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ENZYMES OF COW'S AND BUFFALO'S MILK

I.—Oxidation-Reduction Enzymes Peroxidase, Xanthine Oxidase, Catalase and Cytochrome C Reductase

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The object of this study was to determine the activities and contents of 4 enzymes namely; peroxidase, xanthine oxidase, catalase and cytochrome C reductase found in buffalo and cow milk for comparison. The individuality of the animal effects the value of their activities and contents in milk as they varied widely in the samples tested.

Both milks have approximately similar activities of catalase and cytochrome C reductae, being 10.9 and 12.1 unit/ml milk catalase for buffalo and cow milk respectively, and 1.5 and 1.16 ug/ml cytochrome C reductase for buffalo and cow milk respectively. Buffalo milk has higher peroxidase activity being 343.4 unit/ml milk, but lower xanthine oxidase activity, being 8.6 unit/ml than cow milk being 228.4 and 18.1 unit/ml milk respectively.

Clean milk produced under sanitary conditions from healthy animals contains a number of enzymes which are considered true constituents of milk. Their determination and identification in Buffalo milk lag considerably behind the cow's. Therefore, the object of this study was to determine quantitatively and to compare the activities of oxidation reduction enzymes, such as ,peroxidase, xanthine oxidase, catalase, and cytochrome C reductase in buffalo and local cow milk.

Experimental and Methods

These series of studies were carried out on individual samples of Egyptian buffaloe's and cow's milk.

Thirty samples from each were obtained aseptically from evening milkings. About 250 ml of milk were directly collected in sterile bottles, and 2.5 ml of chloroform were added to each as a preservative. They were immediately transfered to the laboratory in an ice box where they were kept in a refrigerator at 4°C.

1.—Peroxidase activity was determined spectrophotometrically according to Wallerstein et al (1947), using paraphenylenediamine solution, H_2O_2, and butanol as solvent and read at 490 mu wavelength by Jena spectrocolorimeter with 1 cm glass cell: Units of enzyme were calculated from:

$$\text{Units/ml of milk} = \frac{\text{Optical density}}{1.54 \times \text{ml of sample}}$$

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2.—Xanthine oxidase was determined according to Zittle *et al* (1956) using 0.05 M xanthine solution, milk, and triphenyltetrazolium chloride solution in ratio 1 : 5 : 1 V/V. The reaction was carried out at pH 7.5 for 15 min at 50°C. Toluene was used as the extracting solvent and the color intensity was read at 485 mu wavelength in Jena Spectrophotometer using 1 cm^3 glass cells.

3.—Catalase was determined according to iodometric method of Anderson and MacWalter (1937) and the calculation was carried out according to Bergmayer (1963). Since the quantity of the thiosulfate solution is proportional to the amount of H_2O_2 left in the enzymatic reaction mixture, the titration value in ml can be inserted in the following equation:

$$K = \frac{1}{t} \ln \frac{S_1}{S_2} = \frac{2.3}{t} \log \frac{S_1}{S_2} \text{ where}$$

$t = t_2 - t_1$ = the measured reaction time where

t_1 = initial time.

t_2 = terminal time.

S_1 and S_2 = H_2O_2 concentrations at time t_1 and t_2 without further conversion.

The K value used to calculate the catalase units in the enzymatic reaction mixture according to the following equation:

$$1 \text{ unit} = \frac{K \text{ observed}}{6.93 \times 10^{-3}} \text{ where}$$

6.93×10^{-3} = the relationship between the observed half-life time and the enzyme activity.

$$\text{Units/ml of milk} = \frac{K \text{ observed}}{6.93 \times 10^{-3}} \times \frac{1}{20}$$

4.—Cytochrome C reductase was determined by the method of Kaplan *et al* (1952). Exactly 0.05 ml of 5% solution of cytochrome C, 1 ml milk, and 0.1 M potassium phosphate buffer at pH 7.8 were added to make a final volume of 3 ml. An initial reading of optical density was taken at 550 mu wavelength. With optimal conditions the reaction was completed in 30 min. and any increase in its optical density was considered as a measure of the amount of cytochrome C reductase present in 1 ml milk.

Calculation: $E = \frac{O.D.}{C}$ where

E = extention coefficient = 158.

O.D. = optical density

C = mg enzyme.

Results and Discussion

The maximum, minimum, means, standard deviations and standard errors of the peroxidase, xanthine oxidase, catalase and cytochrome C reductase have been shown in table 1.

TABLE 1.—MAXIMUM, MINIMUM, MEANS, STANDARD DEVIATIONS AND STANDARD ERRORS FOR PEROXIDASE, XANTHINE OXIDASE, CATALASE AND CYTOCHROME C REDUCTASE CONTENT OF BUFFALO AND COW MILK.

