Whole blood gas and biochemical reference intervals for Lohmann Silver layers

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ABSTRACT The blood gas and biochemical reference range established with i-STAT clinical analyzer in avian has become common, however, the reference value for various laying hen lines is limited. Therefore, blood gas and biochemical reference intervals will be established for Lohmann Silver layers in the pre- and post-laying periods. The blood sample was collected at a 4-wk interval. A total of 230 Lohmann Silver layers including 80 pullets (5−17 wk) and 150 laying hens (21−37 wk) were collected for whole blood measurement with the i-STAT clinical analyzer. The CG8+ cartridge provides values of the following 13 parameters: sodium (**Na** mmol/L), potassium (**K** mmol/L), ionized calcium (**iCa** mmol/L), glucose (Glu mg/dL), hematocrit (**Hct**% Packed Cell Volume [**PCV**]), pH, partial pressure carbon dioxide (**PCO$_2$** mm Hg), partial pressure oxygen (**PO$_2$** mm Hg), total concentration carbon dioxide (**TCO$_2$** mmol/L), bicarbonate (**HCO$_3$** mmol/L), base excess (**BE** mmol/L), oxygen saturation (**sO$_2$**%), and hemoglobin (Hb g/dL). The correlation of these parameters and the effect of physiological status were investigated. The reference value interval was established with a reference value advisor for pre-laying and post-laying birds. Correlations were found to be statistically significant, especially between BE and HCO$_3$ and TCO$_2$. Besides, values in Na, iCa, K, Hct, Hb, sO$_2$ differed significantly between the pre- and post-laying periods. Data in this study might serve as important information for facilitating the genetic selection and assessing the health of Lohmann Silver laying hens.

Key words: Lohmann Silver laying hen, blood gas and biochemistry, reference interval

INTRODUCTION

The i-STAT clinical analyzer is widely used for blood chemistry in a time-effective manner. In recent years, the device has been implemented in various species including, but not limited to cattle, equine, pigs, fish, cats, dogs, exotic parrots, falcons, and poultry (Matburger et al., 2000; Steinmetz et al., 2007; Martin et al., 2010; Martin et al., 2011; Schaal et al., 2016; Tarbert et al., 2017; Sauer et al., 2019; Sauer et al., 2020). The device has not only been applied in establishing reference intervals for healthy birds (Schaal et al., 2016; Sauer et al., 2019), but also evaluating mechanisms associated with some diseases for unhealthy birds such as Ca tetany in broiler-type birds (Martin et al., 2011), ascites-related traits (Closter et al., 2009; van As et al., 2010), and heat-stress studies (Wang et al., 2018; Barrett et al., 2019; Beckford et al., 2020).

The i-STAT1 technology was earliest validated in Rhode Island Red hens (Steinmetz et al., 2007). Broiler breeder reference intervals have been established (Martin et al., 2011). The reference interval of blood gas and chemistry of four Hyline layer strains was established (Schaal et al., 2016; Sauer et al., 2019). The blood PCO$_2$, iCa, and pH were affected by heat stress (Barrett et al., 2019). The venous blood gas and chemistry components are moderately heritable in commercial white egg-laying hens under acute or chronic heat exposure (Rowland et al., 2019). The significant differences between blood gas and chemistry values in Leghorn and Fayoumi genetic lines were identified (Wang et al., 2018). The blood gas and chemistry profiles varied among distinct Hyline laying birds (Sauer et al., 2019) and productive status (pullets and laying hens) of Hy-Line W-36 (Schaal et al., 2016).

The blood gas and biochemistry values shown in the present study are useful as a guide adjusting nutrition programs and improving feeding techniques for Lohmann Silver laying hens. And these data may help facilitate the genetic selection for various laying hen lines.

Furthermore, these values determined by i-STAT can be applied for assessing physiological disorders and diagnosing clinical diseases in Lohmann Silver laying hens. Although reference ranges of blood gas and chemistry for multiple Hyline laying birds have been established and may provide potential value for egg layer strains, there are differences among genetically distinct laying hen lines. Lohmann Silver is a predominately white feathering layer with the production of uniform brown eggs with small egg sizes. In this study, the blood gas and biochemical parameters were evaluated to investigate the variations and reference intervals for Lohmann Silver laying hens in the pre- and post-laying periods.

