قســـــــم : الصحة ومراقبة الأغذية ـ كلية الطب البيطرى ـ جامعة أسيوط.

رئيس القســـم : أ.د / على يوسف لطفى

التقييم النوعى والكمى لجزئيات سيرم اللبن بطريقة الفصل الكهربائى

فوزى ابو الخير ، راقية الحلوانى ، فـكرى القربى

تم فصل عدد (٣٦) عينة لبن فردية وجماعية لتحديد عدد ونسبة محتويات البروتين الذائب من الجزئيات بطريقة الفصل الكهربائى. وقد أوضح البحث أن سيرم اللبن تجزأ الى أربعة أقســـام (ألبومين السيرم ، ألفالاكتوألبومين ، بيتالاكتوجلوبيلين). مختلفة النسبة حسب الهجـــرة الكهربائية.

وقد أتضح أيضا وجود فروق معنوية فى جزئيات البيتالاكتوجلوبيلين والألفينوجلوبيلين فيمـــا بين اللبن الجاموسى والبقرى. ولم يلاحظ اختلافات معنوية عند مقارنة العينات الفرديـــة والجماعية لكل نوع على حـده.

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QUALITATIVE AND QUANTITATIVE EVALUATION OF SERUM PROTEIN FRACTIONS FROM COW'S AND BUFFALOE'S MILK BY ELECTROPHORETIC TECHNIQUE

(With 3 Tables & One Figure)

By

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SUMMARY

A total of 36 milk samples obtained from Egyptian buffaloe's and Friesian cow's were used to investigate the serum protein fractions. Milk serum protein were obtained after removal of casein from skim milk through precipitation in the isoelectric point. Partition of serum protein by electrophoretic technique revealed four distinct fractions named as serum albumin, beta-lactoglobulin, alpha-lactalbumin and immune-globulins.

Variations in the concentration of serum protein fractions are distinctly present in both cow's and buffaloe's milk specially in beta-lactoglobulin and immune-globulin content. Insignificant differneces could be detected in the concentration of the various fractions between the individual and composite milk samples.

INTRODUCTION and LITERATURE

The true comparative value between the major component of milk proteins depends upon their different amino acids composition, especially their content of essential amino acids. Although the separation of milk proteins into their major componant casein and srum proteins has been investigated extensively, Many researcheers have been attempted to separate them into well defined fractions.

Different methods have been proposed for fractionation of milk proteins such as paper electrophoresis (ZWEIG and BLOCK, 1953, ZDANOVA and VLADOVETS 1959, KARMALEEV 1971, KYGENOV and BARABANCHIK 1973), starch gel electrophoresis (NEELIN, 1964, MAJUMDER and GANGULI 1972, RANDOLPH et al. 1977), column chromatography (LIN and RANDOLPH, 1977).

It has been reported by ZDANOVA and VLADOVETS (1950) that colostrum whey of the first few days of lactation contained over 80 per-cent immune-globulin.

Immune-globulin composed fo euglobulin and psudoglobulin, which carry the immunological properties or antibodies of milk and comprise about 5 per-cent of the total whey proteins (JENNES and BATTON 1969).

Beta-lactoglobulin, the principal milk serum proteins has been the subject of much investigation, since it was obtained in crystaline form. It has been well characterized with respect to electrophoretic mobility and molecular weight (TILIEY 1962). Beta-lactoglobulin is the main source of SH-groups, the cause of cooked flavour of milk, which arises during heat denaturation.

Variations in the relative concentrations of beta-lactoglobulin probably play a major, but yet undetermined role in controlling heat stability of milk (ROSE 1962). In 1963, the same author revealed that there is a significant correlation between maximum heat stability of milk and beta-lactoglobulin content.

Alpha-lactalbumin plays an essential role in biosynthesis of lactose, there is a high homology between this protein and egg white lysozyme (EBBER and BRODBECK 1968).

BOUCHARD and BRISSON (1969) Found that, albumin and beta-lactoglobulin increased greatly during early lactation, and remained relatively constant until the end of lactation. The alpha-lactalbumin on the other hand showed tendency to decrease very slowly through lactation, while immune-globulin was low at the beginning but tend to increase slowly until the end of lactation period.

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DILL et al. (1972) reported that, the concentration of casein and serum proteins in bovine milk decreased when cows were maintained under the prolonged influence of oxytocin.

In clinical pathology, electrophoretic techniques have also been used as a diagnostic procedure of mastitis (RANDOLPH and ERWIN, 1974 and LIN and RANDOLPH, 1977). The protein pattern of sick animals was diffeeent from that of healthy animals, especially in case of whey proteins. The fractions with slow rate of migration namely K-casein, immune-globulin, proteose peptone and serum globulins were found to be more affected in mastitic milk (BURUIANA et al., 1979).

