

Effect of genotype on the hematological parameter of TETRA-SL and Hungarian Partridge coloured chickens at young age

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SUMMARY

Indigenous chickens are well known for their dual-purpose function and palatable meat. However, the information on their hematology parameters is lacking and hence hampering the poultry industry production of local breeds. The main objective of this study was to examine the hematological parameters of Hungarian Partridge Coloured hen (HPC) and TETRA-SL (TSL) genotype and determine the hematological reference interval values. This trial was part of the larger experiment where a total of 200 chicks (HPC + TSL) were reared. For this trial the blood samples were obtained from brachial wing veins of 8 chicks of each genotype for blood hematological analysis. The results indicated that some of the hematological parameters such as red blood cells-RBC, white blood cells-WBC, hemoglobin-Hb, hematocrit-Ht, platelets-Plt, lymphocytes-LYM, and granulocytes-GRAN were significantly affected by the genotypes ($p < 0.05$). The genotype did not affect the mean corpuscular volume-MCV, mean corpuscular hemoglobin-MCH, mean corpuscular hemoglobin concentration-MCHC, and GRAN ($p > 0.05$). The hematological reference interval values were slightly higher in the TSL genotype compared to HPC chicks. It is concluded that genotype has a significant effect on the hematological parameters. The results from this trial will be help and design the baseline reference values for the HPC genotype which will be useful in assessing the health status of these indigenous chickens.

Keywords: Hungarian Partridge coloured hen, Hematology parameter, TETRA-SL, Genotype

INTRODUCTION

Indigenous chickens are well known for their capability to survive harsh conditions and ability to scavenge most of their feed as well as disease resistance that are common in poultry (Melesse, 2014; Maoba et al., 2021). They cover up to 80% of chickens reared in rural areas and play an important role in food security (FAO, 2001). They are the main source of nutrition and economic development of women in rural communities, particularly in developing countries. Hungarian partridge coloured chicken (HPC) is among the indigenous chickens that have been maintained through official gene reserves since 1973 in Hungary (Szalay et al., 2016; Lan Phuong et al., 2014; Spalona et al., 2007). TETRA-SL (TSL) is a brown egg mid-heavy layer kept in cage and alternative systems. TSL are well known for persistent and efficient egg production as well as better internal and external quality due to genetic improvement (TETRA-SL 2021).

Maintaining the layers physiological status at normal range during rearing and laying period is the pre-determinant of their production. Birds' disease and physiological status are well depicted by the hematological parameters, where more attention has been paid nowadays (Hong et al., 2021). The hematological parameters are affected by several

factors such as nutrition, genotypes, sex, age, season as well as management conditions (Irivboje et al., 2020; Hong et al., 2021; Ologbose and Dick, 2021). Several studies have been conducted on the hematological parameters with inconsistent findings on different genotypes of poultry (Peters et al., 2011; Nosike et al., 2017; Ifelayo et al., 2020; Irivboje et al., 2020; Mohanty and GayatriAcharya, 2020; Ologbose and Dick, 2021). In addition, the reference interval values have been shown to differ among the scholarly established values (Jain, 1993; Thrall et al., 2012; Maoba et al., 2021). Moreover, there is no information regarding the hematological parameters and reference interval of the Hungarian breed and TSL which can be used to evaluate the health status and physiological stress status in the farm. Hence, this study intends to determine, whether there is any difference in hematological parameters between HPC and TSL chicks aged 28 days and calculate the indicative reference interval values of each genotype.

MATERIALS AND METHODS

The trial was carried out at the Kismacs Experimental Station of Animal Husbandry of the University of Debrecen under the institute of agricultural research and educational farm between June and July 2021. The experimental protocol was

according to the international, national and institutional guidelines for the use of animals. All the experimental procedures were in accordance with the ethical standards of the institution and approved National Authority (National Scientific Ethical Committee on Animal Experimentation) through the local veterinary ethics committee and the institutional animal care and use committee (6/2021/DEMA'B).

