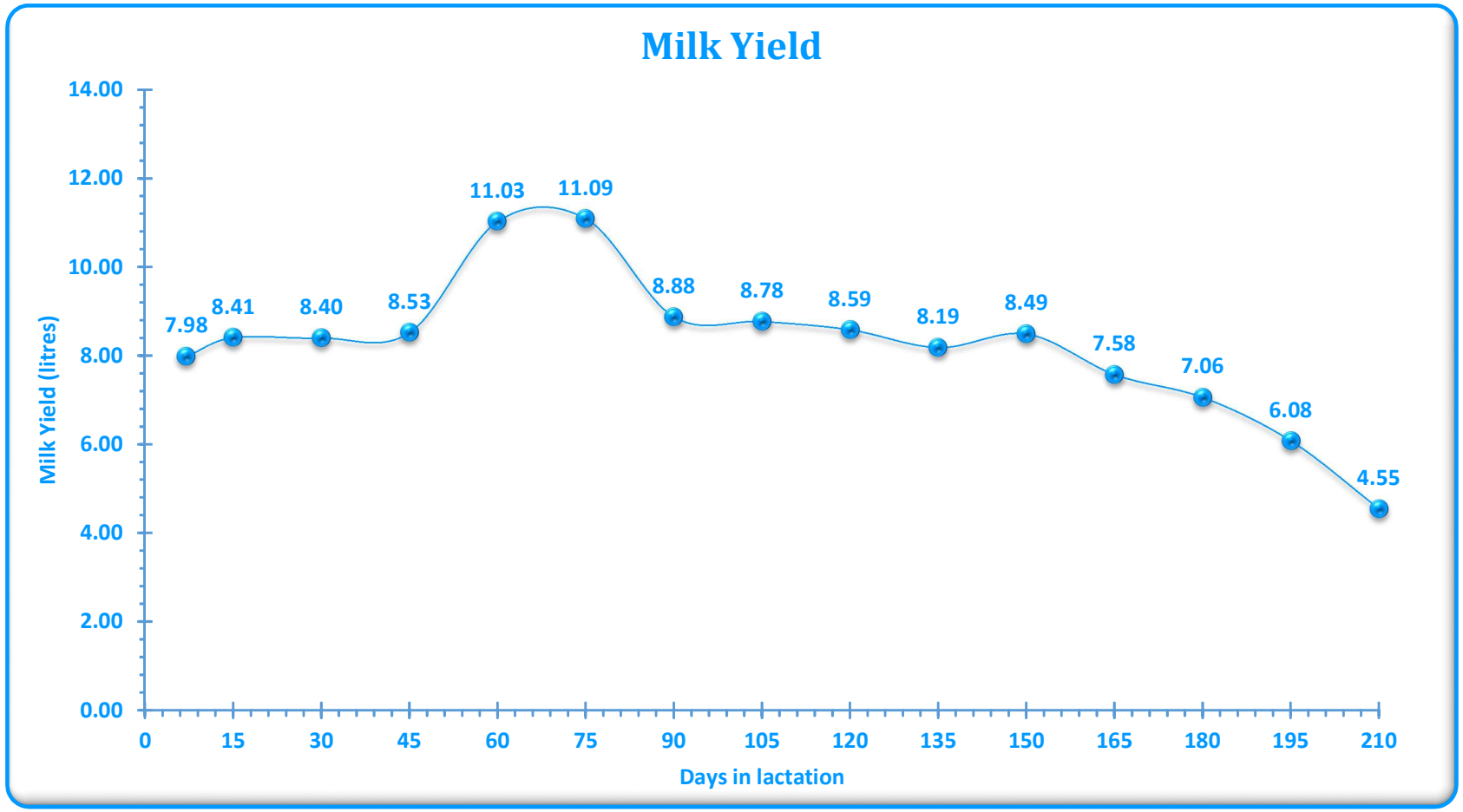

Figure 04. Milk Yield (litres) on day 7th, 15th, 30th, 45th, 60th, 75th, 90th, 105th, 120th, 135th, 150th, 165th, 180th, 195th and 210th of lactation in Murrah buffaloes.

Table 1.01: **Milk yield (litres)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Milk (Litres)
1	07	07.92 ^{bc} ± 0.54
2	15	08.41 ^{bc} ± 0.48
3	30	08.63 ^{b} ± 0.48
4	45	08.53 ^{b} ± 0.50
5	60	11.03 ^{a} ± 0.68
6	75	11.09 ^{a} ± 0.52
7	90	08.88 ^{b} ± 0.36
8	105	08.78 ^{b} ± 0.36
9	120	08.59 ^{b} ± 0.51
10	135	08.19 ^{bc} ± 0.50
11	150	08.49 ^{b} ± 0.43
12	165	07.58 ^{bc} ± 0.65
13	180	07.06 ^{cd} ± 0.42
14	195	06.08 ^{d} ± 0.40
15	210	04.55 ^{e} ± 0.36

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 1.78 CD (0.05) = 1.35

Those means having atleast one common superscript between groups do not differ significantly.

Table 1.02: Analysis of variance of the data of **milk yield** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	595.85	42.56	11.20	0.00
Error	225	855.06	3.80	-	-
Total	239	-	-	-	-

Coefficient of Variation = 23.62

This post conceptional milk yield decline can be attributed reduction in the accessibility of nutrients for milk production due to the hormonal modifications, initiating mammary gland regression to cater to the requirements of the foetal growth demands (Bell *et al.,* 1995).

The Temperature Humidity Index (THI) during this study ranged between 74.94 being the lowest on day 7 (first collection) and 85.96 on day 105 of lactation. Further, the highest average milk yield of 11.09 litres was seen on day 75 when the THI was 81.57. On the days of the highest THI the milk yield was around 8 liters.

Johnson (1985) and Du Preez *et al.,* (1990a) stated that milk production is unaffected by heat stress when mean temperature-humidity index (THI) values are between 35 and 72. On the other hand, both milk production and feed intake decline when THI reaches 72 and this decline is intensified when the THI crosses 75 (Johnson, 1980b).

Farm animals imperiled by rising of environmental temperatures cutback their feed intake and consequently rumination to thwart their metabolic temperature amplification and this eventually led to a drop in the milk produced and diminished milk yield (West, 1999). Aggarwal and Singh, (2010) quantified this production decreased at THI of 80.30 in hot dry climate and 83.60 during hot humid climate in heat stressed Murrah buffaloes.

On the whole, the disparities in the milk yield can be attributed to numerous variances like feed quality and quantity, farm and animal managemental procedures, environmental influences and seasonal deviations.

4.2 Milk Proteins

The mean milk protein percentage from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 2.01, its analysis of variance in Table 2.02 and its graph is illustrated in Figure 5.

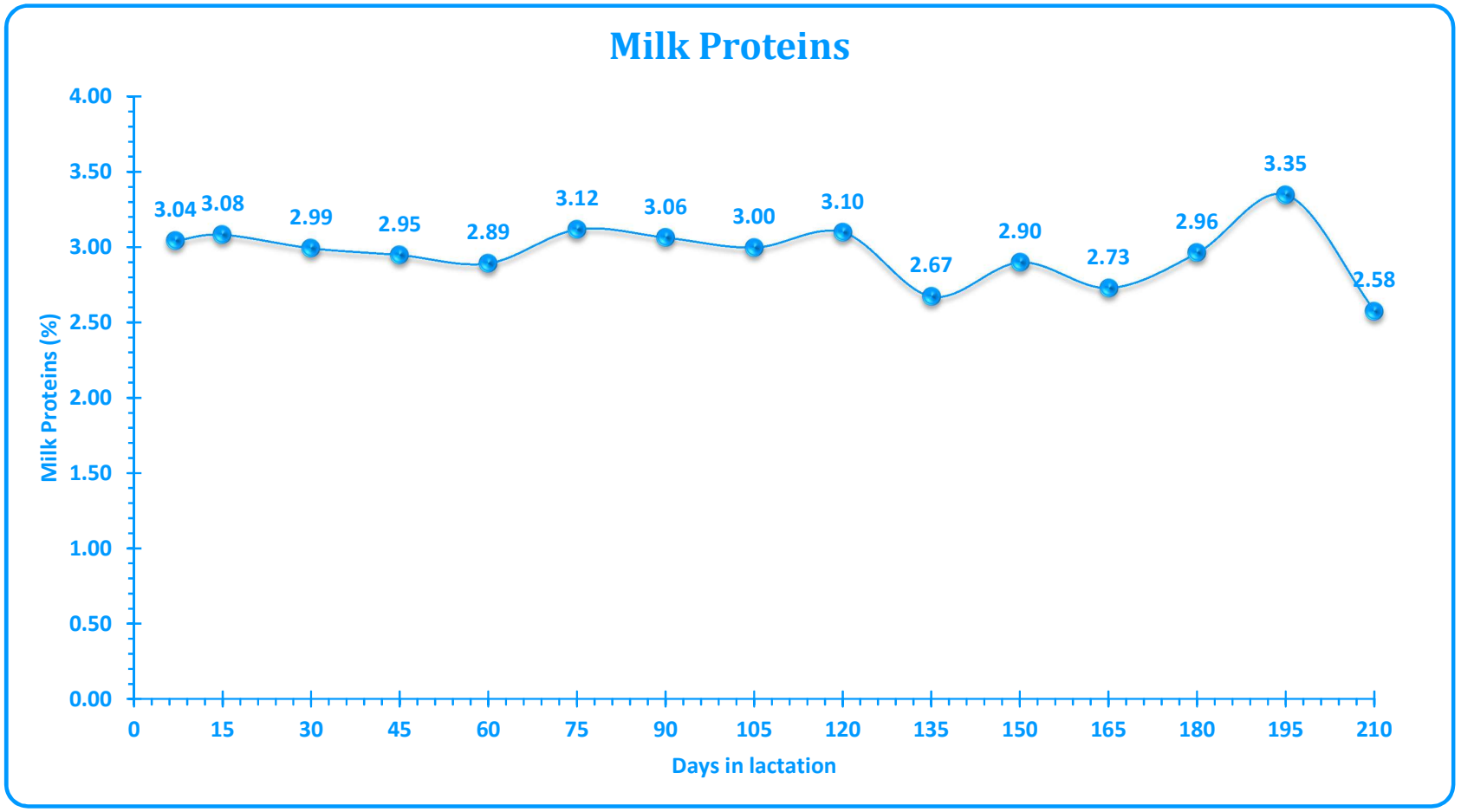

Figure 05. Milk Protein Percentage from day 7 to day 210 of lactation in Murrah buffaloes.

Table 2.01: **Milk proteins (%)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Milk Proteins (%)
1	07	3.04^{b} ± 0.04
2	15	3.08^{b} ± 0.03
3	30	2.99^{b} ± 0.05
4	45	2.95^{bc} ± 0.03
5	60	2.90^{bcd} ± 0.05
6	75	3.12^{ab} ± 0.06
7	90	3.06^{b} ± 0.06
8	105	3.00^{b} ± 0.11
9	120	3.10^{ab} ± 0.13
10	135	2.67^{de} ± 0.08
11	150	2.90^{bcd} ± 0.15
12	165	2.73^{cde} ± 0.10
13	180	3.00^{bc} ± 0.12
14	195	3.35^{a} ± 0.13
15	210	2.58^{e} ± 0.14

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.35 CD (0.05) = 0.26

Those means having atleast one common superscript between groups do not differ significantly.

Table 2.02: Analysis of variance of the data of **milk proteins** percentage from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	8.34	0.60	4.15	0.00
Error	225	32.27	0.14	---	---
Total	239	---	---	---	---

Coefficient of Variation = 12.78

The average milk protein percentage throughout lactation was 2.96 ± 0.09. It ranged between 2.58 ± 0.14 which was the lowest concentration on day 210 to 3.35 ± 0.13, the highest concentration on day 195, both of which are within the normal range for buffaloes. The milk protein concentration with minor fluctuations stayed non-significant from Day 07 to Day 120. It ebbed significantly ($P<0.05$) on Day 135 and remained almost similar upto Day 165. From then on, it waxed significantly ($P < 0.05$) from Day 180 to Day 195 of the study and then waned significantly to the lowest concentration on Day 210.

The protein milk concentration shows comparatively high heritability, and its quantity in milk is virtually almost constant (Meena *et al.,* 2007). The protein content in buffalo milk is normally around 2.7 – 5.2% (El-Salam and El-Shibiny, 2011, Claeys *et al.,* 2014 and Patbandha *et al.,* 2015) which correlates well with the average value for the milk samples analyzed in the present study which was 2.96%.

The values procured in this study are similar to those noted by Meena *et al.,* (2007), Guo *et al.,* (2010), Pawar *et al.,* (2013) and Cinar *et al.,* (2019). Pasquini *et al.,* (2003) found that the lactation milk protein values followed a steady and prolific trend wherein it diminished before mid-lactation and then augmented towards the end of lactation.

Garaniya *et al.,* (2013) did not observe any variation in the milk protein during the different stages of lactation in Jaffrabadi buffaloes. In view of the fact that milk protein percent and milk yield are negatively co-related, the significant yield decline during late lactation might be the cause of elevated milk protein percentages during this period (Friggens *et al.,* 2007 and Ravikala *et al.,* 2014).

On the other hand, higher milk protein percentages than those attained in our findings were reported by Millogo *et al.,* (2009) in dairy cattle and by Pasquini *et al.,* (2003), Garaniya *et al.,* (2013), Mahdi (2014), Silva Juniour *et al.,* (2014), Balusami (2015) and Patbandha *et al.,* (2015) in buffaloes. The protein content in milk augments with good pasture quantity and is affected by the provision of energy and amino acids (Sporndly, 1989 and Kristensen, 1998).

The buffaloes used in this investigation were not inseminated and therefore the protein values are on the lower side of the average range. This corresponds to the observations by AkhandPratap *et al.,* (2014) in cows who stated that the milk protein content is significantly altered in pregnancy and is associated with the overall augmentations in major nutrients anabolism.

The decrease of milk protein percentage could also be a consequence of lower energy input and a lower protein supply in diet, since suitable proteins are either expensive or difficult to acquire due to contamination (Hermansen *et al.,* 1994) and Rai (2011).

Pandey *et al.,* (1986) affirmed multiple factors like lactational stage, calving season and parity played a significant role in milk protein percentages, while Dubey *et al.,* (1997) partially disagreed stating that while they discerned the effects of stage of lactation and year of calving significant, the calving season did not influence the milk protein composition.

Pyne *et al.,* (1990) learnt that the milk produced in winter was significantly richer in protein than that in summer, Gao *et al.,* (2016) ascribed to this theory concluding that heat stress reduced milk proteins.

4.3 Milk Fats

The mean milk fat percentage from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 3.01, its analysis of variance in Table 3.02 and its graph is shown in Figure 06.

The mean milk fat percentage from Day 07 to Day 105 receded significantly and then inflated significantly ($p < 0.01$) upto Day 150 after which there was a gradual slump followed by a mild non-significant cresting. The fat percentage was the highest on Day 150 and lowest on Day 75 of the exploration.

Fat value variations are expected, as it is the most sensitive milk component to a variety of factors, such as food management, genotype, nutrition, lactation, calving phase and parity (Macedo *et al.,* 2001). The significant decline

in milk fat with the advancement of lactation, upto mid lactation followed by a surge in late lactation is in accordance with Sharma *et al.,* (1989), Mahdi (2014), Seerapu (2014), Ashalatha *et al.,* (2015), Ashmawy (2015b), and Patbandha *et al.,* (2015) in buffaloes and Artegoitia *et al.,* (2013) and AkhandPratap *et al.,* (2014) in cows. Shah *et al.,* (1983), Dubey *et al.,* (1997), Kholif (1997), Bhonsie *et al.,* (2003) and Yadav *et al.,* (2013) in buffaloes and Mech *et al.,* (2008) in Mithun cows reported that the fat content builds-up with advanced stage of lactation, which is also in compliance with the results obtained. This escalation in fat percent is a trend demonstrated by the normal inverse relationship between milk yield and fat percent and as the milk yield plateaus, the fat content boosts (Ashalatha *et al.,* 2015).

Haque *et al.,* (2017), Cinar *et al.,* (2015), Simoes *et al.,* (2014) and Araújo *et al.,* (2011) agreed that seasonality altered buffalo milk fat composition as it influences the lipid and fiber content of the ingested vegetation. Mahdi (2014) and Sharma *et al.,* (2000) postulated that the significantly amplified total lipids concentrations in late lactation compared with early lactation could be owing to fatty acids synthesizing enzymes particularly acetyl CoA carboxylase which is a regulatory enzyme in the fatty acid synthesis might have slightly increased in late lactation than early lactation.

Our readings are not favoured by Balusami (2015) who in non-descript buffaloes observed that the fat percentage increased throughout lactation as did Rai (2011). AkhandPratap *et al.,* (2014) did not find significant difference in the fat composition in non-pregnant and pregnant cows. On the other hand Wilcox *et al.,* (1959), Mather *et al.,* (1969), Eckles *et al.,* (1973) and Banerjee (1985) noted that after 4 months post-partum, the yield increases and the percentage composition of fat decreases, due to the pregnancy effects and Bohmanova *et al.,* (2009) reported lower milk fat content in late stage of lactation in Canadian Holstein cows. The average milk fat % realized in this examination throughout lactation was 8.38 ± 0.39 which is similar to those quoted by Patino and Stefani (2005) for Jaffrabadi in Argentina, Meena *et al.,* (2007) in Indian buffalo and Tufarelli *et al.,* (2008) in Italian buffaloes.

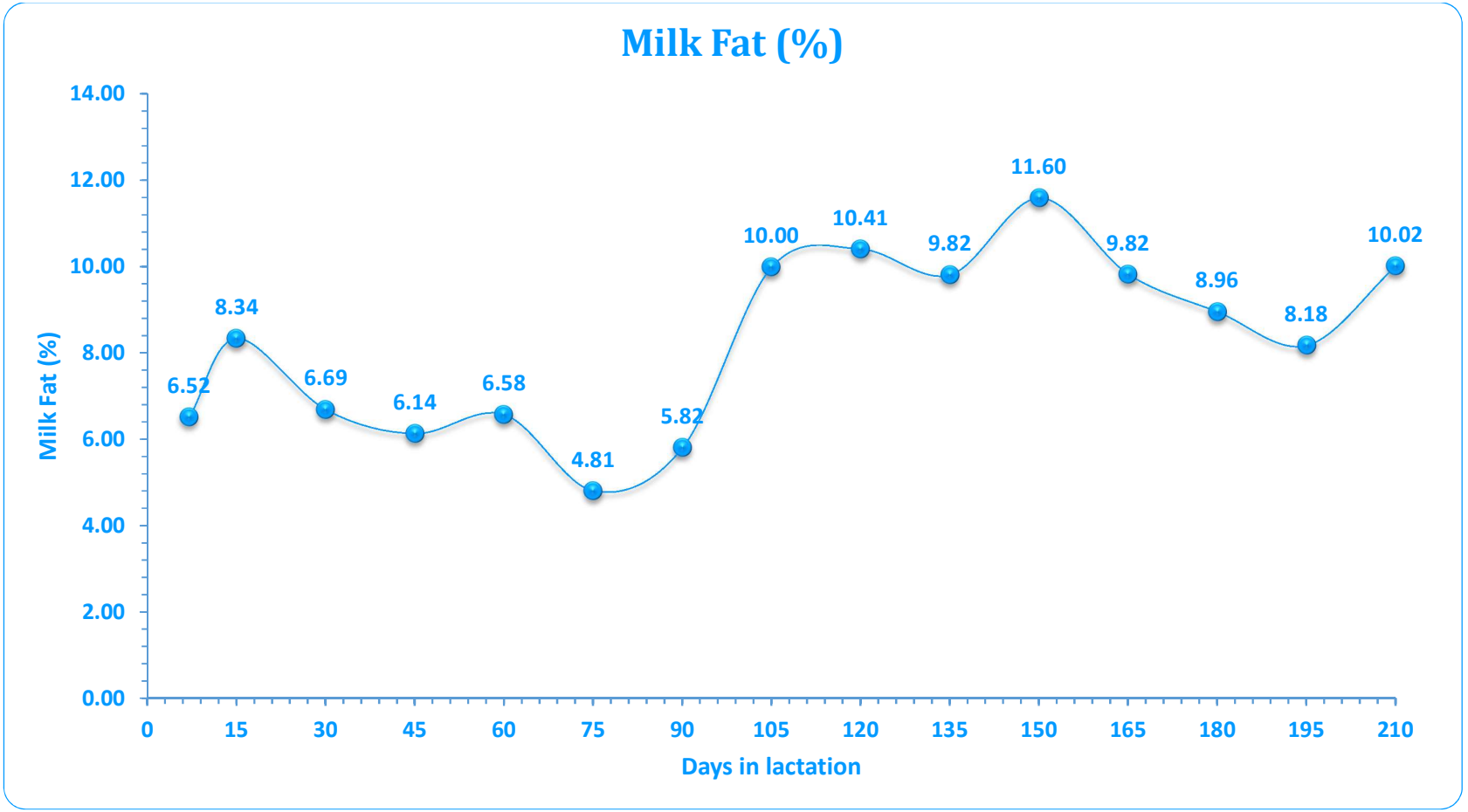

Figure 06. Milk Fat Percentage from day 7 to day 210 of lactation in Murrah buffaloes.

Table 3.01: **Milk fat (%)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Milk Fat (%)
1	07	08.48 ^{d} ± 0.12
2	15	08.34 ^{d} ± 0.10
3	30	06.69 ^{e} ± 0.31
4	45	06.14 ^{e} ± 0.23
5	60	06.58 ^{e} ± 0.39
6	75	04.81 ^{f} ± 0.25
7	90	05.82 ^{ef} ± 0.64
8	105	10.00 ^{bc} ± 0.98
9	120	10.41 ^{ab} ± 0.35
10	135	09.82 ^{bc} ± 0.49
11	150	11.60 ^{a} ± 0.29
12	165	09.82 ^{bc} ± 0.40
13	180	08.96 ^{cd} ± 0.29
14	195	08.18 ^{d} ± 0.47
15	210	10.02 ^{bc} ± 0.60

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 1.64 CD (0.05) = 1.25

Those means having atleast one common superscript between groups do not differ significantly.

Table 3.02: Analysis of variance of the data of **milk fat (%)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	875.83	62.56	19.33	0.00
Error	225	728.04	3.24	-	-
Total	239	-	-	-	-

Coefficient of Variation = 21.47

It was higher than the values proposed in Indian buffaloes by Dubey *et al.,* (1997), Sodi *et al.,* (2008), Balusami (2015), Khedkar *et al.,* (2016) and Yadav *et al.,* (2018).

A possible reason for this variation could be the non-pregnant status of the buffaloes in this study. Khan *et al.,* (2011) confirmed that rise in milk fat was observed with advancement of gestation stage which was previously postulated by Sorensen and Ostergaard (2003) when investigating a herd of HF cows. The fact that the milk fat computed in this examination is steeper than the quoted literature for Murrah buffaloes reasons to state that the milk fat content of non-pregnant buffaloes is higher than pregnant buffaloes (Shah *et al.,* 2009).

4.4 Milk Lactose

The mean milk lactose percentage from day 7 to day 210 of lactation in lactating Murrah buffaloes are represented in in Table 4.01, its analysis of variance in Table 4.02 and its graph is displayed in Figure 07.

The average milk lactose percentage throughout the lactation ranged between 4.63 ± 0.08 and 3.12 ± 0.17. The mean milk lactose concentration from Day 07 to Day 90 did not differ significantly, but displayed some oscillations and escalated in percentage numerically. From Day 105 there was a more or less significant decline upto the end of lactation baring a sudden spike on Day 180.

The significant decline in milk lactose with the advancement of lactation is in accordance with Sharma *et al.,* (2000), Mushtaq (2009), El-Salam and El-Shibiny (2011) and Mahdi (2014) in buffaloes and Sharma *et al.,* (1996) in cows. The decreasing tend in the lactose concentration during late lactation may be due to the fact that the buffaloes on the farm were not inseminated and therefore non-pregnant, right upto the end of the exploration. This decline is due to hormonal changes during lactation (Mushtaq, 2009).

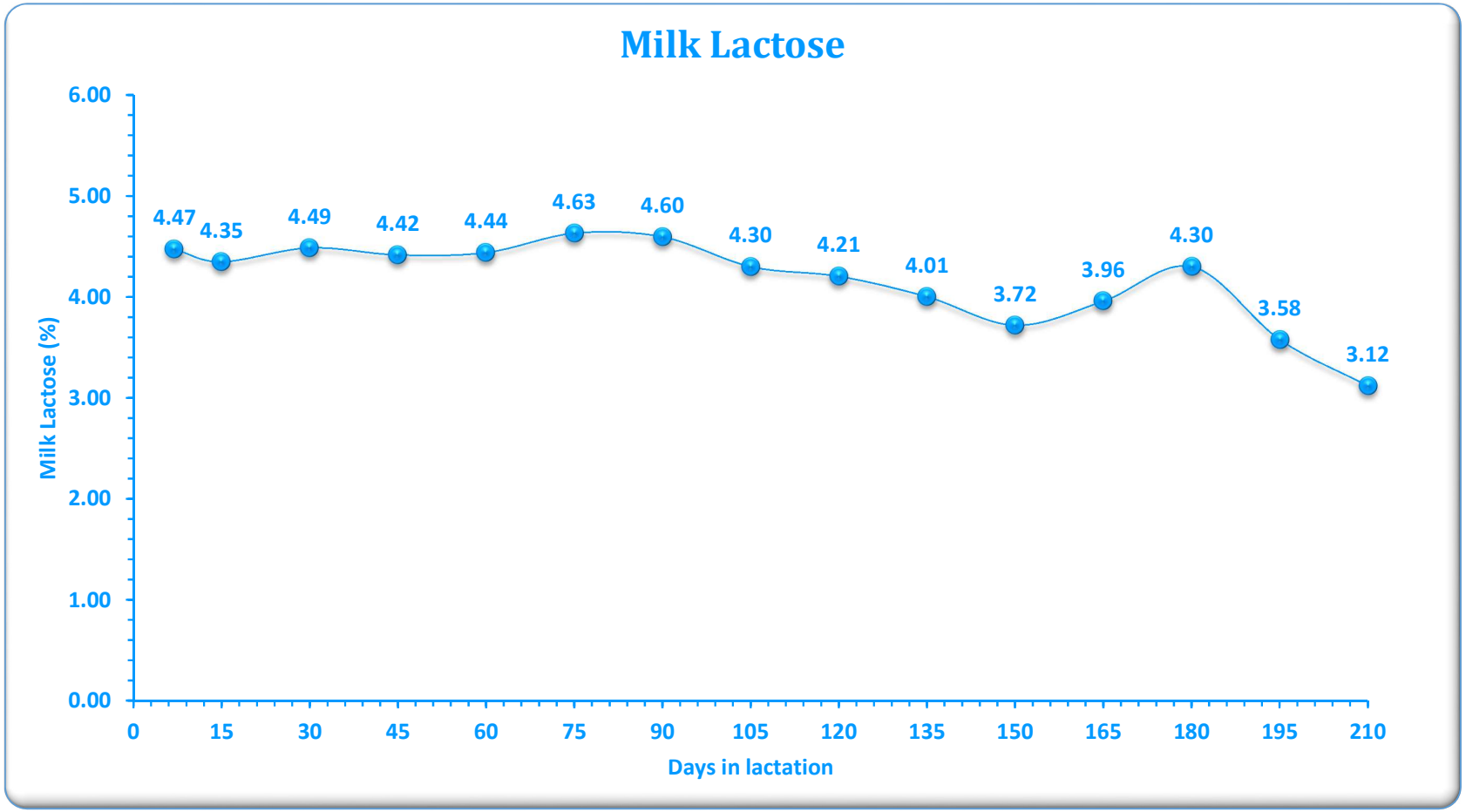

Figure 07. Milk Lactose Percentage from day 7 to day 210 of lactation in Murrah buffaloes.

Table 4.01: **Milk lactose (%)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Milk Lactose (%)
1	07	4.47^{abc} ± 0.09
2	15	4.35^{abc} ± 0.06
3	30	4.49^{abc} ± 0.08
4	45	4.42^{abc} ± 0.04
5	60	4.44^{abc} ± 0.05
6	75	4.63^{a} ± 0.08
7	90	4.60^{ab} ± 0.09
8	105	4.30^{bcd} ± 0.13
9	120	4.21^{cde} ± 0.13
10	135	4.01^{def} ± 0.11
11	150	3.72^{fg} ± 0.11
12	165	3.96^{ef} ± 0.11
13	180	4.30^{bcd} ± 0.19
14	195	3.58^{g} ± 0.12
15	210	3.12^{h} ± 0.17

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.41 CD (0.05) = 0.31

Those means having atleast one common superscript between groups do not differ significantly.

Table 4.02: Analysis of variance of the data of **milk lactose** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	40.26	2.88	14.29	0.00
Error	225	45.29	0.20	-	-
Total	239	-	-	-	-

Coefficient of Variation = 10.75

According to Arora and Bhojak (2013) and Haque *et al.,* (2017) the average lactose percentage may vary according to breed of the animal, lactation stage, feeding habit and season of milking.

The milk lactose percentage recorded in the present study, upto mid lactation, is similar to that reported by Pollott (2004), Rashida *et al.,* (2004), Tsioulpas *et al.,* (2007), Garaniya *et al.,* (2013), Costa *et al.,* (2014), Karan and Shukla (2014), Ashmawy (2015b) Kashwa (2016) in buffaloes and Silva *et al.,* (2018) in cows which remained almost stable with minor fluctuations.

