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≪Research Note≫

Effects of Sampling and Storage Method on Chicken Blood Glucose Measurement

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Glucose is a major circulating carbohydrate in birds and its level in the blood is often used as a biometric indicator in clinical diagnosis and various studies. Notably, hypoglycemia is often associated with Spiking Mortality Syndrome in broilers; therefore, blood glucose levels need to be correctly evaluated in clinical diagnosis. In the present study, we investigated the effect of different blood treatment methods after blood collection on chicken blood glucose measurements. The blood glucose level of plasma separated from blood cell components immediately after blood collection was used as a reference and compared with glucose levels in serum and stored plasma. The mean glucose level in plasma separated from blood cell components immediately after blood collection was 236.1±15.9 mg/dL and remained stable for at least one week in refrigerated storage (between 2℃ and 5℃). However, glucose levels decreased slowly in plasma unseparated from blood cell components in storage with ice water. Mean glucose level in serum separated from blood cell components 1 h after blood collection was 206.4±9.2 mg/dL and fell to 108.3 ±30.0 mg/dL after 24 h. Therefore, the chicken blood serum glucose level was significantly lower than the level in plasma immediately after blood collection, regardless of elapsed time after blood collection. For the measurement of glucose in chicken blood, it is necessary to use refrigeration, use plasma from which blood cell components have been removed, and take measurements within at least 30 min.

Key words: chicken, glucose, plasma, serum, Spiking Mortality Syndrome

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Introduction

Blood glucose concentration is often used as a biological evaluation index in studies that evaluate the effects of dietary components on rearing conditions and health abnormalities. Moreover, in research involving blood glucose, measurements have been performed using whole blood (Burns *et al*., 2002; Bafundo *et al*., 2018), plasma (Smith and Baranowski-Kish, 1976), or serum (Brake *et al*., 1981; Daly and Peterson, 1990; Latour *et al*., 1996; Peebles *et al*., 2012; Sahin *et al*., 2018), and it is difficult to find consistency from blood collection techniques to measurement methods and results. Glucose is the main carbohydrate in circulation in birds and its concentration is controlled by pancreatic hormones, glucagon, and insulin (Scanes, 2015). Typical avian blood glucose levels fall within a range higher than is normal for mammals and are almost unaffected by dietary intake (Stevens, 1996). In broilers, Spiking Mortality Syndrome (SMS) is associated with severe hypoglycemia. In the diagnosis of this syndrome, an abnormally low blood glucose level is an important indicator in addition to typical clinical findings (Davis, 2013). The measured value of glucose in blood biochemical tests is affected by the processing method and processing time after blood collection. After blood collection, glucose in the blood is consumed rapidly by blood cells, mainly for glycolysis (Chan *et al*., 1989). To avoid this effect, it is necessary to examine the blood immediately after collection and to separate the cell components from the liquid component. However, when blood is collected at the site where the chicken is raised, it is not only difficult to carry out the test immediately, but in most cases, it is inevitable

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that the post-treatment process requires a certain amount of time. The glucose value measured in blood immediately after blood collection is lower than that measured using plasma and is affected by the hematocrit value (Kim, 2016). Serum is the liquid component of blood that remains after the cell components have coagulated. In our clinical experiences, for chicken blood samples, the time required for serum to separate out sufficiently exceeds 1 h at ambient temperature (15–35℃). Therefore, when glucose is measured in serum, there is a possibility that levels are lower than those measured in blood and plasma.

In this study, we examined the effect of processing and the elapsed time after blood collection on glucose measurements in chicken blood.

Materials and Methods

Blood Collection, Processing, and Glucose Measurement

In this study, animal handling and sampling were conducted in accordance with the Japan Veterinary Medical Association guidelines for industrial animal medicine.

Blood samples (3–4 mL) were collected individually from twenty-four 41-day-old broiler chickens (ROSS308) via the wing vein. Each blood sample was immediately divided by dispensing half into a Spitz tube that contained lithium heparin and the other half into a plain Spitz tube. Eleven of the lithium heparin samples were immediately gently agitated, centrifuged at 1200×*g* for 10 min, and 0.2 mL of separated plasma from each blood sample was placed in a microtube and chilled with iced water (0 h plasma). The same Spitz tubes (containing the remaining plasma plus blood cell components) were cooled with iced water and 0.2 mL samples of the plasma that had separated from the blood cells were removed at 1 h, 9 h, and 24 h after blood collection and transferred to microtubes (1 h/9 h/24 h plasma). The 11 blood samples in plain Spitz tubes were incubated in a water bath at 37℃ for 1 h and 0.2 mL of each separated serum was placed in a microtube and cooled with iced water (1 h serum). The blood samples that remained in the Spitz tubes were allowed to stand at room temperature (24–28℃), which allowed serum production to continue and the blood clot to become sufficiently retracted. After 9 h and 24 h, serum samples (0.2 mL at each time point) were transferred into microtubes and cooled with iced water (9 h/24 h serum). The plasma and the serum collected in microtubes at each time point were measured for glucose on the day of collection. The 0 h plasma was refrigerated (between 2℃ and 5℃) for 7 d after blood collection and the plasma glucose was measured (0 h＋7 d:rfg plasma). The refrigerated 0 h plasma was frozen at −30℃ for 23 d and thawed at room temperature (23–25℃). After thorough shaking and stirring, the plasma glucose level was measured (0 h＋7 d:rfg＋23d:fz plasma).

