

## Blood Cholinesterase Activities in Cattle, Sheep and Goats Measured by a Modified Electrometric Method

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**Abstract:** Measurement of blood cholinesterase activity is a useful tool for monitoring exposure of animals to organophosphate and carbamate insecticides. A modified electrometric method was described for measurement of normal reference range values of plasma and erythrocyte cholinesterase activities in cattle, sheep and goats of both sexes. The reaction mixture contained 3 mL distilled water, 3 mL barbital-phosphate buffer (pH 8.1), 0.2 mL plasma or erythrocytes and 0.1 mL acetylthiocholine iodide (7.5%) as a substrate. The mixture was incubated at 37°C for 20 min in cattle, 30 min in sheep and 40 min in goats. The pH of the reaction mixture was determined by a pH meter before and after the incubation. The initial pH was measured before the substrate addition. The enzyme activity was expressed as  $\Delta$  pH/incubation time. The mean normal reference range values of plasma cholinesterase activity ( $\Delta$  pH/incubation time) in males and females of the three animal species were as follows, respectively: cattle (0.10 and 0.17), sheep (0.21 and 0.19) and goats (0.22 and 0.22), whereas those of the erythrocyte cholinesterase activity ( $\Delta$  pH/incubation time) were as follows, respectively: cattle (0.91 and 0.89), sheep (0.63 and 0.62) and goats (0.54 and 0.44). The study establishes for the first time by using the described electrometric method normal reference range values of blood cholinesterase activities in cattle, sheep and goats.

**Key words:** Cholinesterase, electrometric method, cattle, sheep, goat, organophosphate

### INTRODUCTION

Measurement of blood (plasma or erythrocyte) and tissue cholinesterase activities is a useful tool for diagnosing poisoning and monitoring exposure of animals to organophosphate and carbamate insecticides<sup>[1-6]</sup>. Various colorimetric and electrometric (potentiometric) methods are available for the determination of blood cholinesterase activity<sup>[2-4]</sup>. One of the main methods for measuring blood cholinesterase activity is the electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid which in turn decreases the pH of the reaction mixture<sup>[2-4]</sup>. The original electrometric method of Michel<sup>[7]</sup> is most commonly used in man<sup>[2-4]</sup>. However, the method is not efficiently applicable to samples from different animal species<sup>[2-4,8,9]</sup>. This is because of the inherent variations in blood or tissue cholinesterase activities of different animal species<sup>[3,4,6,8,9,10]</sup> and the special need for different buffer compositions, reaction temperatures, incubation times and sample volumes<sup>[9,11-13]</sup>. In addition, the original electrometric method cannot be recommended for detection of cholinesterase inhibition caused by carbamate insecticides<sup>[14-16]</sup>. Carbamylated cholinesterase is unstable in the reaction mixture of the electrometric

method of Michel because of considerable sample dilution and long incubation time (totally >60 min)<sup>[3,14-16]</sup>.

Various modifications of the electrometric method are available for measuring blood cholinesterase activity in animals<sup>[3,4,8,9,11,13,15]</sup>. These modifications include increasing sample volume, increasing or decreasing incubation time, increasing incubation temperature or using buffers of different compositions. One simple modification of the electrometric method is that of Mohammad *et al.*<sup>[11]</sup> which was introduced for rapid measurement of erythrocyte and plasma cholinesterase activities in sheep. The method was then applied successfully on several animal species such as mice<sup>[17]</sup>, rats<sup>[18,19]</sup>, rabbits<sup>[20]</sup>, goats<sup>[21]</sup>, chickens<sup>[22,23]</sup>, wild birds<sup>[24]</sup> as well as man<sup>[25]</sup>. The method is characterized by its simplicity, reproducibility, accuracy and one-step short incubation time (20-40 min depending on the animal species)<sup>[11,21,22,25]</sup>. Further, the present method, in contrast to the original electrometric method of Michel<sup>[7]</sup>, can detect cholinesterase inhibition induced by carbamate insecticides such as carbaryl<sup>[22,23,25]</sup> and methomyl<sup>[18]</sup>. The method correlates well with the electrometric method of Michel<sup>[7]</sup> and the colorimetric method of Ellman<sup>[22,24]</sup> in measuring cholinesterase activity. The method also substantially decreases handling of the reaction mixture which is found in other electrometric methods<sup>[3,4,8,13]</sup>.

The purpose of the present study was to establish normal reference range values of blood cholinesterase activities in cattle, sheep and goats using the described electrometric method, because these values have not been reported yet.

