

## Comparative Study of The ATP Level of Swamp Buffalo (*Bubalus bubalis*) and Cow (*Bos indicus*) Red Cells

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### ABSTRACT

The objective of this study was to compare the ATP level of normal swamp buffalo and cow red blood cells. Blood from thirty two swamp buffaloes and thirty indigenous cows from Bangkhen, Bangkok were used for the determination of ATP by the luciferase enzyme. The average ATP level of normal buffalo red blood cells was  $22.870 \pm 5.409 \mu\text{mole}/100 \text{ ml}$ . while that of normal cow was  $35.225 \pm 7.706 \mu\text{mole}/100 \text{ ml}$ .

**Key words :** Adenosine tri phosphate (ATP), swamp buffalo red cells, cow red cells

### INTRODUCTION

Adenosine triphosphate (ATP) is a high energy nucleotide. The ATP content of living cells, including buffalo and cow cells is not high. ATP is repeatedly used and reformed during the course of many catabolic transformation of compounds. Glycolysis is the only source of ATP generation in the mature erythrocyte. ATP is necessary for the initiation of phosphorylation and the subsequent steps in the glycolytic pathway ATP level are higher in young than in old erythrocytes. Brewer and Powell (1966) reported that during reticulocytosis in human the ATP level of the blood increased. No one has reported on the ATP level of the blood of buffaloes and cows. The objective of this investigation was to compare the ATP level of normal buffalo and cow red blood cells in order to understand for the normal physiological function in both animal species.

### MATERIALS AND METHODS

Thirty two swamp buffaloes and thirty indigenous cows from Bangkhen, Bangkok were used. Ten millilitres of blood sample were taken from each animal by the jugular vein puncture using heparin as the anticoagulant.

### Method of extraction of ATP

One volume of heparinized blood sample was precipitated without delay with three volumes of ice-cold 6% (W/V) perchloric acid. After centrifugation aliquots of supernatant were used for the determination of ATP by the luciferase enzyme (Sigma Chemical Company) as described by Stanley and Williams (1969). The light emission produced in the luciferase enzyme reaction was measured by Beckman liquid scintillation counter Model L.S.3801, U.S.A.

The standard solutions of ATP were prepared from stock solution of adenosine-5'-triphosphate disodium salt (Sigma Chemical Company) by diluting 0.05 ml. of stock solution of ATP ( $10^{-7}$  mole/ml) with potassium phosphate buffer to 5 ml. in order to make concentration of  $10^{-9}$  mole/ml. This standard solution was mixed with phosphate buffer to make different ATP concentration in the range of  $10^{-9}$  to  $10^{-10}$  for preparation of a standard ATP curve.

One millilitre of each concentration of the standard solution was added to each vial containing 1.0 ml. arsenate buffer and 1.0 ml. of distilled water. Each of these vials was added with 50  $\mu\text{l}$  of luciferase enzyme solution. The solution was mixed well and the light emission was counted for 6 seconds in the Beckman liquid scintillation counter.

Standard curve of ATP was obtained by plot-

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ting log count of activity per 6 seconds and -log ATP (mole/ml).

## RESULTS AND DISCUSSION

The average ATP level of normal buffalo red cells was  $22.87 \pm 5.41$   $\mu\text{mole}/100 \text{ ml}$  while that of normal cow was  $35.22 \pm 7.71$   $\mu\text{mole}/100 \text{ ml}$ . The average hematocrit of buffalo and cow blood were  $33.67 \pm 7.13$  and  $32.97 \pm 5.73$  % respectively. The average haemoglobin of buffalo and cow blood were  $11.64 \pm 2.62$  and  $10.97 \pm 2.13$  gm % respectively (Table 1 and 2).

**Table 1 ATP levels of the blood of 30 buffaloes with their haematocrit and haemoglobin.**

Buffalo no.	Haematocrit %	haemoglobin gm %	ATP $\mu\text{mole}/100 \text{ ml}$
1	47.00	17.25	18.15
2	40.00	13.75	22.85
3.	38.50	14.75	22.68
4.	47.00	15.10	18.58
5.	45.00	16.75	26.17
6.	40.00	15.00	19.01
7.	37.50	12.50	23.82
8.	32.00	10.40	26.05
9.	32.00	10.80	26.05
10.	35.00	11.65	24.38
11.	39.00	13.00	23.99
12.	25.00	8.55	32.59
13.	26.00	8.75	27.29
14.	33.00	11.60	27.70
15.	24.00	8.50	22.95
16.	30.00	10.75	27.79
17.	29.00	10.25	23.37
18.	34.00	11.25	25.09
19.	31.00	10.75	22.37
20.	42.00	10.25	16.90
21.	21.00	7.50	30.82
22.	39.00	12.50	17.78
23.	31.50	7.00	25.85
24.	37.50	13.80	21.26
25.	31.00	11.10	29.88
26.	37.00	11.75	18.54
27.	27.00	9.75	23.48
28.	25.50	8.50	24.35
29.	29.50	10.25	22.04
30.	34.00	11.50	22.36
N	30	30	30
X	33.67	11.64	22.87
S.D.	$\pm 7.14$	$\pm 2.62$	$\pm 5.41$

Emanuelson et al. (1987) reported that the ATP level in cow milk was increased in the presence of mastitis. The present study is the first report on the ATP level of buffalo and cow red cells. The lower level of ATP in buffalo red blood cells may help to explain why the buffaloes moves more slowly than the cows. In man, American Negroes have a lower mean level of ATP in their red cells than American Caucasians (Brewer and Powell, 1966; Brewer, 1967). The concentration of ATP in human erythrocytes appears to be under genetic controlled, as the same

**Table 2 ATP levels of the blood of 32 cows with their haematocrit and haemoglobin.**

Cow no.	Haematocrit %	Haemoglobin gm %	ATP $\mu\text{mole}/100 \text{ ml}$
1	30	9.50	39.26
2	32	11.00	30.54
3	32	11.75	32.80
4	35	11.27	31.40
5	35	11.75	22.23
6	32.5	10.75	31.56
7	36	11.25	31.24
8	35	10.75	34.43
9	32	9.75	28.57
10	35	11.00	26.12
11	38.5	13.18	24.86
12	31	10.38	48.95
13	29.2	10.10	32.70
14	35.5	11.09	27.59
15	34	11.75	38.86
16	41	13.75	41.18
17	27	9.75	36.28
18	27.5	9.75	36.45
19	33.00	11.75	31.08
20	35.00	11.65	35.64
21	42.50	15.00	20.03
22	41.50	14.25	34.91
23	40.50	13.48	27.14
24	38.00	12.40	37.01
25	37.00	13.75	37.03
26	32.50	11.80	38.83
27	23.00	7.35	50.04
28	22.00	6.75	51.12
29	28.00	8.80	40.17
30	26.00	8.70	35.16
31	18.00	5.25	51.97
32	35.00	11.40	24.38
N	32	32	32
X	32.97	10.97	35.22
S.D.	$\pm 5.73$	$\pm 2.13$	$\pm 7.71$

ATP level in buffalo and cow red cells are likely under genetic influences also.

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