# Behavioral, productive, and reproductive aspects of the water buffalo in Mexico



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Abstract Water buffalo (*Bubalus bubalis*) production in Mexico has generated particular interest in understanding the bases of the development of this species. As a result, alliances have emerged between scientific, governmental, industrial, and productive sectors and other organisms involved with this species nationally and internationally. We must recognize that thanks to specific anatomical characteristics, water buffaloes present a low incidence of complications during calving, though they are not exempt from obstetric and perinatological problems that can end in morbidity and mortality for mother and/or offspring, with losses for both branches of dual-purpose production systems: calves-milk and meat, and draft animals. This article analyzes scientific advances related to water buffalo production in Mexico, including productivity, thermoregulation, behavior, and physiology, and key subthemes like obstetric and perinatological handling, imprinting, milking, and dual-purpose production systems. It highlights features inherent to milk production and the neurophysiological and metabolic processes involved in obtaining meat and milk. Finally, it presents a brief overview of current legislation and summarizes key areas of opportunity.

Keywords: animal production, behavior, Bubalus bubalis, dual-purpose, perinatology, thermoregulation

### 1. Introduction

In recent years in Mexico, the development of water buffalo (Bubalus bubalis) productive systems has gained importance, as has research on this species, especially regarding physiological and behavioral aspects, such as thermographic responses. This has led to the forging national and international alliances between scientific, governmental, industrial, and productive sectors and organisms involved in buffalo production. Water buffaloes have certain characteristics such as rusticity, versatility, and prolificity (Berdugo-Gutiérrez et al 2018; Bertoni et al 2019b; Guerrero-Legarreta et al 2019), in addition to high yields concerning low energy consumption and lower greenhouse gas emissions (Bertoni et al 2022d). Recent scientific publications such as El búfalo de agua en las Américas (The Water Buffalo in the Americas, fourth edition), and Aplicaciones biotecnológicas en la investigación del búfalo de agua (Biotechnological Applications in Research on the Water Buffalo) are compilations of findings from applied research published in high-impact national and international journals that reflect the collaboration of specialists in the main topics addressed.

Analysis of the present review centers on the biological, productive, anatomical, physiological, and behavioral processes of the water buffalo to improve the knowledge about the species and the currently available alternatives for optimizing productive and, especially, reproductive strategies. Obstetric and perinatological handling is another transcendental topic due to its importance in managing water buffaloes raised for any zootechnical purpose. In this regard, this species has anatomical characteristics associated with a low incidence of complications during calving, including a wider pelvis, a fifth free sacral vertebra, a vaginal canal that dilates easily, and long, separate external genital structures (Das et al 2013; Napolitano et al 2018a; Mota-Rojas et al 2022d).

These characteristics increase the reproductive efficiency of buffaloes but do not exempt them from obstetric and perinatological problems that can cause morbidity and mortality of dams and/or their calves. These risks include dystocia caused by maternal or fetal factors (e.g., placental retention, clinical or subclinical uterine infections, and vaginal prolapses). This makes it necessary to develop strategies to prevent lesions and pathologies in both mother and calf during gestation, calving, and the puerperium period. However, this requires a complete understanding of the morphophysiological and behavioral features of eutocic births to reduce the incidence of dystocia (Streyl et al 2012; Mota-Rojas et al 2019c, 2020c, 2021c, 2022d; Napolitano et al 2020b). The objective is to analyze the characteristics of eutocic (normal) births, identify possible causes of labor dystocia-classifying them as maternal and fetal-and include perinatal characteristics

where the main problems seen in newborn water buffaloes can be addressed.

At the end of the second stage of calving, trained personnel must supervise and monitor mother and calf to verify, among other aspects, adequate functioning of the rib cage -no respiratory irregularities- and that the dam approaches the neonate to lick and stimulate it and remove the fetal membranes. Ensuring the correct performance of this stage strengthens the mother-calf bond (Rodríguez-González et al 2022a). It aids the development of expressive behaviors in response to the physiological, endocrine, and emotional changes coordinated by the central nervous system (Coria-Avila et al 2022). In this stage, the mechanoreceptors in the birth canal are stimulated by the transit of the fetus. The afferent signal to the spinal cord and later to the hypothalamus (paraventricular and supraoptic nuclei, generates the release of oxytocin. Oxytocin and dopamine are involved in the mutual recognition of the dam and calf, forming a selective bond known as imprinting. This bonding is fundamental to providing care and food to the newborn calf, characteristics necessary for the survival of the offspring, and adequate productive development in the dam (Orihuela et al 2022).

Another area that has drawn the attention of research groups in Mexico focuses on analyses of the production units. The goal is to incorporate a neurophysiological basis into zootechnical processes, such as handling, to identify key economic factors that impact buffalo production (Bertoni et al 2019c, a, 2022b, c). This considers intrinsic factors such as the genotype associated with reproductive parameters (e.g., age at first calving and parity), estrous behavior, and hormonal and fertility profiles in Mexico and Latin America (Nava-Trujillo et al 2022a). On the other hand, extrinsic factors that impair the productive and reproductive performance of the water buffalo, such as the time of year, the lactation process that delays the onset of postpartum reproductive activity, uncontrolled use of drugs to stimulate ejection milk (oxytocin), type of diet, and diseases established in some herds are other topics of importance (Nava-Trujillo et al 2022b).

Other groups have studied affectations that can arise due to inadequate handling during animal transport, both inside and outside ranches (José-Pérez et al.2022; Napolitano et al 2022f, 2023; Rodríguez-González et al 2022a; Rodríguez-González 2023), and the thermal implications of diverse routines (Bertoni et al 2020c; Mota- Rojas et al 2020; Mota-Rojas et al 2020b, 2021d, e). These lines of research have led, for example, to the identification of the importance of the role of calves in manual milking activities due to the morphophysiological and behavioral characteristics of this species (Bertoni et al 2020b, a; Mota-Rojas et al 2020d, e; Napolitano et al 2020a; Olmos-Hernández et al 2020). It is important to note that this finding emerged when researchers focused their analyses on milking processes, gathering valuable information on the female water buffalo's anatomical, physiological, and behavioral particularities. Findings of this kind have resulted in pertinent recommendations to prevent negative practices and help optimize dairy production. This has also helped to modify the productive characteristics of the buffalo and physicochemical characteristics of the milk by considering elements that can affect its processing and the added value of the products and by-products (Ghezzi et al 2022).

When calves arrive on the scene, decisions must be taken as to the role that they will play as they mature, possibly as draft animals (Mota-Rojas et al 2021a, 2022c), milk producers (Mora-Medina et al 2018a; Mota-Rojas et al 2019a; Rodríguez-González et al 2022a), or meat and meat derivatives producers (Álvarez-Macías et al 2020; Cruz-Monterrosa et al 2020b; Guerrero-Legarreta et al 2020, 2022). Water buffaloes can be bred for one of four objectives, the three mentioned or in dual-purpose units where milk and meat are produced. Whatever the case, the production of water buffalo meat stands out because of its yield about low production costs that reflect, in part, the species' high alimentary efficiency based on its capacity to digest grasses with high lignin content (Bertoni et al 2019c, b; Álvarez-Macías et al 2020).

