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Nejra Hadzimusic, Muhamed Katica, Zijad Muharemovic, Jasmin Mušanovic

Corresponding author: Nejra Hadzimusic (nejra.hadzimusic@vfs.unsa.ba)

Correspondence concerning this article should be addressed to Nejra Hadzimusic, Department of Pathophysiology, Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina; Email: nejra.hadzimusic@vfs.unsa.ba

International Journal of Collaborative Research on Internal Medicine & Public Health Vol. 2 No. 5 (May 2010) Pages 158-166

ISSN 1840-4529

http://www.iomcworld.com/ijcrimph/

**Paper review summary:** Paper submission: April 30, 2010 Revised paper submission: May 24, 2010 Paper acceptance: May 26, 2010 Paper publication: May 26, 2010

## International Journal of Collaborative Research on Internal Medicine & Public Health

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## Effect of Temperature Storage on Hematological Parameters of Avian Turkey Blood

## Nejra Hadzimusic

Department of Pathophysiology Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina Email: <u>nejra.hadzimusic@vfs.unsa.ba</u>

#### **Muhamed Katica**

Department of Pathophysiology Veterinary Faculty, University of Sarajevo, Bosnia and Herzegovina

#### **Zijad Muharemovic**

Department of Biophisycs Medical Faculty, University of Sarajevo, Bosnia and Herzegovina

#### Jasmin Musanovic

Department of Biology and Human Genetics Medical Faculty, University of Sarajevo, Bosnia and Herzegovina

#### Abstract

**Background:** Hematology results are often influenced by the time between blood sampling and measurement, as well as storage conditions (e.g., temperature and time) during sample delivery between laboratories may further affect the resulting data. Hematological changes may occur in the measured parameters as a consequence of delayed analysis and may complicate interpretation of the data. Delayed analysis of blood samples may be caused by restricted access to laboratories. Blood samples collected from turkeys in remote locations (farms) often wait for laboratory processing.

**Objectives:** The aim of this study was to investigate changes that occur in the packed cell volume (PCV), hemoglobin concentration, red blood cell (RBC) counts and mean corpuscular values (MCV) of turkey blood samples due to storage, including: blood samples stored at refrigerator (4°C), laboratory (24°C) and water bath (33°C) temperatures across a storage period of 72 h.

**Methods:** Blood samples were collected from 25 adult turkeys (British United Turkey 600 hybrids – BUT 600). Hematological determinations were carried out on the blood samples immediately upon collection to obtain the baseline value (BV) and thereafter at specific time intervals across the 72-h duration of storage (DOS).

**Results:** Results showed that for the samples stored at 4°C, there were no significant changes at p<0.05 level from the BV in the PCV, hemoglobin concentration and RBC counts, all through the 72-h DOS, but significantly increased values (p<0.05) were obtained when samples stored at 24°C and 33°C.

**Conclusion:** Based on our research results we concluded following: blood samples obtained from turkey stored up to 72 hours at 4°C provides legitimate results for PCV, Hgb concentration and RBC count. MCV value is reliable if blood sample was stored up to 30 hours.

Key words: Turkey blood, Hematology, Duration of storage, Storage temperature

#### Introduction

Avian clinical hematology has become a vital part of a series of routine laboratory tests that aid veterinary clinicians to arrive at a diagnosis, make a prognosis and/ or assess the efficacy of therapeutic interventions in avian clinical practice<sup>1, 2, 3</sup>. Because avian blood does not store well (e.g. during transport) hematologic results obtain soon after collection are preferred over those preferred several hours later<sup>2, 4, 5</sup>. Although it is recommended to perform laboratory analysis immediately upon collection, often it is not possible, especially when blood samples are collected from remotely located farms. Unfortunately, due to often used manual procedure (instead of automated hematology analyzers), blood samples may not be analyzed immediately after arrival at the laboratory. Automated hematology instruments are used for mammalian blood analysis, but there is a lack of accurate automated methods available for avian blood analysis. Manual procedures are commonly used for the determination in avian hematology, because all blood cells are nucleated<sup>1, 2, 3</sup>. It is well known that handling of blood samples, as well as method of keeping and storage can significantly influence the results of hematological determinations. Consequently, results of hematological determinations of improperly stored or handled blood samples can yield misleading results<sup>6, 7, 8, 9, 10</sup>. Butarrello<sup>10</sup> and Goosens<sup>11</sup> in their studies concluded that refrigeration of human blood samples is recommended to stabilize blood and minimize artifactual changes. Research conducted on different animal species showed significant differences in the stability of blood samples stored at room or refrigerator temperatures<sup>12, 13, 14</sup>. Study conducted by Bluel<sup>12</sup> on bovine blood showed that refrigeration had a stabilizing effect on red blood cells count, but led to decrease in white blood cells count during 24 hours of storage. Studies on equine blood conducted by Clarke<sup>15</sup> showed that hematological parameters were more stable in blood samples kept at room temperature than those kept in refrigerator.