Plasma and serum obtained by these treatments were measured for glucose with a dry clinical chemistry analyzer (Spotchem D-00 and D-02, Arkray, Kyoto).

Comparative Analysis of Glucose Measurements

The glucose levels for 1 h, 9 h, and 24 h plasma were compared with those of 0 h plasma to investigate changes over time for plasma stored with iced water without separation from blood cell components. In addition, to investigate the effect of storage by refrigeration and freezing, the glucose levels for 0 h＋7 d: rfg plasma and 0 h＋7 d: rfg＋23d: fz plasma were compared with those of 0 h plasma. To track changes in glucose levels over time for separated serum at room temperature, measurements in 1 h, 9 h, and 24 h serum were compared with those in 0 h plasma.

The statistical analysis in these comparisons used the Wilcoxon signed-rank test.

Results

The glucose levels for 0 h, 1 h, 9 h, and 24 h plasma are shown in Table 1. The mean glucose level of plasma separated from blood cell components immediately after blood collection (0 h plasma) was 236.1±15.9 mg/dL, whereas mean levels in 1 h, 9 h, and 24 h plasma were 229.9±11.1 mg/dL, 224.1±10.3 mg/dL, and 216.7±7.7 mg/dL, respectively. No significant difference was observed between 1 h and 0 h plasma glucose levels ($p \geq 0.05$), but there were significant differences between 9 h and 24 h plasma glucose levels and 0 h plasma (both $p < 0.01$).

The glucose levels for 0h＋7d:rfg plasma and 0h＋7d:rfg ＋3d: fz plasma were 235.7±10.6 mg/dL and 226.3±10.0 mg/dL, respectively (Table 2), with no significant difference ($p \geq 0.05$). However, mean levels in 0 h＋7 d:rfg＋23d: fz plasma were significantly lower than those in 0 h plasma ($p < 0.05$).

The glucose levels for 1 h, 9 h, and 24 h serum are shown in Table 3, with mean glucose levels of 206.4±9.2 mg/dL, 170.7±16.2 mg/dL, and 108.3±30.0 mg/dL, respectively, all significantly different from 0 h plasma (all $p < 0.01$).

Discussion

Avian blood glucose levels are higher than in mammals and steady-state levels are less likely to change, even under starvation (Stevens, 1996). On the other hand, an abnormal drop in blood glucose (hypoglycemia) occurs as one of the clinical signs of SMS in broilers (Brown *et al*., 1991; Davis *et al*., 1995; Burns *et al*., 2002; Davis, 2013). An accurate measurement of blood glucose level is essential for the clinical diagnosis of SMS. Sidebottom *et al*. (1982) suggested that blood samples taken for measuring blood glucose levels should be analyzed promptly; otherwise, the incidence of hypoglycemia might be overestimated. Human medical laboratory tests indicate that whole blood or plasma should be used in the diagnosis of diabetes, where blood glucose measurement results are particularly important clinically (Kim, 2016). However, it is still unclear how differences in post-collection treatment of avian blood affect glucose levels. In general, a considerable amount of time is inevitably required from the time of blood collection in a poultry house until a biochemical test is performed. It may take several hours to catch chickens in a poultry house, collect blood, and transport the samples to a laboratory equipped with biochemical testing equipment. Consequently, for accurate results, it is important to know how differences in processing

Table 1. **The glucose values for each plasma collected over times**

Blood samples	0 h	1 h	9 h	24 h
	mg/dL			
BL01	255	237	226	215
BL02	252	243	243	231
BL03	235	234	220	214
BL04	236	223	216	213
BL05	241	243	214	208
BL06	253	242	242	225
BL07	244	235	230	220
BL08	233	222	222	221
BL09	229	220	218	220
BL10	204	214	214	204
BL11	215	216	220	213
Mean±S.D.	236.1±15.9	229.9±11.1	224.1±10.3	216.7±7.7
Max	255	243	243	231
Min	204	214	214	204
p-value		0.0606	0.0059	0.0020

p-value: Significance between 0 h plasma glucose level.