To encompass all the processes involved in water buffalo production, it is also important to gain familiarity with the legislation applicable in different countries to complement the practices in Mexico. To this end, it is relevant to understand the steps required to enact or apply the laws, norms, and accords that will provide support and options for using practices that will lead to the implementation of procedures and methodologies that improve the productive, reproductive, and behavioral conditions of water buffaloes, based on solid scientific research.

In light of the previous, this review aims to integrate scientific advances related to the water buffalo in Mexico in the following research areas: productivity, thermoregulation, behavior, and physiology. This includes, as primary subtopics, obstetric and perinatal handling, imprinting, milking, dual-purpose production systems, the role of calves in milking, the metabolic and neurophysiological processes that are required to produce meat and milk, and an overview of current legislation and areas of opportunity.

## 2. Obstetric handling of the female water buffalo, eutocic vs. dystocic births and maternal and fetal causes

Gestation in water buffaloes lasts around 300-329 days (average 310-315) (Mota-Rojas et al 2019b; Orihuela et al 2021a; Rodríguez-González et al 2022a). This condition generates biological, productive, and behavioral changes in dams. Studies have documented those ten days prior to calving; farm personnel usually move gestating females to an area specially designed for maternity, keeping them under constant supervision. That space provides comfortable surroundings for normal (eutocic) births (Bertoni et al 2019c; Álvarez-Macías et al 2020; Napolitano et al 2022e; Rodríguez-González et al 2022a). Researchers have verified that calving occurs in response to a cascade of hormonal and physiological actions that generate significant behavioral and morphological changes in the dams. Those events alert trained handlers who can identify that calving is imminent. In zootechnical production, calving is a "gold standard" because it represents the species' reproductive success. Newborn calves may be destined for milk or meat production, but their birth marks the beginning of a new curve of dairy production (Mota-Rojas et al 2019b, 2022e). Therefore, any sign of difficulty in this period must be identified opportunely and monitored by personnel equipped with the necessary technological tools and trained to implement optimal handling strategies (Figure 1).

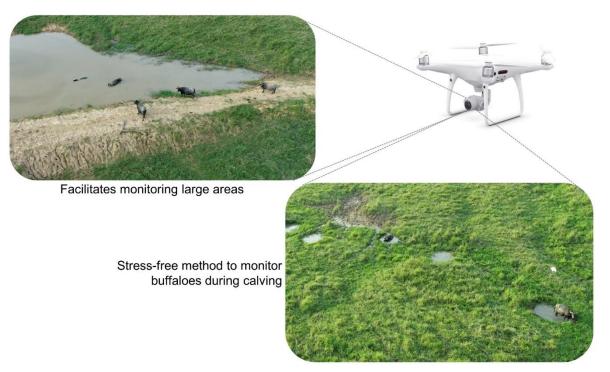


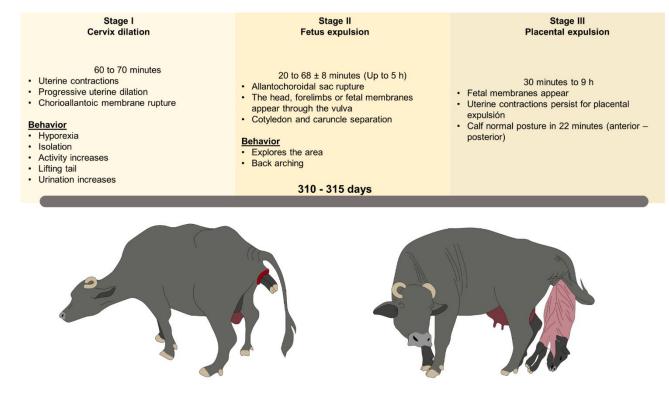
Figure 1 Example of the use of technological tools. In this case, drones monitor production areas with gestating and lactating female buffaloes, others near calving, or fattening animals. These methods minimize human-animal contact during naturally stressful situations, such as calving.

Births are diagnosed as either normal/eutocic or abnormal/dystocia (Napolitano et al 2022e). Normal births are divided into three stages, according to their duration: a) prepartum (60-70 minutes); b) expulsion of the neonate (68 ± 8 minutes); and c) expulsion of fetal membranes (30 min-9 h) (Napolitano et al 2020b; Mota-Rojas et al 2020c, 2022d; Rodríguez-González et al 2022a). The initial stage of calving is marked by uterine contractions, accompanied by the progressive dilatation of the cervix and the correct positioning of the fetus in the birth canal. Contractions may begin 2-3 days before the birth process itself begins. At that point, some indicators are largening of the udder, edematization of the vulva, and signs of restlessness as the female isolates or distances herself from the rest of the herd, a clear signal of the approaching birth. Increased miction may be observed 2 hours before the onset of the prepartum period (Mota-Rojas et al. 2019c). These events are followed by the secretion of fetal cortisol that triggers placental enzymatic synthesis to obtain estrogens, oxytocin, and prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ). These substances act on the actin-myosin system to generate contractions and modify the aggregates of collagen fibers. This triggers regression of the corpus luteum (Young et al 2011; Mota-Rojas et al 2020c), which is associated with behavioral changes like back arching,

flexing of the hocks, and tail-raising or bulging of the base of the tail to relax the pelvic ligaments (Das et al 2013; González-Lozano et al 2020; Napolitano et al 2022e) (Figure 2).

During the expulsion phase of the fetus, abdominal contractions continue, leading to the rupture of the allantochoroidal sac and the release of large amounts of oxytocin (González-Lozano et al 2020; Rodríguez-González et al 2022a). The third stage of calving begins once the fetus has been expelled. In this period, the placenta is expelled, and involution of the uterus occurs (Mota-Rojas et al 2019c). Careful monitoring of each female is essential in this phase to opportunely identify possible placental retention, a problem that occurs in around 23% of births in this species (Napolitano et al 2020b; González-Lozano et al 2020).

Dystocia is calving that exceeds the normal times and/or involves complications that require human intervention, including physical manipulations or even surgical procedures (González-Lozano et al 2020; Napolitano et al 2022e). The incidence of dystocia in water buffaloes is low, around 2% of cases (Napolitano et al 2020b). In Mexico, specifically, Rodríguez-González et al (2022a) identified a rate of just 1.6% of abnormal births in female buffaloes of the Buffalypso breed on dual-purpose production units. Labor dystocia can be caused by factors associated with the dam or the neonate, so it is important to observe individual dams to detect physiological or behavioral anomalies. Once identified, an anomaly must be treated quickly and efficaciously, as this is the only way to increase the possibility of survival of the mother and calf and prevent the development of diseases related to the cause of the problem (Dubey et al 2018; Mora-Medina et al 2018b; Mota-Rojas et al 2019a; González-Lozano et al 2020) (Figure 3).

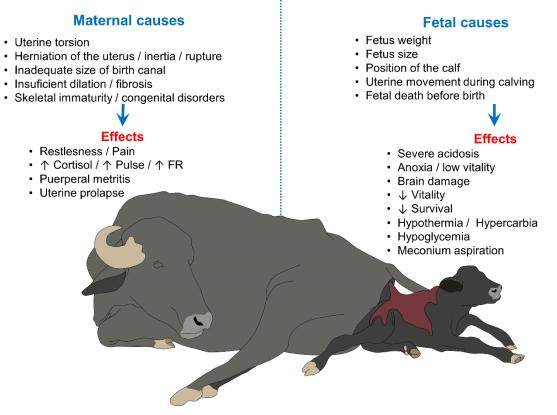


**Figure 2** Eutocic calving with the specific characteristics observed in each phase. Once the fetal hypothalamus reaches maturation, signals are sent to the neurohypophysis for the release of  $PGF2\alpha$ , oxytocin, and relaxin. This paves the way for dynamic uterine stimulation and excitation of the myometrial cells that act on the muscular relaxation and contraction necessary to dilate and expel the fetus and placenta.

