

Research Article



Impact of Oxytocin on Physicochemical Characteristics of Buffalo Milk at Different Lactations

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Abstract | Oxytocin is a hormone that is necessary for lactation and the production of milk. It has frequently been used in the dairy sector to boost the production of animal milk. The goal of this research was to find out how oxytocin affected the physicochemical properties of buffalo milk. Milk samples were collected from thirty lactating buffaloes, divided into a control group and an oxytocin-treated group, and further subdivided by lactation. The samples were analyzed for pH, specific gravity, ash, protein, total solids, fats, lactose, and moisture content. The results showed that oxytocin treatment significantly decreased pH levels in milk from the first lactation, while specific gravity did not differ significantly between the control and oxytocin-treated groups. Total solids, ash, and protein concentration were significantly higher in the oxytocin group, while lactose concentration significantly increased in all lactations of the oxytocin group. Higher milk production was also observed in the third lactation of the oxytocin group. This study provides new insights into the effect of oxytocin on the physicochemical parameters of buffalo milk. This indicates that oxytocin treatment can affect the milk biochemical parameters, along with total solids, ash, and protein concentration, as well as lactose concentration. However, the decrease in pH levels suggests that oxytocin treatment may also affect milk quality. To prevent oxytocin use in the dairy industry from lowering the quality of milk produced, it should be closely regulated.

Keywords | Oxytocin, Buffalo milk, Lactation, Specific gravity, Physicochemical parameter

Received | August 06, 2023; **Accepted** | September 02, 2023; **Published** | September 28, 2023

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Citation | Khan G, Soomro SA, Kabir A, Iqbal A, Marri NM, Khan SA, Khan MR, Rehman A, Khan MZ (2023). Impact of oxytocin on physicochemical characteristics of buffalo milk at different lactations. Res J. Vet. Pract. 11(3): 26-33.

DOI | <http://dx.doi.org/10.17582/journal.rjvp/2023/11.3.26.33>

ISSN | 2308-2798



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INTRODUCTION

Milk synthesis in lactating mammals relies on mammary epithelial cells (MEC) and their efficiency (Li et al., 2017). Milk proteins are synthesized in the rough endoplasmic reticulum using amino acids derived from feed and bacterial protein digestion or limited body pro-

tein catabolism (Neville et al., 2002). Calcium, phosphorus, magnesium, potassium, chloride, and salt are minerals found in milk that is derived from blood and kept in balance by active transport systems (McGuire & McGuire, 2015). Oxytocin is a hormone that plays a significant role in the regulation of milk ejection or milk let-down process in mammals, including buffaloes (Singh & Sharma, 2021).

In response to signs of sucking or milking, the hypothalamus produces it, and the posterior pituitary gland releases it (Singh & Sharma, 2021). When the myoepithelial cells surrounding the mammary gland's alveoli contract as a result of oxytocin, the mammary gland releases its milk into the ducts and cisterns (Kaur, Singh & Sandhu, 2018). In addition, oxytocin controls a range of physiological functions, such as social bonding, uterine contractions, and stress reactions (Carter, 2014).

In many places of the world, especially in Asia and Africa, buffaloes constitute a vital source of milk and meat. Due to their thicker and longer teats, slow milk ejection reflex, and tougher teat sphincter muscles, buffaloes are challenging to milk; as a result, oxytocin injections are utilized before milking in addition to calf suckling (Thomas, 2004). When it comes to buffaloes ejecting milk, oxytocin is crucial. By removing and expelling the gland with the aid of the oxytocin-mediated milk ejection reflex, lowering intra-alveolar pressure, and restoring normal mammary blood flow, oxytocin affects milk production (Belo & Bruckmaier, 2010). Breed, parity, lactation stage, and management techniques are some of the variables that affect the quality of buffalo milk (Khan & Khan, 2015).

According to reports, a buffalo's parity, or how many calves the animal has had, affects the milk's output and composition (An et al., 2023). However, little research has been done on how oxytocin affects the physicochemical properties of milk in buffaloes. Farmers in Pakistan are using exogenous oxytocin to increase milk output. Before milking, oxytocin is given intramuscularly at a dose rate of 10–20 IU. Animals' reproductive and productive abilities are declining as a result of oxytocin use without a veterinarian's prescription (Mustafa et al., 2008). Without considering its effects on the health and production indices of animals, exogenous oxytocin has been utilized in cows and buffaloes as a commonly administered medication before milking for rapid letdown and augmentation of milk production (Abbas et al., 2014). The purpose of the current study was to look at how oxytocin affected the physicochemical characteristics of buffalo milk. Additionally, the outcomes of this study might help veterinarians and dairy producers create more responsible and efficient oxytocin administration techniques.