So far, according to authors' knowledge, there had been no reports on the effects of temperature and duration of storage period on the hematological parameters in turkey blood sample. Therefore, this research has been conducted in order to detect changes in the hematological values of turkey blood samples, stored for up to 72 hours at refrigerator (4°C), room (24°C) and water bath (33°C).

## Material and methods

Blood samples were collected from 25 turkeys (British United Turkey 600 hybrids -

BUT 600). Animals were procured as 1-month old from turkey producer (Jasmin d.o.o., Orašje, Bosnia and Herzegovina). Turkeys were raised in extensive production. A total of 25 blood samples were collected from apparently healthy adult turkeys. Blood samples were collected by ulnar vein-puncture method in tubes  $\acute{a}$  15 ml with anticoagulant. The blood samples were collected in the morning hours. Immediately upon collection hematological determinations were carried out on the blood samples, to obtain the baseline value (BV). Afterwards, the blood sample from each turkey was gently mixed and shared into three equal parts. One part was kept in refrigerator (temperature 4°C), another was kept at room temperature (24°C) while third part was kept in a water bath (temperature 33°C). Hematological determinations were carried out during 72 hours of storage on different temperatures, as follows: firstly, three hour intervals during first 12 hours of storage (hour 3, 6, 9 and 12), then at six-hour intervals during next 24 hours (hours 18, 24, 30 and 36) and at twelve-hour intervals for the remaining 36 hours (hours 48, 60 and 72).

The blood samples were used for hematological parameters including red blood cells (RBC) count, packed cell volume (PCV), hemoglobin (Hgb) concentration, and mean corpuscular volume (MCV). RBC count was determined by using Neubauer hemocytometer (Assistant, Germany). PCV was determined by using microhematocrit method<sup>6</sup> using microhematocrit centrifuge and reader (Hawksley, England), expressed in percents (%). The hemoglobin concentration was determined by using hemoglobinometer (Hawksley, England) and expressed in conventional Units g/dL (Conversion factor to SI Units: x10). MCV was calculated using the standard formula<sup>3</sup>.

Results of the study were presented as mean values with standard errors (SE) of each parameter determined at specific time intervals starting from the BV. Data was analyzed for statistical differences using ANOVA and the result of each determination was compared with BV.

## Results

Mean PCV value in blood samples stored at different temperatures (4°C, 24°C and 33°C) from BV (33,0±0,58%) determined immediately after collection increased to maximum value in hour 72 (36,7±0,54%) in samples stored at 4°C; 43,12±2,5% in samples stored at 24°C and 55,0±1,9% in samples held at 33°C (Fig. 1.). There were no significant differences at p<0.05 between the BV and values determined in samples stored at 4°C during period of 72h (Table 1.). Statistically significant differences (p<0.05) were noticed between BV and 24h from collection in samples stored at 24°C and 33°C (Tab. 1).



Fig. 1: Changes in the PCV of turkey blood samples at different temperatures during 72 hours

There were no significant differences at the p< 0.05 level from BV in the mean hemoglobin concentration of the blood samples stored at 4°C and those kept at 24°C during 72 hours of storage (Table 1). Mean value of hemoglobin concentration in blood samples stored at 4°C decreased slightly from BV, but not significantly (at the p<0.05 level) (Fig. 2.).Mean hemoglobin concentrations in blood samples stored at 24°C decreased as well, but not statistically significant. However, statistically significant decrease of hemoglobin concentration was found in samples stored at 33°C during period of 72 hours. In hour 60, hemoglobin concentration decreased from BV 9,08±0,38 g/dL to 7,1±0,45 g/dL (Fig. 2.).



Fig. 2: Changes in the Hgb concentration of turkey blood samples at different temperatures during 72 hours

RBC count no statistical significance (p>0.05) during 72 hours of storage at 4°C and 24°C (Table 1.). RBC determined as BV was  $2,19x10^{12}\pm1,6/L$  decreased to  $1,95x10^{12}\pm2,1/L$ . However, decrease in RBC count was not statistically significant. RBC count decreased progressively from BV ( $2,19x10^{12}\pm1,6/L$ ) to  $1,2x10^{12}\pm3,2/L$ 

during 72 hours if storage at 33°C (Fig. 3.).



