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REFERENCE VALUES OF HEMATOLOGICAL, BIOCHEMICAL AND ENDOCRINOLOGICAL PARAMETERS IN THE BLOOD OF PIGLETS AGED 1 AND 21 DAYS

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SUMMARY

The purpose of this paper is to determine the reference ranges for hematological, biochemical and endocrinological parameters in the blood of the commercial F1 Landrace x Yorkshire and Yorkshire x Landrace piglets aged 1 and 21 days. A total of 72 animals were enrolled in this study. The following reference ranges were determined for the blood parameters considered (day 1 vs. day 21): WBC 10.0-12.4 vs. 11.5-13.7 $\times 10^9/L$; RBC 3.72-4.06 vs. 5.25-5.73 $\times 10^{12}/L$; HGB 71.4-79.0 vs. 100-110 g/L; HCT 24.4-26.5 vs. 35.7-39.1 %; MCV 64.4-66.0 vs. 72.7-74.8 fL; MCH 20.9-21.7 vs. 24.7-25.6 pg; MCHC 321-329 vs. 367-378 g/L; AST 78.5-98.9 vs. 51.8-66 U/L; ALT 72.5-84.5 vs. 65.9-76.5 U/L; CHOL 2.41-2.71 vs. 3.81-4.27 mmol/L; TGC 1.55-1.93 vs. 1.85-2.25 mmol/L; GLU 5.58-6.24 vs. 5.97-6.67 mmol/L; TPROT 55.2-59.4 vs. 65-69.9 g/L; ALB 13.4-15.1 vs. 27.8-30.9 g/L; GLOB 41.3-44.8 vs. 35.3-40.9 g/L; UREA mmol/L; TBIL 36.6-44.5 vs. 29.4-40.8 $\mu\text{mol}/L$; ALFAAMYLAASE 897-998 vs. 851-949 U/L; LIPASE 74.1-79.7 vs. 88.3-98.1 U/L; CORT 140-196 vs. 41.4-63.8 ng/mL; INSUL 4.04-5.68 vs. 1.71-2.41 $\mu\text{U}/L$. The RBC, HGB, HCT, MCV, MCH, and MCHC values recorded were found to be higher in the piglets aged 21 days than those in the piglets aged 1 day. The total leukocyte count did not differ between the experimental groups. Lower AST, ALT, globulin, cortisol and insulin values were detected in the piglets aged 21 days than those in the piglets aged 1 day. However, the values of triglycerides, cholesterol, total protein, albumin and lipase were found to be higher in the piglets aged 21 days than those in the piglets aged 1 day. The glucose, alpha amylase and total bilirubin (TBIL) values did not differ significantly between the experimental groups. The Kolmogorov-Smirnov test conducted shows that there are significant differences in the frequency distribution of most of the blood parameters tested in the pigs aged 1 and 21 days, which confirms that a separate reference interval must be determined and applied to the respective piglet ages considered. The establishment of reference values for different age categories can facilitate the assessment of health status and productive performance of farm piglets.

Key words: reference population, hematology, biochemistry, hormones, piglets, weaning

INTRODUCTION

Reference values of blood parameters play an important role in assessing the health and productivity of animals. Intense growth of piglets, different breeds and their biological properties, as well as metabolic changes after birth, can significantly affect the blood parameters in pigs. Recent studies have shown that nutrition, weight gain (especially fat gain) and genotype exert significant effects on the metabolic blood parameters of pigs (Abeni et al., 2018; Stevačević et al., 2019; Camacho-Rea et al., 2010; Newell Fugate et al., 2014; Al-Mashhadi et al., 2018). In addition to the influence of race and age, the impact of the farm type on the value of selected blood parameters in pigs has been analyzed and demonstrated in a number of papers (Ježek, 2018). Significant impacts of health, stress and pathogen exposure on blood parameter values have also been reported in the literature (Evans, 1994; Klem et al., 2010; Friendship et al., 1984). Hematological and biochemical parameters in the blood are very important for

quantitative genetic selection and genetic progression. The results of Mpetile et al. (2015) show that 12 out of 15 hematological parameters of the classic blood count in Yorkshire pigs have medium or high heritability. Lingaas et al. (1992) found a heritability of $h^2 > 0.4$ for amylase, total protein, globulin and AST values. Biochemical parameters can be useful for estimating pig growth as certain deviations in these values can indicate smaller and higher birth weights in pigs (Kabalin et al., 2017). Separation (weaning) can lead to significant changes in piglet blood parameters (Yu et al., 2019).

