OPEN ACCESS Journal of Innovative Biology and Environmental Sciences

Research Article

Effect of Oxytocin on Milk Production, its Composition and Hemolytic changes in Buffaloes in District Hafizabad

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Abstract | Present study was conducted at four (A, B, C & D) private dairy/buffalo farms in district Hafizabad, Punjab, Pakistan. The aim of the study was to explore the effect of different dose levels of oxytocin on milk production, its composition, and hemolytic changes in buffaloes. Forty (40) milking buffaloes of age 6-11 years with second to sixth lactation, producing 6-9L of milk daily were included in this study. Oxytocin doses of 0ml, 0.25ml, 0.50ml and 1.0ml were injected to the animals of group-A (control), B, C and D respectively. The milk and blood samples from each animal were collected every week and the samples were brought to the laboratory for the analysis. The experiment lasted 30 days and the animals were kept under similar husbandry and environmental conditions at all four buffalo farms. The results indicated that the animals injected with 1.0ml dose of oxytocin showed the highest per animal average milk yield of (12.0L in Group-D) as compared to per animal average milk production of (6.0L in Group-A). The data further showed that there was a significant increase (p<0.001) in the contents of fat (7.98% vs 3.24%), lactose (7.55% vs 4.58%), protein (4.95% vs 3.44%) and solid not fat (SNF) (12.88% vs 10.21%) in animals of Group-D as compared to animals of Group-A, however this difference was non-significant (p<0.001) for Red Blood cell (RBC mg/dl) level in all groups of animals. The results, further indicated that the milk yield, fat, lactose, protein, and SNF increased (p<0.001) within the treated group of animals as the oxytocin dose level increased from 0.25ml to 1.0ml.

Received | April 19, 2024; **Accepted** | June 6, 2024; **Published** | June, 2024
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Email: iftikharalidr55@yahoo.com
Citation | Ali, I. and Farooqi, G.M.H. 2024. Effect of Oxytocin on Milk Production, its Composition and Hemolytic changes in Buffaloes in District Hafizabad. *Journal of Innovative Biology and Environmental Sciences*, 4(1): 33-46
Keywords | Oxytocin, Buffaloes, Milk yield, Husbandry measures, Composition, SNF, RBC

Introduction

1.1. Oxytocin

Oxytocin is a peptide produced in magnocellular-neurosecretory cells in the paraventricular hypothalamic nuclei, partially stored in the pituitary lobe, and then move into the bloodstream (Renaud *et al*., 1992). Oxytocin is packed in granules, transported to the gland axon, and ejected into the blood only through neurophysins as a carrier protein. The injection of oxytocin causes contractions of myometrium cells, sending suitable stimuli and helps in the transfer of sperm into the oviduct (Carter *et al*., 2020). It also helps with the evacuation of a foetus from the female body. Both males and females also release oxytocin into the bloodstream after sexual orgasm. Oxytocin is released in the

brain and a few other tissues (Ellis *et al.*, 2019). Synthesized oxytocin is utilized both in human and veterinary medicine which is the medication of choice for increasing and inducing regular uterine contractions during delivery labour and is utilized to avoid and regulate the post-abortion and delivery bleeding. It can also be used to manage accidental abortions and to instigate abortions therapeutically. It is also essential to use Oxytocin clinically to promote milk ejection in women who are having problems of breastfeeding. Furthermore, it is used to treat conditions such as breast engorgement and infections (Nucci *et al.*, 2018). The half-life of oxytocin in the bloodstream is 0.56 to 3.5 minutes, implying that it leaves the bloodstream between 2-6 minutes due to the activity of different enzymes (Erdman, 2021).

1.2. Biological importance

Oxytocin stimulates protein-linked receptors in uterine myofibrils by releasing intracellular calcium (Uvnaes *et al.*, 2022). Oxytocin performs on positive feedback when it is elevated and triggers contractions of the muscles of the uterus which boosts the level of oxytocin even more. Oxytocin produces uterine contractions and stimulates cells of myoepithelial cell of the breast of a female to contract (Lollivier and Marnet, 2005). Because contractions cause ejection of milk, positive feedback is provided due to suckling on the mother's breast leads to the release of hormonal oxytocin and milk let-down occurs (Deblon *et al.*, 2011).

Oxytocin enhances cells of myoepithelial cell contraction which affects the alveoli of udder of the female that generates uterine contraction and milk ejection (Mukherjee *et al.*, 2023). During breast feeding, natural oxytocin promotes milk to be released from the udder. In cows and buffaloes, oxytocin is released into the blood and other tissues such as the ovaries, testes, and corpusluteum. Oxytocin promotes and allows the process of milking management with oxytocin injection that increases milk-production in animals (Akhtar *et al.*, 2012). In veterinary medicine, oxytocin is used generally to promote milk let-down and to release residual placenta after birth. In young animals, oxytocin is also utilized to help the birth process in female has who been in labour for an extended amount of time (Ijaz and Aleem., 2006).

1.3. Oxytocin in Pakistan

Oxytocin is commonly used in Pakistan. The hormone is administered primarily to enhance milk output quickly, without considering any impact on the health of animal. The oxytocin injections are purchased commonly from village shops which are easily available in packs with instructions on them (Mustafa *et al.*, 2008; Ahmad *et al.*, 2021). The use of oxytocin injection by dairy producers during milk let down is becoming more common and he impact of a single oxytocin injection last for twenty-four hours. Such behaviour of farmers to use oxytocin in their animals without understanding and ignorance which results in a short benefit, but the regular use of this method extremely damages the fitness of animals (Abbas, and Zahoor, 2014).