As the electrophoretic analysis of milk serum proteins from cows has attracted much attention and as there is no much informations concerning buffaloe's milk, the present study has been undertaken to investigate the electrophoretic pattern of serum proteins from Egyptian buffaloe's and Friesian cow's milk.

MATERIAL and METHODS

A total of 36 individual and composite milk samples from Egyptian buffaloes and Friesian cows were used in this experiment. All milk samples from individual animals were taken three times monthly during fourth, fifth and sixth month of lactation with 10 days interval. Composite milk samples were collected from three selected animals of both species (selection according to the date of last delivery). The dairy animals belong to Karada-Mehalet-Maussa experimental dairy farm's,Animal production research institute, Kafr-El-Shiekh Governorate, Ministry of Agriculture. Both experimental animals were clinically healthy throughout the period of the experiment.

Milk samples were conveyed immediatly to the laboratory and heated at 40°C then deffattened by centrifugation. Casein was precipitated from 100ml skim milk by addition of 20% acetic acid till pH 4.6. The precipitate was separated and the supernatent fluid was filtered to obtain clear acetic acid milk serum.

Milk serum was applied as little as 0.05 ml on Whattman filter paper (No. 1) strips 3 x 40 cm. Veronal buffer (pH 8.6) was used to detect the separate milk serum protein fractions. The protein zones were finally stained with amido black dye, where there were a distinct separation of the fractions (Fig. 1). The quantitative evaluation of each fraction separatly was done according to the method described by KYGENOV and BARABANCHIK (1973).

The statistical analysis of the data was conducted according to SNEDICOR (1955), using "t" test.

RESULTS

The obtained results represented in tables 1 and 2 showed the average concentrations of different milk serum protein fractions from cow's and buffaloe's milk together with the values obtained from analysis of individual and composite samples. The data are expressed in percentage to the total milk serum protein fractions.

DISCUSSION

The separation of milk proteins is based on the fact that the different proteins have different molecular weights and, at a given pH, different net charges and under the influence of an electric field the different proteins will move at different rates depending on their charge and size. On paper electrophoresis at pH 8.6 four protein fractions were obtained from buffaloe's and cow's milk serum (Fig. 1). Milk serum protein was usually fractionated by paper electrophoretic technique according to their mobility into four fractions named as bovine serum albumin, beta-lactoglobulin, alpha-latalbumin and immune-globulin (NAZARKUN, 1966, KARMALEEV, 1977, KYGENOV and BARABANCHIK, 1973, EKERIANKOV, 1974).

The average concentrations of bovine serum albumin fraction (Table 1) in individual and mixed samples were 5.19% and 5.22%, respectively, while those of buffaloe's milk were 6.23 and 5.40 per cent serum albumin (Table 2).

Although buffalo milk contains relatively more concentration of serum albumin than cow's milk,statistical analysis revealed insignificant differences. Higher concentration of serum albumin was also noticed in mixed samples obtained from buffaloe's milk.

Concerning beta-lactoglobulin it was observed that this fraction constitutes the major component of the total milk serum protein fractions in both cow's and buffaloe's milk. The average values for this fraction in cow's and buffaloe's milk were 57.22 and 52.28 per-cent, respectively.

Milk serum proteins from Friesian cows are characterestically rich in beta-lactoglobulin content than buffaloe's milk. On comparing the results obtained from analysis of individual and mixed milk samples from each animal species separetly the authers observed that the variations are mostly present, however statistical analysis revealed insignificant differneces. Regarding the concentration of alpha-lactalbumin it was clear that the value of this fraction is nearly similar in both cow's and buffaloe's milk and constitutes 24.12, 23.77 per-cent, repectively.

A characterestic and important variation is also noted in the immune-globulin fraction. Buffalo milk serum contained a relatively higher concentration than cow's milk. The values obtained were 17.72 and 13.46 per-cent for buffaloe's and cow's milk, respectively.

A similar increase in the immune-globulin and decrease in beta-lactoglobulin fractions was also noticed in mixed samples from buffaloe's milk (Table 2).

In veiw of the obtained results it could be concluded that the basic differences in the milk serum protein fractions between the two animal species confined mainly in the beta-lactoglobulin and immune-globulin content, breed differneces in the concentration of differnet milk serum protein fractions were detected by NAZARKUN 1966, EKERIANIKOV (1974).

The variations in the immune-globulin and beta-lactoglobulin content between cow's and buffaloe's milk serum probably related to the physical condition, feeding systems, and long adaptation of the animals to the surrounding environment.

Table 3. represents the concentration of different milk serum protein fractions obtained by several authors.