MATERIALS AND METHODS

SELECTION OF ANIMALS, PHYSICAL ANALYSIS, COLLECTION AND STORAGE OF MILK SAMPLES

Thirty lactating buffaloes were selected from the vicinity of Tandojam for this study. The buffaloes were divided into three groups based on lactation age: 1st lactation (n=10), 2nd lactation (n=10), and 3rd lactation (n=10). Within

each lactation group, the buffaloes were further divided into a control group (without oxytocin, n=5) and an oxytocin group (n=5). Milk samples were collected aseptically from each buffalo by cleaning the udder and teats with tap water, followed by wiping them with a cloth soaked in 70% ethanol. The first strip of milk was discarded, and 15 mL of milk was then collected directly into a screw-capped sterile polyethylene tube. All milk samples were stored in an icebox to chill and then transported to the Laboratory of Animal Product Technology. The samples were stored at a refrigerator temperature of 4°C until further physicochemical analysis. Using the recommended approach, the physical characteristics of the milk samples were determined by the Association of Official Analytical Chemists (AOAC). The physicochemical analyses included the measurement of various parameters such as pH, specific gravity, ash, protein, total solids, fats, lactose, and moisture content.

DETERMINATION OF PHYSICAL AND CHEMICAL PARAMETERS

Milk's specific gravity (g/cm³) was measured with a pycnometer using the AOAC-recommended technique (2000). The pycnometer was weighed after being filled with regular distilled water. The milk sample was weighed after being filled in the pycnometer to the prescribed level and kept at the specified temperature. The following formula was used to get the specific gravity: The weight of the milk sample divided by the weight of distilled water multiplied by 100 equals specific gravity (g/cm³). A digital pH meter was used to measure the milk samples' pH. The ash content of milk was determined by the process described by AOAC (2000). Five grams of milk were placed in a crucible and heated to (550+250C) for 3–5 hours in a muffle furnace. The crucibles were placed in a desiccator for an hour with moisture absorbent after 3–5 hours. (silica gel). The crucibles were weighed once more, and the following formula was used to determine the ash content: Ash content (%) is calculated as (ash sample weight) / (sample weight) times 100. The amount of milk fat was calculated by the Gerber method according to AOAC. In a nutshell, 10 ml of 90% H₂SO₄ and 1 ml of amyl alcohol were added to 11 ml of milk in a butyrometer. It was then corked shut tightly and spun at 1100 rpm for 3 to 5 minutes in a Gerber centrifuge. Following centrifugation, the butyrometer scale was used to record the fat percentage. The Kjeldahl method, as described by Kjeldahl, was used to determine protein. (1983). In a nutshell, a catalyst was present during the digestion of a 5-gram milk sample. (0.2 gram CUSO₄ and 2 gram K₂SO₄). As an oxidizer, 30 milliliters of H₂SO₄ were employed. The digested samples were distilled in a micro Kjeldahl unit using 250 ml distilled water and 5 ml of 40% NaOH by micro Kjeldahl unit, wherein steam was distilled for 3-5 minutes over 5ml of an indicator (Bromocrystal green) containing 2% boric acid. By utilizing the following

equation: Protein (%) = $(1.4(V_2 - V_1) \text{ Normality of HCL}) / (\text{Weight of sample taken} \times \text{sample used for distillation}) \times 250$, ammonia in boric acid was finally trapped and calculated. To analyze the total soluble solids (TSS), a digital refractometer was used following the method described by AOAC. The refractometer's lens was cleaned, and then it was zeroed out using distilled water. The refractometer prism was coated with a few drops of milk before the lid was set on top of it. The TSS of milk was determined from the digital refractometer measurement. The percentage of milk samples' moisture content was determined by the AOAC method. A five-gram sample of milk was weighed in aluminum dishes and heated for 24 hours at 110°C in a hot air oven. After an hour in desiccators with moisture absorbent (silica gel), the samples were removed and re-weighed. The following formulas are used to calculate the moisture content as measured by weight loss.

