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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 metabolicload 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 (Ingvartsen, 2006; Reinhardt *et al*., 2011; Van Knegsel *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) [16] 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-(ß-Aminoehtyl)-5-hydroxy indole, Thrombotonin)

As one of the crucial monoamines Serotonin ($C_{10}H_{12}N_2O$) with IUPAC ID 3-(2-Aminoethyl)-1*H*-indol-5-o 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 Horseman, 2011) [25-27]. However, enteroepithelial 5-HT secretion represents approximately 90percent 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) [21]. 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) [17] 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) [30]. A mouse model study by Pai and Horseman (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 draining 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 Horseman, 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 Horseman, 2008; Hernandez *et al*., 2011; Collier *et al*., 2012) [35, 31, 36, 16]. A study was undertaken to understand the functional secretory 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 serotonylation, 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 serotonergic 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 5HTR6 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-to-bone 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 Horseman, 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; Horseman and Hernandez, 2014; Zang *et al*., 2018) [45, 46, 47]. As per Zhang *et al*. (2008) [48], the 5-HTR1B genotype 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 serotonergic 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 serotonergic 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) [35]. The study demonstrated the expression of vital 5-HTR7 which regulates the stability of tight junctions among alveolar cells (Stull *et al*., 2007) [35] 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 Wysolmerski (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-Achengli *et al*.,2012; 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) [78, 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 (Davicco *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 Horseman (2008) [31] 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

lactating mammary gland explaining the transient hypocalcaemia. Additionally, postpartum SCH categorization based recent findings of McArt and Neves (2020) [83] suggest that physiological expression of postpartum transient SCH dairy cows are metabolically unconstrained resulting in the least inflictions of postpartum diseases in comparison to postpartum complications much observed in persistent SCH and delayed SCH groups of cows. Finally, serotonin-induced transient hypocalcaemia may have acted as a necessary triggering signal which when sensed via CaSR of mammary gland led to induction of mammary-derived PTHrP, which is critical for PTHrP stimulated calcium liberation from the bone. Thus establishing that transient hypocalcaemia is physiologically an essential innate step to activate the serotonin-PTHrP axis that will regulate circulatory calcium homeostasis in lactating dairy cows. The metabolic capacity of dairy cows to efficiently correct postpartum imbalances in circulating calcium concentrations is negatively correlated to lactation number highlighting the aging factor (Wilkens *et al*., 2020;Caixeta *et al*., 2017) [84, 85]. Since the aging-related, brain-specific decline of neuronal serotonin function is well established as well as supported by various studies (DeKosky and Palmer, 1994; Myers and Badia, 1995) [86, 87], with the same understanding even the Ca homeostasis may become less efficient in subsequent bovine lactations due to weakening of mammary serotonergic functioning. This may cause greater sensitivity towards hypocalcaemia in multiparous cows in comparison to primiparous cows. Lactating women receiving SSRIs based medication for postpartum depression may disadvantage themselves against sustained serotonin signaling, causing prolonged bone resorption activity due to the elevated PTHrP concentrations during lactation(Weaver and Hernandez, 2018) [88].

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) [89, 90] 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) [91]. 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) [92]. 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) [93]. 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) [94, 95]. As suggested by Sordillo *et al*. (2009) [96] amongst the herd, post parturient cows with higher circulating 5-HT experiencing increased milk leukocyte count might have a functional correlative benefit during the peripartum period, when these cows are most susceptible to intramammary infections. Moreover, as reported by Capuco *et al*. (2001) [97] and supported by Annen *et al*. (2007) [98] early lactation corresponds to apoptosis and shedding of mammary epithelial cells into milk at an important rate. Furthermore, Pai and Horseman (2011) [31] demonstrated that serotonergic mechanism augments above-said apoptosis led MECs shedding into the milk which might correspond to the overall elevated SCC found in the milk of cows with higher circulating 5-HT. Studies (Freire-Garabal *et al*., 2003; Ghia *et al*., 2009) [99, 100] demonstrated that 5-HT by activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) stimulates the macrophage IL-1β secretion, which in turn enhances the macrophage phagocytosis via 5-HT receptor 1A. As recently reported by Hernández-Castellano *et al*. (2018) [101] increased mRNA expression of haptoglobin, interleukin-1B, chemokine C-C motif ligand 5 (CCL5) and NF-kB indicates that oral supplementation of 5-HTP might initiate conditioning of the innate and adaptive immune system. This confers additional immuno-protection to the calf through macrophage stimulation and the consequent release of active immune factors. Serotonin seems to be a promising new immunomodulator when it comes to modulating mammary immune responses. The current level of understanding regarding serotonergic immunoregulation suggested that by targeting 5-HTR7, potential therapeutic avenues can be developed for treating inflammatory conditions (Quintero-Villegas and Valdés-Ferrer, 2020) [102].

