Role of mammary serotonin during lactational calcium homeostasis in dairy cows: Review

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Abstract
Lactation is the terminal stage of the bovine reproductive cycle and constitutes an important reproductive strategy to ensure greater survivability of newborn. One of the limitations in the production economy is postpartum disorders as increased lactational performance leads to imposed metabolic load to meet the production requirements. The primary predisposing factor to such disorders is frail calcium metabolism resulting in postpartum subclinical hypocalcaemia. However, during lactation mammary-derived serotonin has been recognized as a calcium regulator biomolecule. In this review, an attempt has been made to describe the functional role of non-neuronal serotonin during lactation in dairy cows.

Keywords: Calcium, homeostasis, lactation, serotonin

Introduction
Higher metabolic plasticity is the key to successful lactation in dairy cows to cope up with robust metabolic demands at the onset of lactogenesis without developing production disorders. The quality and quantity of milk produced are crucial for the survival of the young one. In dairy cows, the levels of metabolic stress especially during the transition period is reflected in the importance given to homeorhetic regulation of nutrient to support lactation (Bauman and Currie, 1980; Bell, 1995; Bruckmaier and Gross, 2017) [1-3]. This is in spite of negative energy balance in the dam due to 30 percent decrease in feed intake near parturition (Hayirli et al., 2002) [4], the beginning of lactation is vital to ensure the survival of newborns as they solely depend on wholesome milk to provide all the nutrients which are, crucial for all kinds of life-sustaining metabolic processes. The dynamic mammary gland is unique as it undergoes remarkable metamorphosis in terms of functional anatomy through repeated cycles of growth, functional differentiation and regression associated with each pregnancy. Advances in the genetic selection of high yielding dairy cows ensured a progressive increment in milk production during recent decades (Akers, 2000; Capper et al., 2009) [5, 6]. However, with improved lactational performance comes the genetically imposed metabolic load of meeting the production requirements. Despite active homeorhesis, many high yielding dairy cows fail to successfully meet with the robust metabolism, thus rendering them susceptible to production diseases (Ingvarssen, 2006; Reinhardt et al., 2011; Van Kneegsel et al., 2014) [7-9]. As concluded by Hadley et al. (2006) [10] to achieve greater production efficiency along with sustainability in dairying, improvements must be fine-tuned targeting lifelong performance and longevity. The two main organs that undergo adjustment during lactation are the mammary gland and the bone. There is a loss of bone minerals especially calcium, to provide calcium for milk production, during lactation (Gustavo and Christina, 2019) [11]. Metabolic disorders are not necessarily associated with lactation efficiency, although the sensitivity to production diseases intensifies with higher performance (Fleischer et al., 2001; Ingvartsen et al., 2003; Mulligan and Doherty, 2008) [12-14]. Monoamine serotonin is one such mammary-derived autocrine-paracrine signaling biomolecule, acting on mammary epithelial cells (MECs) to regulate milk secretion in different species. Pertinent body organ systems, whose basic functional units synthesize serotonin as one of its physiological molecules forms the serotonergic system. In bovines, Bruschetta et al. (2010) [15] reported breed-specific variable concentrations of circulating serotonin in Italian Fresian and Brown Swiss cows in early lactation to be 170 ± 50 (ng/ml) and 334 ± 81 (ng/ml) under healthy physiological conditions.
(Collier et al., 2012) studied dairy cows and found that milk yield that milk yield and composition is affected by 5-HT ligands. Although locally secreted, serotonin through activating specific physiological axis plays a much wider metabolic role in the regulation of the lactating mammary gland, to better coordinate maternal metabolism without compromising maternal health (Hernández-Castellano et al., 2019) [17].

Serotonin

(5-Hydroxytryptamine, Enteramine, Thrombocytin, 3-(β-Aminoethyl)-5-hydroxy indole, Thrombotonin) As one of the crucial monoamines Serotonin (C₁₀H₁₄N₂O) with IUPAC ID 3-(2-Aminoethyl)-1H-indol-5-0 is a phylogenetically conserved biomolecule evolved to its current position across the animal kingdom, influencing the developmental as well as physiological plasticity of various tissues across different species (Turlejski, 1996; Raymond et al., 2001) [18, 19]. Consistent with its evolutionary history, serotonin has culminated in the regulation of a multitude of functions, including physiological mechanisms such as homeostasis, feeding, immunity, energy regulation, cardiovascular function, behaviour, intestinal motility, and reproduction (Gershon and Tack, 2007; Horseman and Collier, 2014; Wyler et al., 2017) [20-22]. The neuronal serotonergic system, since its discovery as a neurotransmitter within CNS in the early fifties, has been the foremost area of research in the field of neurosciences, in aspects of depression, behaviour, and anxiety (Whitaker-Azmitia, 1999; Berger et al., 2009) [23, 24]. It is only in the last decade that the extra-neuronal implications of the serotonergic system specifically in the mammary gland have been elucidated. Although Serotonin does not cross the blood-brain barrier, 5-hydroxy-L-tryptophan(5-HTP) can cross this barrier and create two distinct pools of serotonin in the form of neuronal and peripheral systems in the body(Berger et al., 2009) [24]. Additionally, non-neuronal 5-HT accounts for approximately 98 percent of the body’s total serotonin synthesized in vital organs such as the intestine, lung, pancreas, prostate, thyroid, liver, and the mammary gland (Lauder, 2004; Hernandez et al., 2009; Pai and Horserman, 2011) [25-27]. However, enteroepithelial 5-HT secretion represents approximately 90 percent of the total-body 5-HT biosynthetic capacity (Gershon and Tack, 2007) [20]. Using PRL-knockout (PRL-KO) mice, Matsuda et al. (2004) [28] discovered peripheral serotonin biosynthesis within functional mammary glands. In vivo biosynthesis of serotonin is a two step reaction, starting with L-tryptophan which is converted to 5-HTP under catalysis by rate-limiting enzyme tryptophan hydroxylase 1 (TPH1) in non-neuronal tissues, and TPH2 in neuronal tissues. Furthermore, 5-HTP is catalyzed by enzyme aromatic amino acid decarboxylase to serotonin (Wang et al., 2002) [29].

