Multivariate of physicochemical properties of daily milk throughout milking seasons on Syrian buffaloes (*Bubalus Bubalis*)

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Abstract

Buffalo milk is essential for both human health and manufacturing processes, making it important to improve its productivity and quality. This study applies multivariate analysis to evaluate variations in daily milk yield and its constituents across different milking seasons. The results show that milking seasons significantly affect daily milk and its constituents. Milk, fat, and non-fat solids on test day have the highest variances. Covariances between errors match those of daily milk and its constituents. The highest covariances are between daily milk and non-fat solids, followed by fat, lactose, and protein masses. Most correlations are strong and positive, but there are notable negative correlations between daily milk and fat. Milk density and fat-to-protein ratio have strong negative correlations with fat and protein masses, respectively. Wilks' Lambda, Pillai's trace, Hotelling-Lawley Trace, and Roy's Root indicate significant group variances in multivariate traits related to dependent variables. This study concludes that Syrian Buffalo milk undergoes notable seasonal variations in its physicochemical properties, highlighting the importance of seasonal factors in enhancing daily milk and its quality. Buffalo breeders can improve productivity and benefit the dairy industry by collaborating with nutritionists and selecting animals for milk quality.

Keywords: Physicochemical properties; Seasonal variations; Daily milk; Buffaloes.

Introduction

The global population of buffalo (Bubalus bubalis) stands at approximately 168 million, with over 95% of these animals residing in Asia (Borghese and Mazzi, 2005). Buffaloes are adaptable to various agro-climatic zones and serve as a vital resource for milk production and rural livelihoods across diverse regions, including the arid regions of India (Patel et al., 2016) and the mountainous areas of Nepal (Gautam et al., 2021). Syria is home to approximately 8,000 buffaloes, which contribute more than 620 tons of meat and 6,195 tons of dairy products (FAO, 2018). In Syria, the Al-Ghab Plain is considered an important economic area for food production and buffalo breeding.

According to Javaid et al., (2009), milk possesses significant nutritional value owing to its abundance of essential constituents. To maintain milk quality, it is crucial to determine its fat and non-fat solids content. The rising demand for Buffalo milk can be attributed to its high fat content, substantial calcium content, and low cholesterol levels, as observed by Bilal et al., (2006). Fats and proteins are key constituents of milk solids, and play a vital role in numerous manufacturing processes, as highlighted by Tonhati et al., (2011). Additionally, milk production is considered suitable in terms of quality and sustainability, as Oliveira et al., (2011) stated. Milk is a nutritious and versatile food essential for maintaining good health and producing high-quality dairy products.

For Syrian Buffaloes, calf mortality is high but can be reduced through good management (Al-Najjar, 2022). The season significantly influences the growth traits of Buffalo calves (Elsayed et al., 2021). In Syria, Buffaloes play a crucial role in milk production, as the quality of Buffalo milk is important to both farmers and consumers in general. To gain a deeper understanding of Buffalo productivity, it is necessary to evaluate milk production and its components in the Buffalo herd at the Shatiha Research Station in the Al

-Ghab/Hama area of Syria. Evaluating milk production and its components is essential for understanding the Syrian productivity of Buffaloes.

Seasonal fluctuations in milk constituents of Syrian Buffaloes offer opportunities for improving quality and production. This study measures milk constituent variance and covariance during milking seasons to determine whether they vary between the seasons.

Materials and Methods

Herd of Buffaloes

Al-Ghab Plain is a fertile agricultural region in Syria that provides sustenance and income for the local population. It is situated at 35° latitude and 62° longitude, with an elevation of 270 meters above sea level, and it spans an area of 1,200 square kilometers. The Orontes River flows through the region, forming marshes that have been transformed into irrigated arable land. The temperate climate with four distinct seasons is ideal for breeding Syrian Buffaloes.

Syrian Buffaloes are bred for milk and meat. They roam freely in semi-open yards at the Shetiha station, grazing from morning until evening and cooling off in a pond during the afternoon. At night, they are sheltered in semi-open sheds. Females are milked twice a day, with the calf nearby.

Buffaloes consume a diet of green fodder and concentrated feed, with the amount of concentrated feed varying depending on the individual animal's needs. During milking, concentrated feed is given to support the process. Buffaloes consume about 20 kg of green fodder and 4.5 kg of concentrated feed daily. This feed mixture contains about 12.3% protein and has a TDN value of 64.1%. Buffaloes have continuous access to drinking water.