This may be due to a close relationship between lactose synthesis and the amount of water drawn into milk makes lactose a stable milk component. Lactose synthesis reportedly draws water into milk and is highly correlated, which probably maintains secretion rates of lactose and water nearly constant throughout lactation (Pollott, 2004).

In contrast to this examination, high milk lactose, above 5%, was reported by Jindal and Ludri (1993), Bovera *et al.,* (2002), Vidu *et al.,* (2011), Silva Jr. *et al.,* (2014), Ren *et al.,* (2015), Sales *et al.,* (2018) and Zhou *et al.,* (2018) in buffalo and Tsioulpas *et al.,* (2007) and Thomas (2004) in cow.

This rise in lactose percentage is attributed to the buffaloes being pregnant (Khan *et al.,* (2011), Yadav *et al.,* (2013), AkhandPratap *et al.,* (2014) and Mahdi, (2014); or could be a result of supplemented ration given to pregnant dairy buffaloes Shah *et al.,* (2009) and also may be due to the lactation number as the lactose content increased with the increase in the parity.

4.5 Milk SNF

The mean milk solids-not-fat (SNF) percentage from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 5.01, its analysis of variance in Table 5.02 and its graph is illustrated in Figure 08.

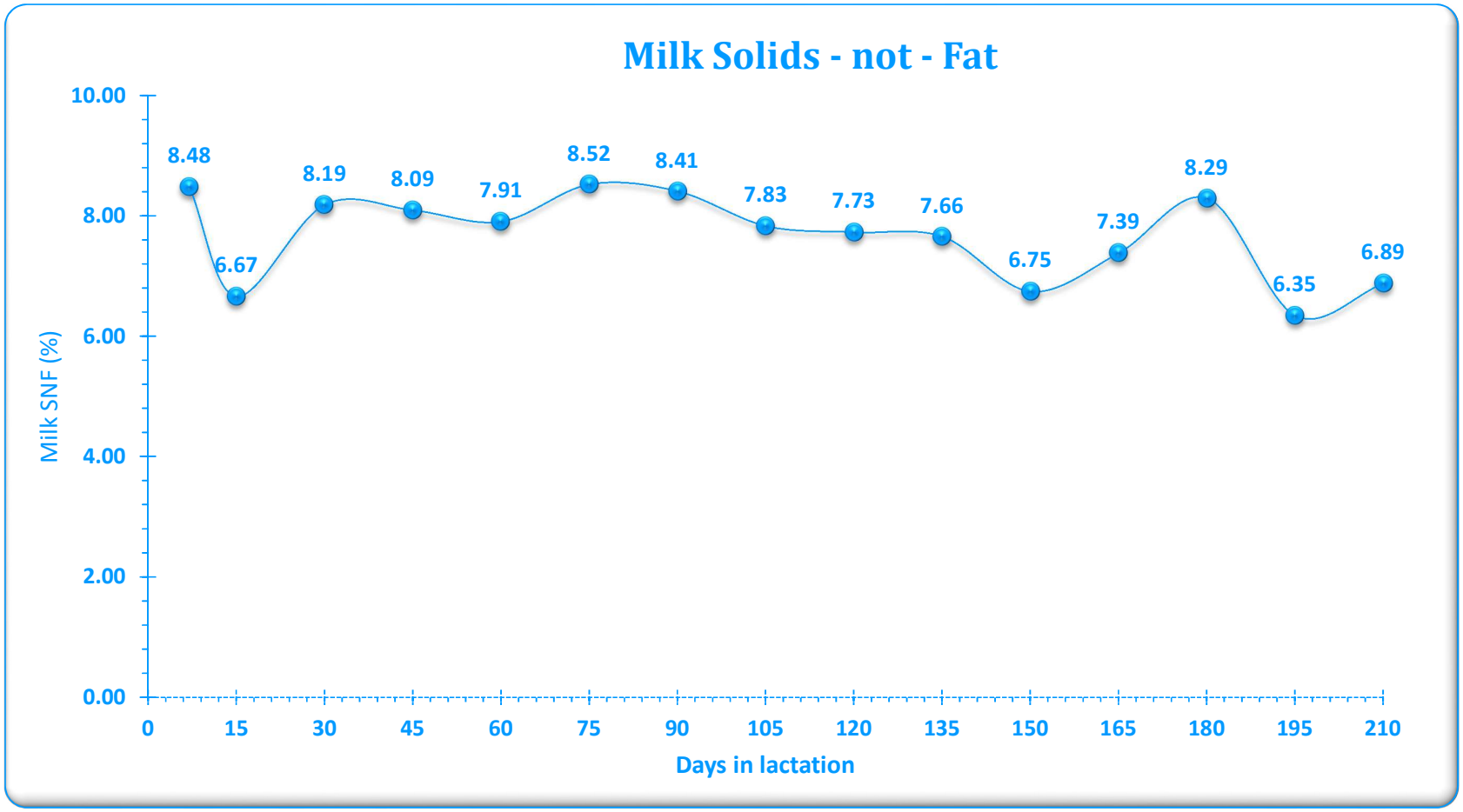

Figure 08. Milk Solids-Not-Fat Percentage from day 7 to day 210 of lactation in Murrah buffaloes.

Table 5.01: **Milk solids-not-fat (%)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Milk SNF (%)
1	07	$8.48^{a} \pm 0.12$
2	15	$6.67^{g} \pm 0.09$
3	30	$8.19^{abcd} \pm 0.15$
4	45	$8.09^{abcd} \pm 0.09$
5	60	$7.91^{abcde} \pm 0.12$
6	75	$8.52^{a} \pm 0.15$
7	90	$8.41^{ab} \pm 0.17$
8	105	$7.83^{bcde} \pm 0.25$
9	120	$7.73^{cde} \pm 0.23$
10	135	$7.66^{de} \pm 0.11$
11	150	$6.75^{g} \pm 0.23$
12	165	$7.39^{ef} \pm 0.21$
13	180	$8.29^{abc} \pm 0.40$
14	195	$6.35^{g} \pm 0.41$
15	210	$6.89^{fg} \pm 0.28$

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.81 CD (0.05) = 0.62.

Those means having atleast one common superscript between groups do not differ significantly.

Table 5.02: Analysis of variance of the data of **milk solids-not-fat** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	114.14	8.15	10.27	0.00
Error	225	178.66	0.79	-	-
Total	239	-	-	-	-

Coefficient of Variation = 11.61

The average milk SNF percentage throughout the lactation ranged between 6.35 ± 0.41 to 8.52 ± 0.15. The high percentage on Day 7, decreased significantly ($P < 0.05$) on Day 15. A significant rise was seen from Day 30 to Day 90 with minor fluxes. From there on there was a deflating trend upto Day 210 of lactation with the exception of a sequentially drop on Day 150 and hike on Day 180 of lactation. The mean milk solids-not-fat throughout the lactation was 7.68 ± 0.20.

The values ascertained in this investigation are lower than those proposed by Dubey *et al.,* (1997) (9.61%), Sarkar *et al.,* (2006) (10.01%), Meena *et al.,* (2007) (8.30%), Sodi *et al.,* (2008) (9.40%) and Balusami (2015) (9.47%) in Murrah buffaloes.

In close agreement with slight differences was the suggestion by Harris and Bachman (2002) that with the onset of lactation SNF content is reasonably elevated in the initial month, plunges in the second and then escalates as lactation progresses.

An opposite pattern was noted by Yadav *et al.,* (1991), Sharma *et al.,* (2000) and Mahdi (2014) wherein SNF remained nearly unchanged throughout the lactation period. Boro *et al.,* (2018) citing Shah *et al.,* (1983), Kholif, (1997), Sarkar *et al.,* (2006), Misra *et al.,* (2008), Sodi *et al.,* (2008) and Yadav *et al.,* (2013) deduced that buffalo milk SNF is affected by breed, stage of lactation, parity and season. He further postulated that among the buffalo breeds, Mehsana is the best performing breed for solids-not-fat quality.

Cheruiyot *et al.,* (2018) in agreement stated that the month of sampling had a significant effect on the content of milk SNF and Haque *et al.,* (2017) marked the milk SNF to be significantly higher during winter season as compared to hot dry and hot humid seasons.

Harris and Bachman (2002) and Mushtaq (2009) opined that since milk SNF content also varies with the quality and quantity of the feed, additional supplementation to high producing cows may improve the SNF percentage and conversely reducing the concentrate ration below requirements or increasing the roughage feed to high producing cows would decrease the milk solids-not-fat.

Further, the changes that occur in SNF are primarily due to changes in the protein and occasionally the lactose content of milk.

Khan *et al.,* (2011) and Gurmessa and Melaku (2012) learnt that the milk SNF contents were higher in milk from pregnant animals than in milk from open ones and this along with low energy and low quality protein feed could be the reasons for the low values obtained in the present investigation as the animals were non-pregnant.

4.6 Serum Total Proteins

The mean serum total protein concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 6.01, its analysis of variance in Table 6.02 and its graph is depicted in Figure 09.

Serum total protein concentrations (g/dl) showed the greatest significant high on day 150 and low on day 15 and 30 of lactation. The serum total protein concentration revealed a declining trend from day 7 upto day 30 followed by an escalation on day 45 of lactation. Subsequently, the values again dipped from day 60 upto day 105 of lactation. Thereafter, the general trend was on the higher side and the values swayed, with days 120, 150 195 being higher and days 135, 165, 180 and 210 being lower. Essentially the serum protein concentrations were stable and abated during peak and mid-lactation but were fluctuatingly loftier around early and late lactation. Correspondingly, the THI levels were also higher during mid-lactation.

A conventional minimal concentration during early and mid-lactation can be attributed to the augmented metabolic demands and nutrient partitioning of serum protein as well as its constituent amino acids for milk protein biosynthesis. The overall late lactation high values could be ascribed to the diversion of body fluid for milk synthesis thus generating a relative upsurge in the serum total protein concentrations.

The average serum total proteins (g/dl) throughout the lactation ranged between 6.84 ± 0.14 to 8.79 ± 0.38. The serum total protein values achieved in this analysis were comparatively in close agreement with slight differences with Jhambh *et al.,* (2016) in dairy animals and Surya Prakash *et al.,* (2018a) in crossbred cows. Yaylak *et al.,* (2009) also reported lower levels of serum protein during early lactation in Holstein cows.

In partial accordance is the investigation in Holstein cows conducted by Dias *et al.,* (2017), wherein he reported higher serum protein levels during late and lower levels during early lactation, but an increase of serum proteins from early to mid-lactation.

This could be due to the reduction of serum proteins owing to higher THI during mid-lactation as seen in the present investigation. Similar results of increasing protein level at later stage of lactation have also been reported in ewes by Antunović *et al.,* (2011) and by Krajnicakova *et al.,* (2003) and Bhat *et al.,* (2016) in lactating goats.

In juxtapose to the present findings, continuous waning of serum total protein concentrations throughout lactation has been observed in Mehsani buffaloes by Das *et al.,* (2017) and Roy *et al.,* (2003). The reason for this difference could be a smaller sample size as compared to our present investigation.

An increased temperature humidity index (THI), synonymous with heat stress, causes a decline in serum protein concentration as observed in the present experiment. This is supported by findings of Ahmed (1990), El-Masery and Marai (1991) and Marai *et al.,* (1997) in cattle and Verma *et al.* (2000) in buffaloes. In contrast few studies have also suggested an increase in serum proteins due to heat stress in buffalo calves (Yousef, 1990) and in Holstein heifers (Rasooli *et al.,* 2004), which may be due an altered physiological stage of the experimental animals used. Raghavan and Mullick (1962) observed little variation in serum protein concentration in buffaloes during spring and summer seasons. Gao *et al.,* (2016) supposed that blood AA utilization is reprioritized away from milk protein synthesis in heat stressed cows.

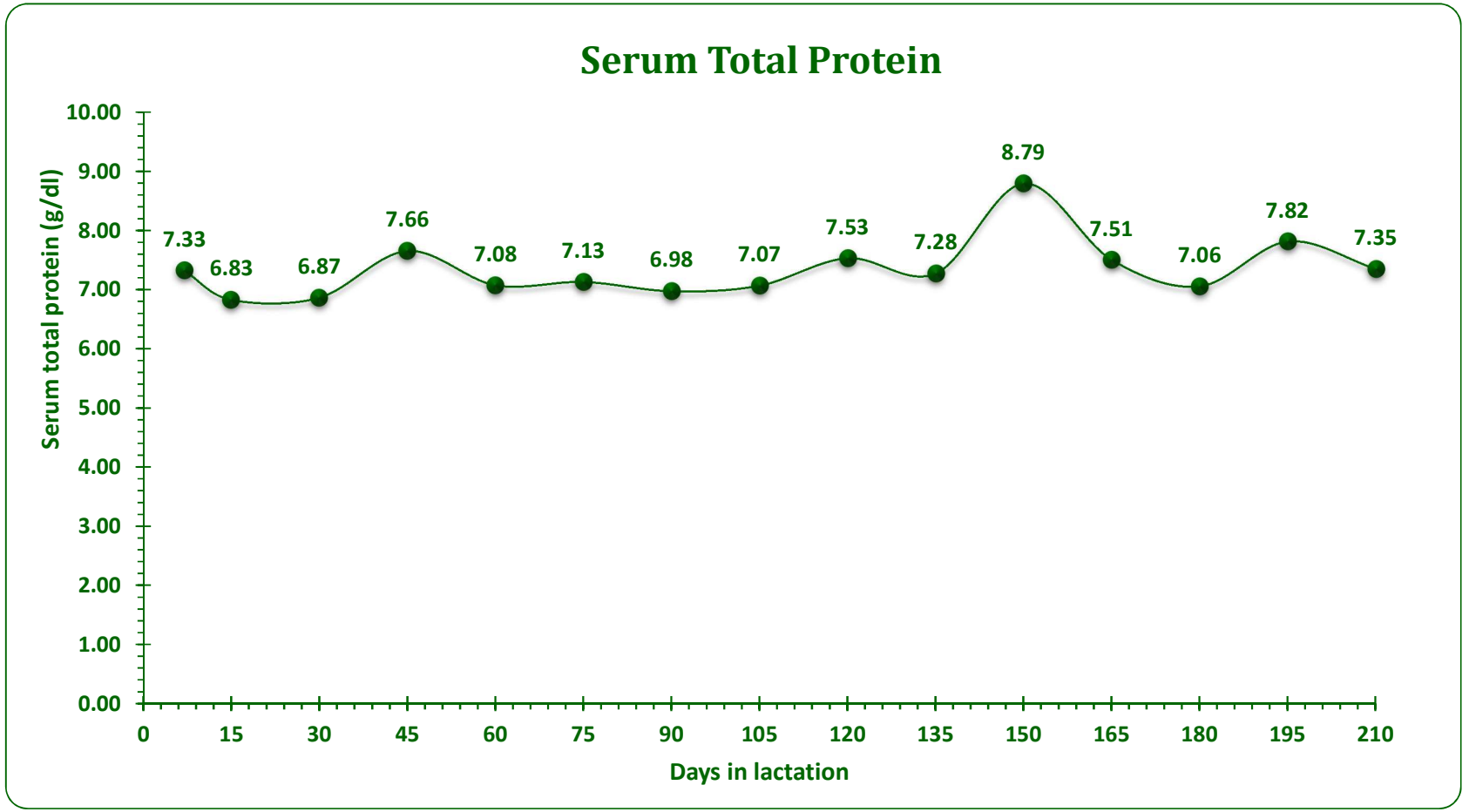

Figure 09. Serum Total Protein (g/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 6.01: **Serum total proteins (g/dl)** concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum Total Protein (g/dl)
1	07	$7.33^{bcde} \pm 0.20$
2	15	$6.84^{e} \pm 0.14$
3	30	$6.87^{e} \pm 0.19$
4	45	$7.66^{bc} \pm 0.20$
5	60	$7.08^{cde} \pm 0.31$
6	75	$7.13^{cde} \pm 0.13$
7	90	$6.98^{de} \pm 0.15$
8	105	$7.07^{de} \pm 0.20$
9	120	$7.53^{bcd} \pm 0.17$
10	135	$7.28^{bcde} \pm 0.27$
11	150	$8.79^{a} \pm 0.38$
12	165	$7.51^{bcd} \pm 0.24$
13	180	$7.06^{de} \pm 0.09$
14	195	$7.82^{b} \pm 0.14$
15	210	$7.35^{bcde} \pm 0.11$

Treatments found significant at 1% and 5% level of significance CD (0.01) = 0.762 CD (0.05) = 0.58.

Those means having atleast one common superscript between groups do not differ significantly.

Table 6.02: Analysis of variance of the data of **serum total proteins concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	54.01	3.86	5.50	0.00
Error	225	157.68	0.70	-	-
Total	239	-	-	-	-

Coefficient of Variation = 11.39

4.7 Serum Albumin

The mean serum albumin concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is represented in Table 7.01, its analysis of variance in Table 7.02 and its graphical representation is depicted in Figure 10.

The average serum albumin (g/dl) throughout the lactation ranged between 3.57 ± 0.09 to 4.85 ± 0.18. The serum albumin concentrations were significantly elevated on day 60 and 180 and the minimal values were discerned on day 15 of lactation. There was hardly any variation in the serum albumin concentrations, however, its values during early lactation *i.e.* upto day 45 of lactation were lower and subsequently rose after day 135 of lactation. From day 45 to 135 of lactation the levels increased from day 60 to 90 and declined from day 105 to 120. The variations in the serum albumin concentrations were analogous to the serum protein concentrations.

Customarily, late pregnant animals are well nourished and sustain physiologically favourable body reserves, thus possessing higher serum albumin concentrations, thereby implying positive protein balance. Early lactation triggers dramatic metabolic vicissitudes causing the serum albumin concentrations to decline. Thereafter, during mid-lactation, albumin concentrations get restored to their physiologically conventional range.

The findings of lowered serum albumin from early to mid-lactation phase in the present study are in coherence with the results attained by Nehra (2016) in Sahiwal cattle. The inferences realised in studies conducted by Onita and Colibar (2009) in dairy cows also stated a decrease of serum albumin after parturition followed by an increase thereafter. Yaylak *et al.,* (2009) also reported lowering of serum albumin during early and late lactation and a mid-lactational surge in dairy cows. Low serum albumin at postpartum and high during the fourth week postpartum has been reported by Abdul – Aziz (2008). Djoković *et al.,* (2013a) specified decreased serum albumin concentrations in Simmental cows during early lactation as compared to the late pregnant stage.

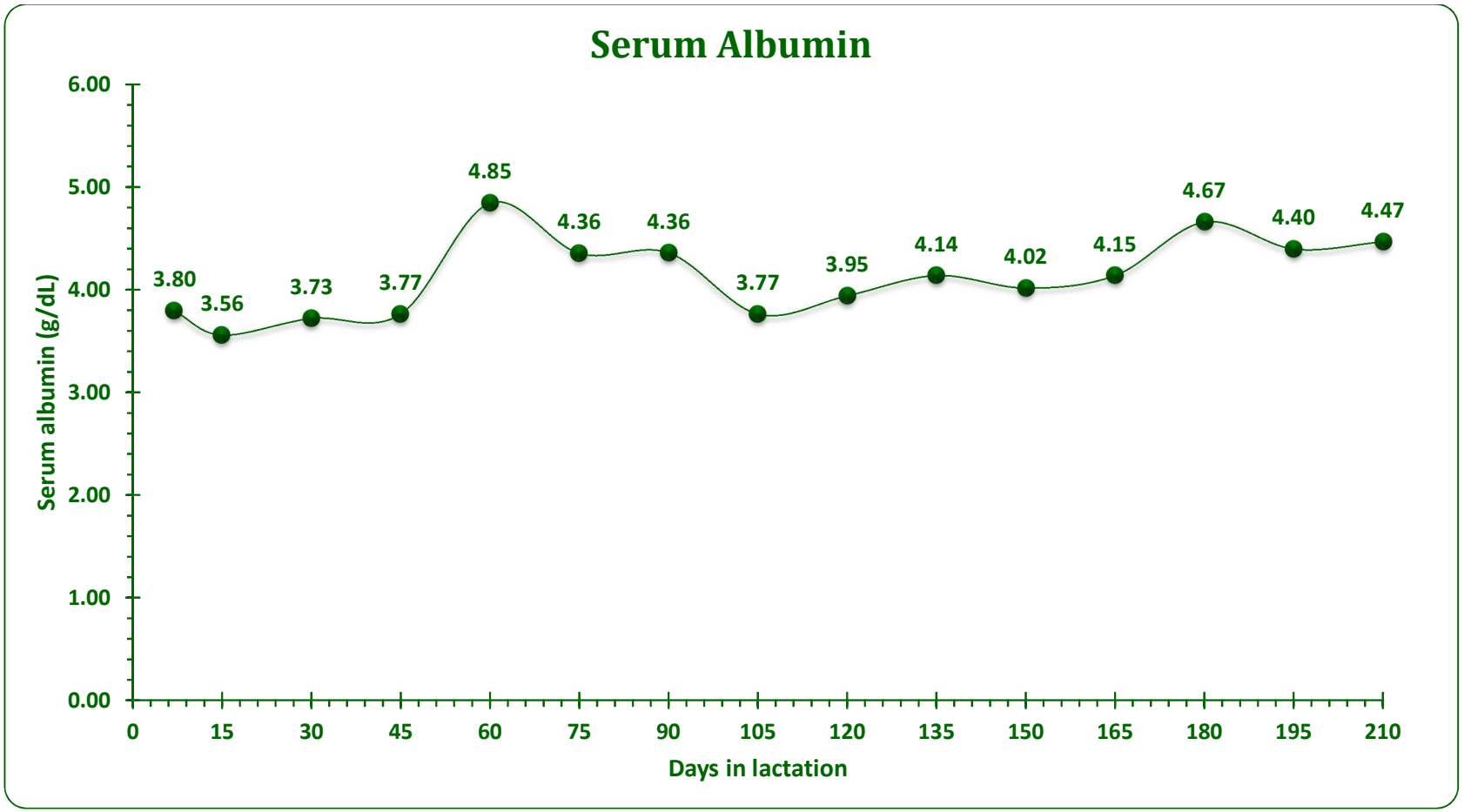

Figure 10. Serum Albumin (g/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 7.01: **Serum albumin (g/dl)** concentration from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum Albumin (g/dl)
1	07	$3.80^{efg} \pm 0.11$
2	15	$3.57^{g} \pm 0.09$
3	30	$3.73^{fg} \pm 0.10$
4	45	$3.77^{efg} \pm 0.12$
5	60	$4.85^{a} \pm 0.18$
6	75	$4.36^{bcd} \pm 0.17$
7	90	$4.36^{bcd} \pm 0.12$
8	105	$3.77^{efg} \pm 0.09$
9	120	$3.95^{ef} \pm 0.11$
10	135	$4.14^{cde} \pm 0.20$
11	150	$4.02^{def} \pm 0.10$
12	165	$4.15^{cde} \pm 0.17$
13	180	$4.67^{ab} \pm 0.17$
14	195	$4.40^{bc} \pm 0.10$
15	210	$4.47^{abc} \pm 0.15$

Treatments found significant at 1% and 5% level of significance CD (0.01) = 0.50 CD (0.05) = 0.38.

Those means having atleast one common superscript between groups do not differ significantly.

Table 7.02: Analysis of variance of the data of **serum albumin concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	31.98	2.28	7.62	0.00
Error	225	67.43	0.30	-	-
Total	239	-	-	-	-

Coefficient of Variation = 13.24

Similar conclusions have also been drawn from the studies of Abdul – Aziz (2008), Djokovic *et al.,* (2015b) and Rossato *et al*., (2001) in dairy cows and Boudebza *et al*., (2014) in ewes. In contrast, Mohamed (2014) reported significantly increased serum albumin during early lactation in dairy cows and Bamerny (2013) also revealed an increase in serum albumin during early post-partum period. These conclusions, which are dissimilar to the present study, could be attributed to the animal's well-nourished status, or blood concentration due to body fluid loss during postpartum and early lactation.

Results of the present experiment also reveal that whenever the THI was higher, serum levels of albumin tended to be lower and vice versa. This could be due to the direct impact of heat stress. Thus the increase in serum albumin during any lactation stage was limited by increase in THI. However in contrast there are also reports in cows (El-Masery and Marai, 1991) and buffalo calves (Koubkova *et al.,* 2002) of relative increase in serum albumin that might be due to dehydration and excess loss of water through urine. This difference from present results may be due to the role of albumin as an antioxidant (Halliwel, 1998 and Rasooli *et al.,* 2004).

4.8 Serum Globulin

The mean serum globulin concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 8.01, its analysis of variance in Table 8.02 and graphical depiction in Figure 11.

The average serum globulin (g/dl) throughout the lactation ranged between 2.23 ± 0.25 to 6.31 ± 0.38 g/dl. Serum globulin levels were significantly high on day 150 of lactation and significantly low on days 60 and 180 of lactation. The variation of serum globulin during the whole lactation was maximum during the mid or initial phase of late lactation especially from day 135 to day 210. While the postpartum levels of serum globulin were generally lower during early lactation dipped on days 60 to 90 & and then rose up to day 150 of lactation. Baring day 180, levels from day 150 of lactation were mostly higher.

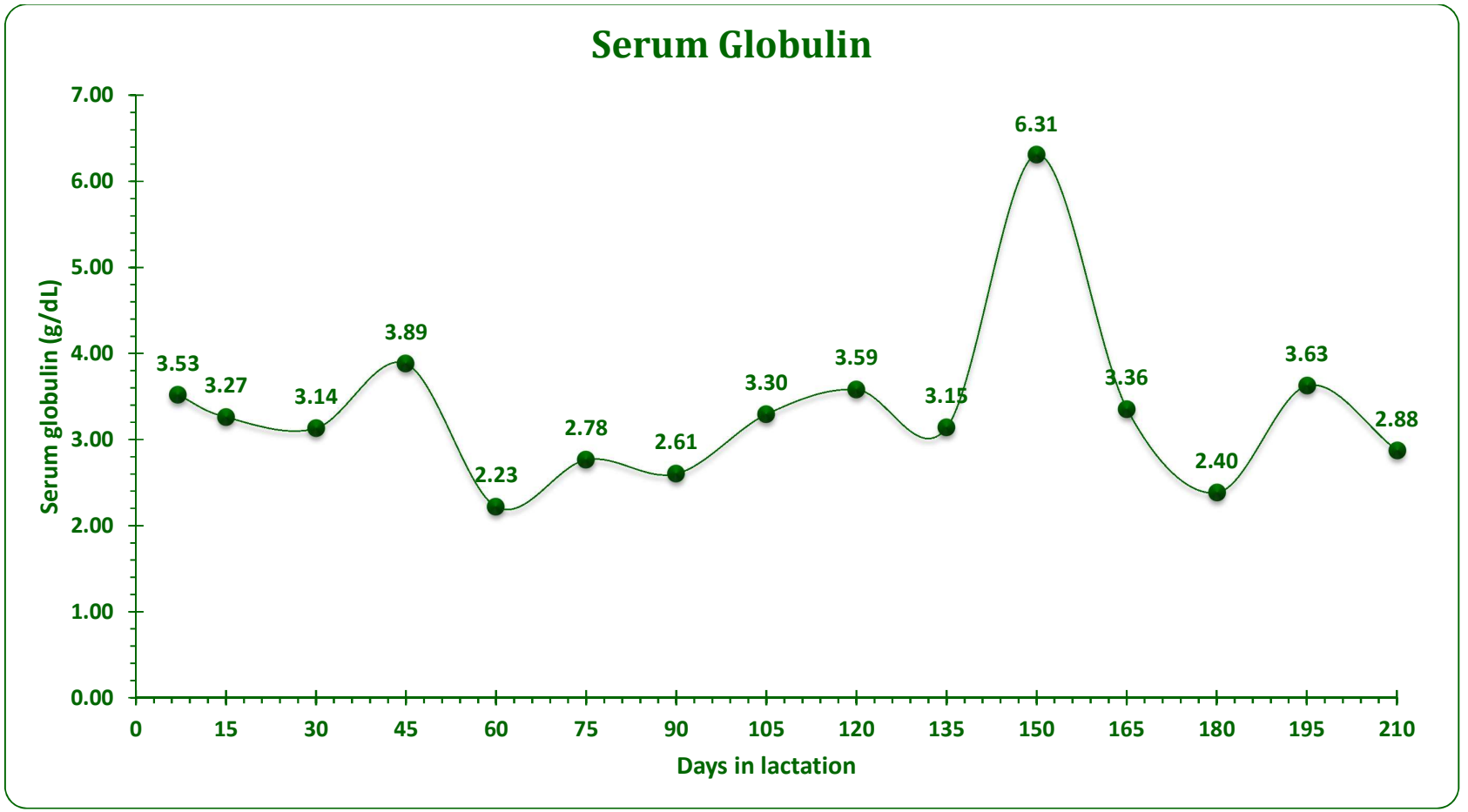

Figure 11. Serum Globulin (g/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 8.01: **Serum globulin (g/dl)** concentration from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum Globulin (g/dl)
1	07	3.53^{bcd} ± 0.20
2	15	3.27^{bcdef} ± 0.18
3	30	3.14^{cdef} ± 0.25
4	45	3.89^{b} ± 0.26
5	60	2.23^{g} ± 0.25
6	75	2.78^{efg} ± 0.17
7	90	2.61^{fg} ± 0.17
8	105	3.30^{bcde} ± 0.18
9	120	3.59^{bc} ± 0.22
10	135	3.15^{cdef} ± 0.34
11	150	6.31^{a} ± 0.38
12	165	3.36^{bcde} ± 0.14
13	180	2.40^{g} ± 0.17
14	195	3.63^{bc} ± 0.32
15	210	2.88^{defg} ± 0.21

Treatments found significant at 1% and 5% level of significance CD (0.01) = 0.87 CD (0.05) = 0.66.

