

## Milk composition and yield changes with advancing pregnancy in dairy buffaloes (*Bubalus bubalis*)

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**Abstract:** The changes in the composition of milk with advancing pregnancy were investigated in Nili-Ravi dairy buffaloes. Forty lactating buffaloes were used, within 2-3 months postpartum. The animals were grouped as high, moderate, and low yielders; synchronized for estrus; and inseminated artificially. Milk yield was recorded daily and sampled fortnightly for analysis. The data were analyzed using the univariate weighted mean and general linear model. Decline in milk yield became significant after the 8th week post-conception. The high yielders had the lowest fat contents. Milk fat increased significantly during succeeding weeks post-conception. The highest fat level (8.5%) was observed in week 22. The solid-not-fat (SNF) was higher during the initial 8 weeks. Protein concentration declined with advancing pregnancy. The mineral contents were lower up to 8 weeks and increased later. It was concluded that milk fat concentrations increased linearly from week 2 to 22, while protein showed the opposite pattern in dairy buffaloes. SNF and lactose initially decreased up to week 14 but increased later on. This study suggests that milk composition from dairy buffaloes changes with advancing pregnancy, making it suitable for various types of human consumers.

**Key words:** Pregnancy, milk yield, milk composition, dairy buffalo

### Introduction

The buffalo holds a significant place in the agricultural economy of South Asian and Mediterranean countries, providing milk and meat for human consumption; traction power for agricultural operations; and hides, skins, and other raw materials for industrial use. The resource-poor families of developing countries in Asia will depend upon this animal in the foreseeable future (1). The fast-growth Asian economies will be supported by this animal through exploitation of its full potential. The buffalo contributes 12.39% of the milk produced from all dairy species at global level. In South Asia 85.4 million tons of buffalo milk is produced, of which

66.7% is contributed by India and 25.2% by Pakistan. Respective figures for buffalo meat production are 65.1%, 2.8%, and 1.1%, respectively (2).

Under the traditional farming system buffaloes are not bred with the fear that the milk production will decline and thus they remain open for a longer period (3). Our group investigated the effect of postpartum breeding interval on productivity of buffalo farms under a peri-urban production system (4). It was concluded that there was a consistent declining trend in milk yield with advancing pregnancy; however, the animals conceiving at a later stage of lactation resulted in a decline in financial return to the tune of 27% conceiving than with those conceiving earlier

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(150 vs. 300 days). Borman (5) reported a decline in the yield of milk, from approximately 90 days in pregnant cows, compared with non-pregnant cows. The difference in production was particularly noticeable during the third trimester of gestation. The report suggests that there is a milk production cost of pregnancy well in advance of 190 days. However, the significance of the difference between milk yields of pregnant and non-pregnant cows was not reported. The greater effect of pregnancy on late lactation was attributed to the decreasing power of galactopoietic hormones as lactation advanced. Sørensen and Østergaard (6) reported that blood concentration of growth hormone (GH) decreased, whereas insulin concentration increased as lactation advanced in dairy cows. Pregnancy also caused a significant decline in milk yield of dairy cows in late lactation from month 5 of gestation onwards (7,8) or from as early as month 3 of pregnancy (3). Placental lactogen peaks during the last trimester of pregnancy and may influence mammatogenesis and lactogenesis, and alter the maternal metabolism to accommodate the growth and development of the fetus (9).

In general, yields declined and percentage levels of these parameters increased after about 3 months of lactation (9). Chloride percentage showed an initial decline and then a rise after 2 months. Stage of pregnancy accounted for a small but significant variation in most traits; variation was 0.2% to 0.4% and <0.1% to 3.0% in yields and percentages for Holsteins, and <0.1% to 0.2% and 0.1% to 1.1% for Jerseys. Interactions between stage of lactation and pregnancy were investigated by response surface methodology and found to be very small compared to those in non-pregnant cows over 305 days. Lee et al. (10) found that pregnant cows produced 265 kg less milk, 9.8 kg less fat, and 9.2 kg less protein than non-pregnant cows.