In modern pig production, the data obtained from laboratory blood tests are rarely used, most likely because of low costs of an individual animal and the fact that such data of great importance cannot be obtained from individual blood tests. However, due to the use of pigs for various scientific purposes and the ability to evaluate health and productivity based on blood parameters, reference values of more important blood parameters should be determined in commercial pigs on the first day of life and at separation for weaning.

MATERIAL AND METHODS

A total of 72 F1 Landrace x Yorkshire and Yorkshire x Landrace piglets were enrolled in the study. Blood samples were taken from *vene jugularis externa (regio colli lateralis)* on days 1 and 21 after birth. Within 2 hours after sampling, the EDTA vacutainer tubes containing the blood samples were sent to the Laboratory for Pathological Physiology of the Faculty of Agriculture in Novi Sad (the Department of Veterinary Medicine) for immediate laboratory processing.

Hematological analyzes were performed using a NIHON KOHDEN analyzer (Japan) with a software for pig blood analysis. A standard blood count was performed.

Using a Chemray-Rayto analyzer (China) and the Byosystems Standard Kit (Spain), the levels of the following blood plasma parameters were determined: glucose, total protein, albumin, globulin, cholesterol, triglycerides, total bilirubin, AST, ALT, urea, alpha-amylase and lipase.

Insulin and cortisol concentrations were determined using a TOSOH AIA 360 immunochemical analyzer (Japan).

The descriptive statistical properties of the data frequency distribution were determined by measuring their central tendencies (mean, median) and variations (standard deviation, variance, minimum, maximum, kurtosis, skewness). The reference values were determined as the 95% CI for each respective parameter considered. Differences in the parameter values between the two experimental age groups of piglets were determined by t-tests. The frequency distribution of the data obtained was presented graphically for each respective piglet age considered to determine visual differences in the parameter value trends between the two age categories.

RESULTS AND DISCUSSION

The following reference ranges were determined for the blood parameters considered (day 1 vs. day 21): WBC 10.0-12.4 vs. 11.5-13.7 $\times 10^9/L$; RBC 3.72-4.06 vs. 5.25-5.73 $\times 10^{12}/L$; HGB 71.4-79.0 vs. 100-110 g/L; HCT 24.4-26.5 vs. 35.7-39.1 %; MCV 64.4-66.0 vs. 72.7-74.8 fL; MCH 20.9-21.7 vs. 24.7-25.6 pg; MCHC 321-329 vs. 367-378 g/L; AST 78.5-98.9 vs. 51.8-66 U/L; ALT 72.5-84.5 vs. 65.9-76.5 U/L; CHOL 2.41-2.71 vs. 3.81-4.27 mmol/L; TGC 1.55-1.93 vs. 1.85-2.25 mmol/L; GLU 5.58-6.24 vs. 5.97-6.67 mmol/L; TPROT 55.2-59.4 vs. 65-69.9 g/L; ALB 13.4-15.1 vs. 27.8-30.9 g/L; GLOB 41.3-44.8 vs. 35.3-40.9 g/L; UREA mmol/L; TBIL 36.6-44.5 vs. 29.4-40.8 $\mu\text{mol/L}$; ALFAAMYLASE 897-998 vs. 851-949 U/L; LIPASE 74.1-79.7 vs. 88.3-98.1 U/L; CORT 140-196 vs. 41.4-63.8 ng/mL; INSUL 4.04-5.68 vs. 1.71-2.41 $\mu\text{U/L}$ (Tab. 1, 2).

The RBC, HGB, HCT, MCV, MCH, and MCHC values recorded were found to be higher in the piglets aged 21 days than those in the piglets aged 1 day. The total leukocyte count did not differ between the experimental groups. Lower AST, ALT, globulin, cortisol and insulin values were detected in the piglets aged 21 days than those in the piglets aged 1 day. However, the values of triglycerides, cholesterol, total protein, albumin and lipase were found to be higher in the piglets aged 21 days than those in the piglets aged 1 day. The glucose, alpha amylase and total bilirubin (TBIL) values did not differ significantly between the experimental groups.

The Kolmogorov-Smirnov test conducted shows that there are significant differences in the frequency distribution of most of the blood parameters tested in the pigs aged 1 and 21 days, which confirms that a separate reference interval must be determined and applied to the respective piglet ages considered. The establishment of reference values for different age categories can facilitate the assessment of health status and productive performance of farm piglets. The statistical significance of all the parameter values tested was $p < 0.001$. As the statistical significance of the GLU, UREA, ALFAAMYLASE and TGC values was not established, no difference was found in the frequency distribution of these parameters relative to the piglet age categories considered.