In Pakistan, the researchers while studying the impact of oxytocin injection on milk production in buffaloes and economic development of rural areas through farmer's education in the Punjab province noticed that the farmers commonly use the oxytocin injections during the milking process in buffaloes which enhanced the milk yield (Bruckmaier, 2003). Oxytocin treatment increased milk production by 11.6% in cattle during a lactation compared to control animals. In oxytocin injected cattle, the entire udder was evacuated during milking and no milk remained in the udder which ensured reduced milk losses (Parodi, 1974). Furthermore, it has been reported that the

milk yield was higher in breastfeeding buffaloes compared to control buffalo's oxytocin injection (Bilal *et al*., 2008).

2. Materials and Methods

2.1. Study site and conditions

The current study was conducted at four different commercial dairy/ buffaloes farms located within a radius of 10 km around Hafizabad city, Punjab Pakistan. Forty (40) milking buffaloes were selected randomly from these four commercial dairy farms keeping in view their age, milk production and lactation. The selected buffaloes were at the age of 6 to 11 years and were in their second to sixth lactation, producing 6 to 9 litres of milk daily. The selected animals were almost of equal body weight of approximately 550kg.

Before the start of the experiment the buffaloes at all four farms were given almost identical feed/fodder, each buffalo was fed 50-55kg green fodder, 5-6kg wheat straw and 3-4kg of concentrate on a regular basis. All the animals were kept/ housed in a similar environmental and husbandry management conditions at the all four buffalo farms for their adjustment and to check their production level for one week. The area veterinary officer/doctor medically checked all the animals at the start of the trial. The owners of all four farms were requested to fix the milking times of experimental buffaloes approximately at the same time to bring uniformity among treatments.

2.2. Experimental design

The selected buffaloes from the four different farms were divided into four groups, having ten buffaloes in each group. These animals were allocated four treatments of oxytocin doses randomly as Group-A (no treatment, control); Group-B (0.25ml of oxytocin per animal per milking); Group-C (0.50ml of oxytocin per animal per milking) and Group-D (1.0ml of oxytocin per animal per milking).

Four oxytocin vials of 50cc were procured from a local medical store and one vial was given to the owner of each farm to use it for the experimental animals. The milking time for each farm was fixed at 5.00am in the morning and 4.00pm in the evening with the consultations of the owners of all four farms.

Four doses of Oxytocin (None, 0.25ml, 0.5ml and 1.0ml) were intramuscularly (I/M) administered to the animals of all four groups at four farms as elaborated above at morning and evening milking times 15 minutes before milking of animal to give reaction time for the injections. The daily milk yield (in liters) of each animal for both milking for each group was measured with calibrated milk utensil commonly used by farmers and at the end of a week (on each Sunday), the composite milk production for each animal of each group was calculated to check the weekly production. The experiment lasted for one month (30 days).

2.3. Sample collection and analysis

Weekly milk samples for each animal of each group for each treatment were taken in a graduated glass tube (100ml capacity) and stored in the refrigerator for laboratory analysis. The milk samples were tested for proteins fats, lactose and Solid Not Fat (SNF) in the Punjab Food Authority (PFA) Laboratory, Hafizabad.

Similarly, the weekly sample of blood from each animal for each group were taken through the Intravenous (I/V) route in 10cc syringes and stored in blue cap tubes having anticoagulants. The blood samples of each animal were separately stored in the refrigerator for the analysis of hemolytic changes, such as hemoglobin level, RBC and WBC counts which were also analyzed in the District Disease Diagnostic Laboratory (DDL), Hafizabad.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to analyse the data by Statistical Packages of Social Sciences (SPSS), where $P < 0.05$ was considered as significant.

3. Results

3.1. Milk yield

The results on the effect of different doses of oxytocin such as 0.00ml, 0.25ml, 0.50ml and 1.0ml on milk yield and their comparison with the control group in 1, 2, 3 and 4 weeks are shown in Table 1, 2, 3 and 4. The results of the study showed that the highest milk yield of 12L was achieved in animals injected with 1.0ml dose of oxytocin at weeks 1, 2, 3 and 4 and a minimum of 6L was recorded in the control group where no oxytocin was injected for the same period as shown at Table 1 with its graphic elaboration at Figure 1. At oxytocin dose level 0.25ml, the average milk yield was 7.3, 8.0, 8.7 and 9.1 liters respectively at weeks 1, 2, 3 and 4 while at oxytocin dose levels of 0.50ml and 1.00ml, the average milk production in animals for the same period was 8.4, 9.9, 10.1, 10.9 liters, 9 liters, and 9.3, 10.6, 11.5 and 12 liters respectively at weeks 1, 2, 3 and 4 as shown at Table 1.

Table 1: Comparison of milk yield between control and experimental groups injected with 0.25ml, 0.50ml and 1.0ml oxytocin after 1, 2, 3 and 4 weeks of the experimental period.

No. of Weeks	No. of Animals	Milk Yield in Control and Experimental Groups in 1,2,3,4 Week			
		Group-A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	5.7	7.3	8.4	9.3
2	10	5.7	8.0	9.9	10.6
3	10	5.7	8.7	10.1	11.5
4	10	5.7	9.1	10.9	12.0
Total	40	22.8	33.1	39.3	42.24
Average		5.7	8.275	9.825	10.85

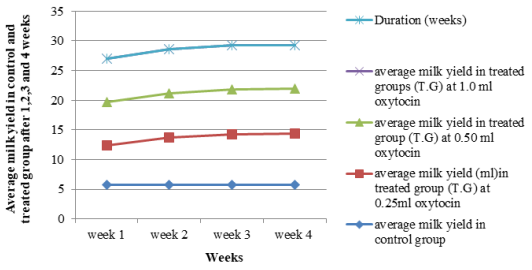

Figure 1: Comparison of milk yield between the control and treated group at 0.25ml, 0.50ml and 1.0ml oxytocin doses after 1, 2, 3 and 4 weeks. Milk yield was higher in the treated group than in the control group. Maximum average milk yield was 7.466ml in the 2nd and 3rd weeks after injecting 1.0 ml oxytocin, while milk yield decreased to 6.7ml in the 1st week after injecting 0.25ml oxytocin. The maximum average milk yield in the control group was 5.7ml.