Samples and editing data

A random sample of 30 Buffaloes at the Shatiha station was selected to measure the daily milk production every two weeks for each Buffalo throughout the milking period in the year 2018. Immediately post-milking, the daily milk yield was weighed in kilograms on an accurate scale, and the volume was measured in liters. Samples of 250 ml of milk were taken under sterile conditions and transported to the laboratory at 4°C.

A milk testing device was employed to analyze 420 milk samples, determining the percentages of milk constituents such as fat, protein, lactose, non-fat solids, and ash, as well as milk density (kilogram /liter). The percentages of milk constituents were converted to masses using the following equation: $\boldsymbol{Z_i} = \left(\frac{\boldsymbol{X_i}}{\boldsymbol{100}}\right) \times \boldsymbol{Y_i}$; where: Z_i represents the mass of (fat, protein, lactose, non-fat solids, and ash) for the ith record. X_i = stands for the percentages of fat, protein, lactose, non-fat solids, and ash for the ith record. Yi signifies the daily milk mass.

Statistical analysis

Data was analyzed using the SAS (2012) program with the generalization of the simple linear regression model. The analysis was conducted to determine the variance and covariance of daily milk and its constituents across milking seasons, utilizing the following multi-traits model. $\boldsymbol{Y} = \boldsymbol{X\beta} + \boldsymbol{\epsilon}$ **(Model, 1**), where Y = a vector-representing observation for traits: daily milk yield, fat, protein, fat-to-protein ratio, lactose, non-fat solids, ash (kilograms), density of milk (kilograms/ liters), and daily milk (liters). X = a matrix of the independent variable including seasons (spring, summer, autumn, and winter). β = a vector of the model parameters. ϵ = a vector of the error terms effects with mean 0 and variance $\sigma^2 e$. Multiple range test was used to identify significant differences between the means of factor effects (Duncan, 1955).

Use the Wilks' Lambda statistic: $\Lambda = \frac{|\mathrm{w}|}{|\mathrm{W+B}|}$ (Model, 2), where $|\mathrm{w}|$ = the determinant of the within-group (error) covariance matrix, $|\mathrm{W} + \mathrm{B}|$ = the determinant of a total covariance matrix, which combines within-group and between-group covariance. Pillai's Trace statistic: $V = \sum_{i=1}^{k} \frac{n_{i-k}}{k(k-1)} \frac{|T_i|^2}{n_i - 1}$ (Model, 3), where k = a number of groups in the independent variable. n_i = a number of observations in ith group. $|T_i|^2$ = a vector of differences in means of dependent variables for the ith group compared to the overall mean. Hotelling-Lawley Trace statistic: $H = \frac{|W - W_0|}{W_0}$ (Model, 4), where W = the determinant of the estimated covariance matrix for all groups combined. W_0 = the determinant of the estimated covariance matrix for the null hypothesis (usually assuming no group differences). Roy's Great Root equation: $\ell\_1 >$ t_α (Model, 5), where ℓ_1 = the largest root of the characteristic equation of test statistic. t_α = the upper α quantile of T distribution with degrees of freedom equal to the difference in degrees of freedom between null and alternative hypotheses. Roy's Great Root Test is a statistical method for comparing covariance matrices. To perform this test, characteristic equations were found for the test statistics, which were done using the formula, $\det(T - \lambda \mathrm{I}) = 0$, where T = test statistic, λ = eigenvalue, and I = identity matrix.

Results and Discussion

Table (1) illustrates the significant impact of milking seasons on milk yields and component masses. This emphasizes the importance of breeders partnering with animal nutrition experts to create customized feeding plans for various milking seasons.

Notably, milk and fat productivity exhibit the highest variability based on F values, underscoring the priority for breeders to enhance these traits during milking seasons.

Environmental conditions are a crucial factor influencing the productivity of Buffalo (Vasantha et al., 2021). Addressing challenging environmental conditions holds significant implications for Buffalo breeding programs (Ayad et al., 2022). The season exerts a considerable influence on milk productivity in Egyptian Buffaloes, with winter-born calves exhibiting the highest milk production, while summer-born calves show the lowest (Fathy et al., 2023). For Syrian Buffaloes, Fatima et al., (2023) confirmed that milk production and composition are affected by seasonal variations, and implementing better management practices could enhance milk quality. Al-Momani et al., (2023) also suggested that improvements in environmental factors have a positive impact on the components of Syrian Buffalo milk. The applied utility of these results is broad and can benefit Buffalo breeders by providing insights into the influence of milking seasons and environmental conditions on Buffalo productivity, enabling more informed decision-making, and the development of strategies to optimize milk production and quality.