The most common maternal cause of dystocia is uterine torsion, which accounted for 83% of all anomalous births in a herd of female Murrah buffaloes (Srinivas et al 2007). This condition starts at the end of gestation, causing severe vascular compression (Mota-Rojas et al 2008, 2022h). It has been suggested that it is caused by macrosomic products and/or a low amount of amniotic fluid in relation to the size of the fetus (Noakes et al 2009; Taverne and Noakes 2019; González-Lozano et al 2020; Mota-Rojas et al 2022d). This type of dystocia negatively impacts the mortality rates of dams and their offspring in proportion to the degree and duration of the condition. It is crucial to provide timely human intervention to prevent hemorrhagic infarctions and ruptured blood vessels (Noakes et al 2009; Tejpal et al 2017; Mota-Rojas et al 2022d). Other maternal causes include pelvic anomalies, vaginal/uterine/ vulvar neoplasias, vaginal or vulvar hypoplasias, sacral dislocations, vaginal cystocele, poor cervical dilatation, and factors like age, inadequate handling, and the mother's genotype. In all cases, obstruction of the birth canal occurs (Mota-Rojas et al 2019c; Napolitano et al 2020b; González-Lozano et al 2020).

In addition to maternal causes, studies have described dystocia of fetal origin. These are usually manifested in prolonged birth processes that exhaust both mother and fetus and reduce the neonate's vigor. For the calf, this represents difficulty standing up and reaching the mother's udder to ingest colostrum. This, in turn, decreases its energy and immunoglobulin intake, which, in the long term, will retard the calf's development. If exhaustion or intense pain occurs, the mortality rate of newborns can increase, mainly if no human attention or intervention is provided (Mota-Rojas et al 2020c; Napolitano et al 2022b, d).

The position of the fetus during expulsion is another factor that can cause dystocia. This condition has an incidence of 4% (Holland et al 1993), mostly when the fetus adopts a posterior dorsal position (72.8% of cases), has unilateral flexion of the shoulder or carpus (11.4%), is in a caudal position (8.2%), or has a flexed head (2.5%), or hocks (1.9%) (Holland et al 1993; Napolitano et al 2020b). During dystocia, these problems trigger an emergency situation when all methods of extracting the fetus have been tried but failed. Those scenarios require physical manipulations that may reach the extreme situation where extracting the fetus requires a surgical procedure called cesarean section. This demands taking decisions concerning survival rates, effects on production curves, and other key economic impacts (Mota-Rojas et al 2022d).



**Figure 3** Principal maternal and fetal causes of dystocic births in female water buffaloes that require monitoring and, when necessary, the support or intervention of a responsible veterinarian.

### 3. Perinatology: the neonate and thermoregulation

When the birth process concludes, the calf experiences a sudden temperature decrease due to the change from the intra- to the extrauterine environment that demands a response by the nervous system to prevent rapid heat loss to values below the animal's thermoneutral zone (Sevegnani et al 2016; Ewart 2020; Mota- Rojas et al 2020; Lezama-García et al 2022a). Several processes exist in neonates for heat production and maintenance of their thermostability. First, they receive information from the thermoreceptors in the dermis. Specifically, transient potential receptors TRPM8 and TRPA1 are activated and send signals along primary sensory fibers to the brain when the ambient temperature falls between 27 and 17°C. This condition can trigger other responses, such as intensified muscular activation -shivering- or the production of metabolic heat through the degradation of brown adipose tissue (BAT). This involves brain structures like the hypothalamus's preoptic area (POA) and the lateral parabrachial nucleus, dorsomedial hypothalamus, raphe pallidus, and motor neurons in the spinal cord. Likewise, thermoregulation in the neonate activates BAT, where POA neurons play a key role (Zhang et al 2011; Almeida et al 2015; Lezama-García et al 2022b; Mota-Rojas et al 2022i; Napolitano et al 2023). Thus, a response coordinated by the hypothalamus produces peripheral vasoconstriction through the action of adrenaline and noradrenaline, modifying the caliber of the surface capillaries to prevent heat exchange

and favor the conservation of the core temperature of central organs like the brain. This prevents a temperature drop from exceeding the calf's heat-producing capacity (Lezama-García et al 2022a). These data suggest that the neonate's thermoregulation mechanism is based on a deviation from its thermoneutral zone that enters into action to prevent heat loss through radiation.

Shivering thermogenesis is another facultative mechanism that combats heat loss under low ambient temperatures. Heat production is triggered as a response to cold, beginning with the action of neurons in the dorsomedial hypothalamic nucleus (DMH) that stimulate the pale raphe (RPa) to send signals to sympathetic preganglionic neurons in the intermediolateral cell column (IML), which increase muscular contractions (Morrison and Nakamura 2019; Lezama-García et al 2022a) (Figure 4).

When cold ambient temperatures are detected, TRPM8 and TRPA1 thermoreceptors are activated, triggering a neuronal response in the spinal cord's dorsal root ganglion (DRG) and cerebral structures. Next, the thermoregulatory center (POA) receives signals from the lateral parabrachial nucleus (LPB). The POA has connections to the dorsomedial hypothalamus (DMH), which, in turn, is connected to neurons of the pale rostral raphe (rRPA) and intermediolateral nucleus (IML). Two responses are produced in the spinal cord through sympathetic efferents: vasoconstriction with heat retention and the sympathetic release of norepinephrine on adrenergic receptors of BAT to produce heat. Shivering is another heat-generating process, but it depends on somatic motoneurons in the spinal cord.

Another mechanism is non-shivering thermogenesis. In this phenomenon, metabolic heat is generated by consuming BAT, an energy-rich tissue whose catabolism is mediated by the norepinephrine release in the nerve endings, which acts on uncoupling proteins-1 (ucp-1) present in mitochondrial adipocytes. They separate ATP synthesis from obtaining heat while simultaneously stimulating fatty acid oxidation and playing a key role in neonates' adaptation to cold temperatures in the new, extrauterine environment they confront (Lezama-García et al 2022b).

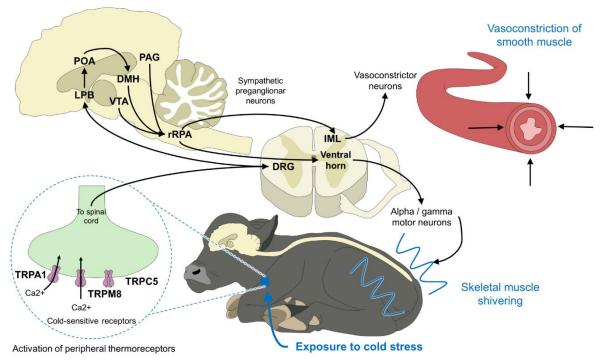


Figure 4 Neonatal thermoregulatory mechanisms in the water buffalo under hypothermic conditions.