Table 2: Statistical analysis of (ANOVA) of milk yield between groups.

ANOVA					
Milk yield in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	52.002	3	17.334	34.798	0.000
Within Groups	5.977	12	0.498		
Total	**57.979**	**15**			

p value is <0.05.

The results indicated that milk yield significantly increased ($p<0.005$) with increased levels of oxytocin doses.

Table 3: Milk yield in group 1, 2, 3 and control group.

Oxytocin dose (ml)	No of Weeks	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0.00	4	5.7000	.00000	.00000	5.7000	5.7000	5.70	5.70
0.25	4	8.0000	.92014	.46007	6.5358	9.4642	6.70	8.70
0.50	4	9.7250	.94296	.47148	8.2245	11.2255	8.40	10.60
1.00	4	10.3500	.50662	.25331	9.5439	11.1561	9.60	10.70
Total	16	8.4438	1.96603	.49151	7.3961	9.4914	5.70	10.70

**Dependent variable: milk yield; Independent variable: Oxytocin*

The standard error of milk yield for Group-1 (Control) at 0 ml Oxytocin dose was = 0.000
The standard error of milk yield for Group-2 (0.25 ml) at Oxytocin dose was = 0.46007
The standard error of milk yield for Group-3 (0.50 ml) Oxytocin dose was = 0.47148
The standard error of milk yield for Group-4 (1.00 ml) Oxytocin dose was = 0.25331

Table 4: Comparison of milk yield between control and treatments.

(I) Control group-1, group-2, group-3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.25	-2.30000*	.49906	0.003	-3.7817	-.8183
	.50	-4.02500*	.49906	0.000	-5.5067	-2.5433
	1.00	-4.65000*	.49906	0.000	-6.1317	-3.1683
.25	.00	2.30000*	.49906	0.003	.8183	3.7817
	.50	-1.72500*	.49906	0.021	-3.2067	-.2433
	1.00	-2.35000*	.49906	0.002	-3.8317	-.8683
.50	.00	4.02500*	.49906	0.000	2.5433	5.5067
	.25	1.72500*	.49906	0.021	.2433	3.2067
	1.00	-.62500	.49906	0.608	-2.1067	.8567
1.00	.00	4.65000*	.49906	0.000	3.1683	6.1317
	.25	2.35000*	.49906	0.002	.8683	3.8317
	.50	.62500	.49906	0.608	-.8567	2.1067

**The mean difference between control and experimental groups at all oxytocin levels is significant at the 0.05level.*

3.2. Fat percentage

The effect of different dose levels of oxytocin 0.25ml, 0.50ml and 1.0ml on milk fat percentage and their comparison among group at 1, 2, 3, and 4 weeks is shown in Table 5, 6, 7, 8 and 9. The results indicated that the highest percentage of milk fat was 7.98% in group-4 with oxytocin dose level of 1.0ml at weeks 1, 2, 3 and 4. A minimum of 3.24% of milk fat was recorded in the control group with no oxytocin dose at weeks 1, 2, 3 and 4 as shown at Table 5 and with its graphic elaboration as Figure 2. The findings indicated that there was no effect on fat percentages between treatments for the experimental period 04 weeks.

Table 5: Comparison of fat percentage between control and experimental groups injected with Oxytocin dose levels of none, 0.25 ml, 0.50 ml, and 1.0 ml oxytocin after 1, 2, 3 and 4 weeks of the experimental period.

No. of Weeks	No. of Animals	Fat% in Control and Experimental Groups in 1,2,3,4 Week			
		Group A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	3.537	5.883	6.088	6.263
2	10	4.243	6.004	6.013	7.143
3	10	3.811	6.168	6.430	6.757
4	10	3.918	5.592	6.585	7.266
Total	40	15.509	23.647	25.116	27.429
Average		3.875	5.9117	6.279	6.8572

Table 6: Statistical analysis of (ANOVA) of milk fat percentage between groups.

ANOVA					
Fat% in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.591	3	9.530	89.893	0.000
Within Groups	1.272	12	0.106		
Total	**29.864**	**15**			

p value is <0.05

The results indicated that fat% increased (p<0.05) with the increased levels of oxytocin at 0.25ml, 0.50ml and 1.00 ml.

Table 7: Comparison of fat percentage between groups

(I) Control group-1, group-2, group- 3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.25	-2.51125*	.23024	.000	-3.1948	-1.8277
	.50	-2.79825*	.23024	.000	-3.4818	-2.1147
	1.00	-3.56175*	.23024	.000	-4.2453	-2.8782
.25	.00	2.51125*	.23024	.000	1.8277	3.1948
	.50	-.28700	.23024	0.611	-.9706	.3966
	1.00	-1.05050*	.23024	0.003	-1.7341	-.3669
.50	.00	2.79825*	.23024	0.000	2.1147	3.4818
	.25	.28700	.23024	0.611	-.3966	.9706
	1.00	-.76350*	.23024	0.027	-1.4471	-.0799
1.00	.00	3.56175*	.23024	0.000	2.8782	4.2453
	.25	1.05050*	.23024	0.003	0.3669	1.7341
	.50	.76350*	.23024	0.027	.0799	1.4471