In the above studies the effects of pregnancy on milk yield and composition were investigated on the basis of data from dairy cows. This information cannot be applied exactly to dairy buffaloes due to the difference in species, climate, and socio-economic conditions of the farmers. In addition, other economic traits indicating the productivity of dairy animals, as affected by pregnancy, need to be documented in buffaloes. The present study was therefore conducted

to investigate the effect of pregnancy on milk yield and composition in dairy buffaloes.

## Materials and methods

A study regarding the effect of pregnancy on milk yield and composition was conducted at a commercial dairy farm located at Peshawar, Pakistan, lying at 31 to 37°N and 65 to 74°E. For this purpose 40 lactating buffaloes, 2-3 months postpartum were selected at the farm and were ear tagged. The selected animals were grouped on the basis of daily milk yield as high yielders (HY), 66 to 75 L/week; moderate yielders (MY), 56 to 65 L/week; and low yielders (LY), 46 to 55 L/week.

Ovarian status of all selected animals was assessed through rectal palpation. For synchronization 5 mL of Lutalyse (Pfizer, Belgium) per animal was injected intramuscularly to all selected buffaloes. Animals showing estrus signs were inseminated using locally available frozen semen. Buffaloes that failed to show estrus signs were provided with a 2<sup>nd</sup> injection after 11 days of the 1<sup>st</sup> injection and were inseminated after 24 h of the 2<sup>nd</sup> injection. Conception in experimental buffaloes was determined through rectal palpation after 2 months of insemination. Animals that remained open were used as controls (Table 1). Daily milk yields of all pregnant as well as non-pregnant animals were recorded and utilized to work out the weekly milk yield. The study continued to week 23 post-conception when most of animals were dry. Milk samples (10 mL each) of all pregnant buffaloes were collected from evening milk after every 15 days until the cessation of lactation. Each milk sample was used for determination of milk composition through a milk analyzer (Ekomilk, Total Ultrasonic Milk Analyzer, Bulltech 2000, Stara Zagora, Bulgaria). Milk fats, protein, lactose, and SNF were determined while ash was calculated.

The data were analyzed using statistical procedures for descriptive statistics and the general linear model. Means were compared through Duncan's multiple range test using SAS (11).

## Results

The dairy buffaloes yielded  $38.56 \pm 11.41$  L/week with a daily equivalent of 5.51 L/day (Table 2). The yield ranged from 11 to 66 L/weeks. Mean fat was

Table 1. Number of experimental animals in various production groups and pregnancy status.

Pregnancy Status	Production group			Total
	HY	MY	LY	
Pregnant	6	11	6	23
Non-pregnant	6	5	6	17
Total	12	16	12	40

HY = High yielders, 66 to 75 L/week; MY = Moderate yielders, 56 to 65 L/week; LY = Low yielders, 46 to 55 L/week

Table 2. Descriptive statistics of various traits in buffalos.

Name of parameter	N	Mean (L/W) $\pm$ SD	Minimum	Maximum
Weekly milk yield	920	38.56 $\pm$ 11.41	11	66
Fat	198	7.47 $\pm$ 0.87	5.7	9.2
SNF	198	9.32 $\pm$ 0.21	8.78	9.81
Lactose	198	5.24 $\pm$ 0.15	4.8	5.6
Protein	198	3.31 $\pm$ 0.13	3.10	3.7
ASH	198	0.77 $\pm$ 0.02	0.68	0.82

7.47  $\pm$  0.87%, ranging from 5.7% to 9.2%. The mean solid-not-fat (SNF) was 9.32  $\pm$  2.21, ranging from 8.78% to 9.81%. The mean lactose was 5.24  $\pm$  0.15% with a minimum value of 4.8 and maximum of 5.6%. Mean protein was 3.31  $\pm$  0.13, ranging from 3.10 to 3.7%. Mean ash was 0.77  $\pm$  0.02 with a range from 0.68% to 0.82%.