Table 2. **Plasma glucose level after refrigerated storage and after frozen storage**

Blood samples	0 h	0 h＋7 d:rfg	0 h＋7 d:rfg＋23d:fz
	mg/dL		
BL01	255	258	241
BL02	252	240	242
BL03	235	228	216
BL04	236	226	235
BL05	241	242	215
BL06	253	245	229
BL07	244	233	226
BL08	233	228	224
BL09	229	239	227
BL10	204	220	212
BL11	215	234	222
Mean±S.D.	236.1±15.9	235.7±10.6	226.3±10.0
Max	255	258	242
Min	204	220	212
p-value		0.8496	0.0186

p-value: Significance between 0 h plasma glucose level.
0 h: Plasma of the day separated immediately after blood collection.
0 h＋7 d:rfg: 0 h Plasma refrigerated for 7 days after blood collection.
0 h＋7 d:rfg＋23d:fz: 0 h Plasma stored for 23 days at −30℃ after refrigeration for 7 days.

methods affect blood glucose levels. In this study, we evaluated the effect of various post-collection blood treatments on glucose levels, by comparing the results against plasma levels measured immediately after collection. Levels in plasma separated from blood cell components were stable for at least 1 week in refrigerated storage. However, after further, frozen, storage at −30℃, mean plasma glucose levels decreased by approximately 4%, which showed that once the plasma was frozen, glucose levels slightly decreased.

Serum is the fluid that remains after blood clots over time at room temperature. Empirically, when chicken blood is placed at room temperature, clot retraction generally progresses slowly and it may take several hours before serum is produced. In this experiment, to accelerate clot retraction, samples were incubated in a water bath (37℃) for 1 h imme-

Table 3. **The glucose values for each serum collected over time**

Blood samples	0 h plasma	1 h	9 h	24 h
	mg/dL			
BL01	255	208	163	131
BL02	252	227	183	108
BL03	235	199	174	134
BL04	236	206	150	96
BL05	241	202	189	94
BL06	253	218	182	127
BL07	244	209	192	128
BL08	233	198	171	117
BL09	229	196	153	63
BL10	204	204	144	50
BL11	215	203	177	143
Mean±S.D.	236.1±15.9	206.4±9.2	170.7±16.2	108.3±30.0
Max	255	227	192	143
Min	204	196	144	50
p-value		0.0020	0.0010	0.0010

p-value: Significance between 0 h plasma glucose level.

diately after blood collection to promote serum production. Even by this time, the mean glucose level had fallen to 206.4 ±9.2 mg/dL, significantly lower than the levels for 0 h plasma (236.1±15.9 mg/dL; $p<0.01$), a decrease of over 10%. Glucose levels in serum produced at room temperature decreased further over time, to less than half the mean levels of 0 h plasma after 24 h. Therefore, we concluded that serum was not suitable for the measurement of chicken blood glucose. The time required for blood clotting in birds, including chickens, is considerably longer than that in mammals, requiring 38–124 min (Scanes, 2015) and the reduction in glucose concentration in human whole blood *ex vivo* averages 5%–7% per hour (Chan *et al*., 1989). The blood-clotting mechanism in birds is presumed to be the same as that in mammals, with prothrombin activated to thrombin by stimulation with surface contact or tissue damage (Scanes, 2015). In human erythrocytes, glucose degradation is promoted by phosphofructokinase activated by stimulation of inorganic phosphate in the blood (Tsuboi and Fukunaga, 1965). Additionally, thrombin and epinephrine induce platelet destruction secondary to aggregation and contraction and increase glucose uptake, thereby promoting glycolysis by hexokinase activity (Karpatkin, 1967). In birds, erythrocytes have phosphofructokinase activity, pyruvate kinase activity, and hexokinase activity and the glycolytic mechanism functions in the same way as human erythrocytes (Kalomenopoulou and Beis, 1990). The decrease in glucose levels in chicken plasma stored for 9–24 h with iced water without separation of blood cell components was less than that in serum and these levels slightly decreased compared with that in plasma separated from blood cell components immediately after blood collection followed by refrigeration. Blood glycolysis can be delayed by treatment with sodium fluoride but progresses to the same extent in blood treated with heparin for 1 h after treatment (Chan *et al*., 1989). In addition, it is necessary to exercise care when using sodium fluoride because hydrogen fluoride may be generated during the measurement of glucose level with the potential to damage the biochemical test apparatus.