It has also been suggested that the body weight of the newborn and the birth process (eutocic or dystocic) impact its thermoregulatory capacity, as there is greater heatproduction per unit of body weight and a smaller surface area in relation to weight. In this regard, a study of 109 calves of different weights by Napolitano et al (2023) concluded that low-weight neonates (Q1=37.8- 46.3 kg) are more susceptible to hypothermia than high-weight calves (Q4= 56.4- 60.3 kg), due to the scarce energy reserves provided by fats. Their findings led the authors to emphasize monitoring temperatures and colostrum ingestion to promote calves' thermoregulation mechanisms as a fundamental aspect of the zootechnical management of neonates (Napolitano et al 2022b, 2023).

The same authors further observed that low-weight neonates presented lower colostrum ingestion than mediumand high-weight calves. This suggests the need to administer foods that help those newborns obtain the energy, protein, vitamin, hormone, and immunological reserves they require to activate their thermoregulatory mechanisms, compensate for passive immunity, and promote vitality and the maintenance of an adequate state of health and development (Guzmán and Olivera-Angel 2020; Napolitano et al 2020b, 2022b; Rodríguez-González et al 2022a; Mota-Rojas et al 2022i). In contrast, low colostrum availability, inadequate ingestion, poor-quality colostrum, and inadequate handling of this food increase calves' morbidity and mortality rates (Barry et al 2019).

These factors also affect the quality of the immunoglobulins in the colostrum by modifying their abundance and bioavailability (Napolitano et al 2022b). In water buffaloes, immunoglobulin G (IgG) (the most abundant type of immunoglobulin in the colostrum of ruminants) is essential for activating response mechanisms to the presence of viruses and bacteria. IgA are necessary for protection against foreign substances in the organism. The IgM type is responsible for responding to infections, so the importance of their ingestion by neonate buffaloes is obvious (Dang et al. 2009; Napolitano et al 2020b, 2022b). In addition, it is essential to provide calves with good-quality colostrum during the first hours of life because the intestinal mucosa undergoes morphological changes in the first 24-36 h postpartum that limit its permeability and absorption (Murray and Leslie 2013; Mora-Medina et al 2018b; Villanueva-García et al 2021).

If perinatal care is performed inadequately, diseases can affect the neonate, causing death in extreme cases. It is well known that the factors which affect dams vary according to the handling of the herd. Still, the main causes of complications are the incidence of dystocia, premature or

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twin births, and macrosomic fetuses in which a substantial difference exists between the diameter of the mother's pelvis and the size of the fetus (Martínez- Burnes et al 2020, 2021). Concerning neonates, in addition to null or deficient monitoring of the birth process, they can die from hypothermia (cause of 10-20% of deaths), neonatal hypoxia that affects their ability to suckle, low birth weight, fatigue from an anomalous birth and/or deficient colostrum consumption (Osilla and Sharma 2019; Mota-Rojas et al 2021e).

The incidence of stillbirths is of multifactorial origin. Some causes have been attributed to characteristics of the fetus due to factors like size, weight, and nutrition. Others involve the mother; for example, the number of births, duration of gestation, genetic factors, and dystocic births. But variables related to the environment and seasonality may also constitute risk factors requiring preventive measures, careful handling, and a safe environment with minimal stressors. It is essential to monitor dams throughout calving and thoroughly analyze the risk factors associated with the incidence of stillbirths and neonatal morbidity and their ginecobstetric consequences (Mota-Rojas et al 2022g).

### 3.1. Imprinting

The calf's first extrauterine contact is with the mother, who immediately becomes its principal source for learning the behaviors necessary for development, and about the physical environment and social conduct. The bond established is called imprinting (Mora-Medina et al 2018b; Orihuela et al 2020), a selective, bilateral bond for learning. Imprinting is associated with the activation and action of neurological mechanisms that promote maternal care (Mota-Rojas et al 2021c; Orihuela et al 2021a). Behaviors in the first hour of life are known to occur during a susceptible period. These include grooming that promotes the mother-calf bond and involves the senses of sight and smell, licking, and vocalization. Imprinting also affects the newborn's thermoregulatory capacity because by cleaning and drying its hide, the mother's actions prevent a sudden drop in surface temperature (Mota-Rojas et al 2020d, f; Orihuela et al 2020; Napolitano et al 2022e) (Figure 5).



**Figure 5** Formation and maintenance of the mother-calf bond. A. Birth of a female buffalo showing the sensitive period for acquiring information from the environment through somatosensorial stimuli. B. Mutual recognition associated with cerebral plasticity involves tactile, visual, auditory, and olfactory stimuli, lasting from a few minutes to a few hours postpartum. C. This bond prevents the development of social and behavioral problems in water buffalo calves.

During imprinting and the sensitive period, the calf acquires knowledge of the physical environment through sensory stimuli and mutual –mother-calf– recognition that depends on cerebral plasticity. Human intervention during this sensitive period can negatively impact imprinting by rupturing this bond and triggering social and behavioral problems in the neonate that may include maternal rejection, which would limit the crucial care and attention she provides, such as providing the colostrum and milking her calf requires for survival and correct growth (Madigan et al 2006; Orihuela et al 2020). The sensitive period is also marked by the opening of a channel of sensory communication that participates in the development of social familiarity. All the animals' senses are involved in this period, including tactile imprinting, which requires physical contact between the mother and calf. For example, by licking her calf, the mother stimulates respiration, blood circulation, and the expulsion of feces and urine. At the same time, her ingestion of the fetal membranes (placentophagy) reduces postpartum pain (Mota-Rojas et al 2020f).

Olfactory imprinting takes place through the capture of volatile chemical substances by G protein-coupled receptors on the principal olfactory epithelium and vomeronasal organ (VNO), which send signals to the olfactory bulb and paraventricular (PVN) and supraoptic nuclei (SON) for oxytocin synthesis and the storage of information in the medial portion of the amygdala (MPOA), actions that stimulate emotional responses to external stimuli (Orihuela et al 2020, 2021a; Rodríguez-González et al 2022a; Mota-Rojas et al 2022a). The goal of visual imprinting is mutual recognition. This interaction projects visual stimuli through the optic nerve to the occipital lobe (LOcc). Those signals are decodified in the lateral geniculate nucleus (LGN) for transformation into physical visual characteristics (Orihuela et al 2020). Auditory communication, meanwhile, produces recognition based on vocalizations with auditory patterns. This process is bidirectional, with participation by an emitter and a receptor of recognizable patterns (Orihuela et al 2020; Rodríguez-González et al 2022a). These sounds are emitted and projected to the auditory thalamus to activate signaling to the PVN and auditory cortex (Mota-Rojas et al 2021c; Orihuela et al 2021b, a).

Descriptions of the mother-calf bond formation also require an endocrine control, including estradiol, progesterone, glucocorticoids, prolactin,  $\beta$ -endorphin, and dynorphin, together with neurotransmitters like epinephrine, dopamine, norepinephrine, gamma-aminobutyric acid glutamate (GABA), and oxytocin. In addition to favoring uterine contractions and the development of maternal behavior during calving, these substances all play essential roles in lactation and milk ejection (Orihuela et al 2020; Mota-Rojas et al 2022e; Napolitano et al 2022e; Rodríguez-González et al 2022b).

