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AMINO ACID PROFILING AND PROTEIN CONTENT DETERMINATION OF BOVINE MILK COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT

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ABSTRACT: Milk and milk products are nutritious food items containing numerous essential nutrients. This study was carried out to determine the influence of variable season on profiling of amino acid and protein content from 90 bovine milk collected (March 2018 to February 2019) samples belongs to four tehsils of Singrauli district. The most abundant amino acid was glutamic acid, followed by proline and leucine. Concentration of amino acid in cow milk was found to be in order of Glycine < histidine < alanine < methionine < isoleucine < phenylalanine < threonine < tyrosine < serine < lysine < valine and aspartic acid < arginine < leucine < proline < glutamic acid. Amino acid status in buffalo milk sample was found in order Glycine < histidine < methionine < alanine < phenylalanine < isoleucine < threonine < arginine < tyrosine < serine and lysine < aspartic acid < valine < leucine < proline < glutamic acid. Average protein content comparison, results, in rainy season, maximum protein content was in Deosar and minimum in Singrauli, In winter protein is higher in Waidhan and minimum in Deosar and maximum protein content was recorded intended for Waidhan and minimum for Deosar in summer. Protein content in cow and buffalo milk was compared seasonally; the average protein content of cow ($3.27 \pm 0.15\%$) and in buffalo ($4.15 \pm 0.16\%$) milk was high in winter followed by rainy and summer.

INTRODUCTION: Milk is one of the most nutritionally complete foods. Bovine milk has long been the prime milk source for human consumption. Milk contains tremendous macro and micronutrients and therefore can play a significant role in serving individuals to meet their nutritional requirements.

Among milk components, proteins are the most important constituents of the human diet, contributing important nutritional, biological and functional properties. Milk is nearly an immaculate natural food consumed comprehensively around the globe for all phases of life ¹. Other than nutrition, some specific milk proteins take part in the early development of immune response and non-immunological defense ².

Since recent years, milk and milk products such as cheese, yogurt, and milk powder have increased as it plays a vital role in human nutrition ³. Regular milk consumption has been widely recommended as bovine milk contains various essential nutrients

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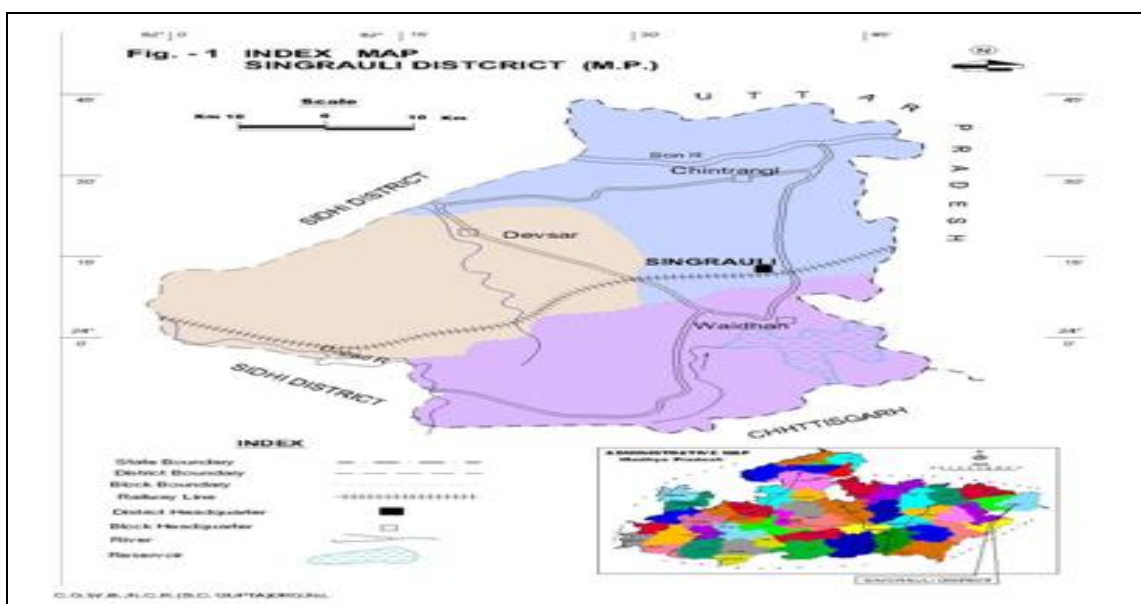
that are critical to maintaining the healthy life of each individual, including humans⁴. Milk allergy is the most frequent food allergy in babyhood along often persists from side to side adult age, forcing an individual to an absolute removal diet. Milk proteins are in attendance in uncounted manufactured goods^{5, 6}. In human nutrition, the amino acid profile of caseins and whey proteins occupies a unique position.