STATISTICAL ANALYSIS

Using the computer program Statistica 8.1, statistical analysis was performed on the data from the physical and chemical analyses. (Statistica, 2006). The means of various lactation ages and oxytocin treatment groups were compared using analysis of variance (ANOVA). At p0.05, the significance level was established. Where necessary, the least significant difference (LSD) test was performed to assess the superiority of therapies.

RESULTS

pH VALUE AND SPECIFIC GRAVITY (g/cm³) OF BUFFALO MILK

When oxytocin was administered, the first lactation had the highest milk pH (6.7), followed by the second and third lactations, which had pHs of 6.4 and 6.3, respectively. Between the first and second lactations as well as between the first and third lactations, there were significant pH variations (p<0.05) found (LSD (0.05) = 0.4612SE = 0.2117). The third lactation had the greatest specific gravity measurement (1.03 g/cm³), with the first and second lactations' specific gravities, respectively, coming in at 1.0316 and 1.0308. Significant differences (p<0.05) were observed in specific gravity between the 3rd and 2nd lactation, as well as between the 3rd and 1st lactation. Oxytocin administration increased the specific gravity of milk during the 3rd lactation. Significant (p<0.05) differences were observed in both pH and specific gravity between control and different lactations (LSD (0.05) = 7.652SE ± = 3.512) (Figure 1, 2).

TOTAL SOLIDS, ASH CONTENTS AND MOISTURE PERCENTAGE IN BUFFALO MILK

The effect of oxytocin on the total solid, moisture, and ash percentages of buffalo milk was recorded. The addition of oxytocin enhanced the milk's total solid content during

the third lactation, with the third lactation recording the greatest total solid content (18.27%), followed by the first and second lactations with respective total solid contents of 17.38% and 17.11%. There was a significant difference in total solid content between the third and second lactation, as well as between the third and first lactation, but not between the first and second lactation (LSD (0.05) = 0.5391SE ± = 0.2474). Similarly, the administration of oxytocin increased the ash content of milk during the third lactation, with the highest ash content recorded in the third lactation (0.86%), followed by 0.83% and 0.84% in the first and second lactations, respectively. There was a significant difference in ash content between the third and second lactation, as well as between the third and first lactation, but not between the first and second lactation (LSD (0.05) = 0.0861SE ± = 0.0395). In contrast, the administration of oxytocin increased the moisture content of milk during the first lactation, with the highest moisture content recorded in the first lactation (82.89%), followed by 82.62% and 81.77% in both the second and third lactations. While there was no significant variation in moisture content between the second and third lactations, there was a substantial difference between the first and third lactations. The LSD test showed a significant difference in total solid, moisture, and ash percentages between the control and different lactations (LSD (0.05) = 0.5404SE ± = 0.2480) (Figure 3,4 and 5).

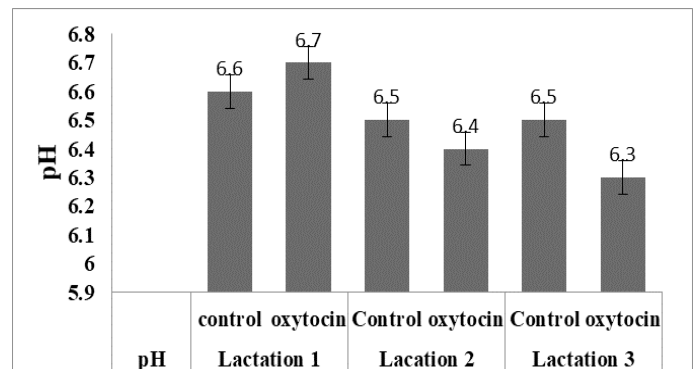


Figure 1: Effects of oxytocin on the pH of Buffalo milk throughout several lactations

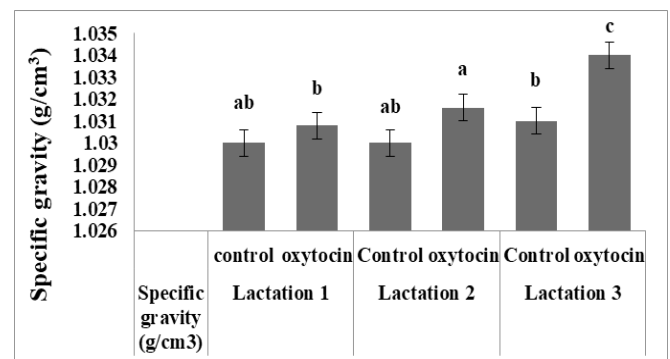


Figure 2: Effects of oxytocin on the specific gravity (g/cm³) of buffalo milk at various time