**The mean difference was significant at the 0.05 level for oxytocin dose levels 0.25ml, 0.5ml, and 1.0 ml. Dependent variable, Fat % for groups 1, 2, 3 and 4 analyzed through Tukey HSD method.*

Table 8: Comparison of fat percentage between groups

(I) Control group-1, group-2, group- 3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.25	-2.51125*	.23024	.000	-3.1948	-1.8277
	.50	-2.79825*	.23024	.000	-3.4818	-2.1147
	1.00	-3.56175*	.23024	.000	-4.2453	-2.8782
.25	.00	2.51125*	.23024	.000	1.8277	3.1948
	.50	-.28700	.23024	0.611	-.9706	.3966
	1.00	-1.05050*	.23024	0.003	-1.7341	-.3669
.50	.00	2.79825*	.23024	0.000	2.1147	3.4818
	.25	.28700	.23024	0.611	-.3966	.9706
	1.00	-.76350*	.23024	0.027	-1.4471	-.0799
1.00	.00	3.56175*	.23024	0.000	2.8782	4.2453
	.25	1.05050*	.23024	0.003	0.3669	1.7341
	.50	.76350*	.23024	0.027	.0799	1.4471

**The mean difference was significant at the 0.05 level for oxytocin dose levels 0.25ml, 0.5ml, and 1.0 ml. Dependent variable, Fat % for groups 1, 2,3 and 4 analyzed through Tukey HSD method.*

Table 9: Descriptive of fat percentage in group 1, 2, 3 and 4.

Duration	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Week 1	4	5.4178	1.27356	0.63678	3.3912	7.4443	3.53	6.26
Week 2	4	5.7098	1.53634	0.76817	3.2651	8.1544	3.53	7.14
Week 3	4	5.9165	1.68718	0.84359	3.2318	8.6012	3.53	7.49
Week 4	4	5.9473	1.69927	0.84964	3.2433	8.6512	3.53	7.48
Total	16	5.7478	1.41100	0.35275	4.9959	6.4997	3.53	7.49

The standard error for fat % at week 1= 0.63678
The standard error for fat % at week 2 = 0.76817
The Standard error for fat % at week 3 = 0.84359
*The Standard error for fat % at week 4 = 0.*84964

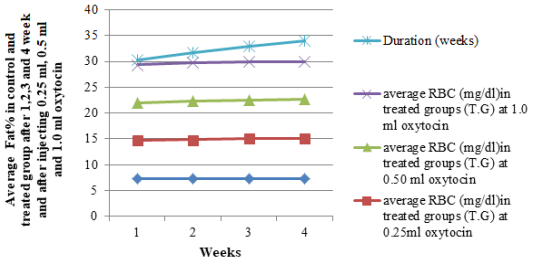

Figure 2: Comparison of fat% between control and treated groups at 0.25ml, 0.50ml and 1.0ml oxytocin after 1, 2, 3 and 4 weeks. Fat% was higher (p<0.05) in the treated group than control group. Maximum average fat% was 7.481% in the 4th week after injecting 1.0ml oxytocin, while decreased fat% of 5.79% was observed in the 1st week with 0.50ml oxytocin dose. The maximum average fat% in the control group was 3.53% without the oxytocin dose.

3.3. Lactose percentage

The data on the effect of different doses of oxytocin, 0.25ml, 0.50ml, and 1.0ml on milk lactose percentage and their comparison with the control group for 1, 2, 3 and 4 weeks is shown in Table 10, 11, 12 and 13. The results of the study showed that the highest lactose percentage of 7.55 was observed in group-4 injected with 1.0ml dose of oxytocin at weeks 1, 2, 3 and 4 and a minimum of 4.58% was recorded in the control group with no oxytocin dose injected at weeks 1, 2, 3 and 4. According to the findings it was observed that different dose levels of oxytocin had no effect on lactose percentage between treatments for the whole duration of experiment (Figure 3).

Table 10: Comparison of lactose percentage between control and experimental groups at 0.25ml, 0.5ml and 1.0ml oxytocin levels after 1, 2, 3 and 4 weeks of the experimental period.

No. of Weeks	No. of Animals	Milk Yield in Control and Experimental Groups in 1,2,3,4 Week			
		Group-A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	5.208	5.828	5.837	5.908
2	10	5.119	5.913	5.799	6.902
3	10	5.226	6.089	5.892	7.016
4	10	5.102	6.11	5.78	7.072
Total	**40**	**20.655**	**21.275**	**23.308**	**26.898**
Average		**5.1637**	**5.3187**	**5.827**	**10.85**

Table 11: Statistical analysis of (ANOVA) of lactose percentage between groups.

ANOVA					
Lactose% yield in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.662	3	0.887	10.032	0.001
Within Groups	1.062	12	0.088		
Total	**3.724**	**15**			

p value is <0.05.
The results indicated that there was no significant (p<.05) lactose% between either control and among treatments. Lactose % slightly increased with the increased levels of oxytocin.

Table 12: Descriptive of lactose percentages in groups 1, 2, 3 and 4.

Oxytocin dose (ml)	No of Weeks	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0.00	4	5.7000	0.00000	0.00000	5.7000	5.7000	5.70	5.70
0.25	4	5.9745	0.11675	0.05837	5.7887	6.1603	5.85	6.08
0.50	4	5.8425	0.03579	0.01790	5.7855	5.8995	5.79	5.87
1.00	4	6.7540	0.58220	0.29110	5.8276	7.6804	5.92	7.23
Total	16	6.0678	0.49827	0.12457	5.8022	6.3333	5.70	7.23

The Standard error of lactose % for Group-1 (Control) with Oxytocin dose was = 0. 00000
The Standard error of lactose % for Group-2 with (0.25 ml) Oxytocin dose was = 0. 05837
The Standard error of lactose % for Group-3 with (0.50 ml) Oxytocin dose was = 0. 01790
The Standard error of lactose % for Group-4 with (1.0 ml) Oxytocin dose was = 0. 29110

Table 13: Comparison of lactose percentage between groups.