Mean comparisons for milk yield and composition across production and pregnancy groups are given in Table 3. The difference among groups was significant ( $P < 0.05$ ). Among the 3 production groups high yielders had the lowest fat contents, followed by moderate and low yielders (7.36%, 7.46%, and 7.58%, for the low, moderate, and high yielders, respectively). The SNF, protein, lactose, and ASH contents did not show any significant difference among the groups.

An overall effect of pregnancy on milk composition is given in Table 3. Pregnant animal has highest fat value than the open animals (7.84% vs. 7.09%,  $P < 0.05$ ). Similarly SNF contents were also higher in milk from pregnant animals than in milk from open ones (9.34% vs. 9.30%,  $P < 0.05$ ). Milk protein, lactose, and ash contents were slightly higher in pregnant animals as compared to milk from open animals.

Pregnancy was associated with a decreased milk yield, detected during week 6 post-conception and decreasing further up to week 22 (Table 4,  $P < 0.05$ ). Milk fat was increased significantly during succeeding weeks post-conception. The highest fat level of 8.5 was observed in week 22, showing a constant increase over the advancing post-conception weeks. This increase in fat contents may be due to the decreasing milk yield resulting in more concentration and fat%. Solid-not-fat (SNF) was higher during the initial 8 weeks and lower later ( $P < 0.05$ ). Protein also showed a declining path in the advancing pregnancy while the ASH contents were lower up to 8 weeks and increased later on.

Looking at Figure 1 the fat concentration increased linearly with the advancing pregnancy from 6.3% during week 2 to 8.5% during week 22. On the other hand, SNF declined constantly and rapidly up to week 14 and slightly but constantly increased later on. Figure 2 shows that protein decreased linearly from month 2 to 22, while lactose increased initially up to 8 weeks, then decreasing rapidly up to 14 weeks and then again increased later on.

Table 3. Mean comparison for milk yield and composition across production and pregnancy groups.

Group	Milk yield (L week)	Fats (%)	Solid-not-fat (%)	Protein (%)	Lactose (%)	Ash (%)
Production group						
HY	49.41 <sup>A</sup>	7.36 <sup>C</sup>	9.30	3.30	5.23	0.771
MY	38.26 <sup>B</sup>	7.46 <sup>B</sup>	9.33	3.31	5.25	0.769
LY	28.08 <sup>C</sup>	7.58 <sup>A</sup>	9.32	3.31	5.24	0.770
Pregnancy group						
Pregnant	37.143 <sup>B</sup>	7.84 <sup>A</sup>	9.34 <sup>A</sup>	3.31 <sup>A</sup>	5.25 <sup>A</sup>	0.771 <sup>A</sup>
Non-pregnant	40.46 <sup>A</sup>	7.09 <sup>B</sup>	9.30 <sup>B</sup>	3.30 <sup>A</sup>	5.23 <sup>A</sup>	0.769 <sup>A</sup>

Means with different superscripts with significant difference ( $P < 0.05$ ); HY = High yielders; MY = Moderate yielders; LY = Low yielders; <sup>A, B, C</sup> The means with different superscripts in the same column are different from each other

Table 4. Mean comparison for milk yield and composition across pregnancy weeks.