Speaking of pharmacokinetics, 5-HT is degraded by monoamine oxidase (MAO) to produce the major non-functional metabolite excreted in urine as, 5-hydroxy indole acetic acid (5-HIAA), which is used as a marker of whole-body serotonin turnover (Horseman and Collier, 2014) [21]. The functional presence of serotonin transporter (SERT) allows two major functions: 5-HT accumulation and 5-HT reuptake. SERT provides a biologic mechanism for 5-HT accretion in those cells not capable of serotonin biosynthesis such as platelets. Additionally SERT presents non physiological accretion by clearing the extracellular space of the secreted 5-HT, which in turn terminates receptor-mediated 5-HT signaling. Serotonin-selective reuptake inhibitors (SSRIs), which constitute a large class of therapeutic drugs, potentiate the biological activity of serotonin by extending its residence time within the extracellular space, thus targeting the additional function of SERT (Horseman, and Collier, 2014) [23]. To demonstrate the serotonergic presence within dairy cows mammary gland, Hernandez et al. (2009) [26] characterized the rate-limiting enzyme TPH1 enzyme and 5-HT receptor subtype expression in bovine mammary tissues. Additionally, as estimated by Hernández-Castellano et al. (2019) [27] blood concentration of circulating 5-HT in newborn calves ranged from 2,500 to 4,000 ng/mL, which when compared to values of lactating cows realized to be 1.5to 2.3fold higher (Moore et al., 2015) [10]. A mouse model study by Pai and Horserman (2008) [31] reported that peripheral serotonin is directly associated with calcium homeostasis based on which they concluded that mammary-derived 5-HT is imperative for the maintenance of blood calcium levels during lactation. With RNA sequencing-based analysis of lactating mammary glands from wild type and TPH1 knockout mice, Laporta et al. (2015) [32] demonstrated the critical implication of the mammary serotonin in mammary gland physiology during lactation. The study revealed a strong association between mammary-derived serotonin and gene ontology pathways within the lactating mammary gland such a calcium homeostasis, lipid metabolism, and hypoxia. Furthermore, Weaver et al. (2017) [33] demonstrated that the mammary-derived serotonin, which when biosynthesized during the lactation period contributes to approximately 50 percent of the circulating serotonin concentrations in mice. Physiologically, the lactating mammary gland is not just a calcium drainage organ system but also a calcium-sensing organ. Integration of mineral sensory inputs via a calcium-sensing receptor (CaSR) localized on mammary epithelial cell (MEC) aids the lactating mammary gland in regulating the influx and efflux of calcium through the innate presence of 5-HT signaling pathway (Pai and Horserman, 2008) [31]. As reported by Toledo et al. (2020) [34] experiments concerning milking frequency in mid-lactating cows did not affect the milk 5-HT concentration. Studies have indicated that the concentration and the time-dependent involvement of the serotonergic system alters the permeability of mammary epithelial cell localized tight junctions (TJ) within the mammary gland of the bovine, mouse, and human (Stull et al., 2007; Pai and Horserman, 2008; Hernandez et al., 2011; Collier et al., 2012) [35, 36, 16]. A study was undertaken to understand the functional secretery difference, which is strongly associated with the dynamics of tight junction permeability in postpartum cows with higher as well as lower circulating 5-HT concentrations in blood (Kessler et al., 2019) [37]. The results demonstrate that the biomarkers for mammary TJ leakiness i.e. lactate dehydrogenase (LDH) activity and serum albumin concentration in milk during the first 6 milking. The levels are significantly higher as compared to the low yield of alpha-lactalbumin in the milk of cows which had high circulatory 5-HT. This is in contrast to these lactating cows having a lower circulating levels of 5-HT. Experiments conducted in vitro on 5-HT and gene expression studies on mammary epithelial cells led to the apropos conclusion of active involvement of 5-HT in suppressing alpha-lactalbumin gene expression within the mammary gland. Thus sustained concentration of 5-HT may down regulate milk protein alpha-lactalbumin gene expression that prevents the biosynthesis of alpha-lactalbumin during the stage of lactogenesis. The collective observations of the above studies also indicate that the failure to the closure of TJs soon
after parturition allowed the subtle continuance of stage one of lactogenesis overlapping the functionalities of stage two lactogenesis within mammary glands of cows with physiologically higher levels of circulating serotonin during the postpartum period.

Serotonin signaling
Apart from serotoninology, the paracrine-autocrine role of serotonin is also elicited through interaction with rhodopsin-like serotonin receptors (5-HTR). These receptors largely belong to the superfAMILY of heptahelical G-protein linked receptors except for the ionotropic type 3 receptors (5-HT3A–E), which are ligand-gated cation channels. Active serotoninergic involvement in diverse physiological functions within the same tissue and also throughout the body demanded adaptive flexibility in terms of its selective action which has caused the formation of different serotonin receptor subtypes (Peroutka and Howell, 1994; Uphouse, 1997) [38, 39]. With diversity in the 5-HT receptors come diverse functional characteristics of multiple receptor subtypes which provide advantages to regulate various physiological mechanisms within a single tissue throughout the body. Moreover, heterogeneity within 5-HT receptors that signal through different mechanisms enables serotonin action to correspondingly vary as per the different physiological status of the animal (Uphouse, 1997) [39]. The knowledge of differential expression patterns and the type of 5-HT receptors localized in tissue is critical to understanding how serotonin regulates a particular tissue’s physiology. All of the serotonin receptor families except for 5HT6 have been identified in the mammary gland (Hernandez et al., 2009; Pai et al., 2009; Stull et al., 2007) [26, 40, 35]. The 5HTR7 regulates the shape and secretory activity of mammary epithelial cell (Pai et al., 2009; Pai et al., 2015; Laporta et al., 2014) [40, 41, 42], whereas 5HTR2b was found to regulate calcium and mammary-tobone signaling during lactation (Stull et al., 2007; Reiter, 1991) [35, 43]. Studies based on immunocytochemistry of human MEC demonstrated that 5-HTR is localized on the MEC basolateral membranes (Pai et al., 2015) [41], and maintains the stability of tight junctions between mammary epithelial cells of mouse origin (Pai and Horsemant, 2011) [27].