(I) Control group-1, group-2, group-3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.25	-.27450	.21032	.577	-.8989	.3499
	.50	-.14250	.21032	.904	-.7669	.4819
	1.00	-1.05400*	.21032	.001	-1.6784	-.4296
.25	.00	.27450	.21032	.577	-.3499	.8989
	.50	.13200	.21032	.921	-.4924	.7564
	1.00	-.77950*	.21032	.014	-1.4039	-.1551
.50	.00	.14250	.21032	.904	-.4819	.7669
	.25	-.13200	.21032	.921	-.7564	.4924
	1.00	-.91150*	.21032	.005	-1.5359	-.2871
1.00	.00	1.05400*	.21032	.001	.4296	1.6784
	.25	.77950*	.21032	.014	.1551	1.4039
	.50	.91150*	.21032	.005	.2871	1.5359

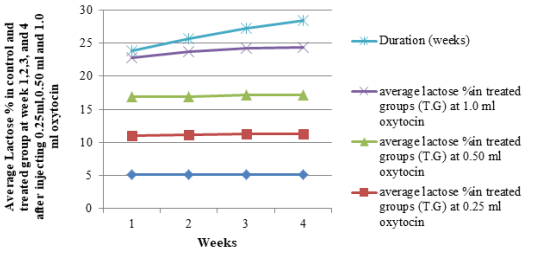

Figure 3: Comparison of lactose% between the control and treated group at 0.25ml, 0.50ml and 1.0ml Oxytocin dose after 1, 2, 3 and 4 weeks. The data was analyzed through Tukey test keeping lactose% as dependent variable and Oxytocin as independent variable.

3.4. Protein percentage

The effect of different doses of oxytocin at 0.25ml, 0.50ml, and 1.0ml on milk protein percentage and their comparison with the control group during four weeks is shown in Table 14, 15, 16 and 17. The results indicated that the highest protein% 4.95 was observed in group-4 with doze oxytocin doze level of 1.00ml during four weeks of study. A minimum of 3.44% was observed in control group with no oxytocin dose level. The data for all groups of protein % is shown at Table 14 and with their graphic elaboration at Figure 4. From the findings of this study, it was observed that there was no significant difference (p<0.05) for protein percentage between treatments for whole duration of experiment (04weeks).

Table 14: Comparison of protein % between the control and experimental groups injected with 0.25 ml,0.50 ml, and 1.0 ml Oxytocin after 1, 2, 3, and 4 weeks of the experimental period.

No. of Weeks	No. of Animals	Protein% in Control and Experimental Groups in 1,2,3,4 Week			
		Group-A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	3.722	4.222	4.145	4.588
2	10	2.858	4.078	4.501	4.666
3	10	3.622	4.228	4.602	4.529
4	10	3.641	4.969	4.69	4.778
Total	**40**	**13.843**	**17.497**	**17.938**	**18.561**
Average		**5.7**	**3.4607**	**4.3742**	**4.484**

Table 15: Statistical analysis of (ANOVA) protein percentage between groups.

ANOVA					
Protein% in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.776	3	0.592	109.89	0.000
Within Groups	.065	12	0.005		
Total	**1.841**	**15**			

***p value is <0.01**, compared to p value 0.000*
The results showed that all four groups indicated statistically significant (p<0.05) difference among groups which showed that with the increased level of Oxytocin, Protein % was significantly increased.

Table 16: Descriptive Protein in group 1, 2, 3 and control group.

Oxytocin dose (ml)	No of Weeks	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.00	4	3.7220	.00000	.00000	3.7220	3.7220	3.72	3.72
.25	4	4.2998	.06574	.03287	4.1951	4.4044	4.22	4.37
.50	4	4.5348	.08537	.04269	4.3989	4.6706	4.43	4.61
1.00	4	4.5400	.09971	.04986	4.3813	4.6987	4.45	4.68
Total	16	4.2741	.35034	.08759	4.0874	4.4608	3.72	4.68

Standard error of Protein % for Group-1 (Control) of Oxytocin dose was = 0. 00000
*Standard error of Protein % for Group-2 (0.25 ml) of Oxytocin dose was = 0.*03287
*Standard error of Protein % for Group-3 (0.50 ml) of Oxytocin dose was = 0.*04269
*Standard error of Protein % for Group-4 (1.00 ml) of Oxytocin dose was = 0.*04986

Table 17: Comparison of milk protein percentages between control treatments.