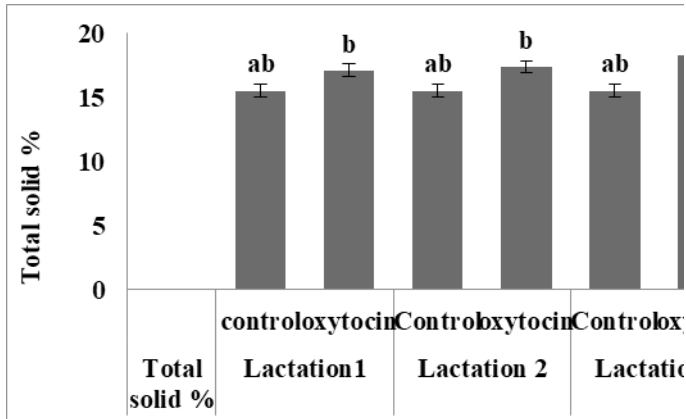


Figure 3: Effects of oxytocin on the total solid (%) of buffalo milk at different lactations

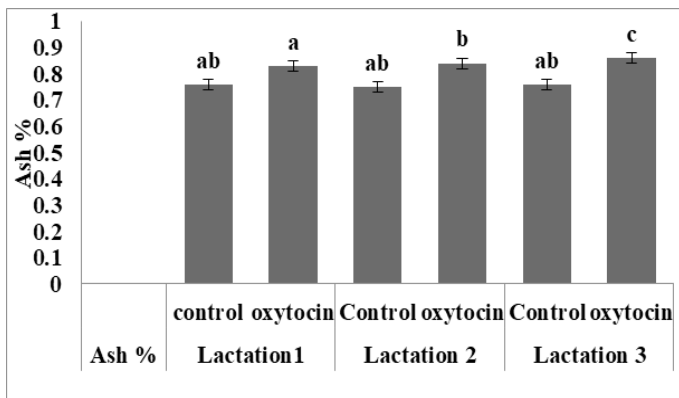


Figure 4: Effects of oxytocin on the Ash content (%) of Buffalo milk at different lactations

BUFFALO MILK'S PERCENTAGES OF PROTEIN, FAT SAND LACTOSE

The effects of oxytocin on the protein, fat, and lactose percentage of buffalo milk throughout various lactations are depicted in Figures 6, 7, and 8. The lactation with the greatest protein percentage was lactation three (4.76), followed by lactations two and one (4.72 and 4.68, respectively). Between lactations 3 and 2 and lactations 3 and 1, there was a statistically significant change in the protein percentage, while between lactations 1 and 2 there was no statistically significant difference. Protein percentage during lactation was raised by oxytocin treatment. The LSD test revealed a difference in protein percentage between the control and various lactations that was significantly different (p0.05) (LSD (0.05) = 0.1868SE = 0.0857). Similarly to this, the injection of oxytocin during lactation 3 enhanced the milk's fat percentage. Lactation 3 had the greatest fat percentage (7.72), which was followed by lactations 2 and 1 with 7.0 and 6.7, respectively. The LSD test revealed a difference in fat% between the control and various lactations that were significantly different (p0.05) (LSD (0.05) = 0.4316SE = 0.1981). The highest lactose content % was observed in lactation-3 (4.88), followed by 4.81 and 4.76 in lactations-2

and 1, respectively. During lactation 3, oxytocin treatment raised the lactose content%. The LSD test revealed a difference in lactose content percent between the control and various lactations that were significantly different (p0.05) (LSD (0.05) = 0.0433SE = 0.0199).