The effect of 5-HT on the mammary gland occurs in two ways. Firstly through milk synthesis and secretion by the two different 5-HT receptor subtypes. Secondly through the stimulation of parathyroid hormone related-protein, a calcium-mobilizing hormone. Though the regulation of 5-HT activity is multifactorial, the important component is the reuptake of 5-HT from the extracellular space following its release. Further, the wide availability of SSRIs allows the manipulation of 5-HT activity in a biological system (Marshall et al., 2014) [44]. Stimulation of Receptor subtype 2 is associated to the biosynthesis and release of mammary specific parathyroid hormone-related protein (PTHrP) into the vascular circulation; as an integral part of physiological axis involved in Ca homeostasis, PTHrP induces bone resorption, causing increased bone calcium turnover which is reflected in serum (Hernandez et al., 2012; Horsemant and Hernandez, 2014; Zang et al., 2018) [45, 46, 47]. As per Zhang et al. (2008) [48], the 5-HTR1B is associated with the milk production traits in dairy cows. Real-time quantitative PCR (RT-qPCR) and in situ hybridization studies demonstrated vital expression of 5-HTR subtypes namely 1B, 2A, 2B, 4 and 7 in epithelial, myoepithelial and vascular endothelial cells of lactating cows mammary tissue (Hernandez et al., 2009) [26]. Pharmacodynamics of SSRIs prolong the histological exposure of bioactive 5-HT by impeding cellular reuptake of serotonin and eventual degradation. Longer tissue exposure allows serotoninergic effects of higher magnitude, resulting in MEC tight junction disruption and contraction in mRNA expression of milk protein across the mouse, human, and bovine species. Additionally, mammary magnification of serotonin concentration via SSRIs or 5-HT precursor 5-hydroxy-l-tryptophan augmented serotoninergic decline in milk secretory function of MECs at dry-off (Collier et al., 2012) [16]. Kessler et al. (2018) [49] studied twelve multiparous Holstein cows during the first 2 weeks of lactation and observed decreased colostrum yield in cows with higher basal 5-HT concentrations. They observed that High Serum Serotonin (HSS) cows produced less milk during the first week of lactation, especially those cows in the second to sixth milking. HSS cows yielded low colostrum which could be related to prolonged effect of 5-HT on colostrum synthesis (Hernández-Castellano et al., 2017) [50]. They further noted that as time passed the 5-HT concentration decreased in both Low Serum Serotonin (LSS) and HSS cows. Further, LSS cows exhibited higher milk 5-HT concentration as compared to HSS cows.In order to comprehensively understand the receptor biology of serotonin across multiple species Suárez-Trujillo et al. (2019) [51] conducted an extensive study across sheep, goat and cows to measure the expression of 5-HTR subtypes and immuno-localize receptor distribution within mammary tissue of lactating and dried off animals (Figures 1, 2). Based on the IHC results they opined that cytoplasmic immunoreactivity for all receptors studied in MEC stand incongruous to the basolateral membrane staining for 5-HTR7 in the MCF10A cells reported by Stull et al. (2007) [15]. The study demonstrated the expression of vital 5-HTR7 which regulates the stability of tight junctions among alveolar cells (Stull et al., 2007) [15] and milk protein synthesis (Hernandez et al., 2009) [26] in the three species studied. Recently, using mouse models Moon et al. (2020) [52] provided vital evidence suggesting the critical role of HTR2B in serotonin signaling to activate pancreatic β cell proliferation during lactation.

Lactational calcium homeostasis
During the transition period, maternal Ca homeostasis is quite challenging to persevere in dairy cows. Post the onset of lactation in conjunction with secreting calcium at the rate of 30 to 50 g/day (Horst et al., 2005) [53], lactating cows also have to maintain total blood calcium within the range of 2.1 to 2.5 mM (Goff, 2008) [54]. As suggested by the estimates, a lactating cow will deplete her Ca reservoir 7 to 10 times per day to meet the demand for peak milk production (Horst et al., 2005) [53]. Ninan (2012) [55] in his work on lactating Gir cows reported the serum calcium levels (mg/dl) to vary from 8.63 ± 0.22 to 9.37 ± 0.23 between the first and third month of lactation. Activation of calciotropic hormones for postpartum maintenance of circulating calcium concentrations is requisite to a successful lactation (Kovacs and Kronenberg, 1997; Weaver et al., 2016) [56, 57]. As opined by Wyolsmers (2012) [58], mammalian species are characterized by increased bone calcium turnover accompanied by loss of bone mass during lactation. The homeostatic regulation of circulating calcium is orchestrated differentially during gestation and lactation when compared to non-pregnant and non-lactating physiological states (Bauman and Currie, 1980; Salari and Abdollahi, 2014) [1, 59]. Serotonin secreted within the mammary gland by mammary epithelial cells has been demonstrated to regulate calcium homeostasis during lactation directly as well as through 5HT- PTHrP axis in humans.
(Modder et al., 2011) [60], mice (Laporta et al., 2014) [44] and dairy cows (Laporta et al., 2013) [61]. During lactation, besides the PTHrP mediated indirect role of serotonin in calcium level maintenance, 5-HT is also found to be produced by and act on osteocytes to reduce osteoblasts (OB) proliferation and initiate osteoclasts (OC) aided calcium resorption. Thus implicating a direct role of serotonin in bone mineral regulation in lactating dam (Modder et al., 2011; Chabbi-Atmani et al., 2013; Ducy, 2011) [60, 62, 63]. Moseley et al. (1987) [64] described a protein isolated from cells of a lung tumor with marked structure and bioactivity similarity to PTH as it utilizes the PTH receptor (Strewler, 2000) [65] which was then named as parathyroid hormone-related protein (PTHrP). In each of the principal stages of mammogenesis, PTHrP appears to serve different functions via cellular signalling (Kovacs, 2020) [66]. Data supporting the notion that PTHrP acts as a signal essential for embryonic mammogenesis in rodents was reported by several studies (Wysolmerski et al., 1998; Dunbar et al., 1999) [67, 68] using PTHrP knockout mouse models. Additionally, based on transgenic mice studies Dunbar et al. (2001) [69] suggested a vital role of PTHrP in the regulation of mammary morphogenesis during puberty. During lactation, homeostatic regulation of circulating calcium is largely regulated by mammary-derived PTHrP (Kovacs, 2011) [70] rather than PTH. Localization studies in rodents and cows have all noted epithelial cells to be the source of PTHrP in the mammary gland during pregnancy and lactation (Liapis et al., 1993; Wojcik, 1998) [71, 72]. With the onset of lactogenesis in mammary tissue, MEC secreted PTHrP ends up in milk, at concentrations exceeding 10000 times higher than in the circulation of non-lactating individuals (Kovacs, 2011) [70]. It is speculated that the tremendous concentrations of this peptide in milk, which provides wholesome nourishment to the newborn may have something to do with neonatal physiology. However, the functional role of PTHrP in a newborn is still unknown. As per Cooke-Hubley et al. (2016) [73] since milk PTHrP is absorbed intact within the neonate's gut rendering it highly bioavailable, PTHrP might therefore have some active role in the calcium metabolism. Physiological implications of enhancing the bioavailability of 5-HT by manipulating the serotonergic metabolism through oral administration of fluoxetine or 5-HTP in pre-weaned dairy calves was recently reported by Marrero et al. (2019) [74]. However, the study revealed no significant difference in physiological parameters, growth, health status, and behaviour of pre-weaned dairy calves, indicating it as a safe approach to increase 5-HT bioavailability. Moreover, the milk concentration of PTHrP is correlated to blood calcium concentrations in lactating cows to coordinate calcium metabolism during lactation (Onda et al., 2006; Kocabagli et al., 1995) [75, 76]. The physiological relationship of PTHrP between lactating mammary gland and maternal bone metabolism was elucidated by VanHouten et al. (2003) [77] using a mouse model devoid of PTHrP gene in mammary epithelial cells. The study documented decreased circulating PTHrP levels, undetectable milk PTHrP, and decreased resorption rate of bone calcium which further diminished bone loss by 50 percent. Elevated PTHrP levels correlate proportionately with biomarkers of bone resorption and inversely with bone mass in lactating mice (VanHouten et al., 2004) [78]. Additionally, unaltered milk calcium concentration was observed which clarified that ablation of mammary specific PTHrP gene did not affect the export rate of mammary calcium into the secreted milk as it is locally regulated by CaSR (VanHouten et al., 2004; Ardeshirpour et al., 2006; Mamillapalli et al., 2013) [79, 80]. The above experiments have thus established the vital role of PTHrP in regulating bone calcium turnover through modulating bone metabolism during lactation. Moreover, apart from its effects on bone calcium studies in the early nineties have also acknowledged that PTHrP increases mammary blood flow during lactation (Davico et al., 1993; Thiede et al., 1992) [81, 82]. As reported by Davicco et al. (1993) [81], an IV injection of amino-terminal fragments of PTHrP into the mammary artery of non-lactating ewes led to enhancement in arterial circulation to mammary glands and overrides the vasoconstrictive effects of vascular endothelin. Research on PTHrP in the dairy cow model is scanty, and this can be further explored based on the rodent literature. Using TPH1 knockout mouse model studies, Pai and Horsemann (2008) [83] demonstrated that physiologic concentration of 5-HT is a prerequisite for the initiation of mammary-derived PTHrP induced calcium homeostasis specifically during lactation. Furthermore, studies by Hernandez et al. (2012) [45] demonstrated mammary gland secreted 5-HT stimulates the production of mammary-derived PTHrP via an autocrine pathway as decreased gene and protein expression of PTHrP was noted in the mammary glands of mice deficient in TPH1. Rodent study based hypothesis relating to 5-HT - PTHrP axis during lactation was cautiously extended onto the related experiments on multiparous transition dairy cows. The experimental correlation data not only did reinforce the presence of an active bovine 5-HT - PTHrP-Ca physiological axis in lactating dams but also highlighted the negative correlation between serotonin and parturient paresis incidence in post parturient cows; which substantiated the interpretations of rodents studies in lactating dairy cows. With the prime objective to study the dynamics of and establish the previously unknown physiological circulatory concentrations of 5-HT in multiparous dairy cows a longitudinal observational study was undertaken on two dairy herd farms. During parturition, progressive decline of 5-HT with total calcium was observed which recovered back around day 3 and day 10 postpartum correspondingly on both the farms. Furthermore, supporting the evidence-based rodent studies bovine blood PTHrP levels were found to be elevated 2-4 days postpartum before which total blood Ca was reduced 1-2 days postpartum. This indicated that the latter event was a lactation induced hypocalcaemia which stimulated MEC biosynthesis of PTHrP via a calcium-sensing mechanism in the mammary gland epithelium. Postpartum accretion of circulatory PTHrP signals its homeostatic role in restraining the calcium supply to the mammary gland without compromising the blood calcium threshold required for maternal physiology. Since the degree of mammary secretion is positively correlated to the healthy udder size and HF cows yield higher milk volume than Jersey cows, the calcium homeostasis was better maintained in HF, reflected by the presence of milk serotonin concentration three fold times in comparison to its Jersey counterpart. As per the study conducted by Laporta et al. (2015) [32] a physiologically stimulated but transient state of circulatory hypocalcaemia was generated in response to IV injection of 5-HTP to non-pregnant late lactation dairy cows. Post 5-HTP administration as expected, a corresponding accretion in circulatory serotonin was measured in all the test cows. Consequently, the enhanced serotonin levels may have exerted their genomic effect leading to increased expression of calcium channel transporters in the mammary gland, which caused the drainage of blood calcium into milk calcium within the
Serotonin as a mammary immunomodulator