These proteins are ranked as quality proteins with the highest biological value, good digestibility (98%), rapid absorption, and utilization in the body. Specifically, casein is an incredibly efficient nutrient supply owing to its providing a sustained and slow release of amino acids into bloodstream⁷. Branched-chain amino acids (BCAAs) present in the highest concentration in milk proteins are important for the maintenance of tissue growth, repair and prevention of catabolic actions during exercise. Similarly, the amino acid cysteine enhances glutathione levels, exhibits strong antioxidant properties and assists the body in combating various diseases⁸. Milk of ruminant species consisting homologous proteins having similar structure and bioactive features⁹. In addition, milk proteins are recognized for their applicability in sports nutrition, baked goods, salad dressings, emulsifiers, infant formulas and medical,

nutritional formulas. Milk plays a vital role in building a healthy society and can be used as a vehicle for rural development. Kinds of milk of various species differ quantitatively and qualitatively according to constituents. Buffalo and cow are the top milk-producing animals in India that can contribute 62% and 34% milk production, respectively¹⁰. Therefore, the present research work was carried out on amino acid profiling of bovine milk (cow and buffalo).

MATERIALS AND METHODS:

Study Area: Present study areas include four different tahsils (Chitrangi, Waidhan, Devsar, and Singrauli) of Singrauli district of Madhya Pradesh, India, which covers a region comprising of the eastern part of the Sidhi district also. Singrauli is fast emerging as an energy hub of India, especially for electric power and coal, and therefore locally, it is also called Urjanchal. Energy generating industries in the city are like Singrauli Super Power Plant (SSPP), Vindhyanchal Super Thermal Power Plant (VSTPP), Northen Coal Limited (NCL), etc. Study area is well known for their higher pollutant especially those which directly release through power generation Industries and was assessed for heavy metal contamination in vegetables, fruits etc but not yet explored for milk contamination.



Collection of Milk Samples: Ninety fresh samples of buffalo and cow (45 samples of each species) were collected in sterile glass bottles from the Dairy Farm and local vendors of four different

tehsils of the Singrauli district. These samples were labeled, ice-packed, and transported to the laboratory. All milk samples were then placed in the refrigerator at 4 °C for further analysis.

Amino Acid Profiling: Sample preparation was done by the method of Moore and Stein (1963)⁷ with some modification. 5g of samples were hydrolyzed with 6NHCl in an electric oven at 110 ± 1 °C for 24 h. After hydrolysis, the sample hydrolysate was made free of excess HCl by the repeated evaporation of the hydrolysate under vacuum over NaOH pellets. Finally, 5 ml water was added to dry samples, and the process of evacuation was repeated twice for the removal of acid. The dried residues were then dissolved in a known quantity of 0.02N sodium acetate buffer (pH 2.20), were decolorized by adding a pinch of activated charcoal, and then filtered through Whatman 42 filter paper to obtain a clean solution of the hydrolysate. An aliquot of hydrolysate was injected into the column (Shim-pack ISC-07/S1504 Na) of the higher performance liquid chromatography based amino acid analyzer (RF-10AXL, Shimadzu Corporation, Tokyo, Japan).