Experimental feeding and facilities

This trial was part of a larger experiment where about 200 chicks for HPC and TSL were raised. The chicks were hatched at the farm after incubation at standard temperature and humidity (37.8 °C and 60% relative humidity, PLM 3600, PL Maschine KFT Budapest-Hungary). Chicks were assigned in groups for experimental feeding based on their body weight immediately after hatching. The chicks were raised in groups with 128 cm square per bird. For TSL chicks, chicks were reared in four replicates with four chicks per replicate while for HPC chicks were reared in two group (larger pen) with 20 chicks each. The TSL group received 100% of the required methionine, which was similar to the methionine content provided for HPC chicks. The pens were bedded with a wood shaving litter with a height of approximately 4 cm. Extra heating in the pen was provided by an infrared lamp (optima plus II 175 W) and the temperature in the pen was maintained according to the poultry standard condition recommendation. The water in the drinkers was changed daily and wood-shaving, as well as droppings of chicks in the feed were removed daily and fresh feed was provided. The HPC chick's diet was designed based on the genotype requirement and it has been used by the farm for feeding of the same genotype. The diet's main composition included maize, soybean meal, and wheat and sunflower meal, and their nutrient content is summarized in *Table 1*. TSL chicks received the diets formulated to meet their requirements with the main components including maize, soybean meal, fish meal, and sunflower oil. The nutrient content of the two diet are summarized in the *Table 1*. All chicks were allowed free access to feed and water for 28 days.

Table 1: Nutrient content summary as used in the trial (% , as-fed basis)

Nutrient ingredient	TSL	HPC
AMEn, MJ/kg	12.35	11.3
CP %	20	18.12
Lys %	1	0.9
Met %	0.4	0.52
Ca %	1	1.02
available P %	0.48	0.59
Na %	0.17	0.15

Abbreviations: AMEn- nitrogen corrected apparent metabolize energy, CP-crude protein, Lys – Lysine, Met- Methionine, Ca – Calcium and Na- Sodium

Blood sample collection.

At 28 days of age, 16 birds (8 birds per genotypic group) were randomly selected for blood sampling, but equally from each pen. Blood was obtained (1 mL) from the brachial vein into a disposable 5 mL tube embedded with anticoagulant EDTA. Blood was collected from two birds from each replicate of the four TSL groups and four birds of the two of HPC groups between 09:00 to 11:00 hours. The blood collection procedure was performed according to Kelly and Alworth (2013). Briefly, the brachial venipuncture was made by two people working together, where the bird was placed in a lateral position with its feet facing the phlebotomist. Then the wing was lifted and gently spread/stretched to expose the blood vein underside of the wing. Then the phlebotomist withdraws the blood by inserting a 26G needle in the vein. The venipuncture site was then gently pressed with cotton wool to stop blood bleeding before returning the bird to the cage. The blood samples were immediately placed in the cooler with ice and transported to the laboratory, where stored in the fridge before analysis. All samples were analyzed within 24 hours from sampling time.

Hematological analysis

The hematological parameters were analyzed by using an automated hematology analyzer (Urit-3000Vet Plus, Orvostechnika Ltd., Budapest). All blood samples were allowed to come to room temperature before analysis. A pre-diluent method was employed, where 20 µL of blood was diluted to 1 mL dilution buffer (Dia-Diluent-D) and then gently mixed by hand shaking before reading (according to UritT-3000Vet Plus Automated Hematology Analyzer Operation Manual). Three technical replicates were measured for each sample. The following were examined; red blood cell (RBC, $10^{12}/L$), hemoglobin (Hb, g/dL), hematocrit (Ht, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), platelet count (PLT, $10^9/L$), white blood cells (WBC, $10^9/L$), lymphocyte percentage and count (LYM % and LYM #), mid-range (eosinophil + basophil) percentage and number (MID % and MID #), and lastly granulocyte percentage and number (GRAN % and GRAN #).

Statistical analysis

Data were checked for normality using Kolmogorov-Smirnov test. All data were analyzed by SPSS version 28 software. Hematological parameters were compared by an independent student t-test between the two genotypes. The significant difference was considered at a $P < 0.05$ significant level. The hematological reference intervals for TSL and HPC chicks were calculated in Microsoft Excel 2013 using Reference Value Advisor V2.1 (Geffré et al., 2011). The Reference Value Advisor includes five methods: the standard, Robust, standard Box-Cox transformation, Robust Box-Cox transformation, and nonparametric. The Box-Cox transformation method is used when the data are neither normal nor symmetric.

In Our study the robust method with a Box-Cox transformation was used to calculate the reference interval since the sample was small ($n < 20$).