## MATERIALS AND METHODS

Venous blood samples of domestic mixed breeds of cattle (1-2 years old), sheep (1-1.5 years old) and goats (1-1.8 years old) of both sexes were obtained at the local slaughterhouse. The animals were apparently healthy and not exposed to any insecticide for at least two weeks before blood sampling. Blood samples were collected using heparinized test tubes<sup>[26]</sup>. Plasma was separated from erythrocytes by centrifugation at 3000 rpm (Centurion, UK.) for 15 min.

The modified electrometric method of Mohammad *et al.*<sup>[11]</sup> was used to measure blood cholinesterase activities of the cattle, sheep and goat. The reaction mixture in a 10-mL beaker contained 3.0 mL distilled water, 0.2 mL plasma or erythrocytes and 3.0 mL barbital-phosphate buffer solution (pH= 8.1). The pH of the mixture (pH<sub>1</sub>) was measured with a glass electrode using a pH meter (Hanna Instruments, Romania). This step was followed by the addition of 0.1 mL of 7.5% aqueous solution of acetylthiocholine iodide (BDH, UK) to the mixture, which was then incubated at 37 °C for 20 min in cattle, 30 min in sheep and 40 min in goats. At the end of the incubation period, the pH of the reaction mixture (pH<sub>2</sub>) was measured. The enzyme activity was calculated as follows:

ChE activity (Å pH/incubation time) = (pH<sub>1</sub> – pH<sub>2</sub>) - Δ pH of blank

The blank was without plasma or erythrocytes. The pH 8.1-buffer solution consisted of 1.237 g sodium barbital (BDH), 0.63 g potassium dihydrogen phosphate (E-Merck, Darmstadt, Germany) and 35.07 g sodium chloride (BDH) dissolved in one liter of distilled water<sup>[11]</sup>.

Preliminary experiments and previous studies<sup>[11,21]</sup> using pooled plasma or erythrocyte samples indicated that an incubation time of 20 min for cattle, 30 min for sheep and 40 min for goats after the addition of the substrate with a sample volume of 0.2 mL were suitable for measuring blood cholinesterase activities. The described electrometric method has been validated for use in ruminants<sup>[11,21]</sup> as well as in other animal species<sup>[17-20,22-25]</sup> and man<sup>[25]</sup>.

The mean, standard deviation, standard error, range and 95% confidence interval of plasma and erythrocyte cholinesterase activities were determined<sup>[27]</sup>. Unpaired Student's-t-test was used to determine the statistical

difference between plasma and erythrocyte cholinesterase activities within each animal species<sup>[27]</sup>. The level of significance was at  $p < 0.05$ .

## RESULTS

Table 1 and 2 show the normal reference range values, 95% confidence interval and related statistics for plasma and erythrocyte cholinesterase activities of the cattle, sheep and goats, respectively. The mean normal reference range values of plasma cholinesterase activity (Δ pH/incubation time) in males and females of the three animal species were as follows, respectively: cattle (0.10 and 0.17), sheep (0.21 and 0.19) and goats (0.22 and 0.22), whereas those of the erythrocyte cholinesterase activity (Δ pH/incubation time) were as follows, respectively: cattle (0.91 and 0.89), sheep (0.63 and 0.62) and goats (0.54 and 0.44). Plasma cholinesterase values were significantly ( $p < 0.05$ ) lower than those of the erythrocytes within each animal species (Table 1 and 2). Plasma cholinesterase activity of the females was significantly higher than that of the males in cattle Table 1. Erythrocyte cholinesterase activity of the females was lower than that of the males in goats (Table 2).

## DISCUSSION

Normal reference range values of plasma and erythrocyte cholinesterase activities of the male and female cattle, sheep and goat are reported for the first time in the present study using the described electrometric method. These values could be reference points for future studies involving monitoring blood cholinesterase activity in such ruminants exposed to cholinesterase inhibiting insecticides. This is important because measurement of blood cholinesterase activities in animals is a non-invasive method for monitoring exposure to organophosphate and carbamate insecticides<sup>[1-4]</sup>.

Plasma cholinesterase values were significantly lower than those of the erythrocytes within each animal species. Lower plasma cholinesterase activity in ruminants was reported earlier by other investigators too using electrometric or colorimetric methods<sup>[9-11,13,15,21]</sup>. This is in contrast to humans and other animal species such as rodents and horses that possess high plasma cholinesterase activity<sup>[2-9,14,15,25]</sup>. However, as in the present study sex differences in blood cholinesterase activities should not be excluded<sup>[2-4,8]</sup>. The differences in blood cholinesterase activities among animal species as well as between sexes could be considered normal physiological differences<sup>[2-4,6-8,12,13]</sup> and they might form the basis of the differential and blood-fraction dependent variations in sensitivity to organophosphates and carbamates<sup>[1-6,15]</sup>.