Weeks post-conception	Milk yield (L/week)	Fats (%)	Solid-not-fat (%)	Protein (%)	Lactose (%)	Ash (%)
2	45.30 <sup>A</sup>	6.36 <sup>I</sup>	9.56 <sup>A</sup>	3.48 <sup>A</sup>	5.036 <sup>A</sup>	0.725 <sup>E</sup>
4	45.9 <sup>A</sup>	6.58 <sup>H</sup>	9.52 <sup>A</sup>	3.42 <sup>AB</sup>	5.34 <sup>A</sup>	0.764 <sup>CD</sup>
6	44.9 <sup>AB</sup>	6.67 <sup>GH</sup>	9.53 <sup>A</sup>	3.38 <sup>BC</sup>	5.38 <sup>A</sup>	0.762 <sup>D</sup>
8	43.55 <sup>B</sup>	6.82 <sup>G</sup>	9.49 <sup>A</sup>	3.35 <sup>C</sup>	5.39 <sup>A</sup>	0.762 <sup>D</sup>
10	42.25 <sup>BC</sup>	7.22 <sup>F</sup>	9.28 <sup>B</sup>	3.32 <sup>CD</sup>	5.17 <sup>CD</sup>	0.781 <sup>AB</sup>
12	40.10 <sup>BC</sup>	7.43 <sup>E</sup>	9.19 <sup>BC</sup>	3.32 <sup>DE</sup>	5.08 <sup>E</sup>	0.789 <sup>AB</sup>
14	37.95 <sup>C</sup>	7.99 <sup>D</sup>	9.12 <sup>C</sup>	3.28 <sup>E</sup>	5.07 <sup>E</sup>	0.782 <sup>AB</sup>
16	36.20 <sup>CD</sup>	8.05 <sup>CD</sup>	9.15 <sup>C</sup>	3.2 <sup>DE</sup>	5.15 <sup>DE</sup>	0.785 <sup>A</sup>
18	33.50 <sup>CD</sup>	8.16 <sup>BC</sup>	9.19 <sup>BC</sup>	3.20 <sup>E</sup>	5.21 <sup>BCD</sup>	0.781 <sup>AB</sup>
20	30.08 <sup>D</sup>	8.32 <sup>B</sup>	9.21 <sup>BC</sup>	3.20 <sup>E</sup>	5.24 <sup>BC</sup>	0.773 <sup>BC</sup>
22	26.20 <sup>D</sup>	8.50 <sup>A</sup>	9.27 <sup>B</sup>	3.23 <sup>E</sup>	5.25 <sup>B</sup>	0.777 <sup>AB</sup>

Means with different superscripts with significant difference ( $P < 0.05$ )

## Discussion

The present study reports a decline in milk yield with advancing pregnancy. In a previous study on pregnant cows (3) a decline in the yield of milk was reported from approximately 90 days. The difference in production was particularly noticeable during the third trimester of gestation. This report suggested that there was a milk production cost of pregnancy

well in advance of 190 days. However, the significance of the difference between milk yields of pregnant and non-pregnant cows was not reported. In Saanan goats (12) pregnancy had no effect on milk yield during the first 8 weeks of pregnancy, but milk yield decreased rapidly thereafter and was 57% of the value of non-pregnant goats in the last week of pregnancy. In another study on goats (13) pregnancy reduced milk yield from week 10 after conceiving onwards in goats.

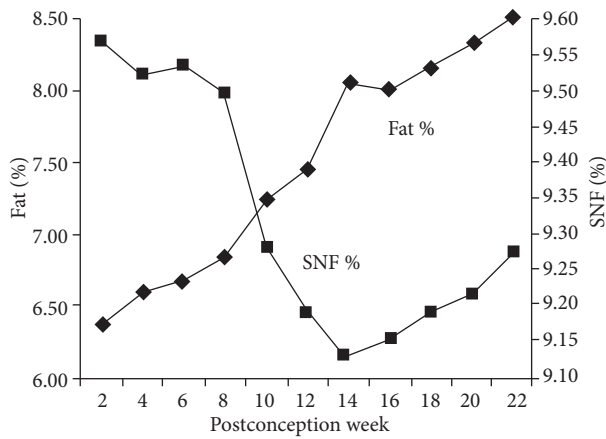


Figure 1. Changes in milk fat and solid-not-fats (SNF) concentrations with advanced pregnancy in dairy buffaloes.

