J. Amin. Prod U.A.R., **4,** No. 2, 121-135 (1964)

THE AMINO ACIDS COMPOSITION OF BUFFALO AND COW MILK CASEINS.

By

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SUMMARY

Acid precipitated caseins of buffalo and cow milk were compared and analysed for amino acids content, elementary analysis and reactive groups. Results showed that both caseins composed of the same amino acids qualitatively and nearly in the quantities estimated. The same was found on comparing the elementary analyses of both caseins.

INTRODUCTION

The chemical nature of casein, particularly the amino acid composition has been subjected to extensive studies (Gordon et al 1949, Khan and Baku 1957, Plimmer et al 1939, Williams 1945, Mednedeva 1958, and Nitolet et al 1942). However, most of these studies were confined to casein from cow milk.

Buffalo milk is an important source of milk proteins in Egypt, India and other countries. This has resulted in initiating research on the chemical and nutritional differences, which may exist between buffalo and cow milk caseines Despite this, not much information is available (Raj et al 1955 and Ganguli et al 1964) and this field remains available for critical studies.

The present paper shows a comparative study on the amino acid composition of local buffalo and cow milk caseins.

MATERIALS AND METHODS

Preparation of casein samples.

Casein was prepared from skimmilk (cow or buffalo) by precipitation at pH 4.6, using 10% HCI. Casein was washed thoroughly with distilled water, and dried with alcohol and ether.

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Acid hydrolysis of casein.

0.2 g of casein was refluxed with 20 ml of 6 N HCl for 20-24 hrs at 120° C. The hydrolysate residue was then evaporated under vacuum at 60° C. to dryness, dissolved in 25 ml of distilled water, stirred with 100 mg charcoal and then filtered. The charcoal was washed several times and the combined filterate was evaporated to dryness under vacuum, and the residue was dissolved in 10 ml of 10% isopropanol.

Alkali hydrolysis of casein.

0.2 g of the casein sample was refluxed with 20 ml of 14% barium hydroxide solution for 20 hrs. at 120° C. The hydrolysate was then neutralized to pH 7 with 10% sulphuric acid, filtered, and the filterate evaporated to dryhess under vacuum at 60° C. The residue was dissolved in 10 ml of 10% isopropanol.

Quantitatine paper chromatography of amino acids.

The method was essentially the same as described by Kuanff et al (1959) The descending technique for paper chromatography was used throughout all the experiments. Two separate solvent systems were used to develop the chromatograms; Butanol : acetic acid : water (4 : 1: 5), and methyl ethyl ketone : pyridine : water (7 : 1.5 : 1.5). The chromatograms were developed with the same solvent three times, which improve the separation of the amino acids. With solvent A and B the chromatograms were developed for 18 and 12 hrs. respectively in each run. The amino acids were separated into the two solvent systmes according to the pattern recorded in table 1.

The chromatograms were sprayed with 0.5 % ninhydrin in butanol and 1% copper nitrate solution as described by Bode et al (1952).

The coloured spots were cut, put in a clean test tube and then extracted with 5 ml methanol. The optical density (0. D.) of the extract was then measured spectrophotometriclly at 515 mu wavelength. The concentration of the amino acides was calculated from standared curves of indivdual amino acids, ranging from 1-10 ug prepared and treated the same as the amino acid mixture.

The concentration of aspartic, glutamic and phenylalanine were determined by calculating the differnce in the optical densities of their mixed spots in the two solvent systems as follows :

0.D. of aspartic = 0.D. of spot 5 (solvent A)
0.D. of spots 5 and 6 (solvent B).

0.D. of glutamic = 0.D. of spot 6 (solvent A)
0.D. of spot 8 (solvent B).

0.D. of phenyl- = 0.D. of spot 13 (solvent B)
alanine = 0.D. of spot 12 (solvent A).

TABLE 1.— **Amino acid pattern of casein in solvent A* and solvent B** arranged in a descending order from the starting line.**

Position	Solvent *A*	Solvent *B*
1	Cystine.	Cystine.
2	Lysine.	Lysine, arginine.
3	Histidine.	Aspartic, glutamic.
4	Arginine.	Histidine.
5	Aspartic, glycine, serine.	Glycine.
6	Glutamic, threonine.	Serine.
7	Alanine.	Alanine.
8	Proline.	Threonine.
9	Tyrosine.	Proline.
10	Valine, methionine.	Valine.
11	Isoleucine, Phenylalanine.	Methionine, tyrosine
12	Leucine.	Isoleucine.
13		Leucine, phenylalanine.