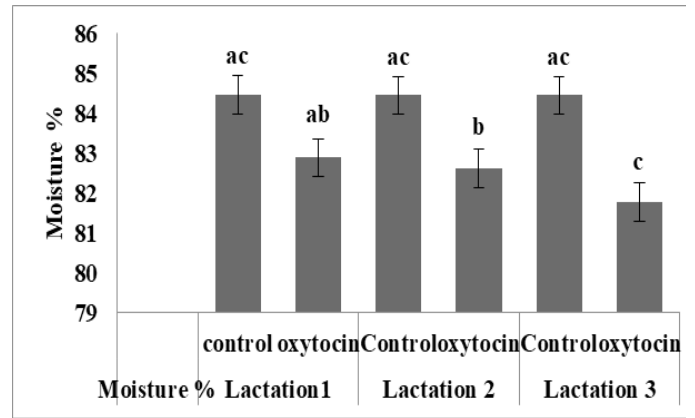


Figure 5: Effects of oxytocin on the moisture content (%) of Buffalo milk at different lactations

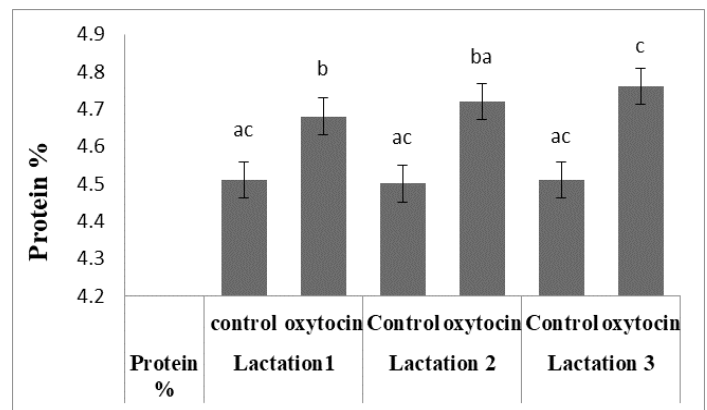


Figure 6: Effects of oxytocin on the protein (%) of buffalo milk at different lactations

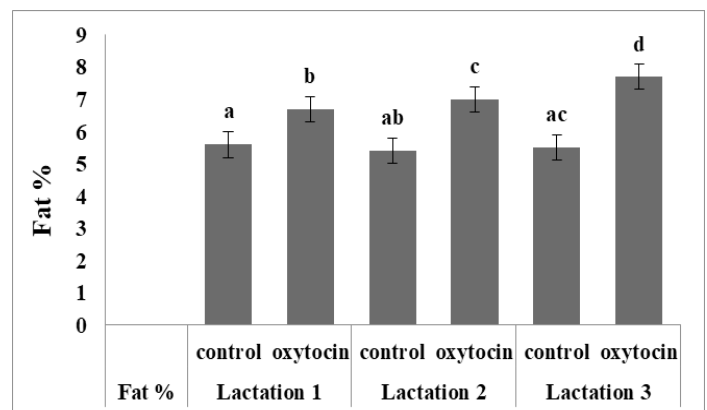


Figure 7: Effects of oxytocin on the fat percentage of Buffalo milk at different lactations

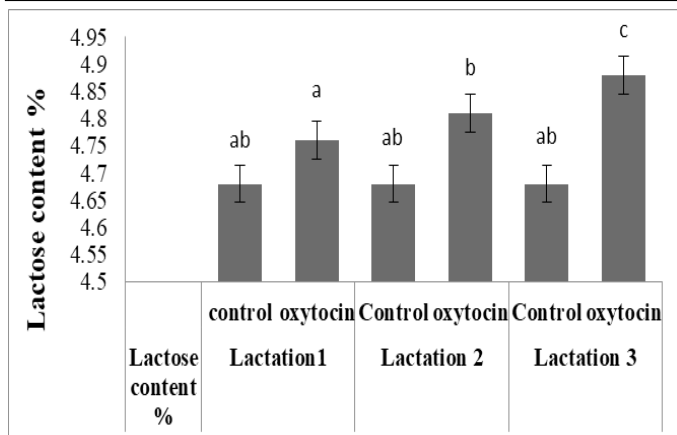


Figure 8: Effects of oxytocin on the lactose content (%) in Buffalo milk at different lactations

PRODUCTION OF BUFFALO MILK IN LITERS

The impact of oxytocin on milk production (L) in buffaloes during different lactations is shown in Figure 9. Our results show that oxytocin-treated buffaloes produced the most milk (L) during lactation 3 (15.4L), followed by 13.6L and 14.4L in the first and second lactations, respectively. When compared statistically, the third lactation and the first lactation both showed significant differences in milk production (L), while the first and second lactations did not show any significant changes. During the third lactation, oxytocin treatment led to an increase in milk production (L). The LSD test also revealed variations between the control and various lactations that were significant (p0.05) (LSD (0.05) = 0.7226SE = 0.3317).