## 4. Importance of the calf in milk production in dual-purpose production systems

During the first lactation and milking of female buffaloes, the calf is often present during manual milking due to its somatosensorial stimulation of the dam that is necessary for releasing oxytocin into the bloodstream during milk ejection. In this activity, the calf impacts its mother in several ways -visual, tactile, auditory, olfactory- to contribute to emptying the alveolar milk and mammary gland (MG) (Napolitano et al 2013, 2022a; Mora-Medina et al 2018a; Costa et al 2020; Bertoni et al 2022c; Rodríguez-González et al 2022a). The presence of the calf during manual milking also reduces the stimulation time required for milk ejection and, therefore, total milking time (the activity that requires the largest time investment in water buffalo production units). An additional benefit is that the calf's presence lowers the incidence of mastitis, a disease that develops when milk remains in the MG due to deficient

ejection (Espinosa et al 2011; Mota-Rojas et al 2019b, 2020e; Bertoni et al 2020b, 2022a; Napolitano et al 2022e).

These benefits for milk ejection capacity and time on dual-purpose production systems are complemented by the fact that the calf's participation positively affects its growth and development while also preventing manifestations of abnormal mechanisms associated with the premature separation from its mother (Mora-Medina et al 2018b). The newborn participates in diverse routines during milking and, in logistical terms, passes much of the day with its mother. The rest of the time is spent among her congeners of similar age that eventually will be identified as replacement females or animals destined for partial fattening or slaughter.

Another critical aspect of buffalo production is zootechnical management. In this regard, Bertoni et al (2022) identified the social and zootechnical aspects with a nutritional, sanitary, productive, reproductive, and genetic approach to managing dual-purpose water buffaloes in Mexico. The study aimed to characterize the previously mentioned levels and provide recommendations and trends to direct investigations in this field.

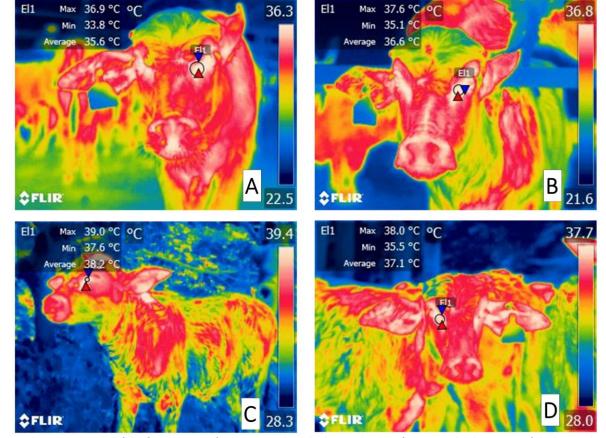
On the other hand, research focused on the methods and practices applied in each stage of the transport or movement of animals from one corral to another, one pasture area to another, or out of the production unit can trigger thermal, behavioral, and physiological problems, especially when efforts are not taken to minimize handling. Moving these animals for zootechnical procedures often induces adverse effects like stress, pain, and anxiety in calves and buffaloes in different stages of development. In this field, Rodríguez-González (2023) evaluated 81 calves divided into two groups: fattening 1 (n=45, less than 2 months of age), and fattening 2 (n=36, over 2 months of age). The animals showed changes in dermal microcirculation in six thermal windows (lacrimal caruncle, periocular lacrimal gland, auditory canal, muzzle, and the thoracic and abdominal regions) during the phases that Buffalypso calves routinely go through during such normal activities as resting in the lairage corral with or without herding or handling before milking, during milking, and post-milking, in the paddock, and while being herded. That study concluded that factors associated with handling, like herding, increased surface temperatures in all the thermal windows examined. At the same time, observation showed that animals in the lairage pen without prior herding had the lowest temperatures, possibly influenced by environmental factors, the hour when readings were taken, and the availability of natural shade for the calves (Figure 6).

This emphasizes the need to ensure adequate handling when moving animals inside the production unit because specific characteristics of the water buffalo –such as low hair density, thick epidermis, and high melanin concentration, coupled with scarce sweat glands– make this species susceptible to thermal stress in humid tropical regions of Mexico. Suppose these features of buffaloes are not considered. In that case, the consequences can include severe economic losses due to low food consumption that decreases the growth rates of calves in the early stages of life (Napolitano et al 2018b; Mota-Rojas et al 2019b, 2022i; Bertoni et al 2020b, c, d).

## 5. The female dairy buffalo: anatomy, physiology, behavior, production, and milking

Lactogenesis is the process of milk production. It occurs in the mammary gland, which is classified as the exocrine type. The function of the MG is to synthesize, store, and secrete milk during lactation (Olmos-Hernández et al 2020). In buffalo herds in Mexico, lactation lasts around 240-270 days (Vázquez-Luna et al 2020; Rodríguez-González et al 2022a). The onset and course of lactation are determined by physical and hormonal stimuli. One particularity observed in the anatomy of the MG of buffalo dams is the ratio between the cisternal (3-5%) and alveolar (92-95%) regions where milk is stored. This feature has productive advantages and disadvantages; for example, female buffaloes require prolonged stimulation (tactile, visual, auditory, and olfactory) to activate the receptors in the epidermis of the inguinal canal that send signals to the dorsal roots of the spinal cord that, in turn, stimulate the hypothalamus to release oxytocin from the posterior lobe of the hypophysis. Oxytocin travels through the bloodstream to the MG. Upon entering this organ, it activates receptors in the alveolar myoepithelial cells that make them contract and release the milk from the alveolar tissue and mammary conducts. In this process, alveolar milk is ejected into the cisternal compartment and teats (Lollivier and Marnet 2005; Espinosa et al 2011; Bertoni et al 2020b; Ghezzi et al 2020; Mota-Rojas et al 2020d; Olmos-Hernández et al 2020; Napolitano et al 2022e). Finally, active ejection is necessary to empty all the milk from the alveolar fraction and prevent sub-milking that can increase susceptibility to mastitis (Olmos-Hernández et al 2020; Mota-Rojas et al 2022f).

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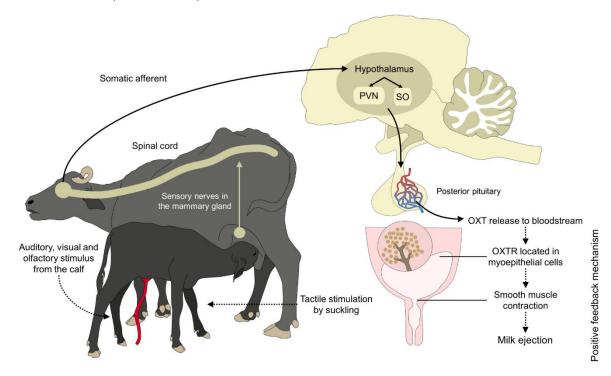


**Figure 6** Radiometric images of the facial region of calves moved inside the ranch. Identification and delimitation of the lacrimal caruncle, an area that makes it possible to evaluate the radiation emitted by the infraorbital artery. This artery has sympathetic branches of the facial nerve. A. A calf in the lairage pen without prior herding (temperatures: max. =  $36.9^{\circ}$ C, min. =  $33.8^{\circ}$ C, average =  $35.6^{\circ}$ C) vs. B. a calf during milking (temperatures: max =  $37.6^{\circ}$ C, min. =  $35.1^{\circ}$ C, average =  $36.6^{\circ}$ C). In comparison, case C shows an increase in temperatures of the lacrimal caruncle: a calf on a trail during mixed herding (temperatures: max. =  $39.0^{\circ}$ C, min. =  $37.6^{\circ}$ C, average =  $38.2^{\circ}$ C); and D. a calf in the lairage pen after herding from the paddock (temperatures: max. =  $38.0^{\circ}$ C, min. =  $35.5^{\circ}$ C, average =  $37.1^{\circ}$ C). The conclusion is that marked surface temperature increases were observed in the lacrimal caruncle after mobilization, compared to those in the resting condition (lairage pen and during milking).

