PRELIMINARY STUDIES INVALIDATE AUTOMATED HAEMO-ANALYZER RESULTS OF AVIAN BLOOD

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ABSTRACT
Experiments were performed with the aim of determining the complete blood cell count of broiler chickens reared on 24-h continuous lighting photoperiod regimens using an automated haemoanalyzer. Six Marshal broiler chickens, randomly selected from a flock of three hundred served as subjects. On the experimental day, 2 ml of blood was collected from the wing vein of each bird at 13:00 h into ethylenediaminetetra acetic acid bottle. The blood was analyzed immediately using automated electronic cell counter for haematocrit, haemoglobin, red blood count, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, thrombocytes, mean thrombocyte volume, thrombocyte distribution width, thrombocyte large cell ratio, total and differential leucocyte counts with their percentages. The automated machine did not process 50 % of the samples for red cell distribution width. Also, 83.33 % of the samples for mean thrombocyte volume, thrombocyte distribution width and thrombocyte large cell ratio were not processed by the machine. It is concluded that results of broiler chicken blood analyzed using automated haemoanalyzer may be accepted, but should be interpreted with caution.

INTRODUCTION
Today, the use of computerized and automated machines in scientific research, including haematological studies has rendered virtually all traditional or conventional manual techniques obsolete. Haemogramme consists of results of laboratory tests used in routine evaluation of erythrocytes and leucocytes, based on complete blood count per microlitre of blood and morphologic evaluation (Gaunt, 2004). Evaluation of leucocytes involves interpretation of leucocyte parameters, including total leucocyte count, differential count and leucocyte morphology (Barger, 2003). Walberg (2001) reported that electronic cell counters are the gold standard in counting of mammalian leucocytes. They are ideal devices to efficiently and accurately count cells in an objective manner. The counters lyse the anuclear mammalian red blood cells and count nuclei. Since avian red blood cells are nucleated, this technique cannot be used to count white blood cells in birds. The leucocyte count in the avian species is obtained using manual techniques because the presence of nucleated erythrocytes and thrombocytes interfere with the counting of leucocytes using electronic cell counters (Campbell, 1995). Knoll and Rowell (1996) and Fudge (2000) considered automated analysis using laser and impedance technologies (Cell Dyn 3500; Abbott Laboratories, North Chicago, IL) the most accurate method of counting avian leucocytes. Unfortunately, this machine, unlike the electronic counters, are not available for use in developing nations of the world, including Nigeria. The three feasible methods of counting leucocytes in Nigeria today involves the use of the "Coulter counter" (Beckman Coulter, Fullerton, CA, USA) (Burgess and Davison, 1999) and, the haemocytometer method; comprising Unopetted technique and Natt-Herrick method (Natt and Herrick, 1956; Dein *et al.*, 1994; Walberg, 2001; Toghyani *et al.*, 2010) as well as the estimated cell count from stained blood smears (Walberg, 2001; Sinkalu *et al.*, 2010). The three methods are very cumbersome, therefore clinicians and researchers in Nigeria and other developing nations practically do not consider avian erythrocyte and leucocyte counts in their diagnostic plans and research. The objective of the present study was to determine the suitability of using an automated haematological analyzer for broiler chickens blood in order to promote research and enhance diagnoses of diseases in developing nations.

MATERIALS AND METHODS
Six Marshal broiler chickens, randomly selected from a flock of three hundred reared in the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, aged 4 weeks and weighing 1.21 ± 0.08 kg served as subjects. The birds were raised on deep litter system and subjected to a 24-h continuous lighting programme. On the experimental day, 2 ml of blood was collected from the wing vein of each bird at 13:00 h into ethylenediaminetetraacetic acid bottle. The blood was analyzed immediately using automated haemoanalyzer (Abbott Haematological Analyzer Cell-Dyn 1700,

Illinois, USA) for haematocrit, haemoglobin, red blood count, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, thrombocyte count, mean thrombocyte volume, thrombocyte distribution width, thrombocyte large cell ratio, total and differential leucocyte counts with their percentages in the haematology unit of Ahmadu Bello University Teaching Hospital, Shika-Zaria (11° 04'N, 7° 42'E), located in the Northern Guinea Savannah zone of Nigeria.

