

**STUDIES ON ENZYMES OF COW'S AND
BUFFALOE'S MILK**

**II.—Esterases Enzymes Alkaline Phosphatase,
Acid Phosphatase, Lipase, and Ribonuclease**

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The individuality of the animal affects the value of the activities and contents of the enzymes in milk as they varied widely in the samples tested.

Buffalo and cow milk have approximately similar activity of lipase, being 16.5 and 14.8 ml 0.01 N NaOH/100 ml milk respectively. Buffalo milk has higher alkaline phosphatase activity, being 1047.5 ug phenol/ml milk but lower acid phosphatase activity, being 57.5 ug phenol/ml than cow milk being 302.3 ug phenol/ml, and 175.8 ug phenol/ml milk respectively.

In this section, studies of the activities and contents of alkaline phosphatase, acid phosphatase, lipase and ribonuclease in buffalo and cow milk in U.A.R.

Experimental and Methods

Samples were obtained and treated as under part I.

1. Alkaline phosphatase activity was determined spectrophotometrically according to Kay and Graham (1935), using buffer substrate tablets contains disodium phenyl phosphate and sodium barbitone, Folin and Ciocalten's reagent in sodium hexametaphosphate and sodium carbonate solution. The optical density of the blue colored solution was measured at 610 mμ wavelength using Jena Spectrocolorimeter, with 1 cm glass cell. Activity of enzyme were calculated as ug phenol/ml milk using calibration curve, from crystalline phenol.

2. Acid phosphatase activity was determined similar to those described under alkaline phosphatase except that buffer substrate was adjusted to pH 4.0 using 0.1 M sodium dihydrogen phosphate (1950).

3. Lipase activity was determined according to Mattick and Kay (1938). using 0.1 N sodium diethyl barbiturate, pH 8.5, 20 ml of milk, 0.5 ml butter oil, and the reaction was stopped by ortho-phosphoric acid. After steam distillation the first 100 ml of distillate were titrated with standard 0.01 N sodium hydroxide solution, using phenolphthalein as an indicator. A control determination was made and the results calculated as ml 0.01 N sodium hydroxide per 100 ml of milk.

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4. Ribonuclease was determined by the method of Tabachnick and Freed (1961), using Michaelis Veronol-acetate buffer, pH 7.5, 1% Nucleic acid solution and milk. The reaction was stopped by glacial acetic acid-butanol solution, centrifuged and the supernatant diluted with butanol in a ratio 1:6, V/V. The optical density was then read at 260 mu wavelength using Unicam SP 500 Spectrophotometer, with 1 cm silica cell. The ug enzyme calculated according to:

$$\text{Ug enzyme/ml of milk} = \text{Optical density} \times 0.3 \times \frac{6}{1}$$

Results and Discussion

The maximum, minimum, means, standard deviations, and standard errors of the alkaline phosphatase, acid phosphatase, lipase, and ribonuclease have been shown in table (1).

TABLE 1.—MAXIMUM, MINIMUM, MEANS, STANDARD DEVIATIONS, AND STANDARD ERRORS FOR ALKALINE PHOSPHATASE ACID PHOSPHATASE, LIPASE ACTIVITIES, AND RIBONUCLEASE CONTENT OF BUFFALO AND COW MILK

Enzyme	Max.	Min.	Mean	S.E.	S.D.
1. Alkaline phosphatase					
Buffalo	39000	240	10475.6	70.2	3735.1
Cow	420	140	302.3	17.8	97.4
2. Acid phosphatase					
Buffalo	208	17	57.5	10.7	5.78
Cow	606	25	175.8	11.04	60.5
3. Lipase					
Buffalo	68.5	3.5	16.5	3.4	16.7
Cow	33.5	3.0	14.8	1.8	10.4
4. Ribonuclease					
Buffalo	495.0	30.0	158.3	6.3	34.6
Cow	465.0	57.0	225.6	6.4	35.3

(1) and (2) ug phenol/ml of milk. (3) ml 0.01 N NaOH/ml of milk. (4) ug/ml of milk.

ENZYMES OF COW'S AND BUFFALO'S MILK

The alkaline phosphatase activity of buffalo milk showed a wide range of variation from a minimum of 240 to a maximum of 39000 ug phenol/ml milk with an average of 10475.6 ug phenol/ml of milk. In cow milk it ranged from 140 to 420 ug phenol/ml milk with an average of 302.3 ug phenol/ml milk.