(I) Control group-1, group-2, group-3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Week 1	Week 2	-.08050	.27558	.991	-.8987	.7377
	Week 3	-.06175	.27558	.996	-.8799	.7564
	Week 4	-.08525	.27558	.989	-.9034	.7329
Week 2	Week 1	.08050	.27558	.991	-.7377	.8987
	Week 3	.01875	.27558	1.000	-.7994	.8369
	Week 4	-.00475	.27558	1.000	-.8229	.8134
Week 3	Week 1	.06175	.27558	.996	-.7564	.8799
	Week 2	-.01875	.27558	1.000	-.8369	.7994
	Week 4	-.02350	.27558	1.000	-.8417	.7947
Week 4	Week 1	.08525	.27558	.989	-.7329	.9034
	Week 2	.00475	.27558	1.000	-.8134	.8229
	Week 3	.02350	.27558	1.000	-.7947	.8417

**Dependent Variable: Protein% in groups 1, 2, 3 and control group*
Tukey HSD

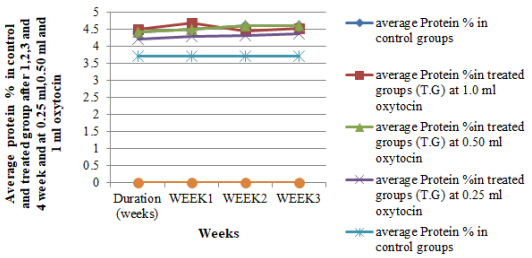

Figure 4: Comparison of protein% between control and treated group at 0.25ml, 0.50ml, and 1.0ml Oxytocin dose level at 1, 2, 3 and 4 weeks. Protein% was higher in 1.00ml oxytocin treated group than all other groups. The data was analyzed through Tukey method keeping protein% as dependent variable and Oxytocin dose as independent variable.

3.5. SNF percentage

The data for the outcome of effect of different doses of oxytocin on milk SNF% during four weeks of trial is shown at Table 18, 19, 20 and 21. The results of the study showed that the highest average of SNF% of 12.08% was observed in the animals injected with 1.0ml dose of oxytocin during four weeks of study and a minimum of 9.30% in animals of group A where no oxytocin was injected. The graphic elaboration of SNF% is in shown at Figure 5. The results of the study for SNF% showed that there was no significant (p<0.05) between control and among treatments.

Table 18: Comparison of SNF% between the control and experimental groups injected 0.25ml, 0.50ml and 1.0ml Oxytocin 4 weeks of the experimental periods.

No. of Weeks	No. of Animals	SNF% Yield in Control and Experimental Groups in 1,2,3,4 Week			
		Group-A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	9.514	10.497	11.397	11.832
2	10	9.297	10.741	11.039	12.291
3	10	9.091	10.685	11.175	12.146
4	10	9.301	11.022	11.121	11.761
Total	40	37.203	43.395	44.732	48.03
Average		9.301	10.848	11.183	12.008

Table 19: Statistical analysis of (ANOVA) of SNF% between groups.

ANOVA					
SNF% in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.804	3	5.935	70.683	0.000
Within Groups	1.008	12	0.084		
Total	**18.811**	**15**			

p value is <0.05.

The results were statistically significant, which indicated that SNF% is significant in different treated groups to whom different Oxytocin levels were given.

Table 20: Descriptive of SNF% in group 1, 2, 3 and control group.

Oxytocin dose (ml)	No of Weeks	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0.00	4	9.5410	0.00000	0.00000	9.5410	9.5410	9.54	9.54
0.25	4	10.7778	0.19584	0.09792	10.4661	11.0894	10.50	10.95
0.50	4	11.4210	0.11616	0.05808	11.2362	11.6058	11.28	11.55
1.00	4	12.4508	0.53291	0.26646	11.6028	13.2987	11.73	12.98
Total	16	11.0476	1.11986	0.27996	10.4509	11.6444	9.54	12.98

*The Standard error of SNF% for Group-1 (Control) (0 ml) Oxytocin dose = 0.*00000
*The Standard error of SNF% for Group-2 0.25 ml Oxytocin dose = 0.*09792
*The Standard error of SNF% for Group-3 0.50 ml Oxytocin dose = 0.*05808
*The Standard error of SNF% for Group-4 98765 1.00 ml Oxytocin dose = 0.*26646

Table 21: Comparison of SNF% between control and treatments.

(I) Control group-1, group-2, group-3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.00	0.25	-1.23675*	.20489	0.000	-1.8450	-.6285
	.50	-1.88000*	.20489	0.000	-2.4883	-1.2717
	1.00	-2.90975*	.20489	0.000	-3.5180	-2.3015
0.25	0.00	1.23675*	.20489	0.000	.6285	1.8450
	0.50	-.64325*	.20489	0.037	-1.2515	-.0350
	1.00	-1.67300*	.20489	0.000	-2.2813	-1.0647
.50	0.00	1.88000*	.20489	0.000	1.2717	2.4883
	0.25	.64325*	.20489	0.037	.0350	1.2515
	1.00	-1.02975*	.20489	0.001	-1.6380	-.4215
1.00	0.00	2.90975*	.20489	0.000	2.3015	3.5180
	0.25	1.67300*	.20489	0.000	1.0647	2.2813
	0.50	1.02975*	.20489	0.001	.4215	1.6380

**Dependent Variable: SNF% in groups 1,2,3 and 4. Tukey HSD*

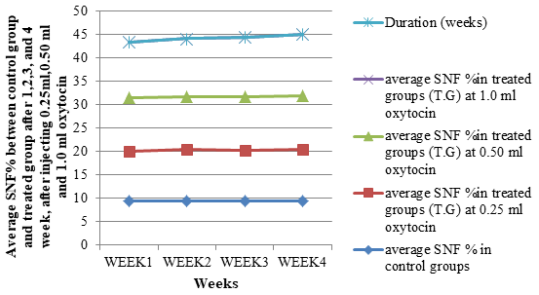

Figure 5: Comparison of SNF % between the control and the treated group at 0.25ml, 0.50ml and 1.0ml oxytocin after 1, 2, 3 and 4 weeks. SNF% was higher in the treated group than in the control group, however this difference was non-significant.