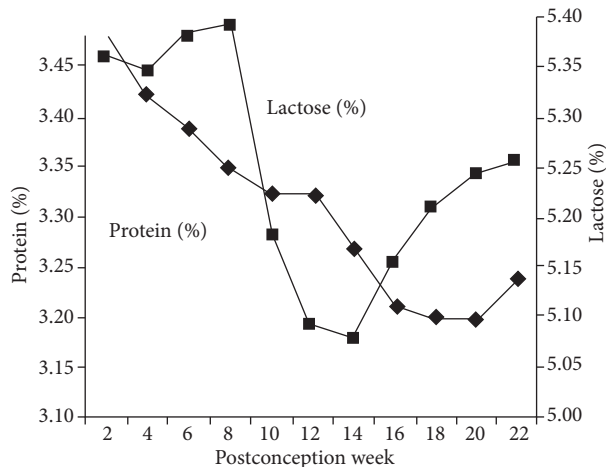


Figure 2. Changes in milk protein and lactose concentrations with advanced pregnancy in dairy buffaloes.

Pregnancy also caused a significant decline in milk yield of dairy cows in late lactation from month 5 of gestation onwards (6,7) or from as early as month 3 of pregnancy (3). Placental lactogen peaks during the last third of pregnancy and may influence mammatogenesis and lactogenesis, and alter the maternal metabolism to accommodate the growth and development of the fetus (8).

Yield losses might be due to the nutritive requirements of the gravid uterus. Energy requirements for pregnancy not only include the energy deposited in the conceptus, but also the energy used for the conceptus metabolism and the energy used by maternal tissues to support the conceptus.

Energy requirements of the pregnant dairy cow after 190 days of gestation was defined and a quadratic equation was developed to describe the daily change in energy content of the gravid uterus (14). Energy requirements directly attributable to pregnancy were presumed to be close to zero (15) up to day 190 of gestation.

In most seasonal calving situations, a lactation length of 305 days is targeted, thereby allowing a 2-month dry period. Therefore, the energy requirements of the gravid uterus would not be expected to have a significant effect on milk production. However, 2 studies have reported (16,17) that an exponential growth of fetal tissues occurs and, therefore, energy demand increased after 90 days of pregnancy. Glucose is the main source of energy for the gravid uterus, and an increase in the net hepatic plasma glucose release in pregnant ewes has been reported from days 40 of pregnancy onwards (18). Despite this hepatic release of glucose, pregnant goats had lower concentrations of blood glucose than non-pregnant goats after 84 days of pregnancy (19). This suggests that there may be competition for glucose between the mammary gland (for lactose synthesis) and the gravid uterus, which would result in milk yield losses during pregnancy.

Increases in milk contents especially milk fats were observed with advancement of gestation stage in the present study. Effect of gestation stage on milk composition was investigated in Holstein-Friesian cows in one herd (6). Gestation stage had a significant effect ( $P < 0.05$ ) on all traits, accounting for 1.38% to 1.69% reduction in total sum of squares for yield traits and  $<0.4\%$  reduction in total sum of squares for content traits.

We found increasing fat and decreasing SNF and protein levels with the advancing pregnancy. Similarly, the ratios of SNF:fat and protein:fat were reported to decline in late pregnancy (9). Another study reported (10) that, in general, yield declined and percentages increased after about 3 months of lactation. Chloride percentage showed an initial decline and then a rise after 2 months. Stage of pregnancy accounted for a small but significant variation in most traits; variation was 0.2% to 0.4% and  $<0.1\%$  to 3.0% in yields and percentages for Holsteins, and  $<0.1\%$  to 0.2% and 0.1% to 1.1% for Jerseys. Another study (3)



reported a decline in the yield of milk, milk fat, and milk protein, from approximately 90 days in pregnant cows, compared with non-pregnant cows.

It has been reported that a decline in milk yield occurs from 126 days of pregnancy in twins that were pregnant and protein and fat concentrations increased in pregnant cows from 77 and 133 days of gestation, respectively (20). The yield of milk fat and protein was not affected by pregnancy until 168 days of gestation, after which pregnant cows produced less

milk fat (0.06 kg/cow per day) and milk protein (0.04 kg/cow per day) compared with their non-pregnant twins.

In conclusion, milk fat concentration increased linearly from week 2 to 22 while protein showed the opposite pattern in dairy buffaloes. SNF and lactose showed a similar pattern of an initial decrease up to week 14 and but increased later on. This study reflects a deterioration of milk quality in dairy buffaloes with advancing pregnancy.

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