Studies have described several productive advantages related to the anatomy of the female buffalo's MG, such as longer teats ( $7.01\pm0.17$  cm anterior,  $8.33\pm0.22$  cm posterior), a thicker muscle layer, and a narrower sphincter, and higher

keratin level s These features protect the MG from the entrance of infectious microorganisms. As a result, research in Mexico reports lower incidences of mastitis in water buffaloes, both clinical and subclinical, than in dairy cattle, though it is still necessary to take preventive measures and apply strategies to prevent the development of mastitis through timely veterinary medical attention (Bertoni et al 2020d; Napolitano et al 2022c, a; Rodríguez-González et al 2022b, a).

Regarding physiology and the hormonal changes associated with milk production and the MG of the water buffalo, reports suggest that they respond to hormonal modifications related to luteinization and ovulation triggered by the action of hormones like prolactin and the growth hormone (secreted by the hypophysis gland). These hormones are necessary for milk synthesis but are complemented by others. Cortisol, for example, is secreted with oxytocin, two biomolecules that are produced and then released into the bloodstream during milking and lactation (Bertoni et al 2022g; Mota-Rojas et al 2022f; Napolitano et al 2022e, a). Anatomically, the alveolar tissue contains lactocytes that are responsible for milk secretion. They are encapsulated in connective tissue that forms the mammary lobules, which are fundamental elements in the formation of the mammary lobes through which milk passes into the milk ducts for ejection through the excretory ducts of the MG (Olmos-Hernández et al 2020) (Figure 7).



**Figure 7** Neurophysiology of milk ejection. During active stimulation of the MG, signals are received in the SON (supraoptic nucleus) and PVN (paraventricular nucleus of the hypothalamus) at in the brain. This triggers the release of oxytocin (OXT) and its action on OXT receptors (OXTR), which travels in the bloodstream to the MG to induce milk ejection.

Proper milk ejection ensures adequate milking, an essential operation in the productive life of dairy buffaloes. Lactation in these animals is influenced by genetic factors and handling, both of which can directly impact milk yields (Bertoni et al 2020b; Mota-Rojas et al 2020d; González-Lozano et al 2020), so it is essential to determine the goals to be achieved, and then plan and implement adequate practices and procedures during lactation and, above all, in the zootechnical activity of milking. The personnel in charge of milking must receive continuous training to provide an environment that prevents the presence of harmful stimuli that could trigger the synthesis and secretion of adrenaline. This hormone produces vasoconstriction in structures of the MG that reduce oxytocin secretion during milking by impeding the contraction of the myoepithelial cells and active milk ejection (Bertoni et al 2020a; Mota-Rojas et al 2020d; Olmos-Hernández et al 2020). Handlers must also consider eliminating harmful external stimuli like excessive noise due to deficient maintenance of milking rooms and machines,

abrupt or continuous changes in the milking room, rotation of personnel, and insufficient training. Generally speaking, when handlers do not treat the female buffaloes well, the animals feel discomfort that can trigger an alert state which negatively affects yields due to milk retention in the MG, a condition that can compromise the welfare of female buffaloes (Polikarpus et al 2014; Bertoni et al 2020b; Olmos-Hernández et al 2020)

As mentioned above, the presence of the calf at her mother's side activates oxytocin secretion and release into the bloodstream, but this can also be stimulated by zootechnical practices like massaging, washing, and drying the udder prior to milking, activities that can favor active milk ejection (Borghese et al 2013; Boselli et al 2014; Mota-Rojas et al 2020e).

When these steps are not followed during milking, the risk that the buffaloes could develop mastitis increases. Mastitis is a common pathology that causes enormous economic losses in dairy production units. In addition, poor milking practices and deficient hygiene in the installations and among handlers favor the entrance of pathogens like *Staphylococcus aureus, Streptococcus agalactiae, S. uberi, Escherichia coli, Klebsiella pneumoniae,* and *Arcanobacter piogenes* into the MG (Fagiolo and Lai 2007; Akhtar et al 2012; Mota-Rojas et al 2019a; Bertoni et al 2020d).

The milking capacity of female buffaloes is influenced by multiple factors. Still, it is also related to the lactation curve, which represents the four phases of milking: ejection, plateau (constant milk flow), decrease, and the blind period when the MG is emptied completely (both the alveolar and cisternal fractions). During these phases, exogenous oxytocin is often administered (especially when mechanical milking is used) because it is not feasible to have the calf at its mother's side, perhaps due to the productive organization of the ranch or space limitations in the installations of the milking room. Applying exogenous oxytocin increases dairy production (by 3-11.6%). Still, it is important to emphasize that it may negatively alter productive and reproductive parameters by reducing the proportions of fat, total solids, magnesium, iron, zinc, protein, and lactose in the milk and prolonging the anestrus period and uterine involution. These latter two changes are observed in the long term, so implementing this practice is very common in buffalo production units (Mota-Rojas et al 2022f; Napolitano et al 2022f, e, a).

## 6. Water buffaloes and meat production, *pre-* and *postmortem* factors that influence the physicochemical characteristics of meat

The facts that buffalo calves reach a weaning weight of around 240 kg thanks to daily weight gains of approximately 0.5 kg and that meat yields at slaughter are around 58% make this species attractive for meat production (Naveena and Kiran 2014; Guerrero-Legarreta et al 2022; Naveena et al 2022). During this productive process, buffaloes develop through several phases and operations to maximize the amount of meat eventually obtained. However, the procedures involved are subject to pre- and postmortem problems that can affect the physicochemical characteristics of the meat. These procedures include transport, stunning, slaughtering, postmortem enzymatic reactions, refrigeration, and maturation. If these successive stages are not managed through well-planned schemes, then production parameters may be modified or altered, generating low yields or deficient characteristics in the commercial or sanitary quality of the final products (Strappini 2012; Fabio et al 2020; Mota-Rojas et al 2020a; Alarcón-Rojo et al 2021). Transport of animals to the abattoir. Once they are judged to have completed the fattening process, it is a crucial operation because numerous problems associated with the environment and the handling of the buffaloes can appear during mobilization. Transport is also deemed a particularly stressful event in the productive life of animals, for even gentle handling can trigger thermal, metabolic, and neurological responses when animals perceive certain aspects of their surroundings as challenging or threatening (Cruz-Monterrosa et al 2020a; GuerreroLegarreta et al 2022; José-Pérez et al 2022; Rodríguez-González 2023).