MATERIALS AND METHODS

Bird Husbandry

A total of 230 commercial Lohmann Silver pullets (at 5, 9, 13, and 17 wk of age, n = 80), and laying hens (21, 25, 29, 33, and 37 wk of age, n = 150) were sampled across 8 mo at multitier chicken layer cage under common diet and management systems according to the breeding manual of Lohmann company. These birds were located in Hebei Province, China. At each sampling for pullets, 20 birds were selected randomly. At each sampling for laying hens, 30 birds were selected randomly.

Blood Collection and Analysis

Blood (0.2−0.3 mL) was collected from the brachial vein into a disposable 1.5 mL tube with anticoagulant heparin lithium. Samples were evaluated with the i-STAT Portable Clinical Analyzer (Abbot Laboratories) and CG8+ cartridges (Abbott Laboratories, Nepean, Canada), which were stored on ice in coolers before use. Blood samples were measured immediately after collection. The needle was removed and one drop of whole blood was dispensed to the CG8+ cartridge, sealing the cartridge port, and inserting the cartridge into the hand-held unit (i-STAT operating manual). The CG8+ cartridge provides values of the following blood gas and biochemical parameters: sodium (Na mmol/L), potassium (K mmol/L), ionized calcium (iCa mmol/L), glucose (Glu mg/dL), hematocrit (Hct% Packed Cell Volume [PCV]), pH, partial pressure carbon dioxide (PCO_2 mm Hg), partial pressure oxygen (PO_2 mm Hg), total concentration carbon dioxide (TCO_2 mmol/L), bicarbonate (HCO_3 mmol/L), base excess (BE mmol/L), oxygen saturation (sO_2%), and hemoglobin (Hb g/dL).

The present study was researched in agreement with the institutional and national guidelines and was supported by the Animal Use and Ethics Committee of the Agricultural University of Hebei (University Identification Number: HB/2019/03).

Statistical Analysis

The normality test was performed with the Shapiro-Wilk normality test. Linear correlation coefficients between traits were evaluated using PROC CORR in SAS 9.4. The resulting principal component analysis was also performed using PROC PRINCOMP in SAS 9.4. The effect size of physiology-related variables was evaluated using PROC GLM in SAS. Reference ranges of blood gas and chemistry were computed in Microsoft Excel 2013 using Reference Value Advisor V2.1. The heatmap for Hyline and Lohnman Silver laying hens was constructed based on mean values of 13 parameters (data of various Hyline lines Schaal et al., 2016; Sauer et al., 2019) with average linkage clustering method and pheatmap package in R version.

RESULTS

Descriptive statistics of data for the blood gas and biochemical parameters in the pre- and post-laying periods are outlined in Table 1.

Reference Interval of Blood Gas and Biochemistry Parameters

The values of blood gas and biochemistry reference intervals of 230 Lohmann Silver laying hens shown in

Table 1. Blood gas and chemistry data from 230 Lohmann pullets (5 to 17 wk), and laying hens (21 to 37 wk).

Analyte	SI units	5-17 wk						21-37 wk					
		N	Mean	Median	SD	Min	Max	N	Mean	Median	SD	Min	Max
pH		80	7.49	7.47	0.08	7.34	7.76	150	7.49	7.49	0.10	7.05	7.71
PCO_2	mm Hg	80	26.85	27.20	5.04	16.50	38.60	150	26.03	26.20	6.12	11.40	42.70
PO_2	mm Hg	80	77.95	69.00	25.95	41.00	156.00	150	67.50	63.00	17.00	46.00	148.00
BE	mmol/L	80	-3.10	-3.00	2.70	-10.00	5.00	150	-3.60	-4.00	2.90	-10.00	6.00
HCO_3	mmol/L	80	20.23	20.40	2.01	14.90	24.40	150	19.66	19.60	2.45	13.00	27.70
TCO_2	mmol/L	80	21.04	21.00	2.01	16.00	25.00	150	20.40	20.00	2.60	13.00	29.00
sO_2	%	80	95.41	95.00	2.49	89.00	100.00	150	93.70	94.00	3.60	82.00	100.00
Na	mmol/L	80	141.27	141.00	2.44	135.00	146.00	150	145.20	144.00	3.50	139.00	154.00
K	mmol/L	80	4.41	4.40	0.31	3.50	5.40	150	4.09	4.10	0.30	2.90	5.10
iCa	mmol/L	80	1.33	1.31	0.10	1.12	1.54	150	1.52	1.53	0.15	1.05	1.97
Glu	mg/dL	80	231.64	225.00	22.14	198.00	292.00	150	231.50	230.00	15.20	193.00	271.00
Hct	%PCV	80	24.45	24.00	2.43	18.00	31.00	150	22.50	22.00	2.30	18.00	31.00
Hb	g/dL	80	8.32	8.20	0.82	6.10	10.50	150	7.65	7.50	0.77	6.10	10.50