RESULT AND DISCUSSION: Food safety of milk and dairy products is a prerequisite for consumption, which must be free from physical, biological and chemical contamination. Chemical contamination of heavy metals is generally derived

from the environment, such as from water, grass, and feed additives. In the present investigation, the established thermal power plants consume a large amount of coal to generate electricity. Due to this coal combustion, a significant quantity and variety of trace elements, some potentially toxic, are transferred to the nearby location through diverse pathways^{11, 12}. In milk, both essential and non-essential amino acid content was determined in cow and buffalo milk samples collected from a tehsil of Singrauli, and the average concentration of individual amino acids was presented graphically in **Fig. 1**.

It was observed that most of the amino acid concentration was high in cow milk compared to buffalo milk. Amount of glutamic acid (22.70 ± 0.17 g/ 100 g) was maximum in both cow (22.70 ± 0.17 g/ 100 g) and buffalo (20.73 ± 0.21 g/ 100 g) milk followed by proline (12.77 ± 0.12 g/ 100 g in cow and 10.70 ± 0.10 g/ 100 g in buffalo) while glycine was present in minimum concentration in both cow (2.03 ± 0.06 g/ 100 g) and buffalo (1.49 ± 0.01 g/ 100 g). The contamination in milk is considered as one of the main dangerous aspects within the last few years.

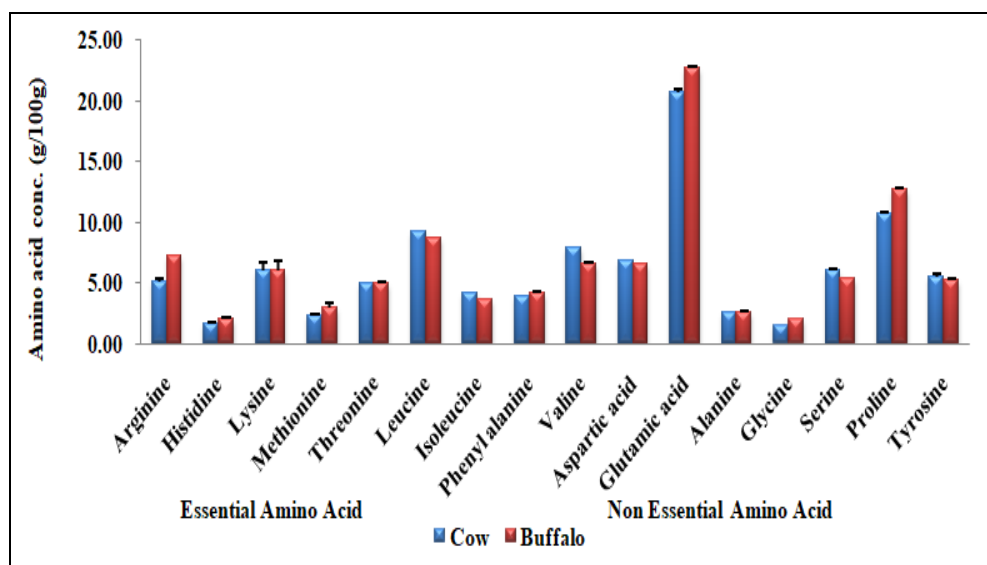


FIG. 1: AVERAGE CONCENTRATION OF AMINO ACIDS IN BOVINE MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT DURING DIFFERENT SEASONS. VALUES WERE EXPRESSED IN MEAN (\pm SD).

Chemical residues which could contaminate milk and milk products include environmental and anthropological factors¹³. Milk composition and factors affecting it in dairy were significantly suggested in the case of buffaloes milk¹⁴. Status of

amino acid in cow milk was found to be in order of Glycine < histidine < alanine < methionine < isoleucine < phenylalanine < threonine < tyrosine < serine < lysine < valine and aspartic acid < arginine < leucine < proline < glutamic acid. Conc. of amino

acid in buffalo milk sample was found in order Glycine < histidine < methionine < alanine < phenylalanine < isoleucine < threonine < arginine < tyrosine < serine and lysine < aspartic acid < valine < leucine < proline < glutamic acid. Milk contamination issues are an extreme concern since milk and milk products have a very special diet of infants, children, and the elderly for whom these are considered perfect natural food. Monitoring of bovine milk samples from different places of the Bundelkhand region in India explores the status of pesticide residues^{15, 16}. The result of the effect of seasonal variation on the amount of amino acid in bovine milk revealed that concentration of