For this reason, ample research in Mexico has described and analyzed the factors that generate stress in buffaloes during road transport (the most common method used in the country), applying methodologies that make it possible to identify and evaluate stress levels. Some studies have measured blood cortisol, protein, and glucose levels. Still, another approach that diverse research groups have adopted is using tools or evaluations that do not require direct interaction with in-transit animals. Because they are non-invasive, these techniques do not aggravate the stress induced by the handling necessary to draw, for example, blood samples prior to, during, and/or after transport. One of the most important tools for assessments of this kind is infrared thermography (IRT). As many studies have this demonstrated, technology permits measuring parameters associated with the stress levels that animals experience under diverse, challenging conditions. These working groups have analyzed existing reports on the frequency of lesions that bovine cattle suffer on foot, during transport, and in the canal to prepare comparative studies of these aspects that have not yet been examined in detail in the water buffalo (Cruz-Monterrosa et al 2020b; Bertoni et al 2022g; Guerrero-Legarreta et al 2022; José-Pérez et al 2022; Mota-Rojas et al 2022b) (Figure 8).

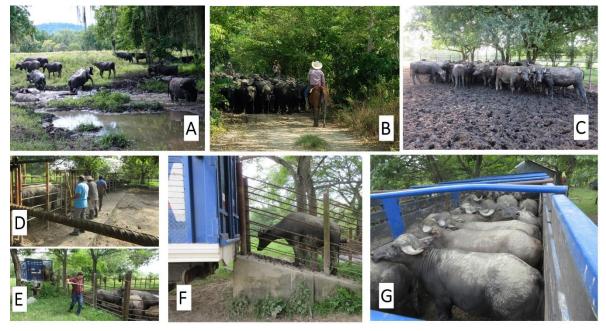
In this area of study, Mota-Rojas et al (2022b) described that IRT makes it possible to observe the effect of peripheral vasodilatation mechanisms in buffaloes transported on short trips as a means of determining the phenomenon of heat dissipation in animals. Those researchers recorded temperature increases in buffaloes' facial and frontoparietal regions, areas that can be considered thermal windows. Rodríguez-González et al (2023) used IRT to evaluate the facial and corporal thermal windows of 624 buffaloes transported for short trips from the paddock through unloading. They found that temperature increases occurred in all the windows assessed during the activities that involved greater human-animal interaction (herding, loading, unloading). Their analysis identified the parieto-frontal window (18.2°C±0.1) as the one that presented the most marked thermal changes (p<0.0001), while the smallest changes were observed in the nasal window (6.8°C±0.4).

A complementary area of interest in the analysis of transport and the degrees of stress that animals experience refers to the incidence of lesions during this activity since this is another indicator of animal welfare. The categories of lesions identified include abrasions, ulcerations, bleeding and penetrating wounds, lacerations, and swelling with hyperkeratosis (José-Pérez et al 2022). Studies in this field have detected the corporal areas that are most susceptible to injury due to the anatomical characteristics of the buffalo and the handling customarily used. The regions identified as suffering the most common lesions are the dorsal and lumbar areas, buttocks, hips, and muzzle. The main cause was identified as the type of transport vehicle used because most trucks are designed to carry conventional cattle (*Bos*) and are poorly-adapted for transporting buffaloes.

Three aspects have been determined as contributing to the incidence of lesions in water buffaloes: anatomical characteristics like narrow bony projections in the lumbar zone and transversal processes, the fact that they are slowmoving compared to bovines, and the type of herding used by stockperson who are rarely well-trained in handling this species, so they use physical and electrical tools in an attempt to speed up loading and unloading. Sadly, lesions frequently occur in buffaloes on foot or in the canal and, in proportion to their type, location, and degree of severity, can affect the appearance and commercial quality of the meat produced. The conditions described above mean that producers must take decisions regarding the final product and consider feedback during the premortem stages to reduce the incidence of lesions (Chandra and Das 2001; Alam et al 2010; Naveena and Kiran 2014; Cruz-Monterrosa et al 2020a; José-Pérez et al 2022; Rodríguez-González et al 2022a).

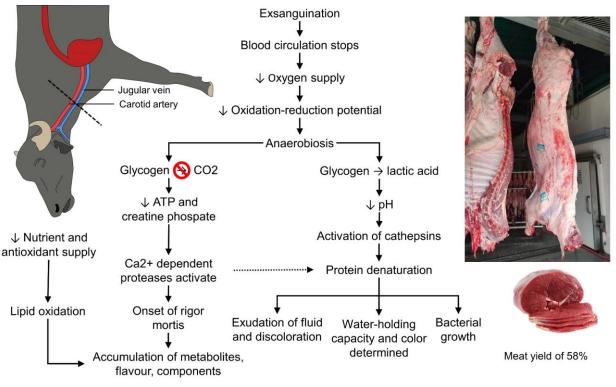
At the end of the transport, the buffaloes arrive at an abattoir for slaughter. In this stage, it is important to ensure that pre-slaughter stunning is performed correctly to induce loss of brain function and unconsciousness. This reduces pain and suffering in the subsequent steps of exsanguination and humanely-induced death (Mota-Rojas et al 2022). Teams of Mexican researchers who have studied these processes describe diverse aspects that can compromise the welfare of buffaloes. These include applying tools like captive bolt pistols that are designed for bovines. When applied to buffaloes in inadequate locations —like frontal positions those pistols often cause severe brain lesions but do not achieve complete stunning or desensitization in buffaloes. One alternative is to apply the pistol to the depression in the buffalo's occipital bone, ventral depression, or the insertion points of the nape to obtain the best results (Napolitano et al 2020; Mota-Rojas et al 2020a, g, 2021b; Naveena et al 2022).

As the information provided in this review suggests, if each phase prior to slaughter, the obtention of the final meat products, and the areas of opportunity detected to date are subjected to rigorous study, it will be possible to propose recommendations that can improve the physicochemical characteristics of buffalo meat and its derivatives. This is especially important because, under adequate conditions, buffalo meat has significant nutritional, physicochemical, and organoleptic values (Cruz-Monterrosa et al 2020a; Guerrero-Legarreta et al 2020).



**Figure 8** Stages and operations involved in mobilizing stock animals: pre-transport, transport, and post-transport. In all three phases, thermographic parameters have indicated that buffaloes experience stress. A. in the paddock; B. during herding; C. in the lairage pen; D. in the handling chute; E-F. during loading; G. transport.

The physicochemical characteristics of buffalo meat are closely related to pH values, so if normal values are modified (5.5-5.7), properties like flavor, aroma, color, and tenderness may be compromised. Changes in pH are often associated with situations of stress. In this regard, challenging environments that provoke chronic stress responses prior to sacrifice (mainly during transport) can trigger muscle and hepatic glycogen catabolism that causes a *postmortem* decrease of lactic acid concentrations in muscles. This condition impedes a decrease in pH during the conversion of muscle-to-meat, primarily during *postmortem* glycolysis, compromises the meat's desirable physical and functional properties, and can threaten food safety. Regarding the issues of food safety and acceptable hygiene of buffalo meat, an adequate concentration of lactic acid in the muscles provides some protection against contamination in the canal and can retard bacterial growth during the various stages of processing: butchering, packaging, labeling, and storage (Strappini 2012; Matarneh et al 2017; Cruz-Monterrosa et al 2020a) (Figure 9).



**Figure 9** Process for obtaining meat. Upon reaching slaughter weight, buffaloes are transported to the abattoir. When transport and exsanguination are performed adequately, anaerobic metabolism of the muscle occurs, followed by the event called *rigor mortis*, which leads to a decrease in pH values (to 5.5-5.7) in the meat. Finally, the action of calpains on filaments of myosin produces desirable modifications in the physicochemical characteristics of the meat.