Transient immunosuppression experienced during the periparturient period makes the cows susceptible to intra-
mammary pathogenesis during early lactation. Postpartum
intra-mammary infections are characterized by elevated pro-
inflammatory cytokines along with phagocytic polymorphonuclear leukocytes such as neutrophils in infected
udders. Immunocharacterization of peripheral circulating
serotonin to which mammary 5-HT belongs has revealed the
presence of an extensive serotonergic network throughout the
immune system (Herr et al. 2017; Ahern, 2011) implicating its functions by modulating innate as well as
adaptive immunity. In 1988, it was demonstrated that cellular
uptake of serotonin in monocytes and macrophages is
mediated through SERT following which it is biodegraded to
5-hydroxy indole acetic acid metabolite (Jackson et al., 1988)
through the stimulation of immune cells such as
monocytes and lymphocytes, circulating serotonin also
influences the secretion of pro-inflammatory cytokines. Store-
operated Ca entry mediated expansion of the intracellular Ca
pool allows diverse neutrophil functions, including cell
degranulation, chemotaxis, reactive oxygen species formation
and phagocytosis (Immler et al., 2018). Moreover, significant neutrophil recruitment to sites of inflammation
was demonstrated in the presence of platelet-derived
serotonin in contrast to weak neutrophil recruitment in the
absence of serotonin (Duerschmied et al., 2013). Given the
immune-physiological implications of transition stress, the
postpartum mammary secretions up to 2 weeks are
characterized by elevated milk SCC consisting of MEC and
immune cells, independent of the potential presence of IMI
(Natzke et al., 1972; Dohoo and Meek, 1982). As suggested by Sordillo et al. (2009) amongst the herd, post
parturient cows with higher circulating 5-HT experiencing
increased milk leukocyte count might have a functional
correlate benefit during the peripartum period, when these
cows are most susceptible to intramammary infections.
Moreover, as reported by Capuco et al. (2001) and
supported by Annen et al. (2007) early lactation