Fig. 3: Changes in the RBC count of turkey blood samples at different temperatures during 72 hours

The MCV of turkey blood samples was found statistically higher (p<0.05) than the BV from hour 36 in blood samples stored at 4°C, as well as from hour 30 in blood samples stored at 24°C and 33°C. The MCV increased from BV (159 $\pm$ 6,2 fl) after 72 hours of storage to 196,34 $\pm$ 26,56 fl (4°C), 220,19 $\pm$ 34,23 fl (24°C) and 289,368,75 fl (33°C), respectively (Fig. 4.).



Fig. 4: Changes in MCV of turkey blood samples at different temperatures during 72 hours

#### Discussion

The PCV and MCV values determined in our research showed increase depending to temperature and storage duration. Such a significant increase can be attributed to the increase in volume of RBC, due to swelling. According to Schalm<sup>6</sup>, Perk<sup>16</sup>, Jandl<sup>17</sup>, Rich<sup>18</sup> and Coles <sup>19</sup>, RBCs swell and increase in size/volume in blood samples kept

for long because of storage-related degenerative changes that occur in the RBCs that lead to widening of the "pores" on the surface of the RBCs, which permit ingress of water into the cells. Increases in PCV and MCV like those mentioned above were reported also in horses<sup>15</sup>, bovine<sup>12, 14</sup> pigs, goats and rats<sup>14</sup> and chicken<sup>20</sup>.

In our study, storage temperature of 4°C significantly decreased changes regards to PCV, as well as MCV. Higher temperatures (24°C and 33°C) yet caused greater RBC swelling. Our findings are consistent with studies conducted on human blood samples<sup>7</sup> as well as cattle<sup>12, 14</sup>, goats, pigs<sup>14</sup> and rats<sup>13</sup>, but in contrast with the study conducted by Clarke<sup>15</sup> on equine blood samples, which showed artifactual changes in stored blood samples. Study showed increased numbers of macrocytic hypochromic RBCs. Changes were, according to Clarke<sup>15</sup> less pronounced in samples stored at 24°C than at 4°C.

Significant decrease of hemoglobin concentrations and RBC counts in blood samples stored at 33°C from BV was noticed after 60 hours from blood collection might be outcome of higher temperature induced conversion of some of the hemoglobin intermediates and due to significant autolysis of the RBCs associated with higher temperatures of storage<sup>8, 21</sup>.

## Conclusion

Based on the results, we concluded that the blood samples obtained from turkeys stored up to 72 hours at 4°C provide legitimate results for PCV, Hgb concentration and RBC count. MCV value is reliable if blood sample was stored up to 30 hours. Blood samples stored at 24 °C can be used for hemoglobin concentration and RBC count up to 72 hours of storage, but for PCV only up to 18 hours and MCV up to 12 hours from sample collection. Samples kept at 33°C give reliable results for MCV up to 12 hours, PCV up to 18 hours and hemoglobin concentration and RBC count up to 48 hours from blood collection.

Results of our research can be used as a guide to determine the appropriate storage and handling of turkey blood samples. We also recommend further research based on shorter intervals during 72 hours of storage as well as further general discussion of sample collection and handling, and discussion on considering some of the routinely encountered problems (and how to avoid them) associated with performing commonly requested tests (e.g. complete blood cell counts, chemistry profiles, etc.).

## List of abbreviations:

BUT 600 - British United Turkey 600 hybrid

BV – baseline value

PCV - packed cell volume

RBC - red blood cell

Hgb – hemoglobin

MCV - mean corpuscular value

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## Tables:

# Table 1: Basal values of hematological parameters of turkey blood samples and<br/>values after 72 hours of storage at different storage temperatures

	BV	4°C	24°C	33°C
PCV (%)	33 ± 0,58 a	$36,7 \pm 0,54$ a	43,2 ± 2,5 b	55,0 ± 1,9 b
Hgb (g/dL)	9,08 ± 0,38 a	8,99 ± 0,42 a	8,95 ± 0,39 a	$6,9\pm0,45~b$
RBC count	2,19x10 <sup>12</sup> ±1,6/L a	$1,95 \times 10^{12} \pm 2,1/L$ a	$1,93 \times 10^{12} \pm 1,7/L$ a	$1,2x10^{12}\pm3,2/L$ b
MCV (fl)	159 ± 5,24 a	196,34 ± 26,56 b	220,19 ± 34,23 c	289,36 ± 98,75 d

a, b, c, d – values with different letter show statistical significance (p<0.05)