* Butanol : acetic acid : water (4 : 1 : 5)..

** methyl ethyl ketone : pyridine : water(7 : 1.5 : 1.5).

Identification of tryptophan.

Tryptophan was identified in the alkali hydolysate of casein by descending paper chromatography, using isopropanol, ammonia, water system (80 5 : 5) for 18 hrs. The chromatograms were dried and sprayed with p-dimethylaminobenzaldehyde as described by Block et al.(1955).

Quantitative determination of tryptophan, tyrosin, and methionine.

Tryptophan, tyrosine and methionine were determined in the alkali hydrolysate of casein by the iodometric method of Barand and Genevois (14).

Quantitative determination of cystine.

0.2 g of caseis was weighed in a test tube, 2 ml of 57% hydroiodic acid were added and the tuve was sealed. The tube was then heated at 100°C for 24 hrs, opened and its contents were made up to 25 ml with distilled water Cystine was determined in the hydrolysate colorimetrically by the method of Kassel and Brand (1938).

Analytical methods.

Nitrogen was determined by the micro Kjeldahl method. (Sing 1957) phosphorus spectrophotometrically as described by Snell and Snell (1949), sulphur gravametrically (Frear 1930), dry weight and ash contents were determined according to the method of Chibnall et al (1943), and the amide nitrogen content as described by Chibnall et al (1985).

Calculation of the reactive groups in casein.

The reactive groups in both caseins were calculated from the amino acid analyses as described by Gordon et al (1949).

RESULTS AND DISCUSSION

The amino acids present in buffalo and cow milk casein were found to be the same Fig 1, 2, 3, 4, and 5, which was in accordance with other investigators Raj, and Joshi (1955) and Ganguli et al (1964). Thus glycine, alanine, valine, leucine, isoleucine, serine threonine glutamic, aspartic, lysine, arginine, histidine, proline, cystine, methionine, tryptophan phenylalanine, and tyrosine were found to be present in both caseins, which agreed with the findings of other workers Gordon et al (1949), Kahn and Baker (1957), Plimmer and Lawton (1939), Williamson (1945), and Ganguli et al (1964),

Elementary analysis of casein showed that buffalo and cow milk caseins had similar composition. The nitrogen content of buffalo and cow milk casein had an average of 15.37% and 15.34% respectively, tabel 2. These figures were lower than that reported by Raj et al (8), 15.67 % and 15.68% respectively, and that for cows' 15.63% Gordon et al(1949). The phosphorus and sluphur content showed similar averages of 0.83% and 0.76% respectively in both caseins table 2. These figures, were comperable to that reported by other workers Gordon et al (1949), Raj and Joshi (1955) and Williamson (1945). In buffalo milk casein the nitrogen to phosphorus ratio had an average of 18.62%, while in cows' it had an average of 18.42%. These figures were comprable to that reported by Raj and Joshi (1955); namely 19.00% and 18.20% respectively. The amide nitrogen content of buffalo and cow milk casein had an average of 1.46% and 1.43% respectively, which was less than that reported by Gordon et al (1949), 1.61