Table (2) shows that Syrian buffaloes produced the largest amount of milk, non-fat solids, and ash masses on daily milk in the spring. This is due to factors such as improved forage quality, hormonal changes, seasonal rearing patterns, and environmental comfort (Bei-Zhong et al., 2007). The observed increase in the amounts of fat, protein, and lactose during the spring and winter seasons can be attributed to seasonal diet changes, better body condition, and the potential influence of cooler temperatures (Chakraborty et al., 2021). Milk density is highest in autumn because cows have high-fat diets and increased prolactin production (Parmar et al., 2020). The lowest fat-to-protein ratio is in the summer due to changes in diet and increased activity. Buffaloes feed more fresh plants with lower fat content and higher protein, and are more active due to warm weather and longer daylight hours (Michele et al., 2019). Buffalo milk's composition shifts with seasons due to environment, management, and Buffaloes' seasonal responses (Aspilcueta-Borquis et al., 2010). Fat-to-protein ratio and milk density in Buffaloes vary with seasons due to diet, hormones, and environment. Temperature, food, and breed help seasonal changes in

Buffalo milk fat and density (Coroian et al., 2013). Ideally, milk's fat and protein content should be balanced (1.0-1.2 ratio). Deviations might signal health or in cows. (Cabezas-Garcia et al., 2021).

Table 1: Analysis variance of some milk traits across milking seasons on Syrian Buffaloes (Model 1)

Milk traits	MS	MSE	F Value	Pr > F
Fat (Kg)	0.359417	0.009596	37.45	0.0001
Protein (Kg)	0.053423	0.002405	22.21	0.0001
Fat to protein ratio	3.500930	0.189303	18.49	0.0001
Lactose (Kg)	0.069216	0.003686	18.77	0.0001
Non-fat solids (Kg)	0.339031	0.012526	27.06	0.0001
Ash (Kg)	0.003618	0.000095	28.01	0.0001
Density of milk (Kg/ L)	0.000028	0.000003	8.61	0.0001
Daily Milk (Kg)	52.668788	1.445047	36.45	0.0001
Daily Milk (L)	50.108687	1.372170	36.52	0.0001

MS = Mean Square; MSE = Mean Square Error; Kg = kilogram; L = liter; Freedom degree for milk traits and errors were 416 and 3, respectively.

Table 2: Least-square means and standard errors of some milk traits/ Kg for Syrian buffaloes.

Milk traits	**Spring**	**Summer**	**Autumn**	**Winter**	**Mean**
Fat (Kg)	0.320^{a} (0.012)	0.198^{b} (0.011)	0.219^{b} (0.013)	0.302^{a} (0.008)	0.266 (0.005)
Protein (Kg)	0.175^{a} (0.006)	0.142^{b} (0.005)	0.125^{c} (0.007)	0.168^{a} (0.004)	0.155 (0.003)
Fat to Protein	1.836^{a} (0.046)	1.438^{b} (0.045)	1.748^{a} (0.047)	1.839^{a} (0.036)	1.733 (0.022)
Lactose (Kg)	0.214^{a} (0.004)	0.172^{b} (0.006)	0.168^{b} (0.007)	0.215^{a} (0.005)	0.195 (0.003)
Non-fat solids (Kg)	0.436^{a} (0.013)	0.325^{c} (0.012)	0.315^{c} (0.011)	0.405^{b} (0.009)	0.376 (0.005)
Ash (Kg)	0.039^{a} (0.001)	0.027^{c} (0.0011)	0.026^{c} (0.0010)	0.035^{b} (0.0007)	0.032 (0.0005)
Density of milk (Kg/ Liter)	1.026b^{c} (0.0001)	1.027^{ab} (0.0002)	1.028^{a} (0.0003)	1.025^{c} (0.0001)	1.027 (0.0001)
Daily milk (Kg)	5.119^{a} (0.127)	3.699^{c} (0.126)	3.528^{c} (0.125)	4.578^{b} (0.098)	4.281 (0.066)
Daily milk (Liter)	4.985^{a} (0.123)	3.602^{c} (0.122)	3.434^{c} (0.121)	4.461^{b} (0.095)	4.169 (0.064)