Those means having atleast one common superscript between groups do not differ significantly.

Table 8.02: Analysis of variance of the data of **serum globulin concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	200.83	14.35	15.67	0.00
Error	225	205.92	0.92	-	-
Total	239	-	-	-	-

Coefficient of Variation = 28.66

The serum globulin pool harbours a major fraction of immunoglobulin that is likely to deplete or increase because of stress generally encountered during parturition and early lactation. Infectious state also determines the level of serum globulin however the increase or decrease depends on exposure duration as being acute or chronic. Mohamed (2014) has observed significant difference in levels of serum globulin between early and late lactation.

Postpartum decrease in globulin levels realized in present research can be attributed to lowered feed intake as well as parturition stress. Similar results were also obtained by Karapehlivan *et al.,* (2007) and Bamerny (2013). Peri-parturient decrease in serum globulin has also been reported by Ghanem *et al.,* (2012).

Heat stress also causes depletion of serum globulin reserves and this was observed in the present study. Further, it was seen that as the THI increases the serum globulin concentrations tend to decrease. These findings are in consensus with by Bernabucci *et al.,* (2014), Das *et al.,* (2016), Garner *et al.,* (2017) and Kekana *et al.,* (2018).

4.9 Serum Albumin Globulin Ratio (A:G)

The mean serum albumin globulin ratio / A:G (AG ratio) from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 9.01, its analysis of variance in Table 9.02 and graph portrayed in Figure 12.

The average serum AG ratio throughout lactation ranged between 0.69 ± 0.06 to 2.88 ± 0.57. It was peaked significantly on days 60 and 180 postpartum and dipped significantly on days 45 and 150 of lactation. Accentuated values of AG ratio on these days corresponded with sagging of serum globulin values and vice versa. During the early lactation the AG ratio was low till day 45 and thereafter it surged up to day 135 lactation. During mid and early phase of lactation from day 135 to 210, it varied without revealing any extricating trend.

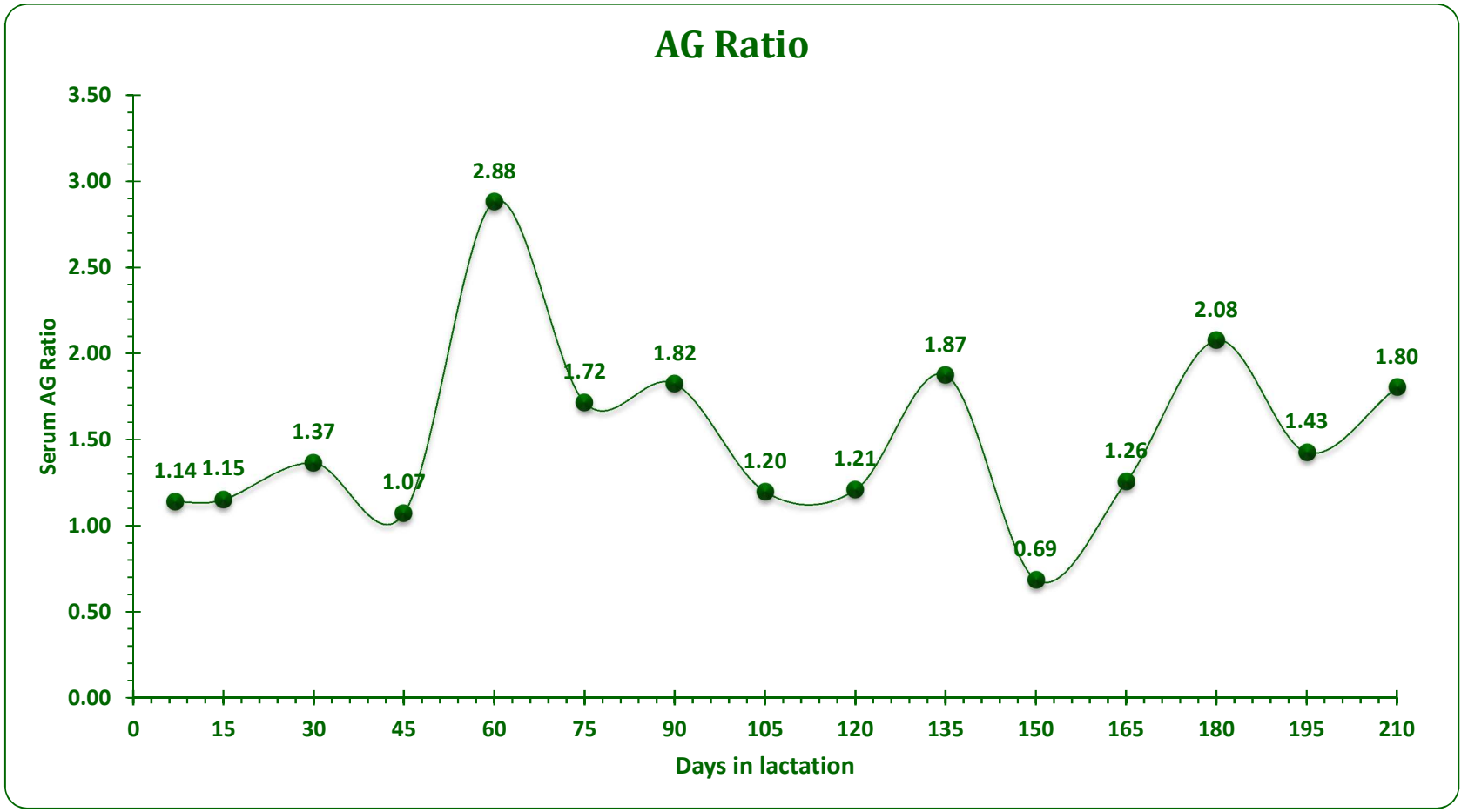

Figure 12. Serum AG Ratio from day 7 to day 210 of lactation in Murrah buffaloes.

Table 9.01: **Serum albumin globulin ratio** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum AG Ratio
1	07	1.14^{efg} ± 0.09
2	15	1.15^{efg} ± 0.08
3	30	1.36^{cdef} ± 0.16
4	45	1.07^{fg} ± 0.11
5	60	2.88^{a} ± 0.57
6	75	1.72^{bcde} ± 0.17
7	90	1.82^{bcd} ± 0.17
8	105	1.20^{defg} ± 0.08
9	120	1.21^{defg} ± 0.13
10	135	1.88^{bc} ± 0.40
11	150	0.69^{g} ± 0.06
12	165	1.26^{cdefg} ± 0.07
13	180	2.08^{b} ± 0.22
14	195	1.43^{cdef} ± 0.18
15	210	1.81^{bcd} ± 0.25

Treatments found significant at 1% and 5% level of significance CD (0.01) = 0.83 CD (0.05) = 0.63.

Those means having atleast one common superscript between groups do not differ significantly.

Table 9.02: Analysis of variance of the data of **serum albumin globulin ratio** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	63.59	4.54	5.52	0.00
Error	225	184.99	0.82	-	-
Total	239	-	-	-	-

Coefficient of Variation = 59.97

During the immediate period following postpartum the stress during early lactation lowered the albumin levels but the lowering of globulin levels might be to some extent nullified by increased gamma globulin synthesis. This could have lowered AG ratio in early lactation. Bhat *et al.,* (2016) also noted similar trend during early, mid and late lactation in Toggenberg goats. Nehra (2016) also reported an increase in A:G during mid-lactation (3 – 5 months) as compared to lower values during early lactation (0 – 2 months) in Sahiwal cattle.

Similar findings of decreased AG ratio upto 35 days postpartum followed by a surge have been reported by Piccione *et al.,* (2011) in Holstein-Friesian cows. Postpartum decrease in A:G in the present finding is also supported by Alberghina *et al.,* (2015) in transition dairy cows while they compared prepartum and postpartum states. The reason implicated for such decrease was also attributed to the utilization of albumin in milk biosynthesis and transfer of immunoglobulin in milk for passive immunity.

When the lactation approaches its peak, there is excess diversion of body fluids for milk volume leading to relatively low plasma volume and subsequently higher relative concentration of albumin. This could also be the reason for increase in A:G during mid-lactation.

Moreover due to heat stress a higher THI is likely to cause reduction in albumin levels leading to low AG ratio during the present investigation. The AG ratio decrease was also learnt during hot dry season in Nili-Ravi buffaloes in the research conducted by Das *et al.,* (2013).

4.10 Serum Urea

The mean serum urea concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 10.01, its analysis of variance in Table 10.02 and the graph is represented in Figure 13.

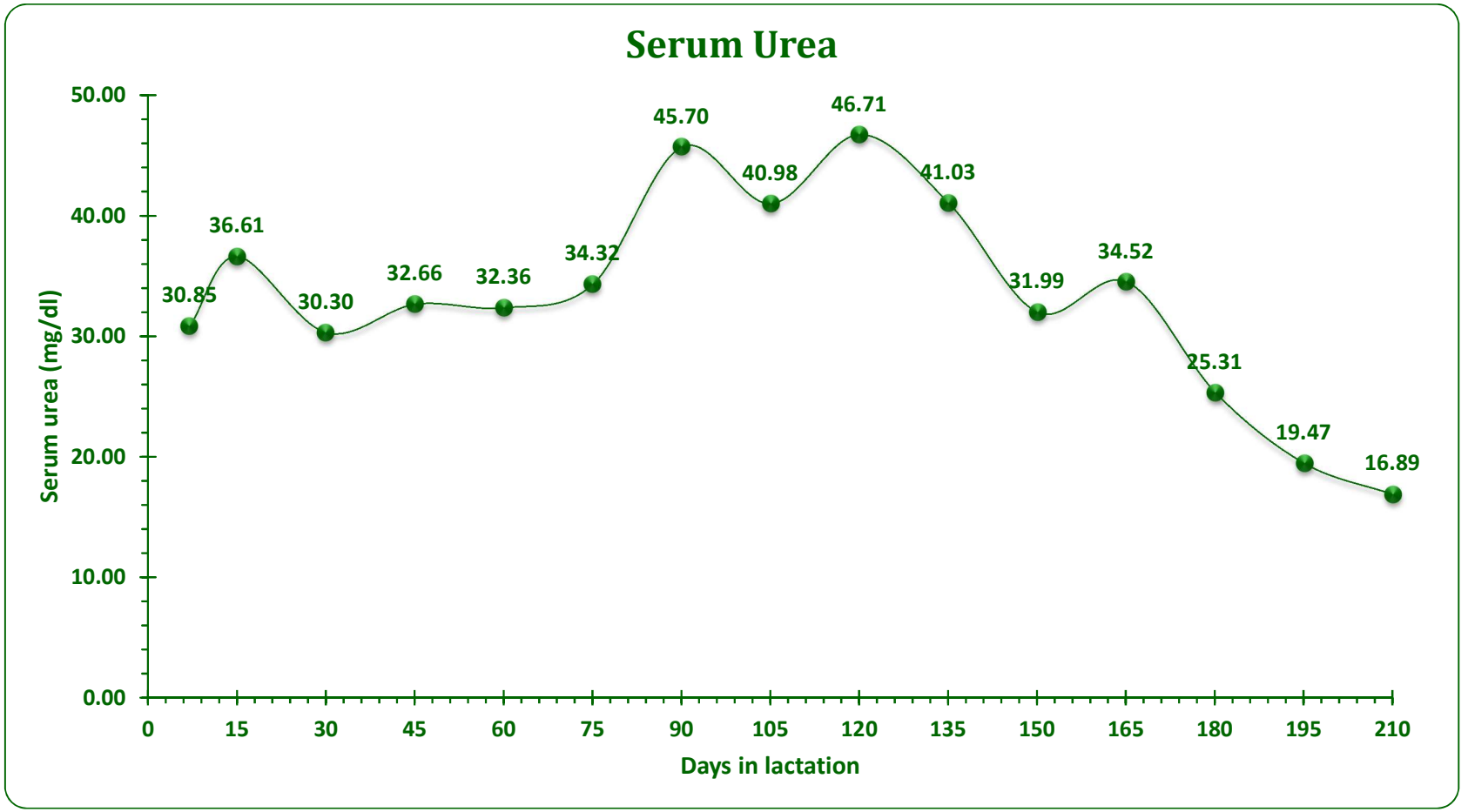

Figure 13. Serum Urea (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 10.01: **Serum urea (mg/dl)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum Urea (mg/dl)
1	07	30.85 ^{cd} ± 3.30
2	15	36.61 ^{bc} ± 2.88
3	30	30.30 ^{cd} ± 1.51
4	45	32.66 ^{cd} ± 2.38
5	60	32.36 ^{cd} ± 1.36
6	75	34.32 ^{bc} ± 1.63
7	90	45.70 ^{a} ± 3.71
8	105	40.98 ^{ab} ± 4.15
9	120	46.71 ^{a} ± 4.77
10	135	41.03 ^{ab} ± 4.36
11	150	31.99 ^{cd} ± 3.27
12	165	34.52 ^{bc} ± 2.04
13	180	25.31 ^{de} ± 1.13
14	195	19.47 ^{ef} ± 0.71
15	210	16.89 ^{f} ± 1.21

Treatments found significant at 1% and 5% level of significance CD (0.01) = 15.639 CD (0.05) = 11.899.

Those means having atleast one common superscript between groups do not differ significantly.

Table 10.02: Analysis of variance of the data of **serum urea concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	16126.72	1151.91	8.80	0.00
Error	225	29437.19	130.83	-	-
Total	239	-	-	-	-

Coefficient of Variation = 34.34

The average serum urea (mg/dl) throughout the lactation ranged between 16.89 ± 1.21 to 46.71 ± 4.77. They were significantly optimized on days 90 and 120 of lactation during later stages of early lactation that was near to peak lactation. Significantly moderate levels were observed on day 210 *i.e.* during late phase of mid lactation. Immediately after parturition on day 7 of lactation, levels were in a normal range and spiralled on day 15 followed by a slackening on day 30. Thereafter the levels of serum urea rose with advancing lactation and reached its zenith after attainment of peak lactation. Thenceforth, serum urea values dwindled until the nethermost value of present investigation *i.e.* day 210 postpartum.

The source of blood urea is either excess protein catabolism or deamination of amino acid during lactational stress and due to pathways of milk biosynthesis. An increase may indicate excess protein breakdown or mobilization for milk constituent synthesis. Stress of parturition and early lactation may lead to increased cortisol secretion causing protein breakdown and increased hepatic deamination (McDonald, 1980).

In the present investigation, from a lower level on day 7 postpartum the levels showed an increasing trend up to mid-lactation. Similar findings have been reported by McDonald (1980) and Naser *et al.,* (2014) in dairy cow and Ouanes *et al.,* (2011) in domestic sheep. Similarly Karapehlivan *et al*., (2007) revealed an increase on day 30 of lactation in serum urea as compared to levels on first day of lactation in dairy cows.

Although the present analysis was up to 210 days postpartum, similar results were also perceived by Nozad *et al.,* (2012) wherein they reported the lowest values of serum urea in lactating cows in tenth month of lactation.

Faith *et al.,* (2017) proposed that higher urea concentration in lactating ewes could be a result of muscles protein catabolization when large amounts of body reserves are mobilized which is in agreement with the ewes BCS and body weight. Caldeira *et al.,* (2007) concluded that ewes with lower BCS have greater urea concentration.

During the present analysis blood urea levels were generally higher when the THI was elevated. Even though the THI values were only slightly high, levels of urea in blood were still increased to some extent except towards the end of study *i.e.* from day 180 to 210 of lactation. The levels were higher from early lactation up to 165 day of lactation.

The THI values were higher throughout the experiment from day 30 except on day 165. Similar findings of increase in blood urea were also observed by Baumgard and Rhoads (2007) and Shwartz *et al.,* (2009) in heat stressed cows during mid-lactation.

In contrast to the moderate levels of serum urea in early lactation in the present study, significant decrease in urea concentration during early lactation in cows by Elitok *et al.,* (2006) and in buffaloes by Hagawane *et al.,* (2009) have been reported.

4.11 Serum Creatinine

The mean serum creatinine concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 11.01, its analysis of variance in Table 11.02 and the graph is depicted in Figure 14.

The average serum creatinine (mg/dl) throughout lactation ranged between 0.19 ± 0.10 to 1.40 ± 0.09. The concentrations were significantly loftier during early lactation on days 7, 15, 45 and 75, but was significantly humbler on days 135, 180 and 195 of lactation. The levels were enlarged immediately postpartum and during early lactation approximately up to the realization of peak lactation yield. Thereafter they faded up to day 105 and kept wavering fortnightly to reach the lowest value on day 195 of lactation.

Early lactation was marked by consistently boosted levels whereas the levels after day 75 were on a capriciously waning trend. Increased nutritional requirement during postpartum and early lactation causes increased protein metabolism.

This increased metabolism acts as a source of serum creatinine. The apparent increase in creatinine level at the early stage of lactation may be ascribed to uterine involution and myometrial protein degradation (Bell *et al.,* 2000).

Intensified serum creatinine values quantified during the lactating postpartum duration as compared to normal range have also been affirmed by Antunović *et al.,* (2011) and Kumar *et al.,* (2015). Lactating ewes also were reported with improved creatinine levels as compared to pregnant ewes (Faith *et al.,* 2017). While reviewing changes of some haematochemical parameters in HF dairy cows, Piccione *et al.,* (2012) revealed serum creatinine levels to be highest at postpartum which then decreased throughout lactation.

Present findings of our research were similar to the observation of Piccione *et al.,* (2012) except that the levels of creatinine during early lactation were consistently high up to day 75 of lactation. This could be because of increasing THI that induced heat stress. During heat stress, protein breakdown may occur for gluconeogenesis to suffice energy demands for thermoregulation. Increased creatinine during most of the lactation in the present experiment was associated with increased THI.

Similar to the findings of present study, increase in creatinine levels were also reported during heat stress by Yadav *et al.,* (2016) in Murrah buffaloes. Higher creatinine levels were also associated with high THI by Kekana *et al.,* (2018) and Chaudhary *et al.,* (2015).

Along with heat stress, the increasing milk yield during early lactation may also lead to reduction in body fluids simulating dehydration like conditions, which is likely to reduce blood flow to kidneys thus decreasing the urine output. Reduction in blood flow to kidney will scale down renal creatinine clearance and retain creatinine in the blood. Thus, relatively higher serum creatinine levels will be observed. In the present research also, the elevated early lactation serum creatinine levels could be associated with the physiological status of increasing milk yield and surrounding climatic factors triggering a surge in the THI.

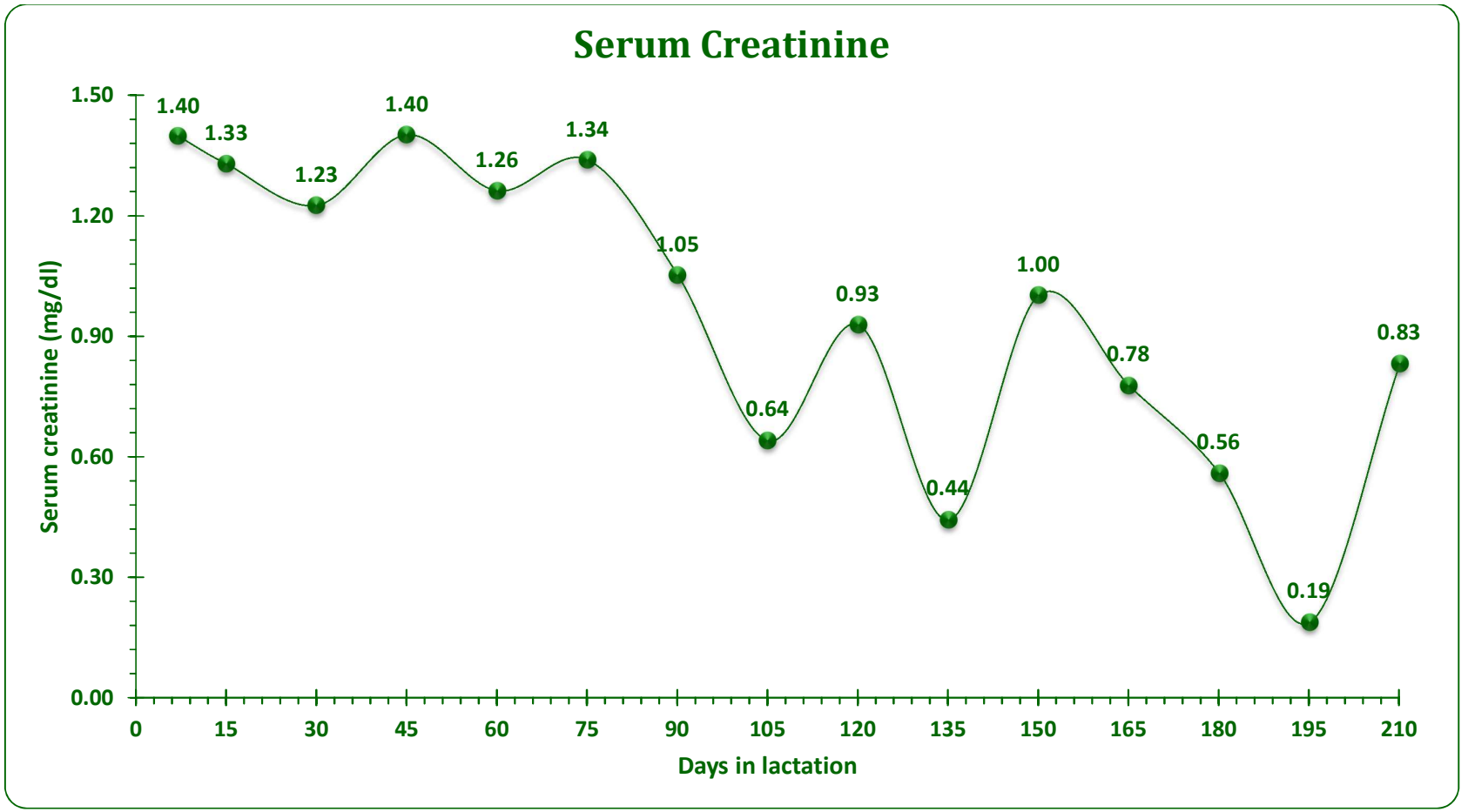

Figure 14. Serum creatinine (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 11.01: **Serum creatinine** (mg/dl) from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum Creatinine (mg/dl)
1	07	$1.40^{a} \pm 0.06$
2	15	$1.33^{a} \pm 0.07$
3	30	$1.23^{ab} \pm 0.06$
4	45	$1.40^{a} \pm 0.09$
5	60	$1.26^{ab} \pm 0.06$
6	75	$1.34^{a} \pm 0.08$
7	90	$1.05^{bc} \pm 0.06$
8	105	$0.64^{fg} \pm 0.12$
9	120	$0.93^{cde} \pm 0.07$
10	135	$0.44^{g} \pm 0.10$
11	150	$1.00^{cd} \pm 0.06$
12	165	$0.78^{ef} \pm 0.07$
13	180	$0.56^{g} \pm 0.04$
14	195	$0.19^{h} \pm 0.01$
15	210	$0.83^{def} \pm 0.12$

Treatments found significant at 1% and 5% level of significance CD (0.01) = 0.280 CD (0.05) = 0.213

Those means having atleast one common superscript between groups do not differ significantly.

Table 11.02: Analysis of variance of the data of **serum creatinine concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	32.11	2.29	24.24	0.00
Error	225	21.29	0.10	-	-
Total	239	-	-	-	-

Coefficient of Variation = 32.073

4.12 Serum Glutamate – Pyruvate Transaminase (SGPT)

The mean serum glutamate – pyruvate transaminase / alanine aminotransferase (SGPT / ALT) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 12.01, its analysis of variance in Table 12.02 and the graph is shown in Figure 15.

The average serum SGPT (IU/L) throughout the lactation were between 24.50 ± 2.59 to 54.59 ± 3.42. The SGPT levels immediately subsequent to parturition were elevated and rose upto day 90 of lactation which showed the significant apex for the SGPT values, and thereafter subsided. The significantly lowest values were seen on day 135 and 150 of lactation. Essentially, the levels upto 3 months *i.e.* early lactation were buffeted and thereafter abated levels were persisted throughout lactation.

This is analogous to Abdulkareem (2013) who reported that SGPT values increased on days 45 and 60 postpartum compared with other periods in buffaloes. SGPT activity surge in buffalo blood during lactation signifies an increase in hepatic metabolism (Ashmawy *et al.,* 2015a). Variations in its enzymatic activities may be due to decreased dry matter intake that impacts hepatic lipidosis to subsequently generate altered liver function (Greenfield *et al.,* 2000).

In the present research SGPT levels were found to be high during early and late lactation but low during mid-lactation. Similar to the present findings Jóźwik *et al.,* (2012) in their investigation revealed that low levels of SGPT occurred on day 60, but the lowest was on day 210.

An opposite pattern was discerned in the work done by Patkowski *et al.,* (2006) wherein they detected lower SGPT activity in sheep blood on the 40th day of lactation. Contradictory observations by Stojevic *et al.,* (2005) and Liu *et al.,* (2012) in Holstein cows and Zvorc *et al.,* (2006) in sows reported significantly higher SGPT activity during mid-lactation than in early lactation. The probable cause for this elevated SGPT activity could be the peak milk yield, which creates a negative energy balance during mid-lactation.

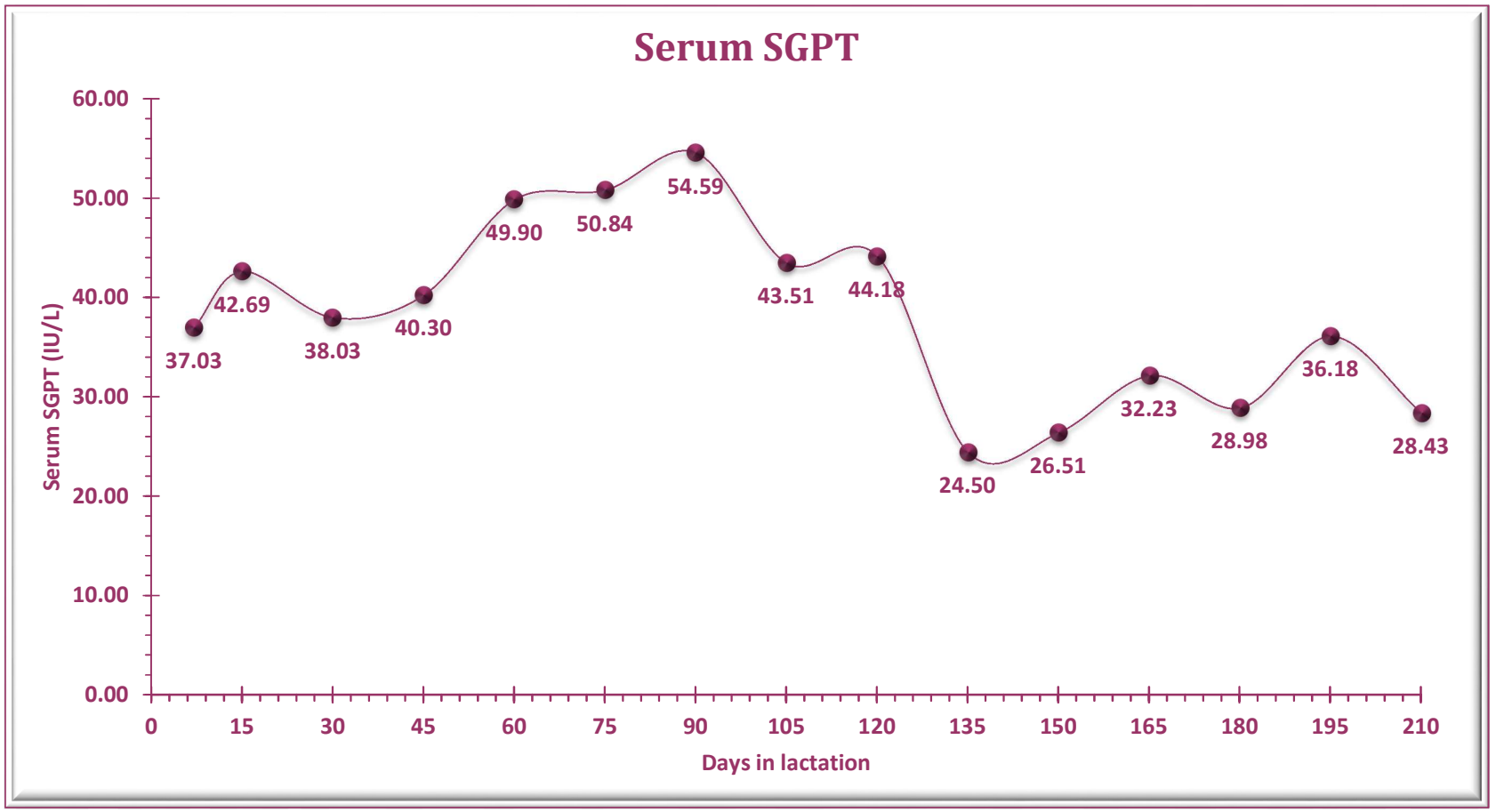

Figure 15. Serum Glutamate – Pyruvate Transaminase (IU/L) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 12.01: **Serum glutamate – pyruvate transaminase (IU/L)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum glutamate – pyruvate transaminase (IU/L)
1	07	37.03 ^{def} ± 1.53
2	15	42.69 ^{cde} ± 1.87
3	30	38.03 ^{def} ± 1.61
4	45	40.30 ^{de} ± 1.76
5	60	49.90 ^{abc} ± 3.04
6	75	50.84 ^{ab} ± 1.39
7	90	54.59 ^{a} ± 3.42
8	105	43.51 ^{bcde} ± 1.26
9	120	44.18 ^{bcd} ± 1.78
10	135	24.50 ^{h} ± 2.59
11	150	26.52 ^{h} ± 4.51
12	165	32.22 ^{fgh} ± 2.54
13	180	28.98 ^{gh} ± 2.94
14	195	36.18 ^{efg} ± 4.86
15	210	28.43 ^{gh} ± 3.78

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 10.27 CD (0.05) = 7.81.