Abbreviations: BE, base excess; Glu, glucose; HCO_3, bicarbonate; Hct, hematocrit; Hb, hemoglobin; iCa, ionized calcium; Na, sodium; k, potassium; PCO_2, partial pressure carbon dioxide; PCV, packed cell volume; PO_2, partial pressure oxygen; sO_2, oxygen saturation; TCO_2, total concentration carbon dioxide.

Table 2. Blood gas and chemistry reference ranges from 230 Lohmann pullets (5 to 17 wk), and laying hens (21 to 37 wk).

Analyte	SI units	5-17 wk			21-37 wk		
		RI	90% CI for lower limit	90% CI for upper limit	RI	90% CI for lower limit	90% CI for upper limit
pH		7.3-7.64	7.27-7.33	7.61-7.68	7.3-7.69	7.05-7.36	7.67-7.71
PCO_2	mm Hg	16.75-36.95	15.08-18.48	35.49-38.63	13.73-38.02	12.41-15.05	36.59-39.36
PO_2	mm Hg	35.65-130.51	32.93-39.83	116.69-144.57	48.8-123.8	46.00-52.00	101-148
BE	mmol/L	−8.59-2.37	−9.47-7.7	1.46-3.23	−9.00-2.20	−10.00-8.00	2.00-6.00
HCO_3	mmol/L	16.2-24.26	15.54-16.88	23.59-24.91	14.28-24.46	13-15.8	23.3-27.7
TCO_2	mmol/L	16.05-25	16.00-18.00	24.00-25.00	15.00-25.2	13.00-16.00	24.00-29.00
sO_2	%	89.1-100	89-91.05	99-100	84.78-99	82-87	99-100
Na	mmol/L	137.00-146	135-137.05	145-146	139-151.2	139-140	151-154
K	mmol/L	3.79-5.02	3.69-3.9	4.92-5.12	3.6-4.9	2.9-3.7	4.6-5.1
iCa	mmol/L	1.12-1.52	1.12-1.18	1.48-1.54	1.22-1.86	1.05-1.29	1.77-1.97
Glu	mg/dL	199-287.65	198-203.05	273.13-291.99	203.6-269.2	193-209	260-271
Hct	%PCV	20.03-29	18-21	28-31	18.8-28	18-19	26-31
Hb	g/dL	6.82-9.9	6.1-7.1	9.5-10.5	6.42-9.5	6.1-6.5	8.8-10.5

Abbreviations: BE, base excess; Glu, glucose; HCO_3, bicarbonate; Hct, hematocrit; Hb, hemoglobin; iCa, ionized calcium; Na, sodium; k, potassium; PCO_2, partial pressure carbon dioxide; PCV, packed cell volume; PO_2, partial pressure oxygen; sO_2, oxygen saturation; TCO_2, total concentration carbon dioxide.

Table 2 are not completely similar to previous studies of different genetic lines, which suggest the necessity to establish an accurate reference interval of blood gas and biochemistry for Lohmann Silver laying hens.

Correlations Among Blood Gas and Biochemistry Parameters

The Pearson correlation of data suggested that many blood gas and biochemistry parameters were related (Supplementary Table 1). The estimated correlation coefficient of BE and HCO_3, BE, and TCO_2 exceeded a value of 0.8. Besides, the correlation coefficient of TCO_2 and HCO_3, and Hb and Hct close to a value of 1. A negative correlation coefficient of pH and PCO_2 close to −0.9.