3.5. RBCs

The data on the effect of different doses of oxytocin on RBC mg/dl for four weeks of

trial is shown at Table 22, 23, 24 and 25. The results showed that the highest average level of RBC 7.89mg/dl was recorded in the animals of group-C that were injected with 0.50ml dose of oxytocin and minimum of 7.36 mg/dl in animals of group-D who were injected with oxytocin dose of 1.0ml. The data is shown at Table 22 with its graphic elaboration at Figure 6. The findings of this study indicated that there was no effect of different dose levels of oxytocin on RBC mg/dl count and the difference was non-significant (p<0.05) between treatments.

Table 22: Comparison of RBC (mg/dl) count between control and experimental groups injected with 0.25ml, 0.50ml, and 1.0ml oxytocin after 1, 2, 3 and 4 weeks of the experimental period.

No. of Weeks	No. of Animals	RBC mg/dl in Control and Experimental Groups in 1,2,3,4 Week			
		Group-A (Control)	Group-B	Group-C	Group-D
		No Oxytocin	0.25ml	0.50ml	1.0 ml
1	10	7.314	7.375	7.511	7.349
2	10	7.468	7.499	7.501	7.666
3	10	7.465	7.509	8.97	7.355
4	10	7.466	7.534	7.581	7.072
Total	40	29.713	29.917	31.56	29.442
Average		7.428	7.479	7.890	7.360

Table 23: Statistical analysis of (ANOVA) of RBC mg/dl between groups.

ANOVA					
RBC (mg/dl) in group 1, 2, 3 and 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.055	3	0.018	1.638	0.233
Within Groups	0.134	12	0.011		
Total	**0.189**	**15**			

p value is <0.05.

The results were statistically non-significant, which indicated that Oxytocin levels had no effect on RBC (mg/dl) count

Table 24: Descriptive of RBC mg/dl of group 1, 2, 3 and control group.

Oxytocin dose (ml)	No of Weeks	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0.00	4	7.3870	0.00000	0.00000	7.3870	7.3870	7.39	7.39
0.25	4	7.5337	0.16434	0.08217	7.2722	7.7953	7.35	7.71
0.50	4	7.4325	0.09840	0.04920	7.2759	7.5891	7.29	7.51
1.00	4	7.3943	0.08921	0.04460	7.2523	7.5362	7.28	7.47
Total	16	7.4369	0.11218	0.02805	7.3771	7.4967	7.28	7.71

The Standard error of RBC for Group-1 (Control) (0 ml) Oxytocin dose = 0.00000

The Standard error of RBC for Group-2 0.25 ml Oxytocin dose = 0.08217

Standard error of RBC for Group-3 0.50 ml Oxytocin dose = 0.04920

Standard error of RBC for Group-4 1.00 ml Oxytocin dose = 0.04460

Table 25: Comparison of RBC mg/dl between control and treatments.

(I) Control group-1, group-2, group-3, group-4	(J) Control group-1, group-2, group- 3, group-4	(I- J) Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.00	0.25	-0.14675	0.07471	0.254	-0.3685	0.0750
	0.50	-0.04550	0.07471	0.927	-0.2673	0.1763
	1.00	-0.00725	0.07471	1.000	-0.2290	0.2145
0.25	0.00	0.14675	0.07471	0.254	-0.0750	0.3685
	0.50	0.10125	0.07471	0.548	-0.1205	0.3230
	1.00	0.13950	0.07471	0.292	-0.0823	0.3613
0.50	0.00	0.04550	0.07471	0.927	-0.1763	0.2673
	0.25	-0.10125	0.07471	0.548	-0.3230	0.1205
	1.00	0.03825	0.07471	0.955	-0.1835	0.2600
1.00	0.00	0.00725	0.07471	1.000	-0.2145	0.2290
	0.25	-0.13950	0.07471	0.292	-0.3613	0.0823
	0.50	-0.03825	0.07471	0.955	-0.2600	0.1835

**Dependent Variable: RBC (mg/dl) in groups 1,2,3 and 4.*

Tukey HSD

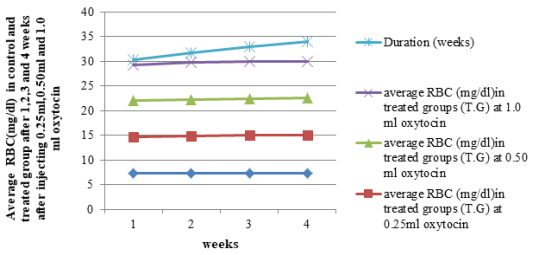

Figure 6: Comparison of RBC (mg/dl) count between control and treated group at 0.25ml, 0.50ml and 1.0ml Oxytocin dose after 1, 2, 3 and 4 weeks.

RBC (mg/dl) count level was higher in the treated group than the control group. Maximum average RBC (mg/dl) was 7.89mg/dl in animals of group-C who were injected 0.50ml dose of oxytocin and lowest of 7.36 in animals of group-D who were given 1.0ml of oxytocin.

4. Discussion

The milk yield increased in treated groups with the increased oxytocin dose level (0.25ml, 0.50ml, 0.1ml) as compared to the control group, which indicated that the increased level of oxytocin dose caused increase in milk yield in animals. The average milk yield in the control group was 6L, whereas 7L in animals of (Group-

B) injected with oxytocin dose of 0.25ml, 8L in animals of group-C Injected with oxytocin dose level of 0.50ml and 12L in animals of group-D who were injected with oxytocin dose level of 1.0ml. The findings of this study matched with the previous research studies that found higher milk yield when dairy cows were given exogenous oxytocin. Exogenous oxytocin also caused boosted milk output in milking buffaloes. The explanation elaborated in their study was that the increase of milk yield might be due to the action of oxytocin that caused the increased contractions of myoepithelial cells that enclose the alveoli, driving milk secretion into milk glands and cisternal cavities (Unal *et al*., 2007; Bilal, 2020; Faraz *et al*., 2021).