Serotonin and Energy Metabolism

The physiological transition from pregnancy to lactation is
categorized by a decrease in the glucose turnover getting
oxidized to carbon dioxide due to enormous demands for
precursors of lactose synthesis. At this point, voluntary feed
intake plummets, and symptoms of negative energy balance
(NEB) are felt that may threaten the lactational efficiency of the
postpartum dams (Grummer, 1995). Ninan et al. (2014) analyzed the data on milk lactose and plasma
glucose during the first three months of lactation, which
revealed that milk lactose levels were non-symmetrically and
positively correlated with plasma glucose (r = 0.277, 0.418,
and 0.413) at all three stages in Gir cows. In Jaffarabadi
buffaloes, they found that the correlation was negative during the
1 m (r = -0.003) and 2 m (r = -0.171) lactation whereas it was positive (r = 0.501) at the 3 m lactation stage. The overall
correlation coefficient between the two variables was non-
significantly positive in both Gir cows (r = 0.385) and
Jaffarabadi buffaloes (r = 0.083). Serotonin has been reported as a potent biomolecule regulating body energy balance since
it induces the much-required proliferation of hepatic parenchyma through cellular hyperplasia and hypertrophy to strongly support the lactational workload of producing copious milk (Oh and Namkung et al., 2015). This hepatic proliferation is mediated mostly by 5HTTR2a family of receptors (Weaver et al., 2017). Dietary studies in rodents have reported that supplementation of 5-hydroxy-L-
tryptophan (5-HTP), a serotonin precursor provided an immediate increase in circulating serotonin level compared to amino acid L-tryptophan (L-TRP) supplement which allowed more time to elevate serotonin level in lactating dams. The
serotonin is known to maintain energy homeostasis by regulating glucose and fatty acid metabolism. Laporta et al. (2013) [107], investigated the correlation between circulating 5-HT, milk fever and the incidence of ketosis and severity in 42 multiparous Holstein cows at the onset of lactation i.e. day 1 of lactation, by analyzing the blood samples for 5-HT, calcium, glucose, and PTHrP. They found that serum 5-HT had a positive correlation with serum calcium and with plasma PTHrP (r>0.37), whereas it had a negative correlation with milk fever incidence and ketosis severity. Further levels of serum Ca and plasma glucose had negative correlation with milk fever and ketosis severity, respectively (r<0.39). This led to the conclusion that 5-HT plays an important role in regulating Ca and glucose during the transition period in cattle. Additionally, higher mRNA expression of GLUT-8 was observed in the mammary glands of 5-HTP supplemented dams for glucose transport from the vasculature to the mammary epithelium during lactation (Laporta et al., 2013) [107]. Furthermore, they also demonstrated that phosphorylated AMP-activated protein kinase (pAMPK), a cellular energy sensor, was elevated in mammary glands of 5-HTP fed dams that switched off biosynthetic pathways to store ATP. Investigations by Laporta et al. (2015) [32] observed that in response to 1.5mg/kg 5-HTP dose administration, the expression of hepatic mRNA array of enzymes like glucose-6-phosphate (G6P), pyruvate dehydrogenase kinase (PDK4), and the fatty acid metabolism enzymes like peroxisome proliferator-activated receptor-α (PPAR-α), carnitine palmitoyl transferase 1 (CPT1) which are involved in gluconeogenesis was highest among administered doses followed by an increase in circulating glucose and NEFA. The mRNA expression of pyruvate carboxylase (PC) and cytosolic phosphoenolpyruvate carboxykinase-1 (PEPCK1) was found to be 6 fold at day one post-partum and 2.5 times increased at two weeks post-partum, respectively when compared to day one post-partum in transition dairy cows (White et al., 2016) [108]. Pyruvate Carboxylase converts pyruvate to oxaloacetate (OAA), an important intermediate product that participates in gluconeogenesis, tricarboxylic cycle and insulin secretion metabolic pathways while PEPCK converts the OAA pool back to pyruvate (White et al., 2016) [108]. Weaver et al. (2017) [106] reported an increase in mRNA expression of PC but not PEPCK2 in Holstein cows infused with 5-HTP compared to control cows which suggested that OAA was enough for gluconeogenesis. However cytosolic PEPCK1 was not measured. Hence, the ratio of PC to PEPCK is significant to maintain liver metabolic homeostasis. They found increased gluconeogenesis as a result of more expression of PC not PEPCK2 in the livers of 5-HTP infused early lactation Holstein dairy cows. Increased concentration of circulating serotonin resulted in increased serum glucose and non-esterified fatty acid level and decreased levels of β-hydroxybutyric acid to improve energy status in 5-HTP infused lactating cows (Laporta et al., 2015) [32] and 5-HT injected wether sheep (Watanabe et al., 2014) [109]. In parturient cows, circulatory concentrations of glucose, plasma insulin, NEFA, and BHBA are to be tightly regulated at the onset of lactation. Supplementation of 5-HTP at optimum dose would elicit a robust response to meet metabolic energy demands in circulation without adverse health outcomes (Weaver et al., 2017) [106]. Thus, well-orchestrated gluconeogenic signals under the influence of serotonin will efficiently allow to meet the copious requirement for milk biosynthesis. It will also sustain the augmented metabolism in the liver and mammary gland.

Conclusion
Mammary gland physiology remains one of the complex physiological organ systems to comprehend, because of its dynamic changes in terms of structure and function. This review has outlined the current state of knowledge regarding mammary-derived serotonin in dairy cows. Extensive knowledge gained over the years relating to the biology of serotonin in mammary glands can effectively be used to devise multi-pronged prophylactic strategies to prevent postpartum hypocalcaemia in dairy cows. However, apart from its role in calcium metabolism during lactation other functional pathways need to be explored concerning lactation function of mammary gland.

Following figures have been data visualized from the tables of original research article of Suarez-Trujillo et al. (2019)

Fig 1: Distribution of serotonin receptor subtypes expression in the lactating mammary gland of cows, goats and sheep (Venn diagram adapted from and conceptualised based on the data given by Suarez-Trujillo et al., 2019)

Fig 2: Specific distribution of serotonin receptor subtypes expression in the lactating mammary gland of cows, goats and sheep (Pie chart adapted from and conceptualised based on the data given by Suarez-Trujillo et al., 2019)

References
1. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science. 1980; 63(9):1514-1529.
2. Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science. 1995; 73(9):2804-2819.

3. Bruckmaier RM, Gross JJ. Lactational challenges in transition dairy cows. Animal Production Science. 2017; 57(7):1471-1481.

4. Hayirli A, Grummer RR, Nordheim EV, Crump PM. Animal and dietary factors affecting feed intake during the refresh transition period in Holsteins. Journal of Dairy Science. 2002; 85(12):3430-3443.

5. Akers RM. Selection for milk production from a lactation biology viewpoint. Journal of Dairy Science. 2000; 83(5):1151-1158.

6. Capper JL, Cady RA, Bauman DE. Demystifying the environmental sustainability of food production. In Proceedings Cornell Nutrition Conference, 2009, 187-203.

7. Ingvartsen KL. Feeding-and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Animal Feed Science and Technology. 2006; 126(3-4):175-213.

8. Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. Prevalence of subclinical hypocalcaemia in dairy herds. The Veterinary Journal. 2011; 188(1):122-124.

9. Van Knegsel ATM, Remmelink GJ, Jorjong S, Fievez V, Kemp B. Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. Journal of Dairy Science. 2014; 97(3):1499-1512.

10. Hadley GL, Wolf CA, Harsh SB. Dairy cattle culling patterns, explanations, and implications. Journal of dairy science. 2006; 89(6):2286-96.

11. Gustavo Canul-Medina and Cristina Fernandez-Mejia. Morphological, hormonal, and molecular changes in different maternal tissues during lactation and post-lactation. Journal of Physiological Science. 2019; 69(6):825-835.

12. Fleischer P, Metzner M, Beyerbach M, Hoedemaker M, Klee W. The relationship between milk yield and the incidence of some diseases in dairy cows. Journal of Dairy Science. 2001; 84(9):2025-2035.