Serotonin and Energy Metabolism

The physiological transition from pregnancy to lactation is characterized 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) [103]. Ninan *et al*. (2014) [104] analyzed the data on milk lactose and plasma glucoseduring the first three months of lactation, which revealed that milk lactose levels were non-significantly 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) [105]. This hepatic proliferation is mediated mostly by 5HTR2 family of receptors (Weaver *et al*., 2017) [106]. 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), whereasit had a negative correlation with milk fever incidence and ketosis severity. Further levels of serum Ca and plasma glucose hada 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 glucogenesis. 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 being data visualized from the tables of original research articleof Suarez-Trujillo *et al*. (2019)

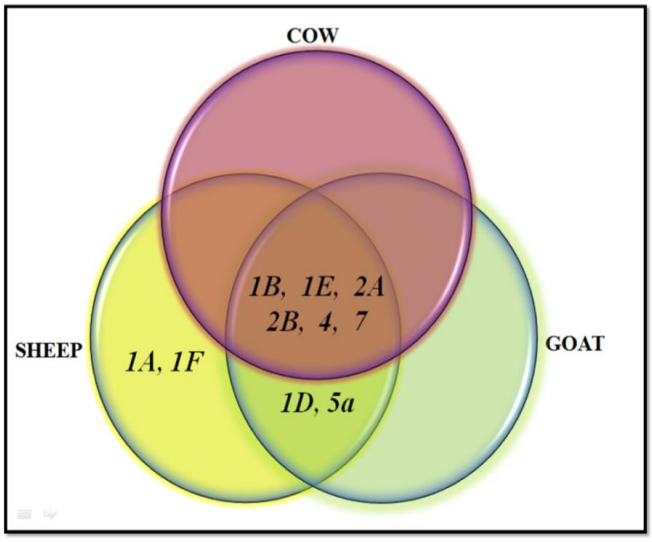

Fig 1: Distribution of serotonin receptor subtypes expression in the lactating mammary gland of cows, goats and sheep (Venn diagram adapted from and conceptualised based on the data given by Suarez-Trujillo *et al*., 2019)

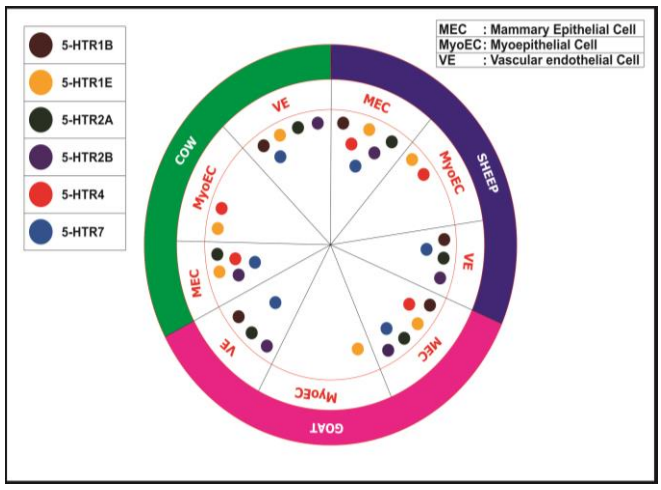

Fig 2: Specific distribution of serotonin receptor subtypes expression in the lactating mammary gland of cows, goats and sheep (Pie chart adapted from and conceptualised based on the data given by Suarez-Trujillo *et al*., 2019)

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