RESULTS AND DISCUSSION

Reference intervals for all hematological parameters were higher in the TSL chicks than that in the HPC chicks except for following MCH, MCHC, LYM, and MID (Table 2). The RBC reference interval was estimated for TSL and HPC in which 2.6 to 3.7 $10^{12}/L$ reference values were obtained for TSL and 2.2 to 3.5 $10^{12}/L$ for HPC chicks (Table 2). The WBC reference intervals value was higher in the TSL chicks

than in the HPC chicks. Interestingly, the 90% confidence interval (CI) for the lower limit of the TSL chicks was higher ($71.4-85.4 \times 10^9 /L$) as compared to that of the HPC chicks ($47.5-70.4 \times 10^9 /L$), but not the same was observed for the 90% CI for the upper limit. Like in the RBC and WBC reference interval values, the same pattern for the Hb, Ht, and MCV has been observed between the TSL chicks and HPC chicks. However, the HPC chicks had higher LYM reference interval values (42.4–54.7%) than the TSL chicks (39.3–40%) (Table 2). Unlike the other hematology parameter, the reference interval of MCHC, MCH and MID % was the similar in the both genotypes.

Table 2: Hematological parameter reference intervals of the TSL and HPC at 28 days of age

Analyte	SI units	RI	90% CI for a		RI	90% CI for a	
			lower limit	upper limit		lower limit	the upper limit
			TSL		HPC		
WBC	$10^9/L$	78.8–104.4	71.4–85.4	97.2–110.5	59–101.3	47.5–70.4	89.7–111.8
RBC	$10^{12}/L$	2.6–3.7	2.3–2.9	3.4–4	2.2–3.5	1.9–2.5	3.1–3.8
Hb	g/dL	10.6–14.4	9.5–11.5	13.3–15.4	8.4–13.8	6.9–9.7	12.2–15.1
Ht	%	34–46.3	30.8–37.3	42.9–49.4	27.5–42.8	23.5–31.6	38.5–46.6
MCV	fL	114.9–141.2	108.2–122.8	134.0–147.7	112.8–136.2	106.8–119.8	129.8–142.1
MCH	pg	36.5–43	34.8–38.2	41.2–44.4	35.8–42.2	34–37.5	40.5–43.7
MCHC	g/dL	30.1–32.1	29.6–30.6	31.5–32.5	30.5–32.2	30–30.9	31.7–32.6
LYM	%	39.3–40	36.9–42	46.4–51.3	42.4–54.7	39.4–46	51.1–57.6
MID	%	11.8–14.1	11.3–12.5	13.5–14.7	12.2–14.3	11.7–12.8	13.7–14.8
GRAN	%	37.1–48.7	34.3–40.4	45.5–51.6	32.1–44.4	28.9–35.4	41.2–47.5
LYM	$10^9/L$	36.2–43.2	34.5–38.2	41.3–44.8	31.7–45.3	28.2–35.3	41.5–48.3
MID	$10^9/L$	10.3–13.3	9.5–11.1	12.4–14.1	7.4–13.8	5.8–9.3	12–15.3
GRAN	$10^9/L$	28–52.3	21.8–34.6	45.8–58.4	19.1–43.1	13.5–25.7	37–49.3

Abbreviations: RBC-red blood cells, Hb-hemoglobin, Ht-hematocrit, MCV-mean corpuscular volume of red blood cell, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, WBC-white blood cells, LYM-lymphocyte, MID-mid-range, GRAN-granulocyte, RI-reference intervals, CI-confidence interval, TSL-TETRA-SL, and HPC-Hungarian partridge coloured chicken.

The TSL chicks’ blood had statistically higher RBC, WBC, Hb, Hct, Plt, and GRAN than HPC chicks ($p < 0.05$) (Table 3). The mean RBC count of TSL chicks ($3.2 \times 10^{12} /L$) differed significantly ($p < 0.05$) from that of HPC chicks ($2.8 \times 10^{12} /L$). The TSL chicks had the higher values of RBC with the maximum value of $3.5 \times 10^{12} /L$ compared to that of HPC chicks where the highest was $3.2 \times 10^{12} /L$. Similarly, the mean values of Hb, Ht, WBC Plt, and GRAN was statistically higher in the TSL chicks than in the HPC chicks ($p < 0.05$). In contrasting, the mean value of HPC chicks LYM ($48.6 \pm 0.886\%$) was higher than of that TSL chicks ($44.1 \pm 0.704\%$) ($p < 0.05$). There was no significant difference in the MCV, MCH, MCHC, and MID mean values between the TSL chicks and HPC chicks ($p > 0.05$) (Table 3).