13. Ingvartsen KL, Dewhurst RJ, Friggens NC. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Science. 2003; 83(2-3):277-308.

14. Mulligan FJ, Doherty ML. Production diseases of the transition cow. The Veterinary Journal. 2008; 176(1):3-9.

15. Bruschetta G, Di Petro P, Sanzarelo LL, Sanzarelo E, Giacoppo, Ferlazzo AM. Plasma serotonin levels in Italian Friesian dairy cows. Veterinary Research Communications. 2020; 34:17-20.

16. Collier RJ, Hernandez LL, Horsemans ND. Serotonin as a homeostatic regulator of lactation. Domestic Animal Endocrinology. 2012; 43(2):161-70.

17. Hernández-Castellano LE, Hernandez LL, Bruckmaier RM. Endocrine pathways to regulate calcium homeostasis around parturition and the prevention of hypocalcaemia in periparturient dairy cows. Animal. 2019; 14(2):330-8.

18. Turlejski K. Evolutionary ancient roles of serotonin: long-lasting regulation of activity and development. Actaanad biotechnaexperimentalis. 1996; 56:619-636.

19. Raymond JR, Mukhin YV, Gelasco A, Turner J, Collinsworth G, Gettys TW et al. Multiplicity of mechanisms of serotonin receptor signal transduction. Pharmacology and Therapeutics. 2001; 92(2-3):179-212.

20. Gershon MD, Tack J. The serotonin signalling system: from basic understanding to drug development for functional GI disorders. Gastroenterology. 2007; 132(1):397-414.

21. Horsemans ND, Collier RJ. Serotonin: a local regulator in the mammary gland epithelium. Annual Review of Animal Biosciences. 2014; 2(1):353-374.

22. Wyler SC, Lord CC, Lee S, Elmsquist JK, Liu C. Serotonergic control of metabolic homeostasis. Frontiers in Cellular Neuroscience. 2017; 11: 277.

23. Whitaker-Azmitia PM. The discovery of serotonin and its role in neuroscience. Neuropsychopharmacology. 1999; 21(1): 2-8.

24. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annual review of medicine. 2009; 60:355-366.

25. Lauder JM. A role for serotonin in the mammary gland. Developmental Cell. 2004; 6(2):165.

26. Hernandez LL, Limesand SW, Collier JL, Horsemans ND, Collier RJ. The bovine mammary gland expresses multiple functional isoforms of serotonin receptors. The Journal of Endocrinology. 2009; 203(1):123.

27. Pai, VP, Horsemans ND. Multiple cellular responses to serotonin contribute to epithelial homeostasis. PLoS One. 2011; 6(2):e17028.

28. Matsuda M, Imaoka T, Vomacka AJ, Gudelsky GA, Hou Z, Mistry M et al. Serotonin regulates mammary gland development via an autocrine-paracrine loop. Developmental Cell. 2004; 6(2):193-203.

29. Wang L, Erlandsen H, Haavik J, Knappskog PM, Stevens RC. Three-dimensional structure of human tryptophan hydroxylase and its implications for the biosynthesis of the neurotransmitters serotonin and melatonin. Biochemistry. 2002; 41(42):12569-74.

30. Moore SAE, Laporta J, Crenshaw TD, Hernandez LL. Patterns of circulating serotonin and related metabolites in multiparous dairy cows in the peripartum period. Journal of Dairy Science. 2015; 98(6):3754-3765.

31. Pai VP, Horsemans ND. Biphasic regulation of mammary epithelial resistance by serotonin through activation of multiple pathways. Journal of Biological Chemistry. 2008; 283(45):30901-30910.

32. Laporta J, Moore SA, Weaver SR, Cronick CM, Olsen M, Prichard AP et al. Increasing serotonin concentrations alter calcium and energy metabolism in dairy cows. Journal of Endocrinology. 2015; 226:43-55.

33. Weaver SR, Prichard AS, Maerz NL, Prichard AP, Endres EL, Hernández-Castellano LE et al. Elevating serotonin pre-partum alters the Holstein dairy cow hepatic adaptation to lactation. PloS one. 2017; 12(9):e0184939.

34. Toledo IM, Zhao X, Lacasse P. Effects of milking frequency and domperidone injections on milk production and prolactin signaling in the mammary gland of dairy cows. Journal of Dairy Science. 2020; 103(2):1969-81.

35. Stull MA, Pai V, Vomacka AJ, Marshall AM, Jacob GA, Horsemans ND. Mammary gland homeostasis employs serotonergic regulation of epithelial tight
junctons. Proceedings of the National Academy of Sciences. 2007; 104(42):16708-13.

36. Hernandez LL, Collier JL, Vomachka AJ, Collier RJ, Horseman ND. Suppression of lactation and acceleration of involution in the bovine mammary gland by a selective serotonin reuptake inhibitor. Journal of Endocrinology. 2011; 209(1):45-54.

37. Kessler EC, Wall SK, Hernandez LL, Gross JJ, Bruckmaier RM. Mammary gland tight junction permeability after parturition is greater in dairy cows with elevated circulating serotonin concentrations. Journal of Dairy Science. 2019; 102(2):1768-1774.

38. Peroutka SJ, Howell TA. The molecular evolution of G protein-coupled receptors: focus on 5-hydroxytryptamine receptors. Neuropharmacology. 1994; 33(3-4):319-324.

39. Uphouse L. Multiple serotonin receptors: too many, not enough, or just the right number? Neuroscience and Biobehavioral Reviews.1997; 21(5):679-698.

40. Pai VP, Marshall AM, Hernandez LL, Buckley AR, Horseman ND. Altered serotonin physiology in human breast cancers favors paradoxical growth and cell survival. Breast Cancer Research. 2009; 11(6):R81.

41. Pai VP, Hernandez LL, Stull MA, Horseman ND. The type 7 serotonin receptor, 5-HT 7, is essential in the mammary gland for regulation of mammary epithelial structure and function. Biomed Research International. 2015; 2015:364736.

42. Laporta J, Keil KP, Vezina CM, Hernandez LL. Peripheral serotonin regulates maternal calcium trafficking in mammary epithelial cells during lactation in mice. PLoS One 2014; 9(10):e110190.

43. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocrine Reviews. 1991; 12:151-80.

44. Marshall AM, Hernandez LL, Horseman ND. Serotonin and serotonin transport in the regulation of lactation. Journal of Mammary Gland Biology and Neoplasia. 2014; 19(1):139-46.

45. Hernandez LL, Gregorson KA, Horseman ND. Mammary gland serotonin regulates parathyroid hormone-related protein and other bone-related signals. American Journal of Physiology-Endocrinology and Metabolism. 2012; 302(8):E1009-15.