Those means having atleast one common superscript between groups do not differ significantly.

Table 12.02: Analysis of variance of the data of **serum glutamate – pyruvate transaminase concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	19172.29	1369.45	10.78	0.00
Error	225	28586.63	127.05	-	-
Total	239	-	-	-	-

Coefficient of Variation = 29.26

Additionally, the physiological status of animals notably influence the serum SGPT levels (Otto *et al.,* 2000). Yaylak *et al.,* (2009) postulated a substantial influence of the animal's lactational stage on the serum SGPT levels and Antunovic *et al.,* (2011) conveyed that an increase in SGPT activity in ewes during lactation is suggestive of hepatic metabolism escalation. Further, SGPT is considered to be an effective biomarker to detect the energetic and mineral imbalance in Saanen dairy goats (Mundim *et al.,* 2007).

Apart from lactation, the rising values of THI in the present research also had an additive effect that resulted in increase of SGPT during early lactation. The effect of THI on SGPT levels seems to be more related during early lactation as high milk yield is likely to be affected more as compared to low milk production. Early lactation is characterized by increasing milk yield up to peak lactation. Higher SGPT values due to summer or heat stress has also been reported by Koubkova *et al.,* (2002), Bhan *et al.,* (2012) and Kalamath (2015).

4.13 Serum Glutamic – Oxaloacetic Transaminase (SGOT)

The mean serum glutamic – oxaloacetic transaminase / aspartate aminotransferase (SGOT / AST) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes is presented in Table 13.01, its analysis of variance in Table 13.02 and the graph is depicted in Figure 16.

The average serum SGOT (IU/L) throughout the lactation ranged between 36.18 ± 3.76 to 165.80 ± 10.53. The loftiest significant SGOT value was witnessed immediately postpartum and comparatively uplifted values were endured upto day 120 of lactation. Thereupon the concentration declined to the significantly baseline value on day 150. After day 150 of lactation, the levels boosted marginally but still were subjacent as compared to early lactation. After the marginal escalation on day 165 these levels waned upto day 210 of lactation. Augmented immediate postpartum SGOT levels might be ascribed to parturition and early lactation stresses. Another factor accountable for the magnified serum SGOT levels was heat stress or enhanced temperature humidity index.

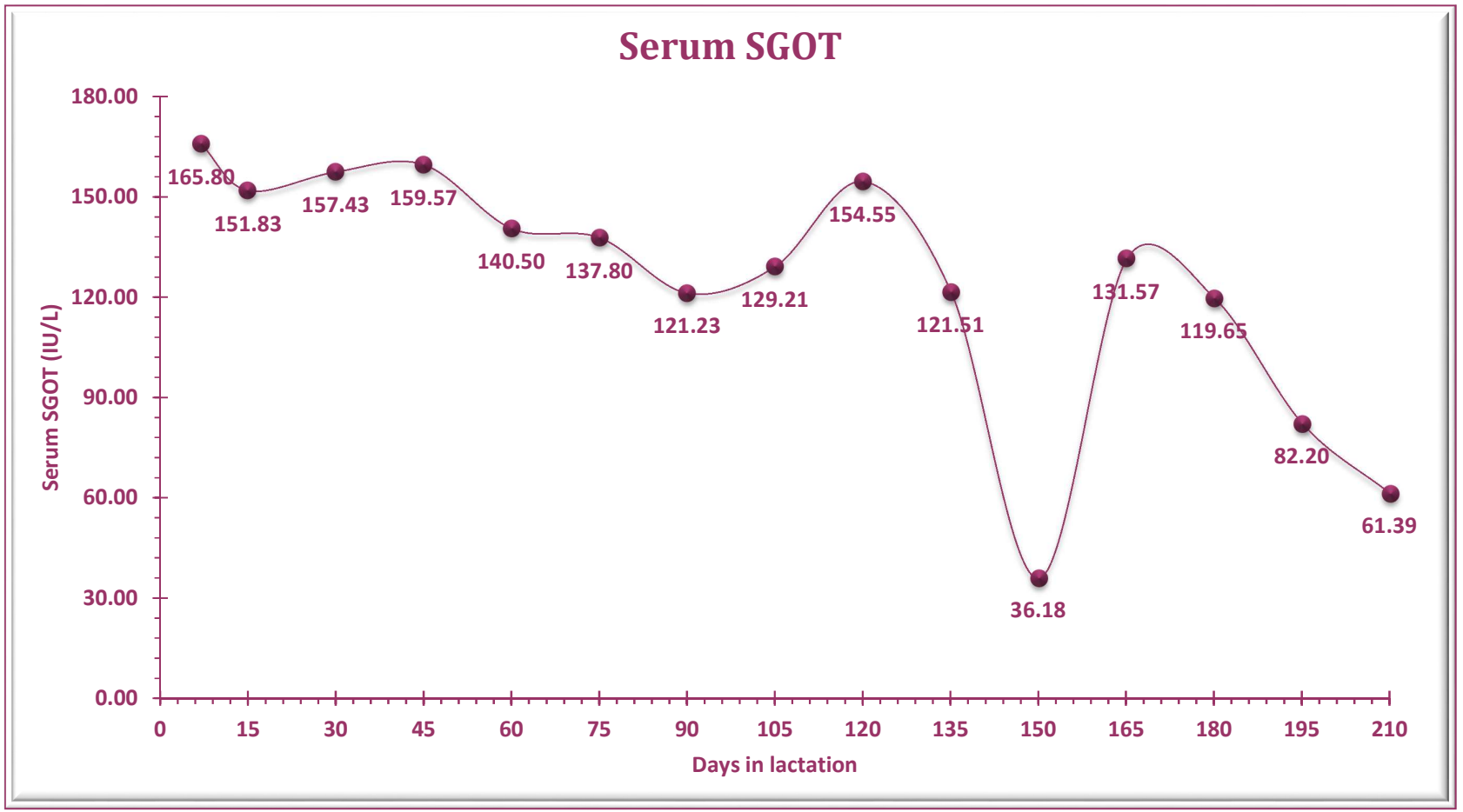

Figure 16. Serum Glutamic – Oxaloacetic Transaminase (IU/L) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 13.01: **Serum glutamic – oxaloacetic transaminase (IU/L)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum glutamic – oxaloacetic transaminase (IU/L)
1	07	165.80 [a] ± 10.53
2	15	151.83 [abc] ± 4.04
3	30	157.43 [ab] ± 6.39
4	45	159.57 [ab] ± 5.75
5	60	140.50 [bcd] ± 4.61
6	75	137.80 [cde] ± 5.76
7	90	121.23 [de] ± 7.77
8	105	129.21 [de] ± 4.23
9	120	154.55 [abc] ± 7.33
10	135	121.51 [de] ± 13.97
11	150	36.18 [h] ± 3.76
12	165	131.57 [de] ± 4.04
13	180	119.65 [e] ± 6.87
14	195	82.20 [f] ± 6.63
15	210	61.39 [g] ± 5.28

Treatments found significant at 1% and 5% level of significance CD (0.01) = 25.45 CD (0.05) = 19.36.

Those means having atleast one common superscript between groups do not differ significantly.

Table 13.02: Analysis of variance of the data of **serum glutamic – oxaloacetic transaminase concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	316632.91	22616.64	28.96	0.00
Error	225	175695.85	780.87	-	-
Total	239	-	-	-	-

Coefficient of Variation = 22.41

The enzyme SGOT is not, however, exclusively a liver enzyme, originating from muscle, as well as from liver and accordingly not exclusive for hepatic function (Cornelius, 1980). An elevation in SGOT implies diminished serum proteins, owing to functional changes in the associated organs (Payne and Laws, 1978 and Todorovic and Davidovic, 2012). Subsequent to parturition, there is loss of body condition and muscle mass, triggering an elevation in this enzyme's concentration (Sattler and Furll, 2004). Additionally, mobilization of muscle protein to liver for gluconeogenesis is perceived (Cardoso *et al.,* (2008). The concurrent lipolysis and ketogenesis that entails metabolic adaptation of liver ultimately leads to SGOT escalation.

High SGOT concentrations during early lactation immediately after parturition are also exemplified by colostrum synthesis that necessitates improved metabolic rate and induces enhanced catabolism for maximum production. Fatty liver syndrome and ketosis signs are also associated with SGOT rise (Stojevic *et al*., 2005 and Ghanem *et al.,* 2012). Antunovic *et al.,* (2004) professed that elevated SGOT levels during the postpartum period rather than at calving, implied hepatic metabolism and tissue catabolism during this period. Investigations in transitional Simmental cows by Đoković *et al.,* (2013) similarly concluded that SGOT levels were higher during early lactation as compared to late lactation.

Elevated SGOT concentrations during early lactation as compared to mid-lactation have been observed by Stojevic *et al*., (2005) and Mohamed (2014) in cows and by Antunović *et al.,* (2011) in ewes. During the mid-lactation the declining levels of SGOT could be elucidated by the optimum care and nutrition given to the lactating dam. Moreover, around mid-lactation, the stress of augmenting milk yield also tempered it down. As compared to the prepartum stage, the levels in postpartum Holstein cows have been perceived to be higher by Reist *et al*., (2003).

Heat stress may induce oxidative stress that may trigger damage to hepatic and other tissues leading to leakage of SGOT enzyme. Furthermore, the amplified metabolic demands for thermoregulation during heat stress may also potentiate enhanced hepatic metabolism and consequently elevated SGOT levels.

In the present research also, THI increase was associated with increased SGOT levels even though it had an additive effect along with early lactation stress. Significant increase in SGOT levels due to increase in THI during heat stress has been conveyed by Kalamath (2015) in Hallikar cattle, Rasooli *et al*., (2004) in Holstein heifers and Mazzullo *et al.*, (2014) in cows.

4.14 Serum Glucose

The mean serum glucose concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 14.01, its analysis of variance in Table 14.02 and the graphical representation is depicted in Figure 17.

The average serum glucose (mg/dl) throughout the lactation ranged between 40.15 ± 2.12 to 90.60 ± 3.17. Serum glucose was significantly moderate during early lactation, especially on day 07 and during mid-lactation on day 195. Serum glucose concentrations were troughed on days 30, 45, 90 and 105 and crested on 150, 165 and 180 days of lactation. Generally, the levels were marginal immediately after parturition at the beginning of lactation and augmented thereafter. Early lactation was marked with subsided and mid lactation by surging serum glucose concentrations.

Reduced serum glucose activity in lactating cows has been observed by Gorski and Saba (2012). Analogous to the present research conclusions, Onita and Colibar (2009), Filipejova and Kovacik (2009), Yaylak *et al.,* (2009), Abdulkareem (2013) and Naser *et al.,* (2014) have communicated a surge in the serum glucose values after parturition, followed by an immediate decline during early lactation.

In ewes also, during first trimester of lactation, the serum glucose levels were reported to be diminutive (Antunovic *et al.,* 2011). Most of the studies are in agreement with the findings of the present analysis.

The stress of parturition and utilization of serum glucose for milk lactose biosynthesis can be attributed to low serum glucose on day 07 postpartum. This is

also significant as glucose delivery and uptake by mammary gland is the rate-limiting step of milk synthesis. In cows yielding 20 liters or more of milk a day, it has been substantiated that the mammary gland requirement for glucose surpasses 1,000 grams (Kronfeld, 1971 and Stamatović *et al.,* 1983).

Dahate *et al.,* (2004) and Hagawane *et al.*, (2009) have revealed that serum glucose concentrations are lower in lactating cows as compared to dry cows and Krsmanović *et al.,* (2013) stated that significantly lower blood glucose in freshly-calved cows may insinuate intensive use of glucose by the mammary gland in early lactation.

Serum glucose concentrations in early lactating cows have enabled Djoković *et al.,* (2013a) to postulate that metabolic disorders are associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. As depicted by Bertoni *et al.,* (1994) glucose is not influenced by lactation most likely due to a proficient glucose homeostasis between the insulin-dependent and insulin-independent pathways during lactation.

The glucose concentration declined with escalating milk yield till the peak yield was attained and this was marked by the vast utilization of glucose for lactose biosynthesis.

After the lactational peak, the serum glucose levels in the present analysis again increased to reach higher values at 150, 165, 180 and 210 days of lactation. This could be explained by the fact that the reducing lactation yield caused a sparing effect on serum glucose levels.

A significant increase in glucose as stage of lactation advances was determined by Sobiech *et al.,* (2008) in Kamieniec ewes. Escalating glucose concentrations may be associated with augmented milk production due to early lactation and improved activity of mammary gland quadrupling the energy needs (Block *et al.,* 2001).

It is established that in lactating ruminants, the tissue needs for insulin are reduced, which can effect a temporary increase in the serum glucose concentration to accordingly stimulate milk production (Szczepański *et al.,* 2005).

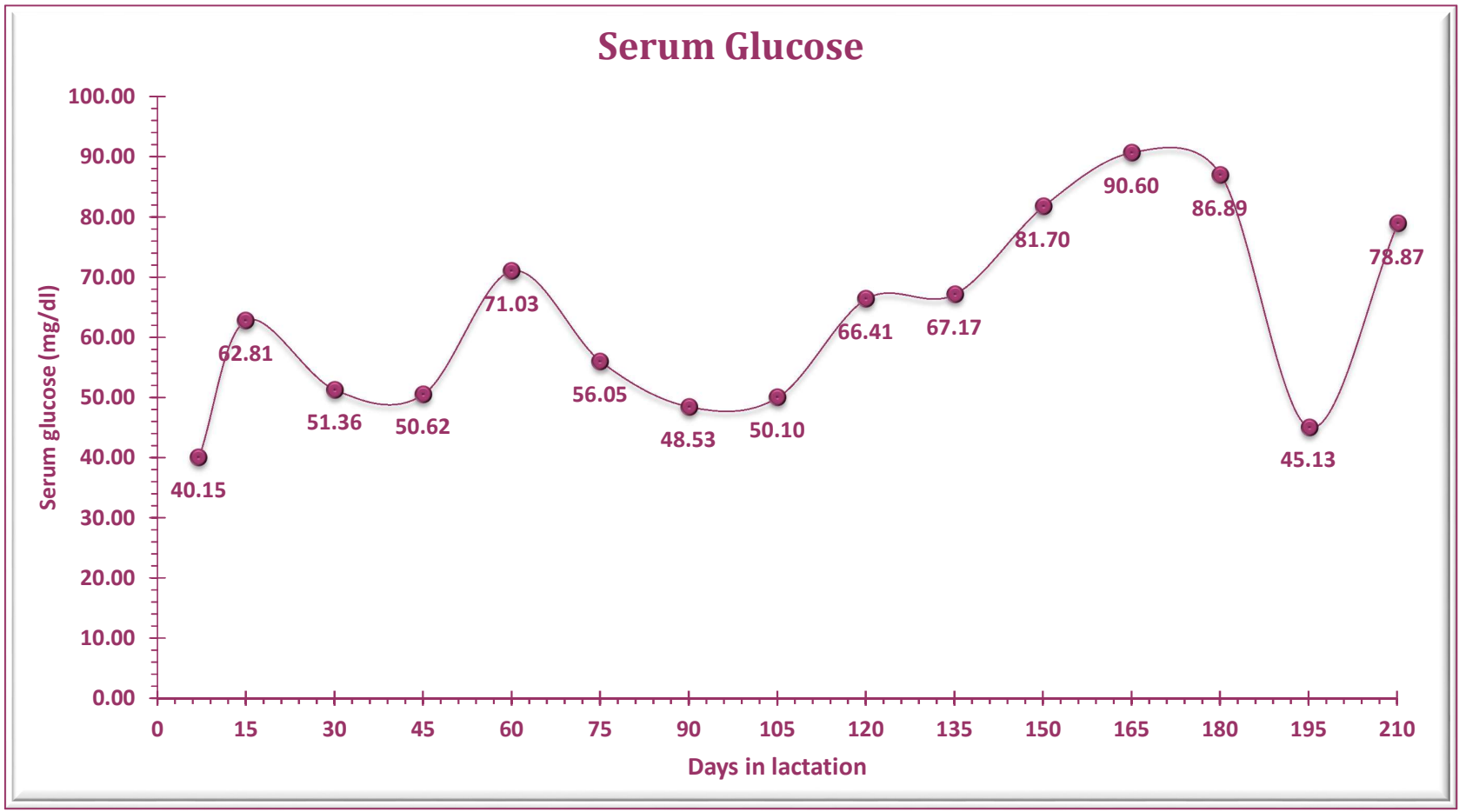

Figure 17. Serum Glucose (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 14.01: **Serum glucose (mg/dl)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Glucose (mg/dl)	
1	07	40.15 ^{h}	± 2.12
2	15	62.81 ^{def}	± 4.58
3	30	51.36 ^{fgh}	± 5.35
4	45	50.62 ^{gh}	± 5.41
5	60	71.03 ^{bcd}	± 5.92
6	75	56.05 ^{efg}	± 4.63
7	90	48.53 ^{gh}	± 3.72
8	105	50.10 ^{gh}	± 3.48
9	120	66.41 ^{de}	± 3.47
10	135	67.17 ^{cde}	± 5.17
11	150	81.70 ^{ab}	± 5.75
12	165	90.60 ^{a}	± 3.17
13	180	86.89 ^{a}	± 3.18
14	195	45.13 ^{gh}	± 3.26
15	210	78.87 ^{abc}	± 2.80

Treatments found significant at 1% and 5% level of significance CD (0.01) = 15.65 CD (0.05) = 11.90.

Those means having atleast one common superscript between groups do not differ significantly.

Table 14.02: Analysis of variance of the data of **serum glucose concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	57304.55	4093.18	13.88	0.00
Error	225	66342.53	294.86	-	-
Total	239	-	-	-	-

Coefficient of Variation = 27.19

During the present experiment serum glucose values maintained an inverse relationship with THI wherein it was perceived that whenever the THI was high, serum glucose was low. Studies done by Abeni *et al.,* (2007), Rhoads *et al.,* (2009), Baumgard and Rhoads (2013) and Guo *et al.,* (2018) are in concurrence with the our finding of heat stress lowering blood glucose. An enhanced effect of lowering the already low blood glucose seems to have transpired on days 45, 75, 90, 105 and vice versa on days 15, 150, 165 and 180 of lactation.

4.15 Serum Calcium (Ca)

The mean serum calcium (Ca) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 15.01, its analysis of variance in Table 15.02 and its graphic portrayal is depicted in Figure 18.

Serum total calcium concentrations showed a significant high on day 7 of lactation, which was followed by a decrease in the serum calcium levels. From day 60 to day 105 of lactation the serum calcium concentration surged non-significantly. Subsequently, there was a small dip and followed by a rise upto day 150 of lactation. Thereafter, the concentration waned till a sudden rise on day 210 of lactation.

The average serum calcium throughout the lactation ranged between 08.30 ± 0.44 to 11.55 ± 0.48 mg/dl which was a little lower than the published range in cows of being between 11.2 – 13.6 mg/dl (Kaneko *et al.,* 2008). It was however in lieu with the concentration (8.96 mg/dl) proposed by Patel *et al.,* (2016) in non-pregnant lactating Banni buffalo but higher than 4.5 – 6.0 (mg/dl) as quantified by Dukes *et al.,* (2015) for cows.

Fundamentally, the serum calcium concentrations were stable and raised during peak and mid-lactation but fluctuating around early and late lactation. The THI levels were also elevated during mid-lactation in our exploration.

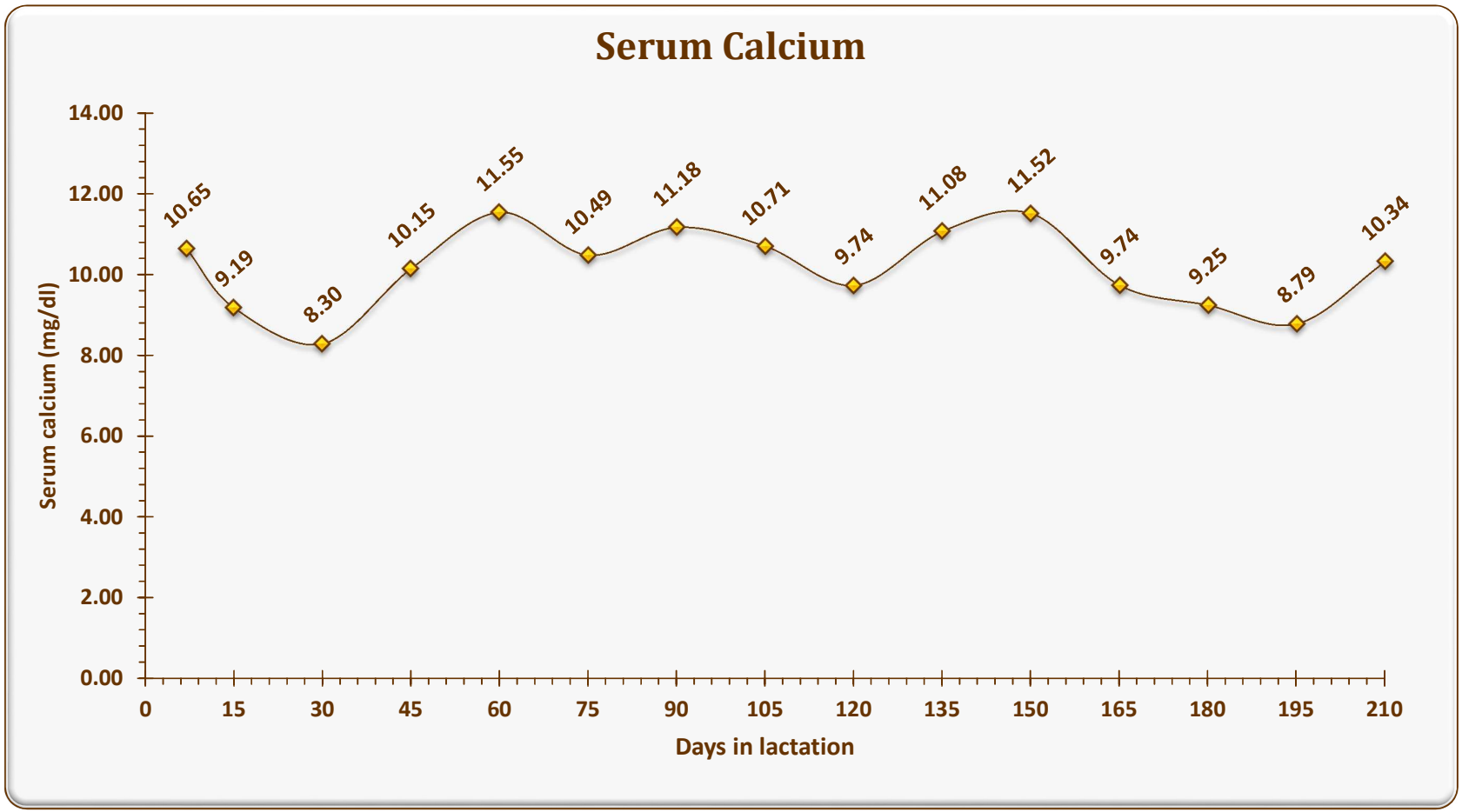

Figure 18. Serum Calcium (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 15.01: **Serum calcium (mg/dl)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum calcium (mg/dl)
1	07	$10.65^{abc} \pm 0.43$
2	15	$09.19^{efg} \pm 0.36$
3	30	$08.30^{g} \pm 0.44$
4	45	$10.15^{bcde} \pm 0.43$
5	60	$11.55^{a} \pm 0.48$
6	75	$10.49^{abc} \pm 0.31$
7	90	$11.18^{ab} \pm 0.46$
8	105	$10.71^{abc} \pm 0.45$
9	120	$09.74^{cdef} \pm 0.38$
10	135	$11.08^{ab} \pm 0.48$
11	150	$11.52^{a} \pm 0.34$
12	165	$09.74^{cdef} \pm 0.29$
13	180	$09.25^{defg} \pm 0.45$
14	195	$08.79^{fg} \pm 0.39$
15	210	$10.34^{bcd} \pm 0.40$

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 1.50 CD (0.05) = 1.14

Those means having atleast one common superscript between groups do not differ significantly.

Table 15.02: Analysis of variance of the data of **serum calcium concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	220.83	15.77	5.84	0.00
Error	225	607.70	2.70	-	-
Total	239	-	-	-	-

Coefficient of Variation = 16.15

During our investigation, there was a drop in calcium level during the early stage of lactation, which corroborates with the findings of Godden and Allcroft (1932), Wilson and Hart (1932), Allcroft and Godden (1934), Kennedy *et al.*, (1939), Hibbs (1950), Blum *et al.,* (1972), Belyea *et al.,* (1975), Wilson *et al.,* (1977), Nazifi and Sami (1997) and Nehra (2016) in cows and Ciani *et al.,* (1994), Sharma *et al.,* (2000), Hussain *et al.,* (2001), Nale, R.A. (2003), Hagawane *et al.,* (2009), Ashmawy (2015a) and Kashwa (2016) in lactating buffaloes. Further, a majority of these authors deliberated that the serum calcium concentrations which declined immediately after calving started to augment around peak lactation.

Since the commencement of lactation, the physiological mechanisms try to compensate for the immense calcium demand by augmented bone mobilization (lactational osteoporosis) and enhance gastrointestinal tract assimilation (Liesegang, 2006). Despite this, nearly all mammals, but especially cows and buffaloes, are in negative calcium balance during early lactation. In dairy cows, near around 13% of bone calcium (800 to 1300 g) is reabsorbed in early lactation, which can be replenished later in lactation if adequately balanced ration is given (Dukes *et al.,* 2015).

Maternal mineral and skeletal homeostasis during lactation is governed by the need of calcium for milk production (Liesegang *et al.,* 2006). The lower calcium levels during early lactation may perhaps be because of voluminous calcium demand and inadequate parathormone (Paul *et al.,* 2011).

On the other hand, Yokus and Cakir (2006) in cattle, Garaniya *et al.,* (2013) in Jaffarabadi buffaloes and Faith *et al.,* (2017) in ewes indicated that calcium remained primarily same throughout lactation, which advocates the existence of efficacious homeostatic mechanisms.

An opposite precedent was gleaned by Larson *et al.,* (1980) Shukla *et al.,* (1983) Mathapati and Bhat (1988) in crossbred cows and Piccione *et al.,* (2012) in HF cows wherein, the serum calcium and amounts were high during in the course of lactation and dwindled when the lactation ceased. Cavestany *et al.,* (2005) in multiparous cows discerned a steady escalation commencing after parturition and ensued by minimal reduction around day 60 of lactation.

Animals kept primarily on high-grain diets are susceptible to calcium deficiency as opposed to those fed forages especially legumes. Unfortunately, even though forages are superior calcium sources, its availability can be low due to the presence of oxalates that render the calcium insoluble. The metabolic and physiological status of the animal governs its ability to uptake and use the available calcium. Calcium assimilation efficacy fades as the animal ages on account of reduced gastrointestinal tract ability to respond to 1,25-$(OH)_2D$ due to the vitamin D receptors declining (Dukes *et al.,* 2015).

4.16 Serum Phosphorus (P)

The mean serum phosphorus (P) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 16.01, its analysis of variance in Table 16.02 and its graph is depicted in Figure 19.

The serum phosphorus concentrations were non-significant throughout lactation. It declined slightly followed by an escalation around day 45. This was followed by a dip in the values with slight upsurges from day 135 and 165 of lactation. Overall the spikes and plunges detected in the serum phosphorus concentrations resembled those seen for the serum calcium concentrations in our analysis, baring the fact that the phosphorous concentrations were non-significant.

The average serum phosphorus throughout the lactation ranged between 5.38 ± 0.25 to 6.28 ± 0.24 mg / dl and within 4 – 8 mg/dl as proposed by Dukes *et al.* (2015) and nearly between 5.6 – 6.5 mg/dl as prescribed Radostits *et al.,* (2007) in buffaloes and Kaneko *et al.,* (2008) in cows.

In our study, there was no significant change in the phosphorus levels throughout lactation. Our findings are in close conformity with the observations of Hagawane *et al.,* (2009) in lactating buffaloes and Yokus and Cakir (2006) and Shrikhande *et al.,* (2008) in the lactating cows and Bamerny (2013) in goats upto 3 weeks post-partum who reported nearly comparable lactational progression.

Overall similar patterns were also documented by Hussain *et al.,* (2001) findings upto two months post-partum and those of Das *et al.,* (2018) in lactating buffaloes are in conjunction with ours but the serum phosphorus concentrations were lower.

Our findings are in contradiction with those of Piccione *et al.,* (2012), Ate *et al.,* (2009) and Nessim (2010) wherein the serum phosphorus values increased during the dairy cows' lactation period and subsided only at the end of lactation. Moderate reduction in the levels of phosphorus might be due to the necessity of it for the colostrum synthesis (Serdaru *et al.,* 2011) and enhanced carbohydrate metabolism. Hagawane *et al.,* (2009) found that the serum phosphorus level in early stage of lactation was significantly ($p<0.05$) lower than the normal healthy control buffaloes.