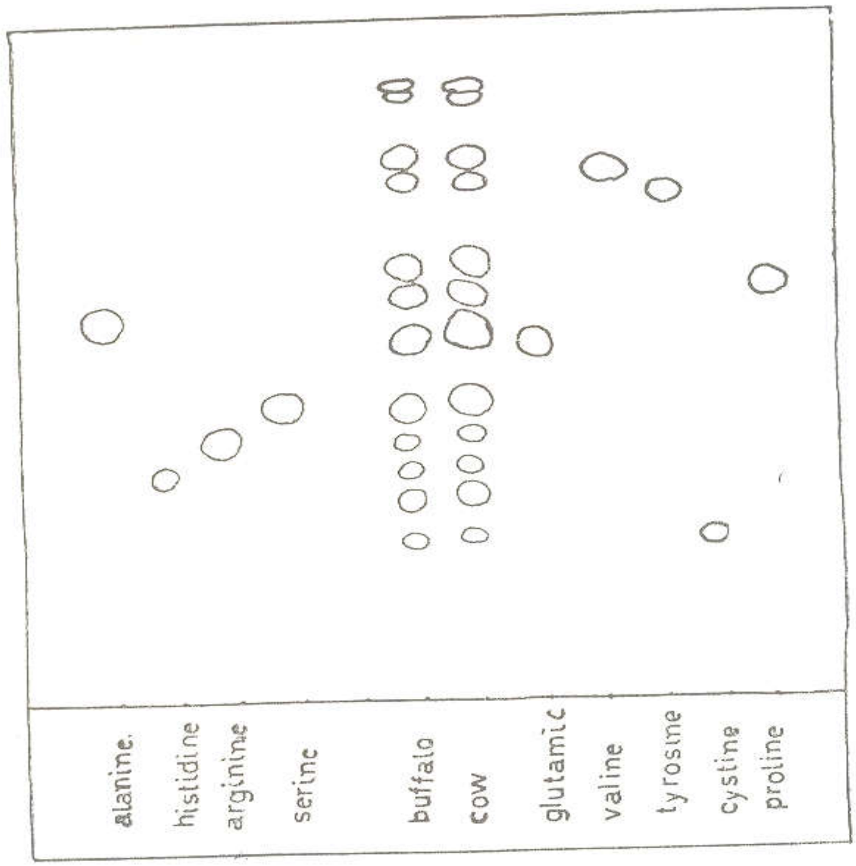

FIG. 1.—Paper chromatography of amino acids of buffalo and cow milk casein using butanol: acetic acid: water (4:1:5) system.

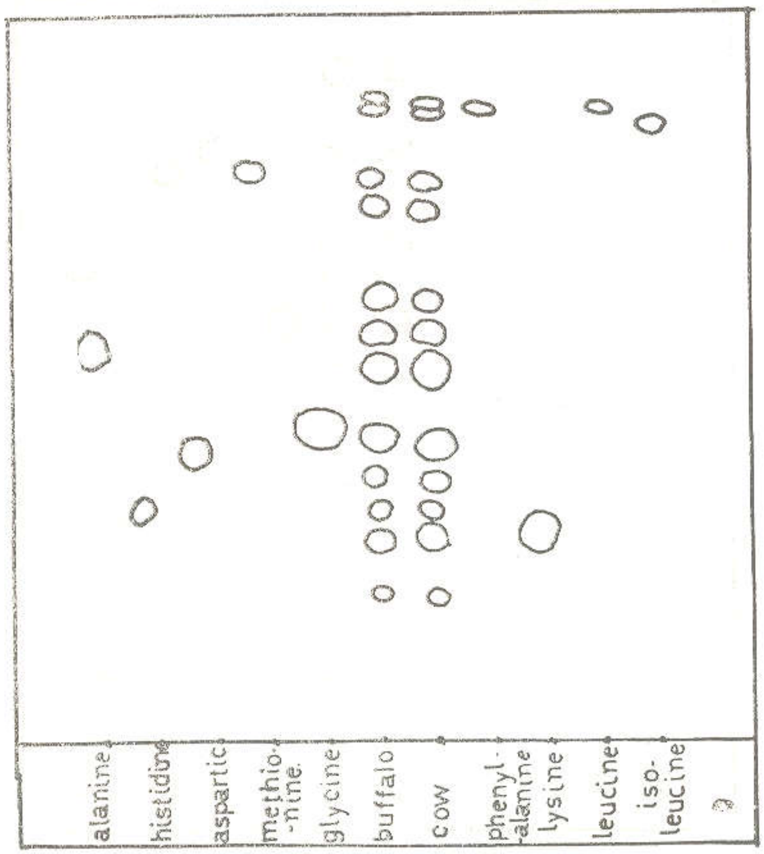

FIG. 2.—Paper chromatography of amino acids of buffalo and cow milk casein using butanol : actic acid : water (4 : 1 : 5) system.