The spectral decomposition of the correlations was implemented to interpret the relationships among multiple variables measured with i-STAT. The principal component (**PC**) analysis indicated that the 3 PC explained 63.8% of the total variance (Table 3). In PC1 eigenvector, the loadings of PO_2, sO_2, Na, K, Hct, Hb showed negative values; while the traits with larger positive values were BE (0.426), HCO_3 (0.504), and TCO_2 (0.501). In PC2 eigenvector, positive loads were largely occupied by pH (0.487) and Glu (0.303). The loadings of Hct (0.306), Hb (0.306), HCO_3 (0.241), and TCO_2 (0.255) in the PC3 eigenvector presented positive values; while the only traits with negative loads were PCO_2 (−0.136), Na (−0.062), and iCa (−0.222).

Difference of Blood Gas and Biochemistry Parameters in Pre-laying and Post-laying Stages

The variations of blood gas and biochemistry between pre- and post-laying periods are shown in Figure 1. Compared with that in the pre-laying period, a highly significant increase in the level of sodium and ionized calcium during the laying period was observed ($P < 0.01$) while there was a highly statistically significant decrease regarding the sO_2, K, Hct, and Hb values ($P < 0.01$).

Effect of Genetic Line on Blood Gas and Biochemistry Parameters

A clustering heatmap of 13 parameters concerning different laying bird lines was presented in Figure 2. Blood gas and biochemistry parameters of 6 Hyline lines or ages were firstly clustered together; while these parameters of the Lohman Silver in the present study were clustered separately.

DISCUSSION

Enhancements in utilizing the i-STAT clinical analyzer which is certainly fast and cost-effective, especially for laying hens, have resulted in some valuable data to predict some diseases related to blood gas and biochemistry parameters. And some reference intervals of blood gas and biochemical parameters for Hyline laying hens

Table 3. Spectral decomposition of correlations between blood gas and chemistry traits, and principal component analysis showing the eigenvalues and loads of the eigenvectors for the first 3 principal components (PC) from 230 Lohmann silver pullets and laying hens.

Trait	PC1	PC2	PC3
pH	0.006	0.487	0.298
PCO_2	0.246	-0.492	-0.136
PO_2	−0.256	−0.061	0.383
BE	0.426	0.178	0.347
HCO_3	0.504	−0.087	0.241
TCO_2	0.501	−0.127	0.225
sO_2	−0.262	0.177	0.466
Na	−0.129	0.166	−0.062
K	−0.01	−0.067	0.244
iCa	0.11	0.189	-0.222
Glu	0.135	0.303	0.032
Hct	−0.189	−0.368	0.306
Hb	−0.19	−0.368	0.306
Eigenvalue	3.2	2.7	2.3
Variance explained (%)	24.9%	21%	17.9%