histidine, leucine, isoleucine, phenylalanine, aspartic acid, alanine, glycine, and serine in buffalo milk is almost the same during rainy, winter and summer season **Table 1, 3**. The concentration of arginine, lysine, methionine, and threonine is more in winter season i.e 5.32 g/100g, 6.5 g/ 100 g, 2.42 g/ 100 g and 5 g/ 100 g respectively while was least in summer 5 g/ 100 g, 5.3 g/ 100 g, 2.2 g/ 100 g and 4.85 g/ 100 g respectively. The concentration of glutamic acid (20.8 g/ 100 g), proline (10.8 g/ 100 g), and tyrosine (5.7 g/ 100 g) were found to be maximum during the rainy season, while the concentration of valine (7.9 g/ 100 g) was same during both rainy and winter season.

TABLE 1: EFFECT OF SEASONAL VARIATION ON CONCENTRATION OF AMINO ACID IN BUFFALO MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT. VALUES WERE EXPRESSED IN MEAN (\pm SD).

The concentration of valine (7.84 g/ 100 g) and

Amino acids	Rainy	Winter	Summer
Arginine	5.2 \pm 0.32	5.32 \pm 0.17	5 \pm 0.28
Histidine	1.7 \pm 0.18	1.7 \pm 0.23	1.6 \pm 0.1
Lysine	6.4 \pm 0.21	6.5 \pm 0.15	5.3 \pm 0.14
Methionine	2.3 \pm 0.12	2.42 \pm 0.24	2.28 \pm 0.21
Threonine	4.9 \pm 0.24	5 \pm 0.18	4.85 \pm 0.25
Leucine	9.2 \pm 0.12	9.25 \pm 0.4	9.2 \pm 0.34
Isoleucine	4.1 \pm 0.27	4.2 \pm 0.1	4.12 \pm 0.2
Phenyl alanine	3.9 \pm 0.36	3.8 \pm 0.28	3.9 \pm 0.14
Valine	7.9 \pm 0.62	7.9 \pm 0.51	7.84 \pm 0.23
Aspartic acid	6.8 \pm 0.49	6.82 \pm 0.12	6.8 \pm 0.4
Glutamic acid	20.9 \pm 0.57	20.8 \pm 0.29	20.5 \pm 0.44
Alanine	2.6 \pm 0.12	2.6 \pm 0.1	2.6 \pm 0.22
Glycine	1.5 \pm 0.09	1.48 \pm 0.13	1.5 \pm 0.15
Serine	6.1 \pm 0.43	6 \pm 0.32	6.1 \pm 0.35
Proline	10.8 \pm 0.49	10.6 \pm 0.37	10.7 \pm 0.3
Tyrosine	5.7 \pm 0.35	5.4 \pm 0.26	5.6 \pm 0.18

TABLE 2: EFFECT OF SEASONAL VARIATION ON CONCENTRATION OF AMINO ACID IN COW MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT. VALUES WERE EXPRESSED IN MEAN (\pm SD).

Amino acids	Rainy	Winter	Summer
Arginine	7.3 \pm 0.3	7.3 \pm 0.18	7.28 \pm 0.21
Histidine	2.2 \pm 0.1	2 \pm 0.2	2 \pm 0.3
Lysine	5.2 \pm 0.25	6.7 \pm 0.3	6.4 \pm 0.2
Methionine	2.8 \pm 0.15	3.5 \pm 0.24	2.82 \pm 0.14
Threonine	5.1 \pm 0.2	4.94 \pm 0.29	5.0 \pm 0.32
Leucine	8.6 \pm 0.39	8.68 \pm 0.21	8.68 \pm 0.1
Isoleucine	3.7 \pm 0.12	3.67 \pm 0.31	3.7 \pm 0.23
Phenylalanine	4.2 \pm 0.1	4.3 \pm 0.42	4.14 \pm 0.17
Valine	6.5 \pm 0.3	6.8 \pm 0.27	6.42 \pm 0.28
Aspartic acid	6.6 \pm 0.45	6.6 \pm 0.23	6.5 \pm 0.1
Glutamic acid	22.6 \pm 0.51	22.9 \pm 0.47	22.5 \pm 0.32
Alanine	2.7 \pm 0.15	2.6 \pm 0.17	2.63 \pm 0.07
Glycine	2 \pm 0.1	2 \pm 0.12	2.1 \pm 0.19
Serine	5.3 \pm 0.41	5.4 \pm 0.22	5.3 \pm 0.38
Proline	12.7 \pm 0.6	12.9 \pm 0.42	12.7 \pm 0.25
Tyrosine	5.2 \pm 0.27	5.4 \pm 0.38	5.2 \pm 0.42