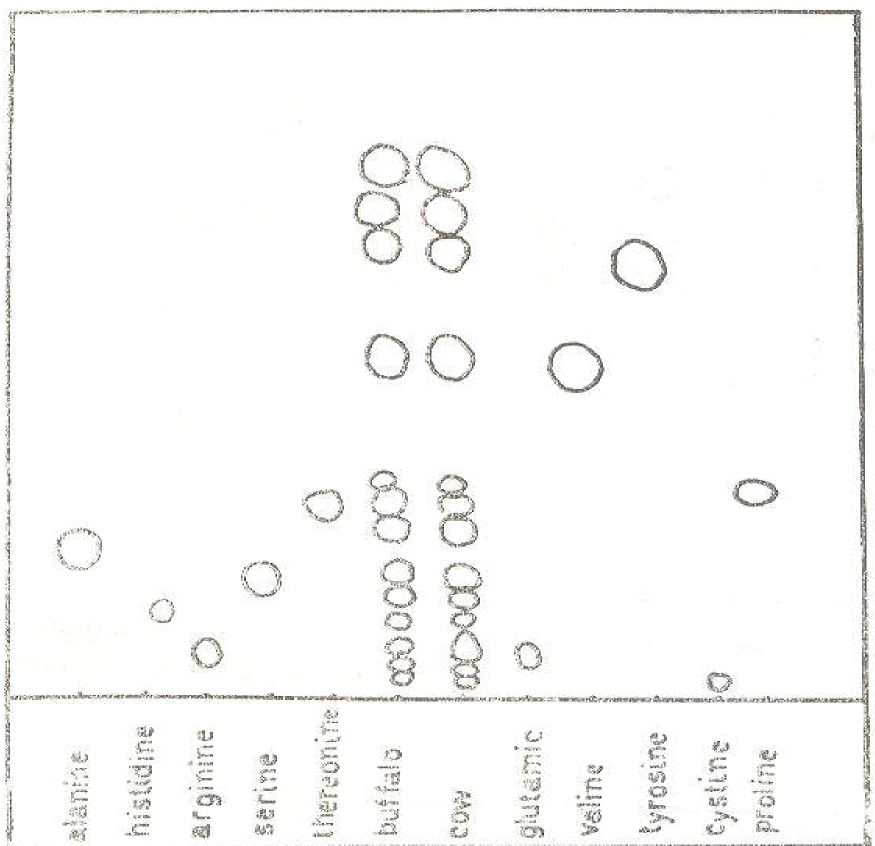

FIG. 3.—Paper chromatography of amino acids of buffalo and cow milk casein using methyl ethyl methyl ketone: pyridine: water (7:1.5:1.5) system.

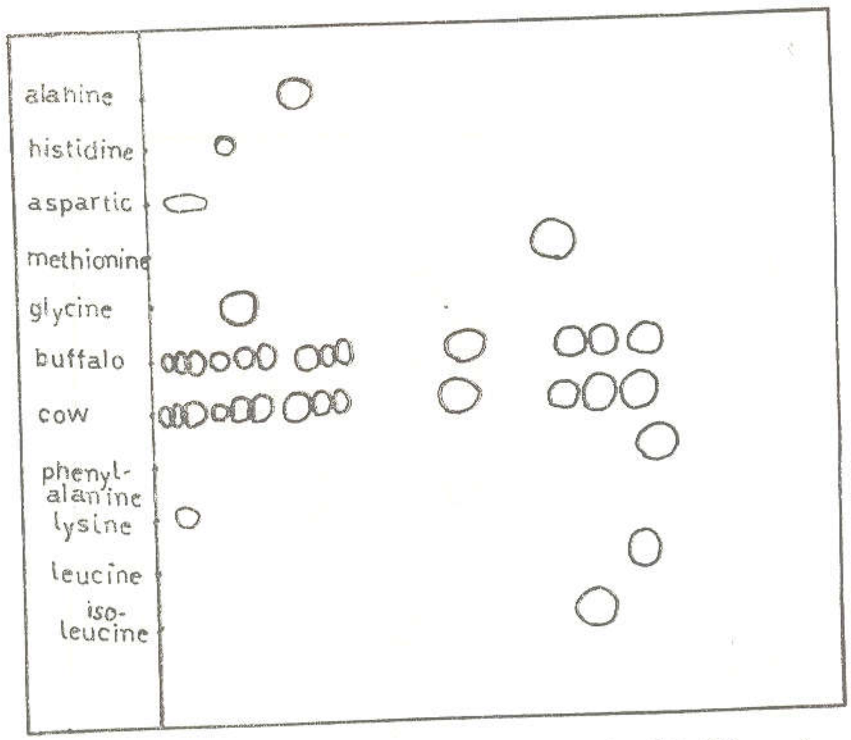

FIG. 4.—Paper chromatoghaphy of amino acids of buffalo and cow milk casein using methyl ketone : pyridine ; water (7 : 1.5 ; 1:5) system.