The resutls obtained from this investigation revealed that milk serum protiens were composed of four different fractions, this agree with the resutls obtained by other authors. Concerning the serum protein fractions obtained from cow's milk the results agree with those reported by ZDANOVA and VLADOVETS (1959). Regarding the fractions obtained from buffaloe's milk, the obtained results differ slightly from those obtained by ASLANYAN (1965), this may be due to different breeds of buffalo. In general, however the variations in the different serum protein fractions are greater among species of dairy animals than that in between individual and mixed samples of milk from the same species separatly.

Table (1): Distribution of milk serum protein fractions from cow's milk

Fraction	Individual samples				Mixed samples			n=9
	Min.	Max.	M_1	SE	Min.	Max.	M_1	SE
Bovine serum albumin	4.80	5.45	5.19	0.10	4.5	6.1	5.22	0.19
Beta-lactoglobulin	51.5	65.0	57.22	1.83	53.0	58.8	55.64	0.61
Alpha-lactalbumin	17.9	29.45	24.12	1.71	22.1	25.2	23.98	0.39
Immune-globulin	11.4	16.2	13.46	0.55	13.0	15.8	15.34	0.61

The data expressed in percentage to the total milk serum protein fractions.

Table (2): Distribution of milk serum protein fractions from Egyptian buffaloe's

Fraction	Individual samples				Mixed samples				n=9
	Min.	Max.	M_2	SE	Min.	Max.	M_2	SE	M_1-M_2
Serum albumin	5.90	6.60	6.23	0.83	4.9	6.1	5.40	0.15	-1.04
Beta-lactoglobulin	47.0	58.5	52.28	1.37	49.0	54.5	52.08	0.76	-4.94*
Alpha-lactalbumin	17.8	26.8	23.77	1.94	20.1	25.2	22.8	0.52	0.35
Immune-globulin	15.8	21.4	17.72	0.62	17.8	21.0	19.72	0.42	-4.26*

The data expressed in precentage to the total serum protein fractions;
* Significant at 0.01 level.

Table (3): Concentration of milk serum protein fractions obtained by several authors

Animal species and breeds	Nomencluture of the fraction				Source of the data
	Bovine serum albumin	Beta-lactoglobulin	Alpha-lactalbumin	Immune-globulin	
Cow's	4.3	60.2	20.4	15.1	Zdanova and Vladovets (1959)
	6.7	59.2	28.3	5.8	Diadchinko (1959)
	2.1 - 6.1	49.1 - 59.8	24,3 - 29.2	14.9 - 14.5	Bernatons et al. (1965)
Friesian	6.4	43.31	26.47	23.82	Nazarkun (1966)
Jersy	5.2	44.1	27.3	23.66	" "
Ayrshire	4.1	55.6	30.2	10.1	" "
	6.4	43.31	26.47	23.2	Karmaleev (1971)
	3.9	48.8	26.4	20.3	Dill et al. (1972)
	6.17	48.19	28.35	16.76	Kygenov and Barabanchik (1973)
	6.99	38.63	32.8	21.58	El-Halawany (1976)
Buffaloe's (indian)	12.1	41.0	20.9	12.3	Aslanyan (1965)

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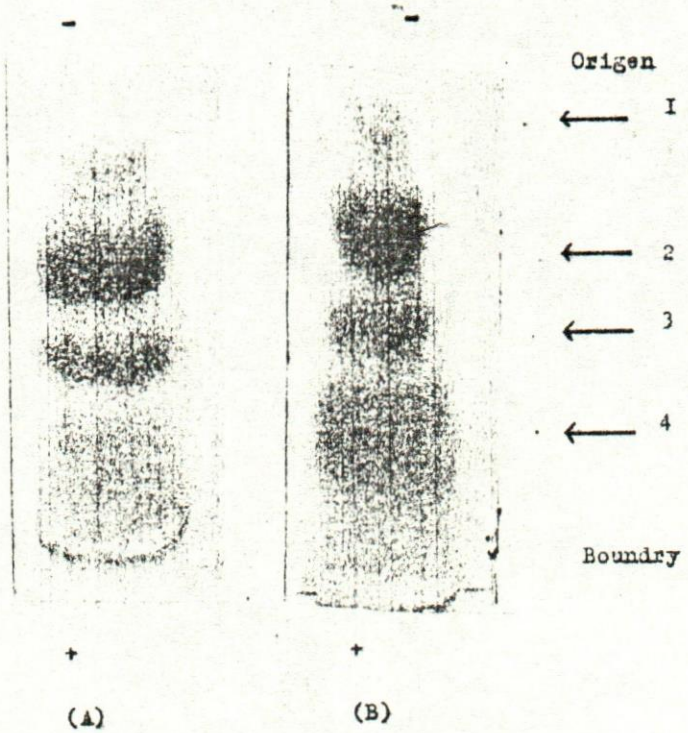

Fig. I Distribution of milk serum protein fractions from cow's and buffaloe's milk.

I. Serum albumin
2. Beta- lactoglobulin
3. Alpha- lactalbumin
4. Immune- globulin