Glutamic acid (20.5 g/ 100 g) was found to be minimum in the summer season, while the concentration of proline (10.6 g/ 100 g) and tyrosine (5.4 g/ 100 g) was minimum in the winter season **Fig. 1, 3**. In parts of Singrauli, fly ash lies in piles five feet thick, consists of fine particles of Ca, S, Mg and Si along with toxic elements like Hg, Pb, As, Se and Cd. These heavy metals can leach into groundwater and soil, causes acid rain, and affect human health through inhalation and through dietary involvement, mostly obtain a dietary product of animals¹⁷. In cow milk, most of the amino acid reported their maximum concentration in the winter season **Table 2**. The concentration of

arginine (7.3 g/ 100 g), aspartic acid (6.6 g/ 100 g), and glycine (2.0 g/ 100 g) were found to be the same during the rainy and winter season while the status of histidine (2.0 g/ 100 g) and leucine (8.68 g/ 100 g) was same during winter and summer season. The concentration of isoleucine (3.7 g/ 100 g), glutamic acid (22.6 g/ 100 g), glycine (2.0 g/ 100 g), serine (5.3 g/ 100 g), proline (12.7 g/ 100 g), and tyrosine (5.2 g/ 100 g) was found to be same during rainy and summer season while there was a slight variation in concentration of the rest of amino acid in rainy, winter and summer season **Table 1 and 2**.

TABLE 3: SEASONAL VARIATION IN PROTEIN CONTENT (%) OF BOVINE MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT.

Sampling Site	Rainy		Winter		Summer	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
Waidhan						
A1	3.32	4.2	3.35	4.25	3.08	3.87
A2	3.14	4.18	3.1	4.34	3.24	3.92
A3	2.98	4.07	3.38	4.12	3.14	3.76
A4	3.17	3.95	3.42	4.02	3.26	4.27
A5	3.12	4.45	3.47	4.37	2.97	3.95
A6	3.38	4.1	3.32	4.21	3.28	4.18
Mean	3.19	4.16	3.34	4.22	3.16	3.99
SD	0.14	0.17	0.13	0.13	0.12	0.19
Chitrangi						
B1	3.14	4.12	3.29	3.91	3.15	3.82
B2	3.36	3.98	3.34	4.35	3.39	3.95
B3	3.1	3.95	3.25	4.24	3.29	3.72
Mean	3.20	4.02	3.29	4.17	3.28	3.83
SD	0.14	0.09	0.05	0.23	0.12	0.12
Deosar						
C1	3.15	4.02	3.04	4.32	3	3.56
C2	3.28	4.1	3.36	4.01	2.92	3.68
C3	3.67	3.99	3.14	3.89	2.87	3.52
Mean	3.37	4.04	3.18	4.07	2.93	3.59
SD	0.27	0.06	0.16	0.22	0.07	0.08
Singrauli						
D1	3.31	3.72	2.97	4.09	3.18	3.41
D2	3.36	3.58	3.4	4.12	2.96	3.67
D3	3.24	3.87	3.27	4.05	3.42	3.43
Mean	3.30	3.72	3.21	4.09	3.19	3.50
SD	0.06	0.15	0.22	0.04	0.23	0.14

Results of the present study revealed that glutamic acid amino acid was the present in greatest concentration in both cow and buffalo milk, followed by proline and leucine. This conclusion was consistent with previous also studies¹⁸. Glutamic acid to be one of the most abundant amino acids in bovine milk samples, whereas Leucine plays a distinct role in protein metabolism and the translation initiation pathway of muscle

protein synthesis¹⁹. It is also involved in reversible phosphorylation of proteins that control mRNA binding to the 40S ribosomal subunit²⁰.