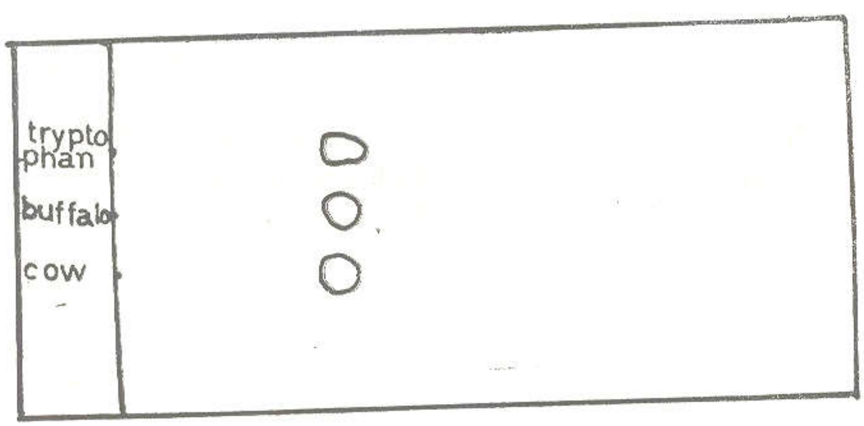

FIG. 5.—Identification of tryptophan in buffalo and cow milk casein by descending paper chro matography.

TABLE 2.—**The chemical compostion of buffalo and cow milk casein***

Constituent	Buffalo**		Cow**		Significance of difference
	range	average	range	average	
Nitrogen . .	15.71–15.10	15.37	15.21–15.10	15.34	—
Phosphorus . .	0.87–0.77	0.83	0.89–0.76	0.83	—
Sulphur . . .	0.75–0.68	0.72	0.74–0.68	0.72	—
N : P ratio . .	19.80–17.58	18.61	20.00–16.99	18.42	—
Amide N . .	1.53–1.45	1.49	1.48–1.38	1.43	—
Glycine . . .	2.50–1.50	2.00	3.14–1.82	2.66	+
Alanine . . .	7.61–5.62	6.49	7.68–5.92	7.02	+
Valine	8.69–7.42	8.15	9.96–8.49	9.38	+
Leucine . . .	7.04–5.77	6.25	7.05–5.96	6.43	—
Isoleucine . .	5.52–3.62	4.51	5.85–3.54	4.81	—
Serine	7.71–5.61	6.54	7.13–5.70	6.72	—
Threonine . .	5.93–4.08	5.18	6.68–5.18	5.96	+
Methionine . .	3.47–2.54	2.98	3.28–2.53	2.93	—
Cystine	0.68–0.48	0.59	0.65–0.46	0.55	—
Aspartic . . .	12.49–10.32	11.73	8.34–7.37	7.92	+
Glutamic . . .	23.43–20.33	22.11	24.39–21.79	22.92	+
Proline . . .	9.80–8.15	8.82	9.16–8.00	8.54	—
Tryptophan . .	1.77–1.48	1.61	1.65–1.39	1.52	—
Lysine . . .	8.14–6.33	7.23	7.73–6.47	7.27	—
Arginine . . .	5.07–3.23	4.03	5.28–3.88	4.44	+
Histidine . . .	3.35–1.93	2.41	3.80–2.02	2.44	—
Tyrosine . . .	7.00–5.38	6.11	6.79–5.15	5.78	—
Phenylalanine .	7.10–5.15	5.95	5.72–4.65	5.06	+

* Analysis of 20 samples buffalo milk casein and 10 samples of cows.