46. Horseman ND, Hernandez LL. New concepts of breast cell communication to bone. Trends in Endocrinology and Metabolism. 2014; 25(1):34-41.

47. Zang WJ, Li H, Zhang ZF, QuZhen R, CuoMu YZ, Zhang DK et al. Serotonin induces parathyroid hormone-related protein in goat mammary gland. Journal of Animal Science. 2018; 96(3):1010-6.

48. Zhang CL, Chen H, Wang YH, Zhang RF, Lan XY, Lei CZ et al. Serotonin receptor 1B (HTR1B) genotype associated with milk production traits in cattle. Research in Veterinary Science. 2008 85(2):265-8.

49. Kessler EC, Wall SK, Hernandez LL, Bruckmaier RM, Gross JJ. Sort communication: Circulating serotonin is related to the metabolic status and lactational performance at the onset of lactation in dairy cows. Journal of Dairy Science. 2018; 101(12):11455-11460.

50. Hernández-Castellano LE, Hernandez LL, Weaver S, Bruckmaier RM. Increased serum serotonin improves parturient calcium homeostasis in dairy cows. Journal of Dairy Science. 2017; 100(27988124):1580-1587.

51. Suarez-Trujillo A, Chen Y, Aduwari C, Cummings S, Kuang S, Buhman KK et al. Maternal high-fat diet exposure during gestation, lactation, or gestation and lactation differentially affects intestinal morphology and proteome of neonatal mice. Nutrition Research. 2019; 66:48-60.

52. Moon JH, Kim YG, Kim K, Osonoi S, Wang S, Saunders DC et al. Serotonin regulates adult β-cell mass by stimulating perinatal β-cell proliferation. Diabetes. 2020; 69(2):205-214.

53. Horst RL, Goff JP, Reinhardt TA. Adapting to the transition between gestation and lactation: differences between rat, human and dairy cow. Journal of Mammary Gland Biology and Neoplasia. 2005; 10(2):141-56.

54. Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcaemia in dairy cows. The Veterinary Journal. 2008; 176(1):50-57.

55. Ninan Jacob. Haematological, biochemical and endocrine parameters at different ages and physiological stages in Gir cattle and Jaffarabadi buffaloes. PhD thesis submitted to Anad Agricultural University, Anand. 2012.

56. Kovacs CS, Kronenberg HM. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocrine Reviews. 1997; 18(6):832-872.

57. Weaver SR, Prichard AP, Endres EL, Newhouse SA, Peters TL, Crump PM et al. Elevation of circulating serotonin improves calcium dynamics in the peripartum dairy cow. Journal of Endocrinology. 2016; 230(1):105-23.

58. Wysolmerski JJ. Parathyroid hormone-related protein: an update. The Journal of Clinical Endocrinology and Metabolism. 2012; 97(9):2947-2956.

59. Salari P, Abdollahi M. The influence of pregnancy and lactation on maternal bone health: A systematic review. Journal of Family and Reproductive Health. 2014; 8(4):135.

60. Mödder UL, Roforth MM, Hoey K, McCready LK, Peterson JM, Monroe DG et al. Effects of estrogen on osteoprogenitor cells and cytokines/bone-regulatory factors in postmenopausal women. Bone. 2011; 49(2):202-7.

61. Laporta J, Moore SAE, Peters MW, Peters TL, Hernandez LL. Short communication: Circulating serotonin (5-HT) concentrations on day 1 of lactation as a potential predictor of transition-related disorders. Journal of Dairy Science. 2013; 96(8):5146-50.

62. Chabbi-Achengli Y, Coudert AE, Callebert J, Geoffroy V, Côté F, Collet C et al. Decreased osteoclastogenesis in serotonin-deficient mice. Proceedings of the National Academy of Sciences. 2012; 109(7):2567-2572.

63. Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodeling and energy metabolism. Diabetologia. 2011; 54(6):1291.

64. Moseley JM, Kubota M, Diefenbach-Jagger H, Wettenhall RE, Kemp BE, Suva LJ et al. Parathyroid hormone-related protein purified from a human lung cancer cell line. Proceedings of the National Academy of Sciences. 1987; 84(14):5048-5052.

65. Strewle, GJ. The physiology of parathyroid hormone–related protein. New England Journal of Medicine. 2000, 342(3):177-185.

66. Kovacs CS. Physiological actions of parathyroid hormone-related protein in epidermal, mammary, reproductive, and pancreatic tissues. In Principles of Bone Biology. 2020; 839-862.

67. Wysolmerski JJ, Philbrick WM, Dunbar ME, Lanske B, Kronenberg H, Brodus AE. Rescue of the parathyroid
hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. Development. 1998; 125(7):1285-94.

68. Dunbar ME, Dann PR, Robinson GW, Hemighausen L, Zhang JP, Wysolmerski JJ. Parathyroid hormone-related protein signaling is necessary for sexual dimorphism during embryonic mammary development. Development. 1999; 126(16):3485-93.

69. Dunbar ME, Wysolmerski JJ. Mammary ductal and alveolar development: lesson learned from genetically manipulated mice. Microscopy Research and Technique. 2001; 52(2):163-70.

70. Kovacs CS. Calcium and bone metabolism disorders during pregnancy and lactation. Endocrinology and Metabolism Clinics. 2011; 40(4):795-826.

71. Liapis H, Crouch EC, Grosso LE, Kitazawa S, Wick MR. Expression of parathyroidlike protein in normal, proliferative, and neoplastic human breast tissues. The American Journal of Pathology. 1993; 143(4):1169

72. Wojcik SF. The role of parathyroid hormone-related protein (PTHrP) in the physiology of the adult mammary gland. PhD thesis submitted to Ohio State University. Ohio, 1998.

73. Cooke-Hubley S, Mugford G, Valcour J, Wahl M, Woodrow J, Adachi JD et al. Trabecular (Spine) bone density increases significantly in the first 6 months after weaning. In43rd Annual European Calcified Tissue Society Congress. Bio Scientifica, 2016, 5.

74. Marrero MG, Dado-Senn B, Field SL, Da Silva DR, Skibiel AL, Laporta, J. Increasing serotonin bioavailability in preweaned dairy calves impacts hematology, growth, and behavior. Domestic Animal Endocrinology. 2019; 69:42-50.

75. Onda K, Sato A, Yamaguchi M, Matsuki N, Ono K, Wada Y. Parathyroid hormone-related protein (PTHrP) and Ca levels in the milk of lactating cows. Journal of Veterinary Medical Science. 2006; 68(7):709-13.

76. Kocabagli N, Riond JL, Spichiger UE, Wanner M, Kocabagli N. Parathyroid hormone-related protein and calcium homeostasis during the periparturient period of dairy cows. American Journal of Veterinary Research. 1995; 56(3):380-385.