Abbreviations: BE, base excess; Glu, glucose; HCO_3, bicarbonate; Hct, hematocrit; Hb, hemoglobin; iCa, ionized calcium; Na, sodium; k, potassium; PCO_2, partial pressure carbon dioxide; PCV, packed cell volume; PO_2, partial pressure oxygen; sO_2, oxygen saturation; TCO_2, total concentration carbon dioxide.
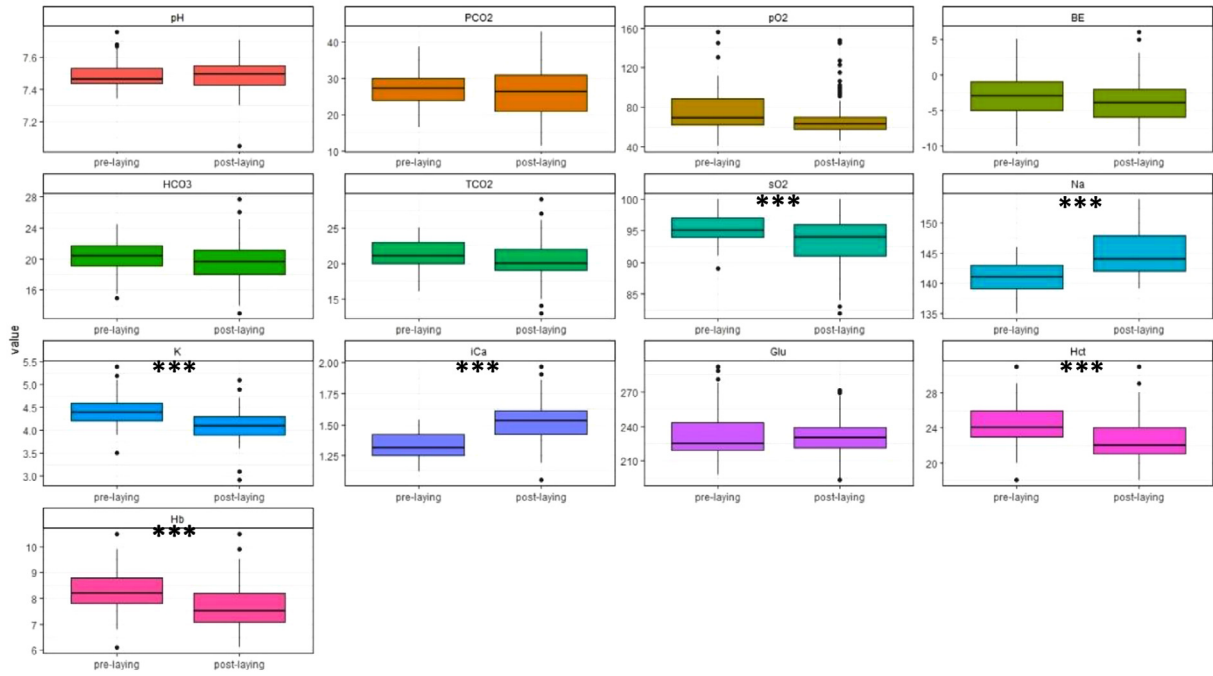
Figure 1. Box and whiskers diagram for the 13 biochemistry parameters between pre- and post-laying period. $^{***}P < 0.001$.

have been established (Schaal et al., 2016; Sauer et al., 2019). Unfortunately, reference intervals for different laying bird lines were diverse and no investigations on blood gas and biochemistry of Lohmann Silver laying hens existed. In this study, accurate reference intervals of blood gas and biochemistry for Lohmann Silver were established to interpret results.

Mean values of 13 parameters in Hyline and Lohmann Silver laying birds varied, which illustrates the necessity of establishing reference rages for Lohmann Silver laying hens. This result is also confirmed by Wang et al. (2018). pH in the blood is the most basic indicator of acid-base imbalance and can guide the treatment of certain pathophysiological processes such as acidosis and alkalosis (Reece et al., 2015). The mean values of pH were within the reference ranges in our study (Steinmetz et al., 2007; Sauer et al., 2019). The strong correlation among BE, HCO3, and TCO2 was in agreement with the previous report (Schaal et al., 2016). This

can be easily explained as most living organisms follow the principle of buffer systems for general acid-base balance (Reece et al., 2015). Alternatively, these parameters are critical for maintaining acid-base balance, which was also reflected in the spectral decomposition of correlations. And they are calculated based on PCO_2 and pH values measured in the i-STAT point-of-care analyzer (Montesinos and Ardiaca, 2013). The HCO_3 is calculated according to the value of pH and PCO_2. The TCO_2 is calculated according to the value of HCO_3 and PCO_2. The BE is calculated with the value of HCO_3 and pH. Due to some uncertain factors including individual metabolic variation and environmental difference, BE values were noted as unreliable in previous findings (Steinmetz et al., 2007; Sauer et al., 2019). Similar results of BE are also reported in this finding.

The correlation value between pH and PCO_2 is -0.825. The similar value is -0.864 and -0.756 in Hyline colored-egg laying lines and W36 white laying hen,
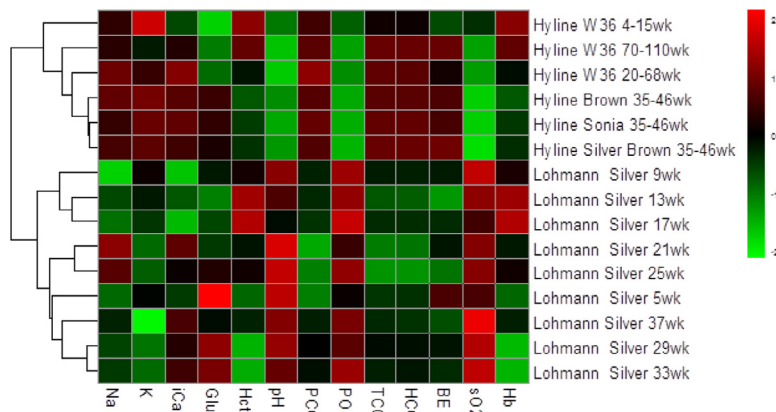