The average serum phosphorus throughout the lactation revealed in our investigation was higher than the concentrations proposed by Pandey *et al.,* (2007), Ashmawy (2015a), Jhambh *et al.,* (2016) and Sateesh *et al.,* (2017) in buffaloes and Yokus and Cakir (2006), Ate *et al.,* (2009), Hadiya *et al.,* (2010) and Piccione *et al.,* (2012) in cows. Flawed feed regime has been linked with low blood phosphorus (Hewett, 1974) or it could be a result of offered salt licks. Low blood phosphorus levels are commonly found in grazing cattle (Parker and Blowey, 1976), and therefore dietary supplement may be essential.

Ahmed *et al.,* (2005), Akhtar *et al.,* (2008) and Patel *et al.,* (2016) in buffaloes and Jhambh *et al.,* (2016) in cattle published serum phosphorus values that were in close agreement with our work. However, our findings disagree with the report of Hussain *et al.,* (2001) in buffaloes and Regmi and Pande (2018) in lactating cross-bred cattle who reported a considerably steeper serum phosphorus range.

Mechanisms for maintaining blood calcium levels perform efficiently most of the time (Goff, 2000). Phosphorus has no direct mechanism of regulation, although calcium-regulating hormones directly affect its blood concentration. The ostensibly depressed phosphorus levels in early and mid-stage of lactation was likely in part due to its removal with milk (Valk *et al.,* 2002).

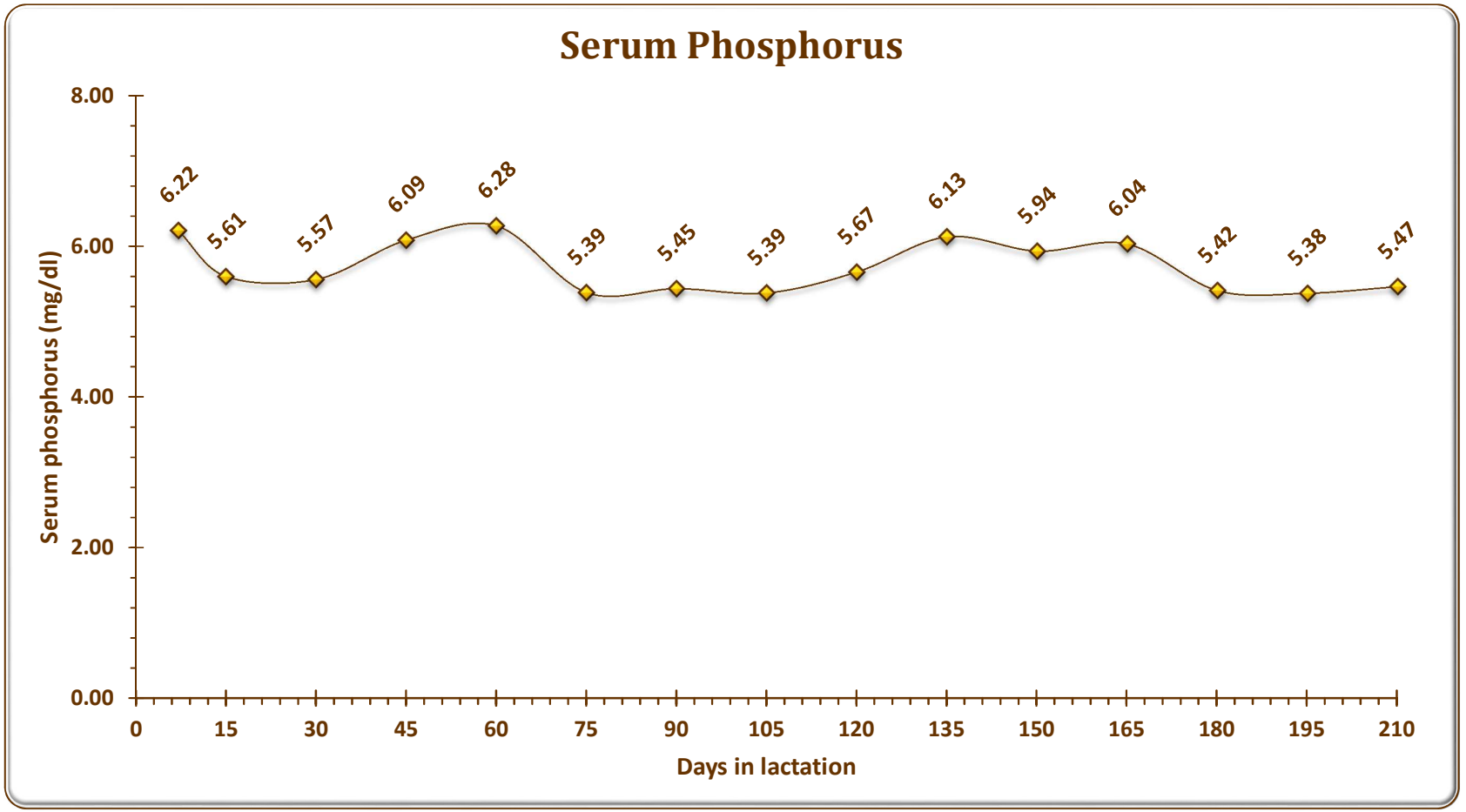

Figure 19. Serum Phosphorous (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 16.01: **Serum phosphorus (mg/dl)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum phosphorus (mg/dl)
1	07	6.22 ± 0.40
2	15	5.61 ± 0.37
3	30	5.57 ± 0.41
4	45	6.09 ± 0.35
5	60	6.28 ± 0.24
6	75	5.39 ± 0.34
7	90	5.45 ± 0.23
8	105	5.39 ± 0.30
9	120	5.67 ± 0.29
10	135	6.13 ± 0.29
11	150	5.94 ± 0.47
12	165	6.04 ± 0.29
13	180	5.42 ± 0.33
14	195	5.38 ± 0.25
15	210	5.47 ± 0.30

Treatments found to be Non-Significant

Table 16.02: Analysis of variance of the data of **serum phosphorus concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	25.77	1.84	0.99	0.47
Error	225	419.93	1.87	-	-
Total	239	-	-	-	-

Coefficient of Variation = 23.818

The additional phosphorus surge, as reported by some authors, perceived during the early lactation may possibly reflect calcium and phosphorus resorption from the bones to cope with the milk production exigencies; the strict hormonal control in calcium levels results in maintenance of its level. Moreover, a large amount of maternal P is transferred to foetus during pregnancy and secreted in milk during lactation (Husnain *et al.,* 1981).

Joshi *et al.,* (2012) concluded that ambient stress during hot environment caused blood phosphorus concentration decline in buffaloes of all physiological states and transformations in the serum phosphorous levels may perhaps be due to heat stress triggering muscular contractions, eliciting carbohydrate metabolism instabilities and ensuing higher serum phosphorous Sreedhar *et al.,* (2013).

4.17 Serum Chloride (Cl)

The mean serum chloride (Cl) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 17.01, its analysis of variance in Table 17.02 and its graph is illustrated in Figure 20.

The serum chloride concentrations were non-significant throughout lactation. The lowest was on day 90 and highest on day 105. It displayed a more or less similar pattern with a sharp decline on day 90. Ensuing this plateau there a steady ascend with intermittent inflation as seen on day 150.

The average serum chloride (mEq/L) throughout the lactation ranged between 87.28 ± 2.14 to 101.76 ± 2.12. It was slightly below the reference values given by Dukes *et al.,* (2015) of 100 – 113 mEq/L and 93 to 107 mmol/l for cows by Winnicka (2015) and 99.0 – 170.0 mEq/L as proposed by Ashmawy (2015a) in lactating Egyptian buffalo, but the concentrations fell within 88 – 119 mEq/L as observed by Ellah (2010) in lactating buffaloes.

Nazifi and Sami (1997) in cows and Patel *et al.,* (2016) in lactating buffalo reported lower serum chloride values.

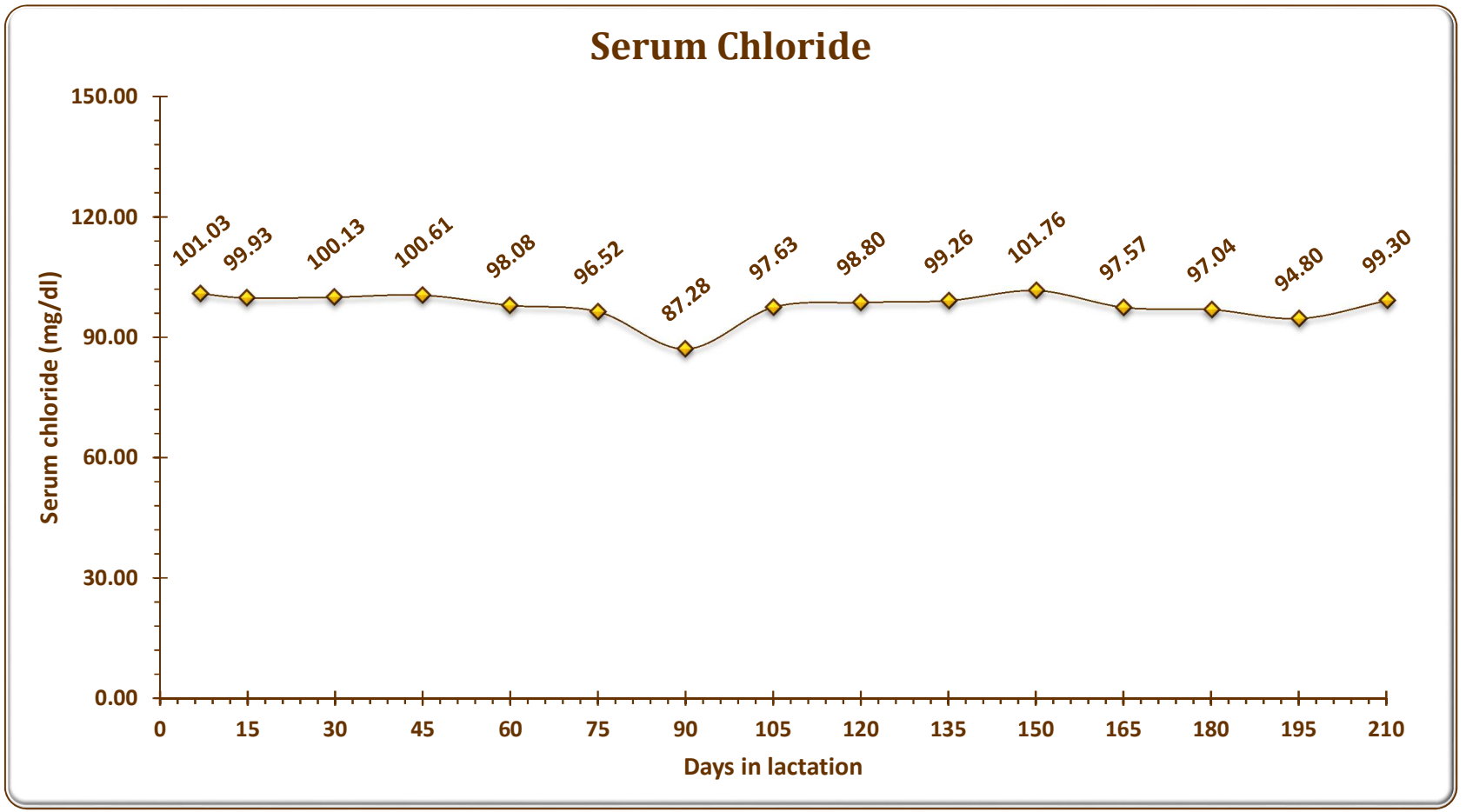

Figure 20. Serum Chloride (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 17.01: **Serum chloride (mEq/L)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum chloride (mg/dl)
1	07	101.03 ± 2.13
2	15	99.93 ± 1.38
3	30	100.13 ± 4.98
4	45	100.61 ± 3.47
5	60	98.08 ± 5.21
6	75	96.52 ± 1.29
7	90	87.28 ± 2.14
8	105	97.63 ± 1.81
9	120	98.80 ± 0.59
10	135	99.26 ± 2.65
11	150	101.76 ± 2.12
12	165	97.57 ± 1.71
13	180	97.04 ± 1.03
14	195	94.80 ± 1.18
15	210	99.30 ± 3.01

Treatments found to be Non-Significant

Table 17.02: Analysis of variance of the data of **serum chloride concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	2926.59	209.04	1.73	0.05
Error	225	27172.58	120.77	-	-
Total	239	-	-	-	-

Coefficient of Variation = 11.26

While Ate *et al.,* (2009), Jarosz (2013) and Kurpinska (2013) in lactating cows observed analogous results, Fettman *et al.,* (1984), Grunwaldt *et al.,* (2005) and Yokus and Cakir (2006) in cattle and Ashmawy (2015a) in lactating Egyptian buffalo obtained higher serum chloride concentrations than those arrived at by us.

The deductions of Yokus and Cakir (2006) in non-mated cattle and Dahima *et al.,* (2016) in Gir cows and Akhtar *et al.,* (2010), Ashmawy (2015a) and Dahima *et al.,* (2016) in buffaloes favoured the present study observations but Kulkarni *et al.,* (1984), Sarwar *et al.,* (2002) and Das *et al.,* (2018) in buffaloes Kupczynski and Chudoba-Drozdowska (2002), Skrzypczak *et al.,* (2008) and Skrzypczak *et al.,* (2014) in cows discerned statistically significant decrease in the serum chloride levels during lactation.

Chloride is involved in the action of hormones and enzymes at sub cellular levels (Arosh *et al.,* 1998). Seeing as the blood chloride concentrations are associated with sodium concentration, fluctuations in sodium concentration may modify the chloride concentration (Batchelder *et al.,* 2007 and Skrzypczak *et al.,* 2008).

Hu and Murphy (2004) noted that since chloride concentration is especially reliant on the nutrient intake, incorrect feeding regime could be concomitant of low levels of chloride, or they may also be supplementary to intermittent offering of salt licks in conjunction with the likelihood of chloride deficient feed.

4.18 Serum Magnesium (Mg)

The mean serum magnesium (Mg) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 18.01, its analysis of variance in Table 18.02 and its graph in Figure 21.

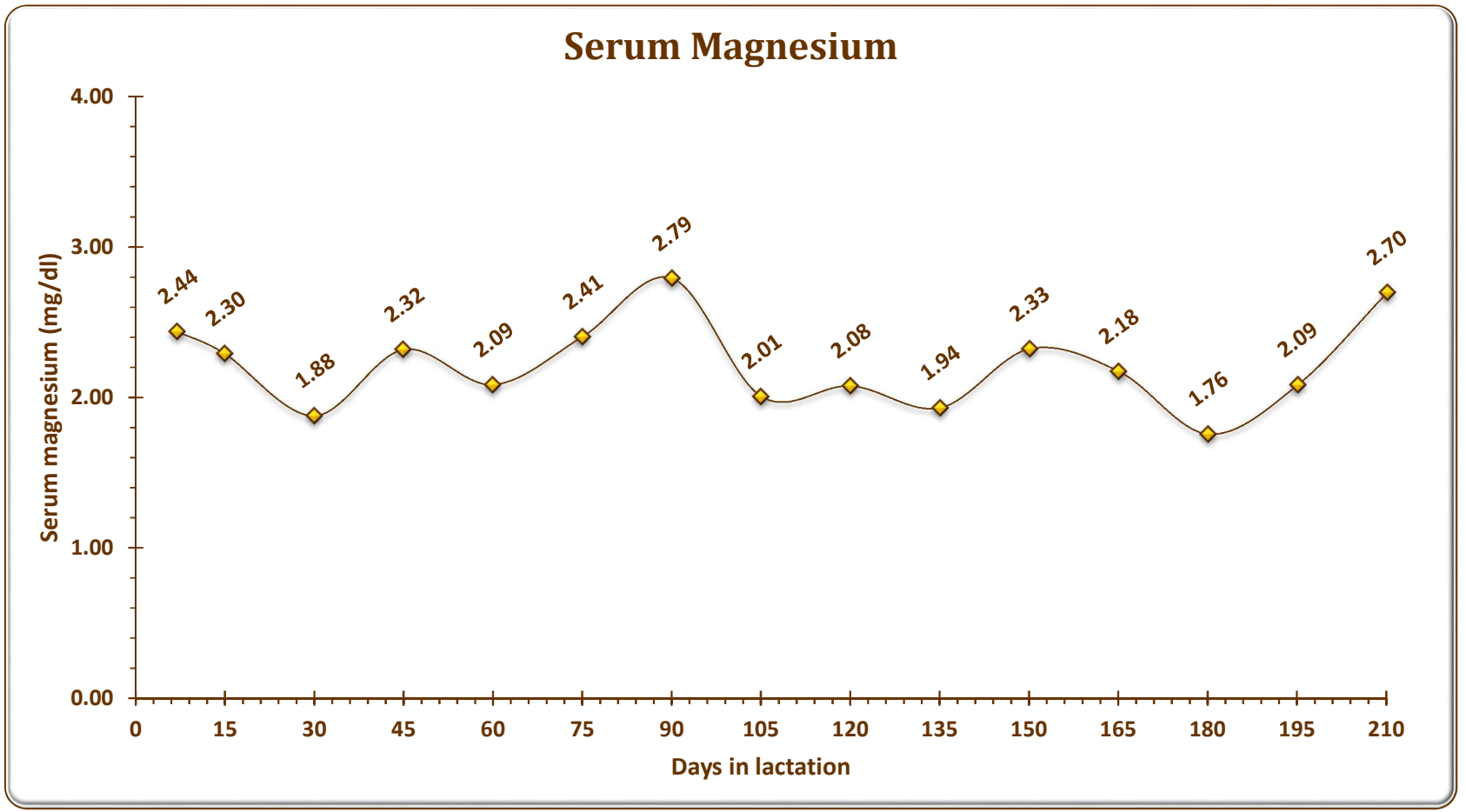

Figure 21. Serum Magnesium (mg/dl) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 18.01: **Serum magnesium (mg/dl)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum magnesium (mg/dl)
1	07	$2.44^{bc} \pm 0.08$
2	15	$2.30^{cd} \pm 0.13$
3	30	$1.88^{fg} \pm 0.11$
4	45	$2.32^{cd} \pm 0.12$
5	60	$2.09^{def} \pm 0.14$
6	75	$2.41^{c} \pm 0.08$
7	90	$2.79^{a} \pm 0.09$
8	105	$2.01^{efg} \pm 0.09$
9	120	$2.08^{def} \pm 0.08$
10	135	$1.94^{efg} \pm 0.06$
11	150	$2.33^{cd} \pm 0.10$
12	165	$2.18^{cde} \pm 0.08$
13	180	$1.76^{g} \pm 0.06$
14	195	$2.09^{def} \pm 0.09$
15	210	$2.70^{ab} \pm 0.11$

Treatments found Significant at 1% and 5% level of significance CD (0.01) 0.36 = CD (0.05) = 0.28

Those means having atleast one common superscript between groups do not differ significantly.

Table 18.02: Analysis of variance of the data of **serum magnesium concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	18.83	1.35	8.47	0.000
Error	225	35.73	0.16	-	-
Total	239	-	-	-	-

Coefficient of Variation = 17.94

Serum total magnesium concentrations showed a significant high on day 7 of lactation, which was followed by a decrease on day 30. From day 45 to day 90 of lactation, it soared, slumped and again soared. Subsequently, there were infrequent spikes till a final significant ascend from days 195 of lactation. The serum magnesium concentrations deduced throughout lactation in this investigation closely emulated the serum calcium concentrations.

The average serum magnesium (mg/dl) throughout the lactation ranged between 1.76 ± 0.06 to 2.79 ± 0.09. It was slightly higher than the reference ranges 1.8 – 2.4 mg/dl recommended by Dukes *et al.,* (2015), 1.8 – 2.3 mg/dl detected by Radostits *et al.,* (2007) in buffaloes and Kaneko *et al.,* (2008) in cows. Ellah (2010) suggested a much broader reference range between 1.6 – 4.4 mg/dl for lactating buffaloes.

Our research showed more or less significant changes in the magnesium concentrations all-through the 210 days study period. Similar trends have been reported by Cavestany *et al.,* (2005), Yokus and Cakir (2006), Piccione *et al.,* (2012) and Nehra (2016) in dairy cows and Hussain *et al.,* (2001) in buffaloes upto two months post-partum. Luke *et al.,* (2019) discerned that serum magnesium concentrations peaked at weeks 3 post-calving, then plateaued.

The results of this investigation diverged from Hagawane *et al.,* (2009), Das *et al.,* (2018) and Kumar *et al.,* (2018) in lactating buffaloes and Nazifi and Sami (1997) and Zidan (2014) in cows who concurred that the serum magnesium levels varied non-significantly during lactation.

The average serum magnesium results measured during the course of lactation in this study corresponded with the previous results of Hussain *et al.,* (2001), Ahmed *et al.,* (2005), Akhtar *et al.,* (2008) and Chaurasia *et al.,* (2010) in buffaloes and Yokus and Cakir (2006), Piccione *et al.,* (2012) and Regmi and Pande (2018) in dairy cows.

Contrasting these results, Hagawane *et al.,* (2009), Patel *et al.,* (2016), Jhambh *et al.,* (2016) and Das *et al.,* (2018) in lactating buffaloes and Rao *et al.,* (1981), Hadiya *et al.,* (2010) and Jhambh *et al.,* (2016) in cows reported higher average serum magnesium concentrations.

Magnesium has a crucial role in the carbohydrate, lipid, nucleic acid and protein metabolism and Mg is required for normal skeletal development and one of the most common enzyme activators (Bamerny, 2013).

While serum calcium and phosphorus have critical bone reserves, there is no fundamental hormonal response for magnesium reparation and its body reserve is primarily minimal (Martens and Schweigel, 2000). Maintenance of normal plasma magnesium concentration is entirely at the mercy of steady dietary magnesium provision (Dukes *et al.,* 2015).

Serum magnesium concentration decline might be attributed to either insufficient consumption or diminished bioavailability of magnesium in the digestive tract (Ghanem *et al.,* 2012). Magnesium, in moderate amount as compared to calcium, is a vital component assimilated in the bone. Zofkova and Kancheva, (1995) hypothesised that its diminished concentrations in non-pregnant buffaloes may be due to Mg inclusion in bone formation of the growing foetus. Further, its apparently low level in early and mid-stage of lactation could likely be in part, due to its inclusion in the milk (Valk *et al.,* 2002).

An added rationale for lower magnesium concentrations in lactating buffaloes could be attributed to its need for colostrum synthesis and superior carbohydrate metabolism (Serdaru *et al.,* 2011).

4.19 Serum Growth Hormone (GH)

The mean serum growth hormone (GH) / somatotropin concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 19.01, its analysis of variance in Table 19.02 and its graph in Figure 22.

The average serum growth hormone (ng/ml) concentrations ranged between 7.05 ± 0.02 to 9.13 ± 0.06 throughout the lactation. They were the highest on day 7 of lactation and lowest on day 210 of lactation. Summing up,

baring a slight flutter on day 45, the growth hormone concentration diminished as lactation advanced and apparently they did not vary with the changes in THI.

Patel (2001) and Mishra *et al.,* (2007) in lactating buffaloes and Vasilatos and Wangsness (1981) and Sartin *et al.,* (1988) in lactating cows recorded higher somatotropin concentrations than those obtained in the present study, but they are higher than those reported by Jindal and Ludri, (1990). Schams *et al.,* (1989) have published that normal plasma of somatotropin concentration in cattle ranges between 3 to 30 ng/ml and these values are determined by the physiological status of the animal.

Growth hormone executes an imperative control in nutrient partitioning in the lactating cow. Bines *et al.,* (1980) concluded that growth hormone fulfils this role by increasing the supply of energy metabolites for milk synthesis, rather than directly controlling the mammary gland. In high yielding dairy cows, hepatic gluconeogenesis is the fundamental glucose source and about 60 – 85% of this glucose is appropriated for milk synthesis.

Further, in lactating dairy cows, variation in glucose availability decides the somatotropin concentrations. Sustenance of metabolic balance, especially during the transition period is facilitated by hormonal fine tuning (Bauman and Currie, 1980). In the course of this phase, there is somatotropin amplification and concurrent mammary gland magnification of IGF and IGF binding proteins, signifying the indispensability of somatotropin in mammogenesis and lactogenesis (Tucker, 1994, Lucy *et al.,* 2001 and Butler *et al.,* 2003). During negative energy balance, GH stimulates lipolysis and milk yield (Bauman and Vernon, 1993).

Growth hormone is a known ontogenic homeostasis metabolism regulator enabling nutrient partitioning during lactation resulting in the availability of these nutrients to the mammary gland where they can be used for milk synthesis (Bonczek *et al.,* 1988, Tucker, 1994, Lucy *et al.,* 2001, Batth *et al.,* 2012 and Butler *et al.,* 2003). In the present experiment, the serum growth hormone concentration was elevated during the early lactation phase that coincided with increasing milk yield as well as the peak milk phase.

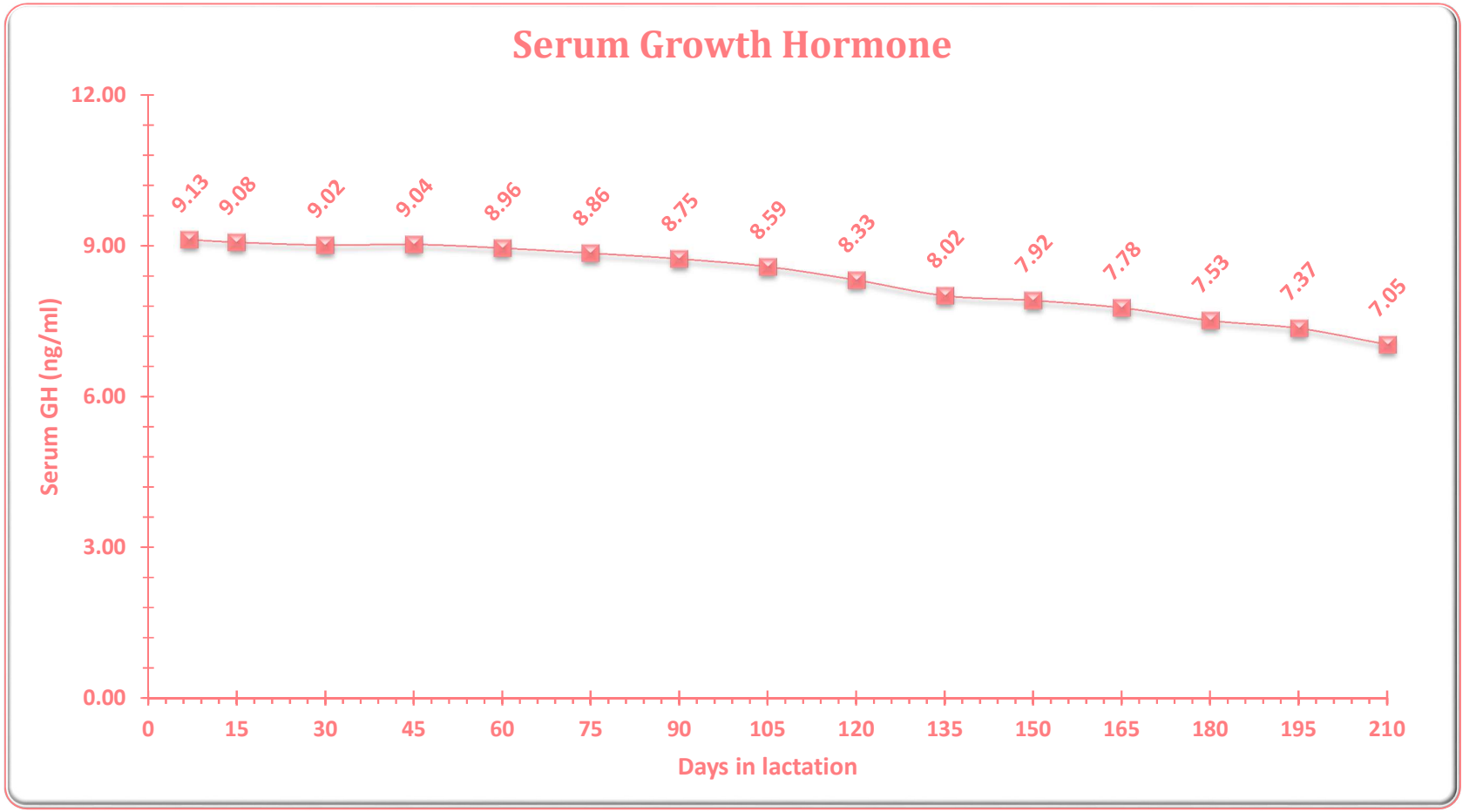

Figure 22. Serum Growth Hormone (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 19.01: **Serum growth hormone (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum growth hormone (mg/dl)
1	07	9.13 [a] ± 0.06
2	15	9.08 [ab] ± 0.05
3	30	9.02 [bc] ± 0.04
4	45	9.04 [bc] ± 0.03
5	60	8.96 [c] ± 0.02
6	75	8.86 [d] ± 0.02
7	90	8.75 [e] ± 0.03
8	105	8.59 [f] ± 0.02
9	120	8.33 [g] ± 0.04
10	135	8.02 [h] ± 0.02
11	150	7.92 [i] ± 0.02
12	165	7.78 [j] ± 0.02
13	180	7.53 [k] ± 0.02
14	195	7.37 [l] ± 0.03
15	210	7.05 [m] ± 0.02

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.12 CD (0.05) = 0.09

Those means having atleast one common superscript between groups do not differ significantly.