In addition, if the processes prior to exsanguination are planned and carried out adequately by trained personnel, it is possible to obtain high-quality products, especially given the fact that buffalo meat has less cholesterol (9%), higher mineral content (10%), and 55-57% fewer calories than beef, as well as a higher percentage of proteins (10%) (Naveena and Kiran 2014; Domaradzki et al 2016). These properties position buffalo meat as a healthier food for human consumption (Cruz-Monterrosa et al 2020a; Guerrero-Legarreta et al 2020). But ensuring these properties requires gentle handling during production and transport and consideration of genetic and alimentary factors that can be gathered under the rubric of "good agricultural practices" and a concern for animal welfare, especially when it comes to the efficacy of stunning and slaughter. It is important, as well, to ensure that *postmortem* factors like refrigeration are performed correctly so that the cold chain is conserved throughout the processing, packaging, distribution, and commercialization of products. This suggests the need for additional research to guarantee the application of the good practices established, with effective supervision by official agencies based on current, applicable legislation.

## 7. Applicable legislation on the breeding, production, transport, and slaughter of water buffaloes

The current development of water buffalo units in tropical regions is a challenge that includes the complexity of the ecosystem to the deficient or null laws and regulations related to productive buffalo activity. Implementing livestock strategies has been observed to improve productive and reproductive characteristics (Álvarez-Macías et al 2022; Bertoni et al 2022f, e). However, it is necessary to establish and apply preventive and corrective measures to ensure the adequate breeding, production, transport, and slaughter of production animals. On this topic, it is important to point out that no consolidated, homologous international norms exist to regulate practices designed to ensure high animal welfare levels during general productive processes, much less for buffalo production. This means that the production chain of this species has not been subjected to modernization processes (Rodríguez-González et al 2022c,d). Although specific standards do not exist, at the international level, the World Organization for Animal Health (WOAH) has issued general guidelines to promote animal welfare through the diverse stages of production. This academic, scientific, and governmental effort, with contributions from the productive sector, holds that it is essential to ensure that animals' needs are satisfied to minimize biological and economic losses due to environmental and physical (handling, infrastructure) factors and the characteristics of each species of productive importance. Concerning the water buffalo, we are dealing with a species that can be profitable despite the challenges of climate change, but this demands ensuring their welfare by performing zootechnical practices that minimize the risks of trauma, disease, pain, fear, and stress (Otter et al 2012; OMSA 2021; Rodríguez-González et al 2022c). Unfortunately, at present, these guidelines are only recommendations, so compliance in producing countries is not obligatory.

In addition, the WOAH publishes and periodically updates the Terrestrial Animal Health Code (TAHC). Based on solid scientific findings, that document proposes the steps to be followed and the events to be considered during the transport of production animals (OMSA 2021). Other initiatives have emerged from private industry, where compulsory measures designed to eradicate poor practices have been established. These developments suggest that greater control and follow-up –both documental and physical– on the processes involved in each phase of the production chain, including transport (Lundmark et al 2014, 2018) and slaughter, are being achieved. They further manifest the importance of the contributions of civil society, the private sector, and final consumers.

In Latin America, specifically, it is clear that the governments of Colombia, Chile, and Argentina have decided to take the TAHC's recommendations as the basis for applying obligatory measures in each stage of the production, transport, and slaughter of water buffaloes. However, countries like Venezuela, a large water buffalo-producing nation, have not contemplated any integral concept of animal welfare applicable to this production species, so legal gaps exist that limit the possibility of conducting adequate inspections and verifications of the quality of buffalo products, even though this raises dangers and risks during commercialization and may jeopardize food safely due to the lack of effective systems for identifying, tracing and, when necessary, coordinating product recalls.

Referring to Mexico, efforts have been made to incorporate aspects related to animal welfare and ensure their application in production units into existing obligatory legal frameworks; however, no decrees in this regard have yet been issued or ratified. Documents like the Federal Animal Health Law and accompanying regulations (Ley Federal de Sanidad Animal y su reglamento) stipulate that the agencies' responsibilities include ensuring that producers procure the quality and innocuousness of their final products and ensure the welfare of their production animals. The Department of Agriculture, Livestock, and Rural Development (SADER, Secretaría de Agricultura, Ganadería y regulates good agricultural Desarrollo Rural) and manufacturing practices in all establishments dedicated to processing animals. However, the water buffalo is not mentioned in any related legal document (DOF 2018).

Even though the water buffalo is not considered specifically in existing legislation in Mexico, this species is not exempt from the controls imposed on transport during campaigns to eradicate diseases (e.g., NOM-041-ZOO-1995 on brucellosis and NOM-031-ZOO-1995 on tuberculosis). In addition, ranchers and shippers must follow the guidelines established in agreements to control, prevent, and eradicate ticks. Producers, therefore, require orientation to become familiar with the requirements for transporting animals on foot. Unfortunately, NOM-051-ZOO-1995, on humane treatment during transport, makes no mention of such important factors as maximum fasting times for large ruminants, so animals can be transported under conditions

that can severely compromise their welfare; for example, allowing a rest time only on trips that exceed 18 hours of travel time. When producers give animals food and water only under these conditions, they run the risk that excessive fatigue, dehydration, and inadequate transport conditions will cause diseases and facilitate their dissemination (DOF 1995a, b, 1996).

These conditions can provoke serious problems during the production and management of water buffaloes in Mexico since the biological structure of this species means that its welfare can be compromised when its zone of thermoneutrality is exceeded. The gaps in laws and regulation thus open areas of opportunity to incorporate contributions, observations, and findings from all the sectors that participate in this species –educational, social, public, and private– not just government agencies, to nourish the legal framework related to the water buffalo.

### 8. Final considerations

This review makes clear that (i) ranchers are increasingly deciding to adopt water buffalo production; and (ii) research on this species is a growing scientific interest. This interest reflects that this is a rustic animal that can utilize low-quality forage as an effective alimentation source. Water buffaloes can be raised for four zootechnical objectives in specific production units because it is a particularly flexible, efficient animals. These factors have led to a marked increase in the number of animals bred in Mexico. We have also seen that the morphology of the water buffalo has advantages and disadvantages; for example, females have a low proportion of dystocic births concerning eutocic births, and fewer problems with the survival of their offspring, compared to dairy cattle. Though all animals subjected to fattening may face challenging environments, if potentially negative stimuli are controlled through adequate training of the personnel involved and efforts are made to improve animal-human interaction, buffalo meat offers enhanced organoleptic and nutritional properties when compared to beef from traditional bovines.

It is necessary to implement non-invasive monitoring strategies with water buffalo herds using technological tools that enable opportunely detect problems and implement corrective handling actions to ensure the survival of mothers and their calves and to guarantee that adverse body temperatures do not compromise the buffaloes' zone of thermoneutrality.

While it is true that studies have been conducted to improve our understanding of the productive, reproductive, and behavioral aspects of the water buffalo in Mexico, as a novel species that can replace the production of traditional bovine cattle, it is necessary to design and carry out detailed research to increase our knowledge of the specific characteristics of this species and, above all, its potential productive benefits. Studies of this kind will also contribute to elaborating public policies that favor the welfare of this species throughout the production chain, from the farm to consumers' tables.

### **Ethical considerations**

Not applicable.

### **Conflict of Interest**

The authors declare that there is no conflict of interest with this work.

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