Protein Content in Bovine Milk: The protein content of the bovine milk sample collected during different seasons of selected sites was presented in **Table 3**. As per PFA, the protein content of cow and buffalo milk sample should be 3.3-3.4% and

3.9-4.3%, respectively. Results revealed that in Waidhan Tehsil, the highest protein content was found during the winter season in cow milk sample (3.47%) and during the rainy season in buffalo milk sample (4.45%) at site A5, respectively. The average protein content of cow milk in rainy, winter, and summer season was 3.19 ± 0.14 %, 3.34 ± 0.13 % and 3.16 ± 0.12 %, respectively, and of Buffalo, milk sample was 4.16 ± 0.17 %, 4.22 ± 0.13 %, and 3.99 ± 0.19 %, respectively. The sources of variation of detailed protein composition predicted from infrared spectra in the milk of dairy furthermore double-principle cattle breeds were also suggested. Results showed that protein fractions were primarily influenced by days in

milk, portions of protein-related through crude protein, total casein, and fat in addition to milk nitrogen. In point of view, mid-infrared calculations of milk portions could be helpful designed for the dairy division to pick up dietary properties of milk^{20, 22}. In Chitrangi tehsil, **Table 3**, the maximum protein content was found during the summer season in cow milk (3.39%) and during winter season in buffalo milk (4.35%) sample at site B2. The average protein content of cow milk in rainy, winter, and summer season was 3.2 ± 0.14 %, 3.29 ± 0.04 %, and 3.27 ± 0.12 %, respectively, and in Buffalo milk sample was 4.01 ± 0.09 %, 4.16 ± 0.22 %, and 3.83 ± 0.11 %, respectively.

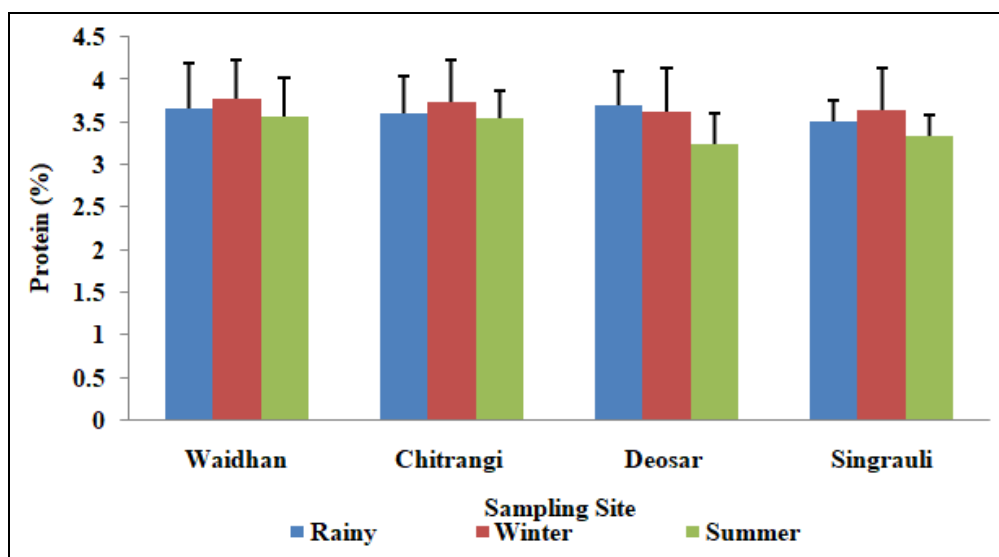


FIG. 2: COMPARISON OF AVERAGE PROTEIN CONTENT (%) OF BOVINE MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT DURING DIFFERENT SEASON. VALUES WERE EXPRESSED IN MEAN (\pm SD).