Table 19.02: Analysis of variance of the data of **serum growth hormone concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	109.62	7.83	482.84	0.00
Error	225	3.65	0.02	-	-
Total	239	-	-	-	-

Coefficient of Variation = 1.52

Similar results have also been observed by Koprowski and Tucker (1973), Smith *et al.,* (1976), Vasilatos and Wangsness (1981), Herbein *et al.,* (1985), Sartin *et al.,* (1988), Jindal and Ludri, (1990) and Djoković *et al.,* (2015b). Growth hormone inhibits apoptosis of alveolar cells. Normal decline of milk production is due to the loss of alveolar cells by apoptosis and this hormone therefore promotes a longer and higher level of milk production (Dukes *et al.*, 2015).

Growth hormone is catabolic as it increases the metabolic rate. Ideally, an increase in THI or environment stress is generally counterbalanced by dwindling the body's metabolic rate to diminish the heat load (Igono *et al.,* 1988). Hence. THI increase or heat stress essentially impacts growth hormone negatively and begets low circulating blood hormone levels (Mitra *et al.,* 1972).

Similar results have also been observed in the present investigation wherein, most of the time, an increase in THI was concurrently witnessed with a decrease in the growth hormone concentration. However further studies are required to elucidate whether the decline in growth hormone was due to advancing lactation of increasing THI.

4.20 Serum Insulin like Growth Factor 1 (IGF1)

The mean serum insulin like growth factor 1 (IGF1) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 20.01, its analysis of variance in Table 20.02 and its graph shown in Figure 23.

The average serum IGF1 (ng/ml) concentrations ranged between 393.64 ± 30.29 to 643.59 ± 42.92 throughout the lactation. They were the highest on day 7 of lactation and lowest on day 75 of lactation. During early lactation, it decreased from day 7 level up to day 30 of lactation significantly and thereafter its concentration in serum remained as such except increasing flutters on days 105, 135 and 165 of lactation.

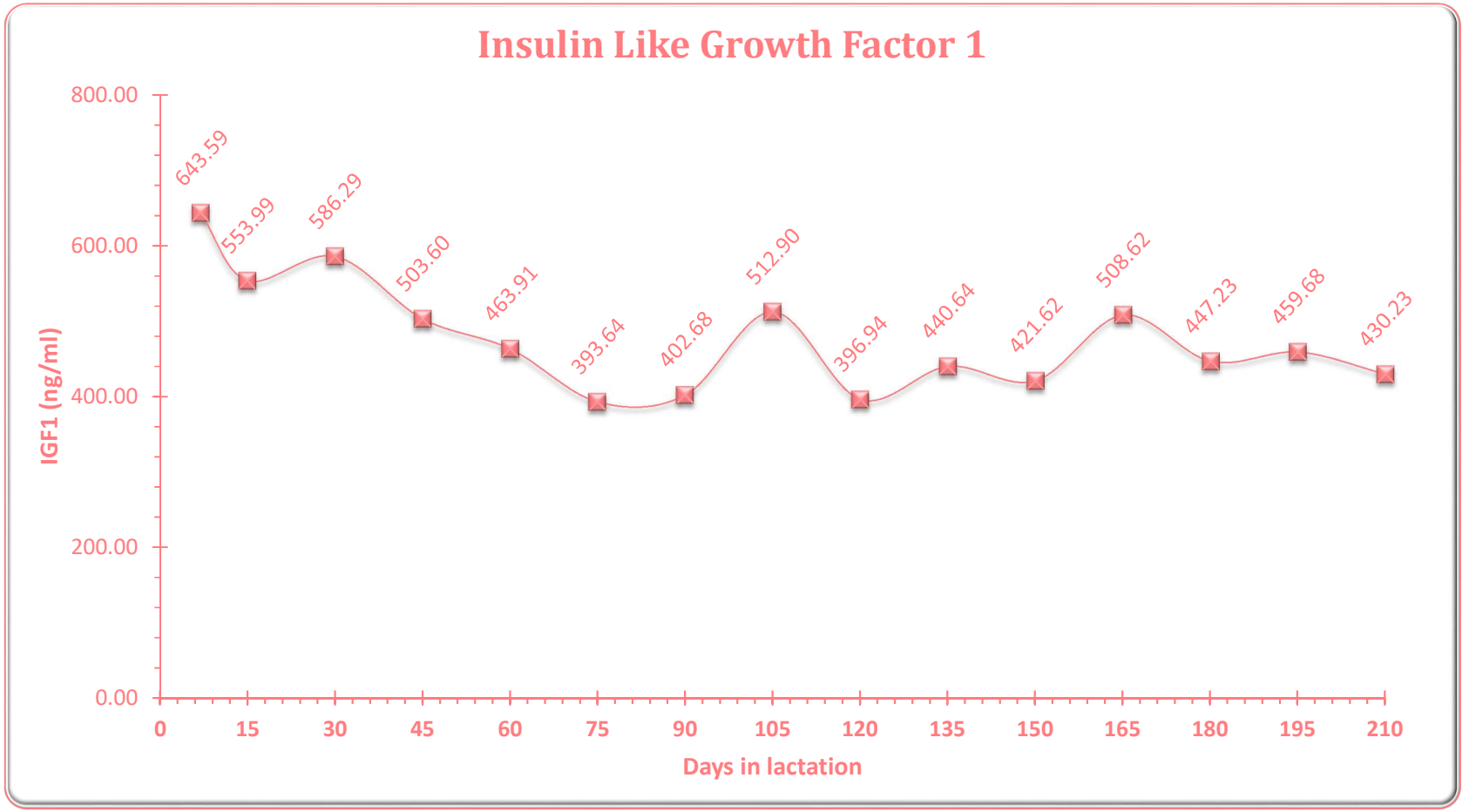

Figure 23. Serum Insulin Like Growth Factor 1 (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 20.01: **Serum insulin like growth factor 1 (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum insulin like growth factor 1 (mg/dl)
1	07	643.59 [a] ± 42.92
2	15	553.99 [abc] ± 36.95
3	30	586.29 [ab] ± 35.35
4	45	503.60 [bcd] ± 38.17
5	60	463.91 [bcd] ± 20.40
6	75	393.64 [d] ± 30.29
7	90	402.68 [d] ± 30.48
8	105	512.90 [bcd] ± 78.20
9	120	396.94 [d] ± 63.86
10	135	440.64 [cd] ± 35.15
11	150	421.62 [d] ± 40.92
12	165	508.62 [bcd] ± 31.17
13	180	447.23 [cd] ± 33.82
14	195	459.68 [bcd] ± 77.68
15	210	430.23 [cd] ± 46.33

Treatments found Significant at 1% and 5% level of significance CD (0.01) 166.68 = CD (0.05) = 126.82

Those means having atleast one common superscript between groups do not differ significantly.

Table 20.02: Analysis of variance of the data of **serum insulin like growth factor 1 concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	1202715.00	85908.21	2.57	0.00
Error	225	7535650.9	33491.78	-	-
Total	239	-	-	-	-

Coefficient of Variation = 38.34

The average mean IGF1 concentrations realized throughout lactation in our trial were lower than those proposed by Talukdar *et al.,* (2017) in buffaloes, in tune with those claimed by but was higher than those postulated by Somal and Aggarwal (2014) and Ashmawy (2015a) in her research as well as the reference ranges quoted by her in buffaloes in dairy cows.

The serum IGF1 concentrations validated in our investigation agreed with Kirovski *et al.,* (2012) in lactating cows.

They disagreed with those reported by Ronge *et al.,* (1988), Vicini *et al.,* (1991), Sharma *et al.,* (1994), Cohick (1998) and Hassan *et al.,* (2014), in lactating cows who ascertained that they were at their minimal during early lactation. In ruminants, the action of growth hormone on the mammary gland is thought to be mediated mainly by the IGF1 signalling axis (Prosser *et al.,* 1987, Winder *et al.,* 1989, Faulkner, 1999 and Etherton, 2004). During the present research also contemporaneous amendments were perceived between growth hormone and IGF1 concentrations. This has been ratified by studies exhibiting improved milk yield when exogenous GH administration stimulated circulatory IGF1 augmentation which in turn acted directly on the mammary gland (Ehrhardt *et al.,* 2000, Lucy *et al.,* 2001 and Taylor *et al.,* 2004). Local IGF production and expression of its receptors are also physiologically orchestrated (Sinowatz *et al.,* 2000 and Plath-Gabler *et al.,* 2001).

An increase in THI or environmental stress as a rule is generally countered by decrease in the dam's BMR to ameliorate the heat load (Igono *et al.,* 1988). To ensure this physiological adaptation, growth hormone being catabolic in nature is trimmed down. It can therefore be reasoned that amplification of THI (heat stress) would imprint growth hormone negatively and muffling it blood circulating concentrations (Mitra *et al.,* 1972). IGF1 levels conventionally emulate GH levels.

In this experiment, although at times IGF1 concentration was low when the THI was high, by and large during other stages no deducible relationship was perceived, validating the fact that the key factor governing the level of IGF1 is the stage of lactation and not the THI.

4.21 Serum 3,5,3'- Tri-iodothyronine (T_3)

The mean serum 3,5,3'-tri-iodothyronine (T_3) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 21.01, its analysis of variance in Table 21.02 and its graphic depiction in Figure 25.

The average serum T_3 (ng/ml) throughout the lactation ranged between 1.51 ± 0.02 to 2.09 ± 0.03. It augmented significantly around day 30, oscillated upto day 75 of lactation, after which there was a steady regression until the end of lactation. T_3 concentration in serum was highest on day 75 of lactation that is almost during peak lactation stage. Amplified T_3 concentrations to fulfil the enhanced exigencies, particularly during early lactation, were perceived from the present study.

A clear drop in T_3, in the early lactation period as seen in our analysis was also observed by Hassan and El-Nouty (1985), Lohan *et al.,* (1989), Singh *et al.,* (1993) and Garg *et al.,* (1997) in buffaloes. Partly comparable to the early trend realized in our investigation, the T_3 concentrations increased and stabilized around the first month of lactation as per Pichaicharnarong *et al.,* (1982), Lohan *et al.,* (1989), Bahga (1989) and Bahga and Gangwar (1989) in buffaloes.

Postpartum reduction in the serum hormones quantity may perhaps be a reflection of the decreased hormone secretion rate due to energy deficit as a result of its large demand by the mammary gland (Fiore *et al.,* 2015b). Additionally, according to Tiiratz, (1997) negative energy balance, which is generally a norm in early lactation, could be accountable for this descent.

An opposite pattern was documented by Hassan *et al.,* (2014) in lactating buffaloes, wherein the plasma T_3 concentrations increased during lactation. This amplification could be ascribed to improved daily milk yield in mid lactation and gestation in late lactation periods.

On the other hand, Fiore *et al.,* (2018) in buffaloes and Mohebbi-Fani *et al.,* (2019) in lactating cows noticed a gradual decline in the serum triiodothyronine concentrations throughout lactation, which could be most likely,

be due to the proliferation of T_3 receptors in the secretory cells of the mammary gland around the commencement of lactopoiesis (Wilson and Gorewit, 1980).

The average mean T_3 concentrations realized throughout lactation in our experiment were lower than those proposed by Ghuman *et al.,* (2011), Hassan *et al.,* (2014) and Ashmawy (2015a) in lactating buffaloes, in tune with those claimed by Habeeb *et al.,* (1996) at 17.5 °C, Aggarwal and Singh (2010), Dalvi *et al.,* (2013), Silva *et al.,* (2014) in buffaloes but was higher than those postulated by Habeeb *et al.,* (1996) at 37.1 °C in lactating buffaloes.

Conversion of T_4 in peripheral tissues produces the active hormone 3,5,3'-tri-iodothyronine (T_3) and reverse T_3 (rT_3) which is thought to be metabolically inactive. T_3 is more potent and has shorter half-life as compared to T_4.

Tucker (2000) hypothesised that throughout lactation the mammary gland is in a euthyroid condition, whereas the rest of the body is in a hypothyroid phase facilitating the advanced transformation of thyroxin to triiodothyronine in the mammary gland supported by its decline in liver and kidneys (Kahl, *et al.,* 1991 and Jack *et al.,* 1994). This mechanism underlines the metabolic significance of mammary gland during lactation.

As an adaptive thermoregulatory response to heat stress, there is a cutback in metabolism and subsequently lesser heat generation. This is facilitated by reduced thyroid hormone secretion, which is responsible for intensifying metabolism.

Temperature escalation precedes hypothalamus releasing hormone suppression generating a downturn in pituitary hormonal secretion and accordingly lessening the thyroid hormones (Habeeb *et al.,* 1992).

In the present study also whenever there was higher THI, for the most part T_3 levels were lower. However, it was difficult to ascertain whether the lowering of T_3 was due to advancing lactation or THI variations.

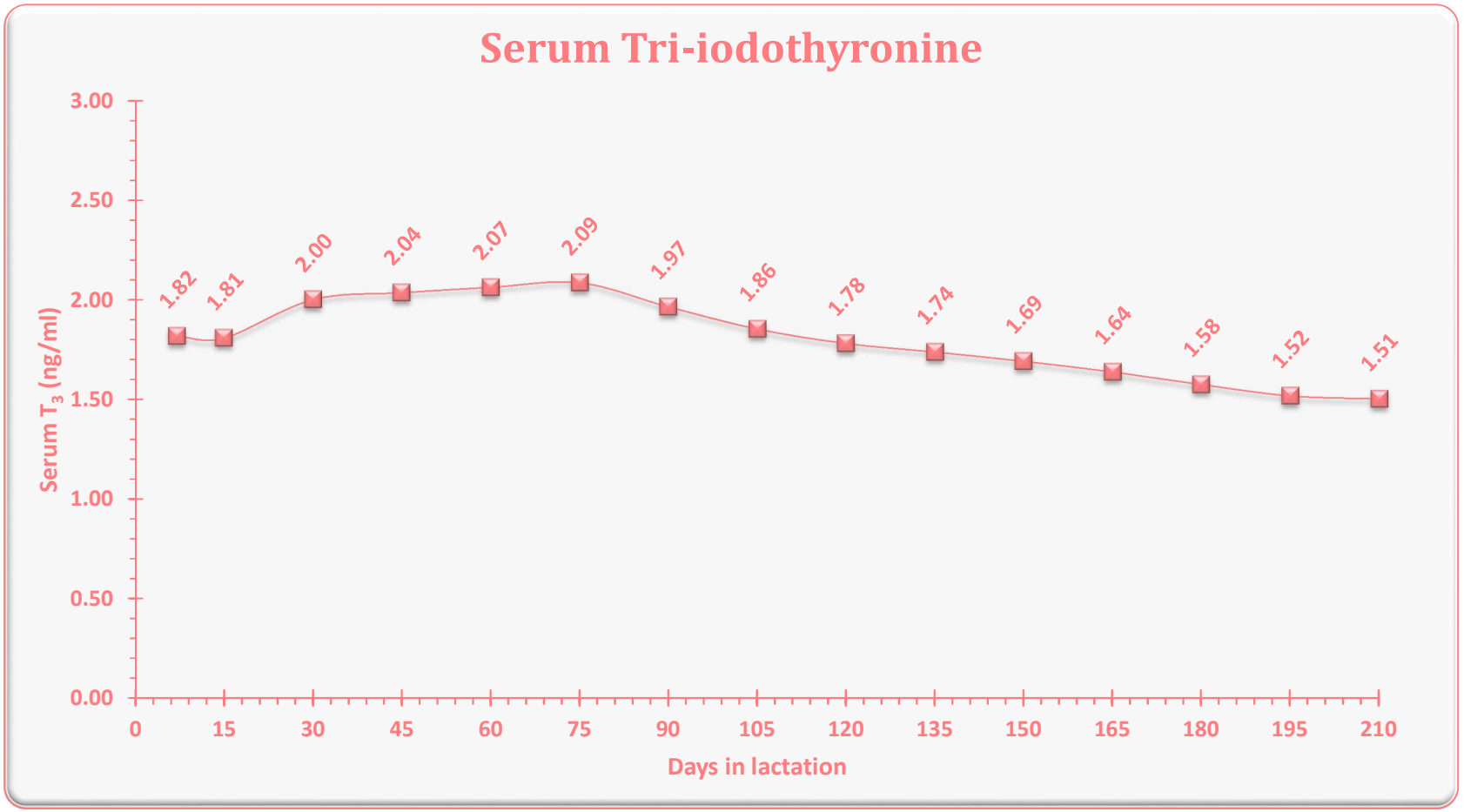

Figure 24. Serum Tri-Iodothyronine (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 21.01: **Serum Triiodothyronine (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum triiodothyronine (ng/ml)
1	07	$1.82^{de} \pm 0.03$
2	15	$1.81^{de} \pm 0.02$
3	30	$2.00^{bc} \pm 0.03$
4	45	$2.04^{ab} \pm 0.02$
5	60	$2.07^{a} \pm 0.02$
6	75	$2.09^{a} \pm 0.03$
7	90	$1.97^{c} \pm 0.01$
8	105	$1.86^{d} \pm 0.02$
9	120	$1.78^{ef} \pm 0.01$
10	135	$1.74^{fg} \pm 0.01$
11	150	$1.69^{g} \pm 0.01$
12	165	$1.64^{h} \pm 0.01$
13	180	$1.58^{i} \pm 0.01$
14	195	$1.52^{j} \pm 0.02$
15	210	$1.51^{j} \pm 0.02$

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.07 CD (0.05) = 0.05

Those means having atleast one common superscript between groups do not differ significantly.

Table 21.02: Analysis of variance of the data of **serum Triiodothyronine concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	8.61	0.62	105.11	0.00
Error	225	1.32	0.01	-	-
Total	239	-	-	-	-

Coefficient of Variation = 4.233

4.22 Serum Thyroxin (T_4)

The mean serum thyroxin (T_4) concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 22.01, its analysis of variance in Table 22.02 and its graph illuminated in Figure 26.

The average serum T_4 (ng/ml) ranged between 50.87 ± 0.35 to 71.00 ± 0.24 throughout the lactation. It augmented significantly around day 30 postpartum and continued this escalation till day 60 which was the highest concentrations in the study and coincided with peak lactation. This was followed by a waning of T_4 until the end of lactation.

In our experiment, serum thyroxin followed a similar, though not exact trend to triiodothyronine which was the consensus of numerous authors (Pichaicharnarong *et al.,* 1982, Hassan and El-Nouty, 1985, Bahga (1989) and Bahga and Gangwar 1989, Lohan *et al.,* 1989, Singh *et al.,* 1993 and Garg *et al.,* 1997) in lactating buffaloes, Bekeova *et al.,* (1993) Karapehlivan et al. (2007) Antunović *et al.,* (2011) in lactating ewes Pezzi *et al.,* (2003) Sinka *et al.,* (2008) Fiore *et al.,* (2015b) and Mohebbi-Fani *et al.,* (2019) in lactating cows.

Thyroid hormone transformations imply the negative energy balance seen during early lactation. The diminishing circulating thyroid hormones in early lactation is the outcome of the mobilization of the body reserves to meet the escalating milk production exigency (Tiirats, 1997 and Huszenicza *et al.,* 2002).

The mammary gland uptake of maternal iodine during lactation can create T_4 deficit (Fiore *et al.,* 2018). In addition, 4 – 7 percentage of the total T_4 required for the maintenance of metabolic functions may be secreted through milk (Akasha and Anderson, 1984).

Further, in case of pregnancy depressed T_4 concentrations could be related to the placental thyroid hormones uptake since the dam is the solitary T_4 source until the foetal thyroid tissues become capable of organogenesis and placental establishment (Escobar, 2001).

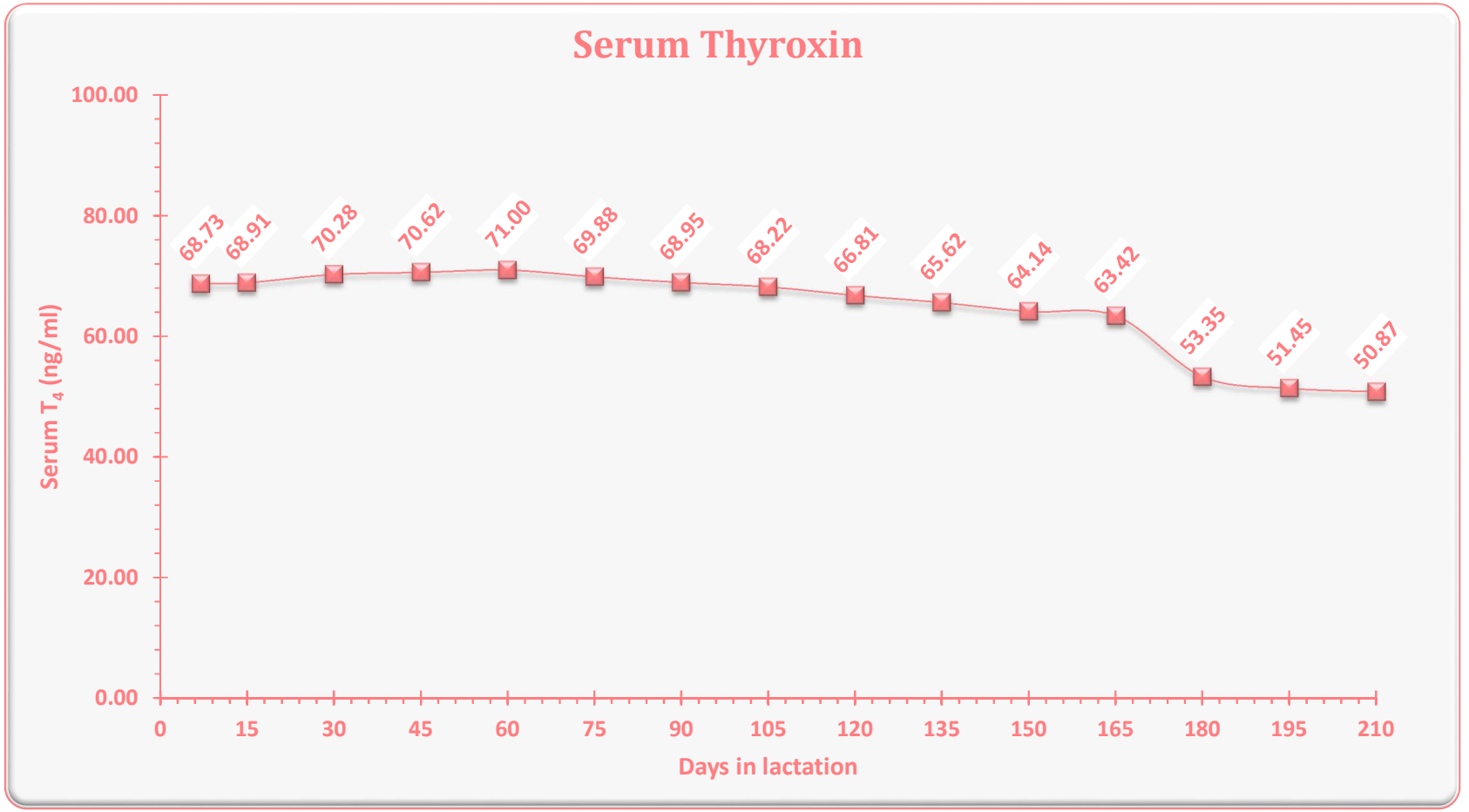

Figure 25. Serum Thyroxin (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 22.01: **Serum tetraiodothyronine or thyroxine (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum thyroxine (ng/ml)
1	07	68.73 [c] ± 0.37
2	15	68.91 [c] ± 0.38
3	30	70.28 [ab] ± 0.23
4	45	70.62 [ab] ± 0.17
5	60	71.00 [a] ± 0.24
6	75	69.88 [b] ± 0.21
7	90	68.95 [c] ± 0.19
8	105	68.22 [c] ± 0.24
9	120	66.81 [d] ± 0.18
10	135	65.62 [e] ± 0.25
11	150	64.14 [f] ± 0.34
12	165	63.42 [f] ± 0.32
13	180	53.35 [g] ± 0.46
14	195	51.45 [h] ± 0.42
15	210	50.87 [h] ± 0.35

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 1.10 CD (0.05) = 0.84

Those means having atleast one common superscript between groups do not differ significantly.

Table 22.02: Analysis of variance of the data of **serum Thyroxine concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	11193.79	799.56	544.38	0.00
Error	225	330.45	1.47	-	-
Total	239	-	-	-	-

Coefficient of Variation = 1.87

However, Gueorguiev (1999) claimed that the T_4 values in early and at peak lactation were significantly lower than in mid and end lactation, when milk production markedly decreased in dairy cows.

The serum thyroxin concentrations observed in our study were similar to those obtained by Gueorguiev (1999) and Singh *et al.,* (2014a) in lactating cows, Antunović *et al.,* (2011) in Tsigai ewes, Aggarwal and Singh (2010) in buffaloes. Reduced blood thyroxin levels are a result of enhanced T_4 expenditure, probably in high yielders giving superior lactational performance (Sharma and Joshi, 2006).

An opposite pattern, wherein the serum T_4 concentrations were far exceeded our values, was found in the study carried by Ghuman *et al.,* (2011) in lactating buffaloes and by Sharma and Joshi (2006), Ali *et al.,* (2011), Jacob (2012) and Surya Prakash *et al.,* (2018b) in cattle. The thyroid hormone escalations might be deemed as marker for tissue protein catabolism (Goldberg *et al.,* 1980).

However, our findings disagree with Ashmawy (2015a) and Dalvi *et al.,* (2013) in lactating buffaloes and Mohebbi-Fani *et al.,* (2019) who reported lower T_4 concentrations in lactating cows.

Lactating buffaloes produce large amounts of heat due to digestion and metabolic processes, and this heat needs to be exchanged with the environment to maintain normal body temperature and this problem of heat stress becomes more acute as the production level increases (Armstrong, 1994).

Thompson (1973) deduced that acclimatisation to elevated temperature brings about decreased thyroid activity. In the present analysis also whenever there were ambient conditions of higher THI, most of the time the level of T_4 was lower.

However, it was difficult to ascertain whether lowering of T_4 was mainly due to advancing lactation or due to variations in THI. Plasma T_4 decreases with increase in THI and at high ambient temperatures plasma T_4 level wanes due to shrinking thyroid activity, moderated gastrointestinal tract motility and rate of passage of ingesta (Thornton *et al.,* 2009).

4.23 Serum Leptin

The mean serum leptin concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 23.01, its analysis of variance in Table 23.02 and its graphical picture in Figure 27.

The average serum leptin (ng/ml) ranged between 6.90 ± 0.66 to 11.17 ± 3.16 throughout the lactation. While the serum leptin concentrations were non-significant throughout lactation, there was a fluctuating peaking and plateauing phenomenon seen. It was truncated during early lactation along with baseline levels observed on days 105, 120 and 150 of lactation, whereas the zenith was on day 135 followed by day 195 of lactation.

The serum leptin values noted in this study were higher than those published by Terzano *et al.,* (2003), Khan *et al.,* (2016) and Nagre and Kuralkar (2017) in lactating buffaloes. Plasma leptin intensities are scrupulously connected to the dam's BCS and nutritional status (Chilliard *et al.,* 2005).

Further, as the hormone is very susceptible to the energy status, diminished levels may possibly be a negative energy balance warning in lactating buffaloes. The rapid post-partum maternal plasma leptin waning accommodates the maternal energy escalation during lactation and counteracts placental leptin shortfall (Schubring *et al.,* 1997).

During galactopoiesis, the ruminant mammary epithelial cells also synthesize leptin, but the plummeting of its circulating levels during lactation is as a result of milk production deficit (Liefers *et al.,* 2003).

Block *et al.,* (2001) in their study on adaptations in lactating dairy cows found that the plasma leptin concentration diminished by around 50% after parturition and this plunge persisted throughout lactation, notwithstanding the reestablishment of a positive energy balance. In most species this leptin decline due to lactational energy debt is not alleviated by increasing food intake too (Macajova *et al.,* 2004).

Houseknecht *et al.,* (1998) classified leptin as a "metabolism modifier" that directs nutrients towards organs or tissues that are metabolically more active. In the present study too, during the demanding first week postpartum and peak lactation periods, lipolysis resulted in BCS decrease to counter the negative energy balance, subsequently leading to a plateau in the circulating leptin levels.

This leptin hormone deficit during early lactation is an advantageous phenomenon as it by encourages feed intake and also has a hand in orchestrating the neuroendocrine adaptations responsible for partitioning energy towards the essential priorities, quelling reproduction and immunity that are dispensable in the short term.

In agreement with this hypothesis are Ahima *et al.,* (1999), Block *et al.,* (2001), Francisco *et al.,* (2002), Gabai *et al.,* (2002), Accorsi *et al.,* (2005a), McDougall *et al.,* (2005), Pinotti and Rosi (2006) and Wylie *et al.,* (2008) who published that plasma leptin concentration are down regulated during early lactation to allow for the energy challenges for copious milk production.

The lactating buffalo, already compromised due to the higher metabolic demands of early lactation is most susceptible to augmented environmental temperatures. An increase in THI causes the dam to reduce its metabolic heat production and enhance heat loss by reducing adipose thickness.

According to Ashour *et al.,* (2007) 40% decrease in voluntary DMI intake is seen during the summer months in buffaloes. This decrease could be credited to the direct effect of elevated temperature on the hypothalamic appetite centre causing spiking of leptin hormones, resulting in diminutive production of VFAs which are the main energy source in ruminants (Baile and Forbes, 1974 and Belhadj *et al.,* 2016).

Especially noteworthy is the fact that during early lactation there is a major paradox as while negative energy balance would induce increase in feed intake, high heat load may cause its decline. However, this contrasting THI trend on leptin concentration was not seen in the present study.