RESULTS AND DISCUSSION

Tables 1 – 3 show that the results obtained on erythrocytic, thrombocytic and leucocytic series were incomplete. The automated machine did not process 50 % of the samples for red cell distribution width (Table 1). Also, 83.33 % of the samples for mean thrombocyte volume, thrombocyte distribution width and thrombocyte large cell ratio were not processed by the machine (Table 2). Table 3 shows that data were not generated from the samples analyzed for monocytes, eosinophils and basophils. The results generated from the machine fell short of providing complete and accurate data on full blood cell count, which is an important and powerful diagnostic tool in human and veterinary medicine (Stacy *et al.*, 2011), used to monitor a patient's response to therapy, and to determine the severity of an illness, or as a starting point for formulating a list of differential diagnoses (Barger, 2003).

Conclusion

It is concluded that results of avian blood analyzed using automated haemoanalyzer may be accepted, but should be interpreted with caution.

Acknowledgement

The authors thank Mr. E. Nwosu of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria for technical assistance.

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Table 1: Erythrocytic indices from preliminary haematological studies of Marshal broiler chickens using electronic cell counter (n = 6)

Parameters	Bird's tag number						Mean ± SEM
	I	II	III	IV	V	VI	
Red blood cell count (x $10^6/\mu L$)	2.29	2.43	2.42	2.27	2.29	2.03	2.29 ± 0.06
Haematocrit (%)	31.8	34.1	32.8	28.4	31.8	29.4	31.38 ± 0.87
Haemoglobin (g/dL)	10.3	11.1	10.5	9.2	9.8	9.3	10.03 ± 0.30
Mean cell volume (fL)	138.9	140.3	135.5	125.1	138.9	144.8	137.25 ± 2.72
MCH (pg)	45	45.7	43.4	40.5	42.8	45.8	43.87 ± 0.84
MCHC (g/dL)	32	32.6	32	32.4	30.8	31.6	31.9 ± 0.26
Red cell distribution width _ SD (fL)	-	-	-	34	28.9	25	14.65 ± 6.66
Red cell distribution width _ CV (%)	-	-	-	16	10.6	8.6	5.87 ± 2.80

Table 2: Thrombocytic indices from preliminary haematological studies of Marshal broiler chickens using electronic cell counter (n = 6)

Parameters	Bird's tag number						Mean ± SEM
	I	II	III	IV	V	VI	
Thrombocytes x $10^3/\mu L$	3	10	9	3.2	16.0	10	8.53 ± 1.99
Mean thrombocyte volume, fL	-	-	-	-	8.6	-	1.43 ± 1.43
Thrombocyte distribution width, fL	-	-	-	-	11.1	-	1.85 ± 1.85
Thrombocyte large cell ratio, %	-	-	-	-	19.8	-	3.3 ± 3.3

Table 3: Leucocytic series from preliminary haematological studies of Marshal broiler chickens using electronic cell counter (n = 6)

Parameters	Bird's tag number						Mean ± SEM
	I	II	III	IV	V	VI	
White blood cell count, x $10^3/\mu L$	219.0	228.6	226.1	229.3	246.9	209.9	226.63 ± 5.03
Lymphocytes, x $10^3/\mu L$	-	-	-	-	240.2	201.3	73.58 ± 46.81
Heterophils, x $10^3/\mu L$	-	-	-	-	6.7	-	1.12 ± 1.12
Lymphocytes, %	95.9	-	-	-	97.3	95.9	48.18 ± 21.55
Heterophils, %	-	-	-	-	2.7	-	0.45 ± 0.45
Monocytes, %	-	-	-	-	-	-	0.00 ± 0.00
Eosinophils, %	-	-	-	-	-	-	0.00 ± 0.00
Basophils, %	-	-	-	-	-	-	0.00 ± 0.00