**Table 1: Normal reference range of the electrometric plasma cholinesterase activity of the cattle, sheep and goat**

| Parameter | Cattle ( $\Delta$ pH 20 min <sup>-1</sup> ) |            | Sheep ( $\Delta$ pH 30 min <sup>-1</sup> ) |            | Goat ( $\Delta$ pH 40 min <sup>-1</sup> ) |            |
|-----------|---|------------|--|------------|---|------------|
|           | Male  | Female     | Male                                       | Female     | Male                                      | Female     |
| N         | 37  | 34         | 35   | 37         | 26  | 47         |
| Mean      | 0.10  | 0.17       | 0.21                                       | 0.19       | 0.22                                      | 0.22       |
| SD        | 0.053                                       | 0.029      | 0.041                                      | 0.063      | 0.084                                     | 0.036      |
| SE        | 0.009                                       | 0.005      | 0.007                                      | 0.010      | 0.016                                     | 0.005      |
| Minimum   | 0.02  | 0.10       | 0.12                                       | 0.06       | 0.04                                      | 0.16       |
| Maximum   | 0.20  | 0.23       | 0.31                                       | 0.33       | 0.38                                      | 0.36       |
| Range     | 0.18  | 0.13       | 0.19                                       | 0.27       | 0.34                                      | 0.20       |
| 95% CI    | 0.08, 0.12                                  | 0.16, 0.18 | 0.20, 0.22                                 | 0.17, 0.21 | 0.19, 0.25                                | 0.21, 0.23 |

N: NO. of animals; SD: Standard Deviation; SE: Standard Error; CI: Confidence Interval. \* Significantly different from the corresponding male value,  $p < 0.05$

**Table 2: Normal reference range of the electrometric erythrocyte cholinesterase activity of the cattle, sheep and goat**

| Parameter | Cattle ( $\Delta$ pH 20 min <sup>-1</sup> ) |            | Sheep ( $\Delta$ pH 30 min <sup>-1</sup> ) |            | Goat ( $\Delta$ pH 40 min <sup>-1</sup> ) |            |
|-----------|---|------------|--|------------|---|------------|
|           | Male  | Female     | Male                                       | Female     | Male                                      | Female     |
| N         | 37  | 35         | 39   | 35         | 26  | 47         |
| Mean      | 0.91  | 0.89       | 0.63                                       | 0.62       | 0.54                                      | 0.44       |
| SD        | 0.212                                       | 0.118      | 0.092                                      | 0.123      | 0.169                                     | 0.071      |
| SE        | 0.035                                       | 0.020      | 0.015                                      | 0.021      | 0.033                                     | 0.010      |
| Minimum   | 0.32  | 0.46       | 0.45                                       | 0.34       | 0.38                                      | 0.29       |
| Maximum   | 1.27  | 1.11       | 0.82                                       | 0.82       | 1.04                                      | 0.68       |
| Range     | 0.95  | 0.65       | 0.37                                       | 0.48       | 0.66                                      | 0.39       |
| 95% CI    | 0.84, 0.98                                  | 0.85, 0.93 | 0.60, 0.66                                 | 0.58, 0.66 | 0.48, 0.60                                | 0.42, 0.46 |

N: NO. of animals; SD: Standard Deviation; SE: Standard Error; CI: Confidence Interval. \* Significantly different from the corresponding male value,  $p < 0.05$

The present electrometric method described for measurement of blood cholinesterase activities in the three ruminants depended mainly on the modifications introduced earlier in sheep<sup>[11]</sup>. The method has been also applied successfully for the determination of blood or tissue cholinesterase activities in other animal species<sup>[17-25]</sup>. The results of the present study further add to and expand previous studies in sheep<sup>[11]</sup> and goats<sup>[21]</sup> that advocated the described electrometric for measurement of blood cholinesterase activities.

The 20, 30 or 40 min one step incubation times and the 0.2 mL sample volume appeared to be suitable for the assay conditions in the three animal species to measure the enzyme activity without interference with the buffering capacity in the reaction mixture. This is in agreement with our earlier findings<sup>[11,22,23,25]</sup>. The one step short incubation time of the described method would be useful in increasing the efficiency of the procedure for multiple samples when compared to more than 60 min of the original Michel's method<sup>[7]</sup>. The method also decreases substantially handling of the reaction mixture as found in other electrometric methods<sup>[3,4,8,9,13]</sup>. Furthermore, the described electrometric method correlated well with the Michel method<sup>[11,26]</sup> and with spectrophotometric method of Ellman<sup>[22,24,28]</sup>.

### CONCLUSIONS

The present study reports normal range values of plasma and erythrocyte cholinesterase activities in cattle,

sheep and goats as described by a simple electrometric method.

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