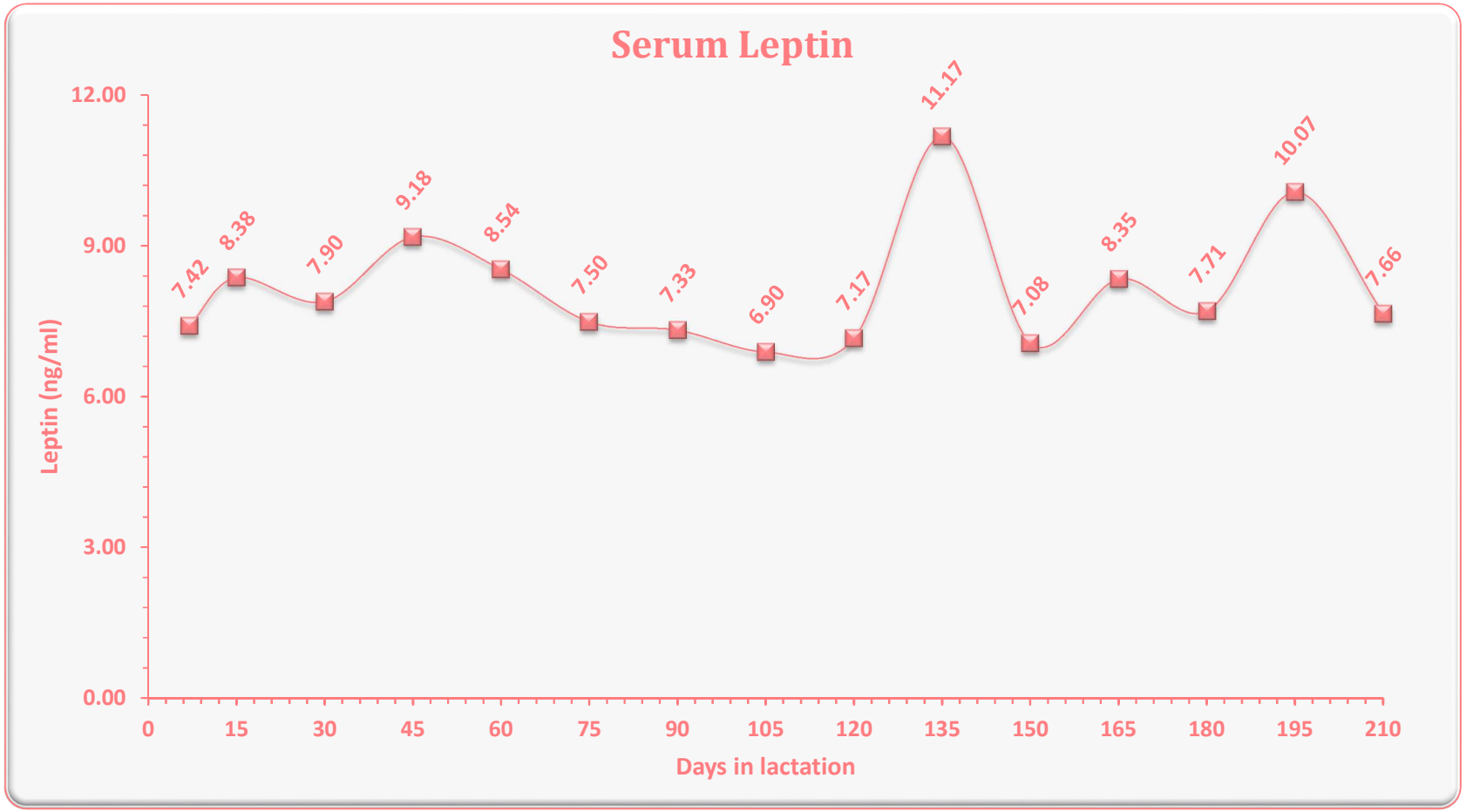

Figure 26. Serum Leptin (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 23.01: **Serum leptin (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum leptin (mg/dl)
1	07	7.42 ± 0.66
2	15	8.38 ± 0.91
3	30	7.90 ± 0.76
4	45	9.18 ± 1.07
5	60	8.54 ± 0.73
6	75	7.50 ± 0.87
7	90	7.33 ± 0.99
8	105	6.90 ± 0.66
9	120	7.17 ± 0.46
10	135	11.17 ± 3.16
11	150	7.08 ± 0.54
12	165	8.35 ± 0.53
13	180	7.71 ± 0.41
14	195	10.07 ± 0.93
15	210	7.66 ± 0.36

Treatments found to be Non-Significant

Table 23.02: Analysis of variance of the data of **serum leptin concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	332.93	23.78	1.26	0.24
Error	225	4258.68	18.93	-	-
Total	239	-	-	-	-

Coefficient of Variation = 53.183

4.24 Serum Cortisol

The mean serum cortisol concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 24.01, its analysis of variance in Table 24.02 and its graph depicted in Figure 27.

The average serum cortisol (ng/ml) ranged between 1.97 ± 0.07 to 5.82 ± 0.09 throughout the lactation. The serum cortisol concentrations waned significantly throughout lactation, with a couple of spikes seen on days 45 and 105 of lactation.

Our results of the serum cortisol range during lactation correspond with the previous results of Eissa *et al.,* (1955), Prakash and Madan (1984) and Heshmat *et al.,* (1985), Aggarwal and Singh (2010), Mondal *et al.,* (2010), Das *et al.,* (2014), Silva *et al.,* (2014) and Talukdar *et al.,* (2017) in lactating buffaloes and Titto *et al.,* (2017) in lactating cows.

They were nevertheless higher than the reference range postulated by Kaneko *et al.,* (2008) in cattle but much lower than those divulged by Vehkataseshu and Estergreeh, Jr. (1970) in non-pregnant lactating dairy cows; Thun *et al.,* (1981), Nessim (2010), Ghanem *et al.,* (2012) and Nehra (2016) in cattle and Habeeb *et al.,* (1996), Bouraoui *et al.,* (2002), Marai and Haeeb (2010) and Chaudhary *et al.,* (2015) in buffaloes.

Our deductions are supported by those given by earlier researchers like Singh and Ludri (2002) and Bhat *et al.,* (2016) in crossbred goats, Nessim (2010) and Nehra (2016) in cows and Prakash and Madan (1984) in buffaloes.

On the other hand our results are not in concurrence with Ninan (2012) and Surya Prakash *et al.,* (2018b) in cows who noted a significantly ascending cortisol trend from the first to second stage of lactation. This contradiction was also chronicled by Mahmoud and Azab (2014) who logged an escalating cortisol trend 2 – 4 weeks postpartum in goats.

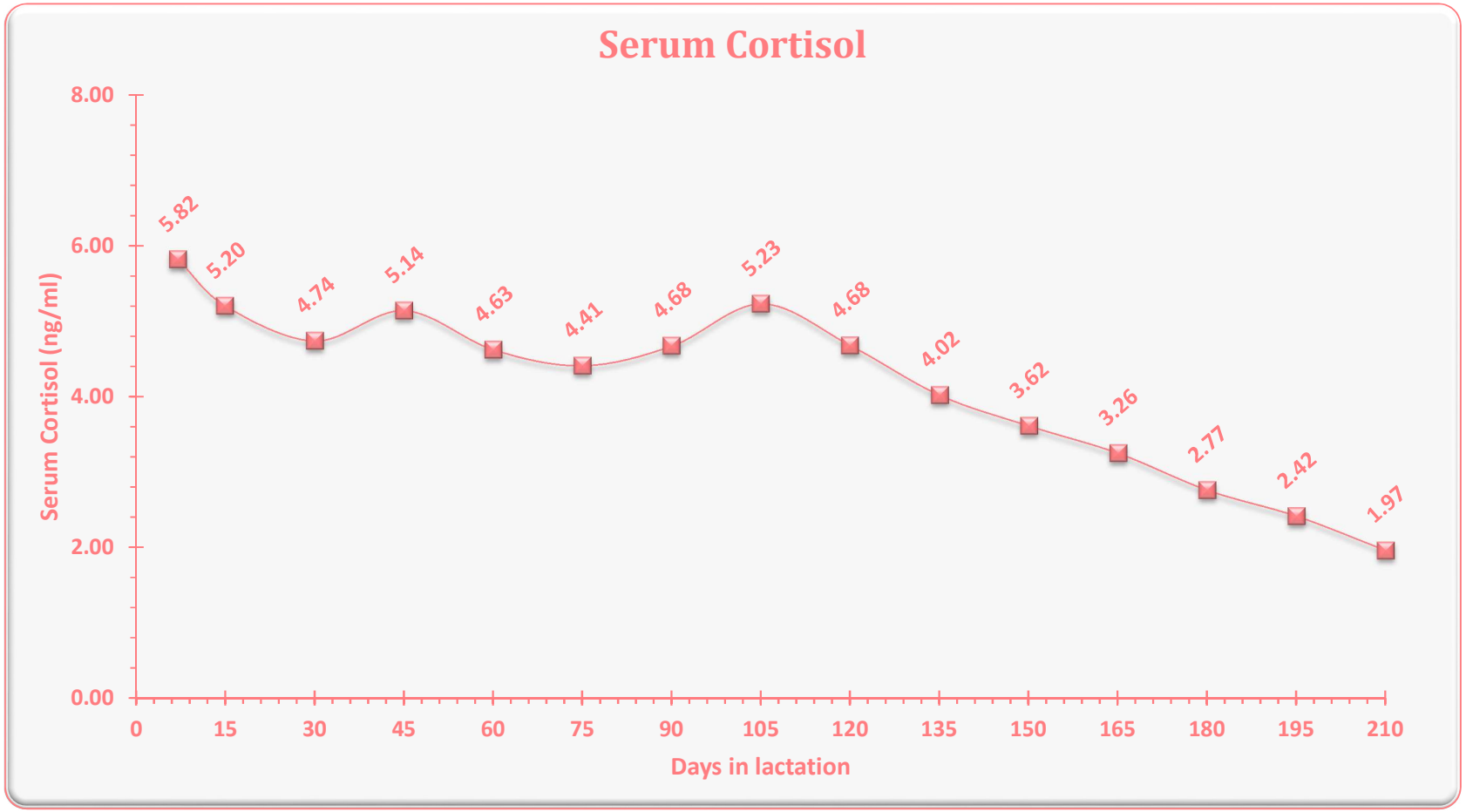

Figure 27. Serum Cortisol (ng/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 24.01: **Serum cortisol (ng/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum cortisol (ng/ml)
1	07	$5.82^{a} \pm 0.09$
2	15	$5.20^{b} \pm 0.11$
3	30	$4.74^{c} \pm 0.09$
4	45	$5.14^{b} \pm 0.07$
5	60	$4.63^{cd} \pm 0.08$
6	75	$4.41^{d} \pm 0.07$
7	90	$4.68^{c} \pm 0.08$
8	105	$5.23^{b} \pm 0.08$
9	120	$4.68^{c} \pm 0.08$
10	135	$4.02^{e} \pm 0.07$
11	150	$3.62^{f} \pm 0.05$
12	165	$3.26^{g} \pm 0.06$
13	180	$2.77^{h} \pm 0.09$
14	195	$2.42^{i} \pm 0.06$
15	210	$1.97^{j} \pm 0.07$

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.278 CD (0.05) = 0.212

Those means having atleast one common superscript between groups do not differ significantly.

Table 24.02: Analysis of variance of the data of **serum cortisol concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	288.66	20.62	221.05	0.00
Error	225	20.99	0.09	-	-
Total	239	-	-	-	-

Coefficient of Variation = 7.319

Cortisol in synchrony with other hypothalamic-pituitary-adrenal (HPA) axis hormones orchestrates a fine balance between survival and procreation (Ricklefs and Wikelski, 2002, Wingfield and Sapolsky, 2003 and Breuner *et al.,* 2008). This survival is supported by modulating fundamental metabolic functions while simultaneously kerbing secondary stressful functions (Sapolsky *et al.,* 2000).

Consequently, it has been projected by many authors that the HPA axis response to stress may possibly be constrained during periods of high investment like production and reproduction, to safeguard against stress-induced disturbances (Ricklefs and Wikelski, 2002, Wingfield and Sapolsky, 2003 and Bókony *et al.,* 2009).

Consistent with this hypothesis, the lactating dams of several species show blunted HPA responses to stress during the lactational period and this phase is a major variable that can impact stress receptivity in lactating females. The lactational phase has therefore been purported to be hyposensitive to stress (Deschamps *et al.,* 2003 and Slattery and Neumann, 2008) and consequently as seen in our study, after a high levels of cortisol during early and peak lactation, the sensitivity is blunted and the serum cortisol levels plateaued.

The high levels of plasma cortisol during early lactation indicates its possible role in peak lactation (Singh and Ludri, 2002) and truncated concentrations during late lactation are coupled with subdued milk yields (Ludri and Sharma, 1985). Cortisol is a lactogenic hormone which influences milk protein formulation (Park and Lindberg, 2005). It can accordingly be deduced that amplified plasma cortisol as the lactation intensifies are an upshot of the udder's cortisol demand for milk synthesis.

Another factor coming into play here, is the diurnal variation in corticoid activity. However, unlike sheep, in cattle and buffaloes seem to show a lack of a well-defined diurnal rhythm related to the sleep-wake schedule which has lead Hudson *et al.,* (1975) to conclude that this is a result of the absence of deep sleep periods in these species.

Research indicates that cortisol is affected by heat stress Bouraoui *et al.,* (2002). Johnson (1980a) has published that serum cortisol concentrations do not consistently increase when animals are exposed to moderate heat, but acute heat will cause it to rise significantly (Abilay *et al.,* 1975), whereas prolonged heat is accompanied by slight declines in plasma level of cortisol (Adeyemo *et al.,* 1981) which could be a result of acclimatisation.

4.25 Serum Insulin

The mean serum insulin concentrations from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 25.01, its analysis of variance in Table 25.02 and its graph portrayed in Figure 28.

The average serum insulin (μU/ml) ranged between 10.18 ± 0.39 to 25.47 ± 0.61 throughout the lactation. The serum insulin concentrations rose steadily upto day 90, remained more or less constant and then started to fall from day 105 of lactation. It however did not go back to its starting minimum concentration.

The serum insulin concentrations realised in our study were higher than the reference values proposed by Kaneko *et al.,* (2008) as well as those proposed by Herbein *et al.,* (1985), Gabai *et al.,* (2002), Abdel-Latif *et al.,* (2016) and Nagre and Kuralkar (2017) in lactating buffaloes and Antunović *et al.,* (2011) in ewes but concurred with those published by Singh *et al.,* (2014a) in buffaloes.

Our results of the measurement of the serum insulin concentrations during the different lactational stages corresponded with the previous results of Phawa (2012), Hassan *et al.,* (2014) and Fiore *et al.,* (2018) in lactating buffaloes, Djokovic *et al.,* (2015b) and Eryavuz *et al.,* (2008) in cows and Antunović *et al.,* (2011) in ewes but diverged from Accorsi *et al.,* (2005b) and Djokovic *et al.,* (2014) in cows who noted that serum insulin remained low nearly throughout the lactation period.

Insulin is an essential metabolic hormone committed to maintaining homeorhesis during lactation Chalmeh *et al.,* (2015). Glucose homeostasis is the core of production physiology wherein the insulin along with a cascade of hormones (growth hormone, cortisol, leptin, T_3 and T_4) adapt to downgrade tissue metabolism to facilitate superior nutrient availability for mammary gland metabolism Fiore *et al.,* (2015a).

Insulin though imperative in overseeing nutrient utilization during lactation does not have any influence on the mammary uptake of crucial metabolites in ruminants (Tucker, 2000). Kahn (1978) elucidating insulin's role in ruminants noted that although many aspects of insulin metabolism, variations in ruminants are due to volatile fatty acids being the major energy source rather than direct glucose sources. Insulin resistance entails changes in the hormone sensitivity (the amount of hormone required to illicit a response) or its responsiveness (the maximum response to a hormone) and similar to its action in humans, in ruminants too insulin resistance can encompass amendments in sensitivity or responsiveness, or both.

Herbein *et al.,* (1985) pronounced that lower insulin in early lactation would facilitate substrate utilization to meet lactational glucose requirements during the negative energy balance. The low insulin concentration seen during early lactation in our experiment can be attributed to the negative energy balance during this phase affirming the contention that cows in early lactation are physiologically under more production stress than in mid or late lactation.

Taking this a step further, Rose *et al.,* (1997) emphasized that transition from using body fat reserves to depositing them could be due to changes in the body tissue's ability to respond to insulin and other such metabolic hormones and this alteration in the response is seen when there is augmentation in nutrient availability and this is seen usually during late lactation. While one school of thought opines that heat stress stimulates the concentrations of insulin (O'Brien *et al.,* 2010 and Wheelock *et al.,* 2010) another contends that feed intake cutback prolongs the negative energy balance period leading to decreased serum insulin concentrations (Rensis and Scaramuzzi, 2003 and Marai *et al.,* 2007).

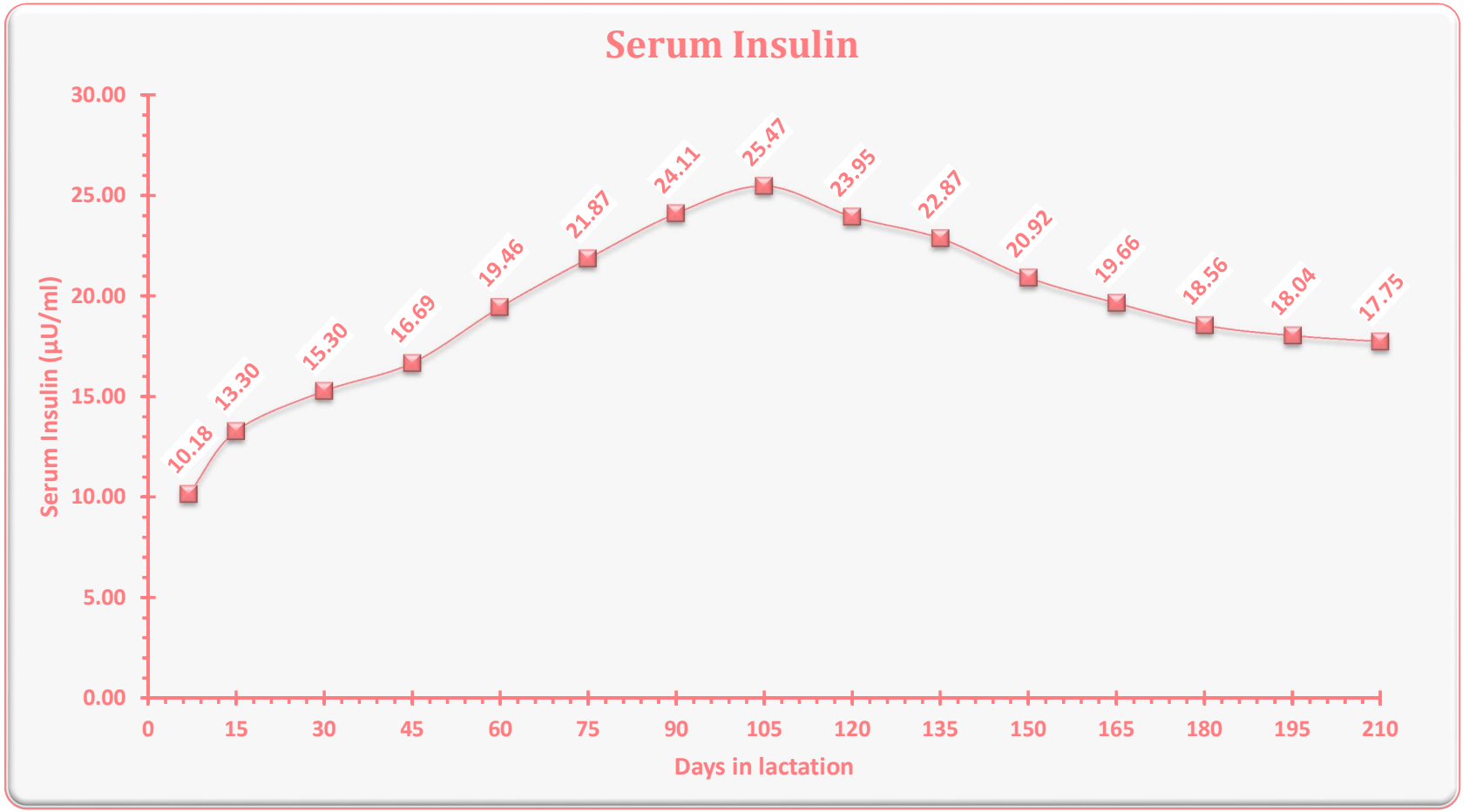

Figure 28. Serum Insulin (µU/ml) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 25.01: **Serum insulin (μU/ml)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Serum insulin (μU/ml)		
1	07	10.18 [l]	±	0.39
2	15	13.30 [k]	±	0.45
3	30	15.30 [j]	±	0.46
4	45	16.69 [ij]	±	0.49
5	60	19.46 [efg]	±	0.63
6	75	21.87 [cd]	±	0.88
7	90	24.11 [ab]	±	0.59
8	105	25.47 [a]	±	0.61
9	120	23.95 [ab]	±	0.91
10	135	22.87 [bc]	±	0.76
11	150	20.92 [de]	±	0.61
12	165	19.66 [ef]	±	0.35
13	180	18.56 [fgh]	±	0.28
14	195	18.04 [ghi]	±	0.25
15	210	17.75 [hi]	±	0.48

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 2.101 CD (0.05) = 1.599

Those means having atleast one common superscript between groups do not differ significantly.

Table 25.02: Analysis of variance of the data of **serum insulin concentration** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	4020.12	287.15	53.96	0.00
Error	225	1197.47	5.32	-	-
Total	239	-	-	-	-

Coefficient of Variation = 12.01

Further, Collier *et al.,* (1982) proclaimed that thermal stress generates negative energy balance triggering lower blood insulin levels and decreased tissue sensitivity to insulin, but this was not observed in this study, wherein there was an increase in the hormone concentrations when the THI was peaking.

4.26 Body Condition Score (BCS)

The mean Body Condition Score (BCS) score from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 26.01, its analysis of variance in Table 26.02 and its graphic depiction in Figure 29.

The average BCS throughout the lactation ranged between 3.50 ± 0.0 to 4.34 ± 0.13 and the mean BCS throughout lactation was 3.85 ± 0.08. The BCS computation was implemented using the chart especially designed for Indian Murrah buffaloes (Anitha *et al.,* 2011), its average range was between 4.34 ± 0.13 to 3.50 ± 0.00 and the mean BCS throughout lactation was 3.85 ± 0.08.

The average BCS at the time of calving was the best and it waned significantly throughout the lactation. As milk production peaks, nutritional support to the animal becomes inadequate and energy deficit spurs a negative energy balance (NEB) in the dams rendering them leaner by marshalling their body lipid stores and thus diminishing the body condition score (Aeberhard *et al.,* 2001, Coffey *et al.,* 2002 and Agenas *et al.,* 2003). Consequently, roughly 33% milk produced in the first month post-partum is sustained by body energy reserves of the dam (Bauman and Currie, 1980).

In agreement with our BCS findings of the mean BCS dipping with advancing lactation were Banu *et al.,* (2012) in buffaloes, Phawa (2012), Doležalová *et al.,* (2013) and Stadník and Atasever (2017) in cows. This BCS deterioration can be attributed to the negative energy balance during this period (De Vries and Veerkamp, 2000).

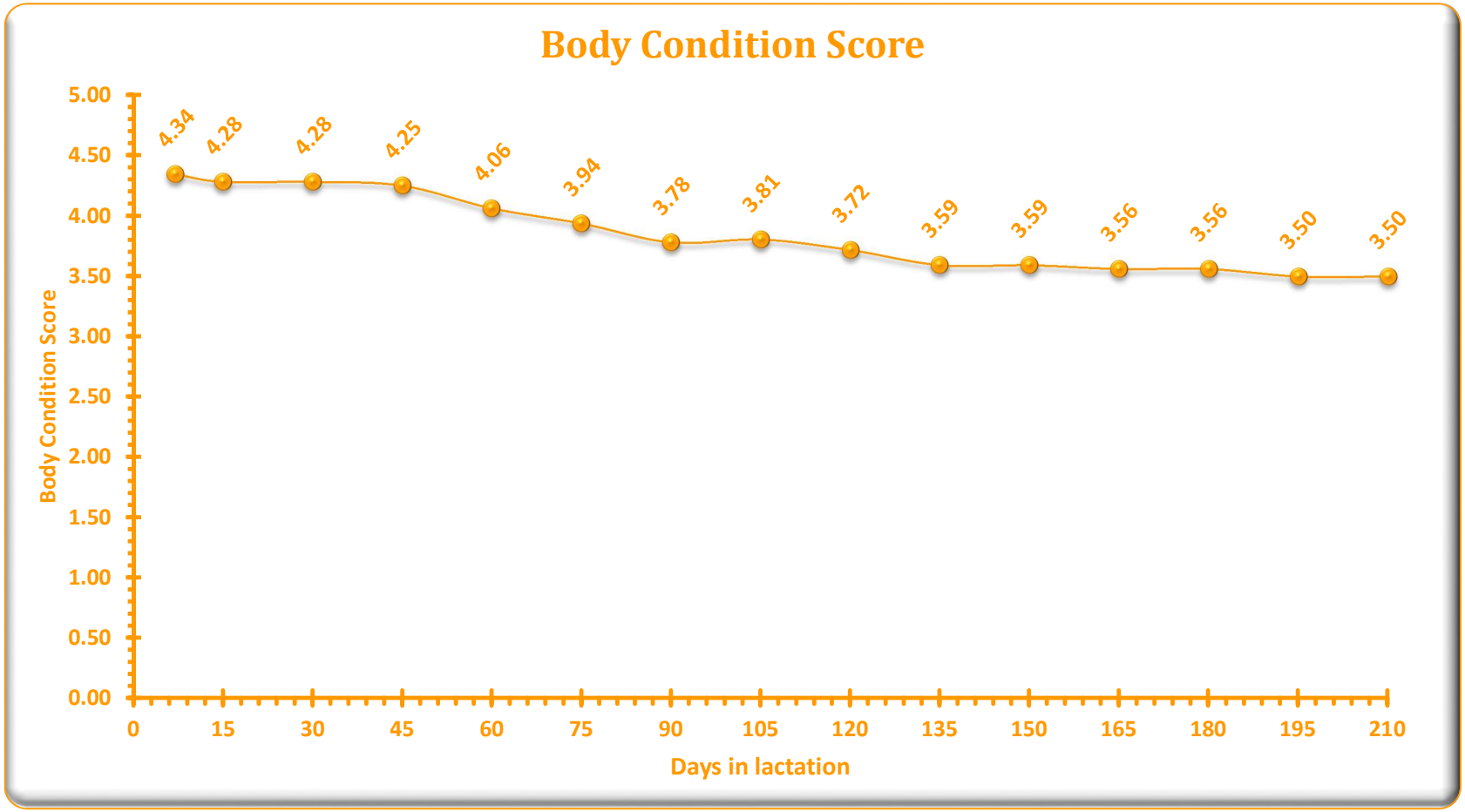

Figure 26. Body Condition Score (BCS) concentrations from day 7 to day 210 of lactation in Murrah buffaloes.

Table 26.01: **Body condition score** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Sr. No.	Days in lactation	Body Condition Score
1	07	4.34 [a] ± 0.13
2	15	4.28 [ab] ± 0.12
3	30	4.28 [ab] ± 0.11
4	45	4.25 [ab] ± 0.10
5	60	4.06 [bc] ± 0.09
6	75	3.94 [cd] ± 0.08
7	90	3.78 [def] ± 0.08
8	105	3.81 [de] ± 0.12
9	120	3.72 [defg] ± 0.08
10	135	3.59 [efg] ± 0.05
11	150	3.59 [efg] ± 0.10
12	165	3.56 [fg] ± 0.04
13	180	3.56 [fg] ± 0.04
14	195	3.50 [g] ± 0.00
15	210	3.50 [g] ± 0.00

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.32 CD (0.05) = 0.24

Those means having atleast one common superscript between groups do not differ significantly.

Table 26.02: Analysis of variance of the data of **body condition score** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	14	22.30	1.59	13.24	0.00
Error	225	27.08	0.120	-	-
Total	239	-	-	-	-

Coefficient of Variation = 9.01

Our inferences were not in line with Mushtaq (2009), Anitha *et al.,* (2010), Ashalatha *et al.,* (2015) and Yadav *et al.,* (2018) who concurred that in the lactating buffaloes BCS troughed upto 2 months postpartum after which it crested as did Gallo *et al.,* (1996) in cows. Darwesh *et al.,* (2013) in black does also similarly ascertained that the initial body condition score at kidding conspicuously subsided during early lactation and then amplified appreciably from mid lactation onwards.

Similarly, Branca and Casu (1989), Atti *et al.,* (1995) in sheep and Cabiddu *et al.,* (1999), Casamassima *et al.,* (2007) in goat, discerned that BCS increased significantly as lactation progressed. This body condition score improvement could be due to the availability of nutrients in the natural pasture as per the seasonal benefit in addition to feeding concentrate. On the other hand Pande *et al.,* (2016) perceived no significant loss in BCS during the first month of lactation and determined that the buffaloes were in a sound nutritional condition.

4.27 Correlation Analysis of Temperature Humidity Index's Effect

The correlation analysis of effect of Temperature Humidity Index (THI) on all the estimated parameters from day 7 to day 210 of lactation in lactating Murrah buffaloes are presented in Table 27.01, its analysis of variance in Table 27.02.

The THI during the study ranged from 74.94 at the commencement of the study to 81.86 at its termination. The maximum THI of 85.96 was noted on day 105 which fell in October. This period also coincided with that stage of lactation when the peak production had plateaued during the persistency period. Since during this stage there is already a moderating effect of the milk production and the buffaloes were therefore not in a huge energy deficit, it is likely that the actual effect of THI was not reflected.