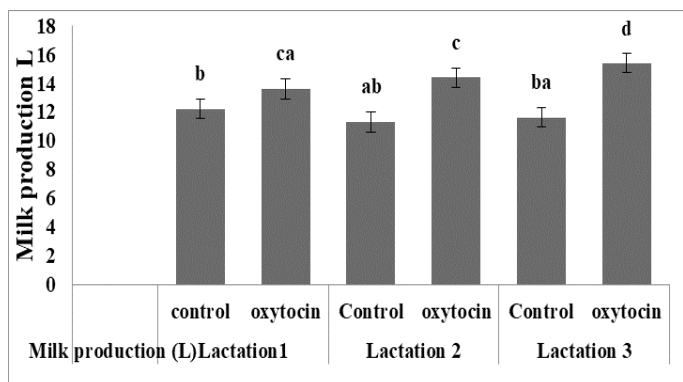


Figure 9: Effects of oxytocin on the amount of buffalo milk produced in liters at different lactations

DISCUSSION

The current study examined the impact of oxytocin administration and the lactation stage on the pH and specific gravity of buffalo milk. Previous research has demonstrated that lactation significantly affects milk pH, which typically varies from 6.5 to 6.6 in buffalo milk (Khan, 2007). In the current study, lactation-1 (6.70) had the highest pH of buffalo milk treated with oxytocin, while lactation-3 (lactation-3) had the lowest pH. (6.36). These results are in

line with earlier research, which found pH values ranging from 6.350.58 to 6.670.86 (Javaid et al., 2009). Similarly to this, prior research has demonstrated that the stage of lactation significantly affects milk-specific gravity, with the usual specific gravity of buffalo milk being 1.033 (Zaman, 2007). In this study, lactation-3 (1.0300) produced buffalo milk with the highest specific gravity when delivered with oxytocin, while lactation-0 (control) produced the lowest specific gravity. (1.0340). These results are in agreement with earlier research, which found that normal specific gravities were typically between 1.031 and 1.033 (Asif & Sumaira, 2010; Mansoor et al., 2012; Perveen et al., 2013). Additionally, another study described the milk sample pH values between 6.44 (Gran et al., 2003). The overall results indicate that the pH and specific gravity of buffalo milk can be influenced by lactation stage and oxytocin administration and that the normal ranges of these parameters are constant throughout investigations.

Buffalo milk typically contains 16.54 to 17.82% total solids (Dubey, 1997), and research has shown that the lactation stage significantly affects milk's total solids. According to our study, lactation 3 was when the largest amount of total solids in buffalo milk given with oxytocin was reported (18.226%). With the addition of 20% H2O (13.52%), 40% H2O (10.13%), and 70% H2O (5.64%), fresh milk's total solids percentage, which was previously reported to be 17.25%, significantly decreased. Our results are consistent with those of Fakhar et al. (2006), who noted a notable rise in the total solids of milk combined with SMP present in regular milk. Buffalo milk typically contains 0.77 to 0.89 percent ash (Sodi, 2008), and research has revealed that the lactation stage significantly affects milk ash levels. According to the results of our study, lactation 3 (0.856), followed by lactation 2 (0.838), and lactation 1 (0.828) had the highest ash content of buffalo milk given with oxytocin. In the control group, the lowest ash% in buffalo milk was noted. (0.756).

Analysis of the data on the amount of ash in buffalo milk given oxytocin revealed a significant difference (p 0.05). Although Enb et al. (2009) claimed that whole milk had a lower ash level (0.65%), our finding contradicts their findings. The stage of infant feeding, which alters the ash content of milk, maybe the cause of this discrepancy. Our research also revealed that lactation-3 (81.774%) had the lowest moisture percentage of buffalo milk, whereas lactation-2 (84.456%) had the highest moisture% of buffalo milk provided with oxytocin. According to Mishra et al. (2008), liquid milk samples contain more moisture than results. On lactation three (0.856), followed by lactation two (0.838) and lactation one (0.828), the ash content of buffalo milk given oxytocin was measured. The control group's minimum buffalo milk ash percentage was noted. (0.756).

Analysis of the data on the amount of ash in buffalo milk given oxytocin revealed a significant ($p < 0.05$) difference.