** All values are expressed in per cent corrected for moisture and ash content.

— (insignificant). + (Significant)

The average glycine content of cow and buffalo milk caseins were 2.00% and 2.66% respectively. These values were comperable to that reported by other workers; namely 2.7% Gordon et al (1949), and 2.03% Kahn and Baker (1957).

Alanine content of buffalo and cow milk had an average of 6.49% and 7.02% respectively, and the difference between the two averages was found to be statistically significant, table 2. These figures were higher than that reported in the literature namely; 3.0% Gordon et al (1949), 3.26% Kahn and Baker (1957), 2.37% and 2.98% Ganguli et al (1964).

Valine content of bufalo and cow milk casein showed averages of 8.15% and 9.38% respectively, and the differnce between the two averages was found to be significant. The average valine content in both caseins was higher than that quoted in the literature namely; 6.72% Williams (1945), 7.27% Kahn and Baker (1957), 7.2% Gordon et al (1949), 5.58% and 6.58% Ganguli et al (1964).

The average leucine and isoleucine contents in buffalo milk casein were 6.25% and 4.51% respectively, table 2, while in cows' their averages were 6.43% and 4.81 respectively. These figures were less than that reported in the literature Gordon et al (1949), Kahn and Baker (1957) Williamson (1945) and Ganguli et al (1964).

The sum values of mono-amino-mono-earboxylic acides gave an average of 27.40% for buffalo milk casein, while cow milk casein had an average of 30.29%. The difference beween the two averages was found to be significant.

Serine and threonine were found to be the only hydroxyacids in casein. The average serine content of buffalo and cow milk casein were 6.54% and 6.72% respectively. These figures were in accrodance to that reported in the literature namely; 6.3% Gordon et al (1949), 7.02% Mednedeva and Kugenev (1958). On the other hand, threonine content in both caseins was higher than that reported by other workers Gordon et al (1949), Kahn and Baker (1957), Williamson (1945), Mednedeva and Kugenev (1958), and Ganguli et al (1964) In the present study the average threonine contents in buffalo and cow milk casein were 5.18% and 5.96% respectively, and the difference between the two averages was significant, table2. The average values of total hydroxy amino acids of cow milk casein (12.68%), was found to be significantly higher than that of buffaloes'(11.73%). These two averages were higher than that reported for foreign cows' namely; 9.05% Nicolet and Shinn (19426).

The acid hydrolysis of casein is known to cause the distruction of cystine accordingly, separate hydrolysis with hydroiodic acid was used for the deermination of cystine. Acid hydrolysis also effects the amount of methionine present in casein, therefore alkali hydrolysis was used for the protein hydrolyss for the determination of methionine iodometrically.

The cystine content of buffalo and cow milk casein had an average of 0.59% and 0.56% respectively, table 2. These figures, were higher than that found by other workers namely; 0.32% Williamson (1945), 0.34% Gordon et al (1949), and 0.40% Sundararagan and Sarma (1957). Buffalo milk casein was found to contain an aver age methionine content of 2.98%

was similar to that of cows namely; 2.93% talbe 2. The verage methionine content in both caseins was in accordance with that quoted in the literature namely; 2.51% Williamson (1945), 2.8% Gordon et al (1949), 3.1% Sundararagan and Sarma (1957), and 2.99% Kahn and Baker (1957). The average total sulphur-containing amino acids in both buffalo and cow milk casein were nearly the same, being 3.63% and 3.49% respectilvely.

Buffalo milk casein was found to contain higher aspartic acid than cow milk casein. On the other hand cow milk casein had a higher avacfage of glutamic acid than that of buffaloes'. In buffalo milk, the average aspartic and glutamic acid contents were 11.73% and 22.11% respectively, while in cows' their averages were 7.92% and 22.92% respectively, table 2. The average aspartic acid content in cow milk casein was comperable to that found by Gordon et al (1949), 7.1% ; and Khan and Baker (1957), 7.34%. The same was found on comparing glutamic acid content in the present study with that reported by other workers Gordon et al (1949) and Kahn and Baker (1957). The total dicarboxylic acids of buffalo milk casein was significantly higher than that of cow's. The average total acidic amino acids of cow milk casein was 30.84%, while that of buffaloes' was 33.48%.