Haab (1958), presented wide range of variation in cow milk, from 238 ug phenol/ml to 8778 ug phenol/ml milk.

Ray and Mullick (1963), Reported averages of 6.71 Bodensky unit/100 ml milk which would equal to 939.4 ug phenol/ml milk and Heyndrick and Peeters (1958), reported even higher average of 1473 ug phenol/ml in cow milk.

In the present study the difference between the two averages was significant as in table (2), the results are contrary to those reported by Safwat and El-Rafeey (1956), who found that the alkaline phosphatase of buffalo milk was much less than that of cow.

Acid phosphatase activity in buffalo milk ranged from 17.0 to 208 ug phenol/ml milk with an average of 57.5 ug phenol/ml milk, while in cow milk it ranged from 25 to 606 ug/ml milk with an average of 175.8 ug phenol/ml milk. The difference between the two averages was significant as shown in table (3).

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

Source of Variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	10475.6	10173.3	3735.1	70.2	2.042	140.4	+
Cow	302.3		97.4	17.8			

TABLE 3.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE ACID PHOSPHATASE ACTIVITY OF BUFFALO AND COW MILK

Source of variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	57.5	118.3	58.7	10.7	2.042	10.13	+
Cow	175.8		60.5	11.04			

Bertran (1952), reported an average activity of 4.0 Bodansky units which equalled to 556.4 ug phenol/ml milk, while Heyndrickx and Peeters (1958), found that it was 370 ug phenol/ml milk.

The lipase activity of buffalo milk ranged from a minimum of 3.5 to a maximum of 68.5 ml 0.01 N NaOH/100 ml milk with an average of 16.5 ml 0.01 N NaOH/100 ml milk, while in cow milk it ranged from 3.0 ml to 33.5 ml 0.01 N/100 ml milk with an average of 14.8 ml 0.01 N NaOH/100 ml milk. The difference between the two averages was insignificant as shown in table (4).

TABLE 4.—SIGNIFICANCE OF DIFFERENCE BETWEEN THE AVERAGE LIPASE ACTIVITY OF BUFFALO AND COW MILK

Source of variation	Means	Difference between means	S.D.	S.E.	T 0.05	T	Sig.
Buffalo . . .	16.5	1.7	16.7	3.4	2.042	0.042	—
Cow	14.8		10.4	1.8			

Pavel (1960), found that the lipase activity for foreign breed cows ranged from 0.31 to 0.40 ml 0.025 N NaOH/2 ml milk according to 38.7 to 50 ml 0.01 N NaOH/100 ml Heyndrickx and Peeters (1958), reported an average of 23 mg NaOH/100 ml which corresponded to 5.75 ml 0.01 N NaOH/100 ml milk.

Ribonuclease content in buffalo milk ranged from 30.0 to 495.0 ug/ml milk with an average of 158.3 ug/ml milk. In cow milk it ranged from 57.0 to 465.0 ug/ml with an average of 225.6 ug/ml milk. The difference between the two averages was significant as shown in table (5).

Bingham and Zittle (1962), stated that the mean content in Skimmilk was 24.9 ug/ml milk.

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انزيمات اللبن البقرى والجاموسى

٢ - انزيمات الـاستر

الفوسفاتيز القلوى والفوسفاتيز الحامضى والليباز والريبونوكليز

الدكتور ابراهيم الدسوقى رفعت - الدكتور جمال الصادق

السيدة عزة عبد العزيز اسماعيل

المـلخص

وجد ان فردية الحيوان لها تأثير على نشاط وكمية الانزيمات الموجودة فى اللبن كما هو ملاحظ فى العينات المختلفة من اللبن .

هذا وقد وجد ان نشاط انزيم الليباز فى اللبن الجاموسى والبقرى تقريبا واحد وهو ١٦ر٥ ، ١٤ر٨ ملليلتر من ٠.١ ر ع ص أيد لكل ١٠٠ ملليلتر لبن على التوالى .

ومن ناحية اخرى وجد ان اللبن الجاموسى يحتوى على انزيم الفوسفاتيز القلوى بكمية اكبر من اللبن البقرى ١٠.٤٧٥٦ ، ٣.٢٣ ميكروجرام فينول لكل ملليمتر لبن ويحتوى على كمية اقل من انزيم الفوسفاتيز الحامضى عن اللبن البقرى ٥٧ر٥ ، ١٧ر٥٨ ميكروجرام فينول لكل ملليلتر لبن على التوالى .