All the reference intervals values of the hematological parameters were within the normal range of the health chicken (Jain 1993; Thrall et al., 2012). The reference interval values of RBC are within the normal reference interval 2.5–3.5 and 1.3–4.5 reported by Jain (1993) and Thrall et al. (2012), respectively.

However, the TSL chicks had statistically higher mean value of RBC ($10^{12}/L$) than HPC chicks. The variation of RBC between the chicken genotypes/strains has also been reported by Ifelayo et al. (2020). But also few experiments had not found any effect of RBC among genotypes (Adeyemo et al., 2018; Baudouin et al., 2021). Moreover, this observed range in this study agrees with the normal range of chickens reported by several studies (Simaraks, et al., 2004; Ding et al., 2019; Maoba et al., 2021). Like RBC, hemoglobin concentration in the blood was significantly different between the genotypes ($p < 0.05$). The TSL chicks had higher hemoglobin concentrations with the ranges of 11.8 to 13.8 g/dL than HPC chicks, whose hemoglobin values ranged from 9.4 to 12.5 g/dL. These results suggest the difference between the two genotypes on oxygen consumption rate, the TSL genotype seem to be improved along with the oxygen consumption for high production performance. This is in line with findings reported by Mohanty and GayatriAcharya (2020), who reported the hemoglobin value ranges from 8.3 to 5.4 for indigenous and broiler chickens respectively.



Table 3: Blood parameters of the TSL and HPC at 28 days of age

Blood parameter	Genotype	Mean	SEM	t	df	p-value																																																																																																																														
RBC ($10^{12}/L$)	TSL	3.2	0.081	2.44	12	0.031*																																																																																																																														
	HPC	2.8	0.100				Hb (g/dL)	TSL	12.5	0.279	2.97	12	0.012*	HPC	11.1	0.385	Hematocrit (%)	TSL	40.2	0.888	3.53	12	0.004*	HPC	35.1	1.106	MCV (fL)	TSL	128.0	1.899	1.40	12	0.187	HPC	124.5	1.692	MCH (pg)	TSL	39.7	0.479	1.10	12	0.293	HPC	39.0	0.476	MCHC g/dL	TSL	31.1	0.144	-1.35	12	0.202	HPC	31.3	0.125	Platele(PLT) ($10^9/L$)	TSL	3.2	0.548	2.43	10	0.035*	HPC	1.7	0.279	WBC ($10^9/L$)	TSL	91.5	1.837	3.19	12	0.008*	HPC	80.1	3.054	LYM%	TSL	44.1	0.704	-3.90	12	0.002*	HPC	48.6	0.886	MID%	TSL	13.0	0.170	-1.26	12	0.233	HPC	13.2	0.151	GRAN%	TSL	42.9	0.830	3.85	12	0.002*	HPC	38.2	0.893	LYM# ($10^9/L$)	TSL	39.7	0.509	1.09	12	0.298	HPC	38.5	0.977	MID# ($10^9/L$)	TSL	11.8	0.216	2.28	12	0.042*	HPC	10.6	0.473	GRAN# ($10^9/L$)	TSL	40.2	1.746	3.69	12
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Abbreviations: RBC-red blood cells, Hb-hemoglobin, Ht-hematocrit, MCV-mean corpuscular volume of red blood cell, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, WBC-white blood cells, LYM-lymphocyte, MID-mid-range, GRAN-granulocyte, TSL-TETRA -SL, and HPC-Hungarian partridge coloured chicken. Data are Mean \pm SEM, n=7. * -significant

Hematocrit (Ht) value (also known as packed cell volume-PCV) concerning the two genotypes was determined, the results indicate a significant difference between the TSL chicks and HPC chicks (40.2 vs 35.1%) ($p < 0.05$). The reference interval for the hematocrit value of TSL chicks was 34–46% while that of HPC chicks was 27.5–42.8%. This reference interval value falls within the normal physiological values reported by (Ladokun et al., 2008; Thrall et al., 2012; Mulatu et al., 2019). The lower value of Ht may suggest the defect in hematopoiesis process. The higher value of Ht in TSL chicks than in HPC chicks corroborates with findings by Baudouin et al. (2021) who found broilers higher value compared to local breeds. In birds the Ht value, are used to examine the anemia and polycythemia condition if the Ht is below 22% in local chicken is considered anemia and below 35% for broilers (Jain, 1993; Abdulazeez et al., 2016; Baudouin et al., 2021). In this study no anemic condition observed, with all birds had the values within the acceptable range. The difference observed here may also explains the difference in their performance and physiological status. Therefore the higher Ht mean value in TSL chicks suggest the higher production

performance traits of the genotype as compared to the HPC chicks.