Proline content of buffalo and cow milk casein showed averages of 8.82% and 8.54% respectively. However, the difference between the two averages was insignificant, table 2. These resluts were lower than that reported in the literature for foreign animals namely; 11.3% Gordon et al (1949), 11.72% Kahn and Baker (1957), and 9.75% — Plimmer and Lawton (1939).

The average tryptophan content of buffalo milk casein was 1.61% while that for cow was 1.52%, and the difference between the two averages was insignificant, table 2. The tryptophan content in both caseins was found to be slightly higher than that reported by other workers namely; 1.32% Williamson (1945) 1.2% Gordon et al (1949), and Kahn and Baker (1957), 1.3% Plimmer and Lawton (1939), and Sundararagan et al (1957), 1.46% and 1.3% Ganguli et al (1964).

Lysine content of buffalo milk casein ranged from 6.33% 8.14% with an average of 7.23%, while in cows' it ranged from 6.47% to 7.73% with an average of 7.27%, table 2. However, the difference between the average lysine content in both caseins was insignificant. These results were lower than that reported by other workers namely; 8.19% 8.2% 8.18% 8.3% 7.56% and 9.47% Gordon et al (1949), Kahn and Baker (1957), Plimmer and Lawton (1939), Williamson (1945), and Ganguli et al (1964).

The average arginine content of buffalo milk casein was 4.03%, while that of cows' was 4.44% and the difference between the two averages was significant, table 2. These figures were in accordance to that quoted in the literature Gordon et al (1949), Kahn and Baker (1957) Plimmer and Lawton (1939), and Sundararagan et al (1957).

Histidine content of cow milk casein randged from 2.02% to 2.80% with an average of 2.44%, while in buffaloes' it ranged from 1.93% to 3.35% with an average of 2.41%. However, the difference between the average histidine content of both caseins was insignificant. These results were less than that reported by Gordon et al (1949), 3.13%, and Khan and Baker (1957), 2.7% and higher than that found by Ganguli et al (1964), 1.62% and 1.38%.

Buffalo and cow milk casein had nearly the same total basic amino acids and statistical analysis showed that the difference between the two averages (13.7% for buffaloes' and 14.15% for cows') was insignificant, table 2.

The tyrosine content of buffalo milk casein had an average of 6.11% and in cows' it had an average of 5.78%, table 2. The difference between the tow averages was insignificant. These results were in acordance to that reported in the literature Gordon et al (1949), Kahn and Baker (1957), and Ganguli et al (1964).

The average phenylalanine content of buffalo milk casein was higher than that of cows' when it was examined statistcally. The averag phenylalanine contents in both caseins were 5.95% and 5.06% respectively, which were comperable to that quoted in the literature Grordon et al (1949), Kohn and Baker (1957), Williamson (1945), and Ganguli et al (1964).

The total aromatic amino acids content of buffalo milk casein was found to be significantly higher than that of cows'. Buffalo milk casein had an average of 12.33%, while in cows' it was 11.25%.

The distribution of the recative groups in buffalo and cow milk casein is shown in thable 3. Buffalo and cow milk casein had nearly the same amount of cationic groups, since the average cationic groups of buffalo milk casein was 93 and of cows' was 94 mole. These values were slightly less than that reported by Gordon et al (1949), being 110, mole per 10^5 g. casein.

The average anionic groups of buffalo and cow milk casein were 140 and 127 mole respectively, which were lower than that reported by Gordon et al (1949), being 157 mole per 10^5 g. casein

TABLE 3.—**Distribution of the reactive groups in bufalo and cow milk casein**

Costituent	Buffalo		Cow		Significance of difference
	Range	Average	Range	Average	
Cationic groups ..	102—85	93	99— 86	94	—
Anionic groups . .	153—125	140	134—115	127	+
Ionic groups . . .	254—211	234	233—214	221	+
Non-ionic polar groups	242—214	227	239—215	227	—
Polar groups . . .	484—440	461	464—440	448	+
Non - polar groups	235—291	305	348—302	320	+

* All values are expressed in mole residue per 105 g casein.
— (insignificant), + (significant).

Consequently, the ionic groups of buffalo milk casein was higher than that of cows'. An average ionic groups of 234 was calculated for buffalo milk casein, while in cows' it was 227. These results were less than that of cows' reported in the literature ; namely 267 Gordon et al (1949).