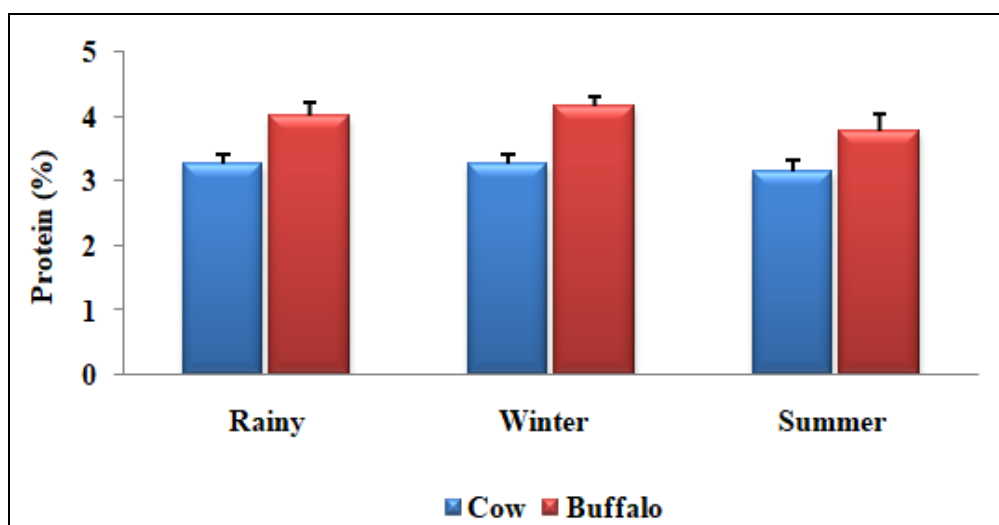


FIG. 3: COMPARISON OF SEASONAL EFFECT ON AVERAGE PROTEIN CONTENT (%) OF BOVINE MILK SAMPLES COLLECTED FROM DIFFERENT TEHSIL OF SINGRAULI DISTRICT. VALUES WERE EXPRESSED IN MEAN (\pm SD).

In Deosar tehsil, the highest protein content was observed during the rainy season in cow milk (3.67%) and during the winter season in buffalo milk (4.32%) samples at sites C3 and C1, respectively. In Singrauli tehsil, the highest protein content was found during the summer season in cow milk (3.42%) and during the winter season in buffalo milk (4.12%) samples at sites D3 and D2, respectively. The average protein content of cow milk in rainy, winter, and summer season was $3.30 \pm 0.06\%$, $3.21 \pm 0.22\%$, and $3.18 \pm 0.23\%$, respectively, and in Buffalo milk sample was $3.72 \pm 0.14\%$, $4.08 \pm 0.03\%$, and $3.50 \pm 0.14\%$, respectively **Table 3**.

The average protein content of the bovine milk sample collected from different tehsil during the different seasons was compared and presented graphically in **Fig. 2**. Results revealed that during the rainy season, the average protein content was maximum in Deosar tehsil ($3.67 \pm 0.52\%$) and minimum ($8.83 \pm 0.43\%$) in Singrauli tehsil. In the winter season, maximum average protein content was found in Waidhan Tehsil ($3.77 \pm 0.47\%$) and minimum in Deosar tehsil ($3.62 \pm 0.51\%$) and during summer season also maximum average protein content was observed in Waidhan Tehsil ($3.57 \pm 0.46\%$) and minimum in Deosar tehsil ($3.25 \pm 0.36\%$). Average protein content in cow and buffalo milk samples during the different seasons was also compared and results are presented graphically in **Fig. 3**. In comparison, it was found that the average protein content of cow ($3.27 \pm 0.15\%$) and buffalo ($4.15 \pm 0.16\%$) milk was high in the winter season followed by the rainy and summer season.

CONCLUSION: It is concluded that cow and buffalo milk samples contained all the amino acids but differed in the contents of certain amino acids and proteins. Such variation is purely biological and genetical and is intended for the nourishment of the young. The current investigation would be valuable for the dairy processing industries to formulate nutritionally improved milk-based functional products for vulnerable segments of the population.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest, financial or otherwise.

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