Graphical representations of the parameter value frequency distribution are shown in Figures 1-21. Each figure consists of two frequency distributions indicating measurements performed on day 1 and 21 after birth. The frequency distribution obtained facilitates determining the exact percentage of piglets with an absolute deviation of the blood parameter values on day 21 after birth compared to those measured on day 1 after birth.

The results obtained show that differences in most of the metabolic parameter values arise as a consequence of the redistribution of data frequency distribution when the values are within the same range. However, in the case of the values that do not overlap in distribution such as RBC, HGB, MCV, MCH, MCHC, CHOL, ALB and insulin values, a reference range must be established for each age category separately. The parameter values with overlapping distribution can be analyzed within the same range by producing a pool of values for both piglet age categories considered. According to Cooper et al. (2014), the hematological and biochemical blood parameters are influenced by various factors such as age, gender, diet, genotype, season and stress.

Table 1. Blood parameters in the piglets aged 1 day

Day 1	Reference range 95% CI	Mean	Std. Error of Mean	Median	Std. Deviation	Variance	Skewness	Kurtosis	Minimum	Maximum
WBC	10.0-12.4	11.20	0.61	9.55	5.14	26.44	1.81	4.88	3.60	33.00
RBC	3.72-4.06	3.89	0.09	3.99	0.75	0.56	-0.32	0.12	2.15	5.80
HGB	71.4-79.0	75.18	1.95	78.00	16.54	273.47	-0.55	-0.04	28.00	106.00
HCT	24.4-26.5	25.42	0.54	25.87	4.58	20.96	-0.60	0.09	13.90	35.85
MCV	64.4-66.0	65.20	0.39	65.00	3.30	10.92	-0.12	0.11	57.50	73.70
MCH	20.9-21.7	21.72	0.21	21.75	1.80	3.24	0.43	0.85	17.10	26.80
MCHC	321-329	324.81	2.16	325.50	18.33	336.10	0.16	0.87	272.00	380.00
AST	78.5-98.9	88.74	4.97	79.00	42.19	1779.96	2.04	5.25	28.00	250.00
TGC	1.55-1.93	1.74	0.10	1.67	0.81	0.65	1.23	2.98	0.50	5.05
CHOL	2.41-2.71	2.56	0.08	2.54	0.66	0.43	0.34	-0.53	1.44	4.06
ALT	72.5-84.5	78.53	3.05	73.00	25.89	670.53	1.69	5.65	34.00	199.00
GLU	5.58-6.24	5.91	0.17	5.90	1.42	2.01	0.49	0.99	3.10	10.70
TPROT	55.2-59.4	57.29	1.07	57.00	9.12	83.20	-0.27	-0.59	35.00	73.00
UREA	3.35-4.41	3.88	0.27	3.35	2.30	5.30	1.26	2.66	0.50	13.10
ALFA AMYLASE	897-998	947.62	25.65	954.15	217.66	47374.03	0.86	2.02	518.10	1782.70
ALB	13.4-15.1	14.22	0.44	14.03	3.70	13.67	0.17	-0.34	6.26	23.15
GLOB	41.3-44.8	43.07	0.90	42.95	7.60	57.74	-0.08	-0.77	25.20	56.35
LIPASE	74.1-79.7	76.93	1.81	76.75	15.32	234.62	0.00	-0.17	41.00	115.90
TBIL	36.6-44.5	40.55	2.57	34.84	21.81	475.76	2.52	6.90	19.00	136.06
CORT	140-196	168.24	18.23	149.97	154.69	23929.10	0.31	-1.33	1.00	492.17
INSUL	4.04-5.68	4.86	0.53	4.50	4.46	19.85	0.98	1.33	1.00	20.90

Table 2. Blood parameters in the piglets aged 21 days (at weaning)