Values in parentheses = standard errors; Number of records = 402; Kg = kilogram

Table 3: Multivariate analysis of covariance was estimated for milk traits (above the diagonal) and errors (below the diagonal) across seasons using the type III SSCP matrix (Model, 1)

Traits of milk	F	PRO	FPR	LAC	NFS	ASH	DEN	DMG	DML
F		0.377	2.826	0.458	1.019	0.103	-0.007	12.463	12.160
PRO	1.474		0.700	0.175	0.392	0.040	-0.003	4.850	4.733
FPR	9.321	-1.155		1.0745	2.279	0.225	-0.016	26.940	26.278
LAC	1.840	1.047	0.006		0.445	0.044	-0.003	5.385	5.257
NFS	3.498	1.864	1.681	2.456		0.104	-0.007	12.616	12.308
ASH	0.306	0.163	0.113	0.219	0.405		-0.001	1.309	1.277
DEN	-0.008	-0.003	-0.019	-0.002	-0.009	-0.001		-0.120	-0.085
DMG	40.848	21.671	18.174	28.183	52.269	4.599	-0.087		154.117
DML	39.805	21.112	17.802	27.447	50.932	4.481	-0.122	585.776	

F = Fat; Pro = Protein; FPR = Fat to Protein ratio; Lac = lactose; NFS = Non-fat Solids; Den = Density of Milk; DMG = Daily Milk (Kg); DML = Daily Milk (Liter).

Table 4: Multivariate analysis of variance was estimated for milk traits and errors across seasons using the type III SSCP matrix (Model 1)

Variances	F	PRO	FPR	LAC	NFS	ASH	DEN	DMG	DML
Traits of milk	1.078	0.160	10.502	0.207	1.017	0.010	0.0001	158.006	150.326
Errors	3.992	1.001	78.750	1.533	5.211	0.039	0.001	601.146	570.823

F = Fat; Pro = Protein; FPR = Fat to Protein ratio; LAC = lactose; NFS = Non-fat Solids; DEN = Density of Milk; DMG = Daily Milk (Kg); DML = Daily Milk (Liter).

Table 5: Manova test criteria and F approximations for the hypothesis of no overall season effect; H = type III SSCP matrix for seasons; E = error SSCP matrix (Models, 2, 3, 4, and 5).

Statistical equations	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.502	11.74	27	1192.2	0.0001
Pillai's Trace	0.591	11.20	27	1230	0.0001
Hotelling-Lawley Trace	0.811	12.23	27	902.51	0.0001
Roy's Greatest Root	0.512	23.33	9	410	0.0001

F Value: F-statistic associated with the test; Num DF: The number of groups being compared in the test; Den DF: Degrees of freedom are associated with the residual variation in the test.

Errors covariances align with studied trait covariances. The most significant covariances were found between daily milk with fat-to-protein, non-fat solids, and fat masses in that order (Table 3). These core constituents closely track variations in daily milk, offering a valuable tool for dairy female breeding programs. Breeders can select females with high milk production, elevated fat content, and increased non-fat solid levels for improved breeding outcomes. This knowledge enables better dairy herd management for a consistent year-round milk supply (Chashnidel et al., 2007; Hyder et al., 2007; Iloeje et al., 1981).

Variances of daily milk and its constituents with errors are consistent (Table 4). Daily milk, fat-to-protein, fats, and non-fat solids show the highest variations, with environmental and dietary factors influencing daily milk and its constituents. Milk variances, influenced by factors like season of calving, offer opportunities to improve Buffalo productivity and herd management (Kumar et al., 2023; Caccamo et al., 2008).

Wilks' Lambda and Pillai's effects indicate a significant difference in the overall variance between groups of variables with multivariate traits. Additionally, the Hotelling-Lawley Trace value provides strong evidence of significant variance between the groups of variables being compared in terms of dependent variables. Furthermore, Roy's root value is of great importance, serving as a significant indicator of the presence of variance between the groups of variables being studied with dependent variables (Table 5). These findings suggest that the milking seasons have a significant effect on the daily milk and its constituents in Syrian Buffaloes.