Table 27: **Temperature Humidity Index (THI)** from day 7 to day 210 of lactation in lactating Murrah buffaloes.

Collection Number	Days post parturition	**THI**
1	7	**74.94**
2	15	**75.52**
3	30	**78.33**
4	45	**83.08**
5	60	**80.63**
6	75	**81.57**
7	90	**83.08**
8	105	**85.96**
9	120	**80.20**
10	135	**82.36**
11	150	**81.64**
12	165	**77.32**
13	180	**78.76**
14	195	**80.20**
15	210	**81.86**

The regression equation formulated by Pawar *et al.,* (2013) propose that milk yield and fat % subsides by 0.028 kg and 0.047% per buffalo per day for each point increase in the THI value beyond 72.

The correlation between THI and milk yield was positive but non-significant. As stated earlier the predominant factor affecting the milk yield was the stage of lactation. Another possible rationale for the largely non-significant result could be the diminutive THI escalation seen during the experimental period. Cincović *et al.,* (2010) also noted that THI rise did not show a significant impact on production and quality of milk, but their findings reflected ours only in the early lactation phase.

Milk yield has been found to plummet with increase in THI of ambient surrounding signifying a negative relationship. Johnson *et al.,* (1963) reported a decline in milk yield at values of THI higher than 72 and this was corroborated by Mallonee *et al.,* (1985) and Du Preez *et al.,* (1990b) in dairy cows. However, positive correlation with THI in the present study indicates that during peak lactation as well as during other phases, the stage of lactation had more profound effect rather than THI on milk yield. Moreover the THI values were not very high in the present study so as to impact the milk yield.

Upon correlation with the milk composition we found that milk fat and SNF were positively correlated but the coefficients were almost negligible. Milk proteins and lactose concentrations were also insignificantly but negatively correlated.

Studies conducted to observe the effect of heat stress on milk composition have concluded that fats decrease in milk during heat stress or higher THI (Knapp and Grummer, 1991, Bouraoui *et al.,* 2002 and Zheng *et al.,* 2009). On the other hand Roman – Ponce *et al.,* (1977) and Rodriguez *et al*., (1985) reported no significant relation between milk fat and heat stress. In the present study even though the milk fat was found to increase with elevating THI, it was not significant and this could be because the THI values were not sufficiently intensified.

Table 28: **Pearson Correlation Coefficient (R)** between the THI and average parameters from day 7 to day 210 of lactation in lactating Murrah buffaloes during that period.

Sr. No.	Parameter	(R)
1	Milk yield	0.130
2	Milk proteins	- 0.142
3	Milk fat	0.006
4	Milk lactose	- 0.124
5	Milk SNF	0.052
6	Serum total protein	0.198
7	Serum albumin	0.175
8	Serum globulin	0.107
9	Serum A:G	0.091
10	Serum urea	0.215
11	Serum creatinine	- 0.274
12	Serum SGPT	0.129
13	Serum SGOT	- 0.366
14	Serum Glucose	-0.079
15	Serum calcium	0.484
16	Serum phosphorus	- 0.228
17	Serum chloride	- 0.277
18	Serum magnesium	0.101
19	Serum growth hormone	- 0.146
20	Serum IGF1	- 0.588*
21	Serum T_3	0.171
22	Serum T_4	0.019
23	Serum leptin	- 0.013
24	Serum cortisol	- 0.088
25	Serum insulin	0.773**
26	Body condition score	- 0.370

**** Correlation is significant at the 0.01 level (2-tailed).**

*** Correlation is significant at the 0.05 level (2-tailed).**

Although studies done to assess effect of heat stress on milk composition have shown varying results but they have commonly demonstrated a decrease in the concentration of milk constituents. Joksimović – Todorović *et al.*, (2011) also noted that heat periods led to decrease in milk fat, SNF, protein as well as lactose. Zheng *et al.,* (2009) suggested that while heat stress has no effect on the milk lactose they observed significant reduction in milk yield and milk fat and protein percentages. In the present study protein and lactose were negatively correlated, but statistically not significant. The reason could be because of low THI values and predominant factor of stage of lactation during the investigation period.

Amongst the serum biochemical parameters the correlation with THI was not significant for any of them. However serums total proteins, albumin, globulin, and albumin: globulin ratio, urea and SGPT were positively correlated to THI and creatinine, SGOT and glucose were negatively correlated with THI. A positive correlation with THI with serum protein in the present study indicates an increase in serum protein whenever there is increase in THI. Similarly other studies have also concluded an increase in serum proteins during heat stress (Raghavan and Mullick, 1962, More *et al.,* 1980, Yousef, 1990, El-Masery and Marai, 1991 and Podar and Oroian, 2003).

The present investigation sheds light on the analogous relation between serum globulin to THI and total protein to THI. Such results are also similar to those found in other studies (Koubkova *et al.,* 2002, Halliwel, 1998 and Rasooli *et al.,* 2004). Similar to the negative correlation obtained between glucose and THI other studies (Baumgard and Rhoads, 2013 and Rhoads *et al.,* 2009) have also reported a decline in blood glucose level with increase in heat stress in dairy animal.

Serum calcium and magnesium were positively correlated and serum phosphorus and chloride were negatively correlated to THI however they were not significant. Kume *et al*., (1986) have demonstrated that higher ambient temperature *i.e.* heat stress may lead to decreased calcium and phosphorus levels which is similar to the phosphorus results in the present investigation but contrary to the findings of calcium in the present exploration.

Amongst serum hormones insulin was significantly and positively correlated to THI and IGF1 was found to have significantly negative correlation with THI. Rest of the hormones were not found to have significant correlation with THI. However growth hormone and cortisol were negatively correlated with THI and T_3 and T_4 were found to have positive correlation with THI.

Heat stress has been found to alter cortisol in heifers (Ronchi *et al*., 2001), increase insulin concentration in cows (Itoh *et al.,* 1998) and cortisol, growth hormone in exercising humans (Hargreaves *et al.,* 1996). Similarly, in the present investigation, positive correlation between insulin and THI could indicate an insulin surge whenever the THI is boosted.

However conflicting findings in growth hormone have also been reported wherein heat stress led to its decline in animals (Bernabucci *et al.,* 2010). This was similar to the result obtained for growth hormone and THI in the present study. Similar to the negative correlation of thyroid hormones with THI in the present investigation, a slump in the thyroid hormones has consistently been reported in several heat stress publications (Magdub *et al.,* 1982, Nardone *et al.,* 1997 and Kahl *et al.,* 2015). IGF1 has also been reported to decrease due to heat stress (Xie, 2015). Similarly in the present study IGF1 was marked to be negatively correlated with THI.

Body condition scoring was negatively correlated but non-significant. Lacetera *et al.,* (1996) have also reported a decline in BCS due to heat stress. The decreased BCS during present study was similar but not significant due to not so higher THI values during the study period.

Summary & Conclusion

SUMMARY AND CONCLUSION

The present study was aimed to investigate the effect of temperature humidity index (THI) on blood metabolites, hormone profile, milk composition and body condition score (BCS) in lactating Murrah buffaloes.

The study was conducted on 15 apparently healthy lactating Murrah buffaloes in their 2^{nd} – 4^{th} lactation, having a minimum BCS score of 3.5. These buffaloes were aged between 5 – 7 years and their average milk yield was 8 – 16 liters per day. They remained healthy and were kept open until the end of the lactation period (210 days).

The average lactational milk yield was 8.24 ± 0.48 litres / day. It was 7.92 ± 0.54 litres on day 07 of lactation and increased non-significantly upto Day 45. Significant ($P < 0.05$) rise (peak yield) was observed from Day 60 to Day 75, which was 11.09 ± 0.52 litres. The persistency period of the lactational curve was reflected from day 90 when there was a significant ($P < 0.05$) dip upto day 165 of lactation. From there on non-significant dwindling of milk yield was observed, followed by highly significant ($P < 0.01$) drop in the milk yield from Day 180 and Day 210 recording the lowest milk yield of 4.55 ± 0.36 litres.

The average milk protein throughout lactation was 2.96 ± 0.09. It ranged between 2.58 ± 0.14 which was the lowest concentration on day 210 to 3.35 ± 0.13, the highest concentration on day 195. The milk protein concentration was non-significant from Day 07 to Day 120. It ebbed significantly ($P < 0.05$) on Day 135 and remained almost similar upto Day 165. From then on, it waxed significantly ($P < 0.05$) from Day 180 to Day 195 of the study and then waned significantly to the lowest concentration on Day 210.

The average milk fat % found in this study throughout lactation was 8.38 ± 0.39. The mean milk fat concentration from Day 07 to Day 105 receded more or less significantly and then inflated significantly ($p < 0.01$) at Day 150 after which there was a gradual slump followed by a mild non-significant cresting. The fat percentage was the highest on Day 150 and lowest on Day 75 of the study.

The average milk lactose throughout the lactation ranged between 3.12 ± 0.17 and 4.63 ± 0.08. The mean milk lactose concentration from Day 07 to Day 90 did

not differ significantly, but displayed some oscillations and escalated in percentage numerically. From Day 105 there was a more or less significant decline upto the end of lactation baring a sudden spike on Day 180.

The average milk SNF throughout the lactation ranged between 6.35 ± 0.41 to 8.52 ± 0.15. The high percentage on Day 7, decreased significantly ($P < 0.05$) on Day 15. A significant rise was seen from Day 30 to Day 90 with minor fluxes. From there on there was a deflating trend upto Day 210 of lactation with the exception of a sequentially drop on Day 150 and hike on Day 180 of lactation. The mean milk SNF throughout the lactation was 7.68 ± 0.20.

The average serum total protein concentrations throughout the lactation ranged between 6.84 ± 0.14 to 8.79 ± 0.38 g/dl. Serum total protein concentration showed the greatest significant high on day 150 and lowest on day 15 and 30 of lactation. Essentially the serum protein concentrations were stable and abated during peak and mid-lactation but fluctuatingly loftier around early and late lactation. Correspondingly, the THI levels were also higher during mid-lactation.

The average serum albumin concentrations throughout the lactation ranged between 3.57 ± 0.09 to 4.85 ± 0.18 g/dl. The variations in the serum albumin concentrations were analogous to the serum protein concentrations. Serum albumin concentrations were significantly elevated on day 60 and 180 and the least were on day 15 of lactation. There was hardly any variation in the serum albumin concentrations, however, its values during early lactation i.e. upto day 45 of lactation were lower, then rose upto day 90, dipped on day 105 and 120 and subsequently elevated upto the end of lactation.

The average serum globulin throughout the lactation ranged between 2.23 ± 0.25 to 6.31± 0.38 g/dl. Serum globulin levels were significantly high on day 150 of lactation and significantly low on days 60 and 180 of lactation. The variation of serum globulin during the whole lactation was maximum during the mid or initial phase of late lactation especially from day 135 to day 210. While the postpartum levels of serum globulin were generally lower during early lactation they showed a lower trend on days 60 to 90 & and increased thereafter up to day 150 of lactation. After day 150 of lactation up to day 210 the levels were generally higher except at day 180.

The average serum A:G throughout lactation ranged between 0.69 ± 0.06 to 2.88 ± 0.57. It peaked significantly on days 60 and 180 postpartum and dipped significantly on days 45 and 150 of lactation. Accentuated values of A: G ratio on these days corresponded with sagging of serum globulin values and vice versa. During the early lactation the A: G ratio was low till day 45 (except day 30) and thereafter it surged up to day 135 lactation. During mid and early phase of lactation from day 135 to 210, the A: G ratio varied without revealing any extricating trend.

The average serum urea (mg/dl) throughout the lactation ranged between 16.89 ± 1.21 to 46.71 ± 4.77. They were significantly optimized on days 90 and 120 of lactation during later stages of early lactation that was near to peak lactation. Significantly low levels were observed on day 210 *i.e.* during late phase of mid lactation. Immediately after parturition on day 7 of lactation, levels were in a normal range and spiralled on day 15 followed by a slackening on day 30. Thereafter the levels of serum urea increased with advancing lactation and reached its maximum levels after attainment of peak lactation. Afterwards a declining trend was observed in values of serum urea until the lowest value of present study *i.e.* day 210 postpartum.

The average serum creatinine (mg/dl) throughout lactation ranged between 0.19 ± 0.10 to 1.40 ± 0.09. Serum creatinine concentrations were significantly loftier during early lactation on days 7, 15, 45 and 75. It was significantly humbler on days 135, 180 and 195 of lactation. The levels were enlarged immediately postpartum and during early lactation approximately up to the realization of peak lactation yield. Thereafter they faded up to day 105 and kept wavering fortnightly to reach the lowest value on day 195 of lactation.

The average serum SGPT (IU/L) throughout the lactation were between 24.50 ± 2.59 to 54.59 ± 3.42. The levels immediately subsequent to parturition were elevated and rose upto day 90 of lactation, which showed the significant top SGPT value and thereafter troughed. The significantly lowest values were observed on day 135 and 150 of lactation. Essentially, the levels upto 3 months i.e. early lactation were elevated and thereafter low levels were sustained

throughout lactation except day 195. SGPT levels were found to be uplifted during early and late lactation but troughed during mid-lactation.

The average serum SGOT (IU/L) throughout the lactation ranged between 36.18 ± 3.76 to 165.80 ± 10.53. The highest significant SGOT value was observed immediately postpartum (165.80 ± 10.53 IU/L) and comparatively raised values were sustained upto day 120 of lactation. Thereafter the concentration declined to the significant lowest value on day 150 (36.18 ± 3.76 IU/L). After day 150 of lactation, the levels increased marginally but still were subjacent as compared to early lactation. After the marginal escalation on day 165 these levels waned upto day 210 of lactation. SGOT concentrations was higher during early lactation as compared to mid- and late-lactation.

The average serum glucose (mg/dl) throughout the lactation ranged between 40.15 ± 2.12 to 90.60 ± 3.17. Serum glucose was significantly low during early lactation, especially on day 07 and during mid-lactation on day 195. Serum glucose concentrations were troughed on days 30, 45, 90 and 105 and crested on 150, 165, 180 and 210 days of lactation. Generally, the levels were marginal immediately after parturition at the beginning of lactation and augmented thereafter. Early lactation was marked with subsided and mid lactation by surging serum glucose concentrations.

The average serum calcium throughout the lactation ranged between 8.30 ± 0.44 to 11.52 ± 0.34 mg/dl. Serum total calcium concentrations showed a significant high on day 7 of lactation, which was followed by a decrease in the serum calcium levels. From day 60 to day 105 of lactation the serum calcium concentration surged non-significantly. Subsequently, there was a small dip and followed by a rise upto day 150 of lactation. Thereafter, the concentration waned till a sudden rise on day 210 of lactation. The serum calcium concentrations were stable and raised during peak and mid-lactation but fluctuating around early and late lactation.

The average serum phosphorus throughout the lactation ranged between 5.38 ± 0.25 to 6.28 ± 0.24 mg/dl. The serum phosphorus concentrations were non-significant throughout lactation. It declined slightly followed by an escalation

around day 45. This was followed by a dip in the values with slight upsurges around from day 135 and 165 of lactation.

The average serum chloride (mEq/L) throughout the lactation ranged between 87.28 ± 2.14 to 101.76 ± 2.12. The serum chloride concentrations were non-significant throughout lactation. The nethermost was on day 90 and uppermost on day 150. It displayed a more or less similar pattern with a sharp decline on day 90. Ensuing this plateau there a steady ascend with intermittent inflation as seen on day 150.

The average serum magnesium (mg/dl) throughout the lactation ranged between 1.76 ± 0.06 to 2.79 ± 0.09. Serum total magnesium concentrations showed a significant high on day 7 of lactation, which was followed by a decrease on day 30. From day 45 to day 90 of lactation, it soared, slumped and again soared. Subsequently, there were infrequent spikes till a final significant ascend from days 195 of lactation. The serum magnesium concentrations attained throughout lactation in this investigation closely emulated the serum calcium concentrations. Our research showed more or less significant changes in the magnesium concentrations all-through the 210 days study period.

The average serum growth hormone (ng/ml) concentrations ranged between 7.05 ± 0.02 to 9.13 ± 0.06 throughout the lactation. They were the highest on day 7 of lactation and lowest on day 210 of lactation. Summing up, baring a slight flutter on day 45, the growth hormone concentration diminished as lactation advanced and apparently they did not vary with the changes in THI. In the present study, the serum growth hormone concentration was elevated during the early lactation phase.

The average serum IGF1 (ng/ml) concentrations ranged between 393.64 ± 30.29 to 643.59 ± 42.92 throughout the lactation. They were the highest on day 7 of lactation and lowest on day 75 of lactation. During early lactation it decreased significantly from day 7 to day 45 of lactation and thereafter its concentration in serum remained as such except increasing flutters on days 105, 135 and 165 of lactation.

The average serum T_3 (ng/ml) throughout the lactation ranged between 1.51 ± 0.02 to 2.09 ± 0.03. It augmented significantly around day 30, oscillated upto

day 75 of lactation, after which there was a steady regression until the end of lactation. T_3 concentration in serum was highest at day 75 of lactation that is almost during peak lactation stage. A clear drop in T_3, in the late lactation period was seen in our study.

The average serum T_4 (ng/ml) ranged between 50.87 ± 0.35 to 71.00 ± 0.24 throughout the lactation. It augmented significantly around day 30 postpartum and continued this escalation till day 60 which was the highest concentrations in the study and coincided with peak lactation. This was followed by a waning of T_4 until the end of lactation.

The average serum leptin (ng/ml) ranged between 6.90 ± 0.66 to 11.17 ± 3.16 throughout the lactation. While the serum leptin concentrations were non-significant throughout lactation, there was a fluctuating peaking and plateauing phenomenon seen. It was truncated during early lactation along with baseline levels observed on days 105, 120 and 150 of lactation, whereas the apex was on day 135 followed by day 195 of lactation.

The average serum cortisol (ng/ml) ranged between 1.97 ± 0.07 to 5.82 ± 0.09 throughout the lactation. The serum cortisol concentrations waned significantly throughout lactation, with a couple of spikes seen on days 45 and 105 of lactation.

The average serum insulin (µU/ml) ranged between 10.18 ± 0.39 to 25.47 ± 0.61 throughout the lactation. The serum insulin concentrations rose steadily upto day 90, remained more or less constant and then started to fall from day 105 of lactation. It however did not go back to its starting minimum concentration.

The average body condition score (BCS) throughout the lactation ranged between 3.50 to 4.34 ± 0.13 and the mean BCS throughout lactation was 3.85 ± 0.08. The average BCS at the time of calving was highest and it showed significant reduction throughout the lactation period.

Amongst serum hormones insulin was significantly and positively correlated to THI and IGF1 was found to have significantly negative correlation with THI. Rest of the hormones were not found to have significant correlation with THI. However growth hormone and cortisol were negatively correlated with THI and T_3 and T_4 were found to have positive correlation with THI.

It is therefore concluded that most of the estimated hormones, a majority of the blood metabolites, milk yield and its constituents along with the BCS, altered significantly, throughout lactation barring serum leptin, phosphorus and chloride, whereas the correlation with THI was not significant except for insulin and IGF1 due to heat stress. These disparities can be attributed to numerous variances like feed quality & quantity, farm & animal managemental procedures, environmental influences & seasonal deviations as well as the fact that the buffaloes were non pregnant and lactating. These results can be used as a reference yardstick for non-pregnant lactating buffaloes during one complete lactation.

Proposed area of future research

PROPOSED AREA OF FUTURE RESEARCH

The buffalo farms in this region are exposed to a climate that promulgates abridged environmental well-being, and this climate transformation is here to stay. Gestation and lactation are both physiological stress inducers and this along with climate extremes make a huge demand on the livestock. In this study, while we were fortunate to get all the animals on the same farm and they were kept open, the study period skipped the most demanding and challenging period, *i.e.* the summer season.

In order to examine the multifarious demands of lactational stage, gestation and climate, a broader study involving more buffaloes and ensuring that all the seasons are accounted for can be conceptualised wherein the buffaloes can be grouped as per stage of lactation, climate and pregnancy status. This would enable a clearer perception of the effect of the various physiological states on the buffalo's milk production and composition during the different periods and stages.

Ideally, this in-depth investigation should encompass the dry period along with the full lactation period to facilitate the all-inclusive comprehension of the body condition fluctuations and the animals' overall sustenance during the next challenge be it lactation and / or gestation.

Since several hormones play an active role in milk synthesis and therefore amendments in the homeorrhetic hormonal controls diverting the nutrients away from the mammary gland could also be factored in while determining the milk yield and its components alterations. Studies to characterize the mechanisms related to changes in hormone secretion in response to heat stress, lactational stress and gestational status are needed. These could incorporate various nutritional levels and organ level studies to ascertain the direct and indirect effects.

Going a step further, the milk carbohydrate, protein and fat chemistry needs to be screened, with regards to season and lactational stage, so the appropriate precursor and enzyme addendums can then be divulged to the livestock breeder for engendering enhanced and wholesome milk.

Finally, all of this information can then be incorporated in the appropriate decision making softwares which gives pivotal guidance taking into consideration all the variables to enable the best possible amelioration strategies which are easy for the farmer to follow.

In conclusion, further studies are recommended for identifying the key biological and management elements for producing healthier milk from dairy animals.

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Abstract

Appendix – G

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	**:**	**EFFECT OF THI ON HORMONE PROFILE, BLOOD METABOLITES, MILK COMPOSITION AND BCS IN LACTATING MURRAH BUFFALOES**
b)	Full name of student	**:**	**BHARUCHA SIMIN VISPI**
c)	Name and address of Major Advisor	**:**	**Dr. S. D. Ingole** Chairman, Advisory Committee Professor of Veterinary Physiology, Mumbai Veterinary College, Mumbai
d)	Degree to be awarded	**:**	**Ph.D.**
e)	Year of award of degree	**:**	**2019**
f)	Major subject	**:**	**Veterinary Physiology**
g)	Total number of pages in the thesis	**:**	**235**
h)	Number of words in the abstract	**:**	**247**
i)	Signature of Student	**:**	
j)	Signature, Name and address of forwarding authority (HOD / SH)	**:**	**Dr. S. D. Ingole**
k)	Signature of the Associate Dean	**:**	

ABSTRACT

In lactating buffaloes responses to THI and lactational stressors are exhibited by alterations in the serum metabolites and hormones and milk constituents along with alterations in the dam's BCS. Since the overall effects of lactation, environment and pregnancy on the buffalo are multifaceted, varied physiological outputs must be studied in order to understand the impact on health and productivity. THI elevations and lactational stages have been found to influence the milk quality and quantity, raising the question of which of the aforementioned variables are causing what effect on the lactating female. Therefore, we negated one variable and kept all the fifteen buffaloes selected from a single farm in Thane District open throughout the 210 days lactational study period. Blood and milk samples were collected on day 7, day 14 and fortnightly thereafter. The analysed data showed significant variations throughout lactation in milk yield and its measured components (protein, lactose, SNF and fat). The blood metabolites panel assessed (total proteins, albumin, globulin, A:G, urea, creatine, glucose, SGPT, SGOT, calcium and magnesium), hormone profile (T3, T4, IGF1, insulin, GH and cortisol) and BCS showed significant variations during the 210 days lactation period, while serum phosphorus, chloride and leptin were non-significant. The correlation between THI and the studied parameters was by and large non-significant, except for insulin (positively correlated) and IGF1 (negatively correlated). This non-significant result could be due to the fact that the lactation period was from July to February and therefore the buffaloes escaped the summer heat stress.

Appendix – G

प्रबंध सारांश

1.	प्रबंधाचे नाव	:	**दुधाळ मुऱ्हा म्हशी मध्ये तापमान आद्रता निर्देशांकामुळे संप्रेरके, रक्त घटक, दुधातील घटक आणि शरिराच्या बांध्यावर/ठेवणीवर होणारा परिणाम अभ्यासणे.**
2.	विद्यार्थ्याचे नाव	:	**भरुचा सिमीन व्ही.**
3.	मार्गदर्शकाचे नाव	:	**डॉ. एस. डी. इंगोले** प्राध्यापक, पशु शरिरक्रियाशास्त्र विभाग, मुंबई पशुवैद्यक महाविद्यालय, परळ, मुंबई 400012
4.	पदवी	:	**आचार्य**
5.	पदवी प्रदान करण्याचे वर्ष	:	**2019**
6.	मुख्य विषय	:	**पशु शरिरक्रियाशास्त्र**
7.	प्रबंधाची एकुण पाने	:	**235**
8.	सारांशाचे एकुण शब्द	:	**255**
9.	विद्यार्थ्याची सही	:	
10.	विभाग प्रमुखाचे नाव, सही आणि पत्ता	:	**डॉ. एस. डी. इंगोले**
11.	सहयोगी अधिष्ठाता **मुंबई पशुवैद्यकिय महाविद्यालय, परळ, मुंबई — 400 012**	:	

प्रबंध सारंश

दुधाळ म्हशी मध्ये तापमान आदत्रत निर्देशांक आणि दूध देण्याच्या कालावधीतील ताणा मुळे रक्त द्रवातील घटक, संप्ररेके, दुधातील घटक आणि शरीराच्या ठेवणीवर फरक पडतो. म्हशीचा दूध देण्याचा कालावधी, वातावरण आणि गाभण कालावधी यां घटकावर परिणाम हा विविध कारणांनी होतो. म्हशीच्या आरोग्य व उत्पादनावर होणारा परिणाम अभ्यासण्यासाठी वेगवेगळ्या शरीरक्रिया घटकांचा अभ्यास करण्यात आला. वाढत जाणारे तापमान आद्रता निर्देशांक आणि दूध उत्पादनाचा कालावधी मुळे दुधातील घटक व दूध वाढीवर परिणाम होतो. याचमुळे दुधाळ म्हशींमध्ये वरील घटकांपैकी कोणता घटक परिणामकारक आहे? हा प्रश्न निर्माण होतो. त्यामुळे आम्ही १५ दुधाळ म्हशींची एकाच तबेल्यातून निवड केली आणि या म्हशीच्या २१० दिवस दूध उत्पादन कालावधीचा अभ्यास केला. या म्हशीचे रक्ताचे व दुधाचे नमुने ७ व १४ व्या दिवशी घेण्यात आले व त्यानंतर दर १५ दिवसांनी घेण्यात आले. अभ्यासाअंती संपूर्ण दूध उत्पादनाच्या कालावधीत दूध उत्पादन व दुधातील घटक प्रथिने, दुग्ध शर्करा, घट्ट चरबी व चरबी यांच्यात लाक्षणिक फरक आढळून आला. रक्तातील चयापचयातील घटक एकूण प्रथिने, अल्बुमिन, ग्लोबुलीन, अ‍ंग प्रमाण नत्र, क्रिएटीन, शर्करा, एस जी पी टी, एस जी ओ टी कॅल्शिअम, स्फुरद संप्रेरके टी ३, टी ४, आय जी एफ १, इन्सुलिन, जी एच आणि कॉर्टिसॉल शारीरिक ठेवण यात २१० दिवसांच्या दूध उत्पादनाच्या कालावधीत लाक्षणिक फरक दिसून आला. परंतु, रक्तजलातील फॉस्फरस, क्लोराईड आणि लेप्टीन यात कोणताही लाक्षणिक फरक आढळून आला नाही. तापमान आद्रता निर्देशांक आणि अभ्यासलेले घटक यांच्यात कोणताही लाक्षणिक फरक आढळला नाही. परंतु, इन्सुलिन मध्ये सकारात्मक सहसंबंध व आय जी एफ १ मध्ये नकारात्मक सहसंबंध आढळून आले. जुलै ते फेब्रुवारी या दूध उत्पादनाच्या कालावधी दरम्यान अभ्यासणाऱ्या म्हशींमध्ये वरील कालावधीत उन्हाचा त्रास कमी जाणवल्यामुळे सदर अभ्यासामध्ये काही घटकामध्ये लाक्षणिक फरक दिसून आला नाही.

Vita Auctoris

VITA

Born on 21st September, 1972 in the "city of dream" then Bombay and now Mumbai, to Bachu Vispi Bharucha (*nee Bachu Sorabji Wadia*) and Vispi Burjorji Bharucha, Simin Vispi Bharucha completed her primary and secondary schooling (SSC) at the Cowasjee Jehangir Primary & Infant School and the J. B. Vachha High School for Parsi Girls, Dadar with a distinction. She concluded her Higher Secondary Course (HSC, Associate Degree) with a first class in the science stream from Jai Hind College, Churchgate.

Simin enrolled in the Bombay Veterinary College (BVC) in 1990 and earned her undergraduate degree (BVSc & AH) with a first class from then affiliated University, the Konkan Krishi Vidyapeeth (KKV). She next secured a first class with distinction Master's Degree (MVSc) in Veterinary Physiology including Biochemistry in 1997 from the same august institutes.

In 2000, Simin joined the Department of Veterinary Physiology, Bombay Veterinary College as a Guest Lecturer and after around four years she was appointed as a Research Associate in the same department. In 2006, she became the Technical Officer to the Associate Dean of the College and continued to intermittently handle that post for the next six years as an additional charge after her selection in April 2008, as Assistant Professor in her Alma Mater.

An empathetic, warm-hearted apprentice of life, an insatiable bibliophile, a lifelong ailurophile and cynophile, a scrupulous gourmet, and an innovative, approachable and splendiferous educator with a finger on the pulse of technological trends could sum her up. Simin is a member of 08 associations and scientific societies, has co-authoured more than 30 publications, and handled a research project. She has been on the dissertation Advisory Committee of around 20 students and has proficiently served on the various committees as formulated by the College and University authorities.