Day 21	Reference range 95% CI	Mean	Std. Error of Mean	Median	Std. Deviation	Variance	Skewness	Kurtosis	Minimum	Maximum
WBC	11.5-13.7	12.62	0.72	11.45	6.14	37.75	1.82	5.30	3.42	38.94
RBC	5.25-5.73	5.49	0.12	5.41	1.02	1.04	-0.05	-0.31	3.04	7.65
HGB	100-110	104.90	2.45	109.44	20.78	431.81	-0.61	0.55	42.92	153.92
HCT	35.7-39.1	37.39	0.88	37.96	7.47	55.82	-0.37	-0.19	18.39	54.49
MCV	72.7-74.8	73.76	0.54	73.43	4.61	21.27	0.32	-0.43	64.79	85.10
MCH	24.7-25.6	25.16	0.24	25.30	2.05	4.22	0.53	0.87	20.70	31.36
MCHC	367-378	372.38	2.73	368.95	23.14	535.54	0.37	1.18	307.36	446.50
AST	51.8-66	58.87	3.63	50.22	30.84	950.84	2.10	5.48	17.36	177.50
TGC	1.85-2.25	2.05	0.10	2.01	0.86	0.75	0.83	1.26	0.57	4.90
CHOL	3.81-4.27	4.04	0.12	3.95	0.98	0.97	0.32	-0.55	2.23	6.29
ALT	65.9-76.5	71.19	2.70	67.59	22.90	524.39	0.77	2.46	3.85	153.22
GLU	5.97-6.67	6.32	0.18	6.28	1.50	2.26	0.36	0.86	3.27	11.30
TPROT	65-69.9	67.45	1.25	68.40	10.64	113.22	-0.25	-0.46	42.00	87.60
UREA	3.32-4.4	3.86	0.27	3.52	2.33	5.41	1.60	5.10	0.47	14.54
ALFA AMYLASE	851-949	900.17	24.97	930.97	211.84	44875.08	-0.49	1.30	155.00	1356.70
ALB	27.8-30.9	29.34	0.79	28.99	6.71	45.07	0.19	0.12	15.05	47.09
GLOB	35.3-40.9	38.11	1.44	39.27	12.25	150.06	0.35	-0.08	12.53	68.38

LIPASE	88.3-98.1	93.23	2.50	95.03	21.20	449.50	-0.05	-0.54	45.92	139.12
TBIL	29.4-40.8	35.07	2.90	30.79	24.58	604.07	2.66	8.62	7.81	149.66
CORT	41.4-63.8	52.63	5.69	52.00	48.31	2333.67	0.55	-0.43	1.00	181.00
INSUL	1.71-2.41	2.06	0.18	2.20	1.51	2.27	0.05	-0.65	1.00	5.90

Figures 1-21. Graphical representations of the parameters tested

The biochemical parameter values obtained are consistent with the reference values found in the literature (Friendship et al., 1984; Klem et al., 2010; Belić & Cincović et al., 2012; Harvey, 2012; Buzzard et al., 2013; Perri et al., 2017). The reference ranges provided by Friendship et al. (1984) show that upper and lower reference limits differ depending on the age and productive category of pigs. Moreover, the reference ranges for hemoglobin concentration, hematocrit, erythrocyte count, the lower reference value of MCV, MCH and MCHC and the mean value of total proteins and albumin were found to increase in weaner pigs, feeder pigs, gilts and sows. Czech et al. (2017) argued that RBC, HGB and Ht value increased linearly with the age of piglets. The linear relationship between red blood cell parameters and body weight can be explained by the increasing blood volume and the amount of hemoglobin and hematocrit in the body (Crichton, 2006). Kotosová et al. (2014) found no significant differences in RBC values measured in different seasons. Hemoglobin and hematocrit are indirect indicators of the iron status of piglets. Iron is of paramount importance to piglet growth, thus iron supplementation is a standard procedure in pig production. Pigs with higher hemoglobin levels have a higher average daily gain (ADG) and a higher average daily food intake (ADFI) at weaning (Gentry et al., 1997). The far-reaching significance of red bloodline in piglet growth has been shown in studies conducted by Peters & Mahan (2008) and Jolliff & Mahan (2011). The authors report that pigs that did not receive iron had a lower body weight at the end of the weaning phase (day 70), which is a consequence of lower ADG levels in the weaning period and less weight at the end of weaning. Chmielowiec-Korzeniowska et al. (2012) argued that protein, creatinine and urea concentrations increase with the age of piglets, whereas no significant changes were recorded in bilirubin, cholesterol, AST, and ALT levels. Lower birth weight (<1000g) piglets were found to have lower glucose and creatinine concentrations 2 and 3 weeks after birth (Kabalin et al., 2017). However, triglyceride and urea concentrations were higher in lighter piglets at 2 weeks of

age, indicating that body weight and weight gain are related to the metabolic status of piglets until weaning (which should be taken into account when interpreting individual parameter values and reference ranges). Separation of piglets is highly stressful so changes in urea, creatinine and fatty acid values, as well as in some immune parameters values (Yu et al., 2019), may be expected shortly after weaning. Accordingly, time must be taken into account when considering reference ranges in the period that has elapsed since separation.

CONCLUSION

The established reference values for the commercial F1 Landrace x Yorkshire and Yorkshire x Landrace piglets aged 1 and 21 days indicate deviations in the piglet age function. Reference values established for different age categories can be helpful in assessing the health status and productive characteristics of farm pigs.

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