Wilks' Lambda, Pillai's Trace, and Roy's Greatest Root tests show similar results, indicating a consistent effect of milking season on milk's physical and chemical properties. However, the Hotelling-Lawley Trace had a higher value, suggesting it was more sensitive to detecting differences between dependent variables.

Asif and Naeem (2011) show that Wilk's lambda test of the physicochemical properties of Buffalo milk is statistically significant, which is useful in monitoring milk quality and food safety in Pakistan. This finding agrees with our results. On the other hand, Fathy et al., (2023) found no significant differences in milk production and lactation length across seasons in Egyptian Buffalo using Wilks lambda, Pillai’s Trace, Hotelling’s Trace tests, and Roy’s Largest Root, contradicting our results. Conducting studies on different breeds of Buffalo, in different environments, or using different methods to collect and analyze data. All of these factors can contribute to different results.

Table (6) shows that most correlations were highly significant and positive. This is likely due to the characteristics of daily milk. Conversely, there were significant negative correlations between daily milk and fat with milk density. This is likely due to the composition and storage of daily milk in the udder affected by feeding practices. This finding agrees with Fatima et al., (2023), who emphasize the negative correlation between daily milk and fat in Syrian Buffaloes. Milk protein and fat-to-protein (FPR) are correlated negatively, meaning as protein increases, FPR decreases. Dairy farmers to indicate energy balance, select animals for milk production traits, and manage dairy animal rations. The correlation between protein and FPR could be used to manage dairy cow rations. By feeding cows a ration that is high in protein, farmers can help to reduce the FPR of their milk. This can improve the overall quality of the milk and make it more valuable to processors.

Gordon et al., (2021) show a relationship between FPR in milk, physiological changes, and energy balance during early lactation. However, FPR is not a robust indicator of energy balance at an individual animal level due to large animal-to-animal variation. The FPR in milk is lowest in summer due to ketosis and reduces acidosis (Fatima et al., 2023). This provides information about nutritional diseases such as ketosis and acidosis (Nicholas et al., 2015). Additionally, milk fat/protein ratios fluctuate due to multiple factors, and farmers must rely on clinical signs, diagnostic tests, and comprehensive health monitoring to manage animal conditions (Atalay, 2019).

Table 6: Partial correlation coefficients between daily milk and some constituents of Syrian Buffaloes milk were calculated from the error SSCP matrix Prob.>|r|. (Model 1).

Milk traits	F (Kg)	Pro (Kg)	FPR	LAC (Kg)	NFS (Kg)	Ash (Kg)	DEN (Kg /L)	DMG (Kg)
Pro	0.74**							
FPR	0.53**	-0.13**						
LAC	0.74**	0.84**	0.001					
NFS	0.76**	0.81**	0.08	0.87**				
Ash	0.77**	0.82**	0.06	0.89**	0.89**			
DEN	-0.11**	-0.08	-0.05	-0.04	0.11**	0.12**		
DMG (Kg)	0.83**	0.89**	0.08	0.92**	0.93**	0.94**	-0.13**	
DML (L)	0.84**	0.88**	0.09	0.93**	0.94**	0.95**	-0.14**	0.99**

F = Fat; Pro = Protein; FPR = Fat to Protein ratio; LAC = lactose; NFS = Non-fat Solids; DEN = Density of Milk; DMG = Daily Milk (Kg); DML = Daily Milk (L); Kg = kilogram; L = Liter. DF = 416.

Conclusions

The study affirms seasonal variations in Syrian buffalo milk production, peaking in winter and decreasing in summer. Significant fluctuations are observed in milk constituents, with strong negative correlations between daily milk fat and density, and milk protein and fat-to-protein ratio. The correlation between daily milk and fat content enables potential improvements in milk production and quality, benefiting farmers, consumers, and the overall dairy industry. These findings emphasize the need to consider seasonal factors to enhance Syrian buffalo milk production and quality.

Recommendations

Buffalo breeders should collaborate with nutritionists for custom feeding plans, address environmental challenges, and select animals for milk quality to increase productivity, benefiting both breeders and the dairy industry.

Author Contributions

All authors made equivalent contributions to the first draft of the manuscript and then participated equally in reviewing and approving the final published version.

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Conflict of Interest

The authors have no financial or non-financial interests that could influence this research.

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