The values of RBC indices such as Hb, and Ht for TSL chicks may reflect their better performance relative to HPC chicks. However, the other RBC indices such as MCV, MCH, and MCHC were not significantly different between the two genotypes. This study corroborates with (Ladokun et al., 2008; Peters et al., 2011), who observed no effect of genotype on MCV, MCH, and MCHC between the Nigerian indigenous naked neck and normally feathered cocks. This is contrary to the findings of Mohanty and GayatriAcharya (2020) who showed genotype influence on the MCV and MCH of the indigenous chicken and broiler. The reference interval of both genotypes in this study falls within the normal ranges of the healthy chicken (Jain, 1993; Simaraks et al., 2004; Maoba et al., 2021). The MCH refers to the blood carrying ability of the RBC carrying capacity, this results suggest that all genotypes had similar respiratory function. (Abdulazeez et al., 2016). The blood platelets were higher in TSL chicks than in HPC chicks. The blood platelets are involved with blood clotting, the lower platelet count indicates the slower

process of blood clotting which can cause excessive loss of blood in case of injury (Etim et al., 2014).

The WBC was determined in this trial, showed that TSL chicks had a higher mean WBC value than HPC chicks (91.5 vs 80.1 x 10⁹/L) and the reference interval values were much higher in TSL (78.8–104.4 x 10⁹/L) than in HPC chicks (59–101.3). However, these reference values are within the normal range of chicken reported by others (Thrall et al., 2012). The higher WBC counts of TSL chicks indicates the immunological status of the genotype is much superior to that of the HPC genotype. The lower the WBC counts the more susceptible to avian diseases (Thrall et al., 2012; Etim et al., 2014). These findings agree with the findings of Chineke et al. (2006) and Nosike et al. (2017). Peters et al. (2011) found no genotype effect on the WBC, however, may be due to different nutrition compositions in their experiment. This may explain the high performance of TSL chicken has been improved along with the immunological capability. Generally high WBC indicates the high capacity of the body to generate the antibody against pathogens.

The WBC indices such as lymphocytes, granulocytes, and mid-range (eosinophil + basophils) were in contradiction with the overall WBC counts pattern between the two genotypes. TSL chicks lower lymphocyte percentage and HPC chicks but not the lymphocyte counts. This indicates that genotypes had an effect on the differential WBC counts, which indicates the type of WBC in the blood. The lymphocytes are types of WBC, which are fractions of natural killer cells that kill abnormal cells of the body or cells that show signs of stress. Lymphocytes play a great role in both the innate and adaptive immunity of animals. The higher lymphocytes mean a stronger and better able to fight viral and bacterial infection as it is involved in both an immediate and delayed role in response to infection (Nosike et al., 2017). Interestingly, the granulocytes (both counts and percentages) were lower in HPC than in TSL chicks. The proportion of mid-range (eosinophils + basophils)

was the same in both genotypes. This indicates the chicks have the same stress exposure as the basophils are associated with stress reactions or reactions to allergies (Nosike et al., 2017).

CONCLUSIONS

The hematological parameters differed between the TSL chicks and HPC chicks at 28 days of age. The TSL genotypes had been improved along with physiological capacity to support their higher production performance with narrow range of reference values which indicates the tolerance to physiological stress. The HPC genotype has conserved their genes responsible for (higher lymphocyte percentage) resistance to avian diseases (bacterial and viral infection) and wide range reference interval values explains their ability to survive in harsh environment (extremely cold and hot weather). The difference in reference interval values suggest more research are required with larger sample size to establish the hematological reference values of the HPC and TSL chicken at different ages that can be used to assess their physiological status.

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Disclosures

The authors declare no conflict of interest.

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