77. VanHouten JN, Wysolmerski JJ. Low estrogen and high parathyroid hormone-related peptide levels contribute to accelerated bone resorption and bone loss in lactating mice. Endocrinology, 2003; 144(12):5521-9.

78. Van Houten J, Dann P, McGeoch G, Brown EM, Krapcho K, Neville M et al. The calcium-sensing receptor regulates mammary gland parathyroid hormone–related protein production and calcium transport. The Journal of Clinical Investigation. 2004; 113(4): 598-608.

79. Ardeshipour L, Dann P, Pollak M, Wysolmerski J, VanHouten J. The calcium-sensing receptor regulates PTHrP production and calcium transport in the lactating mammary gland. Bone. 2006; 38(6):787-793.

80. Mamillapalli, R, VanHouten J, Dann P, Bikle D, Chang W, Brown E et al. Mammary-specific ablation of the calcium-sensing receptor during lactation alters maternal calcium metabolism, milk calcium transport, and neonatal calcium accrual. Endocrinology. 2013; 154(9):3031-3042.

81. Davicco MJ, Roufet J, Durand D, Lefäivre J, Barlet JP. Parathyroid hormone-related peptide may increase mammary blood flow. Journal of Bone and Mineral Research. 1993; 8(12):1519-1524

82. Thiede MA, Grasser WA, Peterson DN. Regulated expression of parathyroid hormone-related protein in mammary blood supply supports a role in mammary blood flow. Bone and Mineral. 1992; 17:71.

83. McArt JAA, Neves RC. Association of transient, persistent, or delayed subclinical hypocalcaemia with early lactation disease, removal, and milk yield in Holstein cows. Journal of Dairy Science. 2020; 103(1):690-701.

84. Wilkens MR, Nelson CD, Hernandez LL, McArt JA. Symposium review: Transition cow calcium homeostasis Health effects of hypocalcaemia and strategies for prevention. Journal of Dairy Science. 2020; 103(3):2909-27.

85. Caixeta LS, Osipa PA, Capel MB, Nydam DV. Association between subclinical hypocalcaemia in the first 3 days of lactation and reproductive performance of dairy cows. Theriogenology. 2017; 94:1-7.

86. Dekosky ST, Palmer AM. Neurochemistry of aging. Clinical neurology of aging. 1994; 2:79-101.

87. Myers BL, Badia P. Changes in circadian rhythms and sleep quality with aging: mechanisms and interventions. Neuroscience and Biobehavioral Reviews. 1995; (19):553-571.

88. Weaver SR, Hernandez LL. Could use of SSRI during lactation cause persistent effects on maternal bone? Journal of Mammary Gland Biology and Neoplasia. 2018; 23:5-25.

89. Herr N, Bode C, Duerschmied D. The effects of serotonin in immune cells. Frontiers in Cardiovascular Medicine. 2017; 4:48.

90. Ahern GP. 5-HT and the immune system. Current Opinion in Pharmacology. 2011; 11(1):29-33.

91. Jackson JC, Walker RF, Brooks WH, Roszman TL. Specific uptake of serotonin by murine macrophages. Life Sciences. 1988; 42(17): 1641-1650

92. Immel R, Simon SI, Sperandio M. Calcium signalling and related ion channels in neutrophil recruitment and function. European Journal of Clinical Investigation. 2018; 48:e12964

93. Duerschmied D, Suidan GL, Demers M, Herr N, Carbo C, Brill A et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. Blood, The Journal of the American Society of Hematology. 2013; 121(6):1008-1015.

94. Natzke RP, Everett RW, Postle DS. Normal milk somatic cell counts. Journal of Milk and Food Technology. 1972; 35(5):261-263.

95. Dohoo IR, Meek AH. Somatic cell counts in bovine milk. The Canadian Veterinary Journal. 1982; 23(4):119.

96. Sordillo LM, Contreras GA, Aitken, SL. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Animal Health Research Reviews. 2009; 10(1): 53.

97. Capuco AV, Wood DL, Baldwin R, Mcleod K, Paape MJ. Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. Journal of Dairy Science. 2001; 84(10):2177-2187.

98. Annen EL, Fitzgerald AC, Gentry PC, McGuire MA, Capuco AV, Baumgard LH et al. Effect of continuous milking and bovine somatotropin supplementation on
mammary epithelial cell turnover. Journal of Dairy Science. 2007; 90(1):165-183.

99. Freire-Garabal M, Nunez MJ, Balboa J, López-Delgado P, Gallego R, García-Caballero T et al. Serotonin upregulates the activity of phagocytosis through 5-HT1A receptors. British Journal of Pharmacology, 2003; 139(2):457-463.

100. Ghia JE, Li N, Wang H, Collins M, Deng Y, El-Sharkawy RT et al. Serotonin has a key role in pathogenesis of experimental colitis. Gastroenterology. 2009; 137(5):1649-1660.

101. Hernández-Castellano LE, Özçelik R, Hernandez LL, Bruckmaier RM. Supplementation of colostrum and milk with 5-hydroxy-L-tryptophan affects immune factors but not growth performance in newborn calves. Journal of Dairy Science. 2018; 101(1):794-800.

102. Quintero-Villegas A, Valdés-Ferrer SI. Role of 5-HT 7 receptors in the immune system in health and disease. Molecular Medicine. 2020; 26(1):1-8.

103. Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science. 1995; 73(9):2820-2833.

104. Ninan Jacob, Arya JS, Gajbhiye PU. Correlation between milk and plasma components in Gir cattle and Jaffarabadi buffaloes during different lactation stages. Indian Journal of Dairy Science. 2014; 67(1):57-61.

105. Oh CM, Namkung J, Go Y, Shong KE, Kim K, Kim H et al. Regulation of systemic energy homeostasis by serotonin in adipose tissues. Nature Communications. 2015; 6(1):1-12.

106. Weaver SR, Jury NJ, Gregerson KA, Horzeman ND, Hernandez LL. Characterization of mammary-specific disruptions for Tph1 and Lrp5 during murine lactation. Scientific Reports. 2017; 7(1):1-8.

107. Laporta J, Peters TL, Merriman KE, Vezina CM, Hernandez LL. Serotonin (5-HT) affects expression of liver metabolic enzymes and mammary gland glucose transporters during the transition from pregnancy to lactation. PLoS One. 2013; 8(2):e57847.

108. White HM, Carvalho ER, Koser SL, Schmelz-Roberts NS, Pezzanite LM, Slabaugh AC et al. Regulation of hepatic gluconeogenic enzymes by dietary glycerol in transition dairy cows. Journal of Dairy Science. 2016; 9(1):812-7.

109. Watanabe H, Saito R, Nakano T, Takahashi H, Takahashi Y, Sumiyoshi K et al. Effect of peripheral 5-HT on glucose and lipid metabolism in wether sheep. PLoS One. 2014; 9(2):e88058.