Enzymes	Max.	Min.	Mean	S.E.	S.D.
1. *Peroxidase*					
Buffalo	853.3	166.5	343.4	26.4	143.90
Cow	353.8	139.4	228.4	10.2	56.02
2. *Xanthine oxidase**					
Buffalo	16.5	1.3	8.6	0.9	4.9
Cow	83.0	0.7	18.1	4.6	25.6
3. *Catalase**					
Buffalo	16.2	8.3	10.9	0.82	4.8
Cow	16.4	8.3	12.1	0.67	3.9
4. *Cytochrome C reductase*					
Buffalo	9.6	0.032	1.5	0.4	2.7
Cow	3.164	0.063	1.16	0.15	0.789

(*) unit/ml of milk. (4) ug/ml of milk.

In buffalo milk, the peroxidase activity showed a wider range of variations than cow milk. It ranged in the former milk from a minimum of 166.5 to a maximum of 853.3 unit/ml with an average of 343.4 unit/ml milk. In cow milk the activity ranged from 139.4 to 353.8 unit/ml with an average of 228.4 unit/ml milk. The difference between the two averages was found to be significant, as shown in table (2).

TABLE 2.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE PEROXIDASE ACTIVITY OF BUFFALO AND COW MILK

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	343.4	115	143.90	26.4	2.042	4.063	+
Cow	228.4		56.02	10.2			

The present results are in accordance with these reported by El-Hagarawy (1959), who found that buffalo milk had higher peroxidase activity than cow milk.

The xanthine oxidase activity in buffalo milk ranged from 1.3 to 16.5 unit/ml with an average of 8.6 unit/ml milk. In cow milk its activity ranged widely from 0.7 to 83.0 unit/ml with an average of 18.1 unit/ml milk. The average xanthine oxidase activity in buffalo milk was less than that in cow milk and the difference between the two averages was significant, as shown in table (3).

TABLE 3.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE XANTHINE OXIDASE ACTIVITY OF BUFFALO AND COW MILK

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	8.6	9.5	4.9	0.9	2.042	4.115	+
Cow	18.1		25.6	4.6			

Pereira *et al* (1962), found that the activity of morning and evening milk, ranged from 33 to 121 unit/ml with an average of 62 unit/ml milk and from 38 to 100 unit/ml with an average of 61 unit/ml milk respectively. On the other hand Modi et al (1959) gave lower values for xanthine oxidase activity of cow milk, the range being 0.08 to 0.56 unit/ml milk.

The catalase activity in buffalo milk ranged from 8.3 to 16.2 unit/ml with an average of 10.9 unit/ml milk. In cow milk it ranged from a minimum of 8.3 to a maximum of 16.4 unit/ml milk with an average of 12.1 unit/ml milk. The difference between the two averages was unsignificant, as shown in table (4).

Wide variations in its activity could be noticed in both milks which was confirmed by Pavel (1960) who reported that catalase activity of cow milk ranged from 1.6 to 15.4 ml 0_2/15 ml milk.

TABLE 4.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE CATALASE ACTIVITY OF INDIVIDUAL BUFFALO COW MILK

Source of variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	10.9	1.2	4.8	0.82	2.042	1.029	—
Cow	12.1		3.9	0.67			

The cytochrome C reductase content of buffalo milk ranged from a minimum of 0.032 to a maximum of 9.6 mg/ml with an average of 1.5 mg/ml milk. In cow milk it ranged from 0.063 to 3.164 mg/ml milk with an average of 1.16 mg/ml milk. The difference between the average contents of the two animals was insignificant, table (5).

TABLE 5.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE CYTOCHROME C REDUCTASE OF BUFFALO AND COW MILK

Source of variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	1.5	0.34	2.7	0.4	2.042	0.704	—
Cow	1.6		0.789	0.15			

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أنزيمات اللبن البقرى والجاموسى
١ ـ الأنزيمات المؤكسدة والمختزلة
أنزيم البيروكسيديز والزانثين أكسيديز والكتاليز والسيتوكروم س ريداكتيز

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الملخص

كان الغرض من هـذه الدراسة معرفة كمية ونشـاط اربع أنزيمات وهى البيروكسيديز والزانثين أكسيديز والكتاليز والسيتوكروم س ريداكتيز فى كل من اللبن الجاموسى والبقرى هذا وقد وجد أن فردية الحيوان لهـا تأثير على كمية ونشاط الأنزيم الموجود فى اللبن وكان هذا واضحا من اختلاف العينات عن بعضها ومن دراسة هذه الأنزيمات وجد أن نشاط انزيم الكتاليز والسيتوكروم س ريداكتيز فى كل من اللبن الجاموسى والبقرى واحد تقريبا فقد كان الكتاليز ١ر١٢ ، ٩ر١٠ وحدة لكل ملليلتر لبن على التوالى وكان السيتوكروم س ريداكتيز ٥ر١ ، ٦٦ر١ ميكروجرام لكل ملليلتر على التوالى .

نشاط أنزيم البيروكسيديز فى اللبن الجاموسى أكثر منه فى اللبن البقرى فقد كان ٤ر٣٤٣ ، ٤ر٢٢٨ وحدة لكل ملليلتر لبن على التوالى .

أما نشـاط أنزيم الزانثين أكسيديز فى اللبن الجاموسى اقل منه فى اللبن البقرى فقد كان ٦ر٨ ، ١ر١٨ وحدة لكل ملليلتر لبن على التوالى .

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