Zhang (1991) estimated that the average fat percentage in swamp buffalo milk was between 8.00 and 10.00 and 9.60, respectively. When compared to cow milk, buffalo milk has almost twice as much fat, which is the main factor in its high nutritional and energy value. Our findings demonstrated that lactation 3 was when the highest fat percentage (7.72) of buffalo milk treated with oxytocin was recorded. However, the minimum fat percentage (5.6) of buffalo milk given with oxytocin was noted in the control group. Our findings conflict with those of Abbas et al. (2014), who claimed that buffaloes given oxytocin injections had reduced milk fat concentration overall. Milk from buffaloes treated with oxytocin contains less fat in it ($P < 0.05$). Because of changes in fat percentage brought on by the lactation stage, our results conflict with those of the prior study. The stage of lactation has a big impact on milk production (Yadav, 2013). Our findings demonstrated that lactation 3 was the time when buffalo milk given with oxytocin produced the most milk (15.4L). However, the minimum milk production was observed on the control (12.6L). When oxytocin is administered, the intra-alveolar pressure rises, which boosts milk production, lowers feedback inhibition, and restores normal lactation-related breast blood flow. Our findings concur with those of Lollivier et al. (2002), who claimed that better milk transport within the mammary gland caused oxytocin to directly stimulate the mammary gland and milk production. Exogenous oxytocin injection increased milk production in dairy cows during milking time, according to a prior study (Ballou et al., 1993).

Ahmad et al. (2013) found that 20% of buffalo milk contains whey proteins, which comprise minor and trace proteins, and that 80% of buffalo milk contains caseins. In addition to normal lactation, milk proteins vary from 2.95 to 3.89% in buffaloes who have had oxytocin injections, while the range for uninjured buffaloes is 3.34 to 3.87%. Beata (2008) revealed that at the beginning of lactation, the milk protein of cows was somewhat lower, as the lactation advances, milk protein content increases and is high between the 200th and 300th day of lactation. The reduction in milk protein content in Sahiwal cows after oxytocin injection was studied by Hameed (2010). Our findings are in contrast to earlier research, which indicated that milk given oxytocin showed a decrease in protein percentage. On lactation three, the highest amount of protein (%) in buffalo milk given oxytocin was noted at 4.766%. But on the control, the minimal amount of protein (%) from buffalo milk was recorded at 4.506%. According to Yadav (2013), the lactation stage has a substantial impact on the amount of lactose in milk up until the sixth month of lactation, at which point lactose% significantly increased. Our find-

ings demonstrated that lactation-3 was the lactose peak at 4.884% for buffalo milk delivered with oxytocin. According to Ahmad et al. (2013), milk from buffalo that had received oxytocin treatment had a greater lactose content. According to Hameed (2010), Sahiwal cattle have a low initial lactose level that increases as lactation progresses. However, our results for lactose content support the prior findings of Misof et al. (2007).

The stage of lactation has a considerable impact on the mineral content of milk, especially in the early stages of lactation (Yadav, 2013). According to our research, lactation 3 had the highest calcium content of buffalo milk supplied with oxytocin (1.204 mg/dL), while lactation 3 had the lowest calcium concentration (1.110 mg/dL). Similar to this, lactation-3 (1.005 mg/dL) had buffalo milk supplied with oxytocin, which had the highest phosphorus concentration, while the control (0.891 mg/dL) had the lowest. These findings are in line with those of Abbas et al. (2014), who discovered that milk from buffaloes given oxytocin included increased levels of calcium and phosphorus.

CONCLUSION

It is concluded from this study that the use of oxytocin for milk letdown can affect the physicochemical characteristics of milk. Use of oxytocin can also be contributing factor of decreased quality of milk.

ACKNOWLEDGEMENTS

We are thankful to the lab technicians for their excellent lab assistance at the Department of Veterinary Physiology Sindh Agriculture University Tandojam, Pakistan.

CONFLICT OF INTEREST

There are no conflicts of interest.

NOVELTY STATEMENT

This study examines the effect of oxytocin on the physicochemical properties of buffalo milk across different lactations. It reveals that oxytocin treatment can increase milk production and alter the biochemical composition of buffalo milk, such as total solids, ash, protein, and lactose. However, it also shows that oxytocin treatment can lower the pH levels of buffalo milk, which may affect its quality and shelf life. This study provides valuable information for the dairy industry and consumers regarding using and regulating oxytocin in buffalo milk production.

We are thankful to the lab technicians for their excellent lab assistance at the Department of Veterinary Physiology Sindh Agriculture University Tandojam, Pakistan.

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