Buffalo and cow milk casein had the same average of 227 mole residue for non-ionic polar groups, which was less than that reported by Gordon et al (1949), namely 250.

The average polar groups in buffalo milk casein was 461, while that of cows' was 448 mole residue. These results were less than that of Gordon et al (1949) ; namely 512.

The sum of all non - polar groups of buffalo milk casein was less than that of cows'. The average non - polar groups of buffalo and cow milk casein was 305 and 320 mole respectively. However, these results were less than that found by Gordon et al (1949), being 376.

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(Printed in 1966)

الأحماض الأمينية المكونة لكازين اللبن الجاموسى والبقرى

محمد الحسينى عبد السلام — ابراهيم الدسوقى رفعت — على حسن فهمى
امين محمد السكرى

الملخص

درس التركيب المقارن لكازين اللبن الجاموسى والبقرى بالنسبة للأحماض الأمينية فى التركيب الأول والمجاميع الفعالة . وقد دلت النتائج على أن نوعى الكازين يتركب من الاحماض الامينية والمجاميع الفعالة الآتية :

جليسين ٢ر٠٠٪ ، ٢ر٦٦٪ — الأنين٦ر٤٩٪ ، ٧ر٢٠٪ — فالين ٨ر١٥٪ ، ٩ر٣٨٪ ، ليوسين ٦ر٥٥٪ — ٦ر٤٣٪ — ابزولوسين ٤ر٥١٪ ، ٤ر٨١٪ — أحماض أمينية ذات مجموعة أمينية كربوسيلية واحدة ٢٧ر٤٠٪ ، ٢٩ر٣٠٪ — سيرين ٦ر٥٤٪ ، ٦ر٧٢٪ — تربتوفان ٥ر١٨٪ ، ٥ر٩٦٪ — أحماض أمينية هيدروكسيلية أمينية كلية ٢٢ر١١٪ ، ٢٢ر٩٢٪ — سستين ٠ر٥٩٪ ، ٠ر٥٦٪ — مثيونين ٢ر٩٨٪ ، ٢ر٩٣٪ — أحماض أمينية محتوية على كبريت ٣ر٦٣٪ ، ٣ر٤٨٪ — وحمض اسبرتيك ١٢ر٧٣٪ ، ٧ر٩٢٪ — حمض جلوتاميك ٢٢ر١١٪ ، ٢٢ر٩٢٪ — أحماض أمينية ثنائية الكربوكسيل ٣٣ر٤٨٪ ، ٣٠ر٨٤٪ — برولين ٨ر٨٢٪ ٨ر٥٤٪ — تربتوفان ١ر٦٧٪ ، ١ر٥٦٪ — أرجنين ٤ر٠٣٪ ، ٤ر٤٤٪ — ليسين ٧ر٢٣٪ ، ٧ر٢٧٪ — هستدين ٢ر٤١٪ ، ٢ر٤٤٪ — أحماض أمينية قاعدية ١٣ر٧٣٪ ، ١٤ر١٥٪ — تيروسين ٦ر٢٣٪ ، ٦ر١٩٪ — فنيل الانين ٥ر٩٥٪ — ٥ر٠٦٪ أحماض أمينية عطرية ١٢ر٣٣٪ ، ١١ر٢٥٪ — كذلك وجدت المجاميع الفعالة التالية معبراً عنها جزىء/$١٠^{٥}$ جم بروتين :

مجموعات موجبة ٩٤ر٩٣ — مجموعات سالبة ١٤٠ ، ١٢٧ — مجموعات أيونية ٣٣٤ ، ٢٢١ — مجموعات غير أيونية قطبية ٢٢٧ فى كلا النوعين — مجموعات قطبية ٤٦١ ، ٤٤٨ ومجموعات غير قطبية ٣٠٥ ، ٣٢٠ .

كلا نوعى الكازين يحتوى على نفس المتوسطات الآتية : ٠ر٨٣٪ فسفورية — ٠ر٧٢٪ كبريت . وكانت نسبة النيتروجين ١٥ر٣٧٪ ، ١٥ر٣٤٪ والرماد١٤٩٪ ، ١٤٣٪ لكازين الجاموسى والبقرى على التوالى .