The fat percentage increased in treated group with the increase of oxytocin level (0.25ml, 0.50ml and 0.1ml) as compared to the control group, which indicated that the increased levels of oxytocin dose effected the increase in fat percentage in animals. The average fat percentage in the control group was 3.24% whereas 5.33% in 0.25ml treated/injected animals (Group-B), 5.89% in 0.50ml injected/treated animals (Group-C) and 7.98% 1.0ml injected/treated animals (Group-D). The results of present study are in agreement with the findings of work done by Ibnelbachyr *et al*. (2015) who found that fat percentage was increased with the increase of oxytocin level in milking buffaloes. Fat content was substantially greater during early lactation and lower fat levels during the first week of mid-lactation, most likely due to decreased fat mobilisation at this time.

The lactose percentage of increased in treated group with the increase of oxytocin levels (0.25ml, 0.50ml and 0.1ml) as compared to the control group, which indicated that the increased level of oxytocin dose effected the increase in lactose percentage in animals. The average lactose percentage in the control group was 4.58% whereas 5.78% in 0.25ml treated/injected animals (Group-B), 6.06% in 0.50ml injected/treated animals 7.23% in1.0ml injected/treated animals (Group-D). The results of this study matched with the findings of previous work (Mahmoud *et al*., 2014; Khan *et al*., 2023), where it was observed that the lactose contents decreased during early lactation and then climbed to a peak between mid- and late lactation. Furthermore, lactose concentrations in West African buffaloes were highest in mid lactation, followed by late lactation, and lowest in early lactation.

The protein percentage increased in treated groups with the increased levels of oxytocin (0.25ml, 0.50ml and 0.1ml) as compared to the control group, which indicated that the increased level of oxytocin dose affected the increases in protein percentage in animals. The average percentage of protein in the control group was 3.71% whereas 4.01% in 0.25ml treated/injected animals (Group-B), 4.55% in 0.50ml injected/treated animals (Group-C), and 4.65% in 1.0ml oxytocin injected/treated animals. These results are in agreement with previous findings (Ibnelbachyr *et al*., 2015; Khan *et al*., 2023).

The SNF percentage increased in treated groups with the increase of oxytocin levels (0.25ml, 0.50ml and 0.1ml) as compared to the control group, which indicated that the increased levels of oxytocin dose affected the increase in SNF percentages in animals. The average SNF percentages in the control was 10.21% whereas 10.77% in 0.25ml treated/injected animals (Group-B), 11.69% in 0.50ml injected/treated animals (Group-C) and 12.88% in 1.0ml injected/treated animals (Group-D). The results of this study confirmed the results of many previous studies which indicated that throughout the week of lactation, the SNF contents changed with no discernible pattern. The

stage of lactation was recognized as to have a large impact on all milk components in milking Buffaloes (Mestawet *et al.*, 2012; Murtaza *et al*,. 2021).

The RBCs (mg/dl) of treated groups did not change with the increase of oxytocin levels (0.25ml, 0.50ml and 0.1ml) as compared to the control group, which indicated that the increased levels of oxytocin doses not affected the presence of RBCs in animals. The average percentage of RBCs in the control group was 7.56mg/dl whereas 7.71mg/dl in 0.25ml treated/injected animals (Group-B), 7.72 mg/dl in 0.50ml treated/injected animals (Group-C) and 7.36% in 1.0ml treated/injected animals (Group-D). The results of this study are in agreement with the findings of many previous works done in this field (Perumal et al., 2020; Zakaria *et al*., 2023).

5. Conclusion

The results on milk yield indicated that the animals injected 1.0ml dose of Oxytocin showed highest average production (12.0 liters) of ten animals in (Group-D) compared to the average production of 6 liters of ten animals in (Group-A) which indicated significant ($p<.0.05$) increase in milk output at this dose level of Oxytocin. The data further showed that there was a significant increase ($P<.0.001$) in fat (7.98% vs 3.24%), lactose (7.55% vs 4.58%), protein (4.95% vs 3.44%), solid not fat (SNF) (12.88% vs 10.21%) in animals of group-D compared to animals of group-A (control) group however this difference was non-significant ($P<0.001$) for Red Blood cell (RBC) (mg/dl) level in all groups of animals. However, the results on milk production, fat, protein, lactose, and SNF percentages showed significant differences ($P<0.001$) among all three treated groups. The results, further showed that the milk yield, Fat, lactose, Protein and Solid Not Fat (SNF) increased ($P<0.05$) within the treated group of animals as the Oxytocin dose level was increased from 0.25ml, to 1.0ml. The results of this study clearly indicated that the Oxytocin dose level of 1.0ml was the most suitable dose for milk let down process in buffaloes. The farmer's community is advised to use this dose level of Oxytocin at their farms.

6. Author's Contribution

Authors contributed equally.

7. Conflict of Interest

No conflicts.

8. Novelty Statement

The novelty of this study lies in its demonstration of the specific effects of varying doses of Oxytocin on milk yield and composition in buffaloes, particularly highlighting that a 1.0 ml dose significantly enhances both the quantity and quality of milk produced. While previous research may have explored the use of Oxytocin in dairy animals, this study provides clear, quantifiable evidence of the optimal dosage for maximizing milk production and improving key components such as fat, lactose, protein, and solid not fat (SNF). Additionally, the study's rigorous statistical analysis ($p<0.001$ and $p<0.05$) adds credibility to its findings, making it a valuable contribution to the field of veterinary science and dairy farming practices. The recommendation for farmers to adopt this specific dosage also emphasizes its practical implications, potentially leading to improved productivity in the dairy industry.

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