In conclusion, when collecting blood to measure blood glucose, the optimal method is to immediately heparinize the sample, remove the blood cell components within at least 30 min after collection, and rapidly store the resulting plasma in a cooler box with iced water or a refrigerator. When collecting a small amount of blood in a poultry house, it is possible to separate plasma and blood cell components immediately after blood collection using a small battery-powered centrifuge. The results in this study were obtained using blood collected from apparently healthy chickens of a single breeding brand, breeding method, and blood collection age. Therefore, it is necessary to further investigate the relationship between different breeding brands, breeding methods, blood collection ages, or differences in the pathophysiological state of chickens and the effects of blood treatment on blood glucose levels and their mechanisms.

Conflicts of Interest

The authors declare no conflict of interest.

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References

Bafundo KW, da Costa MJ and Pesti GM. Blood glucose concentrations in nicarbazin-fed broiler chickens. Avian Diseases, 62: 114–116. 2018.

Brake J, Garlich D, Parkhurst CR, Thaxton P and Morgan GW. Physiological profile of caged layers during one production season, molt, and postmolt: organ weights and blood constituents. Poultry Science, 60: 2157–2160. 1981.

Brown TP, Brunet PY, Odor EM, Murphy DW and Mallinson ET. Microscopic lesions of naturally occurring and experimental "Spiking Mortality" in young broiler chickens. Avian Diseases, 35: 481–486. 1991.

Burns KE, Ruiz J, Opengart K, Hofacre CL, Brown TP and Rowland GN. Hypoglycemia spiking mortality syndrome in broilers with rickets and a subsequent investigation of feed restriction as a contributing factor. Avian Diseases, 46: 735–739. 2002.

Chan AY, Swaminathan R and Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clinical Chemistry, 35: 315–317. 1989.

Daly KR and Peterson RA. The effect of age of breeder hens on residual yolk fat, and serum glucose and triglyceride concentrations of day-old broiler chicks. Poultry Science, 69: 1394–1398. 1990.

Davis JF, Castro AE, de la Torre JC, Scanes CG, Radecki SV, Vasillatos-Younken R, Doman JT and Teng M. Hypoglycemia, enteritis, and spiking mortality in Georgia broiler chickens: experimental reproduction in broiler breeder chicks. Avian Diseases, 39: 162–174. 1995.

Davis JF. Hypoglycemia-Spiking Mortality Syndrome of Broiler Chickens. In: Diseases of Poultry (Swayne DE *et al*., eds.) 13th ed. pp. 1325–1327. John Wiley & Sons. Ames. 2013.

Kalomenopoulou M and Beis I. Studies on the pigeon red blood cell metabolism. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 95: 677–684. 1990.

Karpatkin S. Studies on Human Platelet Glycolysis. Effect of Glucose, Cyanide, Insulin, Citrate, and Agglutination and Contraction on Platelet Glycolysis. The Journal of Clinical Investigation. 46: 409–417. 1967.

Kim HS. Blood glucose measurement: Is serum equal to plasma?. Diabetes & Metabolism Journal, 40: 365–366. 2016.

Latour MA, Peebles ED, Boyle CR, Doyle SM, Pansky T and Brake JD. Effects of Breeder Hen Age and Dietary Fat on Embryonic and Neonatal Broiler Serum Lipids and Glucose. Poultry Science, 75: 695–701. 1996.

Peebles ED, Bafundo KW, Womack SK, Zhai W, Pulikanti R and Bennett LW. Effects of nicarbazin on the blood glucose and liver glycogen statuses of male broilers. Poultry Science, 91: 2183–2188. 2012.

Sahin N, Hayirli A, Orhan C, Tuzcu M, Komorowski JR and Sahin K. Effects of the supplemental chromium form on performance and metabolic profile in laying hens exposed to heat stress. Poultry Science, 97: 1298–1305. 2018.

Scanes CG. Carbohydrate Metabolism. In: Sturkie's Avian Physiology (Scanes CG ed.). 6th ed. pp. 421–441. Academic Press. London. 2015.

Sidebottom RA, Williams PR and Kanarek KS. Glucose determinations in plasma and serum: potential error related to increased hematocrit. Clinical Chemistry, 28: 190–192. 1982.

Smith CJ and Baranowski-Kish LL. The response of chickens to D-mannoheptulose: feeding behavior and blood glucose. Poultry Science, 55: 444–447. 1976.

Stevens L. Avian biochemistry and molecular biology. Cambridge university press. Cambridge. 1996.

Tsuboi KK and Fukunaga K. Inorganic Phosphate and Enhanced Glucose Degradation by the Intact Erythrocyte. Journal of Biological Chemistry, 240: 2806–2810. 1965.