Figure 2. The heatmap of mean values for 13 parameters (CG8+) from variable breeds and ages.

respectively (Schaal et al., 2016; Sauer et al., 2019). PO_2 reflects pulmonary ventilation and pulmonary ventilatory function (Reece et al., 2015). A positive relationship between pH and PO_2 was recorded in this finding. The coefficient reported is 0.071 for Hy-line W-36 laying hen (Schaal et al., 2016).

Some basic blood parameters were significantly affected by physiological status. We found that Hct values markedly decreased during the egg-laying period, which is in line with the data obtained by Schumann et al. (2014). This can be explained by the decrease of erythropoietin under the stressful conditions of egg production (Strakova et al., 2001). A similar trend in Hb was also found, which is mirrored in the strong correlation between Hct and Hb. Hct was reported a lower value using the i-STAT clinical analyzer compared to a conventional reference analyzer (Steinmetz et al., 2007). One potential reason could be the difference in size between avian and human nucleated red blood cells interfering with the use of the machine, which was originally developed for humans (Steinmetz et al., 2007). The decreased level of oxygen saturation was also observed during the laying period. These similar results can explain why parameters related to oxygen transport are particularly susceptible to change during egg production. Although there was a strong positive correlation between sO_2 and PO_2 (0.725), PO_2 values were not changed significantly during the egg-laying period. The similar result was also reported (Sauer et al., 2019). As a result, the data should be interpreted more carefully.

Glu values were not affected by physiology status, which differs from the previous report that the level of Glu decreased significantly during the egg-laying period (Schumann et al., 2014). This might be affected by the time of sampling and various glucose levels in the diet. Electrolyte and acid-base balance are closely linked. Enhancements in sodium and ionized calcium values and a significant decrease in potassium values during egg production were reported (Schaal et al., 2016). This trend is also found in this study. This may be ascribed to the impact of reproduction status and a series of dynamic hormones during the laying period. Alternatively, diet composition and different nutritional levels might also influence the value of these parameters. Sodium is extremely valuable for the cardiovascular system of birds, in particular, reducing and eliminating the effects of water and electrolyte disturbances on physiological functions (Reece et al., 2015). Supplementation diets containing sodium bicarbonate and sodium chloride may also lead to the increased sodium levels during the egg-laying period (Schaal et al., 2016). Decreased values in potassium during the laying period may be related to the lower consumption of water in mature birds (Campbell, 2012). The reduction in water consumption causes the increasing release of aldosterone, which can reabsorb sodium and water in the kidneys in exchange for potassium (Schaal et al., 2016). However, potassium values were reported to be unreliable in previous investigations (Steinmetz et al., 2007; Sauer et al., 2019). Therefore, it should be careful when interpreting these results.

Unlike potassium and sodium, the research on ionized calcium is well established. Ionized calcium values in this study are similar to the data reported by Schaal et al. (2016). On the other hand, higher values in ionized calcium were obtained (Sauer et al., 2019), which was attributed to differences in diet formulation or genetic lines. Significantly increased ionized calcium levels during the laying period can be explained by the increased supplementation of calcium in the diet and mobilization of calcium from bones due to the demand for eggshell production. Calcium assuredly plays a key role in egg production. In summary, the urgent demands of egg-laying metabolism lead to drastic changes in certain indicators in the blood.

CONCLUSIONS

In conclusion, this study provides a more reliable reference range of blood gas and biochemistry for Lohmann Silver laying hens, which makes the utilization of the i-STAT clinical analyzer more credible in the laying industry. The blood parameter values regarding the BE, K, Na, and Glu in the reference interval should be interpreted carefully because some potential factors may exist. Additionally, some blood parameters, especially for ionized calcium, are surely affected by physiological status. Thus, the physiological status should be considered when assessing the blood gas and biochemical parameters in future investigations of laying birds.

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DISCLOSURES

The authors declare no competing financial interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101368.

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